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The Effects of Grass Silage Additive Type and Barley Grain Preservation Method on Rumen Function, Microbial Ecology, and Energy Metabolism of Dairy Cows

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Abstract: The effects of grass silage and barley grain preservation methods on dairy cows were evaluated using four Nordic Red dairy cows placed in respiration chambers in a 4 × 4 Latin square. Silage was conserved using a formic acid-based product (AS) or a homofermentative lactic acid bacteria inoculant (IS), while grains were dried (DB) or crimped and ensiled (EB). Fermentation profile of silages and the chemical composition of the mixed diets were very similar. The dietary treatments did not affect feed intake, milk production, and rumen fermentation except molar proportion of butyrate, and energy metabolism. Digestibility of dry matter and organic matter were higher ($p < 0.05$) and that of crude protein was lower ($p < 0.05$) for AS than IS. Feeding EB compared to DB decreased ($p < 0.05$) diet organic matter and starch digestibility. The cows receiving AS tended ($p = 0.06$) to emit more methane per day than those receiving IS, but methane yield and intensity were not different between dietary treatments. Bacteria alpha diversity was higher ($p < 0.01$) in barley samples than grass silages and was not affected by the diet in rumen samples. All freshly prepared rations were dominated by *Lactobacillaceae*, *Erwiniaceae*, and *Pseudomonadaceae* but rations based on AS than IS remained more stable over 2 days. In conclusion, grass silage and barley grain preservation methods did not affect the measured parameters in dairy cows and the preservation method can be selected based on practical on-farm factors.

Keywords: crimping; high-moisture grain; methane; feed microbiota; formic acid; lactic acid bacteria; rumen fermentation; rumen microbiota; silage fermentation

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1. Introduction

Conservation of feeds for modern ruminant production needs to fulfill many requirements besides providing constant nutrient supply all year around irrespective of varying environmental conditions. Specifically, the efficiency of nutrient utilization by animals, feed and food safety, occupational health of the people involved in the feed chain, minimizing nutrient losses and climate impacts during harvesting and storage of feed, low costs, and low energy use can be mentioned. Conserving forages by ensiling has become the mainstream method at the expense of grazing or drying (hay-making) in most intensive milk production regions [1] and numerous experiments and reviews have been dedicated to improving the preservation characteristics of ensiled forages [2,3]. Northern European livestock production systems are characterized by feeding high-forage diets based on grass silage supplemented with concentrates. Ensiling as a conservation method for forages is a common practice in dairy production. The modifications of grass silage composition [1] caused by different conservation methods can affect animal performance. Restricting the extent of silage fermentation by, e.g., formic acid application increases the voluntary silage dry matter (DM) intake [4] and subsequently milk production [5] of dairy cows. Also, the profile of nutrients provided to the rumen microbiota can vary greatly depending

on the choice of silage additive. Franco et al. [6] demonstrated, in a pilot-scale ensiling experiment, that the same grass material treated with a homofermentative LAB inoculant contained 76 g water soluble carbohydrates (WSC) and 115 g lactic acid per kg DM compared with the respective concentrations of 195 and 2 g/kg DM for a silage treated with a commercial application level of a formic and propionic acid-based additive. Similar effects, although to a smaller extent, can be expected when dry and high-moisture ensiled grains are compared due to a partial conversion of carbohydrates to fermentation end products during preservation of moist grains [7–9]. Depending on the nature of substrates provided in the diet, different fermentation end products produced by the rumen microbes are provided to the host animal [10]. Therefore, it is expected that the extent of feed fermentation during preservation influences the rumen fermentation and subsequently the host animal metabolism. Nevertheless, an understanding of how these microorganisms from interconnected feed-animal ecosystems can contribute to the overall animal production traits is still fragmented and requires further investigation.

Silage inoculants based on lactic acid bacteria (LAB), especially homofermenters, produce primarily lactic acid stimulating the growth of lactic acid utilizing bacteria in the rumen, which increases ruminal production of propionic acid and subsequently decreases hydrogen availability for methane production [11]. In addition, LAB have influenced methane production especially under in vitro conditions [11,12]. The in vivo results indicating the positive effects of silages inoculated with LAB on enteric methane production are limited which warrants conducting such experiments to evaluate the potential of silages inoculated with homofermentative LAB on enteric methane production when compared with silages, where fermentation has been restricted. There is also increasing interest in high-moisture preservation of cereal grains with potential benefits in the nutritional value. Benefits in feed digestibility were observed in a meta-analyses by Ferraretto et al. [13] and Torres et al. [8] without major changes in milk production, while Huuskonen et al. [14] reported increased growth performance of beef bulls receiving high moisture grains compared to dry grains. Further, high-moisture grain preservation can result in substantial economic savings compared to drying under humid harvesting conditions [15].

The same diets as used in the current experiment were used in a parallel milk production trial and the results have been reported by Rinne et al. [16]. The objective of the current experiment was to evaluate the effect of conservation type of both grass silage and barley grains on feed and rumen microbiota, and energy and nitrogen metabolism of lactating dairy cows using respiration chambers. We hypothesized that silages produced by different types of additives (restricting fermentation by formic acid or promoting it by LAB) and barley grain preservation method (ensiled high-moisture or dry grains) will influence diet characteristics and subsequently alter the supply of nutrients to rumen, which will modify the rumen microbiota, methane production, and energy metabolism of the dairy cows.

2. Materials and Methods

2.1. Animals, Experimental Design, and Diets

Four intact multiparous Nordic Red dairy cows (669 $\pm$ 47.7 kg of body weight, 83 $\pm$ 9.6 days in milk, 41.9 $\pm$ 3.15 kg milk/d, and parity 5.0 $\pm$ 1.15) were randomly assigned to treatment sequences in a 4 $\times$ 4 Latin square balanced for carry over effects. Each period lasted 21 d with 14 d for diet adaptation followed by 7 d for data and sample collection.

The same diets were used in a parallel milk production experiment and the details of feed production, dietary treatments, animal feeding, milking, and sampling are reported by Rinne et al. [16]. Briefly, the experimental silages were made from primary growth of a mixed timothy (*Phleum pratense*) and meadow fescue (*Festuca pratensis*) sward at Jokioinen, Finland (60°48′ N, 23°29′ E). The grass was wilted under good drying conditions for 1–3 h, harvested using a precision chopper, and ensiled in horizontal bunker silos. Two silage additives were applied to alternate loads of grass by an applicator attached to the chopper. The additives used were: (I) an organic acid-based product (AIV Ässä Na, Eastman Ltd., Oulu, Finland; containing g/kg 580 formic acid, 200 propionic acid, 52 sodium formate

and 25 potassium sorbate) at a target rate of 5 L/ton fresh matter (AS), and (II) a homofermentative LAB inoculant (Bonsilage, Schaumann GmbH, Pinneberg, Germany; strains included 1k2078 *Lactobacillus plantarum* (DSM 12836) and 1k2103 *Pediococcus pentosaceus* (DSM 12834) in a water solution resulting in a calculated application rate of 10^5 CFU/g fresh material (IS).

The second dietary factor was the preservation method of barley grains. The barley (*Hordeum vulgare*) was harvested at a grain moisture content of 223 g/kg. Part of the grain was dried in a grain drier to reach a moisture concentration of 123 g/kg, milled and pelleted before feeding (DB). The other part was crimped using a farm-scale crimper mill (MD 700 HD, Murska Ltd., Ylivieska, Finland) so that the grains remained whole, but their inner part was exposed. During crimping, a heterofermentative LAB inoculant [SILOMIX® Murske; Agriprep Ltd., Cardiff, UK; strains included 1k20738 *Lactobacillus buchneri* (DSM 22501), 1k20745 *Lactobacillus brevis* (DSM 16680), and 1k1010 *Pediococcus pentosaceus* (DSM 23688) species] was added at a calculated application rate of 7.0×10^5 CFU/g fresh material. The inoculant and tap water were added so that the final moisture content of the crimped grain prior to ensiling was 283 g/kg (EB).

Total mixed rations (TMR) were prepared so that 500 g/kg of the diet DM consisted of either AS or IS silages, 275 g/kg concentrates, and 225 g/kg of barley grain, which was either dried or ensiled, so that four different experimental diets were formed (AD, AE, ID, and IE, respectively). The TMR were fed daily at 0700, 1300, 1600, and 1830 h. In addition, the cows received 0.6 kg/d concentrates in the milking parlor, and during the collection period, the same amount was delivered into the chambers. Leftover feeds were weighed daily at 1200 h before offering fresh feed. At least 5% of refusals was targeted daily to ensure ad libitum feed intake. The cows were housed in an experimental free-stall barn (Jokioinen, Finland), and milked twice daily at 0700 and 1700 h in the milking parlor during the adaptation periods (d 1–16 of each period) and in respiration chambers during d 17–21 of each period. Water and salt blocks were freely available.

2.2. Measurements, Sample Collection, Chemical, and Microbial Analyses

For accurate formulation of the experimental diets and to maintain the predetermined forage to concentrate ratio, DM content of grass silage was analyzed every week during the experiment at 105 °C for 20 h in a forced-air oven. Representative samples of grass silage and concentrate feeds were collected from d 17 to 21 of each experimental period, composited, and stored at −20 °C until analysis for DM and chemical composition. Individual feeds and TMR were sampled for microbial community analysis during the last week of each period. The TMR samples were collected immediately after mixing, and again after 2 days and stored at −80 °C until DNA extraction. Feed intake was measured daily as the difference between offered feed and leftovers. Total feces and urine excreted during three consecutive days starting on d 18 at 1000 h were collected. The sample collection and analysis (DM, ash, crude protein (CP), ether extract, neutral detergent fiber (NDF), starch, and gross energy (GE)) are described by Bayat et al. [17]. In addition, the precipitations in urine samples were collected, stored at −20 °C, and dried at 105 °C for 20 h before nitrogen (N) determination. The collected feed, urine, and fecal samples were analyzed using routine laboratory methods (for details see [17]) of Luke laboratory, which follows the standard SFS-EN ISO/IEC 17025:2017 and is accredited by the Finnish Accreditation Service (#T024, Helsinki, Finland). Fresh silage samples were prepared for pH measurement and analysis of volatile fatty acids (VFA), lactic acid, formic acid, ethanol, water-soluble carbohydrates, soluble N, and ammonia N concentrations as described in [6]. The milk samples were analyzed at a commercial laboratory (Valio Ltd., Seinäjoki, Finland) using an infrared analyzer (MilkoScan FT+; Foss Electric A/S, Hillerød, Denmark). On d 21 of each experimental period after respiratory chamber measurements at 1000 h, samples of rumen liquid (500 mL) were collected by stomach tubing (Ruminator, Profs Products, Wittybreut, Germany). Immediately after collection, the pH was measured using a portable pH meter and two subsamples were taken and prepared for VFA and ammonia N determinations

as described by Bayat et al. [17]. For rumen microbial community analysis, rumen fluid was aliquoted into 2 mL tubes, snap frozen in dry ice, and stored at −80 °C until DNA extraction.

Methane, oxygen, carbon dioxide, and hydrogen exchanges were measured by four open-circuit respiratory chambers for individual animals as described by Bayat et al. [17]. Energy metabolism was measured over a 4 d period starting on d 17 with the first day being considered as acclimatization.

From feed samples, total DNA was extracted from 0.25 g of freeze dried and ground feed as described by Yu and Morrison [18]. Total DNA was extracted from 0.5 mL of rumen liquid following the protocol in [19]. Rumen and feed prokaryotic community composition was determined using universal primers 515F and 806R [20] for 16S rRNA gene V4 region amplicon sequencing. Sequencing libraries were prepared and sequenced in Finnish Functional Genomics Centre (Turku, Finland) on Illumina MiSeq platform by using 2 × 250 bp chemistry. Demultiplexing, adapter removal, and sorting sequences by barcode were performed by the sequencing center. Sequence read quality control was performed using DADA2 [21] following the default settings in Qiime 2 [22] as described in [9] for feed samples and in [16] for rumen samples.

2.3. Calculations and Statistical Analyses

Energy-corrected milk was calculated using the equation suggested by Sjaunja et al. [23] based on milk fat, protein, and lactose yields, and energy secretion (MJ/d) in milk was calculated as 3.14× energy corrected milk (ECM) yield (kg/d). Heat production was calculated according to the Brouwer [24] equation. Nitrogen balance was calculated as the difference between N intake and N excretion in feces, urine, and milk, where milk N was calculated as milk CP/6.38. Total tract apparent digestibility coefficients were calculated as the difference between intake of a nutrient and its fecal output divided by the corresponding intake of a nutrient. Intake of metabolizable energy (ME) was calculated by subtracting energy excretions in feces, urine, and methane from GE intake. Energy loss as methane was calculated using the factor 55.24 kJ/g [25].

The normality of analyzed data was checked using box plot and scatter plot of residuals and fitted values generated using the MIXED procedure of SAS (version 9.4, SAS Institute Inc., Cary, NC, USA). The results were analyzed with a 2 × 2 factorial arrangement of treatments using the MIXED procedure of SAS using individual cow in a period as an experimental unit. The statistical model included fixed effects of period, silage, barley, and silage × barley interaction, while cow was considered as a random effect.

Before feed and rumen microbial community analyses, amplicon sequence variants (ASV) observed in only one sample and ASVs with the total abundance below 10 reads were removed. From feed samples, ASVs affiliated with mitochondria, chloroplast, and Cyanobacteria were also removed. In total, there were 1,667,821 quality filtered reads (average 32,073/sample) for feed samples and 470,711 (average 29,419/sample) for rumen samples available for subsequent alpha and beta diversity analyses, performed as described by Franco et al. [26] and Rinne et al. [16], respectively. Statistical significance was defined as $p < 0.05$, and $0.05 < p < 0.10$ was considered as a trend. Tukey test was used to compare the treatment means when the interaction was significant, and when comparing the fresh and 2-day-old TMR feeds.

3. Results

The experimental feeds and total mixed rations based on them are described in Table 1. The differences in the fermentation profile of the two silages were very small, and they both had a low pH (on average 3.98) indicating good preservation quality. The dry and ensiled barley grains differed slightly in terms of lower starch content of the EB. The chemical composition and feed values of the experimental TMR diets were almost identical. The dietary treatments did not affect feed intake (Table 2) nor milk production (Table 3) except for a tendency ($p < 0.1$) of a higher lactose concentration and a higher ($p < 0.05$) milk urea N

of cows receiving IS when compared to AS. The apparent total tract digestibility of DM and OM were slightly higher ($p < 0.05$) for AS than IS, the opposite being the case for CP ($p < 0.05$; Table 4). Using ensiled rather than dry barley decreased diet DM, OM, and starch digestibility ($p < 0.05$).

Table 1. Characterization of the experimental feeds and diets ($n = 4$ for each feed).

	Grass Silages		Concentrate Feeds				Experimental Total Mixed Rations [3]			
	Acid	Inoculant	MixC [1]	Dry Barley	Ensiled Barley	MPC [2]	AD	AE	ID	IE
Dry matter (DM), g/kg	271	260	871	877	730	877	572	539	567	534
In DM, g/kg										
Ash	73	77	108	30	32	81	73	74	75	76
Crude protein	140	142	240	136	132	184	167	166	168	167
Water soluble carbohydrates	26	41								
Neutral detergent fiber	490	493	251	191	193	204	357	357	359	359
Starch			156	605	585	363	179	175	179	175
Gross energy, MJ/kg DM							17.6	17.6	17.7	17.8
Fermentation profile, g/kg DM										
pH	3.99	3.97			4.42					
Ammonia N, g/kg N	35.8	42.8			19.9					
Ethanol	6.6	6.6			4.6					
Lactic acid	88.8	99.8			14.5					
Acetic acid	16.9	12.5			8.45					
Propionic acid [4]	2.36	0.63			0.05					
Butyric acid	0.36	0.83			0.003					
In vitro organic matter digestibility, g/kg [5]	793	786								
Feed values [6]										
Metabolizable energy, MJ/kg DM	11.7	11.6	11.3	12.9	12.9	12.1	11.9	11.9	11.8	11.8
Metabolizable protein, g/kg DM	87	86	126	110	109	117	102	102	102	102
Protein balance in the rumen, g/kg DM	19	21	65	−23	−26	18	22	21	23	22

[1] Supplementary concentrate. [2] Milking parlor concentrate provided as 0.6 kg as fed per cow/d. [3] AD = organic acid-based additive treated silage and dry barley, AE = organic acid-based additive treated silage and crimped ensiled barley, ID = lactic acid bacteria inoculated silage and dry barley, IE = lactic acid bacteria inoculated silage and crimped ensiled barley. [4] Analysed value including the propionic acid via additive application. When corrected for the added amount, the value equals zero. [5] Concentration of digestible OM based on cellulase solubility in vitro [27]. [6] Calculated according to Luke [28].

Table 2. Feed and nutrient intakes (kg/d, unless otherwise stated) of lactating dairy cows fed the experimental diets ($n = 4$ for each treatment).

Silage Additive (S)	Acid		Inoculant		SEM [1]	*p*-Value		
Barley Preservation (B)	Dry	Ensiled	Dry	Ensiled		S	B	S × B
Dry matter	25.8	26.6	25.5	25.6	0.95	0.13	0.29	0.41
Organic matter	23.9	24.7	23.5	23.6	0.88	0.11	0.30	0.41
Crude protein	4.28	4.39	4.25	4.25	0.153	0.28	0.47	0.45
Ether extract	0.88	0.91	0.87	0.87	0.033	0.16	0.22	0.36
Neutral detergent fiber	9.3	9.6	9.2	9.2	0.333	0.13	0.33	0.39
Starch	4.55	4.62	4.55	4.50	0.188	0.50	0.89	0.51
Gross energy, MJ/d	452	469	452	456	17.2	0.32	0.27	0.42

[1] Standard error of the mean.

Table 3. Milk yield and composition of lactating dairy cows fed the experimental diets (n = 4 for each treatment).

Silage Additive (S)	**Acid**		**Inoculant**		**SEM**	***p*-Value**		
Barley Preservation (B)	**Dry**	**Ensiled**	**Dry**	**Ensiled**		**S**	**B**	**S × B**
Yield, kg/d								
Milk	36.8	37.8	36.5	36.5	1.09	0.28	0.52	0.49
ECM [1]	41.4	42.6	41.2	40.8	2.12	0.33	0.71	0.45
Fat	1.79	1.82	1.77	1.74	0.121	0.33	0.98	0.56
Protein	1.37	1.44	1.38	1.38	0.078	0.40	0.29	0.29
Lactose	1.66	1.70	1.66	1.65	0.050	0.46	0.61	0.49
Total solids	5.20	5.34	5.17	5.13	0.234	0.33	0.63	0.44
Concentration, g/kg								
Fat	48.5	48.3	48.4	47.7	2.55	0.63	0.51	0.65
Protein	37.2	38.1	37.8	37.9	1.43	0.72	0.30	0.42
Lactose	45.2	45.0	45.4	45.3	0.77	0.053	0.34	0.98
Total solids	141	142	142	141	3.6	0.98	0.90	0.31
MUN, mg/100 mL	24.9	26.0	26.9	26.4	1.14	0.047	0.54	0.14
SCC [2], 1000/mL	29.8	28.7	22.4	24.8	7.35	0.10	0.83	0.58
Milk/DMI [3]	1.43	1.42	1.44	1.43	0.042	0.50	0.60	0.97
ECM/DMI	1.60	1.60	1.62	1.60	0.048	0.76	0.53	0.75

[1] ECM, energy-corrected milk yield. [2] SCC, somatic cell count. [3] DMI, dry matter intake.

Table 4. Apparent total-tract nutrient digestibility (g/kg, otherwise stated) of lactating dairy cows fed the experimental diets (n = 4 for each treatment).

Silage Additive (S)	**Acid**		**Inoculant**		**SEM**	***p*-Value**		
Barley Preservation (B)	**Dry**	**Ensiled**	**Dry**	**Ensiled**		**S**	**B**	**S × B**
Dry matter	714	705	704	701	7.4	0.043	0.054	0.30
Organic matter	732	723	723	719	7.7	0.045	0.044	0.41
Crude protein	676	672	679	685	8.1	0.041	0.72	0.13
Ether extract	580	563	570	564	22.3	0.79	0.54	0.76
Neutral detergent fiber	590	597	576	591	14.8	0.38	0.31	0.69
Starch	994	957	994	959	5.2	0.83	<0.01	0.92
Gross energy kJ/MJ	693	684	686	685	7.8	0.39	0.12	0.23

The cows receiving AS tended to emit more methane per day (p = 0.06), but methane yield (g/kg DM intake) and intensity (g/kg milk yield) were not different between the diets (Table 5). The carbon dioxide emissions tended to be higher per kg DM intake and were higher per kg DOMI when IS rather than AS was fed to cows. The hydrogen output was higher per day and per kg DM intake for cows receiving AS, and when dry rather than ensiled barley was used (p < 0.01). The experimental diets resulted in higher molar proportion of butyrate when AS rather than IS and dry rather than ensiled barley was used (p < 0.01; Table 6). Isobutyrate and isovalerate molar proportions were greater for IE compared with other treatments (p < 0.05 for the interaction). Also, the molar proportion of valerate was greater (p < 0.05) for AS compared with IS diets.

Table 5. Enteric methane, carbon dioxide and hydrogen emissions of lactating dairy cows fed the experimental diets (n = 4 for each treatment).

Silage Additive (S)	**Acid**		**Inoculant**		**SEM**	***p*-Value**		
Barley Preservation (B)	**Dry**	**Ensiled**	**Dry**	**Ensiled**		**S**	**B**	**S × B**
Methane								
g/d	521	538	518	513	19.2	0.061	0.41	0.14
g/kg DMI	20.2	20.2	20.4	20.1	0.51	0.92	0.28	0.32
g/kg DOM [1]	29.8	30.1	30.5	30.2	0.55	0.11	0.77	0.18
g/kg Milk	14.2	14.2	14.2	14.1	0.32	0.61	0.89	0.48
g/kg ECM	12.6	12.7	12.6	12.6	0.22	0.70	0.96	0.91
% of GE intake	6.41	6.40	6.39	6.29	0.167	0.13	0.20	0.33
Carbon dioxide								
g/d	13,221	13,449	13,306	13,076	514.9	0.39	0.99	0.19
g/kg DMI	513	505	523	512	9.9	0.069	0.048	0.63
g/kg DOM	756	754	783	770	17.4	0.016	0.31	0.41
g/kg Milk	359	357	364	358	8.0	0.28	0.17	0.53
g/kg ECM	321	317	324	321	11.4	0.50	0.42	0.87
Hydrogen								
g/d	1.33	1.11	0.98	0.65	0.104	<0.01	0.026	0.56
g/kg DMI	0.051	0.042	0.039	0.025	0.0034	<0.01	0.010	0.48

[1] DOM, digested organic matter.

Table 6. Rumen fermentation characteristics of lactating dairy cows fed the experimental diets (n = 4 for each treatment).

Silage Additive (S)	**Acid**		**Inoculant**		**SEM**	***p*-Value**		
Barley Preservation (B)	**Dry**	**Ensiled**	**Dry**	**Ensiled**		**S**	**B**	**S × B**
pH	6.67	6.55	6.51	6.56	0.089	0.42	0.68	0.32
Ammonia-N, mM	3.67	3.24	2.99	4.34	0.817	0.80	0.58	0.30
Total VFA [1], mM	111	115	119	110	4.8	0.82	0.58	0.24
Molar proportions, mmol/mol								
Acetate	607	614	613	625	7.1	0.18	0.17	0.69
Propionate	204	203	207	205	4.8	0.67	0.77	0.92
Butyrate	154	146	147	131	4.9	<0.01	<0.01	0.22
Isobutyrate	6.08 [b]	6.56 [b]	5.94 [b]	7.46 [a]	0.149	0.031	<0.01	<0.01
Valerate	16.3	16.1	15.2	15.4	0.35	0.048	0.98	0.55
Isovalerate	5.90 [b]	6.96 [b]	5.72 [b]	9.19 [a]	0.411	0.034	<0.01	0.017
Caproate	6.97	7.33	6.48	6.98	0.340	0.063	0.060	0.73
Acetate:propionate	2.98	3.03	2.98	3.07	0.097	0.84	0.50	0.83
Acetate + butyrate:propionate	3.74	3.75	3.69	3.71	0.110	0.71	0.90	0.98
Lipogenic:Glucogenic	4.20	4.17	4.14	4.05	0.110	0.42	0.59	0.78

[1] VFA, volatile fatty acids. [a,b] Values with same letter in a row are not significantly different at 5% Tukey test.

Energy and N metabolism of the cows are presented in Table 7. An interaction ($p < 0.01$) was observed for the proportion of energy partitioned to urine as with AE treatment having higher value compared with AD. The cows consuming AS excreted more N in feces as a proportion of N intake ($p < 0.05$) whereas they excreted less N in urine as a proportion of N intake ($p < 0.01$) compared to those fed IS. The N retention tended to be higher for AS than IS fed cows, and an interaction was detected ($p < 0.05$) as AD had greater N retention than ID treatment.

Table 7. Energy and N metabolism of dairy cows fed the experimental diets (n = 4 for each treatment).

Silage Additive (S)	**Acid**		**Inoculant**		**SEM**	***p*-Value**		
Barley Preservation (B)	**Dry**	**Ensiled**	**Dry**	**Ensiled**		**S**	**B**	**S × B**
Energy								
GE intake, MJ/d	263	264	257	259	7.6	0.32	0.27	0.42
ME [1] intake, MJ/d	62.8	63.2	61.5	61.9	1.80	0.27	0.72	0.99
Proportion of energy intake, KJ/MJ								
Feces	307	316	314	315	7.8	0.39	0.12	0.23
Urine	48.7 [b]	53.9 [a]	51.5 [ab]	50.4 [ab]	2.80	0.76	0.11	0.031
Methane	64.1	64.0	63.9	62.9	1.67	0.13	0.20	0.33
Milk	287	285	286	282	8.6	0.65	0.48	0.78
Heat	322	317	325	317	5.7	0.65	0.038	0.58
Milk energy/ME intake	495	505	502	494	14.8	0.84	0.89	0.31
Energy balance, MJ/d	−13.0	−17.6	−18.4	−13.0	5.27	0.92	0.88	0.17
Nitrogen (N)								
N intake, g/d	684	702	680	679	24.5	0.28	0.47	0.45
Proportion of N intake, g/kg								
Feces	324	328	321	315	8.1	0.041	0.72	0.13
Urine	343	350	371	363	13.4	<0.01	0.92	0.16
Milk	314	321	317	319	10.2	0.95	0.36	0.55
N retention, g/d	13.4 [a]	0.8 [ab]	−6.6 [b]	2.9 [ab]	6.63	0.057	0.69	0.028

[1] ME, Metabolizable energy. [a,b] Values with same letter in a row are not significantly different at 5% Tukey test.

3.1. Feed Microbiota

Bacteria alpha diversity was higher ($p < 0.01$) in barley samples than grass silages (Table 8). However, no difference was found in the comparison between AS vs. IS silages or DB vs. EB barley. The alpha diversity of the TMR varied according to the preservation method of the ingredients (Table 9). Both AD and AE showed similar alpha diversity, but IE had lower ($p < 0.01$) Shannon and Simpson indexes when compared to ID. Differences in alpha diversity between freshly prepared and 2-day-old TMR demonstrated an increase ($p < 0.05$) in observed number of ASV in 2-day old AD but reduction ($p < 0.05$) in Shannon and Simpson indexes in 2-day-old IE (Table 9).

Table 8. Alpha diversity estimates of the experimental feed ingredients (n = 4 for each feed ingredient).

	Acid Treated Silage	**Inoculant Treated Silage**	**Dried Barley**	**Ensiled Crimped Barley**	**SEM**	***p*-Value**
Observed ASV [1]	108	86	103	103	16.1	0.819
Shannon	2.03 [c]	2.22 [bc]	3.41 [a]	3.04 [ab]	0.208	0.003
Simpson	0.698 [b]	0.758 [ab]	0.926 [a]	0.901 [a]	0.0408	0.009

[1] ASV, amplicon sequence variants. [a,b,c] Values with same letter in a row are not significantly different at 5% Tukey test.

Table 9. Alpha diversity estimates of fresh and 2-day-old total mixed rations (n = 4 for each treatment).

Silage Additive (S)	**Acid**		**Inoculant**		**SEM**	***p*-Value**		
Barley Preservation (B)	**Dry**	**Ensiled**	**Dry**	**Ensiled**		**S**	**B**	**S × B**
Observed ASV, Fresh	163 [B]	182	181	151	23.9	0.796	0.827	0.334
Observed ASV, 2-day old	189 [A]	176	172	140	23.7	0.295	0.371	0.702
Shannon, Fresh	3.38	3.52	3.65	3.21 [A]	0.116	0.876	0.217	0.034
Shannon, 2-day old	3.46	3.35	3.43	3.00 [B]	0.084	0.051	0.011	0.086
Simpson, Fresh	0.907	0.933	0.929 [A]	0.897 [A]	0.0068	0.35	0.7	0.002
Simpson, 2-day old	0.911	0.924	0.913 [B]	0.879 [B]	0.0067	0.01	0.155	0.006

[A,B] Values with different capital letter in a column within each parameter (i.e., comparison between fresh and 2-day-old total mixed ration) are significantly different at 5% Tukey test.

Observed ASV, Shannon and Simpson were, 119, 3.55, and 0.928 for the supplementary concentrate (MixC), respectively.

The bacterial community structure in the experimental feeds is shown in Figure 1. The PCoA1 separated the grass silages apart from barley and supplementary concentrate, while PCoA2 discriminated between acid vs. inoculant treated grass silage and dried vs. ensiled barley. Each group of feeds tended to form its own cluster showing that the profile of bacterial communities differed between feeds. Considering the bacterial community structure of fresh and 2-day-old TMR (Figure 2), the TMR were separated by ingredient preservation method and according to the production time, but both fresh and 2-day-old samples grouped together.

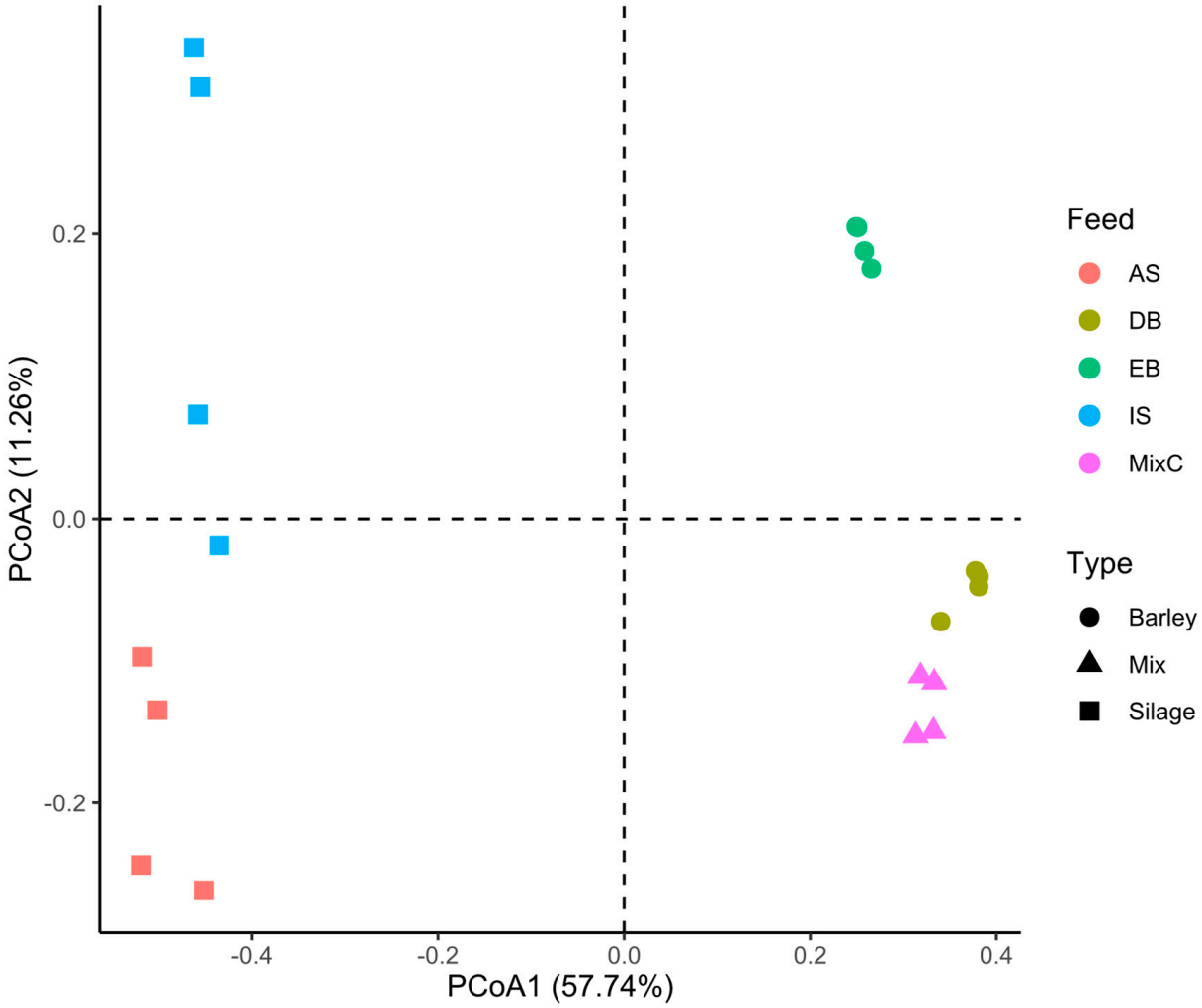

Figure 1. Principal coordinate analysis (PCoA) of the beta diversity analysis of the experimental feeds. AS: acid-treated silage; DB: dry barley; EB: ensiled barley; IS: inoculant-treated silage; MixC: supplementary concentrate.

The relative abundance of bacterial communities in experimental feeds and TMR were affected by the preservation factors (Figures 3 and S1). Both AS and IS silages were dominated by Firmicutes (82%) and Proteobacteria (10–14%) (Figure S1A). Of the Firmicutes-associated sequences, 78–80% were members of *Lactobacillaceae* family, while the Proteobacteria were dominated by *Pseudomonadaceae* (4–10%) (Figure S1B). The barley preservation method had greater impact on microbial composition. The DB was dominated by Proteobacteria (64%), Bacteroidota (19%), Actinobacteriota (10%), and Firmicutes (5%), while in the EB samples both Firmicutes (53%) and Proteobacteria (32%) were predominant. Similarly as for silages, the EB was dominated by *Lactobacillaceae* (51%). The Proteobacte-

ria in both barley types were dominated by *Erwiniaceae* (12–24%) and *Pseudomonadaceae* (7–14%), Bacteroidotoa by *Weeksellaceae* (4–10%) and *Sphingobacteriaceae* (3–6%), and Actinobacteriota by *Microbacteriaceae* (3–6%) families, respectively (Figure S1B). All freshly prepared TMR were dominated by *Lactobacillaceae* (35–61%), *Erwiniaceae* (6–15%), and *Pseudomonadaceae* (7–11%) with the proportions of lower abundance taxa reflecting closely the barley type used for the TMR preparation. The AD and AE remained similar in their bacterial composition irrespective of being freshly prepared or 2-day-old, while ID was less stable. In 2-day-old ID samples, an increase in *Lactobacillaceae* (genera *Lentilactobacillus* and *Lactobacillus*) by 10% and reduction in *Pseudomonadaceae* (genus *Pseudomonas*) by 2% were observed.

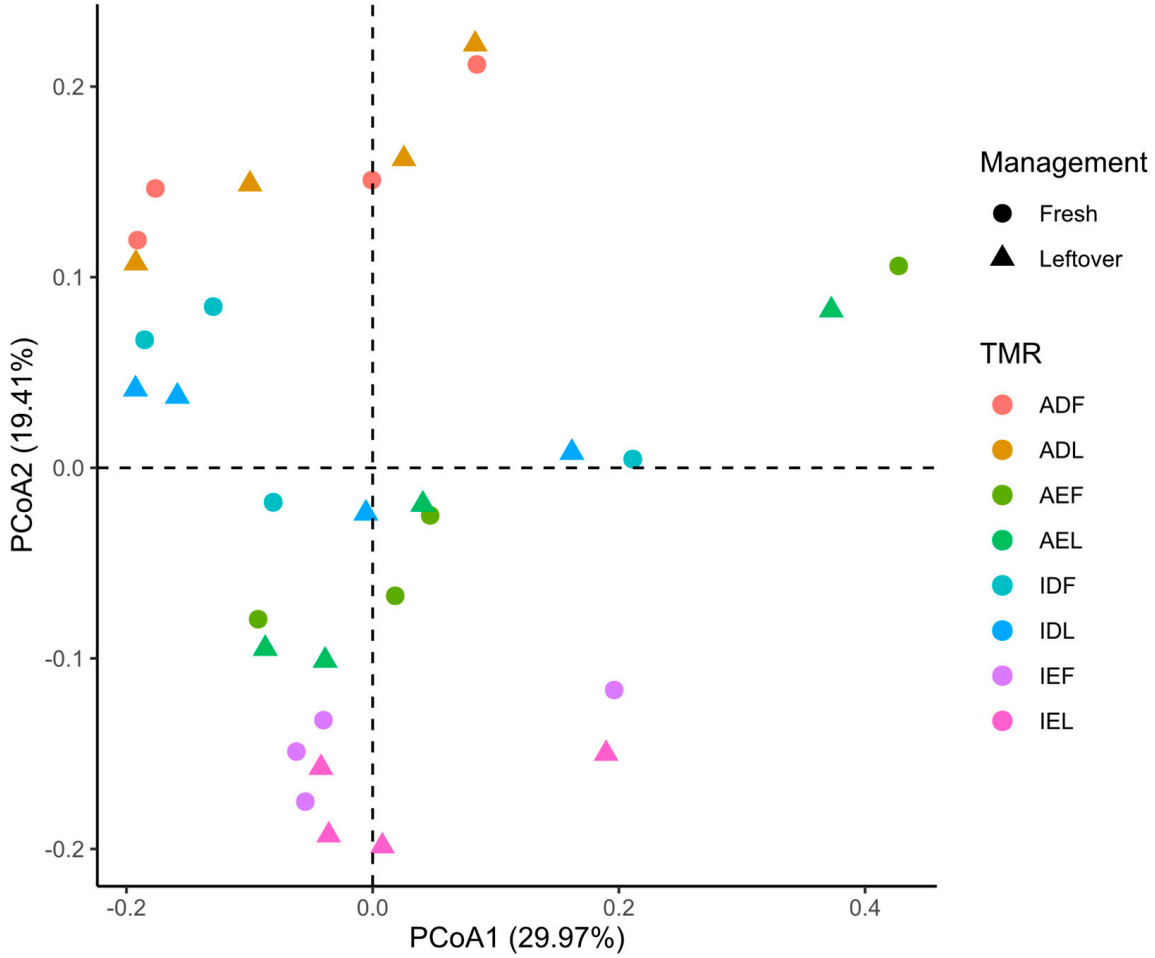

Figure 2. Principal coordinate analysis (PCoA) of the beta diversity analysis of the total mixed rations (TMR). ADF: fresh TMR produced with acid-treated silage and dry barley; ADL: 2-day-old TMR produced with acid-treated silage and dry barley; AEF: fresh TMR produced with acid-treated silage and ensiled barley; AEL: 2-day-old TMR produced with acid-treated silage and ensiled barley; IDF: fresh TMR produced with inoculant-treated silage and dry barley; IDL: 2-day-old TMR produced with inoculant-treated silage and dry barley; IEF: fresh TMR produced with inoculant-treated silage and ensiled barley; IEL: 2-day-old TMR produced with inoculant-treated silage and ensiled barley.

Feed bacterial composition varied greatly at genus level (Figure 3). The AS was dominated by *Lactobacillus* (47%) and *Fructilactobacillus* (24%), while IS had on average higher abundance of *Lentilactobacillus* (26%), *Pediococcus* (17%), *Lactobacillus* (13%), *Pseudomonas* (10%), and *Lactiplantibacillus* (8%). It is to be noted that between sample variation for IS was high. The DB and supplementary concentrate (MixC) were dominated by *Pantoea* (24–27%), *Pseudomonas* (12–14%), and *Chryseobacterium* (3–10%), with many other genera, mainly from

Proteobacteria and Actinobacteriota phyla, detected at lower abundances. On the other hand, the EB showed a high relative abundance of *Lentilactobacillus* (37%), followed by *Pediococcus* and *Pantoea* (12%). At genus level, TMR bacterial community composition and abundances reflected closely those of ingredients. AD was dominated by *Fructilactobacillus* (18%), *Lactobacillus* (16%), *Pantoea* (15%), and *Pseudomonas* (9%), while ID showed larger between sample variation, with *Lentilactobacillus*, *Pantoea* (14%), *Pseudomonas* (11%), and *Lactobacillus* (9%) among the predominant genera. The TMR produced with ensiled barley (AE and IE) were dominated by *Lentilactobacillus* (21–33%), followed by *Lactobacillus* (11%), and *Pediococcus* (9%).

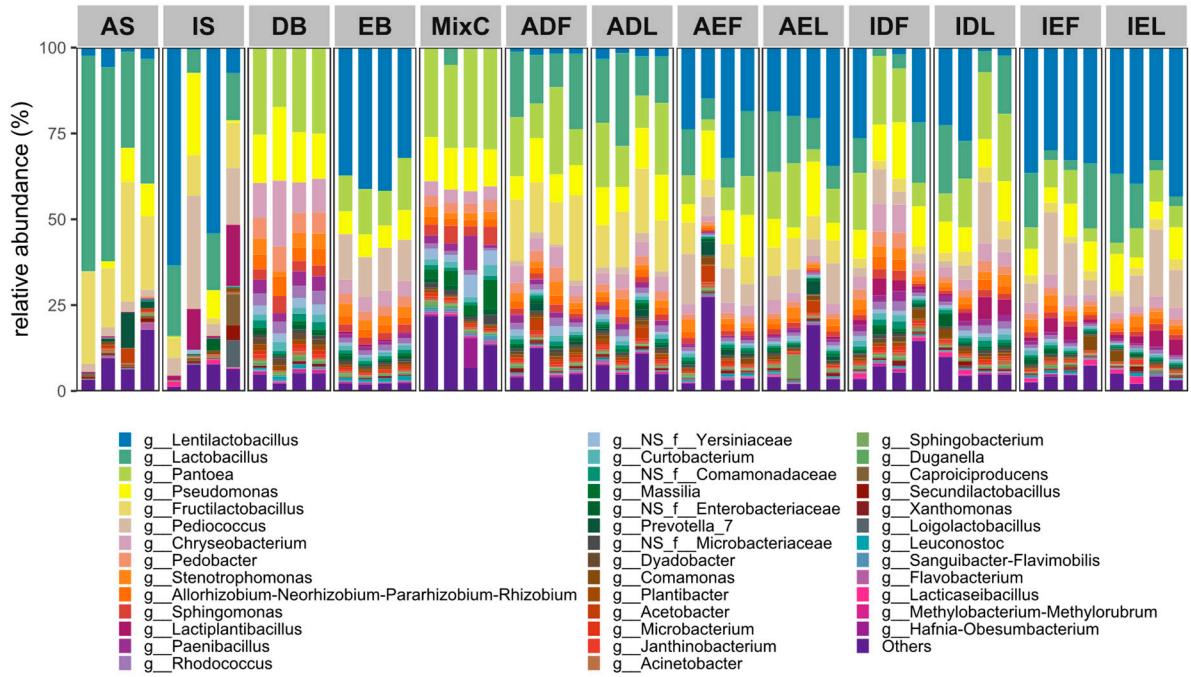

Figure 3. Relative abundance of bacterial communities at genus taxonomical level of the experimental feeds and total mixed rations (TMR). AS: acid-treated silage; IS: inoculant-treated silage; DB: dried barley; EB: crimped and ensiled barley; MixC: supplementary concentrate; ADF: fresh TMR produced with acid-treated silage and dry barley; ADL: 2-day-old TMR produced with acid-treated silage and dry barley; AEF: fresh TMR produced with acid-treated silage and ensiled barley; AEL: 2-day-old TMR produced with acid-treated silage and ensiled barley; IDF: fresh TMR produced with inoculant-treated silage and dry barley; IDL: 2-day-old TMR produced with inoculant-treated silage and dry barley; IEF: fresh TMR produced with inoculant-treated silage and ensiled barley; IEL: 2-day-old TMR produced with inoculant-treated silage and ensiled barley.

3.2. Rumen Microbiota

Alpha and beta diversities of rumen bacteria and archaea were not significantly affected by the diet fed to the animals as TMR, or grass silage and barley preservation methods (Figure S2). The ten most abundant genera that represented 50% of all bacterial genera in the rumen were *Prevotella*, *Lachnospiraceae* NK3A20, *Ruminococcus*, *Succinivibrionaceae* UCG-002, *Christensenellaceae* R-7, *Oscillospiraceae* NK4A214, *Prevotellaceae* UCG-001, *Rikenellaceae* RC9 gut group, and *Clostridia* UCG-014. Among archaea, *Methanobrevibacter gottschalkii* and *Mbb. ruminantium* clades were predominant in all dietary treatments, with *Methanosphaera* ISO3-F5 and *Methanomassiliicoccaceae* Group 10, *Mmc*. Group 12 ISO4-H5 and *Mmc*. Group 5 detected at lower abundances (Figure S3). In both bacteria and archaea, there were several ASV more specific to one of the diets, but when grouped at genus level for bacteria and at species level for archaea, they were shared between the diets (Figure S4). From the bacteria dominating feed samples, only traces of *Lentilactobacillus* (0–0.3%) were detected among rumen microbiotas.

4. Discussion

4.1. Feed Characteristics

Silage additives are widely used in feed production for dairy cows to ensure high preservation quality of the feeds [1,3]. The mode of action of the additives used in the current experiment were opposite, as selected strains of LAB direct and boost lactic acid fermentation, while organic acid-based additives restrict fermentation [3]. These effects have been demonstrated with grass material similar to that used in the current experiment both at farm scale [5] as well as under laboratory conditions [6,29]. However, the differences in the fermentation parameters between formic acid-treated silage and lactic acid bacteria inoculated silage were minor, and in some cases even opposite to what was expected, such as the higher WSC concentration of IS than AS. The lack of response was not due to failures in application of additives or mixing the feeds at TMR preparation, as formic acid was detected in AS and not in IS samples. Based on the formic acid concentration of AS, the level of additive application was 4.3 L/ton, which was only slightly lower than the commercial recommendation (5.0 L/ton). The dose-response to formic acid application has been linear [5] so that even with slightly underdosing, more efficient restriction of fermentation could have been expected.

During preservation of moist crimped grains, some starch is degraded and converted into fermentation end products, mainly lactic and acetic acids. In the current material the reduction in starch during ensiling was 20 g/kg DM, and concomitant formation of fermentation end products (ethanol, lactic acid, and acetic acid) was 28 g/kg DM. The extent of fermentation is highly dependent on the moisture content of the grains [9], and the results obtained in the current experiment can be considered typical for this type of a raw material.

4.2. Feed Microbiota Characteristics

The better understanding of microbiota associated with fresh and ensiled forage crops has an economic value as epiphytic microbiota as well as microbial cultures used for inoculation can affect ensiling performance and feed quality [30,31] and consequently influence dairy production. The microbial communities of the silages were affected by the additive treatments. Both AS and IS were dominated by Firmicutes, but differences between the silages became more obvious when looking at the genus level data. A fresh red clover/timothy grass sample from our previous experiment [26] was dominated by Proteobacteria (82%) and Firmicutes (13%), suggesting that the bacteria of mixed timothy/meadow fescue swards at phylum level could be expected to be similar. A shift in bacterial microbiome from Proteobacteria to Firmicutes is a key to ensure proper conservation of silages, which was the case in this experiment. Not surprisingly, IS had *Lactiplantibacillus* and *Pediococcus* at much higher abundances as compared to low or negligible amounts observed in AS. These genera represent the species used as inoculum and confirm that the ensiling proceeded as expected based on additives administered to them. The IS also demonstrated high abundance of *Lentilactobacillus*. Xu et al. [32] showed that in fresh sweet sorghum forage, *Lentilactobacillus* was present at low abundance, but its proportion increased during the ensiling process. *Lentilactobacillus* has also been noted to become more active in silage during the late fermentation process [33]. These observations suggest that in IS some fermentative activity by *Lentilactobacillus* was going on in the later stage of ensiling process and resulted in higher relative abundances of this microbe in our sequencing data. The AS, on the other hand, was not enriched in the same inoculum species as IS but had high abundance of *Lactobacillus* and *Fructilactobacillus*. Bai et al. [34] evaluated the effect of different LAB inoculants on ensiling properties of alfalfa and demonstrated that keystone microbial taxa present in silage, their metabolism and interaction were LAB inoculant dependent. This could suggest that the environmental conditions caused by organic acid treatment created a niche suitable for fermentative activities of *Lactobacillus* and *Fructilactobacillus* in AS. Among Proteobacteria in both IS and AS the *Pseudomonas* genus was predominant. *Pseudomonas* is detected among the microorganisms of fresh

forages, like red clover/timothy [26] or sweet sorghum [32], and presence of *Pseudomonas* in silage suggests that the microorganism remains viable during ensiling. Despite differences in the microbiota composition of AS and IS silages, the silage fermentation characteristics were similar [16]. Therefore, no analyses of associations between microbiota and silage fermentation parameters were performed.

Barley grain preservation method affected barley associated microbiota. The bacterial composition of fresh crimped barley in our previous study [9] was dominated by Proteobacteria (77%), Actinobacteriota (10%), Firmicutes (9%), and Bacteroidota (3%), which are phyla commonly detected in various seeds [35,36]. The bacterial composition of dried barley in this study resembled the composition of fresh barley and was dominated by Proteobacteria, especially members from *Pantoea* and *Pseudomonas* genera. Drying of seeds has been demonstrated to alter the seed bacterial abundances. For example, a significant decrease in abundance of *Pseudomonas*, *Sphingomonas*, *Massilia*, or *Curtobacterium*, and a significant increase in *Pantoea* was observed in soybean seeds after drying [35]. *Pseudomonas* is a common epiphyte of wheat [37] and barley [38] seeds and together with *Pantoea* have demonstrated plant growth promotion or plant resistance characteristics [39]. The dominance of *Pantoea* in dried barley samples in this study may indicate their resistance to stress is caused due to loss of water. During ensiling process of EB by using heterofermentative LAB mix as inoculant, its bacterial composition shifted from Proteobacteria-dominated to Firmicutes-dominated community. This shift is expected during successful conservation of small grain silages [40]. However, Franco et al. [9] demonstrated that the moisture content during barley ensiling process also plays a significant role in defining the final microbial community composition, with both medium and high moisture contents initiating the shift towards Firmicutes. The EB was dominated by *Lentilactobacillus* and *Pediococcus*, the two genera harboring the LAB species used for inoculation and demonstrated similar replacement of indigenous communities with inoculated species as observed in wilted grass silage [41]. The third species included in the inoculant, *Levilactobacillus* (*Lactobacillus brevis*), on the other hand, was only detected at minor abundance in EB.

The microbial community changes in freshly mixed as compared to 2-day-old TMR diets were preservation method dependent. The changes were minor in AS-based TMR diets but more pronounced in IS-based TMR diets, indicating that risk for spoilage during feed-out could be smaller for AS than for IS. The increase in *Lactobacillaceae* and reduction in *Pseudomonadaceae* abundances in ID could indicate some fermentation activities during aerobic exposure. The same TMR samples were tested for aerobic stability [16] and the clearly faster heating of IS- rather than AS-based TMR (31 vs. 151 h) is in line with the greater changes in the TMR microbiota of IS rather than AS over two days. The poor aerobic stability of IS could be explained by higher yeast count in IS compared to AS (1.2×10^7 vs. 2.0×10^2 colony forming units [16]). Additionally, the larger between-period variation in IS microbial community composition could indicate greater sensitivity of IS than AS in horizontal bunker silos to the environmental factors such as outdoor temperature. The experiment was conducted during January to April 2021 and the average weekly temperature one week prior to sampling was −5.8, −8.2, −3.9, and +3.4 °C for periods 1 to 4, respectively.

4.3. The Association between Feed and Rumen Microbiota

The phenotypic and production traits of dairy cows are influenced by the continuous interactions among the animals, their diets, and the environment. Each of these components possesses specific microbiomes. However, our current understanding of how these microbiomes interact and contribute to the development of specific phenotypes remains limited [42]. For instance, seasonal differences in herd management expose animals to outdoor and indoor environments with distinct microbial ecosystems that could be transferred to and affect raw milk properties [43]. Similarly, the impact of feed-associated microbiota on the subsequent processes related to dairy production remain to be better understood. In this experiment, we examined the microbiota present in both the feed and rumen samples.

Our findings revealed that the predominant bacteria in the feed were not detected among the rumen microbiotas. Only *Lactobacillaceae* family, which accounted for 50–60% of the abundance in TMR samples, exhibited a diminutive presence (0–0.3%) in rumen samples. The second observation was that feeds with different microbiota compositions did not stimulate changes among rumen specific microbiota. This suggests that, while feed-associated microbiota lose ability to function in ruminal environments, they might influence rumen function through microbial-derived feed metabolites rather than the feed microbiota itself.

4.4. Feed Intake and Nutrient Digestion

Voluntary feed intake is a key parameter related to the milk production potential of feeds, and restriction of silage fermentation has resulted in increased feed intake [4]. The slightly higher average DM intake of AS than IS (26.2 vs. 25.6 kg/d) did not reach significance in this trial due to the rather small differences in the fermentation quality of the experimental silages and small amount of cows in the trial, but in the companion milk production trial [16], cows fed AS diets had greater DM intake than those fed IS diet.

The lack of effect of the barley grain preservation method on feed intake is in line with the production trial [16] and a meta-analysis [8] where dry and high moisture cereals were compared. However, the significant reduction of diet OM and starch digestion were contrary to earlier research, where improvements in digestibility have been observed due to crimping and ensiling of mainly corn grains [8,13]. One explanation for the reduced starch digestibility of EB could be unsuccessful breakage of some barley grain kernels, which may have resulted in passage of undigested kernels through the digestive tract. This explanation is supported by the greater OM excretion (6.76 vs. 6.48 kg/d) and rather similar NDF excretion (3.81 vs. 3.85 kg/d) for the cows fed ensiled compared with dry barley. This finding emphasizes the correct adjustments of the crimper mill when cereal grains are ensiled.

4.5. Milk Production and Composition

The lack of diet effects on milk production and milk composition can be explained by the minor changes in the nutrient supply to the cows between the experimental diets. This is in line with the companion milk production trial [16] and regarding grass silages, can be explained by the unexpectedly small differences in the silage fermentation quality despite the use of different additives in silage preparation.

The majority of the published data related to milk production responses to dry vs. high moisture grains are for corn, and similar animal responses have in general been reported irrespective of grain preservation method [8,13,44]. In experiments using barley, Petterson et al. [45] reported a slight decrease, Jaakkola et al. [46] found no difference and Jatkauskas et al. [7] indicated a positive milk production response when ensiled rather than dry barley grains were fed to dairy cows. In addition, improved growth rate of bulls [14] was observed when crimped and ensiled rather than dry barley grains were used in finishing beef cattle diets.

4.6. Rumen Fermentation, Enteric Methane Emissions, and Energy and Nitrogen Utilization

The higher WSC concentration of restrictively fermented silages has resulted in higher lipogenic-to-glucogenic type of rumen fermentation in earlier experiments [47,48], but such an effect was not observed in the current experiment in line with [16] obviously due to the small differences in the fermentation end product profile between AS and IS. The higher proportion of butyrate in total rumen VFA with AS compared to IS was similarly observed in the companion milk production trial [16].

The tendency for lower daily methane emission by feeding IS than AS was mainly caused by the differences in feed intake as methane yield (g/kg DM intake) was not affected by the treatments. This is consistent with the lack of differences in ruminal molar acetate to propionate ratio. The methane conversion factor (methane energy/energy intake × 100) for different diets ranged from 6.29 to 6.41% which is consistent with the value of 6.4%

calculated from an EU database by Niu et al. [49] and 6.42% in a meta-analysis of the previous experiments conducted in Finland [50].

The lack of effects of dietary treatments on energy and N intake are consistent with the minor differences in feed and nutrient intakes. Lower heat production by cows fed EB rather than DB might be related to the lower starch digestibility and the lower microbial fermentation, which also suggests a relation to the lower microbial heat production in the total digestive tract. The rumen microbes produce heat during their maintenance and growth (anabolic functions), and synthesis of reserve carbohydrates and energy spilling (i.e., futile cycles that dissipate heat) [51]. Rumen microbes expend energy for storing energy-accumulating reserve carbohydrates after feeding (during carbohydrate excess) and their mobilization thereafter (during carbohydrate limitation). Protozoa account for most accumulation of reserve carbohydrates, and in competition experiments, protozoa accumulated nearly 35-fold more reserve carbohydrates than bacteria [51]. The lower N balance in cows fed lactic acid bacteria inoculated silage and dried barley compared to formic acid-treated silage and dried barley diet was due to the higher N excretion in urine.

5. Conclusions

The small changes in grass silage fermentation quality, despite contrasting additive treatments and barley grain preservation methods, resulted in only minor differences in fermentation characteristics and chemical composition of the feeds. Similarly, the influence on rumen fermentation was minimal. The energy metabolism of the cows was not affected by the dietary treatments despite some effects from inoculated silage and preserved barley on DM and OM digestibility. Furthermore, grass silage and barley grain preservation methods had a clear influence on silage and barley microbiome with no effect on rumen microbiota which implies the rumen microbial population's resistance to external microbial interventions.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/dairy4040048/s1, Figure S1: Relative abundance of bacterial communities at phylum (A) and family (B) taxonomical levels of the experimental feeds and total mixed rations (TMR). Figure S2: The rumen bacterial (A) and archaeal (B) alpha diversity expressed as observed number of ASVs and Shannon diversity index. Figure S3: The rumen bacterial (A) composition at genus level and archaeal (B) composition at species level. Figure S4: The rumen bacteria (A) at genus level and archaea (B) at species level shared between the diets.

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Institutional Review Board Statement: The experiment was approved by regional State Administrative Agency (ESAVI/32016/2020) in accordance with the guidelines of the European Community Council Directive 2010/63/EU (EU, 2010) for animal experiments complying with the ARRIVE guidelines [52].

Data Availability Statement: Sequencing data are available in the NCBI Sequence Read Archive under BioProject Accession PRJNA796213. Other data can be requested from the authors.

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References

1. Wilkinson, J.M.; Rinne, M. Highlights of Progress in Silage Conservation and Future Perspectives. *Grass Forage Sci.* **2018**, *73*, 40–52. [CrossRef]
2. McDonald, P.; Edwards, R.A.; Greenhalgh, J.F.D.; Morgan, C.A.; Sinclair, L.A.; Wilkinson, R.G. *Animal Nutrition*, 7th ed.; Prentice Hall/Pearson: Harlow, UK, 1991.
3. Muck, R.E.; Nadeau, E.M.G.; McAllister, T.A.; Contreras-Govea, F.E.; Santos, M.C.; Kung, L. Silage Review: Recent Advances and Future Uses of Silage Additives. *J. Dairy Sci.* **2018**, *101*, 3980–4000. [CrossRef] [PubMed]
4. Huhtanen, P.; Rinne, M.; Nousiainen, J. Evaluation of the Factors Affecting Silage Intake of Dairy Cows: A Revision of the Relative Silage Dry-Matter Intake Index. *Animal* **2007**, *1*, 758–770. [CrossRef] [PubMed]
5. Jaakkola, S.; Kaunisto, V.; Huhtanen, P. Volatile Fatty Acid Proportions and Microbial Protein Synthesis in the Rumen of Cattle Receiving Grass Silage Ensiled with Different Rates of Formic Acid. *Grass Forage Sci.* **2006**, *61*, 282–292. [CrossRef]
6. Franco, M.; Tapio, I.; Pirttiniemi, J.; Stefański, T.; Jalava, T.; Huuskonen, A.; Rinne, M. Fermentation Quality and Bacterial Ecology of Grass Silage Modulated by Additive Treatments, Extent of Compaction and Soil Contamination. *Fermentation* **2022**, *8*, 156. [CrossRef]
7. Jatkauskas, J.; Vrotniakienė, V.; Urbšienė, D. Comparison of Ordinary Dried and Ensiled Crimped Barley Grain in Dairy Cattle Diets. 2012. Available online: https://lsmu.lt/cris/handle/20.500.12512/87897 (accessed on 8 August 2023).
8. Torres, R.N.S.; Ghedini, C.P.; Coelho, L.M.; Ezequiel, J.M.B.; Júnior, G.A.A.; Almeida, M.T.C. Meta-Analysis of the Effects of Silage Additives on High-Moisture Grain Silage Quality and Performance of Dairy Cows. *Livest. Sci.* **2021**, *251*, 104618. [CrossRef]
9. Franco, M.; Tapio, I.; Rinne, M. Preservation Characteristics and Bacterial Communities of Crimped Ensiled Barley Grains Modulated by Moisture Content and Additive Application. *Front. Microbiol.* **2022**, *13*, 1092062. [CrossRef]
10. Newbold, C.J.; Ramos-Morales, E. Review: Ruminal Microbiome and Microbial Metabolome: Effects of Diet and Ruminant Host. *Animal* **2020**, *14*, s78–s86. [CrossRef]
11. Astuti, W.D.; Wiryawan, K.G.; Wina, E.; Widyastuti, Y.; Suharti, S.; Ridwan, R. Effects of Selected Lactobacillus Plantarum as Probiotic on In Vitro Ruminal Fermentation and Microbial Population. *Pak. J. Nutr.* **2018**, *17*, 131–139. [CrossRef]
12. Jeyanathan, J.; Martin, C.; Morgavi, D.P. Screening of Bacterial Direct-Fed Microbials for Their Antimethanogenic Potential in Vitro and Assessment of Their Effect on Ruminal Fermentation and Microbial Profiles in Sheep. *J. Anim. Sci.* **2016**, *94*, 739–750. [CrossRef]
13. Ferraretto, L.F.; Crump, P.M.; Shaver, R.D. Effect of Cereal Grain Type and Corn Grain Harvesting and Processing Methods on Intake, Digestion, and Milk Production by Dairy Cows through a Meta-Analysis. *J. Dairy Sci.* **2013**, *96*, 533–550. [CrossRef]
14. Huuskonen, A.; Rinne, M.; Manni, K. Effects of Different Barley Grain Preservation Techniques on Intake, Growth and Carcase Traits of Finishing Dairy Bulls Fed Grass Silage-Based Rations. *J. Agric. Sci.* **2020**, *158*, 748–755. [CrossRef]
15. Jokiniemi, H.T.; Ahokas, J.M. Drying Process Optimisation in a Mixed-Flow Batch Grain Dryer. *Biosyst. Eng.* **2014**, *121*, 209–220. [CrossRef]
16. Rinne, M.; Franco, M.; Tapio, I.; Stefański, T.; Bayat, A.-R.; Mäntysaari, P. Effects of Grass Silage Additive Type and Barley Grain Preservation Method on Rumen Fermentation, Microbial Community and Milk Production of Dairy Cows. *Agriculture* **2022**, *12*, 266. [CrossRef]
17. Bayat, A.R.; Vilkki, J.; Razzaghi, A.; Leskinen, H.; Kettunen, H.; Khurana, R.; Brand, T.; Ahvenjärvi, S. Evaluating the Effects of High-Oil Rapeseed Cake or Natural Additives on Methane Emissions and Performance of Dairy Cows. *J. Dairy Sci.* **2022**, *105*, 1211–1224. [CrossRef]
18. Yu, Z.; Morrison, M. Improved Extraction of PCR-Quality Community DNA from Digesta and Fecal Samples. *BioTechniques* **2004**, *36*, 808–812. [CrossRef]
19. Rius, A.G.; Kittelmann, S.; Macdonald, K.A.; Waghorn, G.C.; Janssen, P.H.; Sikkema, E. Nitrogen Metabolism and Rumen Microbial Enumeration in Lactating Cows with Divergent Residual Feed Intake Fed High-Digestibility Pasture. *J. Dairy Sci.* **2012**, *95*, 5024–5034. [CrossRef]
20. Caporaso, J.G.; Lauber, C.L.; Walters, W.A.; Berg-Lyons, D.; Lozupone, C.A.; Turnbaugh, P.J.; Fierer, N.; Knight, R. Global Patterns of 16S rRNA Diversity at a Depth of Millions of Sequences per Sample. *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 4516–4522. [CrossRef]
21. Callahan, B.J.; McMurdie, P.J.; Rosen, M.J.; Han, A.W.; Johnson, A.J.A.; Holmes, S.P. DADA2: High-Resolution Sample Inference from Illumina Amplicon Data. *Nat. Methods* **2016**, *13*, 581–583. [CrossRef]
22. Bolyen, E.; Rideout, J.R.; Dillon, M.R.; Bokulich, N.A.; Abnet, C.C.; Al-Ghalith, G.A.; Alexander, H.; Alm, E.J.; Arumugam, M.; Asnicar, F.; et al. Reproducible, Interactive, Scalable and Extensible Microbiome Data Science Using QIIME 2. *Nat. Biotechnol.* **2019**, *37*, 852–857. [CrossRef]
23. Sjaunja, L.O.; Baevre, L.; Junkkarinen, L.; Pedersen, J.; Setälä, J. A Nordic Proposal for an Energy Corrected Milk (ECM) Formula. In Proceedings of the 27th Biennial Session of the International Committee for Animal Recording (ICAR), Paris, France, 2–6 July 1990; EAAP Publication No. 50; Centre for Agricultural Publishing and Documentation (Pudoc): Paris, France, 1991; p. 156.
24. Brouwer, E. Report of Sub-Committee on Constants and Factors. In *Energy Metabolism of Farm Animals*; Academic Press: Cambridge, MA, USA, 1965; Volume 11, pp. 441–443.
25. Kriss, M. Quantitative Relations of the Dry Matter of the Food Consumed, the Heat Production, the Gaseous Outgo, and the Insen- Sible Loss in Body Weight of Cattle. *J. Agric. Res.* **1930**, *40*, 283–295.

26. Franco, M.; Tapio, I.; Huuskonen, A.; Rinne, M. Fermentation Quality and Bacterial Ecology of Red Clover Dominated Silage Modulated by Different Management Factors. *Front. Anim. Sci.* **2022**, *3*, 1080535. [CrossRef]
27. Huhtanen, P.; Nousiainen, J.; Rinne, M. Recent Developments in Forage Evaluation with Special Reference to Practical Applications. *Agric. Food Sci.* **2006**, *15*, 293. [CrossRef]
28. *Luke Feed Tables and Nutrient Requirements*; Natural Resources Institute Finland: Helsinki, Finland. 2023. Available online: www.luke.fi/feedtables (accessed on 23 April 2023).
29. Franco, M.; Rinne, M. Dry Matter Content and Additives with Different Modes of Action Modify the Preservation Characteristics of Grass Silage. *Fermentation* **2023**, *9*, 640. [CrossRef]
30. Ali, N.; Wang, S.; Zhao, J.; Dong, Z.; Li, J.; Nazar, M.; Shao, T. Microbial Diversity and Fermentation Profile of Red Clover Silage Inoculated with Reconstituted Indigenous and Exogenous Epiphytic Microbiota. *Bioresour. Technol.* **2020**, *314*, 123606. [CrossRef]
31. Eliasson, T.; Sun, L.; Lundh, Å.; Höjer, A.; Saedén, K.H.; Hetta, M.; Gonda, H. Epiphytic Microbiota in Swedish Grass-Clover Herbage and the Effect of Silage Additives on Fermentation Profiles and Bacterial Community Compositions of the Resulting Silages. *J. Appl. Microbiol.* **2023**, *134*, lxad196. [CrossRef]
32. Xu, H.; Wu, N.; Na, N.; Sun, L.; Zhao, Y.; Ding, H.; Fang, Y.; Wang, T.; Xue, Y.; Zhong, J. Fermentation Weight Loss, Fermentation Quality, and Bacterial Community of Ensiling of Sweet Sorghum with Lactic Acid Bacteria at Different Silo Densities. *Front. Microbiol.* **2022**, *13*, 1013913. [CrossRef]
33. Herrmann, C.; Heiermann, M.; Idler, C. Effects of Ensiling, Silage Additives and Storage Period on Methane Formation of Biogas Crops. *Bioresour. Technol.* **2011**, *102*, 5153–5161. [CrossRef]
34. Bai, J.; Ding, Z.; Ke, W.; Xu, D.; Wang, M.; Huang, W.; Zhang, Y.; Liu, F.; Guo, X. Different Lactic Acid Bacteria and Their Combinations Regulated the Fermentation Process of Ensiled Alfalfa: Ensiling Characteristics, Dynamics of Bacterial Community and Their Functional Shifts. *Microb. Biotechnol.* **2021**, *14*, 1171–1182. [CrossRef]
35. Chandel, A.; Mann, R.; Kaur, J.; Norton, S.; Edwards, J.; Spangenberg, G.; Sawbridge, T. Implications of Seed Vault Storage Strategies for Conservation of Seed Bacterial Microbiomes. *Front. Microbiol.* **2021**, *12*, 784796. [CrossRef]
36. Østlie, H.M.; Porcellato, D.; Kvam, G.; Wicklund, T. Investigation of the Microbiota Associated with Ungerminated and Germinated Norwegian Barley Cultivars with Focus on Lactic Acid Bacteria. *Int. J. Food Microbiol.* **2021**, *341*, 109059. [CrossRef]
37. Hone, H.; Mann, R.; Yang, G.; Kaur, J.; Tannenbaum, I.; Li, T.; Spangenberg, G.; Sawbridge, T. Profiling, Isolation and Characterisation of Beneficial Microbes from the Seed Microbiomes of Drought Tolerant Wheat. *Sci. Rep.* **2021**, *11*, 11916. [CrossRef]
38. Bziuk, N.; Maccario, L.; Straube, B.; Wehner, G.; Sørensen, S.J.; Schikora, A.; Smalla, K. The Treasure inside Barley Seeds: Microbial Diversity and Plant Beneficial Bacteria. *Environ. Microbiome* **2021**, *16*, 20. [CrossRef]
39. Gerna, D.; Roach, T.; Mitter, B.; Stöggl, W.; Kranner, I. Hydrogen Peroxide Metabolism in Interkingdom Interaction Between Bacteria and Wheat Seeds and Seedlings. *Mol. Plant-Microbe Interact.* **2020**, *33*, 336–348. [CrossRef]
40. Duniere, L.; Xu, S.; Long, J.; Elekwachi, C.; Wang, Y.; Turkington, K.; Forster, R.; McAllister, T.A. Bacterial and Fungal Core Microbiomes Associated with Small Grain Silages during Ensiling and Aerobic Spoilage. *BMC Microbiol.* **2017**, *17*, 50. [CrossRef]
41. Parvin, S.; Wang, C.; Li, Y.; Nishino, N. Effects of Inoculation with Lactic Acid Bacteria on the Bacterial Communities of Italian Ryegrass, Whole Crop Maize, Guinea Grass and Rhodes Grass Silages. *Anim. Feed. Sci. Technol.* **2010**, *160*, 160–166. [CrossRef]
42. Attwood, G.T.; Wakelin, S.A.; Leahy, S.C.; Rowe, S.; Clarke, S.; Chapman, D.F.; Muirhead, R.; Jacobs, J.M.E. Applications of the Soil, Plant and Rumen Microbiomes in Pastoral Agriculture. *Front. Nutr.* **2019**, *6*, 107. [CrossRef]
43. Doyle, C.J.; Gleeson, D.; O'Toole, P.W.; Cotter, P.D. Impacts of Seasonal Housing and Teat Preparation on Raw Milk Microbiota: A High-Throughput Sequencing Study. *Appl. Environ. Microbiol.* **2017**, *83*, e02694-16. [CrossRef]
44. Ferland, M.-C.; Guesthier, M.-A.; Cue, R.I.; Lacroix, R.; Burgos, S.A.; Lefebvre, D.; Wade, K.M. Effect of Feeding System and Grain Source on Lactation Characteristics and Milk Components in Dairy Cattle. *J. Dairy Sci.* **2018**, *101*, 8572–8585. [CrossRef] [PubMed]
45. Pettersson, T.; Bernes, G.; Martinsson, K. Ensiled Rolled or Dried Barley Drain and Different Levels of Grass Silage and Hay to Dairy Cows. *Swed. J. Agric. Res.* **1998**, *28*, 99–109.
46. Jaakkola, S.; Saarisalo, E.; Kangasniemi, R. Ensiled High Moisture Barley or Dry Barley in the Grass Silage-Based Diet of Dairy Cows. In *Silage Production and Utilisation, Proceedings of the XIVth International Silage Conference, a Satellite Workshop of the XXth International Grassland Congress, Belfast, UK, 3–6 July 2005*; Park, R.S., Stronge, M.D., Eds.; Wageningen Academic Publishers: Belfast, UK, 2005; p. 184.
47. Saarisalo, E.; Skyttä, E.; Jaakkola, S. Effects of Wilted Grass Silages Varying in Fermentationquality on Rumen Fermentation of Dairy Cows. *J. Anim. Feed Sci.* **2004**, *13*, 199–202. [CrossRef]
48. Halmemies-Beauchet-Filleau, A.; Kairenius, P.; Ahvenjärvi, S.; Crosley, L.K.; Muetzel, S.; Huhtanen, P.; Vanhatalo, A.; Toivonen, V.; Wallace, R.J.; Shingfield, K.J. Effect of Forage Conservation Method on Ruminal Lipid Metabolism and Microbial Ecology in Lactating Cows Fed Diets Containing a 60:40 Forage-to-Concentrate Ratio. *J. Dairy Sci.* **2013**, *96*, 2428–2447. [CrossRef] [PubMed]
49. Niu, M.; Kebreab, E.; Hristov, A.N.; Oh, J.; Arndt, C.; Bannink, A.; Bayat, A.R.; Brito, A.F.; Boland, T.; Casper, D.; et al. Prediction of Enteric Methane Production, Yield, and Intensity in Dairy Cattle Using an Intercontinental Database. *Glob. Chang. Biol.* **2018**, *24*, 3368–3389. [CrossRef] [PubMed]
50. Adjassin, J.S.; Guinguina, A.; Eugène, M.; Bayat, A.R. Models Predicting Methane Emissions and Methane Conversion Factor of Finnish Nordic Red Dairy Cows. In Proceedings of the 74th EAAP Annual Meeting, Lyon, France, 26 Ausust–1 September 2023; p. 899.

51. Hackmann, T.J.; Firkins, J.L. Maximizing Efficiency of Rumen Microbial Protein Production. *Front. Microbiol.* **2015**, *6*. [CrossRef]
52. Kilkenny, C.; Browne, W.J.; Cuthill, I.C.; Emerson, M.; Altman, D.G. Improving Bioscience Research Reporting: The ARRIVE Guidelines for Reporting Animal Research. *PLoS Biol.* **2010**, *8*, e1000412. [CrossRef]

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