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Effects of Grass Silage Additive Type and Barley Grain Preservation Method on Rumen Fermentation, Microbial Community and Milk Production of Dairy Cows

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Abstract: Grass was ensiled using an acid-based additive (AS) or homofermentative lactic acid bacteria (IS). In addition, barley grain was either dried (DB) or crimped and ensiled (EB). The feeds were fed as total mixed rations (TMR) in a 2 × 2 factorial arrangement to 16 Nordic Red dairy cows in four replicated Latin squares. The differences in the fermentation quality of the two grass silages were unexpectedly small. Dry matter intake was higher ($p < 0.01$) for cows fed AS than those fed IS but was not affected by barley preservation method. Ruminal molar proportion of butyrate tended to be higher in cows fed AS rather than IS ($p < 0.10$) in expense of acetate ($p < 0.05$). Barley preservation method did not affect rumen fermentation but modulated rumen bacterial community composition. Milk production was not affected by silage additive but tended ($p < 0.10$) to be higher (39.6 vs. 39.0 kg/d) for cows fed DB rather than EB. However, barley type did not affect energy corrected milk yield due to a tendency ($p < 0.10$) for higher milk fat content of cows fed EB rather than DB. Milk fat yield tended ($p < 0.10$) to be higher for AS-fed cows than IS-fed cows, and milk protein yield was higher for cows receiving DB rather than EB. The AS resulted in more aerobically stable TMR than IS and a minor advantage was found for DB compared to EB.

Keywords: aerobic stability; crimping; ensiled grain; formic acid; grain preservation; high-moisture grain; lactic acid bacteria inoculant; rumen microbiome; silage fermentation

Citation: 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. https://doi.org/10.3390/agriculture12020266

Academic Editors: Elisabet Nadeau, Horst Auerbach and João Daniel

Received: 12 January 2022
Accepted: 11 February 2022
Published: 13 February 2022

1. Introduction

Seasonal feed production makes preservation of both forage and concentrate feeds necessary. Ensiling has become the dominant method of forage preservation in most intensive milk production regions, and several management factors have been greatly developed over the decades, including mechanisation, wilting practices, and use of additives [1]. Grass is more difficult to ensile than, e.g., whole crop maize [2], so that improving fermentation quality of grass silage remains an important target. Different types of additives can be used to facilitate good preservation of grass, such as lactic acid bacteria inoculants (LAB) and organic-acid-based additives [2,3]. Selected LAB strains are widely available in the market and they are used to accelerate and direct the lactic acid fermentation in the silage compared to untreated material, while formic acid (FA)-based additives effectively restrict silage fermentation [2,3].

The differences in the mode of action of these two types of additives induce specific changes in silage composition. Application of FA to grass silage results in a silage with relatively high water soluble carbohydrate (WSC) concentration and low lactic acid and volatile fatty acid (VFA) concentration, the opposite being the case for silages prepared using bacterial inoculants [2,3]. When cows consume these silages, the substrates for rumen microbes also differ substantially, which has been reflected in differences in rumen fermentation pattern and, subsequently, in the metabolism and milk composition of dairy

cows [4–6]. Restriction of silage fermentation has also been shown to increase the voluntary feed intake of dairy cows [7].

Although forages form the basis of ruminant nutrition, modern dairy cows are fed significant amounts of various concentrate feeds to fully exploit their milk production potential. Dry cereal grains, such as barley, wheat, or maize, are predominant energy sources of dietary concentrates but, under humid harvesting conditions, the moisture content of the grain is so high that preservation requires artificial drying or use of alternative methods [8]. Crimping and ensiling, e.g., crushing slightly the moist grains and preserving them air-tight, is a grain preservation method that relies on the same principles as ensiling of forages [9]. Use of high-moisture cereals is a common practice in many areas, as, e.g., Ferland et al. [10] found, in a large dataset collected from commercial farms, that over 60% of the maize for dairy cows in Canada was used as high-moisture.

As a preservation method, ensiling of grains is cost- and energy-effective compared to drying [8]. Generally similar milk production responses have been achieved with dry and moist cereals whether being based on maize [10–13] or barley [14,15], although Pettersson et al. [16] reported a slight decrease in milk production when moist barley compared with dry barley was fed. Moist maize has increased total-tract starch digestibility and affected rumen fermentation pattern when compared with dried maize [11,12], but less information is available on the effects of barley preservation method on these parameters.

The objective of the present study was to evaluate how the grass silage additive choice and barley grain preservation method affect the performance, rumen fermentation, and rumen microbial community of dairy cows. We hypothesised that using different types of silage additives induces clear differences in silage fermentation pattern, which will subsequently affect silage intake, rumen fermentation, and milk production responses of cows, but that barley preservation method has only minor effects on them.

2. Materials and Methods

2.1. Preparation of Experimental Feeds

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) from 4 different parcels during 2 days (15 and 16 June 2020). The botanical composition of the forage was 73% timothy and 27% meadow fescue on dry matter (DM) basis, and ears were emerging in both grass species at the time of harvesting, representing a recommended harvest time in the region. The swards were fertilised with 95 kg N and 4 kg K per hectare in late April using a commercial mineral fertiliser. The grass was wilted in sunny weather for 1–3 h, harvested using a precision chopper (JF FCT 1350, JF-Fabriken—J Freudendahl A/S, Sonderborg, Denmark), and ensiled in horizontal bunker silos. The chopper was equipped with two additive containers and the two experimental additives were applied to alternate loads to ensure identical grass composition and other conditions for both silages. The fresh forage was representatively sampled from all loads delivered to the silos.

The additives used were an organic-acid-based product (AIV Ässä Na, Eastman Ltd., Oulu, Finland; composition: 580 g/kg formic acid, 200 g/kg propionic acid, 52 g/kg sodium formate, and 25 g/kg potassium sorbate) at a target rate of 5 L/ton fresh matter, and a homofermentative lactic acid bacteria (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 an application rate of 1.0×10^5 CFU/g fresh material according to manufacturer recommendations. The experiment started after an ensiling period of 6 months.

The other dietary factor was the preservation method of barley grain. The barley (variety Eversti) was sown on 15 May 2020 and fertilised using a commercial mineral fertiliser (78 kg N/ha). The barley was combine harvested on 24 September 2020 at a moisture content of 223 g/kg. Part of the batch was dried in a grain drier to reach a moisture concentration of 123 g/kg, and milled and pelleted before feeding. The other part of barley

was crimped using a farm-scale crimper mill (MD 700 HD, Murska Ltd., Ylivieska, Finland) so that the grains remained whole but the inner part of the grain 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 an application rate of 7.0×10^5 CFU/g fresh material, exceeding slightly the manufacturer recommendation of 6.0×10^5 CFU/g fresh material. The inoculant was applied as a water solution, and tap water was added simultaneously so that the moisture content of the crimped barley increased to 283 g/kg. The crimped barley was stored outside in a trailer container covered with silo plastic, tarpaulin, and weights, and the feeding experiment started after 3.5 months of storage.

2.2. Animal Care, Experimental Design, and Treatments

The experiment was conducted in the experimental dairy barn of Luke (Jokioinen, Finland). The cows were kept indoors in an insulated barn as a group in a loose-house system and fitted with transponder collars that allowed identification in the milking parlour, weigh scales, and feeding area. The total mixed rations (TMR) were mixed 3 times a week and stored in an uninsulated barn with an approximate temperature of +10 °C. Each cow had an individual feeding place equipped with physical separators between the places. The TMR was delivered to cows daily at 0700, 1300, 1600, and 1830 h by an automatic feeding wagon (TR Feeding Robot, Pellon Group Ltd., Ylihärmä, Finland), which also weighed the amount given to each individual feeding place. Uneaten feed was removed and weighed daily at 1200 h before offering fresh feed. At least 5% of refusals was targeted daily to ensure *ad libitum* feed intake. Cows were milked in a 2 × 6 auto tandem milking parlour daily at 0700 and 1700 h. Water and salt blocks were freely available.

A total of 16 multiparous Nordic Red dairy cows were assigned randomly to four replicated 4 × 4 Latin squares, which were balanced for the sequence of treatments. The length of each period was 21 d with 14 d for diet adaptation and 7 d for data and sample collection. Cows were 64 ± 25.0 days in milk (mean ± standard deviation), with a milk yield of 42.5 ± 5.92 kg/d and body weight of 676 ± 64.7 kg at the beginning of the experiment and their average parity was 3.8 ± 1.89, with no primiparous cows included. The cows were divided into 4 blocks of 4 cows according to parity and calving date. Within the block, the cows were randomly allocated to the 4 dietary treatments. One of the blocks included cows previously fitted with permanent rumen cannulae (Bar Diamond Inc., Parma, ID, USA). The dietary treatments were arranged 2 × 2 factorially and they consisted of two grass silages treated with either organic-acid-based additive (AS) or inoculated with an LAB preparation (IS) and two types of barley grain preservation methods, i.e., dry (DB) or crimped and ensiled (EB).

The experimental diets were fed as TMR comprising 50% grass silage and 22.5% barley grain on DM basis. In addition, a concentrate mixture was included to complement the diets, which contained oats, wheat, sugar beet pulp, rapeseed meal, and mineral and vitamin premix (203, 77, 155, 516, and 49 g/kg on an as-fed basis, respectively). The cows also received 600 g of concentrate daily at the milking parlour, divided into two equal batches containing barley, wheat, sugar beet pulp, rapeseed meal, and mineral and vitamin premix (375, 170, 140, 285, and 30 g/kg on an as-fed basis, respectively). The mineral and vitamin premix used in both concentrate mixtures was Lypsykivennäinen Tiineys+ (Hankkija Ltd., Hyvinkää, Finland; composition: Ca 210 g/kg, P 15 g/kg, Mg 90 g/kg, Na 95 g/kg, Na selenite 3bE8 20 mg/kg, selenomethionine 3b8.11 10 mg/kg, vitamin A 3a672a 250,000 IU/kg, vitamin D_3 3a671 110,000 IU/kg, vitamin E 3a700 all-rac-α-tocoferyl acetate 2000 mg/kg, and D-biotin 3a880 30 mg/kg). All cows were also given a vitamin E supplement (30 mL per week top-dressed on their TMR with Seleeni E liquid (Hankkija Ltd., Hyvinkää, Finland; composition: Na selenite 3bE8 0.02 mg/mL, vitamin E 3a700 all-rac-α-tocoferyl acetate 70 mg/mL, and D-biotin 3a880 0.2 mg/mL)) and they had free access to a mineral supplement (LypsyMelli, Lantmännen Agro Ltd., Raisio, Finland;

composition: Ca 125, P 2, Mg 65, Na 78 g/kg, vitamin A 3a672a 250,000 IU/kg, vitamin D_3 3a671 60,000 IU/kg, and vitamin E 3a700 600 mg/kg) and salt blocks.

2.3. Sampling Procedures

Silage DM concentration was measured twice a week during the whole experiment to facilitate accurate TMR mixing. Silages and all 4 concentrate feeds were sampled daily in the last 7 d of each measurement period. To evaluate the microbial counts in the TMR, samples were taken on d 17 of each period immediately after TMR preparation, and again on d 19 of the same batch after a storage period of 2 d. The amount of milk was gravimetrically recorded (Pellon SAC, Kolding, Denmark) at every milking, and results from the last 7 d of each period were used for calculations. Milk samples were taken at four consecutive milkings, starting in the afternoon of d 18, and preserved with 2-bromo-2-nitropropane-1,3-diol (Bronopol; Valio Ltd., Helsinki, Finland). The cows were weighed every time they left the milking parlour by a walk-through static scale (Pellon Group Ltd., Ylihärmä, Finland). The animals were monitored daily for health problems, and any abnormalities and infections were recorded and treated according to the general barn guidelines. In the current study, all cows completed the whole experiment without symptoms that could be considered to affect the results.

Rumen fluid was sampled from the 4 ruminally cannulated cows using a vacuum pump on d 18 of each period at 1.5-h intervals from 0600 until 1800 h. A sample of approximately 400 mL was obtained by inserting a pipe into several locations in the ventral sac of the rumen. The pH of the rumen fluid was measured immediately after sampling and, after that, the samples were filtered through 2 layers of cheesecloth and prepared for subsequent ammonia (15 mL rumen fluid acidified with 0.3 mL of 50% H_2SO_4) and VFA (5 mL rumen fluid mixed with 0.5 mL saturated $HgCl_2$ solution and 2 mL 1 M NaOH) analyses. For rumen microbial community analysis, 0.5-1 litres of rumen fluid was collected from all cows using an oesophageal stomach tube (Ruminator, Profs Products, Wittybreut, Germany) ca. 3 h after morning feeding. Rumen liquid samples were aliquoted, snap frozen in dry ice, and stored at −80 °C until DNA extraction.

2.4. Analytical Methods and Calculations

Feed and rumen fluid analyses were conducted using routine methods (see Savonen et al. [17] for details) of Luke laboratory, which follows the standard SFS-EN ISO/IEC 17025:2017 and is accredited by the Finnish Accreditation Service (Helsinki, Finland) with a number T024. Starch was analysed as described by Salo and Salmi [18]. Silage FA was analysed according to the instructions of the commercial kit used (Cat. No. 979 732, Roche Diagnostics Ltd., Basel, Switzerland). The equipment used was UV-VIS double-beam UV-1800 spectrophotometer (Schimadzu Co., Kyoto, Japan) and the sample was water extracted 1:15 before analysis. The buffering capacity (BC, g lactic acid/100 g DM) of the fresh forage was determined according to Weissbach et al. [19], and the fermentation coefficient of herbage was calculated using the following formula: DM (%) + (8 × WSC (%)/BC) [19]. Milk samples were analysed for fat, protein, lactose, and urea using an infrared analyser (MilkoScan FT+; Foss Electric A/S, Hillerød, Denmark) at a commercial laboratory (Valio Ltd., Seinäjoki, Finland). The microbial counts of the feeds were analysed by serial dilutions plated on cultivation media as described in detail by Rinne et al. [20]. Due to practical limitations in sample logistics, the samples for microbial analyses were frozen prior to analyses. In order to integrate the individual VFAs into one characteristic, the non-glucogenic to glucogenic VFA ratio (NGR) was calculated according to Abrahamse et al. [21]: (acetic acid + 2 (butyric acid + isobutyric acid) + valeric acid + isovaleric acid)/(propionic acid + valeric acid + isovaleric acid).

Total DNA was extracted from 0.5 mL of rumen liquid as described by Rius et al. [22]. Universal primers 515F and 806R [23] targeting the V4 region of the 16S ribosomal RNA gene (rRNA) were used for bacterial amplicon sequencing. Libraries were prepared following the "16S metagenomics sequencing library preparation" protocol (Illumina) and

sequenced in Finnish Functional Genomics Centre (Turku, Finland) on Illumina MiSeq platform by using 2 × 250 bp chemistry. Demultiplexing of sequences, adapter removal, and sorting sequences by barcode were performed by the sequencing centre. Sequencing data was processed using Qiime 2 [24]. Briefly, quality control, filtering of chimeric reads, and clustering of bacterial sequences into amplicon sequence variants (ASV) were performed using DADA2 [25]. ASVs with less than 10 reads in total were removed. Bacterial ASV taxonomy was assigned using the Silva 138 database [26] and archaeal taxonomy was assigned using the RIM-DB database [27].

The metabolisable energy (ME) concentration of the silages was calculated as the in vitro D-value (the amount of digestible organic matter, g/kg DM) × 0.016. The ME concentration of the concentrate feeds was calculated from digestible nutrients using digestibility coefficients from Luke [28]. The metabolisable protein (MP) concentration and protein balance in the rumen (PBV) were calculated as described in Luke [28]. The energy-corrected milk (ECM) yield was calculated using the equation presented by Sjaunja et al. [29]. The N use efficiency in milk production (NUE) was calculated as: N excreted in milk (kg)/N intake (kg). Energy balance was calculated for each cow by subtracting the energy required for milk production and maintenance from the total energy intake. The ME (MJ) used for ECM production (5.15 × ECM, kg) and for maintenance (0.515 × kg body weight$^{0.75}$) was based on Finnish standards [28].

The aerobic stability of the experimental TMR diets and individual fermented feeds (AS, IS, and ensiled barley) was measured in the laboratory twice during the feeding trial. The TMR was collected from the barn immediately after mixing it on d 17 of periods 1 and 2. The TMR was used as such, and two additional treatments with increased moisture content were prepared by adding tap water into the original TMR. The amount of water was set to obtain a 3% unit difference between the three TMR, which reflected the same difference as TMR based on dry and ensiled barley treatments. Approximately 700 g of each TMR in 3 replicates per period was placed into a polystyrene box. The temperature was measured using a MicroLite USB Data Logger (Fourtec, Chicago, IL, USA), where thermocouple probes inserted into the samples were connected to MicroLite devices. Temperature was automatically recorded at 10-min intervals for a 200-h period. Aerobic stability was defined as the time taken to increase the temperature of the sample for 2 °C above the ambient temperature. The ambient temperature was 21.7 ± 0.60 °C (min. 20.2 and max. 22.6 °C), measured using a similar data logger as for the samples.

2.5. Statistical Analyses

The results of the milk production experiment were analysed as four replicated 4 × 4 Latin squares with a 2 × 2 factorial arrangement of treatments using the MIXED procedure of SAS (release 9.4, SAS Institute Inc., Cary, NC, USA), using individual cows as experimental units. Production data (n = 16 per treatment) were analysed with a model including fixed effects of silage, barley, and their interaction, while square, period, and cow (square) were considered as random effects. The rumen fermentation data from the square of cannulated cows (n = 4 per treatment) were analysed as repeated measurements over time using sampling time, silage, barley, silage × barley, and silage × barley × time as fixed effects, and period and cow(period) as random effects. The covariance structure for repeated measures over time was chosen using the lowest Bayesian information criterion value, with the compound symmetry covariate structure retained in the final model. The normality of the data distribution was inspected using PROC UNIVARIATE (Shapiro–Wilk test). The aerobic stability data were analysed using silage and barley preservation methods, moisture content, and their interactions as fixed effects in the statistical model, while replicate and period were considered as random effects. Regarding the TMR moisture content, the sum of squares was further partitioned into contrasts to evaluate the linear and quadratic effects. A significance level of $p < 0.05$ was considered and trends reported at $0.05 \leq p \leq 0.10$.

For rumen microbial community data after quality control, bacterial sequencing data presented 1,513,107 (mean per sample: 24,404, SD: 6474) sequences and archaeal data presented 24,727 (mean 399, SD 136) sequences in total. Rumen microbial community alpha diversity was calculated using observed ASVs, Shannon and Simpson diversity indexes. For alpha diversity, all samples were subsampled to the same depth, equivalent to the lowest number of 10,000 reads per sample for bacteria and 200 reads for archaea. The significance of differences in alpha diversity between the silage (AS vs. IS) and barley (DB vs. EB) preservation methods, as well as between dietary treatments (AD, AE, ID, IE), was calculated using the nonparametric Wilcoxon signed-rank test, as implemented in *MicrobiotaProcess* R package [30]. To evaluate silage, barley, and dietary treatment effects on the changes in rumen microbial community structure, between sample diversity was calculated as Bray–Curtis dissimilarities following Hellinger transformation and visualised using principal co-ordinate analysis (PCoA). The significance of groups was evaluated by distance-based permutational multivariate analysis of variance (adonis) and defined at $p < 0.05$ level after 999 permutations, as implemented in *vegan* R package [31]. To determine which microbial groups were affected by the silage and barley preservation methods, a linear discriminant analysis was performed as implemented in *MicrobiotaProcess*. First, significantly different taxa among groups ($p < 0.05$) were filtered by using the Kruskal–Wallis and Wilcoxon tests, followed by linear discriminant analysis, which estimated the effect size of these differences. Spearman correlation was applied to explore co-occurrence between rumen microbial genera.

3. Results

The composition of fresh forages, subsequent experimental silages, and the concentrate feeds used are presented in Table 1. The grass raw material nutritional quality was good based on the crude protein (CP) content and in vitro organic matter digestibility. The fresh forages of both additive treatments were similar in DM, ash, and CP concentrations, showing that no major differences in raw material between the two silages were evident. The fermentation quality of the two silages was very similar despite the use of different types of additives. The proportion of ammonia N in total N, and lactic acid concentrations were slightly lower, and that of butyric acid was clearly lower in AS than in IS, but, unexpectedly, WSC was lower and acetic acid higher in AS than in IS. The two experimental barley feeds had very similar chemical composition and feed values. Ensiled barley had lower DM and starch concentration than DB but, on the other hand, EB contained some lactic and acetic acid, as well as ethanol originating from the fermentation during storage. The four experimental TMR diets had only minimal differences between their chemical composition. Figure 1 shows the microbiological quality of the TMR immediately after mixing and after a storage time of 2 days.

Total diet DM intake was higher ($p < 0.01$) for cows fed AS than those fed IS (Table 2), which resulted in a higher intake of nutrients (organic matter, CP, neutral detergent fibre (NDF), and MP) and ME, while barley preservation method did not affect them. On the other hand, starch and PBV intakes were similar between silage treatments, but starch and PBV intakes were higher ($p < 0.01$) for cows receiving dry vs. ensiled barley.

The rumen fermentation of the four ruminally cannulated cows is presented in Table 3. The sampling time significantly affected all rumen fermentation variables but, since no interactions with the dietary treatments were observed, only the average values are presented. Silage additive did not affect ruminal ammonia concentration, but it was higher ($p < 0.05$) for EB than DB. Ruminal molar proportion of butyrate tended to be higher in AS-fed than IS-fed cows ($p < 0.10$) in expense of a lower proportion of acetate ($p < 0.05$). Barley preservation method did not affect the proportions of major VFA in the rumen, but significant ($p < 0.05$) silage additive type $\times$ barley preservation method interactions were observed for propionic, butyric, and isovaleric acid proportions in total VFA, as well as in NGR.

Table 1. Composition of the experimental feeds.

	Fresh Forage		Grass Silage		Concentrate Feeds				Experimental Total Mixed Rations [3]			
	Acid	**Inoculant**	**Acid**	**Inoculant**	**MixC [1]**	**Dry Barley**	**Ensiled Barley**	**MPC [2]**	**AD**	**AE**	**ID**	**IE**
Number of samples	7	5	4	4	4	4	4	4	4	4	4	4
Dry matter (DM), g/kg	259 ± 12.7	258 ± 13.9	271 ± 6.1	260 ± 11.4	871 ± 3.5	877 ± 4.4	730 ± 4.9	877 ± 0.6	401 ± 6.5	397 ± 6.2	396 ± 11.4	389 ± 11.3
Buffering capacity, g lactic acid/100 g DM	6.2 ± 0.30	7.8 + 0.33										
In DM, g/kg												
Ash	76 ± 2.1	76 ± 2.1	78 ± 1.0	81 ± 3.4	108 ± 3.2	30 ± 1.2	32 ± 0.3	81 ± 15.3	76 ± 0.6	76 ± 0.8	77 ± 1.7	77 ± 1.8
Crude protein	137 ± 8.7	135 ± 8.6	149 ± 2.2	150 ± 5.8	240 ± 3.7	136 ± 2.4	132 ± 2.1	184 ± 8.0	170 ± 1.6	169 ± 1.6	171 ± 3.4	170 ± 3.4
Ether extract					36.6 ± 4.6	30.6 ± 1.0	31.3 ± 0.7	28.4 ± 3.9				
Water soluble carbohydrates	141 ± 14.2	128 ± 15.4	26.0 ± 4.0	40.5 ± 10.1								
Neutral detergent fibre	538 ± 16.5	565 ± 17.6	522 ± 17.6	520 ± 17.5	251 ± 8.3	191 ± 9.1	193 ± 8.6	204 ± 4.4	379 ± 9.0	379 ± 12.2	375 ± 9.7	375 ± 12.8
Starch					156 ± 7.6	605 ± 45.9	585 ± 18.3	363 ± 10.5	173 ± 8.7	170 ± 3.1	177 ± 8.1	173 ± 3.2
Fermentation profile, g/kg DM												
pH			3.99 ± 0.307	3.97 ± 0.029			4.42 ± 0.11					
Ammonia N, g/kg N			35.8 ± 2.06	42.8 ± 4.90			19.9 ± 3.1					
Ethanol			6.6 ± 0.75	6.6 ± 0.84			4.6 ± 0.7					
Lactic acid			88.8 ± 4.69	99.8 ± 7.76			14.5 ± 1.4					
Acetic acid			16.9 ± 1.64	12.5 ± 6.18			8.45 ± 1.7					
Propionic acid			2.36 ± 0.102 [4]	0.63 ± 0.307			0.05 ± 0.01					
Butyric acid			0.36 ± 0.069	0.83 ± 0.815			0.003 ± 0.01					
In vitro organic matter digestibility, g/kg	793 ± 10.0	780 ± 10.7	793 ± 2.5	786 ± 5.8								
Feed values												
Metabolisable energy, MJ/kg DM			11.7 ± 0.04	11.6 ± 0.12	11.3 ± 0.03	12.9 ± 0.02	12.9 ± 0.003	12.1 ± 0.21	11.9 ± 0.02	11.9 ± 0.02	11.8 ± 0.06	11.8 ± 0.06
Metabolisable protein, g/kg DM			87 ± 0.5	86 ± 0.9	126 ± 1.2	110 ± 0.6	109 ± 0.5	117 ± 2.1	102 ± 0.4	102 ± 0.4	102 ± 0.5	102 ± 0.6
Protein balance in the rumen, g/kg DM			19 ± 1.7	21 ± 5.2	65 ± 0.3	−23 ± 0.7	−26 ± 0.5	18 ± 0.5	22 ± 1.2	21 ± 1.2	23 ± 2.9	22 ± 2.9
Silage DM intake index [5]			108 ± 0.8	104 ± 2.5								
Microbial counts [6], CFU/g												
Yeasts	1.1×10^5		2.0×10^2	1.2×10^7	3.6×10^2	3.3×10^2	1.6×10^5					
Moulds	3.8×10^4		1.8×10^2	1.0×10^2	3.4×10^2	3.3×10^2	3.7×10^5					
Aerobic bacteria	3.0×10^7		2.1×10^5	1.5×10^7	1.1×10^5	8.1×10^4	5.8×10^7					
Lactic acid bacteria	Not analysed		4.1×10^6	7.4×10^7	2.9×10^4	6.3×10^3	2.2×10^8					

[1] MixC = mixed concentrate for total mixed rations. [2] MPC = milking parlour concentrate. [3] Total mixed rations are coded as follows: 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] Includes the propionic acid provided in the additive. The value corrected for the added amount equals zero. [5] Calculated according to Huhtanen et al. [7]. [6] The barley grains prior to drying or crimping contained 1.64×10^6, 1.01×10^6, and 3.31×10^8 CFU for yeasts, moulds, and aerobic bacteria, respectively.

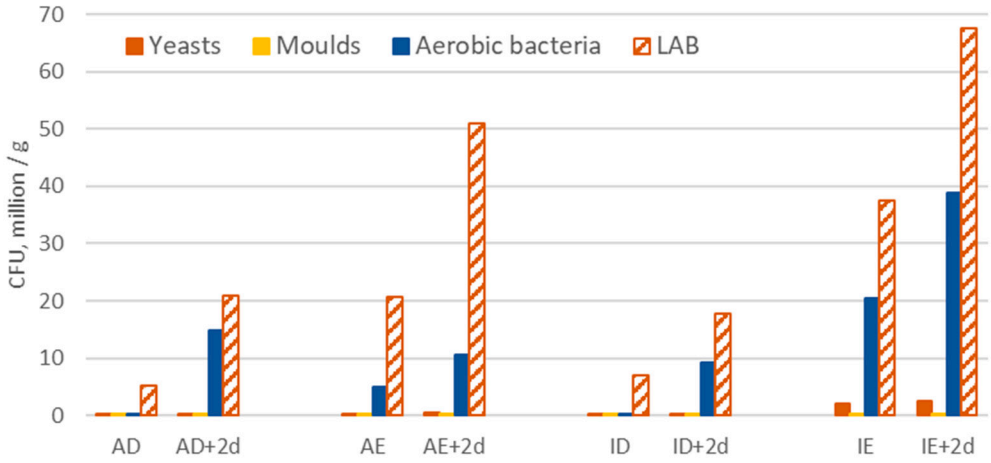

Figure 1. Microbial counts of the experimental TMR immediately after mixing and 2 days after mixing (+2d). The TMR are coded as follows: AD = organic-acid-based additive treated silage and dry barley, AE = organic-acid-based additive treated silage and crimped ensiled barley, ID = lactic acid bacteria inoculated silage and dry barley, IE = lactic acid bacteria inoculated silage and crimped ensiled barley.

Table 2. Feed and nutrient intake (kg per day unless otherwise stated) of the cows fed the experimental diets (n = 16 per treatment).

Silage Additive (S):	**Acid**		**Inoculant**		**SEM**	***p*-Value**		
Barley Preservation (B):	**Dry**	**Ensiled**	**Dry**	**Ensiled**		**S**	**B**	**S × B**
Total dry matter	27.8	27.7	27.3	27.0	0.72	0.005	0.34	0.61
Organic matter	25.7	25.6	25.2	24.9	0.67	0.003	0.32	0.62
Crude protein	4.74	4.70	4.67	4.60	0.140	0.018	0.11	0.65
Neutral detergent fibre	10.5	10.4	10.2	10.0	0.26	<0.001	0.34	0.59
Starch	4.90	4.80	4.92	4.78	0.148	0.91	0.007	0.61
Metabolisable energy [1], MJ	302	301	296	292	7.5	0.001	0.34	0.62
Metabolisable protein	2.85	2.84	2.80	2.76	0.077	0.004	0.27	0.63
Protein balance in the rumen	0.612	0.590	0.623	0.600	0.0366	0.17	0.004	0.88

[1] Daily metabolizable energy intake was corrected using total dry matter intake and concentrations of metabolizable energy and crude protein in the diet according to the correction equation provided by Luke [28].

Table 3. Rumen fermentation of the cannulated cows fed the experimental diets (n = 4 per treatment).

Silage Additive (S):	**Acid**		**Inoculant**		**SEM**	***p*-Value**			
Barley Preservation (B):	**Dry**	**Ensiled**	**Dry**	**Ensiled**		**Time**	**S**	**B**	**S × B**
Ammonia N, mg/dL	7.4	11.1	9.3	10.4	1.34	<0.001	0.55	0.012	0.17
pH	6.05	6.06	6.10	6.11	0.142	0.010	0.54	0.83	0.99
VFA [1], mmol/L	122	123	124	120	3.8	<0.001	0.90	0.63	0.33
In total VFA, mmol/mol									
Acetic acid	618	620	624	630	10.6	<0.001	0.041	0.41	0.51
Propionic acid	204	207	210	198	5.3	<0.001	0.55	0.55	0.022
Butyric acid	142	136	130	134	6.5	<0.001	0.066	0.47	0.001
Isobutyric acid	6.1	6.7	6.6	6.8	0.26	0.31	0.20	0.002	0.16
Valeric acid	15.6	15.3	15.4	14.7	0.57	<0.001	0.19	0.14	0.48
Isovaleric acid	7.2	9.0	8.7	9.1	0.66	<0.001	0.017	0.16	0.034
Caproic acid	6.2	6.7	6.1	6.7	0.38	<0.001	0.66	0.15	0.76
NGR [2]	3.97	3.86	3.80	4.05	0.108	0.002	0.86	0.64	0.010

[1] VFA = volatile fatty acids. [2] NGR = non-glucogenic to glucogenic VFA ratio, calculated according to Abrahamse et al. [21]: (acetic acid + 2 (butyric acid + isobutyric acid) + valeric acid + isovaleric acid)/(propionic acid + valeric acid + isovaleric acid).

Milk production was not affected by the silage additive treatment, but it tended ($p < 0.10$) to be higher (39.6 vs. 39.0 kg/d) for cows fed DB than those fed EB (Table 4). The effect on production, however, disappeared when presented as ECM due to a tendency ($p < 0.10$) for higher milk fat content in the milk of cows fed EB than those fed DB. The milk fat yield tended ($p < 0.10$) to be higher for AS-fed rather than IS-fed cows and that of protein yield was higher for cows receiving DB instead of EB. For milk urea concentration, there was a significant interaction between the dietary treatments ($p < 0.05$) but, numerically, the differences were small. Efficiency of milk production expressed as NUE, or ECM per DM intake or ME intake did not differ ($p < 0.10$) between dietary treatments. However, energy balance was more positive ($p < 0.05$) for AS-fed than IS-fed cows.

Table 4. Milk production and milk composition and efficiency of milk production of the cows fed the experimental diets (n = 16 per treatment).

Silage Additive (S):	**Acid**		**Inoculant**		**SEM**	***p*-Value**		
Barley Preservation (B):	**Dry**	**Ensiled**	**Dry**	**Ensiled**		**S**	**B**	**S × B**
Production per day								
Milk, kg	39.6	39.0	39.5	38.9	2.30	0.89	0.086	0.93
Energy corrected milk, kg	43.3	43.1	42.7	42.6	1.45	0.13	0.76	0.88
Fat, g	1803	1817	1759	1783	53.1	0.079	0.38	0.82
Protein, g	1505	1478	1493	1469	52.7	0.43	0.061	0.91
Lactose, g	1786	1763	1791	1762	105.0	0.88	0.12	0.84
Milk composition, g/kg								
Fat	45.8	46.9	45.0	46.1	1.78	0.17	0.060	0.99
Protein	38.2	38.1	38.1	37.9	1.17	0.30	0.22	0.77
Lactose	45.1	45.2	45.3	45.3	0.29	0.20	0.85	0.37
Total solids	139	140	138	139	3.0	0.12	0.080	0.84
Urea, mg/100 mL	23.3	25.5	24.7	24.5	0.99	0.74	0.057	0.021
Somatic cells, 1000/mL	95	67	89	75	23.9	0.97	0.24	0.70
Efficiency of milk production								
NUE [1]	0.312	0.308	0.313	0.313	0.0082	0.22	0.48	0.45
kg ECM [2]/kg DM [3] intake	1.56	1.56	1.56	1.58	0.031	0.24	0.48	0.38
kg ECM/MJ ME [4] intake	0.143	0.143	0.144	0.146	0.0030	0.10	0.51	0.41
Energy balance [5]	9.56	9.47	6.30	3.41	4.896	0.013	0.41	0.44
Body weight								
Mean, kg	695	694	694	694	16.4	0.69	0.89	0.61
Change, kg/week	2.81	0.77	1.12	1.26	1.163	0.47	0.26	0.20

[1] NUE = nitrogen use efficiency. [2] ECM = energy-corrected milk. [3] DM = dry matter. [4] ME = metabolisable energy. Daily ME intake was corrected using total dry matter intake and concentrations of ME and crude protein in the diet according to the correction equation provided by Luke [28]. [5] Energy balance was calculated for each cow by subtracting the energy required for milk production and maintenance from the total ME intake. The ME (MJ) used for ECM production (5.15 × ECM, kg) and for maintenance (0.515 × kg body weight$^{0.75}$) were based on Luke [28].

In the TMR aerobic stability experiment, the high, medium, and low DM contents of the TMR were 363, 336, and 313 g/kg, and the respective aerobic stabilities were 104, 89, and 81 h (P for the linear effect of moisture level < 0.05). The aerobic stability for the TMR diets AD, AE, ID, and IE was 161, 141, 34, and 28 h, respectively, and both silage additive and barley preservation method affected it significantly ($p < 0.05$). Further, there was an interaction between silage additive treatment and moisture level ($p < 0.05$, Figure 2). The aerobic stability of both silages and ensiled barley as single components was also measured. The ensiled barley and organic-acid-treated silage did not reach the 2 °C threshold during the 200-h follow-up period, but the inoculated silage heated up after an aerobic exposure of 31 h.

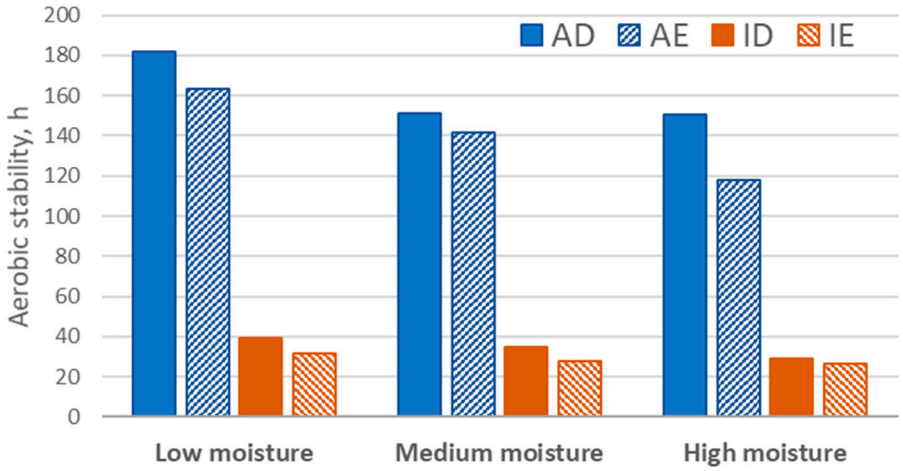

Figure 2. Aerobic stability of the experimental total mixed rations at three different moisture levels ($p < 0.05$ for the interaction of TMR × moisture level). The diets are coded as follows: AD = organic-acid-based additive-treated silage and dry barley, AE = organic-acid-based additive-treated silage and crimped ensiled barley, ID = lactic acid bacteria inoculated silage and dry barley, IE = lactic acid bacteria inoculated silage and crimped ensiled barley.

Amplicon sequencing indicated that rumen bacterial community was dominated by Firmicutes (44–47%) and Bacteroidota (43%). Among less abundant phyla, Proteobacteria was observed at 2–4% and Patescibacteria at 2% abundance, with the remaining 14 phyla detected at an abundance <1%. *Prevotella* (26%), *Rikenellaceae* RC9 (3%), and *Prevotellaceae* UCG-001 (3%) genera were the most abundant among Bacteroidota. Lachnospiraceae NK3A20 (4–6%), *Christensenellaceae* R-7 (3%), *Ruminococcus* (3–4%), and *Oscillospiraceae* NK4A214 genera were the most abundant among Firmicutes, while *Succinivibrionaceae* UCG-002 (1–3%) was the most abundant genus in Proteobacteria (Figure S1).

Grass silage additive treatment (Figure 3A) and barley grain preservation method (Figure 3B) did not have a significant effect on rumen bacterial alpha diversity, calculated as Shannon or Simpson diversity indexes. Similarly, dietary treatments did not have a significant effect on bacterial alpha diversity either (Supplementary Figure S2A).

Rumen bacterial community structure, assessed by PCoA analysis, did not indicate significant differences between two grass silage additive treatments (adonis test $p = 0.5$) (Figure 3C) or four dietary treatments ($p = 0.15$, Supplementary Figure S2B). However, a significant shift in bacterial community structure was observed between diets with DB or EB ($p = 0.015$; Figure 3D). Rumen bacterial community of cows fed DB had significantly more Firmicutes and, at genus level, higher abundances of Lachnospiraceae NK3A20, Acetitomaculum, and Sharpea than those given EB. Diet with EB stimulated a higher abundance of Succinivibrionaceae UCG-002, Lactobacillus, and Selenomonas. Most of these bacteria were present in rumen at low abundance.

To better understand the barley preservation method impact on rumen microbial interactions, Spearman correlations were calculated between microbial genera. When cows received EB, significant positive co-occurrences ($p < 0.05$) were observed between Eubacterium cellulosolvens group, *Butyrivibrio*, *Lactobacillus*, *Shuttleworthia*, *Prevotellaceae* YAB2003, and *Schwartzia*, suggesting that these bacteria share a similar functional niche in the rumen ecosystem and/or benefit from each other's metabolites. Significant negative correlations were observed between *Sharpea* and *Tyzzerella*; *Prevotellaceae* YAB2003 and *Anaerovorax*; or *Succiniclasticum* and *Ruminococcaceae* sp. (Figure 4). When cows received DB, a positive co-occurrence was observed between a different set of bacteria, where *Ruminobacter* correlated positively with *Succinivibrionaceae* UCG-002; *Lachnospiraceae* sp with *Sharpea*; or *Bacteroidales* RF16 group with WCHB1-41 and *Christensenellaceae* R-7 group, among others. *E. ruminantium* and *E. cellulosolvens* groups were negatively associated with several rumen bacteria (Figure 5).

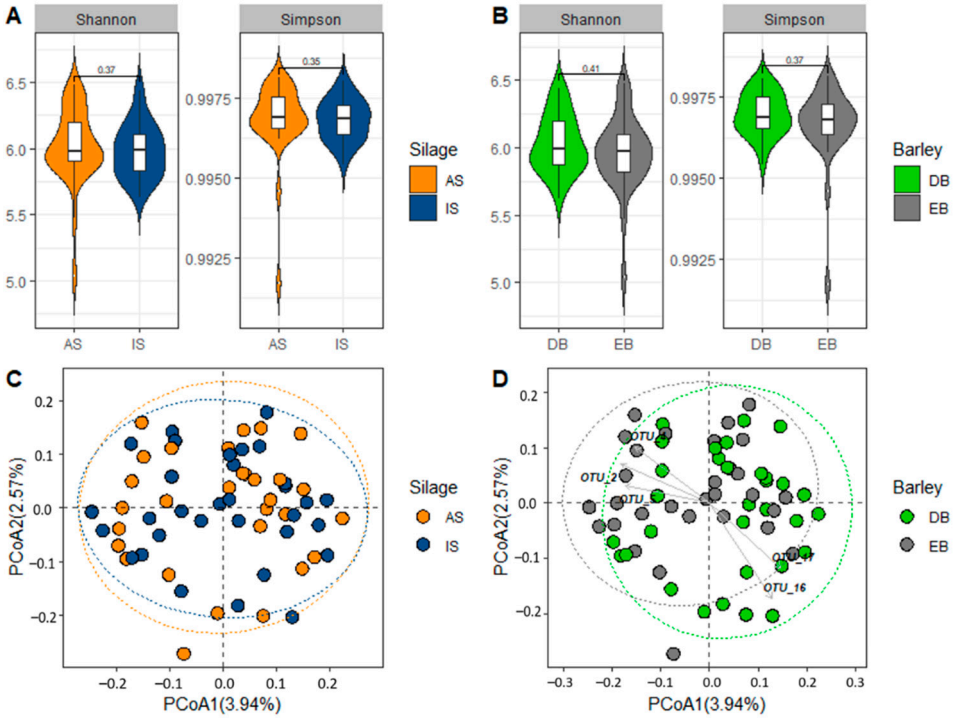

Figure 3. Rumen bacterial alpha and beta diversity estimates for groups based on silage (**A**,**C**) and barley (**B**,**D**) preparation methods. Grass silage preparation method with either organic-acid-based additive (AS) or inoculated with a lactic acid bacteria preparation (IS), and barley grain preservation methods by drying (DB) or crimping and ensiling (EB).

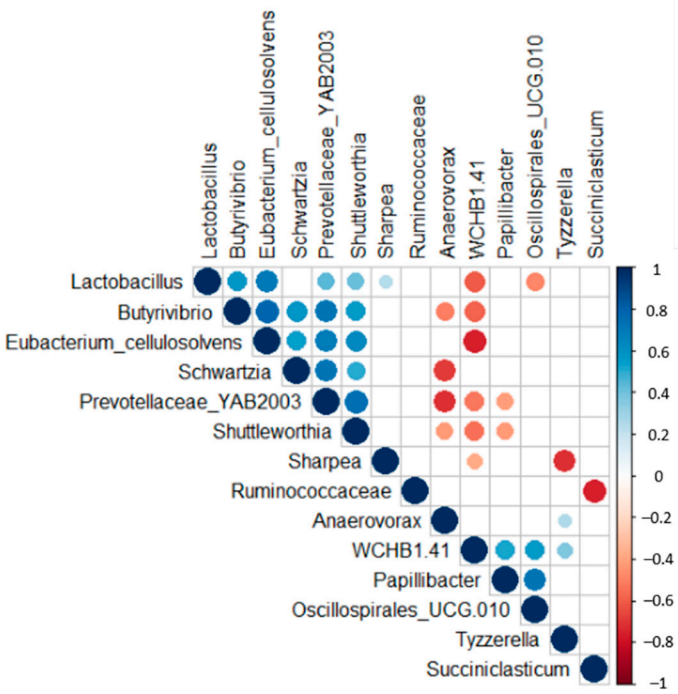

Figure 4. Spearman correlations of ruminal bacteria of cows fed a diet with crimped and ensiled barley. Only genera involved in the strongest ($-0.7 < R > 0.7$) associations and only significant ($p < 0.05$) results are included in the figure. Blue indicates a positive association, while red indicates a negative association.

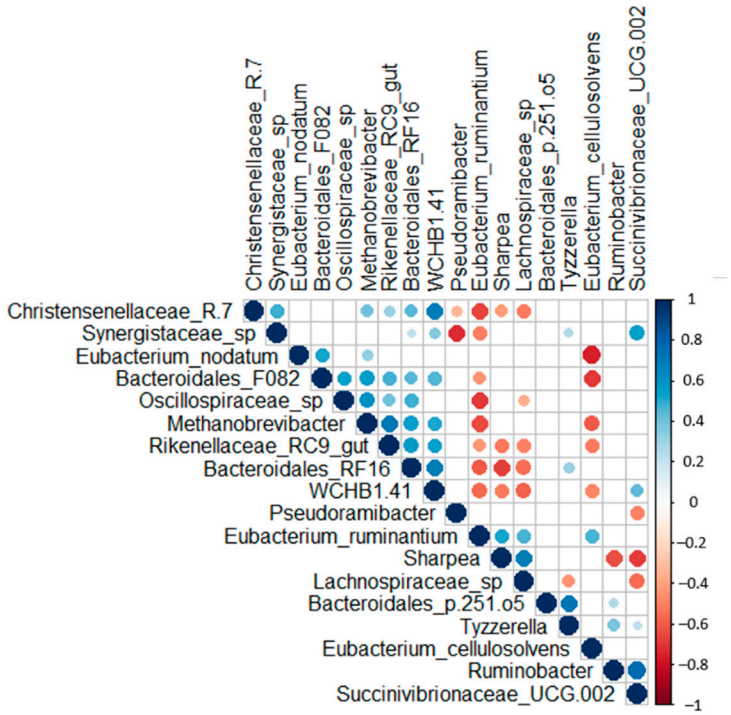

Figure 5. Spearman correlations of ruminal bacteria of cows fed a diet with dried barley. Only genera involved in the strongest ($-0.7 < R > 0.7$) associations and only significant ($p < 0.05$) results are included in the figure. Blue indicates a positive association, while red indicates a negative association.

Rumen archaeal community was dominated by *Methanobrevibacter gottschalkii* (48–54%), *Methanobrevibacter ruminantium* (25–32%) clades, and *Methanosphaera* ISO3-F5 (16–19%). Archaea affiliated with Methanomassiliicoccaceae family Groups 10, 12 ISO4-H5, 3b, 5, and 9 ISO4-G1 were observed at a total abundance of 2–3% (Supplementary Figure S3). Dietary treatments had no significant impact on archaeal alpha or beta diversities (Supplementary Figure S4).

4. Discussion

4.1. Feed Characteristics

The differences in the fresh forage composition between the two additive treatments were small, except for the lower NDF and higher WSC concentrations in AS compared to IS, which may be due to the acid hydrolysis effect of the organic acids applied in AS [2]. The difference in NDF concentration between the silages disappeared during ensiling, as the labile fibre was probably hydrolysed in IS by the acids generated during fermentation.

The lack of differences in the extent of fermentation between the two silage additive treatments was unexpected. Typically, FA-based additives efficiently restrict fermentation, resulting in higher residual WSC concentration and a lower proportion of lactic acid, VFA, and ammonia N in total N than in silages made without additives or inoculated with LAB. This has consistently been found in pilot-scale [32–34], as well as farm-scale experiments [5,35,36].

In the current experiment, the dose of the FA-based additive calculated from the FA concentration analysed in the silage was slightly below the manufacturer recommendation (4.3 vs. 5 litres per ton fresh forage) but, as demonstrated by Jaakkola et al. [37], the acid application restricted fermentation in a linear dose–response manner so that an almost complete lack of effect was not expected in our case. The effects of FA application rates have, however, not always been consistent, as Jaakkola et al. [37] noted a curvilinear effect of FA addition on silage fermentation quality. The effect of FA on the extent of fermentation is typically greater in low DM silages, as low water availability also restricts fermentation [38].

In our case, the silage DM content was relatively low and extensive lactic acid fermentation took place, indicating that fermentation was not restricted by low water activity or any other factor. The FA and propionic-acid-based additive used in the current experiment contained less FA than some of the additives used in the previous experiments, and it was also more buffered. These factors may have contributed to the limited efficiency of it in the plant material used in the current study, although the same product used by Franco et al. [33] resulted in efficient restriction of grass silage fermentation. The FA content was also analysed from LAB-inoculated silage samples and no FA could be detected in them, showing that there was no accidental application of the FA containing additive to the LAB-inoculated silage.

The fermentation coefficients of the fresh forages were 44 and 39 for AS and IS, respectively. According to Weissbach et al. [19], a fermentation coefficient below 35 indicates poor fermentability, while a value above 45 describes a crop easy to ensile. The values from 35 to 45 indicate intermediate fermentability, which represents the raw material of the current experiment. Both silages were relatively extensively fermented, with a high concentration of lactic acid, low pH and proportion of ammonia N in total N, and no signs of secondary fermentation. The fermentation pattern in both silages was so similar that a highly viable epiphytic LAB or some other factor was present in the fresh forage, masking the effects of both additives. Identification of the microbial community in the fresh forage and in final silages by, e.g., sequencing the bacterial DNA present in the materials would provide evidence on that point.

Additives have successfully been used to improve silage fermentation quality, as well as aerobic stability [1]. The product used for AS contains propionic acid, which has been linked with improved aerobic stability, while homofermentative LAB producing only limited amounts of acetic acid is generally expected to be prone to heating up [3]. The clear difference in aerobic stability between AS and IS followed this general trend in line with the previous experiments [33,38]. The high yeast count in IS has probably contributed to its low aerobic stability, as yeasts are known to initiate the aerobic spoilage by assimilating lactic acid [2].

An additional factor of TMR moisture content was included in the TMR aerobic stability evaluation, as water activity is one of the major factors affecting microbial activity [2]. The moisture content of TMR is highly variable depending on the moisture content of the components used, and water is sometimes added to TMR to prevent particle sorting in the feed bunk. The decreased aerobic stability in response to increased moisture content of TMR is in line with our previous experiment [39] and suggests that, in case of unstable TMR, reduction of water addition could be recommended.

Although the aerobic stability of IS-based TMR was clearly lower than those based on AS, heating of the TMR was not observed during the feeding experiment. This was partly alleviated by the low average temperature (−3.8 (SD 6.58) °C measured at the official observatory of Finnish Meteorological Institute located 5 km from the dairy barn) during the experiment in January–April 2021. Although, in particular, the number of both aerobic bacteria and LAB in the TMR increased during the 2-day storage period, the numbers were still tolerable (Figure 1). There is no reason to suspect that the lower aerobic stability of IS would have affected the dairy cow responses in the current experiment, but, under different circumstances, loss of aerobic stability could result in large in-silo losses and reduced feed intake of cows [3].

4.2. Rumen Fermentation

Changes in rumen fermentation induced by changes in silage fermentation have been demonstrated earlier. Shingfield et al. [5] and Saarisalo et al. [35] reported that restrictively fermented silage treated with FA increased the ruminal proportion of acetate and decreased that of propionate compared to the LAB-treated silage, but butyrate was not affected. Halmemies-Beauchet-Filleau et al. [36] compared FA-treated silage with untreated silage and, in their case, rumen acetate proportion was not affected but propionate tended to

decrease, while butyrate increased. In all the above-mentioned studies, the residual WSC concentration was clearly higher in FA-treated silages than in LAB-treated or untreated silages (62 vs. 37 g/kg DM in Shingfield et al. [5]; 94 vs. 51 g/kg DM in Saarisalo et al. [35]; 43 vs. 18 g/kg DM in Halmemies-Beauchet-Filleau et al. [36]), showing that organic-acid-based additives have been able to restrict silage fermentation in contrast to the results of the present experiment. Jaakkola et al. [6,37] conducted two experiments where the level of FA was gradually increased and, in both cases, the rumen fermentation type in more restrictively fermented silages favoured acetate over propionate. Based on all these experiments, the tendency of more lipogenic rather than the glucogenic type of rumen fermentation is to be expected from restricting silage fermentation. However, the NGR was not affected by silage additive type in the current data, obviously due to the lack of major differences in silages treated with different additives. The decrease in ruminal proportion of acetate was, however, compensated by an increase in butyrate, which may have been reflected in the higher milk fat production of AS diets compared to IS diets. It is difficult to explain why replacing DB with EB decreased NGR with AS but increased it with IS, particularly as the differences in the fermentation quality of the silages were minor.

Ensiling of grains converts part of the carbohydrates into fermentation acids, reducing the amount of fermentable substrates for rumen microbes. Further, the profile of fermentation acids in the ensiled grain may influence the rumen fermentation pattern. In a meta-analysis consisting of mostly maize-based material, rumen pH, and ammonia N and acetate concentrations decreased when dry grains were replaced with ensiled ones [13]. In our case, ruminal pH and acetate were not affected but ammonia N concentration increased when DB was replaced with EB.

4.3. Rumen Bacterial Community

Rumen bacterial community was not affected by the silage additive treatment in this experiment due to the very similar silage fermentation pattern in both treatments. However, inclusion of dry vs. ensiled barley into the diet altered several bacterial genera and suggested differences in co-occurrence patterns within the rumen microbial ecosystem. In situ rumen digestibility experiments with barley and maize [40] demonstrated that chemical composition and structure of the grain can affect bacterial community composition involved in particle-associated biofilm formation. The authors showed that 2 h after incubation, *Sharpea* and *Lactobacillus* were more abundant in barley, while *Succiniclasticum*, *Paraprevotellaceae* sp., and *Lachnospiraceae* sp. were more abundant in maize-related biofilms. In our experiment, no particle-associated microbiome was studied but rumen liquid collected 3–4 h after morning feeding suggested a barley form effect on co-occurrences between several bacteria reportedly observed in starch fermentation.

While positive co-occurrences were observed between *Butyrivibrio*, lactate-producing *Lactobacillus*, saccharolytic *Shuttleworthia*, *Prevotellaceae* YAB2003, and succinate-utilising *Schwartzia* in EB, lactate-producing *Sharpea* showed a strong positive correlation with *Lachnospiraceae* sp., and *Ruminobacter* with *Succinivibrionaceae* UCG-002 in DB, suggesting that different bacteria might have played primary and secondary metaboliser roles in both diets. However, despite differences in rumen bacterial community composition, no significant effect was observed in rumen fermentation parameters.

4.4. Feed Intake and Production Responses

Based on a large dataset, Huhtanen et al. [7] quantified the effect of various silage characteristics, including the extent of silage fermentation (concentration of lactic acid + VFA) on voluntary DM intake of dairy cows. Based on that analysis, reducing the fermentation acid concentration by 15 g/kg DM (to a minimum of 40 g/kg DM) increased silage DM intake index by 1 point, which corresponds to a difference of ~0.1 kg DM intake per day for a cow eating 10 kg DM of silage per day. The silage DM intake equation is in line with individual experiments, such as that of Jaakkola et al. [37], where silage fermentation was gradually limited by FA addition and it resulted in respective increases in DM intake,

as well as milk production. The silage DM intake index [7] was 4 points higher for AS than IS due to minor differences in DM content, organic matter digestibility, and extent of fermentation in favour of AS. Indeed, silage intake was also 0.5 kg DM higher for AS compared to IS.

Shingfield et al. [5] and Halmemies-Beauchet-Filleau et al. [36] reported significant increases in ECM production in response to FA treatment of grass silage, but the small differences in the quality of silages used in the current experiment did not affect milk production. The more lipogenic, rather than glucogenic, type of rumen fermentation of cows fed restrictively fermented silages has increased milk fat concentration in previous studies [5,35,36]. Although the tendency of higher fat yield in AS-fed cows compared to IS-fed ones is in line with the studies mentioned above, the minor differences in the extent of silage fermentation did not affect milk composition.

The use of high-moisture grains compared to dry cereals has seldom had large effects on milk production. Most published studies have, however, used maize. A large dataset from commercial Canadian dairy farms revealed that dry and high-moisture maize resulted in similar milk output of dairy cows [10], as well as a meta-analysis of published experiments [11]. Allen and Ying [12] found a slightly lower milk protein content of the high-moisture maize compared to dry maize, while, otherwise, the production results were equal. Torres et al. [13] conducted a meta-analysis comparing dry and moist cereal grains based on 18 studies (17 using maize and 1 using barley grain). They reported no change in milk output, but milk fat concentration and yield were reduced when high-moisture rather than dry grains were used. In studies comparing the milk production potential of dry vs. ensiled barley, Pettersson et al. [16] found a slight decrease, while Jaakkola et al. [14] and Adler and Randby [15] reported no differences. In a recent experiment using beef bulls, crimped ensiled barley resulted in higher feed intake and carcass gain than dry barley [41].

The maize-based dataset of Torres et al. [13] revealed that diet OM and NDF digestibilities increased when moist rather than dry grains were used, but starch digestibility was not affected (0.946 and 0.947 for dry and moist grains, respectively). A recent study by Allen and Ying [42], however, showed that total-tract maize starch digestibility increased from 0.966 in dry grains to 0.981 when high-moisture maize was used. The difference was even greater for ruminal digestibility (0.643 vs. 0.873 for dry and high-moisture maize). In our case, in an unpublished companion study, the diet starch digestibility in dairy cows measured using total faecal collection method decreased in response to ensiling (0.994 for DB and 0.958 for EB; $p < 0.001$). The discrepancies between maize and barley digestion probably originate from different types of starch granules and protein matrix in the grains, which are known to affect the ruminal degradability of starch [41].

The previously published results support the current data that only minor responses to replacing dry with ensiled barley were observed. The magnitude of the effects on milk production has been so small that other practical and economic factors should be considered when deciding on the grain preservation method. Proper attention must, however, be paid to the preservation techniques and choice of additive [9], and the potentially lower aerobic stability in ensiled than in dry cereals must be taken into account when planning the feed-out routines.

Although differences in the feeds of the current experiment were rather small, the importance of feed quality should not be neglected. With larger differences in feed quality, important modifications in milk production can be expected. This is emphasized by the meta-analysis by Huhtanen and Rinne [43] showing that milk, and especially milk fat and protein yields, increased linearly with restriction of silage fermentation by the use of organic-acid-based additives. Additive treatments and other management factors may also affect other important feed traits, such as losses during storage and feed-out in both forage and moist grain preservation [9,33,38].

5. Conclusions

The lack of changes in fermentation quality of the grass silages in spite of contrasting additive treatments was unexpected and against our hypothesis, but, since that was the case, the effects of the silages on rumen fermentation and production responses also remained small. Using a silage treated with formic- and propionic-acid-based additive resulted in clearly more stable TMR than when a homofermentative LAB was used, which needs to be considered if TMR heating is considered a risk. Barley preservation method modified dairy cow responses only to a limited extent. Dry barley resulted in slightly more aerobically stable TMR than when ensiled barley was included as a component. These results suggest that the choice of barley preservation method can be based on practical on-farm factors, such as costs related to drying.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture12020266/s1, Figure S1: Relative abundance of bacterial taxa at genus level in treatment groups AE, AD, IE and ID; Figure S2: Bacterial alpha (A) and beta (B) diversity estimates based on treatment groups AE, AD, IE and ID; Figure S3: Relative abundance of archaea at species level in treatment groups AE, AD, IE and ID; Figure S4: Archaeal alpha and beta diversity estimates for groups based on silage (A and C) and barley (B and D) preparation methods.

Author Contributions: Conceptualisation, M.R.; methodology, M.F. and I.T.; formal analysis, M.F., I.T. and P.M.; investigation, A.-R.B., M.F., I.T. and T.S.; resources, M.R.; data curation, I.T. and P.M.; writing—original draft preparation, M.R. and I.T.; writing—review and editing, M.F., T.S., A.-R.B. and P.M.; visualisation, I.T.; project administration, M.R.; funding acquisition, M.R. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Academy of Finland (grant number 322827).

Institutional Review Board Statement: National Ethics Committee (Hämeenlinna, Finland) approved the experimental procedures prior to the commencement of the experiment with a license number ESAVI-32016-2020 in accordance with the guidelines established by the European Community Council Directive 2010/63/EU.

Data Availability Statement: The rumen bacterial data presented in this study are openly available in the NCBI Sequence Read Archive under BioProject Accession PRJNA796213 with corresponding BioSample Accessions SAMN24829682—SAMN24829697; SAMN24829702—SAMN24829717, SAMN24829722—SAMN24829737, and SAMN24829742—SAMN24829757. For availability of other raw data, contact the authors.

Acknowledgments: We gratefully acknowledge the contribution of M.Sc. student Aino-Maria Mänki (University of Helsinki, Finland). We thank Finnish Functional Genomics Centre supported by University of Turku, Åbo Akademi University and Biocenter Finland (Turku, Finland) for rumen bacterial DNA sequencing.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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