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In vitro rumen fermentation characteristics of substrate mixtures with soybean meal partially replaced by microbially fermented yellow wine lees

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ABSTRACT

This study was conducted to evaluate the effects of replacing soybean meal (SBM) with unfermented and microbially fermented yellow wine lees (YWL) mix on the *in vitro* rumen fermentation characteristics of substrate mixtures. Both types consisting of YWL at 400 g/kg were included in the mixtures at different ratios (1:0, 1:1, 1:2, 1:5 and 0:1, w/w) to replace SBM. Microbial fermentation of YWL did not have a negative impact on gas value ($p > .05$), increased microbial protein (MCP, $p < .01$) and *in vitro* crude protein (CP) digestibility ($p < .01$), and improved the efficiency of nitrogen utilisation ($p < .01$). The ratio of YWL replacing SBM had linear and quadratic effects on gas production (GP, $p < .01$), the rate of GP ($p < .01$), MCP ($p < .01$), total volatile fatty acids (VFAs, $p < .01$), *in vitro* CP digestibility and efficiency of nitrogen utilisation ($p < .01$), with the optimal ratio at 1:1. The *in vitro* digestibilities of dry matter and organic were slightly ($p < .01$) reduced with the increasing ratio of YWL. Besides, positive associative effects were observed on GP parameters, VFA and MCP at some replacement ratios. Considering the *in vitro* rumen characteristics and their associative effects, the optimal ratio at which to replace SBM with microbially fermented YWL was indicated to be 1:1.

HIGHLIGHTS

- The *in vitro* rumen fermentation characteristics performed better with microbially fermented than unfermented yellow wine lees (YWL) replacing soybean meal (SBM).
- The positive associative effects were observed on gas production parameters, volatile fatty acid and microbial protein at replacing ratio of 1:1.
- The optimal ratio at which to replace SBM with microbially fermented YWL was 1:1.

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Associative effects; *in vitro* digestibility; gas production; microbial protein; volatile fatty acids



Introduction

Soybean meal (SBM) is the most important plant protein source in animal industry (Boguhn et al. 2008). In recent years, alternative protein feeds have attracted much interest all over the world, with growing consumer demand for dairy products because of the insufficient supply and high price of SBM. Many studies have been conducted on the utilisation of unconventional feed resources, such as palm meal, distiller's grains and sesame cake, in dairy production (Boguhn et al. 2008). In China, million tons of distiller's grains, by-products of the wine industry, are not well treated, but still contain quite a lot of unused protein, starch, fat and other ingredients which may have feeding value (Schingoethe et al. 2009).

Yellow wine is a traditional Chinese alcoholic beverage made from rice, sorghum or wheat (Hu et al.

2014). The yield of yellow rice wine amounted to 16 billion litres in 2015, generating nearly 1 million tons of yellow wine lees (YWL) as a co-product (Wang 2016). They contain a high content of crude protein (CP, 315–413 g/kg of DM) and the price is one third of that of SBM (Hu et al. 2014). However, YWL has an unbalanced amino acid (AA) profile and is hard to preserve because of its high moisture content (Cheng and Qian 2007). A previous study showed that through microbial pre-treatment, the CP and peptide contents, AA profile and the *in vitro* rumen digestibility of YWL was also improved (Yao et al. 2018), indicating that fermented YWL has potential as protein feed in ruminant rations.

Dairy rations with microbially fermented feed change the rumen fermentation, including increasing rumen microorganisms, enhancing nutrient flow and

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Table 1. Chemical composition of substrate mixtures after replacing soybean meal (SBM^a) with unfermented (UM) or fermented yellow wine lees mix (FM) at various ratios (Dry matter basis).

Items	UM	FM	SBM to UM ratio					SBM to FM ratio				
			1:0	1:1	1:2	1:5	0:1	1:0	1:1	1:2	1:5	0:1
Dry matter, %	89.1	88.3	88.5	88.6	88.7	88.8	88.9	88.5	88.5	88.4	88.4	88.4
Contents ^b , % of DM												
OM	94.9	94.3	92.2	92.3	92.4	92.5	92.5	92.2	92.3	92.3	92.5	92.5
CP	26.3	30.2	18.2	15.9	15.2	14.4	13.7	18.2	16.3	15.6	15.0	14.4
EE	2.4	2.8	2.4	2.5	2.5	2.5	2.6	2.4	2.5	2.5	2.5	2.6
NDF	36.4	41.1	42.0	44.0	44.7	45.4	46.1	42.0	44.5	45.4	46.4	47.1
ADF	14.6	16.8	21.2	22.2	22.5	22.9	23.2	21.6	22.4	22.7	22.9	23.2
NFC	29.8	20.2	29.6	29.9	30.0	30.1	30.1	29.6	29.0	28.8	28.6	28.4

^aSoybean meal accounted for 18% of the substrate mixtures.

^bOM: organic matter; CP: crude protein; EE: ether extracts; NDF: neutral detergent fibre; ADF: acid detergent fibre; NFC: nonfibrous carbohydrate.

improving the digestibility of feeds (Piamphon et al. 2017). In addition, microbially fermented feed can greatly improve the health and production performance of goats and beef cattle (Syngai et al. 2016). The inclusion of microbially fermented feed in a concentrate mixture at a level of 50% significantly improved the nutrient digestibility of feed and the average daily gain of goats and enhanced their disease resistance (Zhang 2017). A diet with yeast cultures could improve milk production and digestibility of CP and DM in lactating cows (Meller et al. 2019). *Bacillus subtilis* probiotics can increase the degradation rate of feed by *in vitro* fermentation, promote the growth of rumen microorganisms and increase the production of microbial protein (MCP) and volatile fatty acids (VFAs) in the rumen (Jia et al. 2018).

A number of studies have indicated the effects of microbially fermented feed on ruminal fermentation and digestion. However, information is limited on the effects of different levels of microbially fermented YWL on rumen fermentation and its associative effects with other ingredients. In this study, an *in vitro* rumen fermentation experiment was conducted to evaluate the effects of replacing SBM with unfermented or microbially fermented YWL on rumen fermentation parameters and digestibility, aiming to identify the optimum replacement level. The hypothesis of this study was that the microbially fermented YWL could partially replace SBM in dairy rations, with better performance than the unfermented YWL.

Materials and methods

Production of fermented yellow wine lees

The solid-state fermentation of YWL mix was conducted following the procedure established in the previous study (Yao et al. 2018). The fermentation substrate consisted of YWL, wheat bran, cassava residue and glucose at a ratio of 400, 500, 50 and

50 g/kg, respectively. Combination of two microorganisms, *C. utilis* KF01 (accession no.: 7691) and *B. subtilis* KF02 (accession no.: 7690), used to treat the YWL. They were provided by Zhejiang Cofine Biotech., Inc., Ltd. (Jiaying, China). The YWL, wheat bran and cassava residue were provided by Zhejiang Huatai Biotech Co., Ltd. (Yiwu, China). After being autoclaved for 20 m, 1500 g of mixed solid-state medium (YWL) was placed on a 45 cm × 25 cm tray. After the medium was cooled, both *C. utilis* KF01 and *B. subtilis* KF02 were inoculated in the solid medium. The quantity of the microorganisms was maintained at 10% (w/v) while the inoculum ratio of the two strains was 1:1 (v/v), and sterilised water was added to the solid medium maintaining a water-to-material ratio of 1:1 (v/w) and a pH value of 6.8–7.2. The medium was placed evenly on the tray with a single layer of sterilised gauze, covered and then placed in a constant temperature incubator (Yao et al. 2018). All media were dried after being incubated at 30 °C for 48 h.

Experimental design and substrate mixtures

The experiment was conducted in a 2 × 5 factorial arrangement, including two substrates (unfermented (UM) and microbially fermented YWL mix (FM)) and five ratios (1:0, 1:1, 2:1, 5:1, 0:1, w/w) of YWL mix replacing SBM.

The forage-to-concentrate ratio was 60:40 for the substrate mixtures (g/kg DM): corn meal 220, SBM with UM or FM 180, corn silage 500 and alfalfa 100. The chemical compositions of the different substrate mixtures are shown in Table 1.

In vitro experiments

The experimental procedures were approved by the Animal Care Committee at Zhejiang University (Hangzhou, China; no. 12401). The inoculum for the *in vitro* incubation was obtained from three donor

Table 2. *In vitro* gas production profiles after replacing soybean meal (SBM^a) with unfermented (UM) or fermented yellow wine lees mix (FM) at various ratios.

Items ^b	SBM to UM ratio					SBM to FM ratio					p Value ^c					
												R				
	1:0	1:1	1:2	1:5	0:1	1:0	1:1	1:2	1:5	0:1	SEM	S	L	Q	S × R	
Gas value, mL	173	181	185	181	180	173	187	185	183	182	6.120	.5200	.0900	.0100	.3200	
<i>a</i> , mL	-2.7000	-6.6000	3.1000	8.1000	19.0000	-2.7000	-1.7000	-0.5800	10.6000	14.3000	0.9600	.6000	<.0100	<.0100	.2300	
<i>b</i> , mL	186	195	185	201	180	186	190	199	177	190	6.0200	.7400	.5800	.0600	.0100	
<i>a</i> + <i>b</i> , mL	184	188	189	209	199	184	188	199	188	205	5.5300	.6500	<.0100	.7500	.1500	
<i>c</i> , %/h	0.0570	0.0640	0.0600	0.0530	0.0420	0.0570	0.0630	0.0600	0.00530	0.0440	0.0008	.0570	<.0100	<.0100	.5800	

^aSoybean meal accounted for 18% of the substrate mixtures.

^bGas production (GP) profiles were estimated by the equation $GP = a + b(1 - \exp^{-ct})$, where *a* or *b* is the GP (mL) from the rapid or slow degradation fraction, *a*+*b* is the potential GP (mL), *c* is the GP rate constant (% h⁻¹) and GP is the cumulative gas (mL) at time *t* (h).

^cS: substrate effect; R: replacement ratio effect; L: linear effect; Q: quadratic effect; S × R: interaction of substrate and replacement ratio.

Hu-sheep (2 years old, body weight of 35 ± 5 kg) fed grass hay and concentrate mixture (60:40 w/w, DM basis) and housed in animal facility of Zhejiang University. Rumen fluid was collected 1 h before the morning feeding and strained through four layers of gauze into prewarmed flasks and stored in an anaerobic environment. Five millilitres of rumen fluid was injected into a 120 mL bottle containing 45 mL of buffer and 500 mg of substrate mixtures at 39 °C. *In vitro* incubations were conducted in two consecutive runs, each involving four replicates. A semi-automated Reading Pressure Technique system (Mauricio et al. 1999) was used to determine the gas production (GP) parameters. The GP was recorded at 2, 4, 6, 9, 12, 24 and 48 h of inoculation. After 48 h of incubation, all the bottles were placed onto ice water to terminate fermentation. Blank correction was conducted for the cumulative GP measurements. Incubations were repeated when gas volumes of the same feedstuffs in the two consecutive runs deviated by more than 10%. The pH was measured by a pH metre (FE20, Mettler Toledo, Zürich, Switzerland). Triplicates of mixed liquor were stored at -20 °C for later analysis of VFA, ammonia nitrogen (N) and MCP. The samples for VFA analysis were acidified with 0.85 M ortho-phosphoric acid containing isocaproic acid as the internal standard before gas chromatography (GC-8A; Shimadzu Corp., Kyoto, Japan) measurement. The MCP yield was estimated according to Makkar et al. (1982). The ammonia-N concentration was assessed by a spectrometer (SpectraMax M5, Molecular Devices, Sunnyvale, CA) using colorimetry (Hu et al. 2005).

The *in vitro* DM digestibility (IVDMD), *in vitro* organic matter digestibility (IVOMD), *in vitro* CP digestibility (IVCPD) and efficiency of N utilisation (ENU) were determined by the differences in DM, OM, CP and N between the medium before fermentation and the residue after 48 h of fermentation (Valentin et al. 1999).

Chemical analyses and calculations

All the samples were ground through a 1-mm screen in a Cyclotec mill (Tecator 1093, Tecator AB, Hoganas, Sweden) before they were analysed for CP (method 988.05; AOAC 1990), ash (method 942.05), ether extract (EE, method 920.39) and acid detergent fibre (ADF, method 973.18). Sodium sulphite and heat-stable amylase were used in analysis of NDF (Van Soest et al. 1991). An ANKOM²⁰⁰⁰ fibre analyser (Ankom Technology Corp., Macedon, NY) was used to extract and filter NDF and ADF. Both NDF and ADF were expressed exclusive of residual ash.

Using Fit Curve software (MLP; Lawes Agricultural Trust 1991), cumulative GP values (corrected for blanks and reference standards) at 2, 4, 6, 9, 12, 24, 36 and 48 h of *in vitro* fermentation were fitted to the equation $GP = a + b(1 - \exp^{-ct})$, where *a* is the rapid degradation fraction of GP (mL), *b* is the slow degradation fraction of GP (mL), *a*+*b* is the potential GP (mL), *c* is the GP rate constant (% h⁻¹) and GP is the cumulative gas (mL) at time *t* (h).

The ENU calculation method was modified according to Bach and Stern (1999) as follows: $ENU = (\text{dietary N} + \text{artificial saliva N} - \text{undegradable N}) / (\text{dietary N} + \text{artificial saliva N})$. The associative effect between YWL or fermented YWL and SBM was calculated according to the equation by Liu et al. (2002):

Associative effect (%) = [observed GP value (mL) - estimated GP value (mL)] / estimated value (mL) × 100, where, the estimated value = (gas value of unfermented or fermented YWL × its proportion) + (gas value of SBM × its proportion).

Statistical analysis

All the *in vitro* fermentation and associative effects data were analysed using the GLM procedure in SAS software version 2000 (SAS Inst. Inc., Cary, NC). For

Table 3. *In vitro* rumen fermentation parameters after replacing soybean meal (SBM^a) with unfermented (UM) or fermented yellow wine lees mix (FM) at various ratios.

Items ^b	SBM to UM ratio					SBM to FM ratio					SEM	p Value ^c			
	1:0	1:1	1:2	1:5	0:1	1:0	1:1	1:2	1:5	0:1		R			
												S	L	Q	S × R
pH	6.64	6.62	6.68	6.64	6.60	6.64	6.64	6.62	6.75	6.70	0.03	.32	.25	.02	.02
NH ₃ -N, mg/dL	15.50	14.50	14.20	13.50	12.90	15.50	14.60	14.50	13.70	13.30	0.28	.28	<.01	.41	.65
MCP, mg/dL	3.52	3.24	3.04	2.88	2.82	3.52	3.49	3.32	3.18	3.16	0.08	<.01	<.01	<.01	.62
Total VFA, mM	83.70	82.10	75.10	74.20	72.20	83.70	82.40	75.70	75.00	73.10	0.470	.40	<.01	<.01	.58
Molar proportion, mM/100 mM															
Acetate (Ac)	59.50	59.90	58.70	59.00	60.70	59.50	59.40	59.40	59.80	59.80	0.43	.70	.08	.11	.27
Propionate (Pr)	26.20	26.50	26.90	27.00	26.20	26.20	26.80	26.50	26.10	26.90	0.19	.32	.70	<.01	.01
Butyrate	9.70	9.60	10.00	9.70	9.10	9.70	9.50	9.80	9.80	9.30	0.43	.75	.01	.01	.63
Isoacids	4.70	4.20	4.50	4.30	4.10	4.70	4.40	4.30	4.40	4.00	0.23	.34	.06	.77	.56
Ac:Pr	2.27	2.26	2.22	2.19	2.29	2.27	2.22	2.24	2.27	2.22	0.03	.70	.09	<.01	<.01

^aSoybean meal accounted for 18% of the substrate mixtures.

^bNH₃-N: ammonia nitrogen; MCP: microbial protein; VFAs: volatile fatty acids

^cS: substrate effect; R: replacement ratio effect; L: linear effect; Q: quadratic effect; S × R: interaction of substrate and replacement ratio.

statistical analyses, the model included substrates, ratio and their interaction as fixed effects. The linear and quadratic effects of the ratio were analysed using orthogonal polynomial contrasts. The cubic effects of the ratio were not examined for inexplicability in biology. Probability values of $p < .05$ were used to define statistical significance, and values of $p \leq .10$ and $p \geq .05$ were regarded as statistical trends.

Results and discussion

Gas production parameters

The cumulative GP was similar between the UM and FM, but differed among the ratios of UM or FM replacing SBM (Table 2). The GP data of both substrates showed a similar quadratic pattern, i.e. initial increase and then decline with decreasing SBM ratio ($p < .01$). No differences were observed between the two substrates in the rapid degradation fraction of the GP ($p > .05$), while linear and quadratic effects of the replacement ratio were observed on the rapid degradation fraction ($p < .01$). No significant difference among substrates existed in the slow degradation fraction of GP. No linear effect of the replacement ratio was observed on the slow degradation fraction, but it tended to be affected ($p = .06$) by replacement ratio in a quadratic way. An interaction effect of the replacement ratio and substrates on slow degradation was observed ($p = .01$). Statistical significance ($p = .05$) was found in the rate of GP between the UM and FM substrates, but the difference was small. Linear and quadratic effects of the replacement ratio existed on the rate of GP ($p < .01$).

The *in vitro* GP reflected the utilisation of substrates by rumen microorganisms, indicating the overall ruminal microbial activity (Rodionow et al. 2006). The

nonfibrous carbohydrate (NFC) concentration plays an important role in generating GP in the rumen. Meanwhile, dairy rations with microbial pre-treatments were confirmed to improve the GP, rate of GP and potential GP (Elghandour et al. 2014). In consistence with the present study, quadratic effects on the GP were observed with the increasing levels of enzymes or fermented feeds inclusion in rations (Togtokhbayar et al. 2015; Elghandour et al. 2017). For the interaction of slowly degradable fraction (*b*), similar results were reported in the study of Elghandour et al. (2014) with supplementation of probiotics to different cereal straws at different levels. Compared with untreated material, YWL fermented with *C. utilis* was expected to increase GP, but the lower NFC and higher CP content in fermented YWL could depress GP and exert a negative impact on the rapid degradation fraction.

In vitro rumen fermentation parameters

The *in vitro* rumen fermentation parameters are listed in Table 3. No difference ($p > .05$) in pH value was observed between the two substrates, but a quadratic effect of the replacement ratio on pH was observed in both substrates ($p = .02$), with significant interaction effects of substrates and replacement ratio on pH ($p = .02$). However, the pH of the culture medium for each treatment was within the normal range of 5.5–7.5 (Zhang et al. 2017).

No differences were observed ($p > .05$) between the two substrates in rumen ammonia-N (Table 3). With increasing levels of UM and FM, the ammonia-N concentration decreased linearly ($p < .01$). Rumen ammonia-N is the final product of the microbial decomposition of N-containing substances (Uyeno et al. 2017). The CP level and digestibility of FM were proved to be higher than those of UM (Yao et al.

Table 4. *In vitro* rumen digestibility after replacing soybean meal (SBM^a) with unfermented (UM) and fermented yellow wine lees mix (FM) at various ratios.

Items ^b	SBM to UM ratio					SBM to FM ratio					SEM	S	<i>p</i> Value ^c		
	1:0	1:1	1:2	1:5	0:1	1:0	1:1	1:2	1:5	0:1			R		
													L	Q	S × R
IVDMD, %	63.80	63.50	62.80	63.10	62.40	63.80	63.40	62.30	62.30	61.70	0.320	0.28	<0.01	0.33	0.38
IVOMD, %	62.60	62.40	61.80	62.00	61.40	62.80	62.30	61.30	61.80	61.00	0.280	0.24	<0.01	0.57	0.47
IVCPD, %	51.60	51.80	52.10	51.20	51.60	51.60	53.80	54.50	53.30	54.20	0.450	<0.01	<0.01	<0.01	0.56
ENU, %	67.30	68.10	66.20	64.30	64.70	67.30	68.80	67.40	65.70	64.90	0.710	0.01	0.09	0.02	0.45

^aSoybean meal accounted for 18% of the substrate mixtures.

^bDMD: *in vitro* dry matter digestibility; IVOMD: *in vitro* organic matter digestibility; IVCPD: *in vitro* CP digestibility; ENU: efficiency of nitrogen utilisation; ENU: (dietary N + artificial saliva N - undegradable N)/(dietary N + artificial saliva N)

^cS: substrate effect; R: replacement ratio effect; L: linear effect; Q: quadratic effect; S × R: interaction of substrate and replacement ratio.

2018), contributing to the higher ruminal ammonia-N level in FM than in UM at each replacement. The MCP concentration was higher in the FM than in the UM ($p < .01$). A linear decline in MCP values was observed with increasing levels of UM or FM ($p < .01$), and quadratic effects of the replacement ratio were observed in both substrates ($p < .01$). Ruminal MCP provides 40–80% of the protein needs for ruminants (Uyeno et al. 2017). The higher MCP with not-different ammonia concentration in the FM compared to the UM indicates that the fermented YWL promoted the ability of rumen microorganisms to synthesise MCP from ammonia-N and enhanced the efficiency of protein synthesis. In the study with cows in mid-to-late lactation by Uyeno et al. (2017), active yeast and its fermented feed improved MCP synthesis and promoted the ability of rumen microbes to use ammonia-N to synthesise MCP.

No differences in VFA values were observed between two substrates ($p > .05$, Table 3). The VFA concentration declined with increasing levels of UM or FM ($p < .01$), and linear and quadratic effects of the replacement ratio were observed in both substrates ($p < .01$). Acetate (Ac) concentration tended to be higher ($p = .08$) with increasing levels of UM and FM. Quadratic effects of the replacement ratio on propionate (Pr) values were observed ($p < .01$). No difference in Ac and Pr existed between two substrates ($p > .05$), but the replacement ratio tended to have linear effect ($p = .09$), and had quadratic effect on the Ac to Pr ratio ($p < .01$). Rumen VFAs are direct indexes to evaluate rumen fermentation and capacity. Addition of a suitable concentration of probiotics can increase the content of VFAs in the rumen, but some studies have showed no effects of adding probiotics on the concentration of VFAs in native cattle (Khampa et al. 2009) and in lactating cows (Uyeno et al. 2017). The rate of GP decreased significantly with the increasing levels of UM or FM, with 35.7 and 29.5% lower rate of GP for UM and FM included only compared to that of

SBM alone (Table 2), which may contribute to the decreased VFA production in the combinations with higher ratio of UM or FM. In the meantime, significant interaction effects existed in the replacement ratio and substrates on Pr ($p = .01$) as well as Ac to Pr ratio ($p < .01$). However, the difference was not large among different combinations (Table 3).

In vitro rumen digestibility

The *in vitro* rumen digestibility of the UM and FM is shown in Table 4. No differences were observed in IVDMD or IVOMD between the two substrates ($p > .05$), which is consistent with the results of *in vitro* GP and ruminal VFA (Table 3). With the increase in the replacement ratio of UM and FM, the values of IVDMD and IVOMD slightly decreased ($p < .01$), probably due to the higher contents of NDF in the mixtures with higher replacing ratio of UM or FM. The IVCPD of FM was higher than that of UM ($p < .01$), with linear and quadratic effects of the replacement ratio on IVCPD observed ($p < .01$), which can probably be attributed to the large number of enzymes and organic acids produced during fermentation by yeast and bacillus that can hydrolyse macromolecules into small molecules, thereby improving the efficiency of nutrient digestion and absorption by rumen microorganisms (Yang and Xie 2010).

The ENU of FM was higher than that of UM ($p = .01$). Replacement ratio tended to have linear ($p = .09$) and had quadratic effects ($p = .02$) on ENU, which is consistent with the ammonia-N and MCP results. Jia et al. (2018) found that multiple bioactive compounds produced by microbes reduce the ammonia-N level and increase the MCP concentration and overall N utilisation of ruminants. The higher ENU of the FM suggests that N was more efficiently utilised than the UM.

Table 5. Associative effects of unfermented (UM) or fermented yellow wine lees mix (FM) and soybean meal (SBM^a) on *in vitro* rumen fermentation variables.

AE, %	SBM to UM ratio			SBM to FM ratio		
	1:1	1:2	1:5	1:1	1:2	1:5
Gas production (GP)						
Gas value	2.6	4.1 [#]	1.2	5.4*	3.4	1.4
Potential GP	-1.8	-2.6	6.4*	-3.3	0.5	-6.7*
Rate of GP	29.2**	28.1**	18.6**	24.2**	24.0**	14.6**
Fermentation variables^b						
MCP	2.2 [#]	-0.4	-1.9	4.5**	1.2	-8.7*
Total VFA	7.9**	2.1	4.5 [#]	5.1**	-1.2	0.2
ENU	3.2 [#]	1.0	-1.3	4.1**	2.6	0.6
<i>In vitro</i> digestibility^c						
IVDMD	0.6	-0.1	0.8	1.0	-0.2	0.4
IVOMD	0.7	-0.0	0.8	0.7	-0.5	0.8
IVCPD	0.4	1.0	-0.8	1.7 [#]	2.2	-0.9

^aSoybean meal accounted for 18% of the substrate mixtures.

^bMCP: microbial protein; VFAs: volatile fatty acids; ENU: efficiency of nitrogen utilisation, ENU=(dietary N + artificial saliva N-undegradable N)/(dietary N + artificial saliva N).

^cIVDMD: *in vitro* dry matter digestibility; IVOMD: *in vitro* organic matter digestibility; IVCPD: *in vitro* CP digestibility.

[#].05 ≤ *p* < .10.

*.01 ≤ *p* < .05.

***p* < .01.

Associative effects on *in vitro* rumen fermentation characteristics

The associative effects of YWL mix and SBM on *in vitro* rumen fermentation variables are shown in Table 5. A positive associative effect on GP was found at 1:1 of SBM to FM (*p* < .05) and a tendency for a positive associative effect was at 1:2 (SBM to UM, *p* < .10). A positive associative effect on the rate of GP was observed for all UM and FM replacement ratios (*p* < .01). Positive associative effects were found in MCP, VFA and ENU at a replacement ratio of 1:1 (*p* < .01). However, no associative effect was observed on the *in vitro* digestibility at all replacement ratios for both substrates (*p* > .10), except for a slightly positive associative effect on IVCPD at the ratio of 1:1 (SBM to FM) (*p* < .10).

The positive associative effects of the YWL mix and SBM on the rate of GP, VFA and MCP at some replacement ratios, indicated their positive effects on improving rumen fermentation and protein synthesis. Positive associative effects exist among corn, distiller's grains and SBM (Lin 2009). In this study, corn and distiller's grain were considered to provide NFC, while the SBM provided N. The proper ratio of NFC to N may promote rumen fermentation and improve N utilisation and feed efficiency. Additionally, the increased AA and CP in microbially fermented YWL may have a positive effect on rumen fermentation, as Ismail et al. (2018) reported that with microbially fermented feather replacing SBM, the *in vitro* rumen fermentation characteristics of dairy rations were improved because of

higher contents of CP and AA. An *in vivo* animal study may be needed to confirm these associative effects.

Conclusions

Compared with unfermented YWL mix consisting of 400 g/kg YWL, replacing SBM with microbially fermented YWL mix increased the rate of GP, improved the MCP and VFA synthesis and enhanced the digestibility of the rations. The replacement ratio had linear and quadratic effects on rumen fermentation characteristics and *in vitro* digestibility. Replacement of SBM with microbially fermented YWL mix at 1:1 had optimal *in vitro* characteristics and a positive associative effect among all the treatments. The results of the present study indicated that microbially fermented YWL could partially replace SBM in dairy rations and performed better than unfermented YWL. *In vivo* studies are needed to evaluate the optimised utilisation of the fermented YWL mix.

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Disclosure statement

No potential conflicts of interest were reported by the authors.

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