Soybean meal substitution with a yeast-derived microbial protein source in dairy cow diets

J. A. Sabbia,* K. F. Kalscheur,*¹ A. D. Garcia,* A. M. Gehman,†² and J. M. Tricaricot³

*Dairy Science Department, South Dakota State University, Brookings 57007

†Alltech Inc., Brookings, SD 57006

ABSTRACT

The objective of this study was to examine the effects substituting soybean meal with a yeast-derived microbial protein (YMP) on rumen and blood metabolites, dry matter intake, and milk production of high-producing dairy cows. Sixteen Holstein cows (12 multiparous and 4 primiparous), 93 ± 37 DIM (mean $\pm$ SD) at the beginning of the experiment, were used in a 4×4 Latin square design with four 28-d periods. Cows were blocked by parity and production, with 1 square consisting of 4 animals fitted with rumen cannulas. Basal diets, formulated for 16.1% crude protein and 1.56 Mcal/kg of net energy for lactation, contained 40% corn silage, 20% alfalfa hay, and 40% concentrate mix. During each period, cows were fed 1 of 4 treatment diets corresponding to YMP (DEMP; Alltech Inc., Nicholasville, KY) concentrations of 0, 1.14, 2.28, and 3.41% DM. Soybean meal (44% CP) was replaced by YMP to attain isonitrogenous and isoenergetic diets. Dietary treatments had no effect on pH and on most ruminal volatile fatty acid concentrations, with the exception of isovalerate, which decreased linearly with the addition of YMP. Rumen ammonia concentration decreased linearly, whereas free amino acids, total amino acid nitrogen, and soluble proteins weighing more than 10 kDa showed a cubic response on rumen N fractionation. A quadratic response was observed in oligopeptides that weighed between 3 and 10 kDa and peptides under 3 kDa when expressed as percentages of total amino acids and total nitrogen. Although nonesterified fatty acid concentration in blood did not differ between treatments, β -hydroxybutyrate and plasma glucose increased linearly as YMP increased. Dry matter intake showed a cubic effect, where cows fed 1.14, and 3.41% YMP had the highest intake. Milk

production was not affected by YMP, whereas a trend was observed for a quadratic increase for 4% fat-corrected milk and energy-corrected milk. Medium- and long-chain fatty acid concentrations in milk increased quadratically, which elicited similar effects on milk fat concentration and yield. Total solids percentage and yield, and milk urea nitrogen also showed quadratic effects as YMP increased in the diet. No effects were observed on feed efficiency, milk protein, and lactose percentage or yield. A complementary *in vitro* study demonstrated a quadratic tendency for apparent and true dry matter digestibility as YMP was added to the diet. It was concluded that the substitution of soybean meal with YMP increased the percentage of total solids in milk and tended to improve energy-corrected and fat-corrected milk production in high-producing dairy cows consuming high-forage diets.

Key words: dairy cow, milk production, nitrogen fractionation, yeast-derived microbial protein

INTRODUCTION

Dairy cow protein requirements should be met by formulating diets with the lowest possible CP percentage that optimizes RDP:RUP balance and meets MP levels that support high milk production and increase N efficiency (Agle et al., 2010). Although microbial CP (MCP) has one of the best AA profiles compared with plant proteins (Clark et al., 1992), the AA requirements of high-producing dairy cows cannot be met by this source only (Brito and Broderick, 2007). According to Satter and Slyter (1974), when the rumen reaches the point of ammonia overflow, the addition of RDP will not yield additional MCP. Meanwhile, with adequate RDP, the inclusion of high-quality RUP will affect the amount of AA that reaches the small intestine to be absorbed and support milk yield and components (Santos et al., 1998; Kalscheur et al., 2006). Thus, it is very important to know the RDP and RUP fractions of the feedstuffs, their AA profile, and AA digestibility to make accurate MP predictions.

Ruminal protein degradation is described oftentimes by first-order mass action models (NRC, 2001). An im-

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¹Corresponding author: kenneth.kalscheur@sdstate.edu

²Present address: 1860 Charter Lane Suite 203, Lancaster, PA 17601.

³Present address: Innovation Center for US Dairy, 10255 W. Higgins Road Suite 900, Rosemont, IL 60018-5616.

portant feature of these models is that they consider the CP of feedstuffs as consisting of multiple fractions that differ widely in degradation rates, and their ruminal disappearance results from 2 simultaneous activities: degradation and passage (NRC, 2001). The most used model to describe in situ ruminal protein degradation divides feedstuffs into fractions A, B, and C (Ørskov and McDonald, 1979). The NRC (2001) states that the data obtained from in situ, in vitro, and enzymatic digestions generally fits the model that divides feed CP into these fractions. However, Broderick et al. (1991) suggested that the in situ technique has theoretical limitations that call its accuracy into question. The major concerns are that the rates of ruminal degradation of the soluble N fraction (fraction A) from all feeds incubated in situ are assumed to be infinite, and that the proportion of dietary protein that is degraded in the rumen (fraction B) is assumed to be entirely used for microbial protein synthesis, production of ammonia and carbon skeletons, or both (Reynal et al., 2007). However, if a portion of these fractions (A and B) escapes from ruminal degradation as soluble protein, peptides, and free AA, the validity of these assumptions may not hold true (Reynal et al., 2007). Furthermore, Hedqvist and Udén (2006) stated that feeds high in soluble protein should be studied in vitro, not in situ, because the latter method cannot measure the degradation rates of soluble proteins.

In dairy cows fed alfalfa hay or silage diets, feed-soluble NAN (**FSNAN**) represented 30 to 35% of the total N pool in the rumen and had a very high out-flow rate (Hristov et al., 2001). Similarly, it has been reported that the contribution of FSNAN represented a significant component of the flow of NAN entering the omasal canal (Choi et al., 2002a; Reynal et al., 2007). In addition, omasal flow measurements, in vitro studies, and studies using ^{15}N -labeled feeds have also shown considerable flow of FSNAN, with peptides being quantitatively the most important component (Huhtanen and Hristov, 2009). These studies suggest that a portion of the FSNAN is not completely degraded and utilized in the rumen, as assumed by models estimating RDP and RUP from degradation kinetics. Furthermore, when dealing with feedstuffs containing high proportions of FSNAN, the passage rate of this fraction should be considered equal to that of the liquid phase. The rate of passage of the liquid phase of high-producing dairy cows can vary between 12%/h (Agle et al., 2010) and 17%/h (Choi et al., 2002b).

It was hypothesized that this yeast-derived microbial protein (**YMP**) moves with the liquid phase of the rumen (as FSNAN) at a high rate of passage. As a result, a large proportion of YMP should escape ruminal fermentation and reach the small intestine,

providing a high-quality and absorbable source of AA to support milk production. The aim of this study was to determine the animal response when this microbial protein source substituted soybean meal (**SBM**) in high-producing dairy cow diets.

MATERIALS AND METHODS

Animals and Diets

This experiment was conducted at the Dairy Research and Training Facility at South Dakota State University (Brookings), and all procedures were approved by the South Dakota Institutional Animal Care and Use Committee. Sixteen Holstein dairy cows (12 multiparous and 4 primiparous) at 93 ± 37 DIM (mean $\pm$ SD) were used in a 4×4 Latin square design with four 28-d periods. Cows were blocked by parity and milk production with 1 square consisting of 4 ruminally cannulated cows. During each period, cows were fed 1 of 4 treatments where YMP (**DEMP**; Alltech Inc., Nicholasville, KY) replaced SBM in isonitrogenous and isoenergetic diets as follows: control (0 g of YMP/cow per day) and 300, 600, and 900 g of YMP/cow per day, for a cow consuming 26.5 kg according to NRC (2001). This corresponds to 0, 1.14, 2.28, and 3.41% of YMP DM for each respective diet (Table 1). Basal diets contained 40% corn silage, 20% alfalfa hay, and 40% concentrate mix, and were formulated for 1.56 Mcal of NE_L /kg and 16.1% CP (Table 1). In addition, the diets were isonitrogenous and the RDP and RUP fractions were very similar; NRC (2001) predicted a decrease in the MP supply when YMP was increased (Table 1).

Forages were premixed in a vertical mixer and blended with concentrates in a Calan Data Ranger (American Calan Inc., Northwood, NH). Cows were individually fed for ad libitum intake once daily (0900 h) using Calan Broadbent individual animal feeders (American Calan Inc.). Orts were weighed once daily and the diet offered was adjusted to ensure 10% feed refusal. Weeks 1 and 2 of each period were used for diet adjustment, and wk 3 and 4 for data collection. Cows had unlimited access to water and feed during the day except when they were milked. All cows received recombinant bovine somatotropin (Posilac; Monsanto, St. Louis, MO) every 14 d according to normal farm protocol.

Measurements and Sampling

Feed intakes and orts for individual cows were recorded daily using a Calan Data Ranger (American Calan Inc.). The DM content of corn silage and alfalfa hay was determined weekly, and diets were adjusted to

Table 1. Ingredient and nutrient composition of the experimental diets formulated for increasing concentration of yeast-derived microbial protein (YMP)

Item	Treatment ¹			
	Ctrl	300	600	900
Ingredient composition, % of DM				
Corn silage	41.6	41.6	41.6	41.6
Alfalfa hay	18.9	18.9	18.9	18.9
Corn ground dry	17.0	17.0	17.0	17.0
Soybean meal (46.5% CP)	9.09	9.09	9.09	9.09
Dried distillers grains with solubles	4.91	4.91	4.91	4.91
Soybean meal (44% CP)	3.41	2.28	1.14	0.0
Yeast-derived protein ²	0.0	1.14	2.28	3.41
Soybean hulls	1.89	1.89	1.89	1.89
Tallow	0.94	0.94	0.94	0.94
Calcium carbonate	0.57	0.57	0.57	0.57
Limestone	0.38	0.38	0.38	0.38
Sodium bicarbonate	0.36	0.36	0.36	0.36
Salt	0.34	0.34	0.34	0.34
Dicalcium phosphate	0.23	0.23	0.23	0.23
Magnesium oxide	0.15	0.15	0.15	0.15
Vitamin premix ³	0.09	0.09	0.09	0.09
Vitamin E ⁴	0.02	0.02	0.02	0.02
Nutrient composition ⁵				
DM, % of diet	48.1	48.3	47.7	48.2
CP, % of DM	16.1	16.0	16.0	15.9
RDP, % of DM	10.0	10.0	10.0	10.1
RUP, % of DM	6.10	6.00	5.90	5.80
Microbial CP, % of DM	2.21	2.21	2.21	2.21
RUP, % of DM	1.61	1.59	1.57	1.55
MP, % of DM	2.88	2.86	2.84	2.81
MP balance, % of DM	17	-4	-25	-46
NE _L , Mcal/kg	1.56	1.56	1.56	1.56
NFC, % of DM	40.0	40.2	40.3	40.5
NDF, % of DM	34.7	34.6	34.5	34.5
ADF, % of DM	22.3	22.2	22.1	22.1
Starch, % of DM	23.7	23.7	23.6	23.6
Fat, % of DM	3.70	3.70	3.70	3.60
Ca, % of DM	0.90	0.90	0.90	0.90
P, % of DM	0.40	0.40	0.40	0.40

¹Ctrl = control (0 g of YMP/cow per day); 300 = 1.14% YMP (300 g of YMP/cow per day); 600 = 2.28% YMP (600 g of YMP/cow per day); 900 = 3.41% YMP (900 g of YMP/cow per day).

²DEMP (Alltech Inc., Nicholasville, KY).

³Contained 4.3% Zn, 3.8% Mn, 7,800 mg of Cu/kg, 900 mg of I/kg, 315 mg of Co/kg, 220 mg of Se/kg, 5,632,000 IU of vitamin A/kg, 1,408,000 IU of vitamin D₃/kg, 18,700 IU/kg of vitamin E (Vita Plus Corp., Madison, WI).

⁴Contained 20,000 IU/kg.

⁵Calculations based on individual ingredients analysis.

⁶Estimated from NRC (2001) according to nutrient composition of the analyzed ingredients.

maintain the same forage:concentrate ratio during the experiment.

Samples of alfalfa hay, corn silage, concentrate mix, YMP, SBM, and TMR of each treatment were collected on 3 consecutive days during wk 4 of each period, frozen, and stored at -20°C until analysis. Additional TMR samples were taken during wk 4 to determine particle size using the Penn State Particle Separator (**PSPS**; The Pennsylvania State University, University Park).

Ruminal fluid was sampled from the fistulated cows on d 27 of each period over 9 time points, including

before feeding, and 2, 4, 6, 8, 10, 12, 16, and 24 h after feeding. Ruminal fluid samples were collected using a 50-mL syringe and a stainless steel suction device to collect 10 mL from 5 separate locations throughout the rumen for a total of 50 mL. Samples were immediately measured for pH, and 10-mL aliquots of rumen fluid were placed in scintillation vials, one containing 50% (vol/vol) sulfuric acid and another containing 25% (wt/vol) metaphosphoric acid. Samples were frozen and stored at -20°C for ammonia and VFA analysis.

Blood was collected by venipuncture of the tail vein approximately 3 h after feeding on 2 consecutive days

during wk 4 of each period. Blood was drawn into 10-mL Vacutainer tubes containing K₃-EDTA anticoagulant (Becton Dickinson and Co., Rutherford, NJ).

Cows were milked 3 times daily (0600, 1400, and 2100 h). Milk from individual cows was sampled at each milking on 2 consecutive days on wk 3 and 4 for milk composition analysis. An additional sample was taken on d 1 of wk 3 for FA analysis.

Body weights were recorded on 3 consecutive days at the beginning of the experiment and at the end of each period. Body condition score was assessed on a 1 to 5 scale (Wildman et al., 1982) by 3 independent observers at the beginning of the experiment and at the end of each period.

Laboratory Analysis

All feed and TMR samples were composited by period, dried for 48 h at 55°C in a Despatch oven (style V-23; Despatch Oven Co., Minneapolis, MN), ground through a 4-mm screen using a Wiley mill (model 3; Arthur H. Thomas Co., Philadelphia, PA), and then further ground through a 1-mm screen (Brinkman ultracentrifuge mill; Brinkman Industries Co., Westbury, NY). Approximately one-third of the samples ground at 4 mm were saved for *in vitro* digestibility analysis. Subsamples of feed composites were dried at 105°C for 3 h for DM determination (Shreve et al., 2006).

Composites of corn silage, alfalfa hay, YMP, concentrate mix, customized SBM, and TMR, dried at 55°C, were sent to Dairyland Laboratories Inc. (Arcadia, WI) for composition analysis by wet chemistry methods for the following nutrients: DM (105°C for 24 h), CP (method 955.04; AOAC International, 2006), crude fat (method 920.39; AOAC International, 2006), ADF, NDF, and lignin (Goering and Van Soest, 1970). Ash, Ca, P, Mg, and K were analyzed according to AOAC International procedures (AOAC International, 2006). Starch was determined with an YSI 2700 Select Biochemistry Analyzer (application note 322; YSI Inc., Yellow Springs, OH; method 996.11; AOAC International, 2006). Composites of YMP were analyzed for AA composition according to AOAC International [method 982.30 E (a,b,c); AOAC International, 2006]. Particle size distribution of the diets was determined by the 4-screen PSPS (Kononoff et al., 2003) using fresh feed samples.

Composites of YMP were used for N fractionation in a modified procedure from Reynal et al. (2007). Five grams of YMP was dissolved in distilled water to produce a 5% solution (wt/vol). Proteins have traditionally been classified into fractions according to their solubility in various solvents: albumins in water, globulins in salt solution, prolamines in aqueous alcohol, and

glutelins in diluted alkali (Blethen et al., 1990). Thus, using water, only a portion of the total N was obtained, but it was considered the first approach to estimate the protein composition of YMP. An aliquot was filtered through a 0.1- μ m filter (Millex-VV, no. SLVV033RB, PVDF filter; Millipore Corp., Billerica, MA), and 4 samples of 1 mL were stored at -20°C for later total N analysis. Four 10-mL aliquots of filtrate were pipetted into a 10-kDa molecular weight (MW) cut-off ultracentrifugation filter with a cellulose membrane (Amicon-Ultra, no.UFC901024, Millipore, Billerica, MA), and centrifuged at 5,000 $\times g$ at 5°C for 30 min. Two 1-mL aliquots of each filtrate were stored at -20°C for later N determination of oligopeptides of 3 to 10 kDa. A 5-mL aliquot of each filtrate was transferred to a 3-kDa MW cut-off ultracentrifugation filter with a cellulose membrane (Centriprep-3, no. 4302; Millipore Corp.), and centrifuged once at 3,000 $\times g$ at 5°C for 65 min, and then 2 more times for 20 min at the same speed and temperature. Two 1-mL aliquots of each filtrate were stored at -20°C for later N analysis of free AA and peptides of less than 3 kDa. Nitrogen analysis was conducted at Alltech Laboratories (Alltech Inc.) using a TruSpec Carbon Nitrogen Determinator (LECO Corp., St. Joseph, MI). Soluble protein concentration >10 kDa was calculated by the difference between total CP concentration minus oligopeptides plus peptides and free AA concentration [soluble proteins = total CP - (oligopeptides + peptides + free AA)].

Milk samples were analyzed by Heart of America DHIA Laboratory (Manhattan, KS) for composition analysis according to approved procedures of AOAC International (2006). Fat, crude protein, lactose, and SNF were analyzed with mid-infrared spectroscopy (Bentley 2000 infrared milk analyzer; Bentley Instruments Inc., Chaska, MN); somatic cells were counted by laser technology (Soma Count 500; Bentley Instruments Inc.), and MUN concentration was determined using chemical methodology based on a modified Berthelot reaction (ChemSpec 150 analyzer; Bentley Instruments Inc.).

Milk composites were used for FA analysis, and prepared as butyl esters in an adapted method of Sukhija and Palmquist. (1988) as described in Abdelqader et al. (2009). The butyl esters were analyzed by gas chromatography equipped with a flame ionization detector and an autoinjector (HP Agilent 6890 GC; Hewlett-Packard, Palo Alto, CA).

Plasma was collected after centrifuging blood samples at 2,000 $\times g$ for 20 min at 5°C (CR412 centrifuge; Jouan Inc., Winchester, VA) and frozen until analysis. Plasma glucose concentration was determined by glucose oxidase reaction (Trinder, 1969) with a glucose kit (glucose kit, code 439-90901; Wako Chemicals USA

Inc., Richmond, VA). The concentration of BHBA in plasma was determined with a BHBA kit (BHBA kit, catalog no. 2440-058; Stanbio Laboratory, Boerne, TX) according to Williamson et al. (1962). Plasma was analyzed for NEFA using a NEFA kit (NEFA kit, code 434-91795; Wako Chemicals USA Inc.) following specifications of Johnson and Peters (1993). Blood glucose, NEFA, and BHBA kit preparations were read in a microplate reader (Cary 50 MPR; Varian Inc., Lake Forest, CA).

Rumen samples conserved with metaphosphoric acid were centrifuged at $12,500 \times g$ for 15 min at 4°C (accuSpin Micro 17R; Fisher Scientific Inc., Denver, CO); subsamples of the centrifuged rumen fluid were sent to Alltech Laboratories (Alltech Inc.) for VFA analysis. Gas chromatography (HP Agilent 6890 GC, Hewlett-Packard) was used to analyze VFA as described by Erwin et al. (1961) using Chromosorb WAW in a 1.83 m $\times$ 4-mm glass column (Supelco Inc., Bellefonte, PA). Ammonia nitrogen concentration and nitrogen fractions were determined in rumen samples conserved with sulfuric acid. Rumen subsamples were centrifuged and analyzed for ammonia-N concentration as described by Weatherburn (1967). Rumen fluid obtained from the 9 time points were composited per cow for rumen N fraction analysis following a modified procedure of Reynal et al. (2007). The determination of free- α AA analysis on hydrolyzed fractions was determined following a specification described in AOAC International (2006). Nitrogen fractions were classified according to Reynal et al. (2007), as free AA, small soluble peptides <3 kDa, soluble oligopeptides between 3 and 10 kDa, soluble proteins >10 kDa, total AA-N, NH_3 -N, and total N.

An *in vitro* study for measuring fermentation gas production kinetics, NDF digestibility (NDFD), DM apparent digestibility (DMAD), DM true digestibility (DMTD), and rumen VFA production was conducted following a procedure similar to that described by Adesogan et al. (2005). Samples of TMR were ground to 4 mm, composited across periods, and their DM calculated after drying for 24 h at 105°C. Rumen fluid was collected from a fistulated lactating dairy cow fed a high-producing diet, and pH was measured immediately before incubation started. Five replicates of approximately 0.5 g each of TMR composite samples were placed in acetone-washed and sealed bags (Ankom F57; Ankom Technology, Macedon, NY). Bags were incubated (Incubator 6LM; Thermo Fisher Scientific, Marietta, OH) at 39°C for 48 h in culture bottles containing 100 mL of inoculant consisting of rumen fluid plus *in vitro* incubation medium (20:80 rumen fluid:medium). The incubation medium was prepared as described by Mould et al. (2005). Gas production was continuously measured with individual wireless infrared sensors and

automatically recorded (PointWare; Point Six Inc., Lexington, KY). At the end of the fermentation period, 10 mL of gas from each bottle was collected with a syringe into a Vacutainer tube (BD, Franklin Lakes, NY) for headspace gas analysis using a gas chromatograph (HP 6850 GC; Hewlett-Packard). A Vacutainer containing 20% methane was analyzed twice as standard reference. Gas accumulation data was interpreted using the bacterial growth-based model, in the form of a modified logistic equation, described by Schofield et al. (1994). Final pH was measured, and a 1-mL aliquot was preserved with 25% (wt/vol) metaphosphoric acid and stored at -20°C until VFA analysis. An aliquot of inoculant was preserved in similar conditions. Rumen fluid and inoculant samples were analyzed as described previously. Bags were dried for 24 h at 105°C for DM, DMAD, and later NDF determinations. Another set of 3 replicate bags (Ankom F57; Ankom Technology) containing 0.5 g of composite feed samples was used as control for NDFD and DMTD calculations. The NDF content of all bags was analyzed using an Ankom 200 fiber analyzer (Ankom Technology) using sodium sulfite in a modified procedure described in method 6 (Ankom Technology, 2011).

Data Analysis

All data were analyzed with the MIXED procedure of SAS (SAS Institute, 2001). Means of DMI, milk yield, milk composition, milk FA, plasma metabolites, BCS, and BW were analyzed using the following fitted model:

$$Y_{ijkl} = \mu + T_i + P_j + C_{k(Sl)} + S_l + \varepsilon_{ijkl},$$

where Y_{ijkl} is the dependent variable, μ is the overall mean, T_i is the effect of treatment i ($i = 1$ to 4), P_j is the effect of period j ($j = 1$ to 4), $C_{k(Sl)}$ is the effect of cow k ($k = 1$ to 4) nested with square l , S_l is the effect of square l ($l = 1$ to 4), and ε_{ijkl} is the residual error. The experimental design used cow as experimental unit and cow (square) as random variable.

Nitrogen fractions of the rumen fluid collected on d 27 and composited per cow were analyzed using the fitted model

$$Y_{ijk} = \mu + T_i + P_j + C_k + \varepsilon_{ijk},$$

where Y_{ijk} is the dependent variable, μ is the overall mean, T_i is the effect of treatment i ($i = 1$ to 4), P_j is the effect of period j ($j = 1$ to 4), C_k is the effect of cow k ($k = 1$ to 4), and ε_{ijk} is the residual error. All terms were considered fixed except for cow (C_k), which was considered random.

Table 2. Analyzed nutrient compositions of concentrate mixes, corn silage, alfalfa hay, yeast-derived microbial protein (YMP), and soybean meal (44% CP) used during the lactation study

Item	Concentrate mix	Corn silage	Alfalfa hay	YMP	Soybean meal
DM, %	96.3	93.1	95.6	97.3	96.3
Nutrient, % of DM					
CP	22.3	7.31	17.4	44.1	50.3
NDF	11.0	51.3	42.1	5.00	10.4
ADF	6.21	30.9	32.7	1.51	7.68
Starch	34.9	21.7	1.52	4.72	2.53
Fat	6.42	2.78	1.86	0.50	1.12
Ash	8.39	4.97	8.98	6.54	6.73
Ca	1.48	0.30	1.45	0.07	0.55
P	0.60	0.28	0.22	0.78	0.74

A repeated measures model was used to evaluate ruminal parameters (pH, NH₃, and VFA), using the model

$$Y_{ijm} = \mu + T_i + P_j + \varepsilon_{ij} + H_m + HT_{mi} + \omega_{ijm},$$

where Y_{ijm} is the dependent variable, μ is the overall mean, T_i is the effect of treatment i ($i = 1$ to 4), P_j is the effect of period j ($j = 1$ to 4), ε_{ij} is the whole plot error, H_m is the effect of time m ($m = 1$ to 9), HT_{mi} is the interaction between time m and treatment i , and ω_{ijm} is the subplot error. The covariance structure with the lowest value according to Akaike's information criterion was selected (Littell et al., 2006).

In vitro gas production parameters, NDFD, DMAD, DMTD, and in vitro VFA concentration were analyzed using the fitted model

$$Y_{ijk} = \mu + T_i + \varepsilon_{ijk},$$

where Y_{ijk} is the dependent variable, μ is the overall mean, T_i is the effect of treatment i ($i = 1$ to 4), and ε_{ijk} is the residual error.

Polynomial orthogonal contrasts were used to test the linear, quadratic, and cubic effects of increasing dietary inclusion of YMP. Significance was declared at $P \leq 0.05$, and tendencies were discussed at $0.05 \leq P \leq 0.10$. Interactions that were deemed insignificant were removed from the models.

RESULTS AND DISCUSSION

Nutrient Content of Diets

The ingredient and nutrient composition of the experimental diets is shown in Table 1. Diets were formulated to be isoenergetic (1.56 Mcal of NE_L/kg) and isonitrogenous (16% CP); however, dietary CP concentrations decreased slightly as YMP replaced SBM because the CP concentration of the customized SBM

was higher than YMP (Table 2). The differences in MP values (Table 1) could be related to overestimated rumen degradability and underestimated passage rate of YMP. The NRC (2001) estimated the same MCP supply for all treatments but a decrease in RUP (kg/d) based on the decrease of the RUP (% CP) when YMP increased in the diet (Table 1), affecting the MP predictions. Dietary treatments were similar in ADF, NDF, ether extract, Ca, and P. The essential AA profile as a percentage of total essential AA (Table 3) showed that YMP had a similar profile to MCP for many AA (Clark et al., 1992) and higher concentrations for Met and Lys compared with SBM. According to Schwab et al. (1992) Met and Lys are the most limiting AA for milk production.

Particle size measured with the PSPS showed no difference between treatment diets, and retention in the screens was according to recommendations of Kononoff et al. (2003). The means for upper, middle, lower, and bottom screens were 4.1, 42.0, 37.6, and 16.3% respectively.

The intention was to balance diets with 60% forage inclusion and between 32 and 35% NDF that would

Table 3. Essential AA composition of yeast-derived microbial protein (YMP), soybean meal, and microbial CP (MCP)

AA, % of total essential AA	YMP	Soybean meal ¹	MCP ²
Arg	10.9	16.2	10.2
His	5.1	6.1	4.0
Ile	11.1	10.1	11.5
Leu	17.6	17.2	16.3
Lys	16.0	13.9	15.8
Met	3.6	3.2	5.2
Phe	9.6	11.6	10.2
Thr	10.0	8.7	11.7
Trp	2.9	2.8	2.7
Val	13.4	10.2	12.5
Total essential AA, % of CP	44.0	45.3	—

¹Table 5-10 (NRC, 2001).

²Clark et al. (1992) and NRC (2001).

Table 4. Effect of increasing concentration of yeast-derived microbial protein (YMP) on ruminal pH, NH₃ concentration, and VFA concentration

Rumen parameter	Treatment ¹				SEM	Contrast ² (<i>P</i> -value)		
	Ctrl	300	600	900		L	Q	C
pH	6.42	6.46	6.38	6.41	0.51	0.33	0.96	0.16
NH ₃ , mg/dL	14.1	11.9	13.1	11.7	2.54	0.09	0.64	0.11
Acetate, mM	97.2	95.9	94.6	96.5	2.24	0.68	0.37	0.66
Propionate, mM	30.7	30.4	31.6	31.0	2.32	0.47	0.78	0.31
Isobutyrate, mM	2.10	1.99	2.06	2.09	0.08	0.85	0.25	0.48
Butyrate, mM	16.2	15.6	16.3	16.6	0.70	0.31	0.31	0.33
Isovalerate, mM	3.22	2.83	2.71	2.84	0.22	<0.001	0.001	0.98
Valerate, mM	3.14	2.91	2.94	2.86	0.39	0.12	0.48	0.51
Total VFA, mM	152.5	149.7	150.2	151.9	4.90	0.93	0.41	0.86
Acetate, %	64.0	64.2	63.3	63.6	1.31	0.18	0.81	0.13
Propionate, %	19.9	20.3	20.7	20.4	0.99	0.16	0.20	0.51
Isobutyrate, %	1.40	1.37	1.39	1.41	0.04	0.77	0.48	0.68
Butyrate, %	10.6	10.3	10.8	10.9	0.20	0.11	0.48	0.16
Isovalerate, %	2.12	1.91	1.85	1.88	0.12	<0.001	0.005	0.79
Valerate, %	2.00	1.92	1.92	1.86	0.19	0.11	0.80	0.61
Acetate:propionate	3.28	3.22	3.17	3.16	0.21	0.12	0.66	0.83

¹Ctrl = control (0 g of YMP/cow per day); 300 = 1.14% YMP (300 g of YMP/cow per day); 600 = 2.28% YMP (600 g of YMP/cow per day); 900 = 3.41% YMP (900 g of YMP/cow per day).

²L = linear; Q = quadratic; C = cubic.

support high solids composition in milk without decreasing milk yield or DMI (Beckman and Weiss, 2005). High forage inclusion could also allow for greater fluid passage rates (Church, 1988), and if, as hypothesized, YMP is diluted in the liquid fraction, then a larger amount of small peptides, oligopeptides, and soluble proteins should escape ruminal fermentation and reach the small intestine. By doing so, a high-quality source of absorbable AA (Table 3) would reach the intestine to support milk production.

Nitrogen fractionation of YMP showed that oligopeptides that weigh between 3 and 10 kDa are the main fraction and account for 72.7% of the N. Peptides <3 kDa and free AA accounted for 19.4 and 7.8% of the YMP N, respectively. It is known that peptides stimulate bacterial growth (Chikunya et al., 1996), are highly absorbed by the portal-drained viscera (Tagari et al., 2008), particularly by intestinal cells (Gilbert et al., 2008), and contribute to protein synthesis in the mammary gland (Mabjeesh et al., 2005).

Ruminal Fluid Analysis

Concentrations and proportions of VFA were not different between treatments, except for isovalerate ($P < 0.001$; Table 4). Branched-chain VFA, isobutyrate, isovalerate, and 2-methylbutyrate are produced by oxidative deamination of the branched-chain AA, Val, Leu, and Ile, respectively (Hoover, 1986). The linear decrease in isovalerate concentration and proportion could be explained as a result of lower Leu availability for ruminal fermentation due to greater ruminal escape of YMP to the small intestine and portal-drained vis-

cera through the rumen wall. The fact that in vitro fermentation is not affected by rate of passage and ruminal absorption could explain the differences on the isovalerate response between in vivo and in vitro results. Greater rate of passage of peptides in YMP diets could also explain the linear decrease on ruminal ammonia concentrations in vivo ($P = 0.09$).

A cubic effect of increasing YMP concentration was observed on free AA ($P = 0.02$) and a tendency for total AA-N ($P = 0.09$) in rumen fluid, soluble proteins greater than 10 kDa ($P = 0.08$), and total N ($P = 0.09$; Table 5). A quadratic effect of increasing YMP concentration on <3-kDa peptides and on 3- to 10-kDa oligopeptides (Table 5) was observed when they were expressed as a percentage of AA-N, and as percentage of total N. The average percentages for AA in soluble proteins >10 kDa, 3- to 10-kDa oligopeptides, <3-kDa peptides, and free AA were 26.8, 19.2, 45.2, and 8.8%, respectively, with the peptide fraction being the largest one (Figure 1). In contrast, Reynal et al. (2007) found different soluble AA proportions in omasal samples using a similar N fractionation technique. In their study, the biggest fraction was the 3- to 10-kDa oligopeptides, accounting for 71% of the soluble AA, with small peptides representing 17.5%, and free AA only 1.6%. According to these results, Reynal et al. (2007) concluded that hydrolysis of the 3- to 10-kDa oligopeptides was the rate-limiting step during microbial degradation of soluble proteins and that small peptides and free AA were rapidly utilized by ruminal microbes and did not accumulate in the rumen. Results of the current study were similar to those described by Choi et al. (2002a) on omasal samples and on rumen concentration (Choi et

Table 5. Fractionation of soluble N fractions from ruminal fluid of cows fed increasing concentration of yeast-derived microbial protein (YMP)

Item, ¹ mg/L	Treatment ²					Contrast ³ (<i>P</i> -value)		
	Ctrl	300	600	900	SEM	L	Q	C
Total AA-N	141.6	130.3	146.2	136.8	9.2	0.95	0.87	0.09
>10 kDa	38.6	34.4	40.1	38.6	2.8	0.52	0.47	0.08
3–10 kDa	20.8	28.4	28.5	25.2	3.1	0.37	0.12	0.78
<3 kDa	70.0	57.1	63.5	61.9	5.3	0.40	0.25	0.22
Free AA	12.1	10.4	14.1	11.2	0.9	0.82	0.50	0.02
NH ₃ -N	90.6	69.6	85.5	75.0	17.2	0.44	0.55	0.14
Total N	232.2	199.8	231.7	211.8	25.9	0.63	0.64	0.09
% AA-N								
>10 kDa	26.5	25.7	27.2	27.7	1.5	0.45	0.69	0.62
3–10 kDa	14.6	22.8	19.7	18.9	1.8	0.27	0.05	0.15
<3 kDa	49.8	43.4	43.4	45.2	1.7	0.11	0.05	0.55
Free AA	9.1	8.1	9.6	8.2	0.7	0.74	0.77	0.15
% Total N								
Total AA-N	62.0	65.1	63.8	64.8	3.0	0.26	0.45	0.27
>10 kDa	16.5	16.7	17.6	18.2	1.5	0.36	0.90	0.87
3–10 kDa	9.10	14.9	12.3	12.0	1.3	0.32	0.05	0.11
<3 kDa	30.8	28.3	27.7	29.4	1.4	0.21	0.03	0.94
Free AA	5.7	5.3	6.1	5.3	0.6	0.90	0.67	0.24
NH ₃ -N	38.0	34.9	36.2	35.2	3.0	0.26	0.45	0.27

¹>10 kDa = soluble proteins greater than 10 kDa; 3–10 kDa = soluble oligopeptides between 3 and 10 kDa; <3 kDa = small soluble peptides less than 3 kDa.

²Ctrl = control (0 g of YMP/cow per day); 300 = 1.14% YMP (300 g of YMP/cow per day); 600 = 2.28% YMP (600 g of YMP/cow per day); 900 = 3.41% YMP (900 g of YMP/cow per day).

³L = linear; Q = quadratic; C = cubic.

al., 2002b), even though those authors used a different N fractionation procedure that uses trichloroacetic acid precipitation. Choi et al. (2002a,b) reported a higher proportion of <3-kDa peptides, averaging 63 and 59% of soluble AA, respectively, in each study.

Results of the present experiment suggest that the hydrolysis of 3- to 10-kDa oligopeptides to <3-kDa peptides, and that of <3-kDa peptides to free AA were affected by diet, and that the hydrolysis of <3-kDa peptides to free AA by rumen microbes was the rate-

limiting step in ruminal protein degradation. Furthermore, these results may suggest that ruminal protein degradation rate may depend not only on the degradability (Broderick and Wallace, 1988) and AA profile of the protein (Yang and Russell, 1992), but also on the dietary combination of different protein sources that provide the best AA balance.

Blood Analysis

Plasma NEFA concentrations were not affected by increasing concentration of YMP; however, BHBA ($P = 0.04$) and glucose ($P = 0.06$) concentrations both increased linearly (Table 6). It is well established that the main source of ketone bodies is liver oxidation of NEFA (Bergman, 1971; Drackley et al., 1992). Although no difference in NEFA concentration was observed in the present study, a linear increase in BHBA occurred. Even-chained VFA are considered the second main source of ketone bodies, with butyrate as principal precursor when metabolized to BHBA in the rumen wall (Yamdagni et al., 1968). In this study, neither butyrate nor acetate concentration in the rumen fluid supported the linear increase in plasma BHBA.

Catabolism of certain AA carbon skeletons is another source of ketone bodies (McKee and McKee, 2003). Surplus AA that are not used as building blocks for protein synthesis, are degraded in the liver, producing

Figure 1. Nitrogen fractions comparison expressed as percentage of soluble AA (% AA-N). Ctrl = control (0 g of YMP/cow per day); 300 = 1.14% YMP (300 g of YMP/cow per day); 600 = 2.28% YMP (600 g of YMP/cow per day); 900 = 3.41% YMP (900 g of YMP/cow per day).

Table 6. Effect of increasing concentration of yeast-derived microbial protein (YMP) on plasma metabolites

Plasma metabolite	Treatment ¹					Contrast ²		
	Ctrl	300	600	900	SEM	L	Q	C
NEFA, mM	0.15	0.15	0.15	0.14	0.01	0.38	0.90	0.88
Glucose, mg/dL	63.7	59.7	68.9	68.5	2.81	0.06	0.52	0.08
BHBA, mg/dL	11.5	11.8	12.9	13.1	0.68	0.04	0.88	0.52

¹Ctrl = control (0 g of YMP/cow per day); 300 = 1.14% YMP (300 g of YMP/cow per day); 600 = 2.28% YMP (600 g of YMP/cow per day); 900 = 3.41% YMP (900 g of YMP/cow per day).

²L = linear; Q = quadratic; C = cubic.

acetyl-CoA, α -ketoglutarate, succinyl-CoA, or oxaloacetate (McKee and McKee, 2003). Ketogenic and glucogenic AA produce acetyl-CoA and intermediates of the citric acid cycle as end products, respectively. From the data in this experiment, it can be hypothesized that as YMP increased, a greater concentration of ketogenic AA in blood was absorbed from the intestine and non-mesenteric drained viscera that could lead to a linear increase in BHBA. Similarly, a higher concentration of glucogenic AA forming α -ketoglutarate (Gln, Glu, Pro, Arg, and His), succinyl-CoA (Met, Ile, and Val), or oxaloacetate (Asp) could explain a linear response in blood glucose. Propionate is the major precursor, assuming that approximately 80% of this VFA is converted to glucose (Sharp et al., 1982). In this experiment, however, no difference was observed in propionate concentration or proportion between dietary treatments (Table 4). To further test these hypotheses, additional investigation is warranted, including AA absorption and blood AA pool size within treatments.

Intake, BCS, and BW

No treatment effect on BCS was observed, although cubic effects on DMI ($P = 0.04$) and BW change ($P = 0.05$; Table 7) were observed. Cows fed treatments 300 and 900 g of YMP/cow per day resulted in the highest DMI and BW change. It is likely that the positive effect on intake resulted in a greater increase on rumen fill and body reserves. The control and treatment 600 g of YMP/cow per day had lower intakes but still had a positive effect on BW change, averaging 3.13 and 6.23 kg, respectively.

According to Oba and Allen (1999), higher forage NDF digestibility could help explain the effects on DMI. Those authors reported that a 1-U increase in NDF digestibility in vitro or in situ was associated with an increase of 0.17 and 0.25 kg in DMI and 4% FCM, respectively. Conversely, in the present study, although there was no statistical difference in NDFD in vitro (Table 8), the treatments with the highest numerical values for NDFD, control, and 600 g of YMP/cow per

day, had the lowest values for DMI. Short-term regulation of feed intake is complex and involves more than 1 mechanism that interacts to affect satiety (Allen, 2000). On the basis of the data collected in the current study, it is difficult to interpret mechanisms responsible for the greater DMI observed in cows fed treatments 300 and 900 g of YMP/cow per day compared with the control and 600 g of YMP/cow per day and warrants further investigation.

Milk Production and Composition

Milk production averaged 41.1 kg and was not affected by dietary treatments (Table 7). Milk fat percentage ($P = 0.06$) and yield ($P = 0.07$) tended to show a quadratic increase. Milk FA concentrations also showed quadratic responses ($P = 0.02$) for mid-chain (C10 to C16) and long-chain FA ($\geq$ C17), whereas no difference was observed in short-chain FA (C 4 to C9; Table 9). It can be assumed that no differences existed in FA mobilization from body reserves or in their utilization by the mammary gland because no differences existed in plasma NEFA concentrations between treatments (Table 6). About 50% of the milk fat is synthesized de novo in the mammary gland from precursors (Akers, 2002); therefore, it can be hypothesized that the higher fat yields observed for treatments 300 and 600 g of YMP/cow per day resulted from greater synthesis in the mammary gland. In ruminants, the major precursors for milk fat synthesis are acetate, BHBA, triacylglycerides (mostly very-low-density lipoproteins in blood), and NEFA (Akers, 2002). Dietary AA, in particular Met and Lys, may sometimes increase fat content of the milk (NRC, 2001). Although not well established, these AA could be involved in de novo synthesis of short- and medium-chain FA in the mammary gland (NRC, 2001). Pisulewski et al. (1996) observed that the infusion of Met into the duodenum of early lactation cows increased proportions of short- and medium-chain FA and decreased proportions of long-chain FA in milk.

Milk protein percentage and yield did not differ between treatments (Table 7). The NRC (2001) indicates

Table 7. Effect of increasing concentration of yeast-derived microbial protein (YMP) on DMI, milk yield, milk composition, BW, and BCS

Item	Treatment ¹					Contrast ² (<i>P</i> -value)		
	Ctrl	300	600	900	SEM	L	Q	C
DMI, kg/d	25.9	27.1	25.9	26.6	0.73	0.57	0.59	0.04
Milk, kg/d	40.3	41.5	41.2	41.4	1.23	0.20	0.33	0.37
Fat, %	3.53	3.66	3.62	3.53	0.14	0.82	0.06	0.70
Fat, kg/d	1.35	1.45	1.49	1.42	0.06	0.26	0.07	0.89
Protein, %	3.09	3.12	3.10	3.12	0.05	0.60	0.78	0.38
Protein, kg/d	1.23	1.27	1.27	1.26	0.04	0.39	0.42	0.73
Lactose, %	4.81	4.85	4.86	4.82	0.04	0.73	0.12	0.96
Lactose, kg/d	1.94	2.02	2.00	1.99	0.07	0.36	0.15	0.40
TS, %	12.3	12.5	12.5	12.3	0.16	0.95	0.02	0.45
TS, kg/d	4.87	5.11	5.12	5.03	0.15	0.24	0.08	0.72
MUN, mg/dL	13.4	13.7	13.5	12.9	0.53	0.08	0.05	0.90
SCC, 10 ⁵ cells/mL	3.23	2.49	1.78	2.17	1.22	0.44	0.20	0.57
4% FCM, ³ kg/d	36.3	38.4	38.8	37.8	1.14	0.20	0.08	0.91
ECM, ⁴ kg/d	39.5	41.5	41.8	41.0	1.22	0.20	0.09	0.87
ECM/DMI	1.52	1.55	1.60	1.56	0.04	0.28	0.29	0.44
BW, kg	716.0	717.5	713.0	715.0	11.3	0.61	0.94	0.39
BW change, kg/d	0.11	0.60	0.22	0.49	0.17	0.31	0.52	0.05
BCS ⁵	3.23	3.29	3.27	3.27	0.04	0.51	0.20	0.39
BCS change	0.03	0.10	0.09	0.11	0.04	0.13	0.28	0.45

¹Ctrl = control (0 g of YMP/cow per day); 300 = 1.14% YMP (300 g of YMP/cow per day); 600 = 2.28% YMP (600 g of YMP/cow per day); 900 = 3.41% YMP (900 g of YMP/cow per day).

²L = linear; Q = quadratic; C = cubic.

³4% FCM = $[0.4 \times \text{milk yield (kg)}] + [15 \times \text{fat yield (kg)}]$.

⁴ECM = $[0.327 \times \text{milk yield (kg)}] + [12.95 \times \text{fat yield (kg)}] + [7.20 \times \text{protein yield (kg)}]$. Adapted from Orth (1992).

⁵Body condition scores: 1 = emaciated to 5 = obese (Wildman et al., 1982).

that the content and yield of milk protein can be influenced by the profile of AA in MP, by the amount of surplus protein, and by fermentable carbohydrates in the diet. In contrast, numerous reports exist in the literature of small or often no increases in milk yield and

protein content when dietary protein, AA, or energy are supplemented (Bequette et al., 1998), especially when energy and protein are not limiting production (Brun-Lafleur et al., 2010). Such observations serve to highlight that the knowledge is incomplete concerning

Table 8. Effect of increasing concentration of yeast-derived microbial protein (YMP) on in vitro gas production fermentation, DM apparent digestibility (DMAD), DM true digestibility (DMTD), and NDF digestibility (NDFD)

Item	Treatment ¹					Contrast ² (<i>P</i> -value)		
	Ctrl	300	600	900	SEM	L	Q	C
DMAD, %	51.2	52.9	53.2	49.3	1.56	0.44	0.09	0.70
DMTD, %	62.9	64.3	65.6	61.4	1.38	0.62	0.06	0.40
NDFD, %	16.1	15.9	21.0	13.2	2.59	0.79	0.22	0.16
Gas pool size, mL/g of OM	235.6	233.8	229.6	226.8	7.60	0.85	0.62	0.67
Methane, %	12.2	11.9	11.4	11.2	0.55	0.20	0.99	0.88
Fermentation rate 1, /h	0.16	0.16	0.15	0.16	0.008	0.99	0.63	0.59
Fermentation rate 2, /h	0.033	0.035	0.034	0.033	0.0007	0.69	0.05	0.61
Lag phase, h	0.41	0.43	0.36	0.48	0.09	0.76	0.59	0.47
pH	6.46	6.46	6.47	6.46	0.02	0.90	0.93	0.88
Total VFA, mM	30.8	28.9	31.0	24.3	3.11	0.19	0.41	0.35
Acetate, %	48.6	49.0	50.4	48.6	2.39	0.88	0.62	0.68
Propionate, %	20.4	21.0	22.6	20.6	0.90	0.55	0.14	0.25
Butyrate, %	17.4	16.8	15.4	17.0	1.13	0.58	0.29	0.46
Isovalerate, %	5.40	5.50	4.40	5.40	0.42	0.52	0.26	0.08
Valerate, %	5.00	5.00	4.60	5.20	0.44	0.91	0.48	0.47
Acetate:propionate ratio	2.39	2.36	2.25	2.38	0.17	0.85	0.62	0.67

¹Ctrl = control (0 g of YMP/cow per day); 300 = 1.14% YMP (300 g of YMP/cow per day); 600 = 2.28% YMP (600 g of YMP/cow per day); 900 = 3.41% YMP (900 g of YMP/cow per day).

²L = linear; Q = quadratic; C = cubic.

Table 9. Effect of increasing concentration of yeast-derived microbial protein (YMP) on milk FA composition

FA ¹ (g/100 g of total FA)	Treatment ²					Contrast ³ (<i>P</i> -value)		
	Ctrl	300	600	900	SEM	L	Q	C
C4:0	2.75	2.90	2.83	2.87	0.12	0.50	0.53	0.43
C6:0	2.02	2.01	1.94	1.97	0.07	0.32	0.74	0.48
C8:0	1.28	1.27	1.23	1.25	0.04	0.34	0.63	0.48
C10:0	3.15	3.15	3.02	3.09	0.12	0.32	0.59	0.29
C11:0	0.03	0.03	0.04	0.04	0.11	0.26	0.25	0.61
C12:1	0.12	0.10	0.10	0.11	0.006	0.63	0.06	0.76
C13:0	0.12	0.10	0.11	0.12	0.007	0.43	0.05	0.37
C14:0	11.6	11.8	11.6	11.6	0.22	0.52	0.34	0.26
C14:1	1.46	1.52	1.57	1.54	0.12	0.35	0.55	0.83
C15:0	1.21	1.19	1.27	1.28	0.06	0.12	0.69	0.35
C16:0	32.7	33.8	34.2	33.0	0.76	0.40	0.001	0.61
C16:1	1.72	1.73	1.80	1.80	0.13	0.41	0.99	0.70
C17:0	0.54	0.54	0.55	0.58	0.02	0.15	0.50	0.98
C18:0	9.34	9.15	8.99	9.19	0.38	0.58	0.41	0.75
C18:1 <i>cis</i> -9	22.1	21.0	21.3	21.5	0.63	0.40	0.08	0.35
C18:1 <i>trans</i> -11	1.39	1.55	1.44	1.65	0.12	0.16	0.78	0.21
CLA 18:2 <i>cis</i> -9, <i>trans</i> -11	0.43	0.42	0.45	0.45	0.03	0.41	0.80	0.49
C18:2 <i>cis</i> -9, <i>cis</i> -12	2.95	2.75	2.77	3.03	0.11	0.25	<0.001	0.97
C18:3 α	0.51	0.50	0.51	0.54	0.01	0.05	0.09	0.94
C20:0	0.13	0.12	0.13	0.14	0.01	0.48	0.26	0.64
C20:1	0.08	0.05	0.06	0.08	0.01	0.83	0.01	0.50
C20:4	0.14	0.10	0.10	0.10	0.02	0.13	0.21	0.66
SCFA	6.09	6.21	6.03	6.11	0.21	0.89	0.87	0.38
MCFA	56.1	57.4	57.5	57.2	0.86	0.65	0.02	0.95
LCFA	37.6	36.2	36.3	37.3	0.91	0.73	0.02	0.73

¹CLA = conjugated linoleic acid; SCFA = short-chain FA (C4 to C9); MCFA = medium-chain FA (C10 to C 16); LCFA = long-chain FA ($\geq$ C17).

²Ctrl = control (0 g of YMP/cow per day); 300 = 1.14% YMP (300 g of YMP/cow per day); 600 = 2.28% YMP (600 g of YMP/cow per day); 900 = 3.41% YMP (900 g of YMP/cow per day).

³L = linear; Q = quadratic; C = cubic.

how dietary nutrients influence or limit milk protein synthesis. It is important to notice that milk protein percentage (3.10%), and yield (1.26 kg/d), could be considered high for midlactation cows fed a 60% forage diet, explaining the lack of response to dietary treatments.

Although a linear increase in plasma glucose was observed (Table 6), no differences in milk lactose percentage or yield were observed (Table 7). The absence of a positive response in milk yield to glucose administration can be explained by other factors (other than whole-body glucose rate appearance) that limit lactose synthesis and milk yield (Rigout et al., 2002). Rigout et al. (2002) reported similar values in plasma glucose and milk lactose when increasing amounts of glucose were infused in the duodenum. Furthermore, similar to the results of the present study, the authors observed a linear increase in plasma glucose and no effect on lactose percentage. An increase in glucose utilization in insulin-dependent tissues may reduce glucose availability to the mammary gland (Lemosquet et al., 1997). A downregulation of glucose utilization by the mammary gland may also be responsible. Furthermore, limited availability of nutrients other than glucose, such as AA (Clark et al., 1977), might limit milk synthesis.

Total solids percentage ($P = 0.02$), and TS yield ($P = 0.08$) responded quadratically to dietary treatments (Table 7), which was mainly explained by a similar trend in milk fat percentage and yield. Similarly, yields for ECM and 4% FCM showed the same quadratic trend ($P = 0.09$ and $P = 0.08$, respectively). Feed efficiency, expressed as ECM/DMI, was not affected by treatments.

Ruminal ammonia concentration ($P = 0.05$; Table 4) tended to decrease linearly, whereas MUN showed a quadratic response ($P = 0.05$; Table 7). Based on the hypothesis that higher absorption and metabolism of surplus AA could lead to linear responses in plasma glucose and BHBA, a linear increase in MUN would be expected because liver AA deamination contributes further to the urea pool (Lapierre and Lobley, 2001). Milk urea nitrogen is an indicator of urea production by the liver, but also can be influenced by N recycling from the animal tissues to the rumen (NRC, 2001). The clearance of plasma urea to the rumen is inversely proportional to the concentration of rumen ammonia (Kennedy and Milligan, 1980). The linear tendency in rumen ammonia concentration could lead to a higher N recycling to the rumen, thus reducing the concentration of urea-N in the blood and, consequently, in milk.

In Vitro Study

In vitro DMAD ($P = 0.09$) and DMTD ($P = 0.06$) exhibited a tendency for quadratic increase, whereas NDFD was unaffected when dietary concentration of YMP increased (Table 8). Observed values of NDFD were lower than expected, particularly considering the NFC/NDF relation of the diet (Huhtanen et al., 2009).

Total gas pool size, methane production, and lag phase were not affected by YMP concentration in the diet. Fermentation rate of the first (fast) pool did not respond to dietary treatments, whereas the fermentation rate of the second (slow) pool showed a quadratic increase ($P = 0.05$). The first and second fermentation pools and their rates correspond to rapid and slow-digesting components in the diet, respectively (Schofield et al., 1994). No differences were observed for cumulative gas production, pH, total VFA concentrations, VFA proportions, or acetate:propionate ratio.

CONCLUSIONS

The substitution of SBM by YMP resulted in a quadratic effect on TS percentage, whereas a quadratic trend was observed on milk fat percentage and yield, TS yield, ECM, and 4% FCM. Rumen ammonia tended to decrease linearly as YMP in the diet increased, supporting the hypothesis of a higher YMP contribution to RUP, possibly because of greater rate of ruminal escape of this ingredient with the liquid fraction. Further investigation is needed to answer some questions and hypotheses arising from this experiment. It was concluded that the substitution of soybean meal with YMP tends to improve ECM and FCM production in high-producing dairy cows consuming high-forage diets.

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