fermentation

MDPI

Article

The Effect of Forage Source and Concentrated Liquid Feedstuff Supplementation on Improving the Synchronization of Ruminant Dietary Energy and Nitrogen Release In Vitro

Yi-Hsuan Chen [1,2], Ching-Yi Chen [1] and Han-Tsung Wang [1,*]

[1] Department of Animal Science and Technology, National Taiwan University, Taipei 10672, Taiwan
[2] Conservation Research Center, Taipei Zoo, Taipei 116016, Taiwan
* Correspondence: htwang@ntu.edu.tw

Abstract: This study aimed to investigate the effect of supplementation with a mixture of molasses and condensed molasses fermentation solubles (M-CMS) in different synchronization diets formulated with different forage sources in an attempt to improve the fermentation efficiency of diets by M-CMS. In the first experiment, three levels of M-CMS (N: 0%; L: 1.75%; and H: 3.50%) were supplied to diets with or without corn silage to evaluate the supplementation effect on the diet with a synchrony index (SI) of 0.80. In the second experiment, diets containing different corn silage levels (60 or 30% of the forage source) were used to evaluate the effects of M-CMS supplementation on higher SI (at 0.88). The in vitro digestibility, fermentation products, microbial crude protein (MCP), and gas kinetic parameters were determined after 48 h of fermentation. The results demonstrated that M-CMS supplementation improved MCP synthesis in both diets with low and high SI, but did not enhance digestibility. M-CMS supplementation was beneficial to the fermentation stability and extent. It also affected the gas kinetic parameters of the fast- and slow-degradation fractions during fermentation. M-CMS supplementation improved MCP synthesis in diets containing less corn silage. The forage source and degradation rate of individual ingredients should be considered simultaneously to enhance the rumen fermentation efficiency. M-CMS provided a practical choice to further improve MCP synthesis and fermentation stability, even in a diet with high SI.

Keywords: forage source; liquid feedstuff; synchronization; microbial protein synthesis; degradation kinetics; rumen fermentation

Citation: Chen, Y.-H.; Chen, C.-Y.; Wang, H.-T. The Effect of Forage Source and Concentrated Liquid Feedstuff Supplementation on Improving the Synchronization of Ruminant Dietary Energy and Nitrogen Release In Vitro. *Fermentation* **2022**, *8*, 443. https://doi.org/10.3390/fermentation8090443

Academic Editor: Qing Zhang

Received: 22 August 2022
Accepted: 5 September 2022
Published: 6 September 2022

1. Introduction

Microbial crude protein (MCP) is a high-quality protein source with a good amino acid composition for ruminants and is synthesized in the rumen to supply the majority of absorbable amino acids to the small intestine. A key objective of protein feeding strategies is maximizing the utilization of rumen-degradable protein (RDP) for conversion into MCP and reducing the loss of ruminal ammonia [1].

During fermentation, matching both RDP and energy (mainly from rumen-fermentable carbohydrates) to the rumen improves microorganism use, simultaneously resulting in higher MCP synthesis [2]. However, the availability of carbohydrates or nitrogen is more likely to limit fermentation performance than improve protein and carbohydrate synchronization. An in vitro study with ruminal fluid inoculation by Henning et al. [3] showed that the bacterial cell production efficiency increased, with a rapid rate of energy availability. Determining the relative rates of the ruminal degradation of protein and energy sources is required to establish the desired dietary amounts of synchronous substrates [4]. To quantify ruminal synchronization, Sinclair [5] introduced the synchronization index (SI) for nitrogen to carbohydrate or organic matter (OM) and reported a figure of 32 g N/kg carbohydrate or 25 g N/kg OM degraded in the rumen. An SI value of 1.0 represents perfect synchrony between energy and N supply throughout the day, while values of <1.0

indicate the degree of asynchrony. The kinetics of feedstuff degradation can be determined from the fermentative gas production; the gas measurement also provides useful data on the digestion kinetics of both soluble and insoluble fractions of feedstuffs [6]. A suitable model could link the gas production technique to animal performance by deriving an expression for the extent of ruminal degradation [7]. Combining the in vitro digestibility and fermentation products (e.g., volatile fatty acids and ammonia) results with the gas kinetic data could be a useful tool to investigate the mechanism of ruminant diet utilization.

Corn silage, grass hay, and legume hay are the major forage sources used in dairy cattle feeding systems' total mixed ration (TMR). However, the degradation rate and degradable protein ratio of feeds are quite different. The quality of hay and corn silage also undulates [8]. This results in the inability to effectively synchronize the rumen's energy and nitrogen source release. Consequently, the MCP synthesis efficiency cannot achieve its full potential.

Molasses, a commonly used liquid feed additive rich in water-soluble carbohydrates (WSC), has been used as an energy and mineral supplement to improve the production performance and rumen health of cattle fed with lower-quality forage [9]. Condensed molasses fermentation solids (CMS) from the monosodium glutamate industry are a residual product of microbial fermentation, produced after the fermentation of monosodium glutamate from molasses. CMS is rich in proteins, amino acids, organic acids, vitamins, minerals, biochemical fulvic acid, and unknown growth factors that are synthesized by microorganisms during the fermentation process [10]. CMS shares some physical characteristics with molasses, but contains higher levels of crude protein (CP), sodium, and chloride. Previous studies reported that CMS supplementation in ruminant diets stabilized the rumen pH, all CP from CMS was soluble and could be degraded in the rumen, and CMS also contains high non-protein nitrogen (approximately 30% CP). CMS also benefits the rumen cellulolytic bacterial population and fiber digestibility [11]. It should be noted, however, that the excessive use of CMS could reduce the digestibility of dry matter (DM), organic matter (OM), and neutral detergent fiber (NDF) and tends to decrease intake and growth performance [12].

Previous studies indicated that pure CMS contains very high amounts of NPN (about 30% of CP) and chloride ion (about 16.5% DM), but a lower amount of WSC (270 g/kg) than sugar cane molasses (450 g/kg) [12,13]. Besides the effect of the high chloride ion content on the cation–anion balance, the lower WSC to non-protein nitrogen (NPN) may indicate a defect in the optimal synchronization for MCP synthesis in the early stage of fermentation. A decrease in animal production was also reported in diet supplementation with CMS at a high level [11], but a mixture of molasses and pure CMS could relieve the negative effect under high-level supplementation [9]. Based on the CP characteristics and WSC levels of CMS and molasses, the mixture of molasses and CMS has a high potential for use as a feed supplement to enhance nitrogen and energy synchronization. However, the appropriate proportion of molasses and CMS mixture product supplementation in the diet needs to be evaluated.

The objective of this study was to investigate the supplementation effect of the molasses–CMS mixture product (M-CMS) under diets with different SI values formulated with different proportions of hay and corn silage as forage sources in an attempt to improve the synchronization efficiency of diets by M-CMS during fermentation. In order to investigate our hypothesis, two in vitro experiments were done. The first experiment determined the effect of M-CMS supplementation on diets with or without corn silage at a lower SI value (0.80). The second experiment determined the effect of M-CMS supplementation on diets with higher SI values (0.88) and different corn silage levels. In vitro digestion and gas production experiments were performed to evaluate the fermentation performance and MCP synthesis efficiency of the diets. Owing to the M-CMS was fermented very fast in the early stage and different forage source compositions also vary in the degradation during fermentation, we try to use the two-phase exponential model to investigate the mechanism of the M-CMS supplementation effect.

2. Materials and Methods

2.1. Chemical Composition of M-CMS and Calculation of Synchrony Index

According to the previous animal feeding and in vitro study review on the CMS product [12], the liquid M-CMS used in this study was a mixture of sugarcane molasses and condensed molasses fermentation solubles in a 1:1 ratio. The chemical composition of M-CMS was as follows: moisture content, 278 g kg^{-1}; CP content, over 220 g kg^{-1}; minor amounts of crude fat, 12 g kg^{-1}; and no crude fiber. In M-CMS with a high ash content (over 231 g kg^{-1}), chloride is the most common mineral (76 g kg^{-1}), followed by potassium (33 g kg^{-1}) and sodium (11 g kg^{-1}). The total amino acid content was approximately 53 g kg^{-1}, with more than 32 g kg^{-1} glutamic acid, 7 g kg^{-1} alanine acid, and 7 g kg^{-1} aspartic acid. M-CMS also contained over 350 g kg^{-1} of WSC. The chemical composition of M-CMS was assayed according to AOAC methods [14]. The CP, EE, fiber, mineral, and amino acids were assayed by methods used to evaluate animal feed and fertilizers. The moisture, total sugar, and ash were assayed by methods used to evaluate molasses and molasses products.

The synchrony indices of the experimental diets in this study were calculated from the OM and CP fractions of each feed ingredient. Values that were determined in previous studies [1,15,16] and the degradation rates of OM and CP fractions were acquired for NRC [17]. The SI of N for OM was calculated as described by Sinclair et al. [5]:

$$\text{Synchrony index (SI)} = \frac{25 - \sum \sqrt{(25 - \text{hourly N/ OM})^2}/24}{25} \quad (1)$$

where 25 indicates 25 g of N/kg of OM being truly digested in the rumen.

2.2. Preparation of Experimental Diets

All diets in this study were formulated using the recommendation from the National Research Council [17] for a multiparous lactating dairy cow (650 kg of BW) producing 30 kg/d of milk containing 3.0% true milk protein and 3.5% milk fat. The difference in minerals and vitamins between the experimental diets was made up via the formula of premix to meet the requirement of dairy cattle.

2.2.1. Experiment 1: Effect of M-CMS Supplementation on Diets with or without Corn Silage

Six TMRs with similar CP and RDP ratios (accounting for 60% of CP), and different forage sources (T: forage source without silage and S: forage source containing silage) and M-CMS supplementation levels (N: 0%; L: 1.75%; H: 3.50%) were used in this experiment. The SI of the diets was adjusted to 0.8 in experiment 1 and the concentrated part of all TMRs was formulated with the same ingredients. The composition and nutrient content of the experimental diets are shown in Table 1.

2.2.2. Experiment 2: Effect of M-CMS Supplementation on Corn Silage Level under High Synchrony Index

Four TMRs had similar crude protein and RDP ratios (accounting for 60% of CP) with high (HS) or low (LS) levels of corn silage (accounting for 60 or 30% of forage source). Different M-CMS supplementation levels (N: 0%; V: 4.42%) were applied in experiment 2. The SI of the diets was adjusted to 0.88 in experiment 2 and the concentrated part of all TMRs was formulated with the same ingredients. The composition and nutrient content of the experimental diets are shown in Table 2.

2.3. In Vitro Rumen Digestibility and Gas Production Assay

Two rumen-fistulated dry cows with a body weight of about 650 kg were used as rumen fluid donors. Rumen fluid was collected from animals that were fed 16 kg of diet per day. A forage-to-concentrate ratio of 55:45 (300 g/kg Bermuda hay, 250 g/kg alfalfa

hay, and 450 g/kg commercial concentrate) provided 150 g/kg CP and 1.6 Mcal/kg NE_L (DM basis). Two dry cows were housed in individual pens and had continuous access to fresh water. Both cows were fed the same diet for 14 days before rumen fluid collection. On the sampling day, the rumen liquor was collected through the cannula of each animal 2 h after the morning feeding and was strained through four layers of cheesecloth into a 40 °C pre-warmed serum bottle gassed with CO_2 before use.

Table 1. Composition of diets for Experiment 1 (M-CMS supplementation to diets with or without corn silage).

	Treatment [1]					
	T			**S**		
Item	**N**	**L**	**H**	**N**	**L**	**H**
	Ingredient (% Dry matter, DM)					
Oat hay	27.07	26.80	26.16	12.53	12.53	12.53
Pangolagrass hay	0.00	0.00	0.00	3.08	3.08	3.08
Alfalfa hay	24.11	24.12	24.34	19.28	19.27	19.27
Corn silage	0.00	0.00	0.00	20.03	20.03	20.03
Steam-flaked corn	25.99	24.80	23.87	19.85	18.92	17.26
Soybean meal	14.51	14.08	13.62	16.77	15.76	15.58
Soybean hull	3.86	3.97	4.06	5.78	5.98	6.08
Bypass lipid	2.15	2.21	2.25	1.09	1.08	1.08
Sodium bicarbonate	0.80	0.80	0.80	0.86	0.86	0.86
Calcium carbonate	0.18	0.10	0.00	0.35	0.35	0.35
Dicalcium phosphate	0.43	0.44	0.45	0.18	0.18	0.18
Bypass methionine	0.17	0.18	0.18	0.00	0.00	0.00
Magnesium oxide	0.17	0.18	0.18	0.00	0.00	0.00
Vitamin premix	0.21	0.22	0.23	0.20	0.20	0.20
Salt	0.34	0.35	0.36	0.00	0.00	0.00
M-CMS	0.00	1.75	3.50	0.00	1.75	3.50
	Calculated parameter [2]					
Forage %	51.18	50.92	50.50	54.92	54.92	54.92
SI	0.81	0.81	0.81	0.79	0.80	0.80
RDP (%CP)	60.40	61.30	62.14	60.25	61.00	62.01
RUP (%CP)	39.60	38.70	37.86	39.75	39.00	37.99
	Chemical composition [3] (% DM)					
OM	93.44	93.15	92.88	93.43	93.10	92.74
CP	15.21	15.18	15.18	15.79	15.54	15.60
EE	4.32	4.34	4.35	3.52	3.48	3.43
NDF	35.49	35.21	34.83	38.30	38.18	38.01
ADF	20.99	20.91	20.78	22.74	22.73	22.73
Ash	6.56	6.74	6.89	6.57	6.79	7.04
NSC	38.42	38.42	38.53	35.81	35.90	35.70
NE_L(Mcal/kg)	1.70	1.69	1.68	1.68	1.67	1.67

[1] T: forage source without silage; S: forage source containing silage; N: 0% M-CMS; L: 1.75% M-CMS; H: 3.50% M-CMS. M-CMS: molasses + condensed molasses fermentation solubles (1:1 mixture). [2] SI: synchrony index; RDP: rumen-degradable protein; RUP: rumen-undegradable protein. [3] OM: organic matter; CP: crude protein; EE: ether extract; NDF: neutral detergent fiber; ADF: acid detergent fiber; NSC: non-structure carbohydrate; NE_L: net energy of lactation.

Samples of each experimental diet (800 mg) were weighed into 100 mL serum bottles (six replicates per treatment) for the gas production assay. Ruminal fluid from each animal was mixed with the artificial saliva solution described by Menke and Steingass [6] at a ratio of 1:4 (v/v) at 39 °C under continuous flushing with CO_2. Eighty milliliters of the rumen inoculum mixture were added to each serum bottle under CO_2 flushing. Following inoculation, the serum bottles were connected to an ANKOM pressure sensor module and gas was produced (every 2 min during fermentation) using a wireless ANKOM RF gas-production system (Ankom Technology) [18]. Fermentation was performed in a 39 °C

water bath for 48 h. To determine the effect of M-CMS and corn silage on fast and slow degradation, a two-stage model [7] was applied in this study. At the end of the incubation period, the cumulative gas production data were fitted to a two-phase exponential model as follows:

$$y(t) = a + b_1(1 - e^{-c_1 t}) + b_2(1 - e^{-c_2 t}) \quad (2)$$

where a is the gas production from the immediately soluble fraction, b_1 is the gas production from the fast-degradation fraction, b_2 is the gas production from the slow-degradation fraction, c_1 is the gas production rate constant for the fast-degradation fraction (b_1) shown as K-fast, c_2 is the gas production rate constant for the slow degradation fraction (b_2) shown as K-slow, t is the incubation time, $(a + b_1 + b_2)$ is the potential extent of gas production, and y is the gas produced at time t. The half-times of the fast ($t_{1/2}$-fast) and slow proportion ($t_{1/2}$-slow) are computed as $\ln(2)/c_1$ and $\ln(2)/c_2$, respectively. Span-Fast and Span-Slow are the fractions of the potential extent of gas production accounted for by the two faster and slower components, respectively.

Table 2. Composition of diets for Experiment 2 (M-CMS supplementation with different corn silage levels).

Item	Treatment [1]			
	HSN	**HSV**	**LSN**	**LSV**
	Ingredient (% Dry matter, DM)			
Corn silage	29.77	29.77	14.42	14.42
Oat hay	6.37	6.37	20.93	20.00
Alfalfa hay	14.33	14.33	14.88	15.81
Corn	13.74	17.74	14.02	19.14
Soybean meal	3.02	3.49	3.72	2.93
Soybean hull	17.24	8.64	16.31	8.03
DDGS	14.83	14.92	14.92	14.92
Premix	0.33	0.33	0.33	0.33
Bypass lipid	0.37	0.00	0.47	0.00
M-CMS	0.00	4.42	0.00	4.42
	Calculated parameter [2]			
Forage %	50.47	50.47	50.23	50.23
SI	0.88	0.87	0.88	0.87
RDP (%CP)	59.34	61.27	58.86	60.81
RUP (%CP)	40.66	38.73	41.14	39.19
	Chemical composition [3] (% DM)			
OM	94.82	94.16	94.05	94.43
CP	15.22	15.28	15.07	15.07
EE	3.98	3.95	3.86	3.85
NDF	44.81	44.26	42.38	41.64
ADF	26.60	22.32	26.57	22.42
Ash	5.18	5.84	5.95	5.57
NSC	30.81	30.67	32.74	33.87
NE_L (Mcal/kg)	1.59	1.60	1.63	1.63

[1] HSN: high-level (accounts for 60% of forage) corn silage diet; HSV: high-level corn silage diet + M-CMS; LSN: low-level (accounts for 30% of forage) corn silage diet; LSV: low-level corn silage diet + M-CMS; M-CMS: molasses + condensed molasses fermentation solubles (1:1 mixture). [2] SI: synchrony index; RDP: rumen-degradable protein; RUP: rumen-undegradable protein. [3] OM: organic matter; CP: crude protein; EE: ether extract; NDF: neutral detergent fiber; ADF: acid detergent fiber; NSC: non-structure carbohydrate; NE_L: net energy of lactation.

The in vitro degradability was determined using an Ankom Daisy^{II} Incubator (Ankom Technology, Macedon, NY, USA) for 48 h. Digestion and sample collection were performed as described by Spanghero et al. [19]. The percentage weight loss was determined and presented as the in vitro DM degradability (IVDMD). Residual NDF was assayed to calculate the in vitro NDF degradability (IVNDFD).

2.4. Sample Collection and Chemical Analysis

After fermentation, the ANKOM pressure sensor module was removed from the serum bottle and the pH of the fluid was measured using a pH meter (F-71, HORIBA Scientific, Kyoto, Japan). After the pH was determined, the serum bottle was capped again and placed in an ultrasonicator containing cold water (about 4 °C) for 5 min and then sonicated under cold water for 15 min to remove the attached microbes on the residual substrates. The sonicated fermented fluid was centrifuged at 400× *g* at 4 °C for 5 min. In this step, half of each supernatant fraction was collected, and lyophilization for the analysis of MCP was conducted according to the purine content method described by Zinn and Owens [20]. The MCP data from the blank group of the fermentation experiment (without substrate but with rumen inoculum mixture added) were deducted when MCP synthesis was calculated. The other half of the supernatant fraction was further centrifuged at 13,500× *g* and 4 °C for 15 min to analyze volatile fatty acids (VFAs) and ammonia. The ammonia concentration was determined immediately using a colorimetric method [21]. The samples for the VFA assay were acidified with 25% meta-phosphoric acid (4:1) and filtered using a 0.22 µm filter (Millipore Co., Bedford, MA, USA). All VFA samples were stored at −20 °C until analysis. The VFA concentration was determined using high-pressure liquid chromatography (LC-4000 system, Jasco, Tokyo, Japan) with a Rezex ROA-Organic Acid H^+ (8%) column (300 mm × 7.8 mm, Phenomenex, Torrance, CA, USA). The DM, OM, and NDF contents of the samples before and after in vitro digestion were determined using the AOAC method [14]. The MCP synthesis efficiency was presented as the MCP (mg) production per gram of digested OM.

2.5. Statistical Analysis

Statistical analyses were performed using SAS software (version 14.1; SAS, Cary, NC, USA). Data of the in vitro fermentation results from experiments 1 and 2 were compared using the mixed model procedure in SAS for 2 × 3 and 2 × 2 factorial treatment arrangements, respectively. The ANOVA included the main effects of the forage source, M-CMS supplementation, and forage source × M-CMS supplementation interaction. The statistical model used was as follows:

$$Yij = \mu + \alpha i + \beta j + (\alpha\beta)ij + eij \quad (3)$$

where Yij is the measured variable, μ is the overall mean, αi is the effect of forage source, βj is the effect of M-CMS supplementation, $(\alpha\beta)ij$ is the effect of the interaction between forage source and M-CMS supplementation, and eij is the random error term. The mean values and pooled standard errors of the mean values were reported. A non-linear regression following Huhtanen et al. [7] was used for curve-fitting using GraphPad Prism 9. The separation of the treatment means was carried out using the Tukey–Kramer procedure. The probability level ($p < 0.05$) was identified as statistically significant for all variables.

3. Results

3.1. Effect of M-CMS Supplementation on Diets with or without Corn Silage

The VFA production, NH_3 concentration, in vitro digestibility results, and MCP synthesis efficiency in experiment 1 are shown in Table 3. No significant differences were observed in the total VFA concentration, butyrate proportion, and BCVFA proportion between all treatments, but diets with silage had a higher acetate proportion and lower propionate. The forage source affected the proportion of propionate in the VFA composition, but M-CMS supplementation did not affect the propionate proportion. NH_3 concentration increased as M-CMS supplementation increased, but diets without silage and supplementation with high M-CMS resulted in the highest NH_3 concentration among all treatments. Under the same SI conditions, a high M-CMS supplementation level resulted in lower in vitro digestibility of DM, OM, and NDF. However, M-CMS supplementation significantly enhanced the MCP synthesis efficiency in both types of forage sources. The pH values of

the tested diets are provided in Figure 1. M-CMS supplementation in the diets resulted in a higher pH in the fermentation fluid during the 12 h after fermentation, especially in diets containing corn silage (Figure 1b). The pH decreased rapidly after rumen fluid inoculation when the diet contained silage as a forage source, but M-CMS supplementation resulted in a higher pH during fermentation. During the 48 h fermentation period, M-CMS supplementation also maintained a higher pH when diets without silage were used as a forage source (Figure 1a). This suggests that M-CMS supplementation had the benefit of stabilizing fermentation and providing more suitable conditions for rumen health.

Table 3. The effect of M-CMS supplementation to diets with or without corn silage on fermentation parameters and in vitro digestibility (Experiment 1).

	Treatment [1]									
Item	**T**			**S**				**Effect (*p*-Value) [2]**		
	N	**L**	**H**	**N**	**L**	**H**	**SEM**	**F**	**C**	**F × C**
Total VFA (mM)	60.55	61.52	62.04	59.18	63.17	60.74	0.35	0.773	0.241	0.492
VFA (%) [3]										
Ac	52.71 ^{bc}	51.63 ^{c}	52.09 ^{bc}	54.40 ^{a}	53.31 ^{ab}	53.63 ^{ab}	0.37	<0.001	0.029	0.973
Pr	25.66 ^{a}	24.59 ^{ab}	23.80 ^{ab}	23.72 ^{ab}	22.64 ^{b}	22.66 ^{b}	0.59	0.003	0.060	0.737
Bu	18.62	19.16	19.99	18.00	19.26	18.89	0.47	0.177	0.062	0.455
BCVFA	3.03	4.62	4.12	3.88	4.79	4.82	0.62	0.272	0.131	0.850
A/P ratio	2.05 ^{c}	2.10 ^{c}	2.19 ^{bc}	2.29 ^{ab}	2.35 ^{ab}	2.37 ^{a}	0.06	<0.001	0.233	0.836
NH_3 (mM)	25.75 ^{d}	27.68 ^{bc}	33.22 ^{a}	26.64 ^{cd}	27.57 ^{bc}	28.77 ^{b}	0.45	0.006	<0.001	<0.001
In vitro digestibility (%)										
DM	63.59 ^{abc}	61.89 ^{cd}	61.57 ^{d}	65.00 ^{a}	62.30 ^{bcd}	63.74 ^{ab}	0.53	0.003	0.011	0.327
OM	68.07 ^{abc}	66.47 ^{cd}	66.39 ^{d}	69.56 ^{a}	66.89 ^{bcd}	68.63 ^{ab}	0.57	0.020	0.032	0.060
NDF	62.86 ^{a}	61.61 ^{a}	57.64 ^{b}	61.66 ^{a}	57.24 ^{b}	58.66 ^{b}	0.76	0.076	0.001	0.002
MCP synthesis (mg/g OMD) [4]	59.57 ^{b}	75.37 ^{a}	76.79 ^{a}	56.88 ^{b}	70.93 ^{a}	74.75 ^{a}	1.31	0.118	<0.001	0.874

^{a,b,c,d} Within a row, values without a common superscript differ ($p < 0.05$). [1] T: forage source without silage; S: forage source containing silage; N: 0% M-CMS; L: 1.75% M-CMS; H: 3.50% M-CMS. M-CMS: molasses + condensed molasses fermentation solubles (1:1 mixture). [2] F: forage source; C: M-CMS supplementation level; F × C: interaction between forage source and M-CMS supplementation level. [3] VFA, volatile fatty acids; Ac: acetate; Pr: propionate; Bu: butyrate; BCVFA: branch chain VFA; A/P ratio: acetate to propionate ratio. [4] MCP: microbial crude protein; OMD: organic matter digested.

These data indicate that, even under the same SI conditions, both forage source and M-CMS level had an effect on the fermentation product and in vitro digestibility. In this study, the M-CMS supplementation could enhance the MCP synthesis efficiency under the lower SI conditions (SI = 0.8) and fixed RDP conditions, especially at the lower supplementation level (1.75% DM).

The cumulative gas production curves for the six test diets are compared in Figure 2. The diets with corn silage as a forage source showed similar curves during 48 h of fermentation (Figure 2b), but M-CMS supplementation increased gas production from diets without corn silage (Figure 2a). The gas kinetics results are presented in Table 4. A lower potential extent of gas production and the fast-fermentation fraction (fast%) was observed in the diet without corn silage and M-CMS supplementation. Interestingly, fast% and span-fast increased with the M-CMS supplementation levels when the diet without corn silage was used as the forage source. Contrary to the diets without corn silage, the data from diets with corn silage as a forage source showed higher fast% and span-fast values after M-CMS supplementation. Diets with corn silage as the forage source showed a lower degradation rate in both the fast- and slow-degradation fractions (K-fast and K-slow). The higher span-slow value of the diet with M-CMS supplementation suggests that M-CMS supplementation may be beneficial for the fermentation stability and extent. The gas kinetic results indicated that forage source and M-CMS supplementation had effects on the kinetic parameters, except for the fast% and span-fast values.

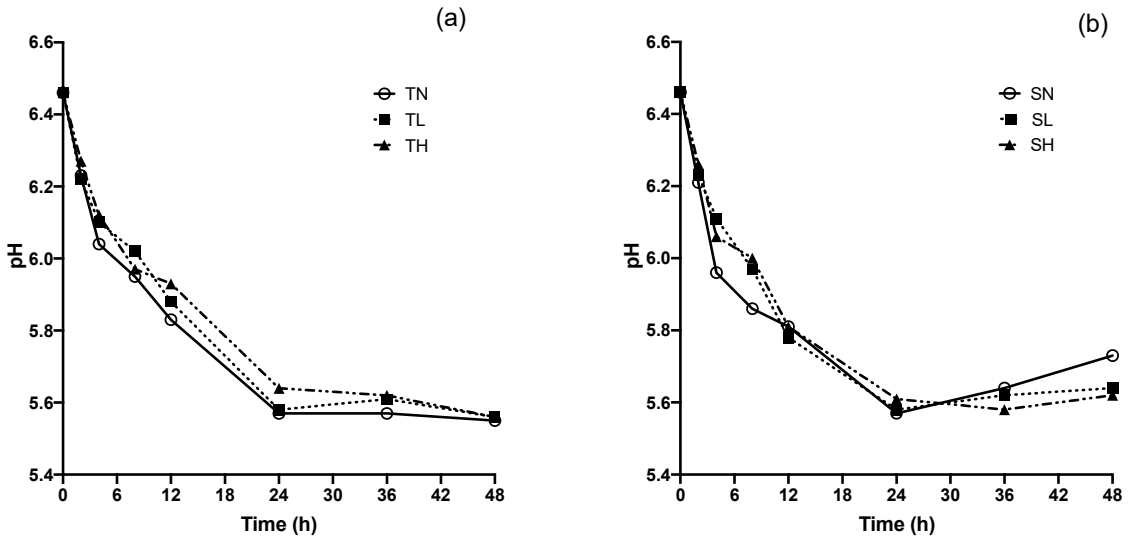

Figure 1. The effect of M-CMS supplementation to diets with or without corn silage on pH during fermentation (Experiment 1). (**a**) Diets without corn silage; (**b**) diets with corn silage. T: forage source without silage; S: forage source contain silage; N: 0% M-CMS; L: 1.75% M-CMS; H: 3.50% M-CMS; M-CMS: molasses + condensed molasses fermentation solubles (1:1 mixture).

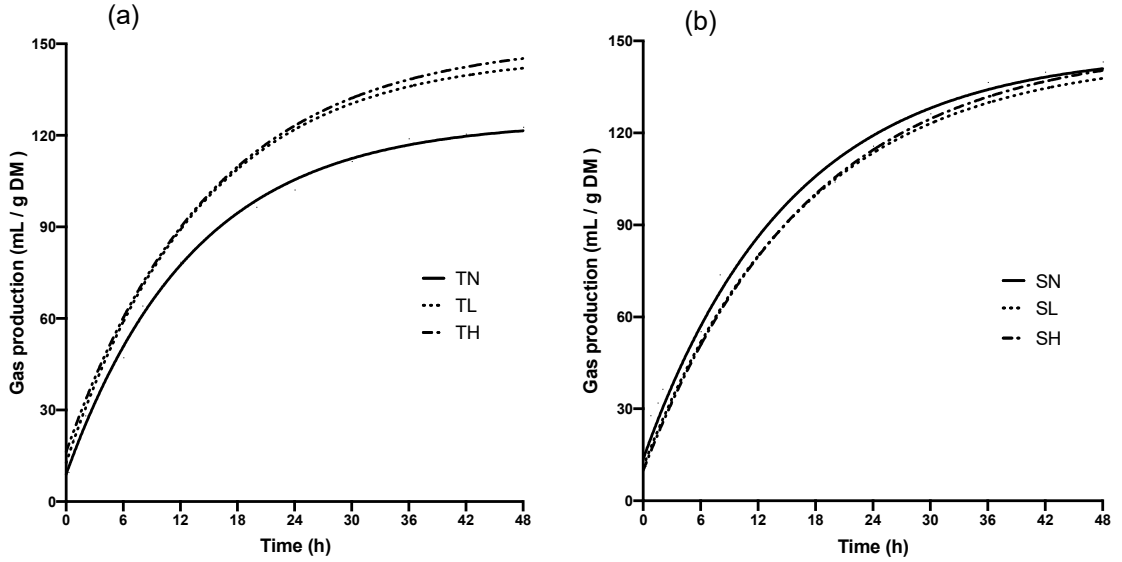

Figure 2. The effect of M-CMS supplementation to diets with or without corn silage on gas production (Experiment 1). (**a**) Diets without corn silage; (**b**) diets with corn silage. T: forage source without silage; S: forage source contain silage; N: 0% M-CMS; L: 1.75% M-CMS; H: 3.50% M-CMS; M-CMS: molasses + condensed molasses fermentation solubles (1:1 mixture).

3.2. The M-CMS Supplementation Effect on Difference Corn Silage Levels under High Synchrony Index

The VFA production, NH_3 concentration, pH, in vitro digestibility results, and MCP synthesis efficiency from experiment 2 are provided in Table 5. The total VFA concentration was similar among all diets. The forage source and M-CMS supplementation did not affect the final VFA concentration. However, a higher acetate and lower propionate proportion were shown in the high-silage diet without M-CMS supplementation (HSN diet). The pH value at the end of fermentation was higher in diets with low silage (LSN and LSV), but a high-silage diet with M-CMS supplementation resulted in a lower pH value after fermentation. The NH_3 concentration showed no difference among all diets under higher-

SI conditions (SI = 0.88) in experiment 2. Although the NSC content was higher in diets containing low silage (LSN and LSV in Table 2), IVDMD and IVOMD were higher in diets containing high levels of silage (HSN and HSV in Table 2). However, IVNDFD was not affected by the forage source or M-CMS supplementation. M-CMS supplementation significantly improved MCP synthesis in low-silage-level diets, indicating that M-CMS supplementation improved the MCP synthesis efficiency, even in diets with high SI.

Table 4. The effect of M-CMS supplementation to diets with or without corn silage on the gas kinetic parameters (Experiment 1).

	Treatment [1]									
Item [3]	**T**			**S**				**Effect (*p*-Value) [2]**		
	N	**L**	**H**	**N**	**L**	**H**	**SEM**	**F**	**C**	**F × C**
GP (mL/g DM)	166.56 ^{b}	187.92 ^{a}	193.94 ^{a}	189.41 ^{a}	190.23 ^{a}	192.57 ^{a}	0.94	0.005	<0.001	<0.001
Fast %	10.40 ^{c}	13.29 ^{b}	16.49 ^{a}	15.97 ^{a}	12.38 ^{bc}	11.18 ^{bc}	0.75	0.735	0.423	<0.001
K-fast (h^{-1})	3.60 ^{b}	3.33 ^{bc}	4.39 ^{a}	2.51 ^{d}	2.97 ^{c}	3.06 ^{c}	0.13	<0.001	0.000	0.003
K-slow (h^{-1})	0.066 ^{a}	0.058 ^{b}	0.056 ^{b}	0.051 ^{c}	0.050 ^{c}	0.051 ^{c}	0.001	<0.001	0.005	0.003
$t_{1/2}$ -fast (h)	0.19 ^{c}	0.23 ^{b}	0.16 ^{d}	0.27 ^{a}	0.23 ^{bc}	0.23 ^{b}	0.01	<0.001	<0.001	<0.001
$t_{1/2}$ -slow (h)	11.07 ^{d}	11.92 ^{cd}	12.45 ^{bc}	14.14 ^{a}	13.85 ^{a}	13.55 ^{ab}	0.38	<0.001	0.576	0.057
Span-fast (mL)	15.21 ^{d}	25.02 ^{b}	32.19 ^{a}	30.80 ^{a}	23.53 ^{bc}	20.70 ^{c}	1.15	0.954	0.110	<0.001
Span-slow (mL)	146.32 ^{d}	163.28 ^{bc}	163.11 ^{bc}	158.89 ^{c}	166.85 ^{bc}	173.79 ^{a}	2.08	<0.001	<0.001	0.126

^{a,b,c,d} Within a row, values without a common superscript differ ($p < 0.05$). [1] T: forage source without silage; S: forage source containing silage; N: 0% M-CMS; L: 1.75% M-CMS; H: 3.50% M-CMS. M-CMS: molasses + condensed molasses fermentation solubles (1:1 mixture). [2] F: forage source; C: M-CMS supplementation level; F × C: interaction between forage source and M-CMS supplementation level. [3] GP: gas production after 48 h of fermentation; Fast%: percentage of fast-degradation fraction; K-fast: degradation rate of fast-degradation fraction; K-slow: degradation rate of slow-degradation fraction; $t_{1/2}$ -fast: time at which half of the fast-degradation fraction gas production asymptote was reached; $t_{1/2}$ -slow: time at which half of the slow-degradation fraction gas production asymptote was reached; Span-fast: gas production from the fast-degradation fraction; Span-slow: the gas production from slow degradation fraction.

Table 5. The effect of M-CMS supplementation with different corn silage levels on fermentation parameters and in vitro digestibility (Experiment 2).

	Treatment [1]					Effect (*p*-Value) [2]		
Item [3]	**HSN**	**HSV**	**LSN**	**LSV**	**SEM**	**SL**	**C**	**SL × C**
pH	5.62 ^{b}	5.57 ^{c}	5.68 ^{a}	5.66 ^{ab}	0.01	<0.001	0.005	0.001
Total VFAs (mM)	89.04	88.48	89.00	85.60	2.85	0.620	0.501	0.625
VFAs (%)								
Ac	57.67 ^{a}	56.71 ^{b}	57.04 ^{b}	55.10 ^{b}	0.95	0.260	0.154	0.614
Pr	26.02	29.68 ^{ab}	30.06 ^{ab}	31.64 ^{a}	1.27	0.035	0.061	0.428
Bu	13.71	13.61	12.90	13.26	0.34	0.111	0.712	0.508
BCVFA	2.61	2.69	2.62	2.52	0.13	0.561	0.956	0.488
Ac/Pr ratio	2.23 ^{a}	1.96 ^{ab}	1.90 ^{ab}	1.75 ^{b}	0.13	0.058	0.128	0.671
NH_3 (mM)	34.47	35.50	35.47	34.19	0.46	0.852	0.880	0.163
In vitro digestibility								
DM%	66.89 ^{a}	64.19 ^{b}	63.08 ^{bc}	62.04 ^{c}	0.76	<0.001	0.010	0.207
OM%	71.70 ^{a}	68.80 ^{a}	67.61 ^{b}	66.49 ^{ab}	0.82	0.001	0.013	0.257
NDF%	62.86	59.64	62.35	63.19	0.74	0.607	0.647	0.913
MCP synthesis (mg/g OMD) [4]	75.81 ^{c}	78.23 ^{c}	85.80 ^{b}	92.19 ^{a}	0.92	<0.001	0.001	0.053

^{a,b,c} Within a row, values without a common superscript differ ($p < 0.05$). [1] HSN: high-level (accounts for 60% of forage) corn silage diet; HSV: high-level corn silage diet + M-CMS; LSN: low-level (accounts for 30% of forage) corn silage diet; LSV: low-level corn silage diet + M-CMS; M-CMS: molasses + condensed molasses fermentation solubles (1:1 mixture). [2] SL: corn silage level; C: M-CMS supplementation; FL × C: interaction between corn silage level and M-CMS supplementation. [3] VFAs, volatile fatty acids; Ac: acetate; Pr: propionate; Bu: butyrate; BCVFAs: branch-chain VFAs; A/P ratio: acetate to propionate ratio.[4] MCP: microbial crude protein; OMD: organic matter digested.

The gas production curves and gas kinetic results of experiment 2 are presented in Figure 3 and Table 6, respectively. Diets with low silage levels (LSN and LSV) had lower total cumulative gas production and spin-slow values. This suggests that a lower silage level might result in less degradation of the insoluble fractions. However, the spin–fast value trend was different in the diets with two silage levels after M-CMS supplementation. The interaction between forage source and M-CMS supplementation was also shown in fast% and span-fast. M-CMS supplementation exerted an effect on the K-slow and half-time of the slow-degradation fraction ($t_{1/2}$-slow), and increased K-slow. This implies that M-CMS supplementation could increase the degradation rate of the insoluble fraction under the same SI conditions. Compared with the gas kinetic data in experiment 1 (Table 4), the forage source seemed to have less effect on the degradation and fermentation model under higher-SI conditions. Notably, M-CMS supplementation had the benefit of enhanced MCP synthesis efficiency under both low- and high-SI conditions.

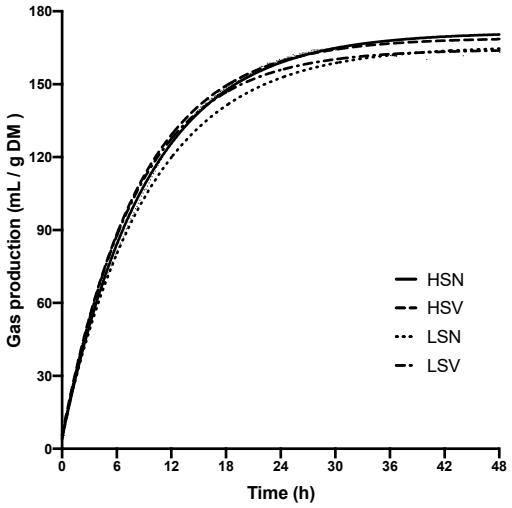

Figure 3. The effect of M-CMS supplementation with different corn silage levels on gas production (Experiment 2). HSN: high-level (accounts for 60% of forage) corn silage diet; HSV: high-level corn silage diet + M-CMS; LSN: low-level (accounts for 30% of forage) corn silage diet; LSV: low-level corn silage diet + M-CMS; M-CMS: molasses + condensed molasses fermentation solubles (1:1 mixture).

Table 6. The effect of M-CMS supplementation with different corn silage levels on gas kinetic parameters (Experiment 2).

	Treatment [1]					Effect (*p*-Value) [2]		
Item [3]	**HSN**	**HSV**	**LSN**	**LSV**	**SEM**	**SL**	**M-CMS**	**SL × C**
GP (mL/g DM)	176.60 ^{a}	172.96 ^{ab}	170.20 ^{b}	167.55 ^{b}	0.72	0.001	0.049	0.736
Fast %	5.32 ^{ab}	6.20 ^{a}	6.13 ^{a}	5.07 ^{b}	0.27	0.583	0.737	0.004
K-fast (h^{-1})	2.080 ^{b}	2.695 ^{a}	2.134 ^{b}	2.155 ^{b}	0.16	0.151	0.068	0.087
K-slow (h^{-1})	0.097 ^{c}	0.107 ^{b}	0.094 ^{c}	0.113 ^{a}	0.001	0.321	<0.0001	0.005
$t_{1/2}$ -fast (h)	0.34 ^{a}	0.26 ^{b}	0.33 ^{a}	0.32 ^{a}	0.02	0.263	0.084	0.132
$t_{1/2}$ -slow (h)	7.12 ^{a}	6.47 ^{b}	7.36 ^{a}	6.14 ^{b}	0.09	0.631	<0.0001	0.008
Span-fast (mL)	9.43 ^{b}	10.71 ^{a}	10.44 ^{a}	8.51 ^{b}	0.45	0.219	0.482	0.004
Span-slow (mL)	177.39 ^{a}	173.36 ^{b}	170.46 ^{bc}	167.86 ^{c}	1.44	0.001	0.040	0.628

^{a,b,c} Within a row, values without a common superscript differ ($p < 0.05$). [1] HSN: high-level (accounts for 60% of forage) corn silage diet; HSV: high-level corn silage diet + M-CMS; LSN: low-level (accounts for 30% of forage) corn silage diet; LSV: low-level corn silage diet + M-CMS; M-CMS: molasses + condensed molasses fermentation solubles (1:1 mixture). [2] SL: corn silage level; C: M-CMS supplementation; FL × C: interaction between corn silage level and M-CMS supplementation. [3] GP: gas production after 48 h of fermentation; Fast%: percentage of fast-degradation fraction; K-fast: degradation rate of fast-degradation fraction; K-slow: degradation rate of slow-degradation fraction; $t_{1/2}$ -fast: time at which half of the fast-degradation fraction gas production asymptote was reached; $t_{1/2}$ -slow: time at which half of the slow-degradation fraction gas production asymptote was reached; Span-fast: gas production from the fast-degradation fraction; Span-slow: gas production from the slow-degradation fraction.

4. Discussions

4.1. The Interaction of Forage Source and M-CMS Supplementation

This study was conducted to evaluate the effects of M-CMS supplementation on diets with the same synchronization index. The synchronization of dietary N and energy has been suggested as a useful method to improve the ruminal MCP synthesis efficiency, reduce the excretion of N in urine and feces, and improve animal production [13].

The acetate concentration and the A:P ratio were higher when corn silage was the forage source in experiment 1, which could be explained by the higher NDF content in these diets. A lower propionate concentration was also observed in these treatment groups (SN, SL, and SH), which may have resulted from the lower NSC levels in these diets compared with diets without corn silage (TN, TL, and TH). A previous study indicated that a higher NDF level was related to a higher acetate concentration during rumen fermentation, and higher NSC or starch also resulted in higher propionate production [6].

In this study, the in vitro digestibility of DM and OM was not affected by forage source and M-CMS supplementation. Previous in vitro digestion studies manipulated the same SI in diets by changing the ingredients and showed similar results [22]. Compared with the in vitro digestibility results in experiments 1 and 2, the higher SI condition (SI = 0.88, Experiment 2) resulted in higher DM and OM digestibility. The increase in SI had a positive effect on DM and OM digestibility according to previous studies [22,23]. This suggests that, under the same SI conditions, DM and OM digestibility might be similar, even in diets containing different forage sources. A previous study reported that manipulating dietary synchronization by changing ingredients had no significant effect on digestibility [24].

A previous study showed that lower ruminal NH_3-N was correlated with higher usage of NH_3-N for MCP synthesis [1]. In this study, the SH treatment exhibited a similar NH_3-N concentration to the SL treatment group after 48 h of fermentation. However, compared with the MCP synthesis efficiency results in the HL and SL groups, greater improvement in MCP synthesis was observed in the SH group than in the HL group (Table 3). This may have resulted from the higher soluble nitrogen and free amino acids in the M-CMS product. Because M-CMS contains a high level of non-protein nitrogen (approximately 30% CP) and over 25% WSC, the fast-soluble energy and nitrogen source showed benefits for MCP synthesis in the early stage of fermentation [25]. Another reason for the better MCP synthesis efficiency in M-CMS treatments could be related to the fact that M-CMS supplementation tended to increase the BCVFA concentration. The increase in BCAA in the diet was caused by rich BCAA from the M-CMS product. The higher BCVFA produced during fermentation and BCAA in the diet is beneficial for MCP synthesis [26].

In Experiment 1, the IVDMD and IVOMD decreased slightly after M-CMS was supplied, but the MCP synthesis efficiency was still higher after M-CMS supplementation. However, low or high M-CMS supplementation levels resulted in no differences in IVDMD and IVOMD. Broderick and Radloff [27] observed that, under a diet, CP was 15.6% (close to diets in the present study). The higher molasses product supplementation that increased sugar (>5% DM) in the diet resulted in decreases in DM and NDF digestibility. The study supplemented different CMS in dairy cow diets with high corn silage (35.8% DM) and reported that DM and OM digestibility decreased under high CMS supplementation levels [11], which was in line with our results.

The supplement type, nutrient profile, and degradation rates are often prime considerations associated with nutrient synchrony in different levels of forage diets [28], suggesting that the synchronization manipulation effect may differ depending on the ingredient source and its degradation rate. Forage quality also plays an important role in successful nutrient synchrony. High-quality forage may contain an excessive amount of N compared with energy, which results in lower efficiency in supporting nutrient synchrony [28]. In Experiment 1, the K-fast value (Table 4) was significantly affected by forage source and M-CMS supplementation, but the fast% and span-fast values showed the opposite trend between the two forage source groups. Diets without silage as the forage source (HN, HL, and HH groups) had a higher NSC. The CNCPS fraction [29] indicated that oat hay

in the forage source had a higher CHO-A fraction (sugar) and CHO-B1 fraction (starch and pectin) ratio in the CHO composition than corn silage. The degradation rates of the CHO-B1 and CHO-B2 fractions (hemicellulose and cellulose) were similar between oat hay and corn silage, but the degradation of the CHO-A fraction was much lower in corn silage. Previous studies have also indicated that the available energy or CHO was more effective than synchrony in fermentation and MCP synthesis [30,31].

The MCP synthesis efficiency was positively related to the K-fast value (rate of fast-degradation fraction), but not to fast% (% of the fast-degradation fraction) or t1/2-fast (half-time of the fast-degradation fraction) in experiment 1. The M-CMS supplementation level increased as the K-fast value increased. This suggests that M-CMS supplementation may result in more balanced energy and nitrogen release conditions during the early fermentation stages. M-CMS is rich in sucrose, free amino acids, and NPN, providing ready-to-use energy and nitrogen sources simultaneously. According to the pH data in Figure 1, the high ammonia concentration in M-CMS also prevented the rapid pH decrease caused by fast fermentation in the early stage. Under lower-SI conditions, the forage quality or other feedstuff degradation rates also affected the synchronization between CHO and N release and the rumen microorganism's N utilization [22]. An early in vitro study indicated that molasses in combination with roughage could increase the efficiency of MCP synthesis [32]. Trevaskis et al. [33] indicated that ruminal sucrose infusion was more effective in stimulating microbial protein formation than other sugars when synchronized with the ammonia peak occurring 1 to 2 h after feeding.

According to the effect of M-CMS supplementation on diets with different forage sources, ruminal fermentation might be limited by nitrogen or energy availability, rather than by the lack of synchronization of the release of these nutrients. Different NSC and protein sources may confound the effects of synchronization, and the nitrogen recycling in ruminants also confounds the synchronization situation. This has led to a lesser effect on improving the total tract digestibility and production performance through dietary synchronization in vivo [34].

4.2. The M-CMS Supplementation Effect on Different Corn Silage Levels under High Synchrony Index

M-CMS supplementation or decreasing corn silage levels in diets resulted in lower IVDMD and IVOMD in experiment 2. A partial explanation for this may be in line with the fact that high molasses and CMS products decreased the digestibility [11,27], especially under high-SI conditions [23].

The corn silage contains high NPN (approximately 45–50% of total N), but the NPN in corn silage is a slow-release form [35]. This may lead to a delay in N release and cannot meet the fast release of carbohydrates from forage and other feedstuffs in the early stages of fermentation. Owing to the much lower degradation rate of the CHO-A fraction in corn silage than in oat hay, diets containing high amounts of oat hay (LSN and LSV) may provide more available soluble carbohydrates and result in better microbial growth during the early fermentation stage.

Compared with experiment 1, experiment 2 applied higher-SI diets (SI = 0.88); the higher SI diets resulted in a higher propionate proportion and MCP synthesis efficiency, but no significant effect was observed on NDF digestibility in vitro. These results are consistent with those reported by Cabrita et al. [23]. A higher proportion of propionate resulted in increased energy capture relative to acetate. Therefore, diets with a low A:P ratio should have higher nutritive value than those with higher ratios [36]. These VFA patterns also appeared in experiment 2 of this study. The A:P ratio was negatively correlated with the MCP synthesis efficiency.

Diets with lower corn silage and higher oat hay contents (LSN and LSV) showed better MCP synthesis efficiency in experiment 2. Additionally, M-CMS supplementation exhibited advanced benefits for MCP synthesis under high-SI conditions. Although M-CMS contained high NPN in its N source composition, the NH_3 concentration showed no difference

after 48 h of fermentation among the four diets. This suggests that energy and nitrogen supplementation remained balanced during the late stage of fermentation. Rotger et al. [37] also reported that the synchronization of NSC and protein sources for both rapid and slow fermentation tended to result in greater VFA production and flow of microbial N in a dual-flow continuous culture.

The total gas production was lower when the diet had a lower corn silage level. Supplementation with M-CMS may be associated with the lower A:P ratio in these diets. According to the stoichiometry of the fermentation of hexose to the VFAs, a higher propionate ratio resulted in lower CO_2 and CH_4 production and improved the efficiency of the conversion of hexose energy to VFA [38]. The improvement in energy conversion also increased the ATP supply and enhanced MCP synthesis [39]. In experiment 2, the increase in K-slow (rate of the slow-degradation fraction) and the shorter $t_{1/2}$-slow (half-time of the slow-degradation fraction) after M-CMS supplementation (diet HSV and LSV) implied that the degradation rate of the insoluble fraction (mainly consisting of fiber) was improved. A previous study reported that CMS product supplementation in the ruminant diet stabilized rumen pH. Additionally, it increased both the rumen cellulolytic bacterial population and fiber digestibility [40]. The opposite fast% and Span-fast (gas production volume from the fast-degradation fraction) trends of M-CMS supplementation for different corn silage diets (HSN vs. HSV and LSN vs. LSV) are shown in experiment 2. This may be caused by the interaction between soybean hull and corn silage in the experimental diet. In this study, diets supplemented with M-CMS (HSV and LSV) decreased the soybean hull in the diet formula to adjust the SI of the diets. However, over 65% of the carbohydrate proportion of the soybean hull was insoluble (CHO-B2 and undigestible fraction in CNCPS 5.0), but M-CMS only contained the rapidly soluble fraction (CHO-A fraction in CNCPS 5.0). Moreover, the CHO fraction degradation rate of corn silage and oat hay also affected the total soluble CHO released from the diets. The carbohydrate and protein fraction data of corn silage and oat hay from the CNCPS manual [41] indicated that the degradation rate of corn silage was low in the CHO-A fraction (10% h), but high in the Protein-B1 fraction (300% h). However, the oat hay data showed that the degradation rate of the CHO-A fraction (250% h) was high and that of PB1 (135% h) was much lower than that in corn silage. The high corn silage level may have contributed to the unstable results when the diet was supplied with M-CMS due to the CHO and protein degradation characteristics of corn silage. The similar K-fast and $t_{1/2}$-fast values shown in LSN and LSV could prove this assumption. A previous study on feeding two levels of molasses (4 and 8%) with a diet containing low or high silage (35 or 65%) also reported inconsistent effects on digestibility and milk production [42].

The MCP synthesis and digestibility data in experiment 2 suggested that M-CMS with rapidly released energy and N source could be a valuable tool to manipulate rumen fermentation with different forage combinations. However, the level of corn silage in the diet formula should be considered when M-CMS is supplemented in the synchronous diet to improve MCP synthesis. The SI value in this study was calculated based on the OM and CP fractions of each feedstuff in the diet. However, the OM degradation rate may be affected by both CHO and CP sources [43]. This suggests that manipulating the CHO and CP degradation rates through a feedstuff source could further improve MCP synthesis.

5. Conclusions

In this study, the synchrony of energy and nitrogen supply was improved by increasing SI-enhanced VFA production and MCP synthesis. However, even under the same dietary SI, the variation in the diet ingredients or forage source level affected the MCP synthesis efficiency. The optimization of the combination of CHO and N degradation rates in the diets with the same SI could further enhance rumen microorganism energy utilization and MPC synthesis. During the early stage of rumen fermentation after feeding, supplementation with a liquid feedstuff rich in highly soluble CHO and N, similar to the M-CMS product, could help enhance microbial growth. Although M-CMS supplementation did not improve digestibility in the energy and nitrogen synchronization diet, it continued to demonstrate

the ability to enhance MCP synthesis efficiency, even in a diet with high SI. According to the results of this study, M-CMS supplementation improved MCP synthesis in diets containing less corn silage. This suggests that utilizing the properties of the forage source and degradation rate of individual feedstuff in the diet formula at the same time could be a profitable manipulation to enhance the rumen MCP synthesis efficiency. The M-CMS-like product also provided a practicable ingredient choice to further improve MCP synthesis, even when the diet had high SI.

Author Contributions: Conceptualization, C.-Y.C. and H.-T.W.; methodology, H.-T.W.; software, Y.-H.C.; validation, Y.-H.C., C.-Y.C. and H.-T.W.; formal analysis, Y.-H.C.; investigation, Y.-H.C. and H.-T.W.; resources, C.-Y.C. and H.-T.W.; data curation, Y.-H.C.; writing—original draft preparation, Y.-H.C.; writing—review and editing, C.-Y.C. and H.-T.W.; visualization, C.-Y.C.; supervision, H.-T.W.; project administration, H.-T.W.; funding acquisition, H.-T.W. All authors have read and agreed to the published version of the manuscript.

Funding: This study was supported by the Ministry of Science and Technology, Taiwan (MOST 110-2622-B-002-006).

Institutional Review Board Statement: All animal care procedures used in this study were approved by the Institutional Animal Care and Use Committee of the National Taiwan University (approval no: NTU-110-EL-00068).

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: The authors would like to thank all colleagues and students who contributed to this study.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Kaswari, T.; Lebzien, P.; Flachowsky, G.; ter Meulen, U. Studies on the relationship between the synchronization index and the microbial protein synthesis in the rumen of dairy cows. *Anim. Feed Sci. Technol.* **2007**, *139*, 1–22. [CrossRef]
2. Herrera-Saldana, R.; Gomez-Alarcon, R.; Torabi, M.; Huber, J.T. Influence of synchro- nizing protein and starch degradation in the rumen on nutrient utilization and microbial protein synthesis. *J. Dairy Sci.* **1990**, *73*, 142–148. [CrossRef]
3. Henning, P.H.; Steyn, D.G.; Meissner, H.H. The effect of energy and nitrogen supply pattern on rumen bacterial growth *in vitro*. *Anim. Sci.* **1991**, *53*, 165–175. [CrossRef]
4. Hall, M.B.; Huntington, G.B. Nutrient synchrony: Sound in theory, elusive in practice. *J. Anim. Sci.* **2008**, *86*, E287–E292. [CrossRef] [PubMed]
5. Sinclair, L.A.; Garnsworthy, P.C.; Newbold, J.R.; Buttery, P.J. Effect of synchronizing the rate of dietary energy and nitrogen release on rumen fermentation and microbial protein synthesis in the sheep. *J. Agric. Sci.* **1993**, *120*, 251–263. [CrossRef]
6. Menke, H.H.; Steingass, H. Estimation of the energetic feed value obtained from chemical analysis and in vitro gas production using rumen fluid. *Anim. Res. Dev.* **1988**, *28*, 7–55.
7. Huhtanen, P.; Seppälä, A.; Ahvenjärvi, S.; Rinne, M. Prediction of in vivo neutral detergent fiber digestibility and digestion rate of potentially digestible neutral detergent fiber: Comparison of models. *J. Anim. Sci.* **2008**, *86*, 2657–2669. [CrossRef]
8. Peltekova, V.D.; Broderick, G.A. In vitro ruminal degradation and synthesis of protein on fractions extracted from alfalfa hay and silage. *J. Dairy Sci.* **1996**, *79*, 612–619. [CrossRef]
9. Bowman, J.G.P.; Sowell, B.F.; Patterson, J.A. Liquid supplementation for ruminants fed low-quality forage diets: A review. *Anim. Feed Sci. Tech.* **1995**, *55*, 105–138. [CrossRef]
10. Scull, I.; Savon, L.; Gutierrez, O.; Valino, E.; Orta, I.; Mora, P.O.; Orta, H.; Yusmely, R.; Molineda, A.; Coto, G.; et al. Physio-Chemical composition of concentrated vinasse for their assessment in animal diets. *Cuba. J. Agric. Sci.* **2012**, *46*, 385–389.
11. Ma, J.; Ma, C.; Fan, X.; Shah, A.; Mao, J. Use of condensed molasses fermentation solubles as an alternative source of concentrates in dairy cows. *Anim. Biosci.* **2021**, *34*, 205–212. [CrossRef] [PubMed]
12. Stemme, K.; Gerdes, B.; Harms, A.; Kamphues, J. Beet-vinasse (condensed molasses solubles) as an ingredient in diets for cattle and pigs-nutritive value and limitations. *J. Anim. Physiol. Anim. Nutr.* **2005**, *89*, 179–183. [CrossRef] [PubMed]
13. Henning, P.H.; Steyn, D.G.; Meissner, H.H. Effect of synchronization of energy and nitrogen supply on ruminal characteristics and microbial growth. *J. Anim. Sci.* **1993**, *71*, 2516–2528. [CrossRef]
14. Association of Official Analytical Chemists. *Official Methods of Analysis*, 17th ed.; AOAC: Washington, DC, USA, 2000.

15. Lei, Y.G.; Li, X.Y.; Wang, Y.Y.; Li, Z.Z.; Chen, Y.L.; Yang, Y.X. Determination of ruminal dry matter and crude protein degradability and degradation kinetics of several concentrate feed ingredients in cashmere goat. *J. Appl. Anim. Res.* **2018**, *46*, 134–140. [CrossRef]
16. Seo, S.; Tedeschi, L.O.; Schwab, C.G.; Garthwaite, B.D. Fox, D.G. Evaluation of the passage rate equations in the 2001 dairy NRC model. *J. Dairy Sci.* **2006**, *89*, 2327–2342. [CrossRef]
17. National Research Council. *Nutrient Requirements of Dairy Cattle*, 7th rev. ed.; National Academy of Sciences: Washington, DC, USA, 2001.
18. Ankom Technology. In Vitro True Digestibility Using the DaisyII Incubator. Available online: https://www.ankom.com/sites/default/files/document-files/Method_3_Invitro_D200_D200I.pdf (accessed on 21 December 2018).
19. Spanghero, M.; Boccalon, S.; Gracco, L.; Gruber, L. NDF degradability of hays measured in situ and in vitro. *Anim. Feed Sci. Technol.* **2003**, *104*, 201–208. [CrossRef]
20. Zinn, R.A.; Owens, F.N. A rapid procedure for purine measurement and its use for estimating net ruminal protein synthesis. *Can. J. Anim. Sci.* **1986**, *66*, 157–166. [CrossRef]
21. Chaney, A.L.; Marbach, E.P. Modified reagents for determination of urea and ammonia. *Clin. Chem.* **1962**, *8*, 130–132. [CrossRef]
22. Seo, J.K.; Kim, M.H.; Yang, J.Y.; Kim, H.J.; Lee, C.H.; Kim, K.H.; Ha, J.K. Effects of synchronicity of carbohydrate and protein degradation on rumen fermentation characteristics and microbial protein synthesis. *Asian-Austral. J. Anim. Sci.* **2013**, *26*, 358–365. [CrossRef]
23. Cabrita, A.R.J.; Dewhurst, R.J.; Abreu, J.M.F.; Fonseca, A.J.M. Evaluation of the effects of synchronising the availability of N and energy on rumen function and production responses of dairy cows–a review. *Anim. Res.* **2006**, *55*, 1–24. [CrossRef]
24. Kolver, E.; Muller, L.D.; Varga, G.A.; Cassidy, T.J. Synchronization of ruminal degradation of supplemental carbohydrate with pasture nitrogen in lactating dairy cows. *J. Dairy. Sci.* **1998**, *81*, 2017–2028. [CrossRef]
25. Zali, A.; Eftekhari, M.; Fatehi, F.; Ganjkhanlou, M. Effect of vinasse (condensed molasses solubles) on performance and meat chemical composition of Holstein male calves. *Ital. J. Anim. Sci.* **2017**, *16*, 515–520. [CrossRef]
26. Sannes, R.A.; Messman, M.A.; Vagnoni, D.B. Form of rumen-degradable carbohydrate and nitrogen on microbial protein synthesis and protein efficiency of dairy cows. *J. Dairy Sci.* **2002**, *85*, 900–908. [CrossRef]
27. Broderick, G.A.; Radloff, W.J. Effect of molasses supplementation on the production of lactating dairy cows fed diets based on alfalfa and corn silage. *J. Dairy Sci.* **2004**, *87*, 2997–3009. [CrossRef]
28. Hersom, M.J. Opportunities to enhance performance and efficiency through nutrient synchrony in forage-fed ruminants. *J. Anim. Sci.* **2007**, *86*, E306–E317. [CrossRef]
29. Tedeschi, L.O.; Chalupa, W.; Janczewski, E.; Fox, D.G.; Sniffen, C.; Munson, R.; Kononoff, P.; Boston, R. Evaluation and application of the CPM dairy nutrition model. *J. Agric. Sci.* **2008**, *146*, 171–182. [CrossRef]
30. Yang, J.Y.; Seo, J.; Kim, H.J.; Seo, S.; Ha, J.K. Nutrient Synchrony: Is it a suitable strategy to improve nitrogen utilization and animal performance? *Asian Australas. J. Anim. Sci.* **2010**, *23*, 972–979. [CrossRef]
31. Witt, M.W.; Sinclair, L.A.; Wilkinson, R.G.; Buttery, P.J. The effects of synchronizing the rate of dietary energy and nitrogen supply to the rumen on milk production and metabolism of ewes offered grass silage based diets. *Anim. Sci.* **2000**, *71*, 187–195. [CrossRef]
32. El Khidir, O.A.; Thomsen, K.V. The effect of high levels of molasses in combinations with hay on digestibility of organic matter, microbial protein synthesis and volatile fatty acid production in vitro. *Anim. Feed Sci. Technol.* **1982**, *7*, 277–286. [CrossRef]
33. Trevaskis, L.M.; Fulkerson, W.J.; Gooden, J. Provision of certain carbohydrate-based supplements to pasture-fed sheep, as well as time of harvesting of the pasture, influences pH, ammonia concentration and microbial protein synthesis in the rumen. *Aust. J. Exp. Agric.* **2001**, *41*, 21–27. [CrossRef]
34. Valkeners, D.; Tahewis, A.; Piron, F.; Beekers, Y. Effect of imbalance between energy and nitrogen supplies on microbial protein synthesis and nitrogen metabolism in growing double muscled Belgian Blue bulls. *J. Anim Sci.* **2004**, *82*, 1818–1825. [CrossRef] [PubMed]
35. Buchanan-Smith, J.G.; Yao, Y.T. Non-protein nitrogen in corn silage: A partial characterization, its utilization in the rumen and effect upon digestibility and retention of nitrogen in lambs. *Can. J. Anim. Sci.* **1978**, *58*, 681–690. [CrossRef]
36. Wischer, G.; Boguhn, J.; Steingass, H.; Schollenberger, M.; Hartung, K.; Rodehutscord, M. Effect of monensin on in vitro fermentation of silages and microbial protein synthesis. *Arch. Anim. Nutr.* **2013**, *67*, 219–234. [CrossRef] [PubMed]
37. Rotger, A.; Ferret, A.; Calsamiglia, S.; Manteca, X. Effects of nonstructural carbohydrates and protein sources on intake, apparent total tract digestibility, and ruminal metabolism in vivo and in vitro with high-concentrate beef cattle diets. *J. Anim. Sci.* **2006**, *84*, 1188–1196. [CrossRef]
38. Whitelaw, F.G.; Eadie, J.M.; Bruce, L.A. Shand WJ. Methane formation in faunated and ciliate-free cattle and its relationship with rumen volatile fatty acid proportions. *Br. J. Nutr.* **1984**, *52*, 261–275. [CrossRef]
39. Münnich, M.; Klevenhusen, F.; Zebeli, Q. Feeding of molassed sugar beet pulp instead of maize enhances net food production of high-producing Simmental cows without impairing metabolic health. *Anim. Feed Sci. Technol.* **2018**, *241*, 75–83. [CrossRef]
40. Weidmeier, R.D.; Tanner, B.H.; Bair, J.R.; Shenton, H.T.; Arambel, M.J.; Walters, J.L. Effects of a new molasses by-product, concentrated separator by-product, on nutrient digestibility and ruminal fermentation in cattle. *J. Anim. Sci.* **1992**, *70*, 1930–1940. [CrossRef]
41. Van Amburgh, M.E.; Collao-Saenz, E.A.; Higgs, R.J.; Ross, D.A.; Recktenwald, E.B.; Raffrenato, E.; Chase, L.E.; Overton, T.R.; Mills, J.K.; Foskolos, A. The cornell net carbohydrate and protein system: Updates to the model and evaluation of version 6.5. *J. Dairy Sci.* **2015**, *98*, 6361–6380. [CrossRef]

42. Morales, J.L.; Van Horn, H.H.; Moore, J.E. Dietary interaction of cane molasses with source of roughage: Intake and lactation effects. *J. Dairy Sci.* **1989**, *72*, 2331–2338. [CrossRef]
43. Bach, A.; Calsamiglia, S.; Stern, M.D. Nitrogen metabolism in the rumen. *J. Dairy Sci.* **2005**, *88*, E9–E21. [CrossRef]