Effects of feeding lauric acid on ruminal protozoa numbers, fermentation, and digestion and on milk production in dairy cows

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Effects of feeding lauric acid on ruminal protozoa numbers, fermentation, and digestion and on milk production in dairy cows[1]

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ABSTRACT: The objectives of this study were 1) to determine the level of lauric acid (LA) addition to the diet necessary to effectively suppress ruminal protozoa (RP) to the extent observed when a single dose was given directly into the rumen, 2) to assess LA effects on production and ruminal metabolism, and 3) to determine the time needed for RP to reestablish themselves after LA is withdrawn from the diet of lactating dairy cows. In Exp. 1, 2 Holstein cows fitted with ruminal cannulae were used in a split-plot design pilot study. Both cows consumed the same level of LA, starting with 0 g/d and increasing to 129, 270, and 438 g/d mixed into the diet. Diets were fed as total mixed ration (TMR) and contained (DM basis) 30% corn silage, 30% alfalfa silage, and 40% concentrate. Lauric acid intake linearly decreased DMI ($P = 0.03$), RP numbers ($P < 0.01$), ruminal acetate molar proportion ($P = 0.03$), and ruminal ammonia concentration ($P = 0.03$). Lauric acid intake linearly increased ruminal valerate molar proportion ($P = 0.02$). A quadratic response of LA consumption was observed on total ruminal VFA concentration ($P < 0.01$) and propionate molar proportion ($P < 0.01$), with maximum responses at 270 g/d of LA intake. A quadratic response of LA consumption was also observed on total ruminal free amino acid (TAA) concentration ($P < 0.01$), with minimum concentration at 270 g/d of LA intake. After withdrawing the greatest LA dose from the diet, RP returned to their original numbers in 12 d. In Exp. 2, 48 multiparous Holstein cows (8 with ruminal cannulae) were blocked by days in milk into 12 blocks of 4 cows (2 blocks of cannulated cows) and randomly assigned within replicated 4 × 4 Latin squares to balanced dietary treatment sequences. Diets were fed as TMR and contained (DM basis) 36% corn silage, 29% alfalfa silage, and 35% concentrate, and LA intake levels were 0, 220, 404, and 543 g/d mixed in the TMR. In Exp. 2, LA linearly reduced RP ($P < 0.01$), ruminal ammonia ($P < 0.01$), and total free AA concentration ($P < 0.01$); however, dietary LA also linearly decreased DM intake ($P < 0.01$). Intake of LA linearly reduced ruminal total VFA concentration ($P < 0.01$); DM, OM, NDF, and CP digestibility ($P < 0.01$); and milk production and milk components ($P < 0.01$). Therefore, LA does not appear to be a feasible RP suppressant for feeding in practical diets.

Key words: dairy cow, lauric acid, protozoa

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INTRODUCTION

Reducing ruminal protozoa (**RP**) might improve N utilization in the rumen (Jouany, 1996; Hristov and Jouany, 2005). However, reducing RP in a practical and effective way has been a challenge. In a previous study (Faciola et al., 2013), lauric acid (**LA**), a saturated medium-chain fatty acid (C12:0), showed considerable antiprotozoal activity when a single dose of 160 g/d was given via ruminal cannulae, reducing the RP numbers by approximately 90% within 2 d of treatment. In the same study, concentration of ruminal am-

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monia and total free AA (**TAA**) was reduced by 60% and 40%, respectively. In a second experiment, LA fed in the total mixed ration (**TMR**) at 160 and 240 g/d in the TMR of dairy cows reduced RP by 25% and 30%, respectively, and no changes in ruminal fermentation and milk production were observed.

Those results indicated that the levels of LA in the diet were not sufficient to achieve a concentration within the rumen that provided the anti-RP effect. Therefore, the objectives of the current study were 1) to determine the level of LA in the diet necessary to effectively suppress RP to the extent observed when a single dose was given within the rumen, 2) to assess the changes in ruminal fermentation patterns associated with suppression of RP, 3) to determine the time needed for the RP population to reestablish itself after LA treatment is withdrawn, and finally 4) to assess changes on DMI, apparent digestibility, ruminal microbial changes, rumen fermentation, and milk production and composition because of feeding LA to dairy cows. Objectives 1 through 3 were the aim of Exp. 1, and objective 4 was the main goal of Exp. 2. On the basis of the results from a previous study (Faciola et al., 2013), we hypothesized that intakes greater than 243 g/d of LA would be required to achieve a suppression in RP numbers necessary to increase N utilization in the rumen.

MATERIALS AND METHODS

Care and handling of all experimental animals, including ruminal cannulation, were conducted under protocols approved by the University of Wisconsin Institutional Animal Care and Use Committee.

Experiment 1

To run a large reversal trial with enough animals to provide sufficient statistical power, a key point needed to be addressed: the time necessary for RP to reestablish themselves in the rumen after withdrawing the LA treatment, which is important to avoid carryover effects because of incomplete RP establishment between periods. In addition, we wanted to evaluate the LA level in the TMR needed to obtain adequate suppression of RP over time. To address these 2 issues, a preliminary study was designed to test different levels of lauric acid in the TMR of lactating dairy cows that were as effective in reducing protozoal population as levels previously delivered via rumen cannula as a single dose (Faciola et al., 2013) and to assess the time needed by RP to reestablish themselves after LA treatment withdrawal. For this pilot trial, 2 ruminally cannulated Holstein cows averaging 697 kg BW, 164 d in milk (**DIM**), and 25.3 kg/d of milk were used in a split-plot design.

Table 1. Chemical composition of fermented feeds fed in both experiments

Item	Alfalfa silage	Corn silage	HMSC[1]
DM, %	37.4	36.7	73.9
CP, % DM	23.2	6.7	9.1
Ash, % DM	10.5	4.1	1.8
NDF, % DM	41.7	33.9	10.0
ADF, % DM	32.9	17.1	2.5
NDIN, % total N	7.0	5.8	6.9
ADIN, % total N	3.1	1.0	3.8
NPN, % total N	61.0	59.8	50.5
Ammonia N, % total NPN	6.8	8.9	3.8
Total free AA N, % total NPN	32.4	30.5	22.6
Unidentified N, % total NPN	21.8	20.4	24.1
pH	4.81	4.22	4.75

[1]HMSC = high-moisture shelled corn.

Both cows were fed the same level of LA (KIC chemicals Inc., Armonk, NY), starting with 0 g/d and increasing stepwise to 160, 320, and 480 g/d mixed into the TMR. The composition of the fermented feeds fed in this study and the next experiment is presented in Table 1. Diets were fed as TMR and contained (DM basis) 30% corn silage, 30% alfalfa silage, and 40% concentrate (Table 2). Both cows were injected with bovine ST (500 mg of Posilac, Elanco Animal Health, Greenfield, IN), and injections were synchronized such that animals received a full dose of bovine ST on d 1 and at 14-d intervals throughout the trial. Cows were housed in tie stalls and had free access to water during the trial.

Each experimental period lasted 7 d. Diets were offered once daily at 1000 h. Orts were collected and weights were recorded at 0900 h, and feeding rate was adjusted daily to yield orts of about 5% to 10% of intake. Weekly composite samples of corn silage, alfalfa silage, TMR, and orts were taken from daily subsamples of about 0.5 kg that were stored at −20°C. Weekly samples were also taken of ground corn grain and solvent-extracted soybean meal and stored at room temperature. The DM was determined in weekly composites of corn silage and alfalfa silage by drying at 60°C for 48 h and in weekly samples of corn grain and soybean meal at 105°C (AOAC, 1980). Weekly samples of feed ingredients were also analyzed for total N using a combustion assay (FP-2000 N Analyzer; Leco Instruments Inc., St. Joseph, MI).

Ingredient DM and N contents were used to adjust dietary composition weekly. Intake of DM was computed on the basis of the 60°C DM determinations for TMR and orts. After drying, ingredients and TMR were ground through a 1-mm screen (Wiley mill, Arthur H. Thomas, Philadelphia, PA). Samples were analyzed for total N, DM at 105°C, ash, and OM (AOAC, 1980) and sequentially for NDF and ADF using heat-stable α-amylase and Na_2SO_3 (Van Soest et al., 1991; Hintz et

Table 2. Composition of diet (Exp. 1)

Item	Content
Ingredient, % DM	
Alfalfa silage	30.0
Corn silage	30.0
Solvent-extracted soybean meal	9.5
Corn grain, ground dry	29.0
Sodium bicarbonate	0.73
Limestone	0.33
Salt	0.13
Dicalcium phosphate	0.23
Vitamin-mineral premix[1]	0.08
Composition	
DM, %	49.5
OM, % of DM	92.5
CP, % DM	16.5
NDF, % DM	31.2
ADF, % DM	19.6
NFC[2]	41.0
RDP,[3] % DM	11.0
RUP,[3] % DM	5.5
NE_l,[4] Mcal/kg DM	1.56
NDIN, % total N	5.90
ADIN, % total N	2.53
Fraction B3,[5] % total N	3.37
NPN,[6] % total N	18.6
Ether extract, % DM	3.8

[1]Provided (per kg DM): 56 mg of Zn as zinc oxide, 46 mg of Mn as manganous oxide, 22 mg of Fe as ferrous sulfate, 12 mg of Cu as copper sulfate, 0.9 mg of I as potassium iodide, 0.4 mg of Co as cobalt oxide, 0.3 mg of Se as sodium selenite, 6,440 IU of vitamin A, 2,000 IU of vitamin D, and 16 IU of vitamin E.

[2]NFC = nonfiber carbohydrates: NFC = 100 − (% NDF + % CP + % fat + % ash) + % NDIN × 6.25 according to the NRC (2001) model and using fat contents of individual dietary ingredients from NRC (2001) tables.

[3]Predicted from the NRC (2001) model.

[4]Computed by discounting dietary energy based on actual DM intakes (NRC, 2001).

[5]Fraction B3 = NDIN (% total N) − ADIN (% total N) (Fox et al., 2004).

[6]Proportion of total N soluble in 10% (wt/vol) trichloroacetic acid (Muck, 1987).

al., 1995). Composite samples of TMR were also analyzed for NPN (Muck, 1987; FP-2000 N Analyzer; Leco Instruments Inc.). For calculation of BW change, BW was measured 3 consecutive days at the beginning of the experiment and at the end of each 7-d period.

On d 7 of each period, about 100 to 200 mL of digesta were collected from 3 locations in the ventral rumen at 0 (just before feeding), 1, 2, 4, 8, 12, 18, and 24 h after feeding and strained through 2 layers of cheesecloth, and pH was measured immediately using a glass electrode. Two 10-mL samples of ruminal fluid were then preserved in scintillation vials by addition of 0.2 mL of 50% H_2SO_4 and stored at −20°C. Just before analysis, samples were thawed and centrifuged (15,300 × *g* for 20 min at 4°C), and flow injection analyses (QuikChem 8000; Lachat Instruments, Milwaukee, WI) were applied to supernatants to determine ammonia using a phenol-hypochlorite method (method 18-107-06-1-A; Lachat Instruments) and free TAA using a fluorimetric procedure based on the reaction with *o*-phthaldialdehyde (Roth, 1971). Leucine was the standard in the *o*-phthaldialdehyde assay, and free TAA are reported in leucine equivalents. Samples also were thawed and centrifuged (28,000 × *g* for 30 min at 4°C) for determination of individual and total ruminal VFA using a modification of the GLC method for FFA (Bulletin 855B; Supelco Inc., Bellefonte, PA) with flame-ionization detection. Standards or supernatants (0.5 or 1μL) were injected onto a ZB-FFAP capillary column (30 m × 0.53 mm × 1.0 μm; number 7HK-G009-22; Phenomenex Inc., Torrance, CA) with helium carrier gas at 100 kPa and a flow rate of 20 mL/min. Column oven temperature was 100°C at injection. After 2 min, the temperature was increased to 130°C at 10°C/min. Injector and detector temperatures were 230°C and 250°C. The method did not resolve isovalerate and 2-methylbutyrate.

Protozoal counts were done 3 times weekly in a sample composed of subsamples collected at 8 different time points after feeding (0, 1, 2, 4, 8, 12, 18, and 24 h) at each level of LA as described by Dehority (1993). Moreover, at the end of period 4, after LA was withdrawn from the TMR, RP counts were done on d 3, 6, 9, 12, 15, and 18 to determine the time needed for RP to reestablish.

Experiment 2

Forty-eight multiparous Holstein cows (8 fitted with permanent ruminal cannulas), averaging 2.6 ± 1.8 parity, 71 ± 39 DIM, 43 ± 8 kg of milk/d, and 628 ± 45 kg BW at the beginning of the study, were blocked by DIM into 12 blocks of 4 cows (2 blocks of cannulated cows) and used in a replicated 4 × 4 Latin square study. Cows were randomly assigned within squares to balanced dietary treatment sequences (i.e., with each diet coming after every other diet once in each square over the course of the trial). Diets were fed as TMR and contained (DM basis) 36% corn silage, 29% alfalfa silage, and 35% concentrate. The LA target intakes were 0, 240, 480, and 720 g/d mixed in the TMR (Table 3).

All cows were housed, managed, and fed similarly to Exp. 1. Each experimental period lasted 28 d and consisted of 21 d for adaptation and 7 d for collection of intake and production data. The 21-d adaptation period was based on results from Exp. 1, which showed that a minimum of 12 d was required for RP numbers to reestablish after changing dietary LA concentration. Weekly composites of corn silage, alfalfa silage, high-moisture shelled corn, TMR, and orts were taken daily and processed as in Exp. 1.

Table 3. Composition of diets (Exp. 2)

	Actual lauric acid intake, g/d			
Item	0	220	404	543
Ingredient, % DM				
Alfalfa silage	28.8	28.8	28.8	28.8
Corn silage	36.0	36.0	36.0	36.0
High-moisture shelled corn	7.5	7.5	7.5	7.5
Solvent-extracted soybean meal	6.0	6.0	6.0	6.0
Dry molasses	8.0	8.0	8.0	8.0
Corn grain, ground dry	12.2	11.2	10.2	9.2
Lauric acid	0.00	0.97	1.95	2.92
Sodium bicarbonate	0.75	0.75	0.75	0.75
Limestone	0.36	0.36	0.36	0.36
Salt	0.16	0.16	0.16	0.16
Dicalcium phosphate	0.24	0.24	0.24	0.24
Vitamin-mineral premix[1]	0.08	0.08	0.08	0.08
Composition				
DM, %	48.5	49.2	48.8	49.5
OM, % DM	93.1	93.1	93.1	93.0
CP, % DM	15.6	15.6	15.6	15.6
NDF, % DM	29.6	29.5	29.4	29.3
ADF, % DM	18.2	18.2	18.2	18.1
Ether extract, % DM	4.1	5.8	7.6	9.1
NFC[2]	41.3	39.7	38.1	36.7
RDP,[3] % DM	10.4	10.6	10.5	10.6
RUP,[3] % DM	5.2	5.0	5.1	5.0
NE_l,[4] Mcal/kg DM	1.57	1.57	1.57	1.57
NDIN, % total N	6.25	6.24	6.24	6.23
ADIN, % total N	2.58	2.59	2.58	2.58
Fraction B3,[5] % total N	3.67	3.65	3.66	3.65
NPN,[6] % total N	20.3	20.5	20.4	20.5

[1]Provided (per kg DM): 56 mg of Zn as zinc oxide, 46 mg of Mn as manganous oxide, 22 mg of Fe as ferrous sulfate, 12 mg of Cu as copper sulfate, 0.9 mg of I as potassium iodide, 0.4 mg of Co as cobalt oxide, 0.3 mg of Se as sodium selenite, 6,440 IU of vitamin A, 2,000 IU of vitamin D, and 16 IU of vitamin E.

[2]NFC (nonfiber carbohydrates) = 100 − (% NDF + % CP + % fat + % ash) + % NDIN × 6.25 according to the NRC (2001) model and using fat contents of individual dietary ingredients from NRC (2001) tables.

[3]Predicted from the NRC (2001) model.

[4]Computed by discounting dietary energy based on actual DM intakes (NRC, 2001).

[5]Fraction B3 = NDIN (% total N) − ADIN (% total N) (Fox et al., 2004).

[6]Proportion of total N soluble in 10% (wt/vol) trichloroacetic acid (Muck, 1987).

Cows were milked twice daily at 0600 and 1700 h, and milk yield was recorded at each milking in all experimental periods. Milk samples were collected at 2 consecutive (afternoon and morning) milkings on d 24 and 25 of each period and were analyzed for fat, true protein, lactose, and solids not fat (**SNF**) by infrared analysis (AgSource, Verona, WI) with a spectrum analyzer (FT6000; Foss North America Inc., Eden Prairie, MN) using AOAC (1990) method 972.16. For milk urea N (**MUN**) determination, 5 mL of milk from each milking were treated with 5 mL of 25% (wt/vol) trichloroacetic acid. Samples were vortexed and allowed to stand for 30 min at room temperature before filtering through Whatman No. 1 filter paper. Filtrates were stored at −20°C until being thawed and analyzed for MUN by an automated colorimetric assay (Broderick and Clayton, 1997) adapted to flow injection (Lachat Quik-Chem 8000 FIA, Lachat Instruments). Concentrations and yields of fat, true protein, lactose, and SNF and MUN concentration were calculated as weighted means from morning and afternoon milk yields on each test day. The yield of 3.5% fat-corrected milk (**FCM**; Sklan et al., 1992) was also calculated. The efficiency of conversion of feed DM was calculated for each cow over the last 2 wk of each period by dividing mean yield of actual milk and FCM by mean DMI.

Apparent N efficiency (assuming no retention or mobilization of body N) was also calculated for each cow by dividing mean milk N output (milk true protein/6.38) by mean N intake. On d 26 and 27 of each period, 2 spot urine and 2 spot fecal samples were collected from all 28 cows at 6 and 18 h after feeding. Fecal samples were dried in a forced-draft oven (60°C for 72 h) and then ground through a 1-mm screen (Wiley mill, Arthur H. Thomas). Equal DM from each fecal subsample was mixed to obtain 1 composite sample for each cow in each period. Fecal samples were analyzed for total DM, ash, OM, N, NDF, and ADF as described earlier. Indigestible ADF (ADF remaining after a 12-d in situ incubation; Huhtanen et al., 1994) was used as an internal marker to estimate both apparent nutrient digestibility and fecal output (Cochran et al., 1986). Urine samples were acidified immediately after collection by diluting 1 volume of urine with 4 volumes of 0.072 *N* H_2SO_4 and stored at −20°C. Later, urine samples were thawed at room temperature and filtered through Whatman No. 1 filter paper. Filtrates were analyzed for creatinine using a picric acid method (Oser, 1965) adapted to flow injection analysis (QuikChem 8000; Lachat Instruments), for total N (Leco FP-2000 N Analyzer; Leco Instruments Inc.), and for urea with the colorimetric method also used for MUN. Daily urine volume and excretion of urea N and total N were calculated from urinary creatinine concentration and BW assuming a creatinine excretion rate of 29 mg/kg of BW (Valadares et al., 1999). Purine derivatives (allantoin and uric acid) were analyzed according to Vagnoni et al. (1997). On d 27 of each period, about 100 to 200 mL of digesta were collected from the ruminally cannulated cows, as well as samples for RP counting, and were analyzed as described in Exp. 1.

Statistical Analysis

In Exp. 1, data were analyzed using the mixed procedure (SAS Inst. Inc., Cary NC). Protozoa numbers were analyzed in an ANOVA with autoregressive cova-

riance structure within cow. A split-plot design was used with cow as whole plot and LA dose as subplot. This model was used to fit the data to assess effects of LA dose on RP numbers:

$$Y_{ij} = \mu + Cow_i + D_j + E_{ij},$$

where cow was considered random effect, D is LA dose effect, which was considered fixed, and E is the error term. Ruminal variables, for which there were repeated measurements over time (pH, ammonia, TAA, individual, and total VFA), were analyzed in a 2-way repeated measures ANOVA with time nested within periods and compound symmetry correlation structure using the split-plot model:

$$Y_{ijk} = \mu + Cow_i + D_j + E1_{ij} + T_k + TCow_{ik} + TD_{jk} + E2_{ijk},$$

where D and T are LA dose and time, respectively, and E1 and E2 are error terms. All terms were considered fixed, except for Cow_i, E1, and E2, which were considered random. Values reported are least squares means that were separated into significant main effects using Fisher's protected least significant difference. Differences among treatments were considered to be significant when $P \leq 0.05$.

In Exp. 2, data were analyzed using the MIXED procedure of SAS as a replicated 4 × 4 Latin square design. This model was fitted to all variables that did not have repeated measurements over time:

$$Y_{ijkl} = \mu + Sq_i + Per_j + V_{k(i)} + T_l + SqT_{il} + E_{ijkl},$$

where Y_{ijkl} = dependent variable, μ = overall mean, Sq_i = effect of square i, Per_j = effect of period j, $V_{k(i)}$ = effect of cow k (within square i), T_l = effect of treatment l, SqT_{il} = interaction between square i and treatment l, and E_{ijkl} = residual error. All terms were considered fixed, except for $V_{k(i)}$ and E_{ijkl}, which were considered random. The interaction term SqT_{il} was removed from the model when $P > 0.25$. Differences between least squares means were reported only if the F test for treatment was significant at $\alpha = 0.05$. This model was used for ruminal variables for which there were repeated measurements over time (pH, ammonia, TAA, individual, and total VFA):

$$Y_{ijklm} = \mu + Sq_i + Per_j + V_{k(i)} + T_l + SqT_{il} + E1_{ijkl} + Z_m + ZT_{ml} + E2_{ijklm},$$

where Y_{ijklm} = dependent variable, μ = overall mean, Sq_i = effect of square i, Per_j = effect of period j, $V_{k(i)}$ = effect of cow k (within square i), T_l = effect of treatment l, SqT_{il} = interaction between square i and treatment l, $E1_{ijkl}$ = whole-plot error, Z_m = effect of time m, ZT_{ml} = interaction between time m and treatment l, and $E2_{ijklm}$ = subplot error. The spatial covariance structure was used for estimating covariances, and the subject of the repeated measurements was defined as cow (square × period × treatment). All terms were considered fixed, except for $V_{k(i)}$, $E1_{ijkl}$, and $E2_{ijklm}$, which were considered random. The interaction SqT_{il} was removed from the model when $P > 0.25$. Differences between least squares means were reported only if the F test for treatment was significant at $\alpha = 0.05$. Orthogonal polynomial contrasts were used to examine the responses (linear, quadratic, and cubic) to increasing levels of LA in the TMR in both experiments; quadratic and cubic responses with P values greater than 0.10 were removed from the tables. In orthogonal polynomial analysis, coefficients were corrected because of unequal spacing of treatments.

RESULTS AND DISCUSSION

Experiment 1

In a previous study (Faciola et al., 2013), LA appeared to be promising because it was found to have strong antiprotozoal activity when a single dose of 160 g/d was given via ruminal cannulae, reducing the RP numbers by approximately 90% within 2 d of treatment. However, in a follow-up experiment reported by Faciola et al. (2013), LA consumed at 164 and 243 g/d in the TMR of dairy cows reduced RP by only 25% and 30%, respectively. This indicated that to be effective as a RP suppressant agent, the level of LA inclusion in the TMR would have to be much greater than the level given as a single dose through ruminal cannulae. It is likely that at a normal rate of DMI during a 24-h period, the cows never achieved a ruminal concentration of LA needed to suppress RP. In Exp. 1, we tested 4 levels of LA mixed in the TMR; with target intakes of 0, 160, 320, and 480 g/d of LA, cows were allowed ad libitum feed intake, and actual LA intakes were 0, 129, 270, and 438 g/d (Table 4). Intake of DM was linearly reduced by LA consumption ($P = 0.03$). Because both cows received the same treatment and there was no switchback, we were not able to test for period effect. However, overall, this was not a crucial constraint because the variables of interest (e.g., DMI, RP counts, and ruminal metabolites) were not likely affected by time over the duration of the experiment. Moreover, elevated environmental temperatures during period 3 (average high: 31°C), when 270 g/d of LA was consumed, might have contributed to the observed decrease in DMI during that period. In the

Table 4. Effects of feeding lauric acid (LA) on DMI and ruminal variables (Exp. 1)

	Actual LA intake, g/d					*P*-value		
Item	0	129	270	438	SEM	LA	Linear	Quadratic
DMI, kg/d	15.90	14.85	12.37	16.04	0.30	<0.01	0.03	0.11
Protozoa, $\times 10^6$ cells/mL	6.22	3.92	2.05	1.24	0.44	<0.01	<0.01	0.09
pH	6.25	6.16	6.23	6.31	0.07	0.32	0.28	0.31
Total VFA, m*M*	107.7	115.6	116.3	95.1	5.4	<0.01	0.14	<0.01
Acetate (A), % total VFA	64.1	64.9	55.1	57.4	3.3	<0.01	0.03	0.21
Propionate (P), % total VFA	19.8	19.0	28.5	21.9	3.1	<0.01	0.19	<0.01
A:P	3.24	3.41	1.93	2.63	0.10	<0.01	0.15	0.02
Butyrate, % total VFA	11.5	11.3	10.6	13.6	2.1	0.45	0.44	0.49
Valerate, % total VFA	1.74	1.47	2.89	4.09	0.15	<0.01	0.02	0.23
Isobutyrate, % total VFA	1.04	1.12	0.96	1.20	0.17	0.38	0.40	0.43
Isovalerate, % total VFA	1.88	2.22	1.86	1.98	0.19	0.56	0.59	0.51
NH_3, m*M*	4.71	4.90	2.44	2.77	0.42	<0.01	0.03	0.23
Total free AA, m*M*	3.35	2.24	1.65	3.42	0.61	<0.01	0.21	<0.01

next week, however, temperatures moderated (average high: 27°C) and DMI increased. Consumption of 129, 270, and 438 g/d of LA linearly reduced RP population ($P < 0.01$) by 37%, 67%, and 80%, respectively (Table 4). That contrasted with our previous results where RP population was reduced by only 25% and 30% when LA intake was 164 and 243 g/d, respectively (Faciola et al., 2013). One variable that might explain that observation was that DMI in our previous study was approximately 10 kg/d greater than in Exp. 1; thus, LA probably was more concentrated in the rumen of the cows in Exp. 1 of the current study. Moreover, initial RP population in Exp. 1 was greater than observed previously (Faciola et al., 2013), which would magnify the drop in RP count. Body weight changes were not observed in Exp 1.

Lauric acid linearly reduced ruminal ammonia concentration ($P = 0.03$). Intakes of 270 and 438 g/d reduced ruminal ammonia concentration by 50% and 40%, respectively (Table 4 and Fig. 1). Ruminal ammonia concentration is a good indicator of N utilization within the rumen (Hristov et al., 2004), and reduction in ruminal ammonia concentrations is often reported in studies where RP have been eliminated (Hsu et al., 1991; Williams and Coleman, 1992; Jouany, 1996). Reduction in ruminal ammonia is believed to occur due to a decrease of bacterial N turnover within the rumen, which is a consequence of decreased bacterial predation by RP. Moreover, diets typically low in N content might provide the best opportunity to examine this phenomenon (Leng and Nolan, 1984). Reducing RP population might play an important role in increasing N utilization, which has the potential to decrease the environmental impact of dairy production and decrease feeding cost.

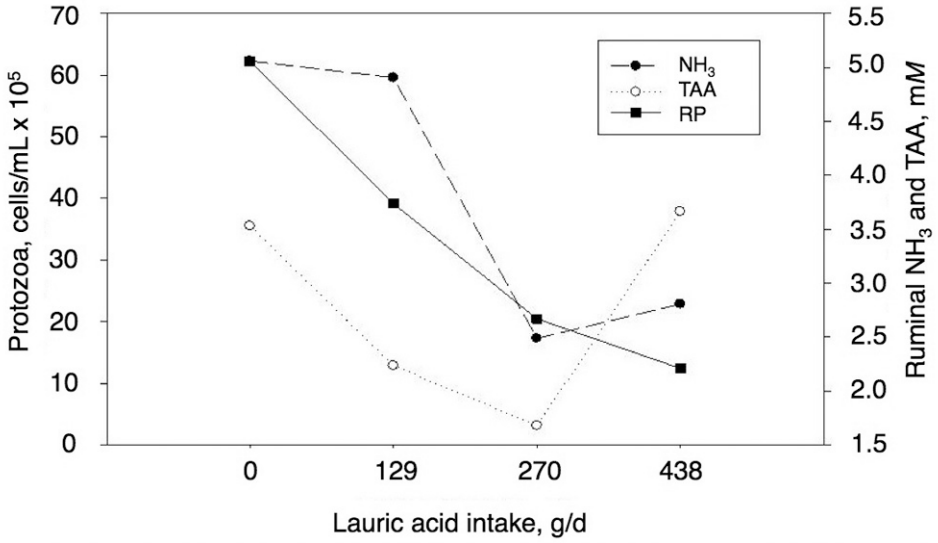

Figure 1. Least squares means of rumen protozoal numbers (RP) and ruminal concentration of ammonia and free total AA (TAA) at different lauric acid intakes (Exp. 1).

A quadratic response of LA consumption on ruminal free TAA concentration was observed ($P < 0.01$), with minimum concentration at 270 g/d of LA intake, which reduced TAA concentration by 49% (Table 4 and Fig. 1). Reduction of ruminal free TAA also has been observed in studies where RP have been eliminated (Jouany, 1996). Most of the NPN in alfalfa silage is present as free TAA and small peptides, which can be rapidly degraded to ammonia (Broderick, 1995). Moreover, Broderick et al. (1988) indicated that free peptides originating from dietary protein degradation might accumulate in the rumen and be available for protozoal attack, which might contribute to free TAA accumulation in the rumen. In the presence of RP because of active protozoal proteases (Naga and el-Shazly, 1968; Coleman, 1983; Forsberg et al., 1984; Nagasawa et al., 1994) and peptidases (Newbold et al., 1989), dietary proteins and peptides are more susceptible to cleavage, which will release free AA into the medium. Coleman (1975) proposed that RP utilize only about one-half of their ingested N; the rest are expelled as short-chain peptides and free TAA. This could explain why free TAA concentration in the rumen decreased with partial RP suppression. Furthermore, reduction of RP might increase bacterial growth and thus bacterial assimilation of free TAA available in the rumen. Reduced free TAA concentration in the rumen might contribute to reduced N loss as ammonia, which could increase N utilization and decrease N excretion. Therefore, reductions in both ruminal ammonia and free TAA can be taken to represent a step toward increasing N utilization efficiency in the rumen.

A quadratic response of LA consumption on total ruminal VFA concentration was observed ($P < 0.01$), with maximum concentration at 270 g/d of LA intake (Table 4), indicating that RP suppression might increase ruminal fermentation up to a point, but at greater levels of suppression, that could depress fermentation, mostly likely because of decreases in DMI (Hristov et al., 2011), fiber digestion (Faciola et al., 2013), and microbial growth (Hristov et al., 2011). Most studies have reported a decrease in ruminal total VFA concentration as a consequence of RP elimination (Williams and Coleman, 1997). In Exp. 1, LA intake linearly decreased ruminal acetate molar proportion ($P = 0.03$) and linearly increased ruminal valerate molar proportion ($P = 0.02$). A quadratic response of LA consumption on propionate molar proportion was observed ($P < 0.01$), with maximum responses at 270 g/d of LA intake. Similarly, a quadratic response of LA consumption on ruminal acetate to propionate ratio was observed ($P = 0.02$), with minimum ratio at 270 g/d of LA intake (Table 4). Lee et al. (2011) also observed an increase in ruminal propionate and a decrease in A:P ratio when RP numbers were reduced by feeding coconut oil, which is rich in LA. Acetate and butyrate are produced by ruminal ciliates during carbohydrate fermentation (Williams and Coleman, 1997); therefore, reducing RP might favor propionate formation in the rumen. Total ruminal VFA concentrations in Exp. 1 should be interpreted with caution because it was a preliminary trial and therefore short in duration and with fewer animals, which might explain inconsistencies with Exp. 2. Long-term experiments with more observations would be valuable to understanding how RP would affect VFA fermentation and individual VFA molar proportions.

The most critical objective of Exp. 1 was to assess the time needed for RP to reestablish their populations after LA was withdrawn from the TMR. That information is necessary to design more statistically powerful reversal experiments with RP suppression using larger numbers of animals, which has not been reported in the literature. In Exp. 1, we observed that RP required 12 d to return to the basal numbers observed before LA feeding (Fig. 2).

Experiment 2

Actual LA intakes were 220, 404, and 543 g/d when LA was added to the TMR, and LA substantially reduced DMI ($P < 0.01$) in a linear fashion (Table 5). Faciola et al. (2013) observed a trend for reduced RP population with increasing levels of LA in the TMR; however, the levels fed in that study were less than those fed in Exp. 2. Because Exp. 2 was conducted as a Latin square, we anticipated greater accuracy in detecting smaller differences in variables such as DMI and milk yield. Moreover, in Exp. 2, even greater differences among treatments were observed. Faciola et al. (2013) did not detect a reduction in DMI when 243 g/d of LA was fed in the TMR; however, in Exp. 2, consumption of 220 g/d of LA was found to decrease DMI (Table 5). Even though both experiments used the same source of LA and diets were stored, mixed, and handled in the same way, this observation was not expected. Hristov et al. (2011) observed a reduction in DMI of about 25% (about 7 kg/d) when 240 g/d of LA were dosed directly into the rumen of cows right before feeding time. Ruminal protozoal count in Exp. 2 was linearly reduced ($P < 0.01$) by 28%, 49%, and 64% when 220, 404, and 543 g/d of LA were consumed, and these RP numbers are consistent with previous findings (Hristov et al., 2004; Hristov et al., 2009; Faciola et al., 2013). Although these reductions were slightly smaller than those observed in Exp. 1, lower intakes in Exp. 1, with concomitantly greater LA concentrations in the rumen, might explain the greater reductions in RP counts. Changes in BW were not observed in Exp. 2 either.

Intake of LA linearly reduced yields of milk, 3.5% FCM, fat, protein, lactose, and SNF ($P < 0.01$). Furthermore, LA intake linearly reduced fat, lactose, and SNF content in milk ($P < 0.01$; Table 5). In contrast, Faciola et al. (2013) did not observe a reduction in yield of milk,

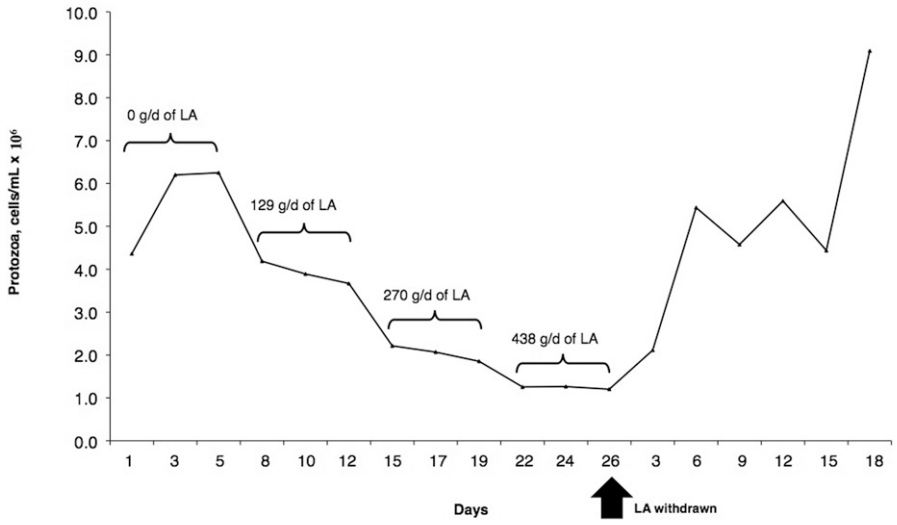

Figure 2. Ruminal protozoa numbers during lauric acid (LA) feeding and after withdrawal of the greatest level of LA from the diet (Exp. 1).

3.5% FCM, and milk components when 243 g/d of LA was fed in the TMR. In that same study, consumption of 243 g/d of LA in the TMR reduced RP by 30%. Hristov et al. (2009) observed no effect on milk yield, 4% FCM yield, apparent OM digestibility, and DMI when 240 g/d of LA was dosed via rumen cannula before feeding in 6 rumen-cannulated cows. However, Hristov et al. (2011) did observe about a 20% (about 9 kg/d) reduction in milk yield when 240 g/d of LA were dosed into the rumen. We believe that the depression observed in the yield of milk and components in Exp. 2 might have resulted from a combination of factors, the major ones being the reduction in DMI and the decrease in apparent digestion of nutrients ($P < 0.01$; Table 5). Lauric acid intake linearly increased efficiency of milk yield when it is expressed as a function of DMI ($P < 0.01$; Table 5); however, this is probably due to the decrease in DMI, which dropped more than 20% when 543 g/d of LA was consumed, combined with a drop in milk components, which could indicate an energy shortage for milk synthesis. However, BW was not affected by treatment during this experiment, indicating that large amounts of tissue energy were not mobilized to support lactation.

Intake of LA linearly reduced ruminal ammonia and TAA concentration ($P < 0.01$; Table 6). Furthermore, dietary LA linearly reduced total ruminal VFA concentration ($P < 0.01$), indicating that under the conditions of this second trial, dietary LA negatively affected ru-

Table 5. Effects of different levels of dietary lauric acid (LA) on DMI, milk production and composition, and protozoal numbers (Exp. 2)

	Actual LA intake, g/d					*P*-value	
Item	0	220	404	543	SEM	LA	Linear
DMI, kg/d	24.4	22.7	20.7	18.6	0.5	<0.01	<0.01
Milk yield, kg/d	33.6	32.7	31.3	28.8	1.0	<0.01	<0.01
3.5% fat-corrected milk, kg/d	34.7	33.5	29.8	26.6	1.0	<0.01	<0.01
Milk yield/DMI	1.36	1.43	1.51	1.60	0.04	<0.01	<0.01
Milk N/N intake, %	26.2	26.3	28.5	28.4	0.7	<0.01	<0.01
Fat, %	3.74	3.73	3.25	3.19	0.10	<0.01	<0.01
Fat yield, kg/d	1.23	1.16	1.00	0.86	0.04	<0.01	<0.01
Protein, %	3.10	3.04	3.07	3.10	0.05	0.09	0.14
Protein yield, kg/d	1.02	0.95	0.94	0.84	0.03	<0.01	<0.01
Lactose, %	4.85	4.75	4.65	4.56	0.04	<0.01	<0.01
Lactose yield, kg/d	1.60	1.51	1.44	1.25	0.04	<0.01	<0.01
Solids not fat (SNF), %	8.84	8.68	8.62	8.55	0.06	<0.01	<0.01
SNF yield, kg/d	2.92	2.75	2.66	2.34	0.08	<0.01	<0.01
Milk urea N, mg/dL	13.8	14.7	16.0	16.7	0.3	<0.01	<0.01
Blood urea N, mg/dL	15.9	16.5	18.0	18.2	0.3	<0.01	<0.01
Protozoa, $\times 10^6$ cells/mL	7.12	5.11	3.61	2.53	0.23	<0.01	<0.01

Table 6. Effect of different levels of lauric acid (LA) on ruminal variables (Exp. 2)

	Actual LA intake, g/d					*P*-value	
Item	0	220	404	543	SEM	LA	Linear
Total VFA, m*M*	121.6	115.9	106.6	100.0	5.2	<0.01	<0.01
Acetate (A), % total VFA	63.1	60.8	58.5	59.2	2.3	<0.01	0.05
Propionate (P), % total VFA	20.1	22.0	22.5	23.3	1.3	<0.01	<0.01
A:P	3.14	2.76	2.60	2.54	0.12	<0.01	<0.01
Butyrate, % total VFA	12.3	12.2	12.9	11.6	0.6	0.11	0.14
Valerate, % total VFA	1.90	2.17	2.97	2.74	0.14	<0.01	0.04
Isobutyrate, % total VFA	1.09	1.07	1.11	1.14	0.04	0.08	0.09
Isovalerate, % total VFA	1.58	1.66	1.84	2.00	0.05	<0.01	0.10
NH_3, m*M*	7.54	6.20	4.10	5.15	0.42	<0.01	<0.01
Total free AA, m*M*	10.05	8.36	7.82	6.53	0.55	<0.01	<0.01
pH	6.23	6.13	6.20	6.20	0.11	0.65	0.69

minal fermentation. Consistent with observations from this study, Hristov et al. (2011) observed a reduction in total VFA concentration when 240 g/d of LA were dosed into the rumen. Molar proportion of ruminal acetate ($P = 0.05$) and acetate to propionate ratio ($P < 0.01$) linearly decreased with dietary LA. Molar proportion of ruminal propionate ($P < 0.01$), valerate ($P = 0.04$), and isovalerate ($P = 0.10$) were increased by dietary LA. Effects of RP elimination on individual VFA have been variable, although experimental results frequently have shown an increase in molar proportion of propionate at the expense of butyrate and acetate (Veira, 1986; Ushida et al., 1991; Lee et al., 2011). Literature data on the effects of RP suppression on branched chain VFA are scarce. However, because of the reduction in proteolysis and TAA concentration in the rumen commonly reported after RP elimination, it is expected that the suppression of protozoa would reduce ruminal branched-chain VFA. Veira (1986) suggested that because of the inconsistency of the results of RP elimination on VFA proportions, it should not be assumed that RP per se are responsible for the observed differences and changes in the bacterial population probably also play a role in the pattern of VFA concentration.

Lauric acid intake linearly decreased apparent digestibility of DM, OM, NDF, ADF, and CP ($P < 0.01$; Table 7). Faciola et al. (2013) observed a reduction in apparent NDF digestibility when dairy cows were fed LA at 83 g/d and further reductions when LA was fed at 164 and 243 g/d. However, in that study, there were no reductions in DM, OM, ADF, and CP apparent digestibility at any level of LA tested. Diet composition, especially type and quality of forage included, is likely to influence the effects observed in studies where RP have been eliminated. However, TMR fed in Exp. 2 and results reported by Faciola et al. (2013) were similar in ingredient and nutrient composition. Suppression of RP is often associated with decreasing NDF digestibility (Jouany et al., 1988; Williams and Coleman, 1992). Changes in ruminal pH were not observed in the present trial (Table 6); therefore, that does not explain the reduction of fiber digestion. Changes in bacterial population and thus ruminal fermentation might play a role in alteration of apparent digestion. It is possible that at greater levels of LA intake rumen function has been compromised. Hristov et al. (2011) reported a decrease in NDF digestibility after LA treatment, which agrees with the findings of this study.

Purine derivatives (allantoin plus uric acid) and estimated microbial N flow were linearly reduced when LA was consumed ($P < 0.01$; Table 7). This agrees with the findings reported by Hristov et al. (2011), indicating that not only RP but also bacterial growth were impaired in the rumen. Reduced microbial yields in Exp. 2 would result from decreased DMI and decreased supply of fermentable substrate available in the rumen.

Urine volume was not affected by any level of LA inclusion; however, total urine N and urinary urea N were linearly reduced when LA was consumed ($P < 0.01$; Table 7). Jouany (1996) summarized 7 studies that showed decreased N excretion in urine after RP suppression, and the author suggested that this was associated mainly with decreased ruminal ammonia concentration and possibly with increased capture of recycled blood urea for microbial synthesis. On the other hand, Jouany (1996) reported that fecal N excretion increased after RP suppression. The most acceptable explanation is that the reduced ruminal digestion of structural carbohydrates usually observed after RP suppression is compensated for by greater digestion in the large intestine (Ushida et al., 1991). As a consequence, there is more microbial protein formed in the large intestine and greater fecal excretion of microbial N. Thus, as discussed by Jouany (1996), differences in total N losses between animals, with or without RP, largely disappear, and the net effect of RP suppression is to shift N excretion from the urine to the feces. However, changing the pattern of N excretion from urine to feces, even with the same amount of N excretion, might be use-

Table 7. Effects of different dietary levels of lauric acid (LA) on apparent digestibility and N excretion (Exp. 2)

	Actual LA intake, g/d					*P*-value	
Item	0	220	404	543	SEM	LA	Linear
Apparent digestibility, %							
DM	63.9	60.3	56.5	55.4	1.01	<0.01	<0.01
OM	66.1	61.6	59.5	58.7	1.02	<0.01	<0.01
NDF	44.6	39.5	31.0	30.3	1.03	<0.01	<0.01
ADF	48.2	44.3	35.5	34.6	1.01	<0.01	<0.01
CP	64.5	62.2	61.6	60.0	1.03	<0.01	<0.01
Excretion							
Urine volume,[1] L/d	25.0	24.5	25.5	23.8	1.31	0.13	0.15
Urinary urea N, g/d	148	139	145	134	3.30	<0.01	<0.01
Total urinary N, g/d	184	181	193	176	3.14	<0.01	<0.01
Urea N/total urinary N, %	80.5	76.7	75.1	76.3	2.75	0.22	0.27
Allantoin, mmol/d	400	403	372	351	7.24	<0.01	<0.01
Uric acid, mmol/d	45.2	37.5	31.8	34.0	4.15	<0.01	<0.01
Purine derivatives,[2] mmol/d	445.2	440.5	403.8	385.0	14.81	<0.01	<0.01
Microbial N flow,[3] g/d	256	253	225	211	12.0	<0.01	<0.01
Fecal N/urinary N	1.17	1.18	1.03	1.06	0.30	<0.01	<0.01
Milk N/fecal plus urinary N	2.69	2.60	2.46	2.52	0.04	<0.01	<0.01
Apparent N balance,[4] g/d	49	23	−22	−29	8.1	<0.01	<0.01

[1]Estimated from creatinine concentration in spot urine samples assuming an excretion of 29 mg creatinine/kg BW (Valadares et al., 1999).

[2]Allantoin plus uric acid.

[3]Estimated from urinary excretion of purine derivatives according to Vagnoni et al. (1997).

[4]N intake − (urinary N + fecal N + milk N).

ful because environmental problems related to fecal N concentration are of less concern than the N emissions from urinary N, which is the most environmentally labile form of excreted N (Varel et al., 1999).

Under the conditions of Exp. 1, consumption of LA in the TMR substantially suppressed RP and might have increased N utilization in the rumen, as indicated by reductions observed in ruminal ammonia and TAA. After withdrawing LA from the TMR, RP numbers were reestablished in about 12 d. Under the conditions of Exp. 2, LA was effective in reducing RP when added to the TMR; however, dietary LA also substantially decreased DMI. Consumption of LA reduced yield of milk, FCM, and milk components. In addition, LA intake reduced nutrient digestibility, especially NDF digestibility. Therefore, on the basis of the results from these 2 experiments, we reject our hypothesis that suppressing RP would improve N use mainly because of its negative effects on DMI, milk yield, and digestibility. This large feeding trial with 48 lactating cows has shown that feeding LA mixed in the TMR is not a practical method of reducing RP because if its negative effects on DMI. Strategies to overcome this negative effect will be crucial if this approach is to be used to successfully suppress RP and improve nutrient efficiency of milk production.

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