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J ANIM SCI 2013, 91:363-373.
doi: 10.2527/jas.2012-5168 originally published online October 24, 2012

Effects of lauric acid on ruminal protozoal numbers and fermentation pattern and milk production in lactating dairy cows[1]

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ABSTRACT: The objectives of this study were to evaluate lauric acid (LA) as a practical ruminal protozoa-suppressing agent and assess effects of protozoal suppression on fermentation patterns and milk production in dairy cows. In a pilot study, 6 lactating Holstein cows fitted with ruminal cannulae were used in a randomized complete-block design trial. Cows were fed a basal total mixed ration (TMR) containing (DM basis) 15% alfalfa silage, 40% corn silage, 30% rolled high moisture shelled corn, and 14% solvent soybean meal, and assigned to 1 of 3 treatments: 1) control, 2) 160 g/d of LA, or 3) 222 g/d of sodium laurate, which is equimolar to 160 g/d of LA, all given as a single dose into the rumen via cannulae before feeding. Both agents showed high antiprotozoal activity when pulse dosed at these amounts via ruminal cannulae, reducing protozoa by 90% ($P < 0.01$) within 2 d of treatment. Lauric acid reduced ruminal ammonia concentration by 60% ($P < 0.01$) without altering DMI. Both agents reduced ruminal total free AA concentration ($P < 0.01$) and LA did not affect ruminal pH or total VFA concentration. In a large follow-up feeding trial, 52 Holstein cows (8 with ruminal cannulae) were used in a randomized complete-block design trial. Cows were assigned to 1 of 4 diets and fed only that diet throughout the study. The TMR contained (DM basis) 29% alfalfa silage, 36% corn silage, 14% rolled high moisture shelled corn, and 8% solvent soybean meal. The 4 experimental diets were similar, except part of the finely ground dry corn was replaced with LA in stepwise increments from 0 to 0.97% of dietary DM, which provided (as consumed) 0, 83, 164, and 243 g/d of LA. Adding these amounts of LA to the TMR did not affect DMI, ruminal pH, or other ruminal traits, and milk production. However, LA consumed at 164 and 243 g/d in the TMR reduced the protozoal population by only 25% and 30% ($P = 0.05$), respectively, showing that these levels, when added to the TMR, were not sufficient to achieve a concentration within the rumen that promoted the antiprotozoal effect of LA.

Key words: dairy cow, lauric acid, protozoa

J. Anim. Sci. 2013.91:363–373
doi:10.2527/jas2012-5168

INTRODUCTION

Improving N utilization is a major challenge in ruminant nutrition research. Overfeeding of protein to dairy cows is commonly reported in high producing herds (Shaver and Kaiser, 2004; Huhtanen and Hristov, 2009), which is uneconomical and results in excessive urinary N, the most environmentally labile form of excreted N (Varel et al., 1999).

Microbial protein synthesized in the rumen and RUP are the sources of α-amino N absorbed by ruminants. Ruminal protozoa have a negative effect on protein utilization in ruminants by reducing ruminal outflow of both microbial protein plus RUP (Jouany,

[1]The authors thank the farm crew for harvesting and storing the feedstuff used in this trial, and J. Davidson and the barn crew for feeding and animal care at the U.S. Dairy Forage Center Research Farm (Prairie du Sac, WI), W. Radloff, M. Becker, and S. Reynal (U.S. Dairy Forage Research Center, Madison, WI) for conducting laboratory analyses and assisting with sampling collection, and P. Crump (University of Wisconsin, Madison) for assisting with statistical analyses.

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Received January 30, 2012.
Accepted October 6, 2012.

1996). Protozoa possess protease (Forsberg et al., 1984), peptidase (Newbold et al., 1989), and deaminase (Itabashi and Kandatsu, 1975) activity; therefore, they actively degrade dietary protein. Moreover, protozoa briskly consume bacteria (Coleman, 1975) and in vitro studies indicated that they are the main contributors to bacterial protein turnover in rumen fluid (Wallace and McPherson, 1987), which increases ruminal ammonia concentration. These are central factors in determining the economic cost and environmental impact of ruminant production (Hristov et al., 2004).

Removal of protozoa appears to reduce ruminal pH stability (Veira et al., 1983), fiber digestion, and total VFA production (Jouany et al., 1988). This perhaps explains the inconsistent effects on animal performance reported from protozoal removal studies. However, partial suppression of protozoal populations has received little attention.

Most techniques used to eliminate protozoa are harmful to animals and are not applicable under practical feeding conditions. Medium chain fatty acids, such as lauric acid (**LA**; C12:0), have been shown to have potent antiprotozoal effects (Newbold and Chamberlain, 1988; Matsumoto et al., 1991; Soliva et al., 2003; Hristov et al., 2004). Furthermore, they can be used routinely in farm operations and they have the potential to replace hazardous antiprotozoal chemicals.

The current state of knowledge regarding partial suppression of protozoal populations in the rumen does not allow for conclusive understanding of its effects on milk production and efficiency of N utilization. More importantly, literature on effects of partial suppression of protozoa on milk production, using large-scale feeding trials involving lactating dairy cows, is lacking. The current and follow-up reports (Faciola and Broderick, 2013), derived from 2 large feeding trials (52 and 48 cows, respectively), were both preceded by 2 pilot studies that assessed such effects. We hypothesized that successful partial suppression of the protozoal population would improve N utilization without being harmful to carbohydrate fermentation, thus enhancing animal performance. Hence, the aims of this study were to: 1) evaluate LA as a practical protozoal-suppressing agent that could be fed routinely, and 2) assess the effects of partial suppression of protozoa on fermentation patterns and milk production in dairy cows.

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

A pilot study was conducted to evaluate LA as a practical alternative to sodium laurate as a protozoal-suppressing agent. In this study, 6 multiparous Holstein cows, averaging 660 ± 46 kg BW, 3.5 ± 1.0 parity, 102 ± 23 d in milk (**DIM**), 40.4 ± 6.2 kg/d of milk, and fitted with ruminal cannulae, were blocked into groups of 3 by DIM to give 2 blocks in a trial of randomized complete-block design. Before starting the experimental phase of the trial, all cows were fed the basal diet for a 2-wk covariate period and DMI and protozoal counts were determined for use in statistical analysis. Cows within blocks were then randomly assigned to 1 of the 3 treatments, on which they remained throughout the entire study. Treatments were: 1) control, 2) 160 g/d of LA (KIC chemicals Inc., Armonk, NY), or 3) 160 g/d of LA equivalent, given in the form of 222 g/d of sodium laurate (**NaLA**), prepared as described by Hristov et al. (2004). Both LA and NaLA were given in a single dose into the rumen via cannulae, daily, just before feeding. The basal diet was fed as a total mixed ration (**TMR**) and is shown in Table 1.

Cows were housed in tie stalls bedded with straw and had free access to water throughout the trial. Cows were milked twice daily at 0500 and 1700 h. Individual milk yields were recorded at each milking. Milk samples were collected at 2 consecutive (afternoon and morning) milkings midway through wk 2 of the covariate period and once at wk 1, 2, and 3 of the experimental phase, and 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 both milkings was 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 MUN analysis by an automated colorimetric assay (Broderick and Clayton, 1997), adapted to flow injection (Lachat QuikChem 8000 FIA; Lachat Instruments, Milwaukee, WI). Concentrations and yields of fat, true protein, lactose, and SNF and MUN concentration were computed as the weighted means from morning and afternoon milk yields on each test day. Yields of 3.5% fat corrected milk (**FCM**) were also computed, according to Sklan et al. (1992). Conversion efficiency of feed DM was calculated for each cow by dividing mean yield of actual milk and FCM by mean DMI. Similarly, use efficiency of feed N was computed for each cow by dividing mean milk N output (milk true protein/6.38) by mean N intake, assuming no net deposit or mobilization of N from body tissues. Body weights were measured on 3 consecutive days at the start and end of the experimental phase to

Table 1. Composition of the diet (Exp. 1)

Item	Content
Ingredient, % DM	
Corn silage	40.0
Alfalfa silage	15.0
Rolled high moisture shelled corn	29.6
Solvent soybean meal	13.9
Sodium bicarbonate	0.50
Salt	0.30
Limestone	0.30
Dicalcium phosphate	0.20
Vitamin-mineral mix[1]	0.20
Composition	
DM, %	49.6
CP, % DM	16.1
Ash, % DM	7.2
Fat, % DM	3.8
RDP,[2] % DM	10.6
RUP,[2] % DM	5.5
NFC,[3] % DM	50.1
NE_l,[4] Mcal/kg of DM	1.58
NDF, % DM	24.8
ADF, % DM	14.1
Starch, % DM	24.5
NDIN, % total N	6.20
ADIN, % total N	2.61
Fraction B3,[5] % total N	3.59
NPN,[6] % total N	20.3
True protein-N,[7] % non-urea N	79.7

[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 selenium selenite, 6,440 IU of vitamin A, 2,000 IU of vitamin D, and 16 IU of vitamin E.

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

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

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

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

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

[7]Proportion of total N detected as 17 protein AA in acid hydrolysates of each diet.

compute BW change. All cows were injected with bovine somatotropin (500 mg of Posilac; Elanco Animal Health, Greenfield, IN), beginning on d 1 of the trial and at 14-d intervals throughout the trial. Cows were fed the basal TMR for ad libitum intake once a day at 0800 h and orts were collected and weighed daily at 1900 h of the next day. The feeding rate was adjusted daily to yield orts of ~10% of intake.

Weekly composites of the TMR, orts, alfalfa silage, corn silage, and high moisture shelled corn (**HMSC**) were collected from daily samples of ~0.5 kg and stored at –20°C. Weekly samples of the solvent-extracted soybean meal (**SSBM**) and LA were stored at 21 to 24°C. Proportions of each ration ingredient on an as-fed basis were adjusted weekly, based on total N content and DM determined by drying weekly composites at 60°C (48 h) for alfalfa silage, corn silage, and HMSC, and at 105°C (AOAC 1980) for SSBM and LA; salt, sodium bicarbonate, dicalcium phosphate, and mineral and vitamin supplements were assumed to have 100% DM. Dry matter intake was computed based on the 60°C DM values for TMR and orts. After drying, major dietary ingredients and TMR were ground through a 1-mm screen (Wiley mill; Arthur H. Thomas, Philadelphia, PA) and then analyzed for DM at 105°C, ash, and OM by AOAC (1980) method, total N by combustion assay (Leco 2000; Leco Instruments, Inc., St. Joseph, MI), and sequentially for NDF and ADF, using heat-stable amylase (Van Soest et al., 1991) and Na_2SO_3 (Hintz et al., 1995) during the NDF step. Composition data in Table 1 were from analysis of ingredient composites. At the end of the trial, weekly composites of alfalfa silage and corn silage were thawed, and water extracts were prepared, deproteinized, and then analyzed for NPN (Muck, 1987), using a combustion N assay (Mitsubishi TN-05 Nitrogen Analyzer; Mitsubishi Chemical Corp., Tokyo).

About 100 to 200 mL of digesta were collected from 4 different sites in the rumen of cows at 0 (just before feeding), 1, 2, 4, 8, 12, 18, and 24 h after feeding on d 8 and 16 of the experimental phase, strained through 2 layers of cheesecloth, and pH measured immediately by glass electrode. One sample from each cow was preserved at each time point by adding 0.2 mL of 50% (vol/vol) H_2SO_4 to 10 mL of strained ruminal fluid and storing samples at –20°C. Just before analysis, samples were thawed and centrifuged (15,300 × g for 20 min at 4°C). Flow-injection analyses (Lachat Quick-Chem) were applied to supernatants to determine ammonia, using a phenol-hypochlorite method (Method 18–107–06–1-A; Lachat) and total AA, using a fluorimetric procedure based on reaction with o-phthalaldehyde (Roth, 1971). Leucine was the standard in the o-phthalaldehyde assay and total AA are reported in Leu equivalents. Also, samples 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 free fatty acids, as described (Supelco Bulletin 855B; Supelco Inc., Supelco Park, Bellefonte, PA), with flame-ionization detection. Standards or ruminal supernatants (1 µL) were injected onto a capillary column (ZB-FFAP, 30 m × 0.53 mm × 1.0 µm, and No. 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 ramped up to 130°C at 10°C/min. Injector and detector temperatures were 230 and 250°C,

respectively. Response areas from standards were used to compute VFA concentrations in ruminal samples. The method did not resolve isovalerate and 2-methylbutyrate. Individual VFA are reported in concentration units, rather than molar proportions.

Total protozoa numbers in rumen contents were determined on d 1, 2, 3, 4, 8, 11, and 17, as described by Dehority (1993). Briefly, ruminal fluid was collected from 4 different sites in the rumen of cows at 8 time points after feeding (0, 1, 2, 4, 8, 12, 18, and 24 h), squeezed through 1 layer of cheesecloth, and then 10 mL of rumen fluid was mixed with 10 mL of 50% formalin (20% wt/vol formaldehyde). Two drops of brilliant green dye were added to 1-mL aliquots and allowed to stand overnight. After staining, 9 mL of 30% glycerol solution was added and the diluted samples were pipetted into a Sedgewick-Rafter counting chamber (1 cm^3 volume). Further dilutions were made with 30% glycerol, when needed, to allow a maximum of 50 cells per slide. Protozoa were counted at a 100× magnification. Counts were done in duplicates; and when they differed by >5%, another slide was made until duplicates were within 5% difference.

At the end of wk 1, 2, and 3 of the experimental phase, 2 spot urine samples were collected from all cows at ~6 and 18 h after feeding. Fresh urine was acidified by diluting 15 mL of urine with 60 mL of 0.072 N H_2SO_4 and stored at –20°C until analyzed. At the end of the trial, all urine samples were thawed at room temperature and filtered through Whatman No. 1 filter paper. Filtrates were analyzed for creatinine, using a picric acid assay (Oser, 1965), adapted to the flow injection analyzer (Lachat Quick-Chem; Lachat Instruments), for total N using a N analyzer (Mitsubishi, Tokyo), for allantoin using the method of Vogels and van der Grift (1970), adapted to a 96-well plate reader, for uric acid using a commercial kit (No. 683–100P; Sigma Chem. Co., St. Louis, MO), and for urea with the colorimetric method used for MUN. Daily urine volume and excretion of urea N, total N, and purine derivatives (allantoin plus uric acid) were estimated from mean urinary concentrations, assuming a creatinine excretion rate of 29 mg/kg of BW (Valadares et al., 1999).

Experiment 2

Thirty-two multiparous Holstein cows (8 with ruminal cannulae), averaging 2.6 ± 1.0 parity, 633 ± 52 kg BW, 152 ± 72 DIM, and 43.1 ± 11 kg milk/d, and 20 primiparous Holstein cows, averaging 566 ± 39 kg BW, 123 ± 42 DIM, and 40.5 ± 2.6 kg milk/d were blocked into groups of 4 by DIM to give 8 multiparous blocks (2 cows with ruminal cannulae) and 5 primiparous blocks in a trial of randomized complete-block design. Cows within blocks of 4 were then randomly assigned to 1 of 4 diets and fed only that diet during the remaining 8 wk of the study. Chemical composition of fermented feeds used in Exp. 2 is shown in Table 2. The 4 experimental diets were similar, except that some of the finely ground dry corn was replaced with LA in stepwise increments from 0 to 0.97% of dietary DM, which resulted in LA intakes of 0, 83, 164, and 243 g/d, respectively (Table 3). Lauric acid was first blended with finely ground dry corn to prepare a premix, which was then mixed into the TMR.

Table 2. Chemical composition of fermented feeds (Exp. 2)

Item	Alfalfa silage	Corn silage	HMSC[1]
DM, %	43.3	42.2	73.1
CP, % DM	24.6	7.3	8.3
Ash, % DM	10.7	4.2	1.9
NDF, % DM	35.9	36.2	8.2
ADF, % DM	26.8	19.0	2.2
N fractions			
NDIN, % total N	7.2	5.8	7.1
ADIN, % total N	3.3	1.2	4.2
NPN, % total N	60.1	61.1	50.6
Ammonia-N, % total N	6.48	8.72	3.67
Total free AA-N, % total N	29.3	31.5	21.0
Unidentified N, % total N	24.3	20.9	25.9
pH	4.73	3.92	4.32

[1]HMSC = high moisture shelled corn

Cows were housed, managed, fed, and milked as described in Exp. 1. Milk samples were collected at 2 consecutive milkings midway through wk 2, 4, 6, and 8 of the experimental phase and analyzed as described in Exp. 1. Body weights were measured on 3 consecutive days at the end of wk 4 and 8 of the experimental phase to compute BW change. At the end of wk 4 and 8 of the experimental phase, urine samples also were collected from all cows at ~6 and 18 h after feeding. Urine samples were collected, managed, and processed as described in Exp. 1. About 100 to 200 mL of ruminal digesta were collected from cannulated cows on the last day of wk 4 and 8, and analyzed as described in Exp. 1. Ruminal samples for total protozoa numbers were taken on d 1, 14, 28, 42, and 56, and processed as described in Exp. 1.

Statistical Analysis

Data from both experiments were analyzed as a randomized complete-block design, using the mixed procedure (SAS Inst. Inc, Cary, NC), with a model that included the covariate mean for each trait for each cow, plus block, week, treatment, and the interaction of week and treatment. The following model was used for production traits measured in all cows:

$$Y_{ikl} = \mu + \beta V_k + B_i + W_j + C_{k(i)} + T_l + WT_{jl} + E_{ijkl},$$

Table 3. Composition of the diets to provide lauric acid (Exp. 2)

	Actual lauric acid intake, g/d			
Item	0	83	164	243
Ingredient, % DM				
Alfalfa silage	28.8	28.8	28.8	28.8
Corn silage	35.7	35.7	35.7	35.7
Rolled high moisture shelled corn	14.1	14.1	14.1	14.1
Solvent soybean meal	7.59	7.59	7.59	7.59
Sodium bicarbonate	0.75	0.75	0.75	0.75
Limestone	0.36	0.36	0.36	0.36
Salt	0.19	0.19	0.19	0.19
Dicalcium phosphate	0.24	0.24	0.24	0.24
Vitamins and minerals[1]	0.08	0.08	0.08	0.08
Corn grain ground dry	12.2	11.8	11.5	11.2
Lauric acid	0.00	0.32	0.65	0.97
Composition				
DM, %	49.6	49.9	48.4	49.1
CP, % DM	15.5	15.7	15.9	16.1
Ash, % DM	7.3	7.5	7.4	7.3
RDP,[2] % DM	10.5	10.6	10.8	10.8
RUP,[2] % DM	5.0	5.1	5.1	5.3
NE_l,[3] Mcal/kg DM	1.55	1.58	1.58	1.58
NDF, % DM	28.7	28.8	28.8	28.8
ADF, % DM	17.4	17.5	17.5	17.6
NFC,[4] % DM	45.0	44.3	44.0	43.7
Fat, % DM	3.5	3.7	3.9	4.1
NDIN, % total N	6.20	6.22	6.22	6.23
ADIN, % total N	2.60	2.61	2.64	2.62
Fraction B3,[5] % total N	3.60	3.61	3.58	3.61
NPN,[6] % total N	20.3	20.4	20.2	20.3

[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 selenium selenite, 6,440 IU of vitamin A, 2,000 IU of vitamin D, and 16 IU of vitamin E.

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

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

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

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

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

where Y_{ikl} = dependant variable, μ = overall mean, β = regression coefficient, V_k = covariate measurement, B_i = effect of block i, W_j = effect of week j, $C_{k(i)}$ = effect of cow k within block i, T_l = effect of treatment l, WT_{jl} = interaction between week and treatment, and E_{ijkl} = residual error. All terms were considered fixed, except for $Ck_{(i)}$ and E_{ijkl}, which were considered random.

The following model was used for ruminal traits for which there were repeated measurements over time [pH, ammonia, Total AA (**TAA**), and VFA]:

$$Y_{iklm} = \mu + \beta V_k + B_i + W_j + C_{k(i)} + T_l + WT_{jl} + E_{ijkl} + Z_m + ZT_{ml} + S_{ijklm}$$

where Y_{iklm} = dependant variable, μ = overall mean, β = regression coefficient, V_k = covariate measurement, B_i = effect of block i, W_j = effect of week j, $C_{k(i)}$ = effect of cow k within block i, T_l = effect of treatment l, WT_{jl} = interaction between week and treatment, E_{ijkl} = whole plot error, Z_m = effect of time m, ZT_{ml} = interaction between time m and treatment l, and S_{ijklm} = subplot error. The spatial covariance structure SP was used for estimating covariances and the subject of the repeated measurements were defined as cow (block × week × treatment). This approach resulted in the lowest Akaike's information criterion corrected values, indicating the model with the smallest number of parameters. All terms were considered fixed, except for $Ck_{(i)}$, E_{ijkl}, and S_{ijklm}, which were considered random. For all models used, the interaction term WT_{jl} was removed from the model when $P \geq 0.25$. The PDIFF option was used to test treatment differences among least squares means within sampling time protected by the overall F-test and the SLICE option was used to analyze for differences among weekly treatments means. In Exp. 1, treatment effects were further separated into single degree of freedom comparisons by orthogonal contrasts. The comparisons were control vs. others (effect of protozoal suppression; C1) and NaLA vs. LA (effect of protozoal-suppressing agent; C2). In Exp. 2, orthogonal polynomial contrasts were used to examine the responses (linear, quadratic, and cubic) to increasing levels of LA in the diets. Cubic responses were not significant; therefore, they were not presented in the results. In orthogonal polynomial analysis, coefficients were corrected because of unequal spacing of treatments. Differences were considered significant at $P \leq 0.05$. All reported values are least squares means.

RESULTS AND DISCUSSION

Experiment 1

In this experiment, a pulse dose of 160 g/d of LA or an equimolar amount of NaLA administered into the rumen via cannulae daily before feeding reduced protozoa rapidly and dramatically to <10% of the original number within 2 d. Both LA and NaLA were equally effective on reducing protozoa numbers ($P < 0.01$; Table 4). This indicated that such a dose, when administered into the rumen as a pulse dose, was sufficient to achieve protozoal suppression. Hristov et al. (2004, 2011) observed reductions of 91% and 96% of protozoa, respectively, when 240 g/d of LA was dosed via rumen cannulae, which agrees with the findings of the present experiment. Be-

Table 4. Effects of ruminal dosing of lauric acid (LA) or sodium laurate (NaLA) on ruminal traits (Exp. 1)

					P-value[1]	
Item	Control	LA	NaLA	SEM	C1	C2
Ammonia, mM	6.6	2.6	4.6	0.5	<0.01	<0.01
Total free AA, mM	10.4	6.6	3.9	0.8	<0.01	<0.01
pH	6.25	6.38	6.69	0.09	0.14	0.08
Total VFA, mM	75.6	71.6	63.6	13.5	0.83	0.72
Acetate, mM	42.6	38.0	38.6	7.5	0.80	0.90
Propionate, mM	18.7	20.0	15.4	5.1	0.82	0.67
Butyrate, mM	10.0	8.0	5.7	0.8	0.09	0.08
Isobutyrate, mM	0.98	0.91	0.92	0.10	0.80	0.89
Isovalerate, mM	1.53	1.92	1.71	0.11	0.18	0.23
Valerate, mM	1.76	2.66	1.23	0.37	0.22	0.15
Protozoa, × 10^6 cells/mL	5.90	0.37	0.51	0.34	<0.01	0.12

[1]C1 = control vs. LA + NaLA, and C2 = LA vs. NaLA.

cause this was a preliminary trial with a limited number of animals, results must be interpreted with caution, especially for traits that were only moderately influenced by treatments. For these traits, the low number of observations may restrict the interpretation of results. Nevertheless, the magnitude of the main finding of this pilot trial (protozoal reduction) was so large that increasing the number of animals at the level of significance used herein ($P \leq 0.05$) would not have changed the inference concerning protozoal suppression because the change in statistical power would be negligible for this particular trait. Moreover, reversal experiments, such as Latin squares, were not feasible due to possible cross contamination and carryover. Those issues have been addressed in our companion paper (Faciola and Broderick, 2013).

Other studies have reported the inhibitory activity of LA on protozoa (Matsumoto et al., 1991; Dohme et al., 2000; Machmuller et al., 2001, 2002; Soliva et al., 2003; Hristov et al., 2004, 2009, 2011). It is well accepted that lipids, in general, possess antiprotozoal effects. However, the mechanisms by which these occur are still unclear. Jenkins (1993) suggested that antimicrobial effects of lipids in the rumen may have similarities to cytotoxic effects of fatty acids on biological membrane functions, such as oxidative phosphorylation. Other mechanisms for lipid toxicity that have been suggested were: perturbation of ether-lipid metabolism, inhibition of an enzyme involved in lipid-remodeling (Lux et al., 2000), and inhibition of methylation of phosphatidylcholine, which would block de novo synthesis (Lira et al., 2001). However, these were studied in flagellated protozoa, which have different membrane structure than rumen ciliates; nevertheless, these mechanisms may shed light into possible lipid toxicity mechanisms in rumen ciliates.

In this study, protozoal enumeration was done on composites composed of subsamples collected at 8 different time points of the feeding cycle (0, 1, 2, 4, 8, 12, 18, and 24 h postfeeding); this approach likely reduced daily variation associated with diurnal changes in protozoal numbers. Multiple daily counts for each time point and genera separation would not be practical using this procedure due to its laborious and time-consuming nature. Recent studies using a different protozoa quantification method have examined inhibition of different genera and have found no specific genera effects (Hristov et al., 2011; Lee et al., 2011).

In the present study, ruminal ammonia decreased sharply with both the LA and NaLA treatments; furthermore, LA was more effective ($P < 0.01$) at reducing ruminal ammonia than NaLA (Table 4). A reduction in ruminal ammonia concentration is often reported in studies where protozoa have been eliminated (Ushida et al., 1986; Williams and Coleman, 1992; Jouany, 1996). This is mainly associated with the ability of protozoa to ingest and degrade bacterial proteins (Broderick et al., 1991; Ushida et al., 1991) and protozoal deaminase activity (Wallace et al., 1987). Cows on the control treatment showed slightly less than expected ruminal ammonia concentration; nevertheless, both agents were effective in further reducing ruminal ammonia concentration. Lauric acid reduced ruminal ammonia by 60%, whereas NaLA reduced it by 30% (Table 4). Ruminal ammonia concentration observed in the LA treatment was even less than the minimum concentration of 5 mg N/dL (3.6 mM) proposed by Satter and Slyter (1974), which may have impaired ruminal bacterial growth.

Feeding studies have confirmed that alfalfa silage protein often is poorly utilized by lactating dairy cows (Broderick, 1985, 1995b), mainly because of its high NPN content and excessive protein degradation, which is wasteful because of excessive ammonia formation in the rumen (Broderick et al., 2004). Suppressing protozoal population, therefore, would potentially improve N utilization in the rumen by reducing microbial N recycling and increasing net bacterial growth (Jouany, 1996), which uses a large proportion of the ammonia N typically found when alfalfa silage is fed (Hristov and Broderick, 1996).

In the current study, both LA and NaLA treatments reduced the concentration of ruminal total free AA (**TAA**, NaLA was more potent ($P < 0.01$) in reducing TAA than LA (Table 4). Most of the NPN in alfalfa silage is present as TAA and small peptides (Broderick, 1995a). Broderick et al. (1988) indicated that free peptides originating from dietary protein degradation accumulate in the rumen, becoming available for protozoal attack. In the presence of protozoa, because of active protozoal peptidases (Newbold et al., 1989) and proteases (Naga and el-Shazly, 1968; Coleman, 1983; Forsberg et al., 1984; Nagasawa et al., 1994), dietary proteins and peptides are more susceptible to cleavage, which will

Table 5. Effects of ruminal dosing of lauric acid (LA) or sodium laurate (NaLA) on DMI and milk production and composition (Exp. 1)

					P-value[1]	
Item	Control	LA	NaLA	SEM	C1	C2
DMI, kg/d	25.3	23.8	21.7	0.5	0.08	0.07
Milk yield, kg/d	30.5	29.5	28.7	1.0	0.17	0.15
3.5% FCM,[2] kg/d	33.0	31.7	30.8	1.0	0.15	0.12
Milk yield/DMI	1.21	1.24	1.32	0.06	0.09	0.08
3.5% FCM/DMI	1.30	1.33	1.42	0.05	0.07	0.08
Milk N/N intake, %	24.2	24.7	26.4	0.7	0.11	0.14
Milk true protein, %	3.23	3.21	3.21	0.04	0.15	0.16
Milk true protein, kg/d	0.99	0.95	0.92	0.04	0.12	0.11
Milk fat, %	4.00	3.96	3.95	0.06	0.14	0.12
Milk fat, kg/d	1.22	1.17	1.13	0.03	0.13	0.11
Milk lactose, %	4.65	4.66	4.67	0.04	0.50	0.53
Milk lactose, kg/d	1.42	1.37	1.34	0.06	0.15	0.12
Milk SNF,[2] %	9.04	9.10	9.04	0.08	0.22	0.24
Milk SNF, kg/d	2.76	2.68	2.59	0.07	0.17	0.15
MUN,[2] mg/dL	11.4	11.5	11.3	0.1	0.23	0.22
BUN,[2] mg/dL	13.5	13.4	13.3	0.1	0.21	0.23
BW change, kg/d	0.20	0.10	0.10	0.11	0.33	0.37

[1]C1 = control vs. LA + NaLA, and C2 = LA vs. NaLA.

[2]FCM = fat corrected milk; SNF = solids not fat; MUN = milk urea N; BUN = blood urea N.

Table 6. Effects of ruminal dosing of lauric Acid (LA) or sodium Laurate (NaLA) on apparent digestibility and N excretion (Exp. 1)

					P-value[1]	
Item	Control	LA	NaLA	SEM	C1	C2
Apparent digestibility, %						
DM	62.3	62.2	61.3	1.00	0.13	0.12
OM	62.6	62.9	62.1	1.01	0.12	0.11
NDF	36.9	35.6	35.0	1.09	0.10	0.12
ADF	40.3	39.8	38.9	1.07	0.11	0.13
CP	55.6	54.6	54.5	1.00	0.32	0.35
Excretion						
Urine volume, L/d	22.1	24.3	19.8	1.21	0.14	0.10
Urinary urea-N, g/d	139	135	141	3.20	0.20	0.18
Total urinary N, g/d	163	163	166	3.34	0.17	0.15
Urea-N/total urinary N, %	85.3	82.9	84.8	2.73	0.41	0.39
Allantoin, mmol/d	398	403	385	7.20	0.22	0.20
Uric acid, mmol/d	41.2	37.6	32.9	4.10	0.33	0.36
Purine derivatives, mmol/d	439	441	418	15.2	0.60	0.55
Microbial N flow,[2] g/d	252	253	236	16.0	0.35	0.31
Fecal N/urinary N	1.8	1.7	1.5	0.31	0.20	0.19
Milk N/fecal plus urinary N	2.18	2.15	2.19	0.05	0.63	0.62

[1]C1 = control vs. LA + NaLA; C2 = LA vs. NaLA.

[2]Estimated from urinary purine derivatives excretion, according to Valadares et al. (1999).

release free AA into the medium. Moreover, Coleman (1975) proposed that protozoa utilize only about one-half of their ingested N, the rest being expelled as short-chain peptides and free AA. This may explain why TAA concentration in the rumen decreased with partial suppression of protozoa. There was no difference in total ruminal VFA concentration among treatments (Table 4).

Dosing LA at 160 g/d or NaLa at 222 g/d per cow intraruminally did not reduce DMI (Table 5). Hristov et al. (2004) found no reduction in DMI when 240 g/d of NaLa, divided into 2 equal doses of 120 g, was added directly to the rumen. These authors also reported that cows went off feed on 320 g/d of NaLA, even when divided into 2 daily doses.

Neither LA nor NaLA affected milk production and composition in this study (Table 5). We also did not observe changes in apparent digestibility of nutrients (Table 6). Suppression of protozoa is often associated with decreased NDF digestibility (Jouany et al., 1988; Williams and Coleman, 1992); however, this was not observed, which may explain why total VFA were not reduced. It is likely that ruminal function was not disrupted by the reduction in protozoal population in Exp. 1. The main objective of this study was to test LA as an antiprotozoal agent in vivo, which was possible due to the large magnitude (90% reduction) of the reductions observed. However, the experimental design and limited observations of this trial precluded accurate assessment of changes in variables such as digestibility, milk production, and composition; those were the main objectives of the second experiment.

Experiment 2

The main objective of Exp. 2 was to test dietary LA as a practical protozoal suppressant in a large feeding trial, information that has not been published previously. In this experiment, protozoa were linearly reduced ($P = 0.05$) by LA (Table 7). Dietary intakes of 164 and 243 g/d of LA only reduced protozoal numbers by 25% and 30%, respectively, and 83 g/d of LA had no effect. Despite having only 2 ruminally cannulated cows per treatment (and 11 noncannulated), results from this experiment are clear that at these levels of intake, LA was not effective in reducing the protozoal population. It is likely that a greater suppression is needed to have an impact in ruminal fermentation. Compared with daily dosing LA directly into the rumen in Exp. 1, LA intake in the TMR up to 243 g/d did not achieve the same level of protozoal inhibition. Also, the feeding pattern of the cows may have had an impact on the response. Friggens et al. (1998) observed that cows eating a diet with 50% concentrate (DM basis) made ~30 visits to the feedbunk per day, which indicates that a greater

Table 7. Effects of feeding lauric acid (LA) on ruminal traits (Exp. 2)

	Actual LA intake, g/d					*P*-value[1]		
Item	0	83	164	243	SEM	LA	Linear	Quadratic
Ammonia, mM	6.11	6.24	6.57	7.51	0.60	0.40	0.18	0.31
Total free AA, mM	11.6	9.5	12.4	9.43	1.00	0.23	0.40	0.33
pH	6.56	6.60	6.51	6.40	0.06	0.27	0.22	0.38
Total VFA, mM	101.5	82.7	93.9	97.3	7.6	0.44	0.48	0.40
Acetate, mM	64.7	55.2	60.0	60.8	4.1	0.52	0.32	0.51
Propionate, mM	20.7	15.6	19.6	21.6	2.1	0.31	0.41	0.28
Butyrate, mM	10.5	8.3	9.8	9.9	1.0	0.52	0.51	0.32
Isobutyrate, mM	1.54	1.12	1.31	1.35	0.14	0.28	0.25	0.22
Isovalerate, mM	1.99	1.31	1.71	2.05	0.29	0.34	0.33	0.29
Valerate, mM	1.88	1.15	1.56	1.66	0.25	0.39	0.38	0.42
Protozoa, $\times 10^6$ cells/mL	5.05	5.16	3.81	3.43	3.76	0.05	0.05	0.18

[1]Overall effect of LA or a linear or quadratic effect of LA.

dose of LA may have been necessary to match the effect obtained with a pulse dose. It appears that, in our second trial, the concentration of LA within the rumen did not reach the same critical concentration obtained with the pulse dose of 160 g/d, which was equivalent to ~0.2% of ruminal contents (assuming a total ruminal volume of 80 kg). It also appears that, if a certain degree of protozoal suppression is not achieved, the remaining protozoa are able to re-establish the previous population by replication and growth. Greater doses of LA in the TMR were tested in our follow-up study (Faciola and Broderick, 2013).

Ankrah et al. (1990) used steers to evaluate 2 protozoal-suppressing protocols as follows: 2 d of dosing 40 g/d of dioctyl sulfosuccinate (**DSS**) directly into the rumen via cannulae or 10 d of feeding 40 g/d of DSS. Dosing DSS directly into the rumen was effective in reducing protozoa, whereas feeding DSS did not reduce protozoal numbers. These results corroborate with our findings and show that the antiprotozoal effects differ between infusing directly into the rumen and feeding such agents.

Ruminal pH and ruminal concentration of NH_3, TAA, and VFA were not different among treatments (Table 7). This confirmed that the doses of LA used, when fed in the TMR, were not high enough to reach a critical concentration of LA within the rumen to reduce the protozoal population to the magnitude expected, based on Exp. 1. Also, there was no effect of LA on DMI, milk production, and composition (Table 8), indicating that inclusion of LA to provide 83, 164, and 243 g/d did not affect diet palatability but also did not improve N utilization. Apparent NDF digestibility was linearly reduced by LA ($P < 0.01$;

Table 8. Effects of feeding lauric acid (LA) on DMI and milk production and composition (Exp. 2)

	Actual LA intake, g/d					*P*-value[1]		
Item	0	83	164	243	SEM	LA	Linear	Quadratic
DMI, kg/d	26.6	25.5	25.3	25.0	0.6	0.14	0.16	0.22
Milk yield, kg/d	35.3	36.1	35.8	36.5	0.8	0.74	0.66	0.62
3.5% FCM,[2] kg/d	38.1	38.4	37.0	37.8	0.7	0.64	0.55	0.61
Milk yield/DMI	1.33	1.42	1.41	1.46	0.04	0.16	0.18	0.14
3.5% FCM/DMI	1.43	1.51	1.46	1.51	0.05	0.13	0.15	0.11
Milk N/N intake, %	27.4	28.4	27.4	28.6	0.6	0.21	0.31	0.19
Milk true protein, %	3.20	3.15	3.08	3.16	0.06	0.57	0.37	0.45
Milk true protein, kg/d	1.16	1.19	1.12	1.09	0.05	0.47	0.43	0.51
Milk fat, %	3.99	3.89	3.71	3.72	0.16	0.43	0.33	0.41
Milk fat, kg/d	1.46	1.47	1.35	1.28	0.08	0.26	0.31	0.24
Milk lactose, %	4.86	4.86	4.87	4.82	0.07	0.88	0.78	0.85
Milk lactose, kg/d	1.76	1.84	1.81	1.67	0.08	0.51	0.52	0.45
Milk SNF,[2%]	8.93	8.90	8.85	8.89	0.07	0.89	0.81	0.85
Milk SNF, kg/d	3.23	3.37	3.26	3.08	0.13	0.52	0.46	0.41
MUN,[2] mg/dL	15.4	15.0	16.1	15.7	0.2	0.81	0.60	0.76
BUN,[2] mg/dL	17.2	16.7	17.9	17.3	0.2	0.71	0.81	0.77
BW change, kg/d	0.25	0.40	0.36	0.22	0.17	0.41	0.33	0.41

[1]Overall effect of LA or a linear or quadratic effect of LA.

[2]FCM = fat corrected milk; SNF = solids not fat; MUN = milk urea N; BUN = blood urea N.

Table 9. Effects of feeding lauric acid (LA) on apparent digestibility and N excretion (Exp. 2)

	Actual LA intake, g/d					*P*-value[1]		
Item	0	83	164	243	SEM	LA	Linear	Quadratic
Apparent digestibility, %								
DM	66.2	64.3	64.0	64.4	1.0	0.16	0.18	0.23
OM	68.5	66.1	66.4	65.6	1.0	0.13	0.15	0.14
NDF	46.3	43.9	41.0	36.1	1.1	<0.01	<0.01	0.21
ADF	46.0	46.2	43.8	40.9	1.0	0.12	0.11	0.14
CP	59.7	58.0	61.7	60.0	1.0	0.35	0.39	0.29
Excretion								
Urine Volume, L/d	24.0	22.3	24.5	19.8	1.2	0.11	0.12	0.24
Urinary urea-N, g/d	145	132	140	150	3	0.21	0.27	0.22
Total urinary N, g/d	163	164	167	163	3	0.19	0.21	0.18
Urea-N/total urinary-N, %	89	81	84	92	3	0.44	0.51	0.40
Allantoin, mmol/d	390	403	382	380	7	0.21	0.22	0.19
Uric acid, mmol/d	42.3	35.2	32.9	36.2	4.2	0.35	0.41	0.29
Purine derivatives, mmol/d	432	438	415	416	15	0.66	0.59	0.55
Microbial N flow,[2] g/d	247	251	234	235	17	0.42	0.43	0.45
Fecal N/Urinary N	1.63	1.64	1.47	1.58	0.31	0.33	0.35	0.37
Milk N/Fecal plus urinary N	2.64	2.63	2.66	2.74	0.04	0.54	0.55	0.51

[1]Overall effect of LA or a linear or quadratic effect of LA.

[2]Estimated from urinary purine derivatives excretion, according to Valadares et al. (1999).

Table 9). This effect has often been reported in protozoal suppression studies and its cause is believed to be due to the ability of protozoa to digest fiber and their effects on pH stabilization in the rumen (Jouany et al., 1988; Williams and Coleman, 1992). Despite decreased NDF digestibility, apparent digestibilities of DM, OM, ADF, and CP were not affected by LA feeding in this trial.

Both LA and NaLA had high antiprotozoal activity when pulse dosed via ruminal cannulae, 160 and 222 g/d, respectively, reducing protozoa by 90% within 2 d of treatment. Lauric acid reduced ruminal ammonia concentration by 60% without depressing DMI. When dosed directly into the rumen, both agents reduced ruminal TAA concentrations. Lauric acid did not affect ruminal pH or reduce total ruminal VFA concentration. Under the conditions of the first trial, LA was a potent protozoal-suppressing agent, obviating the need to convert it to the sodium salt. Therefore, LA appeared to have high antiprotozoal activity and its use could potentially increase N utilization in the rumen. Lauric acid consumption at 83, 164, or 243 g/d mixed in the TMR when fed to 52 lactating dairy cows did not affect DMI, milk production, ruminal pH, or ruminal traits. Consumption of LA at 164 and 243 g/d in the TMR of dairy cows reduced the ruminal protozoal population by only 25% and 30%, respectively, indicating that these levels, when added to the diet, were not sufficient to achieve a critical concentration within the rumen to promote the antiprotozoal effect sufficient to alter nutrient utilization. Therefore, based on the results from these 2 experiments, we reject our hypothesis that partial suppression of protozoa would improve N utilization and animal performance, mainly because the degree of suppression achieved in our lactation trial was insufficient to promote such results.

Further studies are necessary to determine the concentration of LA in the TMR required to promote high protozoal suppression and identify a practical way to obtain that level. Moreover, knowing the time needed by protozoa to re-establish themselves in the rumen, after they have been suppressed, would allow use of more statistically powerful reversal trials. In addition, the degree of protozoal suppression needed to be beneficial for nutrient use in the dairy cow is still unknown.

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