Effects of dietary protein concentration and coconut oil supplementation on nitrogen utilization and production in dairy cows

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ABSTRACT

The objective of this study was to investigate the effect of metabolizable protein (MP) deficiency and coconut oil supplementation on N utilization and production in lactating dairy cows. The hypothesis of the study was that a decrease in ruminal protozoal counts with coconut oil would increase microbial protein synthesis in the rumen, thus compensating for potential MP deficiency. The experiment was conducted for 10 wk with 36 cows (13 primiparous and 23 multiparous), including 6 ruminally cannulated cows. The experimental period, 6 wk, was preceded by 2-wk adaptation and 2-wk covariate periods. Cows were blocked by parity, days in milk, milk yield, and rumen cannulation and randomly assigned to one of the following diets: a diet with a positive MP balance (+44 g/d) and 16.7% dietary crude protein (CP) concentration (AMP); a diet deficient in MP (–156 g/d) and 14.8% CP concentration (DMP); or DMP supplemented with approximately 500 g of coconut oil/head per day (DMPCO). Ruminal ammonia tended to be greater and plasma urea N (20.1, 12.8, and 13.1 mg/dL, for AMP, DMP, and DMPCO diets, respectively) and milk urea N (12.5, 8.3, and 9.5 mg/dL, respectively) were greater for AMP compared with DMP and DMPCO. The DMPCO diet decreased total protozoa counts (by 60%) compared with DMP, but had no effect on the methanogens profile in the rumen. Total tract apparent digestibility of dry matter and CP was decreased by DMP compared with AMP. Fiber digestibility was lower for both DMP and DMPCO compared with AMP. Urinary N excretion was decreased (by 37%) by both DMP and DMPCO compared with AMP. The DMP and DMPCO diets resulted in greater milk N efficiency compared with AMP (32.0 and 35.1 vs. 27.6%, respectively). Milk yield was decreased by both DMP and DMPCO compared with AMP (36.2, 34.4, and 39.3 kg/d, respectively) and coconut oil supplementation suppressed feed intake and

caused milk fat depression. Coconut oil supplementation decreased short-chain fatty acid (C4:0, C6:0, and C8:0) concentration and increased medium-chain (C12:0 and C14:0) and total *trans* fatty acids in milk. Overall, the MP-deficient diets decreased N losses, but could not sustain milk production in this study. Coconut oil decreased feed intake and similar to DMP, suppressed fiber digestibility. Despite decreased protozoal counts, coconut oil had no effect on the methanogen population in the rumen.

Key words: metabolizable protein, coconut oil, rumen protozoa, dairy cow

INTRODUCTION

Providing dietary protein to meet protein requirements is one of the critical factors in dairy nutrition necessary to maximize production and minimize protein input and manure N output. Protein feeds are among the most expensive ingredients in animal diets and accumulation of manure N is a primary concern in terms of environmental impact of animal production, such as surface water eutrophication, aerosol formation (specifically, particles with aerodynamic diameter less than or equal to 2.5 μm ; i.e., $\text{PM}_{2.5}$), and soil acidification (USEPA, 2004). In spite of these concerns, however, protein is often overfed to lactating cows. For example, the average CP content of the lactating cow diets in high-producing western dairy herds was 17.6% (Hristov et al., 2006). Recent meta-analysis suggested deficiencies in the current NRC (2001) protein model, potentially leading to overfeeding of dietary protein (Huhtanen and Hristov, 2009). Cyriac et al. (2008) concluded that ruminal microbial RDP requirements could be met with RDP levels below those recommended in the current NRC (2001). Recently, Agle et al. (2010) reported that lower dietary RDP concentration (7.1 vs. 10.3%) decreased urinary N excretion and ammonia emission from manure without affecting milk production. The main reason for over-estimation of RDP requirements in the current NRC (2001) might be the lack of provision for hepatic urea N recycling. A review by Reynolds and Kristensen

Received October 1, 2010.

Accepted August 1, 2011.

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(2008) indicated that total urea production that is being returned to the gut decreases with increasing dietary CP concentration. Thus, low-CP diets may stimulate urea recycling and provide additional RDP to the rumen microbes when dietary RDP supply is low. Feeding diets deficient in MP and RDP, however, may result in decreased milk and milk protein yields (Cressman et al., 1980; Wu and Satter, 2000; Frank and Swensson, 2002). Therefore, the environmental and economic benefits of decreased dietary N input have to be balanced against potential negative effect on cow productivity and farm profitability.

Another opportunity to optimize N efficiency is to maximize microbial protein synthesis (MPS) in the rumen and microbial MP supply to the small intestine. Defaunation has been suggested as an effective method for increasing MPS and microbial outflow from the rumen (Hristov and Jouany, 2005). It was reported that a single protozoon is able to engulf 10^2 to 10^4 bacteria hourly in vitro (Coleman, 1975). Although the magnitude of bacterial predation by protozoa in vivo is less than that in vitro (Coleman and Sandford, 1979), the reported increase in MPS in defaunated animals (Eugène et al., 2004) is logical. Medium-chain saturated fatty acids (MCSFA) are potent antiprotozoal agents. Dohme et al. (2000) reported that coconut oil (composed of about 45% C12:0 and 20% C14:0) eliminated about 93% of ciliate protozoa in vitro. In vitro and in vivo experiments (Hristov et al., 2004a,b, 2009a, 2011) demonstrated strong antiprotozoal properties of C12:0 and coconut oil. In addition, defaunation has resulted in elimination of specific rumen methanogens due to the endosymbiotic relationship between protozoa and methanogens (Dohme et al., 1999; Karnati et al., 2009), which may contribute to improved efficiency of feed energy utilization and decreased methane emissions from ruminants.

Therefore, the objective of this study was to test the production effects of diets supplying MP below NRC (2001) requirements with or without supplementation of an antiprotozoal agent, coconut oil. We hypothesized that a decrease in ruminal protozoal counts would increase microbial protein outflow from the rumen, thus compensating for dietary MP deficiency.

MATERIALS AND METHODS

Animals involved in this study were cared for according to the guidelines of the Pennsylvania State University Animal Care and Use Committee. The committee reviewed and approved the experiment and all procedures carried out in the study.

Animals and Experimental Design

Thirteen primiparous and 23 multiparous Holstein cows including 6 ruminally cannulated (10-cm cannulas; Bar Diamond Inc., Parma, ID); average parity, 2.1 lactations (SD = 1.0); BW, 618 kg (SD = 84); 132 DIM (SD = 7); and milk yield of 44 kg/d (SD = 9.6) at the beginning of the trial, were blocked into 12 blocks of 3 cows each by parity, DIM, milk production, and rumen cannulation. Cows within each block were randomly assigned to one of the following diets fed as TMR: (1) a diet with positive MP balance (AMP; NRC, 2001); (2) a diet deficient in MP (DMP); or (3) the DMP diet supplemented with approximately 500 g of coconut oil/head per day (DMPCO; Table 1). The feeding level of coconut oil (containing 46 g of C12:0 and 20 g of C14:0/100 g; GloryBee Foods Inc., Eugene, OR) was chosen based on previous studies (Hristov et al., 2009a). Although C12:0 and C14:0 were the major fatty acids (FA) in coconut oil, some other minor FA, such as C10:0 (see Table 1 footnote) possess strong antiprotozoal properties (Hristov et al., 2004b) and may have contributed to the effect of coconut oil in this experiment. The duration of the experiment was 10 wk, with the first 2 wk being a covariate period and the second 2 wk an adaptation period. Data were collected from wk 5 through 10 (i.e., 42 d). Cows were fed the control (AMP) diet during the covariate period. The diets in this experiment were formulated to meet the nutrient requirements, except MP, of a lactating Holstein cow yielding 43 kg/d of milk with 3.6% milk fat and 3.02% milk true protein at 25 kg of DMI/d (NRC, 2001). According to NRC (2001), the AMP diet met the MP requirements of the cows (at actual DMI and milk yield) and the DMP and DMPCO diets were deficient in MP. All diets supplied NE_L in excess of requirements: +2.2, +3.2, and +2.3 Mcal/d for AMP, DMP, and DMPCO, respectively.

The TMR were mixed (model 1050 TMR mixer; I. H. Rissler Mfg. LLC, Mohnton, PA) every morning and fed to the cows twice daily (at 0800 and 1500 h, approximately one-third in the morning and two-thirds in the afternoon). Coconut oil (solid at room temperature) was placed in an oven at 50°C to melt for at least 12 h before feeding. The liquid coconut oil was mixed with the morning portion of the TMR only. All diets were fed ad libitum, targeting about 5 to 10% refusals. Because of the ad libitum feeding and depression of DMI with the DMPCO diet, the actual inclusion level of coconut oil was lower than planned and varied during the trial and between individual cows. The average coconut oil intake throughout the trial was 499 g/head per day ($n = 516$; SD = 88.5, minimum = 138, and maximum =

Table 1. Ingredient and chemical composition (% of DM) of the diets fed in the trial

Item	Diet ¹		
	AMP	DMP	DMPCO
Ingredient			
Corn silage ²	26.0	25.1	25.2
Alfalfa haylage ³	18.8	18.8	18.8
Grass hay ⁴	5.0	5.0	5.0
Forages	49.8	48.9	49.0
Corn grain, ground	14.3	19.5	19.9
Bakery byproduct meal ⁵	7.3	7.3	7.3
Canola meal (solvent extracted)	11.9	11.2	11.0
Cottonseed, hulls	5.3	6.2	6.1
Soybean seeds, whole, heated ⁶	4.5	0	0
Megalac ⁷	2.6	2.6	0
Coconut oil ⁸	0	0	2.3
Corn dry distillers grains with solubles	1.5	1.5	1.5
Molasses ⁹	1.5	1.5	1.5
Minerals and vitamins premix ¹⁰	1.5	1.5	1.5
Composition, % of DM ¹¹			
CP	16.7	14.8	14.7
RDP, ¹² %	10.6	9.8	9.9
RUP, ¹² %	6.1	4.9	4.8
MP, ¹² %	10.8	9.7	9.5
RDP balance, ¹² g/d	141	−42	−35
RUP balance, ¹² g/d	56	−206	−381
MP balance, ¹² g/d	44	−156	−288
NDF	31.7	31.9	31.8
ADF	21.1	21.2	21.1
NE _L , Mcal/kg	1.64	1.64	1.64
NE _L balance, ¹² Mcal/d	2.2	3.2	2.3
TNC ¹³	27.4	30.5	29.6
Ether extract	5.8	5.7	5.8
Ca	1.06	1.04	0.81
P	0.45	0.42	0.42

¹AMP = diet adequate in MP, containing 16.7% CP; DMP = diet deficient in MP, containing 14.8% CP; DMPCO = the DMP diet, supplemented with coconut oil.

²Corn silage was 34.1% DM and (% of DM): 39.1% NDF and 8.3% CP.

³Alfalfa haylage was 44.5% DM and (% of DM): 35.1% NDF and 20.5% CP.

⁴Grass hay contained (% of DM): 76.8% NDF and 5.0% CP.

⁵Bakery byproduct meal (Bakery Feeds Inc., Honey Brook, PA) contained (% of DM): 13.5% CP, 8.3% ether extract, and 13.8% NDF.

⁶Contained 46.9% CP (% of DM).

⁷Megalac (Church & Dwight Co. Inc., Princeton, NJ) contained 85% fat.

⁸Coconut oil (GloryBee Foods Inc., Eugene, OR) contained 46 g of lauric acid and 20 g of myristic acid/100 g. Other major fatty acids (FA) in coconut oil were (per 100 g of FA): C16:0, 11 g; monounsaturated FA, 7 g; C8:0, 6.1 g; C10:0, 5.5 g; C18:0, 3 g; and polyunsaturated FA (PUFA), 2 g.

⁹Molasses (Westway Feed Products LLC, Tomball, TX) contained (% of DM): 3.9% CP and 66% total sugar.

¹⁰The premix contained (% as-is basis): trace mineral mix, 0.88; MgO (54% Mg), 8.3; NaCl, 6.4; vitamin ADE premix, 1.73; limestone, 35.8; selenium premix, 1.09; and dry corn distillers grains with solubles, 45.8. Composition: Ca, 14.9%; P, 0.37%; Mg, 4.84%; K, 0.44%; S, 0.32%; Se, 7.04 mg/kg; Cu, 377 mg/kg; Zn, 1,146 mg/kg; Fe, 191 mg/kg; Se, 6.67 mg/kg; Co, 5.4 mg/kg; vitamin A, 125,875 IU/kg; vitamin D, 31,418 IU/kg; and vitamin E, 946 IU/kg.

¹¹Calculated from analyzed composition of individual feed ingredients (Cumberland Valley Analytical Services, Maugansville, MD), unless otherwise indicated.

¹²RDP, RUP, MP, and NE_L percentages and balances (supply – requirement) were estimated using NRC (2001) guidelines and individual cow DMI, BW, milk yield, and composition.

¹³Total nonstructural carbohydrates; composite TMR samples were analyzed for TNC (see Materials and Methods).

748 g/head per day). Coconut oil inclusion rate was more consistent and averaged 2.3% (dietary DM basis) with standard deviation = 0.12, minimum = 2.1%, and maximum = 2.6%. Cows were housed in a tie-stall barn

during the trial and were exercised for 1.5-h periods before each milking, which was twice daily. Cows had free access to fresh water and received recombinant bST (Posilac; Elanco Co., Greenfield, IN; 500 mg, i.m.)

every other week. As all cows were treated with bST at the same intervals during the trial, we assumed our results were not affected by this treatment.

Sampling and Measurements

Feed intake was monitored and recorded daily. Averaged individual cow intake and milk yield data from wk 5 through 10 (i.e., average of 42 d) were used for the statistical analysis and to calculate feed efficiency (milk yield $\div$ DMI). Individual forage and concentrate ingredients samples were collected once weekly and TMR and refusal samples were collected twice weekly. Samples were composited (equal weight basis), dried to a constant weight at 65°C in a forced-air oven for 48 h, ground in a Wiley Mill (A. H. Thomas Co., Philadelphia, PA) through a 1-mm sieve, and submitted to Cumberland Valley Analytical Services (Maugansville, MD) for wet chemistry analyses of CP, NDF, ADF, ether extract, Ca, and P and estimated NE_L. Samples were analyzed for total nonstructural carbohydrates (TNC) according to Smith (1981, except ferric cyanide was used as a colorimetric indicator; see Hristov et al., 1999).

Fecal and urine samples were collected twice during the experiment, at wk 5 and 7. Fecal samples (400 g per sampling) were collected from the rectum during the first 2 d of each sampling week at 0700 and 1500 (d 1) and 1100 h (d 2). Samples were dried at 65°C in a forced-air oven for 48 h, composited per animal and sampling period, and then ground in a Wiley mill through a 1-mm sieve. Samples were analyzed for DM, OM (AOAC, 2000), CP, and NDF and ADF (Ankom A200 fiber analyzer; Ankom Technology, Macedon, NY, and Van Soest et al., 1991). Amylase and sodium sulfide were used in the NDF analysis. Samples were analyzed for TNC with the same procedures as described for the TMR. Samples were pulverized using a Mixer Mill MM 200 (Retsch GmbH, Haan, Germany) for N analysis. Nitrogen was analyzed on a Costech ECS 4010 C/N/S elemental analyzer (Costech Analytical Technologies Inc., Valencia, CA) and multiplied by 6.25 to obtain CP. Indigestible NDF was used as an intrinsic digestibility marker and was analyzed as described by Huhtanen et al. (1994), except 25- μ m pore size filter bags (Ankom Technology) were used for the ruminal incubation. Fecal and TMR samples collected during the sampling weeks were analyzed for indigestible NDF and digestibility was calculated separately for each sampling week. Digestibility data for wk 5 and 7 were averaged for the statistical analysis.

Spot urine samples (300 mL per sampling) were collected at the time of fecal collections (wk 5 and 7) by massaging the vulva. Urine samples were acidified with

2M H₂SO₄ to a pH <3.0, diluted 1:10 with distilled water, and stored frozen (−20°C) for later analysis of allantoin (Chen et al., 1992), uric acid (Stanbio Uric Acid Kit 1045; Stanbio Laboratory Inc., San Antonio, TX), urea N (UUN; Stanbio Urea Nitrogen Kit 580; Stanbio Laboratory Inc.), and creatinine (Stanbio Creatinine Kit 0400; Stanbio Laboratory Inc.). Total N concentration in urine was analyzed as in feces. Daily urine volume and excretion of UUN and total N were estimated from urinary creatinine concentration, assuming a creatinine excretion rate of 29 mg/kg of BW (Hristov et al., 2011). Urinary purine derivative (allantoin and uric acid) excretion was used to estimate duodenal microbial N flow, as described in Hristov et al. (2009a). A ratio of purine N to total N in rumen microorganisms of 0.134 was assumed based on the data of Valadares et al. (1999). Urine samples were analyzed separately for each sampling week. Urine data for wk 5 and 7 were averaged for the statistical analysis.

Whole ruminal contents were collected at 2, 4, and 6 h after the morning feeding from the ruminally cannulated cows (2 cows per treatment) in 2 consecutive days during each sampling week (wk 5 and 7). Ruminal samples were collected from 4 locations in the rumen and the reticulum (1 sample from the ventral sac, 2 samples from the feed mat, and 1 sample from the reticulum; approximately 250 g each). Sample processing and analyses [pH, ammonia, total free amino acids (TFAA), VFA, protozoal counts, and polysaccharide-degrading enzyme activities (PSDA)] were as described elsewhere (Hristov et al., 2011). Time samples were analyzed individually for pH, ammonia, and VFA; composited per-cow and sampling-week samples (equal weight or volume basis) were analyzed for PSDA, protozoal counts, and TFAA. The effect of diet on ruminal methanogen populations was analyzed using denaturing gradient gel electrophoresis (DGGE) analysis. Rumen samples were composited per cow (equal weight basis) and used for DNA extraction using the repeated bead beating plus column (RBB+C) method (Yu and Morrison, 2004), and PCR thermoprofiles; DGGE conditions and cluster analysis of the banding patterns were as explained before (Hristov et al., 2009a). The DGGE banding patterns were then used for cluster analysis and dendrogram construction.

Blood samples from the tail vein or artery were collected 2 h after the morning feeding on 2 consecutive days during each sampling week (wk 5 and 7). Samples were centrifuged at 1,500 $\times$ g for 20 min at 4°C and frozen at −40°C for later analysis of BUN (Stanbio Urea Nitrogen Kit 580; Stanbio laboratory Inc.) and glucose (Stanbio Glucose kit 1075; Stanbio Laboratory Inc.).

Milk yield was recorded daily. Data from wk 5 through 10 (i.e., 42 d) were averaged per cow and

used in the statistical analysis and to calculate feed efficiency. Milk samples for analysis of milk fat, true protein, lactose, and MUN (Pennsylvania DHIA, University Park, PA) and milk FA composition (Hristov et al., 2010) were collected at 2 consecutive milkings (p.m. and a.m.), twice per week during each sampling week (wk 5 and 7). Consecutive milking samples were composited according to milk yield and analyzed individually for milk composition (i.e., 4 samples per cow). Data were averaged for the statistical analysis. Milk samples were composited per cow (equal volume basis) and one sample was analyzed for FA.

Body weight was recorded throughout the duration of the trial. Cows were weighed using the Afifarm 3.04E scale system (S.A.E. Afikim, Rehovot, Israel) while exiting the milk parlor. Averaged BW data from wk 5 through 10 (i.e., 42 d) were used in the statistical analysis.

Statistical Analysis

All data were analyzed using PROC MIXED of SAS (SAS Institute, 2003; SAS Inst. Inc., Cary, NC). All data, except protozoal counts, were normally distributed (Shapiro-Wilk test from the UNIVARIATE procedure of SAS; $W \geq 0.90$). Milk yield, DMI, feed efficiency, and BW data were collected from wk 5 through 10 of the experiment (i.e., 42 d), averaged, and the average values were used in the statistical analysis. Nutrient intake and digestibility, urine excretion, and milk composition data collected during the 2 sampling periods (wk 5 and 7) were first analyzed with sampling week and sampling week $\times$ treatment interaction in the model. The effect of sampling week and interaction were not significant for any of the variables and were, therefore, removed from the final model; data were averaged per cow and the average values were used in the statistical analysis. All data, except rumen pH, ammonia, and VFA were analyzed using the following model:

$$Y_{ijk} = \mu + B_i + C(B)_{ij} + \tau_k + e_{ijk},$$

where Y_{ijk} is the dependent variable, μ is the overall mean, B_i is the block, $C(B)_{ij}$ is the cow within block, and τ_k is the k th treatment, with the error term e_{ijk} assumed to be normally distributed with mean = 0 and constant variance. Block and cow within block were random effects, whereas all other factors were fixed. The DMI, milk yield, and milk composition models included a term for the covariate measurement.

Rumen pH, ammonia, and VFA data were analyzed as repeated measures assuming an ar(1) covariance structure. The following model was used:

$$Y_{ijk} = \mu + B_i + \tau_j + B\tau_{ij} + D_k + \tau D_{jk} + e_{ijk},$$

where Y_{ijk} is the dependent variable, μ is the overall mean, B_i is the block, τ_j is the treatment, $B\tau_{ij}$ is the block $\times$ treatment interaction, D_k is the time of sampling effect, and τD_{jk} is the treatment $\times$ time of sampling interaction, with the error term e_{ijk} assumed to be normally distributed with mean = 0 and constant variance. Block and block $\times$ treatment interactions were random effects, whereas all other factors were fixed. The effect of sampling week and the week $\times$ treatment interaction were not significant for any of the rumen variables and were, therefore, not included in the final model.

Significant differences were declared at $P \leq 0.05$. Differences at $P \leq 0.10$ were considered a trend toward significance. Means are presented as least squares means. When the main effect of treatment was significant, means were separated by pairwise t -test (diff option of PROC MIXED).

RESULTS

The nutrient composition of the diets (Table 1) was calculated based on the chemical analyses of the individual feed ingredients and known inclusion rate in the TMR. Intake and balance of the protein fractions and NE_L were calculated based on actual DMI, milk yield, and milk composition and calculated TMR composition. Both DMP and DMPCO were estimated to be deficient in MP, RDP, and RUP, whereas AMP slightly exceeded the MP requirements of the cows (NRC, 2001). All diets supplied NE_L in excess of requirements (NRC, 2001). Concentrations of fiber fractions and ether extract were similar among diets, but DMP and DMPCO had slightly higher TNC content compared with AMP. Due to the inclusion of Megalac, AMP and DMP diets had higher Ca content compared with the DMPCO diet, but all diets supplied Ca in excess of requirements (from +14 to +68 g/d).

The 2 MP-deficient diets increased ($P = 0.007$) rumen pH and tended to decrease ($P = 0.09$) ammonia concentration in ruminal fluid compared with the AMP diet (Table 2). Ammonia concentration decreased ($P = 0.04$) with time after feeding and was consistently lower for the MP-deficient diets throughout the sampling period (Figure 1). No effect of time ($P = 0.22$) on ruminal pH was observed. No treatment $\times$ time of sampling interaction existed for pH and ammonia concentration ($P = 0.50$ and 0.48 , respectively). Concentrations of TFAA did not differ among treatments. The DMPCO diet decreased ($P = 0.02$) the proportion of acetate compared with the AMP and DMP diets and increased

($P = 0.05$) the proportion of propionate in the total VFA compared with the AMP diet. As a consequence, the acetate:propionate ratio was decreased ($P = 0.04$) by DMPCO compared with the control. No other effects of treatment on rumen VFA were observed. The concentration of VFA decreased ($P = 0.08$ to 0.007 ; data not shown) with time after feeding and no treatment $\times$ time of sampling interaction ($P = 0.14$ to 0.69) was observed for VFA. The DMPCO diet decreased ($P = 0.05$) total protozoal and *Entodinium* spp. counts compared with the DMP diet, but not compared with the AMP diet. Carboxyl methyl cellulose, xylanase, and amylase activities of ruminal contents were not affected by treatment. Blood plasma glucose concentration was not affected by treatments, but BUN was decreased ($P < 0.001$) by the DMP and DMPCO diets compared with the AMP diet. Urinary allantoin, uric acid, and total purine derivative excretion and the estimated microbial N flow were not different among treatments.

The effect of MP supply and coconut oil supplementation on the methanogen population in the rumen was analyzed using DGGE. About 12 to 13 bands were amplified from composited samples (Figure 2). No effect of treatment on the clustering of the DGGE banding patterns was found in the dendrogram. The addition of coconut oil did not affect the number or relative abundance of bands in the DGGE gel.

Intake of CP during the sampling periods (wk 5 and 7) was lower ($P = 0.001$) for the DMP and DMPCO diets compared with the AMP diet (Table 3). Intake of DM, NDF, and ADF tended ($P = 0.09$ to 0.10) to be lower for the DMP diet and particularly for the DMPCO diet compared with the AMP diet. Intake of TNC tended to be higher ($P = 0.10$) for the DMP diet compared with the DMPCO and AMP diets. Apparent total tract digestibility of DM was decreased ($P = 0.04$) by the DMP diet compared with the AMP diet. Digestibility of CP was lower ($P = 0.01$) for both the DMP and DMPCO diets compared with the AMP diet. Digestibility of NDF and ADF was decreased ($P < 0.001$) by the DMP and DMPCO diets compared with the AMP diet. Digestibility of TNC was not affected by treatment.

As indicated earlier, N (or CP) intake was greater ($P = 0.001$) for the AMP diet compared with the DMP and DMPCO diets (Table 4). No differences in milk N secretion occurred among treatments. Urinary N and UUN excretions were higher ($P < 0.001$) for cows fed the AMP diet compared with cows fed the DMP and DMPCO diets, with no difference between the DMP and DMPCO diets. As a proportion of total urinary N, UUN concentration was the highest ($P < 0.001$) for the AMP diet, followed by the DMPCO and DMP diets. Fecal N excretion was lower ($P = 0.01$) for the

DMPCO diet compared with the AMP and DMP diets. Nitrogen excreted with feces and urine was lower ($P < 0.001$) for the DMP and DMPCO diets compared with the AMP diet. As a proportion of N intake, milk N secretion was increased ($P = 0.001$) by the MP-deficient diets compared with the AMP diet. Urine N excretion as a proportion of N intake was the highest ($P < 0.001$) for the AMP diet, followed by the DMPCO and DMP diets. The AMP diet had lower ($P = 0.002$) fecal N excretion as a proportion of N intake compared with the DMP and DMPCO diets. Total excreta N losses and N recovered in excreta and milk as a proportion of N intake were similar among diets.

Dry matter intake during the experimental period (wk 5 through 10) was similar between the AMP and DMP diets (only numerically lower for the DMP diet), but was decreased ($P = 0.002$) by the DMPCO diet (Table 5). Milk yield was decreased ($P = 0.01$) by both the DMP and DMPCO diets compared with the AMP diet. Milk fat concentration and yield and 4% FCM were lower ($P = 0.01$ to 0.001) for the DMPCO diet than the AMP and DMP diets. Milk urea N concentration was decreased ($P < 0.001$) by both the DMP and DMPCO diets compared with the AMP diet. Feed efficiency (milk yield $\div$ DMI), milk true protein concentration and yield, lactose concentration, milk NE_L and NE_L efficiency (milk $NE_L \div NE_L$ intake), BW, and BW change were not affected by treatment.

Few changes in milk FA composition were observed when MP supply was below NRC (2001) requirements, but C18:1, *cis*-9 and C18:2, *cis*-9, *cis*-12 were reduced ($P < 0.001$) by the DMP diet compared with the AMP diet (Table 6). As expected, dietary addition of C12:0 and C14:0 from coconut oil significantly increased ($P < 0.001$) their content in milk fat. Fatty acids $<C10:0$ were generally decreased ($P = 0.05$ to <0.001) by the DMPCO diet compared with the DMP diet. Additionally, C18:1, *cis*-9 and C18:2, *cis*-9, *cis*-12 contents were decreased ($P < 0.001$) with inclusion of coconut oil. In contrast, most *trans* FA were increased ($P = 0.03$ to <0.001) by the DMPCO diet compared with the DMP diet, with the exception of C18:1 *trans*-11, which was decreased ($P = 0.001$). *Cis*-9, *trans*-11 CLA was not influenced by dietary treatment, but *trans*-10, *cis*-12 CLA was increased ($P < 0.001$) to detectable levels with the DMPCO diet compared with the DMP and AMP diets.

DISCUSSION

The reduction in MP supply with the MP-deficient diets was achieved mostly by replacing the whole soybeans (and some of the canola meal) in the AMP diet with corn grain. This resulted in slightly higher TNC concentration in the MP-deficient diets. This difference

Table 2. Effect of MP supply and coconut oil on rumen fermentation, blood metabolites, and microbial protein synthesis in dairy cows

Item	Diet ^{1,2}			SEM	P-value
	AMP	DMP	DMPCO		
Rumen					
pH	6.20 ^b	6.45 ^a	6.58 ^a	0.080	0.007
Ammonia, mM	9.74	3.88	3.92	0.979	0.09
TFAA, ³ mM	3.04	2.36	1.41	0.542	0.25
Total VFA, ⁴ mM	163.7	143.1	127.1	10.51	0.06
As % of total					
Acetate	63.5 ^a	63.3 ^a	60.4 ^b	0.27	0.02
Propionate ⁵	20.1 ^b	21.3 ^{ab}	23.4 ^a	0.62	0.05
Butyrate	12.3	10.9	10.8	0.42	0.13
Isobutyrate	0.84	0.99	1.13	0.069	0.13
Valerate	1.91	2.07	2.43	0.201	0.44
Isovalerate	1.30	1.44	1.88	0.136	0.22
Acetate:propionate ⁶	3.16 ^a	2.98 ^{ab}	2.61 ^b	0.099	0.04
Total protozoa, ⁷ × 10 ⁴ /mL	94.2	144.5	58.6	16.70	
	(5.96 ^{ab})	(6.16 ^a)	(5.71 ^b)	(0.123)	0.05
<i>Entodinium</i> spp.	81.2	141.9	57.4	16.51	
	(5.90 ^{ab})	(6.15 ^a)	(5.70 ^b)	(0.123)	0.05
<i>Isotricha</i> spp.	1.0	0.4	0.5	0.49	0.39
<i>Dasytricha</i> spp.	1.9	0.6	0.2	0.13	0.07
<i>Epidinium</i> spp.	3.5	ND	ND	1.96	0.15
<i>Ophryoscolex</i> spp.	0.4 ^a	ND ^b	ND ^b	0.14	0.05
<i>Diplodinium</i> spp.	6.2 ^a	1.5 ^a	0.5 ^b	2.75	0.002
PSDA ⁸					
CMCase ⁹	244.6	254.8	192.2	57.21	0.73
Xylanase	318.3	291.9	254.5	41.98	0.61
Amylase	66.2	75.9	83.5	9.56	0.52
Blood plasma, mg/dL					
BUN	20.1 ^a	12.8 ^b	13.1 ^b	0.79	<0.001
Glucose	60.2	60.8	60.0	1.57	0.93
Urinary PD, ¹⁰ mmol/d					
Allantoin	522.2	558.2	511.3	35.93	0.62
Uric acid	48.5	50.8	42.7	3.73	0.28
Total PD	570.8	609.1	553.9	38.61	0.58
Microbial N flow, ¹¹ g/d	386.1	414.6	373.7	28.11	0.58

^{a,b}Within a row, means without a common superscript letter differ ($P < 0.05$).

¹Least squares means; n = 36 for rumen pH, ammonia, and VFA data; n = 12 for rumen TFAA, protozoa, and PSDA data; n = 72 for blood plasma and urinary purine derivative (PD) data; n represents number of observations used in the statistical analysis.

²AMP = diet adequate in MP, containing 16.7% CP; DMP = diet deficient in MP, containing 14.8% CP; DMPCO = the DMP diet, supplemented with coconut oil.

³Total free amino acids.

⁴AMP versus DMPCO, $P = 0.02$.

⁵AMP versus DMP, $P = 0.06$.

⁶DMP versus DMPCO, $P = 0.10$.

⁷Actual protozoal counts were log₁₀ transformed for the statistical analysis. The standard errors of the means for the log₁₀-transformed data are given in parentheses. ND = not detected (assumed 0).

⁸Polysaccharide-degrading activities (expressed as nmol of reducing sugars as glucose released per milliliter of ruminal fluid per minute).

⁹Carboxymethylcellulase.

¹⁰Excretion of urinary PD.

¹¹Estimated microbial N outflow from the rumen (based on urinary PD excretion).

may have resulted in a higher concentration of ruminally available energy with the MP-deficient diets. It is unlikely, however, that dietary energy was limiting microbial protein synthesis or production in this study, as NE_L supply exceeded requirements (according to NRC, 2001) for all diets.

The ruminal effects reported in this study have to be interpreted with caution due to the very low number of experimental units (2 ruminally cannulated cows per treatment). One of the significant effects of coconut oil in this experiment was on rumen protozoa. In previous studies, intraruminal administration of 530 g of coconut

Figure 1. Effect of MP supply and coconut oil on ruminal ammonia concentration in dairy cows (means $\pm$ SE; effect of treatment, $P = 0.09$; effect of time of sampling, $P = 0.006$; treatment time interaction, $P = 0.32$). AMP = diet adequate in MP, containing 16.7% CP; DMP = diet deficient in MP, containing 14.8% CP; DMPCO = the DMP diet, supplemented with coconut oil.

oil eliminated approximately 83% of ruminal protozoa (Hristov et al., 2009a). A similar dose of coconut oil decreased protozoal counts by only 38% (compared with the control diet, AMP) in the present experiment. As discussed previously (Hristov et al., 2009a), this discrepancy is likely due to the application method of coconut oil (intraruminal vs. TMR). In the current experiment, coconut oil would have been introduced into the rumen gradually through the TMR consumed, resulting in a lower ruminal concentration of C12:0 (and other coconut oil FA) throughout the feeding cycle. As coconut oil was delivered with the morning portion of the TMR, however, it is likely that ruminal concentrations of coconut oil FA might have been slightly higher for a portion of the feeding cycle compared with mixing the oil with the entire TMR. A similar effect of lauric acid application mode was suggested by Faciola et al. (2008).

The concept of this experiment was that the potential decrease in MP supply when cows are fed a decreased-CP diet may be compensated for by increased microbial protein production and outflow from the rumen due to the defaunating effect of coconut oil (Hristov and Jouany, 2005; Hristov et al., 2009a). In this experiment, we

did not directly determine the effect of diet on bacterial population in the rumen, but pH, acetate:propionate ratio, and some numerical differences in PSDA could be interpreted as signs of depressed rumen bacterial activities by the DMP diet. Coconut oil supplementation seemed to further depress rumen fermentation, based on the numerical decreases in total VFA and acetate proportion, which is in agreement with the literature (Dong et al., 1997; Hristov et al., 2009a). In spite of the decrease in protozoal counts in the DMPCO diet, microbial N flow was not different among treatments. Lauric acid numerically (Hristov et al., 2004a; 2009a), or significantly (Hristov et al., 2011) decreased microbial N flow in our previous experiments, but no such effect was observed for coconut oil (Hristov et al., 2009a).

The DGGE analysis showed no specific effect of addition of coconut oil on the methanogen populations in the rumen. Methane-suppression effects of coconut oil have been reported both in vitro (Dong et al., 1997; Machmüller et al., 1998; Dohme et al., 2004) and in vivo (Machmüller and Kreuzer, 1999; Hollmann et al., 2009; Hristov et al., 2009a). Based on dot-blot analyses, it was previously proposed that association with protozoa accounted for 90% of the total ruminal methanogen

Table 3. Effect of MP supply and coconut oil on intake of nutrients and total tract apparent digestibility in dairy cows

Item	Diet ^{1,2}			SEM	P-value
	AMP	DMP	DMPCO		
Intake, ³ kg/d					
DM	24.0	23.4	20.8	1.07	0.09
OM	22.1	21.7	19.3	0.98	0.11
CP	4.0 ^a	3.5 ^b	3.1 ^b	0.16	0.001
NDF	7.6	7.5	6.6	0.34	0.10
ADF	5.1	5.0	4.4	0.23	0.09
TNC ⁴	6.6	7.1	6.2	0.32	0.10
Apparent digestibility, %					
DM	69.7 ^a	68.4 ^b	69.0 ^{ab}	0.37	0.04
OM	70.8	69.4	69.6	0.41	0.06
CP	66.8 ^a	63.6 ^b	65.6 ^b	0.63	0.01
NDF	54.0 ^a	49.2 ^b	48.1 ^b	0.68	<0.001
ADF	48.7 ^a	41.5 ^b	41.6 ^b	0.98	<0.001
TNC	85.7	87.3	87.5	0.66	0.11

^{a,b}Within a row, means without a common superscript letter differ ($P < 0.05$).
¹Least squares means; n = 36; n represents number of observations used in the statistical analysis.
²AMP = diet adequate in MP, containing 16.7% CP; DMP = diet deficient in MP, containing 14.8% CP; DMPCO = the DMP diet, supplemented with coconut oil.
³Intake during sampling weeks.
⁴Total nonstructural carbohydrates.

population (Sharp et al., 1998). Therefore, elimination of ruminal protozoa had been proposed as a strategy to decrease methane production in the rumen. However, more recent studies have revealed that ruminal

methanogens are much more diverse than previously thought, whether assessed by a procedure similar to DGGE (Nicholson et al., 2007) or by a more complete analysis based on clone libraries (Wright et al., 2007).

Figure 2. Denaturing gradient gel electrophoresis profiles of methanogens produced from community DNA samples from whole ruminal contents from cows fed: AMP = diet adequate in MP, containing 16.7% CP; DMP = diet deficient in MP, containing 14.8% CP; DMPCO = the DMP diet, supplemented with coconut oil. The lanes are labeled with the cow number, period number (P represents the sampling week: P1 = wk 5 and P2 = wk 7), and treatment. The calculated similarity coefficients determined using BioNumerics software (Applied Maths Inc., Austin, TX) are shown on the top left-hand side.

Table 4. Effect of MP supply and coconut oil on milk N secretion and urinary and fecal N losses in dairy cows

Item	Diet ^{1,2}			SEM	P-value
	AMP	DMP	DMPCO		
N intake, ³ g/d	640.7 ^a	554.6 ^b	488.9 ^b	25.93	0.001
N secretion and excretion, g/d					
Milk N	174.7	174.3	169.6	8.47	0.90
Urine N	193.7 ^a	122.2 ^b	122.5 ^b	8.77	<0.001
UUN, ⁴ g/d	153.1 ^a	69.3 ^b	80.0 ^b	5.06	<0.001
UUN ÷ total urinary N, %	80.3 ^a	56.7 ^c	66.4 ^b	2.41	<0.001
Fecal N	213.2 ^a	207.0 ^a	174.7 ^b	9.43	0.01
Total excreta N ⁵	406.7 ^a	329.2 ^b	298.6 ^b	16.88	<0.001
As proportion of N intake, %					
Milk N	27.6 ^b	32.0 ^a	35.1 ^a	1.36	0.001
Urine N	30.1 ^a	22.4 ^c	25.1 ^b	1.06	<0.001
Fecal N	33.2 ^b	37.9 ^a	35.7 ^a	0.83	0.002
Total excreta N	63.3	60.3	61.3	1.55	0.37
Total N recovered in excreta and milk ⁶	92.2	94.2	95.3	2.22	0.52

^{a-c}Within a row, means without a common superscript letter differ ($P < 0.05$).

¹Least squares means; n = 36; n represents number of observations used in the statistical analysis.

²AMP = diet adequate in MP, containing 16.7% CP; DMP = diet deficient in MP, containing 14.8% CP; DMPCO = the DMP diet, supplemented with coconut oil.

³Crude protein intake during sampling weeks ÷ 6.25.

⁴Urinary urea nitrogen.

⁵Total excreta N losses = urine N + fecal N.

⁶Total N recovered in excreta and milk = urine N + fecal N + milk N.

The effect of the MP-deficient diets and coconut oil supplementation on total tract fiber digestibility was substantial. This depression in digestibility is likely a reflection of RDP deficiency and depressed ruminal microbial activities. The numerical trend for decreased total VFA concentration, particularly with the DMP diet, is indicative of depressed carbohydrate fermenta-

tion in the rumen. The numerically decreased ammonia concentration with the MP-deficient diets is also indicative of potential RDP deficiency. These trends did not reach statistical significance due to the very low number of experimental units for the ruminal fermentation data. Ruminal cellulolytic bacteria such as *Fibrobacter succinogenes*, *Ruminococcus albus*, and

Table 5. Effect of MP supply and coconut oil on milk yield and composition in dairy cows

Item	Diet ^{1,2}			SEM	P-value
	AMP	DMP	DMPCO		
DM intake, ³ kg/d	24.7 ^a	23.8 ^a	21.6 ^b	0.60	0.002
Milk yield, ³ kg/d	39.3 ^a	36.2 ^b	34.4 ^b	1.06	0.01
Milk ÷ DMI	1.61	1.56	1.61	0.04	0.60
Milk fat, %	3.61 ^a	3.73 ^a	3.05 ^b	0.11	0.001
Yield, kg/d	1.40 ^a	1.33 ^a	1.08 ^b	0.06	0.001
4% FCM, kg/d	36.7 ^a	34.4 ^a	30.3 ^b	1.25	0.01
Milk true protein, %	2.97	3.03	3.04	0.04	0.38
Yield, kg/d	1.11	1.13	1.07	0.05	0.82
Milk lactose, %	4.72	4.71	4.73	0.03	0.93
Milk NE _L , ⁴ Mcal/d	25.4	25.2	22.1	1.35	0.17
Milk NE _L as % of intake	65.4	63.2	62.0	2.82	0.63
MUN, mg/dL	12.5 ^a	8.3 ^b	9.5 ^b	0.34	<0.001
BW, kg	648	640	649	4.9	0.27
BW change, kg	18.2	19.6	22.2	8.68	0.90

^{a,b}Within a row, means without a common superscript letter differ ($P < 0.05$).

¹Least squares means; n = 36; n represents number of observations used in the statistical analysis.

²AMP = diet adequate in MP, containing 16.7% CP; DMP = diet deficient in MP, containing 14.8% CP; DMPCO = the DMP diet, supplemented with coconut oil.

³Averaged DMI and milk yield for the experimental period (42 d).

⁴Milk NE_L (Mcal/d) = kg of milk × (0.0929 × % fat + 0.0563 × % true protein + 0.0395 × % lactose) (NRC, 2001).

Table 6. Effect of MP supply and coconut oil on milk fatty acid composition (g/100 g of total fatty acids) in dairy cows

Fatty acid	Diet ^{1,2}			SEM	P-value
	AMP	DMP	DMPCO		
C4:0	4.16 ^a	3.83 ^a	2.88 ^b	0.214	<0.001
C6:0	1.82 ^a	1.87 ^a	1.36 ^b	0.121	0.01
C8:0	0.88 ^{ab}	0.97 ^a	0.73 ^b	0.067	0.05
C10:0	1.74	2.04	1.77	0.146	0.27
C12:0	1.96 ^c	2.46 ^b	7.25 ^a	0.156	<0.001
C14:0	6.91 ^c	7.99 ^b	11.54 ^a	0.183	<0.001
C14:1	0.71 ^b	0.99 ^b	1.66 ^a	0.115	<0.001
C15:0	0.56 ^b	0.62 ^b	0.73 ^a	0.021	<0.001
C16:0	26.5 ^b	27.7 ^a	25.6 ^b	0.35	<0.001
C16:1	1.45 ^b	1.82 ^b	2.43 ^a	0.154	<0.001
C17:0	0.34 ^a	0.32 ^a	0.29 ^b	0.007	<0.001
C18:0	9.82 ^a	8.67 ^a	6.46 ^b	0.468	<0.001
C18:1, <i>trans</i> -6-8	0.74 ^b	0.75 ^b	1.01 ^a	0.076	0.03
C18:1, <i>trans</i> -9	0.52 ^b	0.53 ^b	0.71 ^a	0.049	0.02
C18:1, <i>trans</i> -10	0.89 ^b	1.29 ^b	5.55 ^a	0.635	<0.001
C18:1, <i>trans</i> -11	1.76 ^a	1.50 ^a	1.05 ^b	0.124	0.001
C18:1, <i>trans</i> -12	0.88	0.91	0.84	0.036	0.39
C18:1, <i>cis</i> -9	30.3 ^a	28.4 ^b	21.4 ^c	0.58	<0.001
C18:2, <i>cis</i> -9, <i>cis</i> -12	4.23 ^a	3.36 ^b	2.59 ^c	0.104	<0.001
C20:0	0.16 ^a	0.15 ^a	0.11 ^b	0.006	<0.001
C18:3	0.35	0.31	0.27	0.035	0.25
CLA- <i>cis</i> -9, <i>trans</i> -11	0.81	0.80	0.68	0.067	0.38
CLA- <i>trans</i> -10, <i>cis</i> -12	ND ^b	ND ^b	0.04 ^a	0.004	<0.001
Σ unidentified	2.58 ^b	2.69 ^b	3.14 ^a	0.090	<0.001
Σ saturated fatty acids ³	54.8	56.7	58.7	1.09	0.05
Σ <i>trans</i> fatty acids	5.59 ^b	5.77 ^b	9.86 ^a	0.778	0.001
Σ MUFA ⁴	37.2	36.2	34.6	0.94	0.15
Σ PUFA ⁴	4.58 ^a	3.67 ^b	2.85 ^c	0.117	<0.001

^{a-c}Within a row, means without a common superscript letter differ ($P < 0.05$).

¹Least squares means; n = 36; n represents number of observations used in the statistical analysis; ND = not detected.

²AMP = diet adequate in MP, containing 16.7% CP; DMP = diet deficient in MP, containing 14.8% CP; DMPCO = the DMP diet, supplemented with coconut oil.

³AMP versus DMPCO, $P = 0.02$.

⁴MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids.

R. flavefaciens require a certain level of degradable N (especially ammonia N) to effectively ferment carbohydrates in the rumen (Atasoglu et al., 2001). Therefore, the tendency for lower ammonia concentration in the DMP and DMPCO diets may be responsible for the depressed total tract fiber digestibility with these diets. Requirements for ammonia of the fibrolytic species and ruminal carbohydrate availability may be interrelated. Erdman et al. (1986), for example, demonstrated that the minimum ammonia concentrations required to maximize digestion depended on feed fermentability; if readily degradable carbohydrates were available, higher rumen ammonia N concentration was needed for maximum digestion. The MP-deficient diets in this experiment contained a slightly higher concentration of TNC and may have required greater ammonia concentration for maximum ruminal digestion compared with the AMP diet. Broderick (2003) and Colmenero and Broderick (2006) also reported linear increases in NDF and ADF digestion with increasing dietary CP levels.

The greater apparent CP digestibility with the AMP diet can be explained by dilution of metabolic fecal N and more digestible CP content of the adequate- versus MP-deficient diets (Broderick, 2003; Hristov et al., 2004a). Coconut oil supplementation did not decrease fiber digestibility compared with the DMP diet. Others have reported decreased fiber digestibility with coconut oil supplementation both in vitro (Dong et al., 1997; Dohme et al., 2000) and in vivo (Sutton et al., 1983; Machmüller and Kreuzer, 1999). Rumen protozoa play an important role in fiber degradation in the rumen (Williams and Coleman, 1985) and decreased protozoal population was perhaps the reason for the lowered total tract fiber digestibility in these studies. This effect, however, is not consistent, and in some studies, a drastic decrease in protozoal population due to coconut oil or C12:0 supplementation did not result in depressed fiber digestibility, suggesting perhaps a significant compensatory digestion of dietary fiber in the lower tract (Hristov et al., 2004a, 2009a).

Due to overall depression in DMI and lower dietary CP concentration, N intake was decreased by the MP-deficient diets. Depression of DMI with C12:0 or coconut oil was reported by Faciola et al. (2005, 2008), Hristov et al. (2011), Machmüller and Kreuzer (1999), Hollmann and Beede (2008), and Hollmann et al. (2009). In other studies, however, C12:0 and coconut oil had no effect on DMI (Hristov et al., 2009a). Again, the application method (feeding with the TMR vs. intraruminal administration) is the likely explanation for these different responses.

Urinary N losses are more responsive to N intake than fecal N losses, or milk N secretion (Kebreab et al., 2002). The reduction in fecal N losses with the DMPCO diet resulted from reduced DM and N intakes. Similar to the current study, Colmenero and Broderick (2006) also observed gradual increases of urinary N and urea N excretion with increasing dietary CP concentrations. Urinary urea is the main precursor for ammonia emitted from manure (Hristov et al., 2009b) and it was expected that DMP-diet manure would have lower ammonia emission than AMP-diet manure. Indeed, our companion study showed that cumulative ammonia emissions from manure were decreased 45% ($P < 0.001$) by the DMP diet compared with the AMP diet (Lee et al., 2010). Ammonia produced by protein breakdown, microbial lysis, tissue protein metabolism, or from urea recycled and unutilized in the rumen is primarily excreted in urine (Muck, 1982; Reynolds and Kristensen, 2008). Reynolds and Kristensen (2008) showed that the amount of N recycled back to the rumen is inversely related to the amount of dietary N consumed. Apparently, compared with the AMP diet, the MP-deficient diets resulted in lowered N intake, a more efficient utilization of dietary protein, and likely lowered ruminal ammonia production, which led to lower urinary N losses and greater milk N efficiency. More efficient utilization of dietary N with decreasing dietary CP concentration was also reported by Cyriac et al. (2008).

The effects of dietary CP concentration on dairy cow performance have been well documented. In some studies (Leonardi et al., 2003; Colmenero and Broderick, 2006), relatively low CP diets ranging from 13.2 to 15.1% CP, did not affect production compared with diets ranging in CP from 16 to 18%. In other studies, however, decreased dietary CP concentration decreased milk production or milk protein content (Cressman et al., 1980; Wu and Satter, 2000). In the present experiment, the DMP diet decreased milk yield by about 3 kg compared with the AMP diet. A similar effect of lowering dietary CP was reported by Cyriac et al. (2008). The numerical decrease in DMI with the DMP diet (about 1 kg/d) was partially responsible for the

decreased milk yield in the current experiment because DMI is one of the most important factors in controlling milk yield (Hristov et al., 2004c). Further decreases of milk (numerical) and 4% FCM yields caused by the DMPCO diet can be also explained by the significant decrease in DMI. Diet palatability may be partially responsible for the decreased DMI with the DMPCO diet, but depressed rumen fermentation also can cause decreased feed intake (Firkins et al., 1986; Allen, 2000). It is worth pointing out that NRC (2001) predicted, based on actual nutrient intake and milk composition, MP-allowable milk yields of 38.8 ± 5.9 , 29.6 ± 4.8 , and 26.2 ± 4.5 kg/d for AMP, DMP, and DMPCO diets, respectively. This represented underestimation of the actual milk yield of the cows by 0.8, 7.1, and 8.5 kg/d, respectively. Although the model apparently predicted closely milk yield of the cows on the AMP diet, the underprediction for the MP-deficient diets was large. This confirms previous discussions about deficiencies of the NRC (2001) protein model, particularly when dietary protein (or MP) intake is below requirements. Inaccurate estimation of feed RDP, and perhaps overestimation of RDP requirements, in addition to unaccounted physiological mechanisms such as urea recycling (Huhtanen and Hristov, 2009), are likely responsible for the gross underprediction of milk yield for the MP-deficient diets in this experiment.

Milk urea N concentration was decreased with the MP-deficient diets in this experiment. Milk urea N has been extensively studied as a tool to predict feed N utilization efficiency and losses in dairy cows (Kauffman and St. Pierre, 2001) and is clearly related to dietary CP concentration (Colmenero and Broderick, 2006). In agreement with Agle et al. (2010) and Colmenero and Broderick (2006), milk protein content and yield were not altered by treatments.

The DMPCO diet significantly depressed milk fat percentage and yields compared with the other diets. The milk fat depressing effect of MCSFA has been consistently reported. Hristov et al. (2009a) and Faciola et al. (2008) observed numerical or statistically significant decreases in milk fat yield caused by C12:0 or coconut oil. Myristic acid, the other major MCSFA in coconut oil, has no effect on milk fat content (Hristov et al., 2011) and it is apparent that the milk fat depression observed in the current experiment was caused by the C12:0 in coconut oil. Much larger quantities (710 g/d) of C14:0 may also cause milk fat depression (Odongo et al., 2007). Other ruminal mechanisms, however, cannot be excluded. Shingfield and Griinari (2007), for example, demonstrated that milk fat depression can be caused by (1) depressed fiber digestion with low acetic acid production, thus limiting precursors for de novo synthesis of shorter-chain milk FA, and (2) incomplete

biohydrogenation in the rumen and enhanced rumen production of some CLA isomers causing milk fat depression. Bauman and Griinari (2003) and Baumgard et al. (2001) reported that biohydrogenation intermediates such as *trans*-10,*cis*-12 CLA are potent inhibitors of milk fat synthesis. In the present experiment, both decreased fiber digestion and significant increase of *trans*-10 C18:1 and *trans*-10,*cis*-12 CLA in milk were observed for the cows fed the DMPCO diet.

Changes in dietary protein had minimal influence on milk FA composition. Total polyunsaturated FA concentration was greater with the AMP diet compared with the DMP diet and this could be related to the inclusion of whole, heat-treated soybeans. The additional fat contained in this feed ingredient and its minimal processing would facilitate increased C18:1, *cis*-9 and C18:2, *cis*-9,*cis*-12 for absorption (Solomon et al., 2000). Addition of coconut oil increased the fat content of the diet and increased milk fat content of C12:0 and C14:0, the major FA constituents of the oil. Alterations in biohydrogenation are also evident from increases in the concentrations of *trans* FA and the presence of *trans*-10,*cis*-12 CLA in the milk fat of cows fed the DMPCO diet. Reductions in short-chain FA synthesis de novo were detected and would be related to the milk fat depression observed with coconut oil inclusion and production of biohydrogenation intermediates known to inhibit milk fat synthesis by mammary epithelial cells.

CONCLUSIONS

In this experiment, MP-deficient diets depressed fiber digestibility and decreased milk production compared with an MP-adequate diet. The NRC (2001) model severely underpredicted (7 to 8 kg/d) milk yield on the MP-deficient diets. Lowering the MP supply and dietary CP considerably decreased urinary N and total excreta N losses and increased milk N efficiency. Supplementing the MP-deficient diet with coconut oil decreased ruminal protozoal counts, but depressed DMI and cow productivity. In addition, coconut oil caused milk fat depression. Coconut oil supplementation had no effect on the methanogen population in the rumen. As expected, coconut oil supplementation increased milk C12:0 and C14:0 content.

REFERENCES

- Agle, M., A. N. Hristov, S. Zaman, C. Schneider, P. Ndegwa, and V. K. Vaddella. 2010. The effects of ruminally degraded protein on rumen fermentation and ammonia losses from manure in dairy cows. *J. Dairy Sci.* 93:1625–1637.
- Allen, M. S. 2000. Effects of diet on short-term regulation of feed intake by lactating dairy cattle. *J. Dairy Sci.* 83:1598–1624.
- AOAC. 2000. Official Methods of Analysis. 17th ed. Association of Official Analytical Chemists, Arlington, VA.
- Atasoglu, C., C. J. Newbold, and R. J. Wallace. 2001. Incorporation of [¹⁵N]ammonia by the cellulolytic ruminal bacteria *Fibrobacter succinogenes* BL2, *Ruminococcus albus* SY3, and *Ruminococcus flavefaciens* 17. *Appl. Environ. Microbiol.* 67:2819–2822.
- Bauman, D. E., and J. M. Griinari. 2003. Nutritional regulation of milk fat synthesis. *Annu. Rev. Nutr.* 23:203–227.
- Baumgard, L. H., J. K. Sangster, and D. E. Bauman. 2001. Milk fat synthesis in dairy cows is progressively reduced by increasing supplemental amounts of *trans*-10, *cis*-12 conjugated linoleic acid (CLA). *J. Nutr.* 131:1764–1769.
- Broderick, G. A. 2003. Effects of varying dietary protein and energy levels on the production of lactating dairy cows. *J. Dairy Sci.* 86:1370–1381.
- Chen, X. B., Y. K. Chen, M. F. Franklin, E. R. Orskov, and W. J. Shand. 1992. The effect of feed intake and body weight on purine derivative excretion and microbial protein supply in sheep. *J. Anim. Sci.* 70:1534–1542.
- Coleman, G. S. 1975. The inter-relationships between rumen ciliate protozoa and bacteria. Pages 149–164 in *Digestion and Metabolism in the Ruminant*. I. W. McDonald and A. C. Warner, ed. University of New England, Armidale, New South Wales, Australia.
- Coleman, G. S., and D. C. Sandford. 1979. The engulfment and digestion of mixed rumen bacteria and individual bacteria species by single and mixed species of rumen ciliate protozoa grown *in vivo*. *J. Agric. Sci.* 92:729–742.
- Colmenero, J. J., and G. A. Broderick. 2006. Effect of dietary crude protein concentration on milk production and nitrogen utilization in lactating dairy cows. *J. Dairy Sci.* 89:1704–1712.
- Cressman, S. G., D. G. Grieve, G. K. Macleod, E. E. Wheeler, and L. G. Young. 1980. Influence of dietary protein concentration on milk production by dairy cattle in early lactation. *J. Dairy Sci.* 63:1839–1847.
- Cyriac, J., A. G. Rius, M. L. McGilliard, R. E. Pearson, B. J. Bequette, and M. D. Hanigan. 2008. Lactation performance of mid-lactation dairy cows fed ruminally degradable protein at concentrations lower than National Research Council recommendations. *J. Dairy Sci.* 91:4704–4713.
- Dohme, F., A. Machmüller, B. L. Estermann, P. Pfister, A. Wasserfallen, and M. Kreuzer. 1999. The role of the rumen ciliate protozoa for methane suppression caused by coconut oil. *Lett. Appl. Microbiol.* 29:187–192.
- Dohme, F., A. Machmüller, F. Sutter, and M. Kreuzer. 2004. Digestive and metabolic utilization of lauric, myristic and stearic acid in cows, and associated effects on milk fat quality. *Arch. Anim. Nutr.* 58:99–116.
- Dohme, F., A. Machmüller, A. Wasserfallen, and M. Kreuzer. 2000. Comparative efficiency of various fats rich in medium-chain fatty acids to suppress ruminal methanogenesis as measured with RUSITEC. *Can. J. Anim. Sci.* 80:473–482.
- Dong, Y., H. D. Bae, T. A. McAllister, G. W. Mathison, and K.-J. Cheng. 1997. Lipid-induced depression of methane production and digestibility in the artificial rumen system (RUSITEC). *Can. J. Anim. Sci.* 77:269–278.
- Erdman, R. A., G. H. Proctor, and J. H. Vandersall. 1986. Effect of rumen ammonia concentration on *in situ* rate and extent of digestion of feedstuffs. *J. Dairy Sci.* 69:2312–2320.
- Eugène, M., H. Archimède, and D. Sauvant. 2004. Quantitative meta-analysis on the effects of defaunation of the rumen on growth, intake and digestion in ruminants. *Livest. Prod. Sci.* 85:81–97.
- Faciola, A. P., G. A. Broderick, A. N. Hristov, and M. I. Leão. 2005. Effect of feeding different levels of lauric acid on ruminal protozoa, and milk production in dairy cows. *J. Dairy Sci.* 88(Suppl. 1):178 (Abstr.)
- Faciola, A. P., G. A. Broderick, A. N. Hristov, and J. A. Pires. 2008. Effect of four levels of lauric acid on ruminal protozoa, milk production and composition in dairy cows. *J. Dairy Sci.* 91(Suppl. 1):76 (Abstr.)
- Firkins, J. L., L. L. Berger, N. R. Merchen, G. C. Fahey Jr., and D. R. Nelson. 1986. Effects of feed intake and protein degradability

- on ruminal characteristics and site of digestion in steers. *J. Dairy Sci.* 69:2111–2123.
- Frank, B., and C. Swensson. 2002. Relationship between content of crude protein in rations for dairy cows and milk yield, concentration of urea in milk and ammonia emissions. *J. Dairy Sci.* 85:1829–1838.
- Hollmann, M., and D. K. Beede. 2008. Dietary coconut oil and animal fat blend decrease lactational performance of Holstein cows fed a high starch diet. *J. Dairy Sci.* 91(E-Suppl. 1):331 (Abstr.)
- Hollmann, M., W. J. Powers, A. Fogiel, N. M. Bello, J. S. Liesman, and D. K. Beede. 2009. Increasing dietary concentration of coconut oil reduces enteric methane emission from lactating Holstein cows. *J. Dairy Sci.* 92(E-Suppl. 1):343.
- Hristov, A. N., K. L. Grandeen, J. K. Ropp, and M. A. McGuire. 2004a. Effect of sodium laurate on ruminal fermentation and utilization of ruminal ammonia nitrogen for milk protein synthesis in dairy cows. *J. Dairy Sci.* 87:1820–1831.
- Hristov, A. N., W. Hazen, and J. W. Ellsworth. 2006. Nitrogen, phosphorus, and potassium balance and potentials for reducing phosphorus imports in Idaho dairy farms. *J. Dairy Sci.* 89:3702–3712.
- Hristov, A. N., M. Ivan, and T. A. McAllister. 2004b. In vitro effects of individual fatty acids on protozoal numbers and on fermentation products in ruminal fluid from cattle fed a high concentrate, barley based diet. *J. Anim. Sci.* 82:2693–2704.
- Hristov, A. N., and J.-P. Jouany. 2005. Factors affecting the efficiency of nitrogen utilization in the rumen. Pages 117–166 in *Nitrogen and Phosphorus Nutrition of Cattle and Environment*. A. N. Hristov and E. Pfeffer, ed. CAB International, Wallingford, UK.
- Hristov, A. N., C. Lee, T. Cassidy, M. Long, K. Heyler, B. Corl, and R. Forster. 2011. Effects of lauric and myristic acids on ruminal fermentation, production, and milk fatty acid composition in lactating dairy cows. *J. Dairy Sci.* 94:382–395.
- Hristov, A. N., T. A. McAllister, F. H. Van Herk, K. J. Cheng, C. J. Newbold, and P. R. Cheeke. 1999. Effect of *Yucca schidigera* on ruminal fermentation and nutrient digestion in heifers. *J. Anim. Sci.* 77:2554–2563.
- Hristov, A. N., W. J. Price, and B. Shafii. 2004c. A meta-analysis examining the relationship among dietary factors, dry matter intake, and milk and milk protein yield in dairy cows. *J. Dairy Sci.* 87:2184–2196.
- Hristov, A. N., M. Vander Pol, M. Agle, S. Zaman, C. Schneider, P. Ndegwa, V. K. Vaddella, K. Johnson, K. J. Shingfield, and S. K. Karnati. 2009a. Effect of lauric acid and coconut oil on ruminal fermentation, digestion, ammonia losses from manure, and milk fatty acid composition in lactating cows. *J. Dairy Sci.* 92:5561–5582.
- Hristov, A. N., G. Varga, T. Cassidy, M. Long, K. Heyler, K. R. Karnati, B. Corl, C. J. Hovde, and I. Yoon. 2010. Effect of yeast culture on ruminal fermentation and nutrient utilization in dairy cows. *J. Dairy Sci.* 93:682–692.
- Hristov, A. N., S. Zaman, M. Vander Pol, P. Ndegwa, L. Campbell, and S. Silva. 2009b. Nitrogen losses from dairy manure estimated through nitrogen mass balance and chemical markers. *J. Environ. Qual.* 38:2438–2448.
- Huhtanen, P., and A. N. Hristov. 2009. A meta-analysis of the effects of protein concentration and degradability on milk protein yield and milk N efficiency in dairy cows. *J. Dairy Sci.* 92:3222–3232.
- Huhtanen, P., K. Kaustell, and S. Jaakkola. 1994. The use of internal markers to predict total digestibility and duodenal flow of nutrients in cattle given six different diets. *Anim. Feed Sci. Technol.* 48:211–227.
- Karnati, S. K. R., Z. Yu, and J. L. Firkins. 2009. Investigating unsaturated fat, monensin, or bromoethanesulfonate in continuous cultures retaining ruminal protozoa. II. Interaction of treatment and presence of protozoa on prokaryotic communities. *J. Dairy Sci.* 92:3861–3873.
- Kauffman, A. J., and N. R. St-Pierre. 2001. The relationship of milk urea nitrogen to urine nitrogen excretion in Holstein and Jersey cows. *J. Dairy Sci.* 84:2284–2294.
- Kebreab, E., J. France, J. A. Mills, R. Allison, and J. Dijkstra. 2002. A dynamic model of N metabolism in the lactating dairy cow and an assessment of impact of N excretion on the environment. *J. Anim. Sci.* 80:248–259.
- Lee, C., A. N. Hristov, C. Dell, G. Feyereisen, J. Kaye, and D. Beegle. 2010. Effect of dietary protein concentration on ammonia emission from dairy manure. *J. Dairy Sci.* 93(Suppl. 1):690 (Abstr.)
- Leonardi, C., M. Stevenson, and L. E. Armentano. 2003. Effect of two levels of crude protein and methionine supplementation on performance of dairy cows. *J. Dairy Sci.* 86:4033–4042.
- Machmüller, A., and M. Kreuzer. 1999. Methane suppression by coconut oil and associated effects on nutrient and energy balance in sheep. *Can. J. Anim. Sci.* 79:65–72.
- Machmüller, A., D. A. Ossowski, M. Wanner, and M. Kreuzer. 1998. Potential of various fatty feeds to reduce methane release from rumen fermentation in vitro (Rusitec). *Anim. Feed Sci. Technol.* 71:117–130.
- Muck, R. E. 1982. Urease activity in bovine feces. *J. Dairy Sci.* 65:2157–2163.
- NRC. 2001. *Nutrient Requirements of Dairy Cattle*. 7th rev. ed. Natl. Acad. Sci., Washington DC.
- Nicholson, M. J., P. N. Evans, and K. N. Joblin. 2007. Analysis of methanogen diversity in the rumen using temporal temperature gradient gel electrophoresis: Identification of uncultured methanogens. *Microb. Ecol.* 54:141–150.
- Odongo, N. E., M. M. Or-Rashid, E. Kebreab, J. France, and B. W. McBride. 2007. Effect of supplementing myristic acid in dairy cow rations on ruminal methanogenesis and fatty acid profile in milk. *J. Dairy Sci.* 90:1851–1858.
- Reynolds, C. K., and N. B. Kristensen. 2008. Nitrogen recycling through the gut and the nitrogen economy of ruminants: An asynchronous symbiosis. *J. Anim. Sci.* 86(E. Suppl.):E293–E305.
- SAS Institute. 2003. *SAS User's Guide: Statistics*. SAS Inst. Inc., Cary, NC.
- Sharp, R., C. J. Ziemer, M. D. Stern, and D. A. Stahl. 1998. Taxon-specific associations between protozoal and methanogen populations in the rumen and a model rumen system. *FEMS Microbiol. Ecol.* 26:71–78.
- Shingfield, K. J., and J. M. Griinari. 2007. Role of biohydrogenation intermediates in milk fat depression. *Eur. J. Lipid Sci. Technol.* 109:799–816.
- Smith, D. 1981. Removing and analyzing carbohydrate from plant tissue. Wisconsin Agric. Exp. Sta. Rep. No. R 2107. University of Wisconsin, Madison.
- Solomon, R., L. E. Chase, D. Ben-Ghedalia, and D. E. Bauman. 2000. The effect of nonstructural carbohydrate and addition of full fat extruded soybeans on the concentration of conjugated linoleic acid in the milk fat of dairy cows. *J. Dairy Sci.* 83:1322–1329.
- Sutton, J. D., R. Knight, A. B. McAllan, and R. H. Smith. 1983. Digestion and synthesis in the rumen of sheep given diets supplemented with free and protected oils. *Br. J. Nutr.* 49:419–432.
- USEPA (United States Environmental Protection Agency). 2004. *National Emission Inventory—Ammonia Emissions from Animal Husbandry Operations*. US EPA, Washington, DC.
- Valadares, R. F. D., G. A. Broderick, S. C. Valadares Filho, and M. K. Clayton. 1999. Effect of replacing alfalfa silage with high moisture corn on ruminal protein synthesis estimated from excretion of total purine derivatives. *J. Dairy Sci.* 82:2686–2696.
- Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* 74:3583–3597.
- Williams, A. G., and G. S. Coleman. 1985. Hemicellulose-degrading enzymes in rumen ciliate protozoa. *Curr. Microbiol.* 12:85–90.
- Wright, A.-D. G., C. H. Auckland, and D. H. Lynn. 2007. Molecular diversity of methanogens in feedlot cattle from Ontario and Prince Edward Island, Canada. *Appl. Environ. Microbiol.* 73:4206–4210.
- Wu, Z., and L. D. Satter. 2000. Milk production during the complete lactation of dairy cows fed diets containing different amounts of protein. *J. Dairy Sci.* 83:1042–1051.
- Yu, Z., and M. Morrison. 2004. Comparisons of different hypervariable regions of *rrs* genes for use in fingerprinting of microbial communities by PCR-denaturing gradient gel electrophoresis. *Appl. Environ. Microbiol.* 70:4800–4806.