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Effect of Low Level Monensin Supplementation on the Production of Dairy Cows Fed Alfalfa Silage*

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ABSTRACT

Effectiveness of low level monensin supplementation on N utilization in lactating dairy cows fed alfalfa silage was assessed using 48 multiparous Holsteins. Cows were fed a covariate diet [% of dry matter (DM): 56% alfalfa silage, 39% ground high moisture corn, 3% soybean meal, 1% ground corn, 1% vitamin-mineral supplements] for 2 wk, then grouped by days in milk into blocks of 4. Cows were randomly assigned within blocks to 1 of 4 diets that were fed for 10 wk: 1) control (covariate diet), 2) control plus 3% fish meal (replacing DM from high moisture corn), 3) monensin (10 mg/kg DM), and 4) monensin plus 3% fish meal. Diets 1 and 3 averaged 16.7% crude protein (25% from free AA in alfalfa silage); diets 2 and 4 averaged 18.5% crude protein. Monensin intake averaged 16 mg/d on diets 1 and 2 (due to contamination) and 248 mg/d on diets 3 and 4. There was no effect of fish meal or monensin on DM intake. However, weight gain and yield of milk, protein, and SNF increased with fish meal feeding, indicating metabolizable protein limited production. Feeding monensin increased blood glucose but reduced yield of 3.5% fat-corrected milk, milk fat content and yield, and milk protein content and yield. Apparent N efficiency was greatest on monensin (diet 3) but lowest on monensin plus fish meal (diet 4). Fish meal reduced blood glucose concentration and apparent N efficiency, and increased concentrations of milk and blood urea. Monensin increased ruminal propionate concentration and decreased concentration of acetate and butyrate and acetate:propionate in ruminally cannulated cows fed the experimental diets. However, these changes were small, suggesting that too little monensin was fed. Fish meal reduced ruminal total amino acid (AA) but monensin did not alter ruminal NH_3 or total AA. Both fish meal and monensin increased NH_3 formation from casein AA using ruminal inoculum from the cannulated cows. There was no evidence from this trial that feeding 250 mg of monensin per day to lactating cows improved N utilization by reducing ruminal catabolism of the large amounts of free AA in alfalfa silage.
(**Key words:** monensin, fish meal, milk yield, N efficiency)

INTRODUCTION

Upon ensiling, a large proportion of the CP in haycrop silages is converted to NPN; typically, 50 to 60% (Broderick et al., 1990; Luchini et al., 1997) of the total N in alfalfa silage is in this form. Conversion of true protein to NPN substantially reduces efficiency of CP utilization in lactating cows (Nagel and Broderick, 1992; Broderick, 1995). Most of the NPN in alfalfa silage is present as free AA and small peptides (Broderick, 1995b). In the rumen, α-amino N in the NPN fraction is converted to NH_3 when AA deamination exceeds microbial incorporation, and there is probably little escape of free AA (Choi et al., 2002). Ammonia overflow from the rumen can lead to the excessive loss of urinary N by dairy cows fed alfalfa silage as their principal forage. Yang and Russell (1993) found that monensin feeding reduced in vitro and in vivo ruminal NH_3 formation from protein hydrolysates by suppressing certain bacteria with high deamination activity. Thus, monensin supplementation may improve N efficiency by increasing gut absorption of α-amino N. Because of the high levels of free AA, monensin might be particularly effective in lactating cows fed alfalfa silage. Ruiz et al. (2001) observed that adding monensin to the diet reduced ruminal NH_3, suggesting a N-sparing effect. Phipps et al. (2000) reported that feeding monensin at 150, 300, and 450 mg/d improved milk yield, with the greatest effect at 150 mg/d, in cows averaging 26.7 kg milk/d and fed diets containing 64 to 74% forage. Large field studies showed that prepartum treatment with a slow-release monensin supplement increased serum glucose and reduced serum ketones (Duffield et al., 1998) and reduced incidence of clinical ketosis (Duffield et al., 2002), all indicating improved glucogenic status. Seal and Parker

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(1996) observed that propionate infusion increased AA leaving the portal-drained viscera, possibly by reducing the amounts of AA catabolized for energy by the gut. Thus, monensin supplementation also may improve N efficiency in the lactating cow through positive effects on increased formation of ruminal propionate.

The objectives of these trials were to determine if monensin supplementation of dairy cows fed all their forage as alfalfa silage would: 1) improve the yield of milk and milk components and 2) improve N efficiency and reduce the need for RUP supplementation.

MATERIALS AND METHODS

Lactation Trial

Two sets of 24 multiparous Holstein cows, averaging (±SD) 610 (±54) kg of BW, parity 3.4 (±1.2), 53 (±17) DIM, and 39 (±4) kg/d of milk, were blocked by DIM into 2 sets of 6 groups of four in a trial of randomized complete block design. The experimental phase for the first set was during period 1 (wk 1 to 10) and for the second set was during period 2 (wk 7 to 16) of the trial. Cows were housed in tie stalls with free access to water and were not injected with rBST during the trial. Second-cutting alfalfa that was field wilted to about 40% DM, chopped to a theoretical length of 2.9 cm, and ensiled in 2 bunker silos was the sole forage fed. The 4 diets used in the trial were formulated from this alfalfa silage, high moisture ear corn (ground through a 10-mm screen when removed from the silo; Ekinci and Broderick, 1997), solvent-extracted soybean meal, low-solubles fish meal ("Sea-Lac," Zapata Protein, Hammond, LA), plus minerals and vitamins (Table 2): 1) control, 2) control plus 3% fish meal (DM basis), 3) 12 mg of monensin/kg of DM, and 4) monensin plus fish meal. Monensin was incorporated into the diets by adding 1% of a supplement made from a blend of Rumensin (Elanco Animal Health, Greenfield, IN) premix plus ground shelled corn as carrier formulated to 1200 mg of monensin/kg of DM; control diets were supplemented with ground shelled corn only. Weekly samples of these supplements were collected and stored at 20 to 25°C. At the end of the trial, all 16 weekly samples were analyzed for monensin content (by Elanco Animal Health). Determined monensin contents averaged 1061 mg/kg DM in the supplement and 10.2 mg/kg DM in the 2 monensin diets (Table 3). Control diets were formulated to contain 0 monensin; however, a second batch of ground shelled corn (fed from wk 11 to 16) was contaminated and averaged 220 mg of monensin/kg DM (Table 3). Thus, monensin content of the control diets was 0 only during period 1 and the control diets averaged 1.3 mg/kg of DM during period 2. Overall mean monensin content of control diets was 0.6 mg/kg DM over the 2 periods of the trial (Table 3). The determined rather than formulated monensin concentrations will be referred to in subsequent discussions.

All cows were fed the control diet for a 2-wk covariate period prior to starting the experimental period and production of milk and milk components was determined for use in statistical analysis. Cows were then randomly assigned within blocks of 4 to 1 of the 4 diets and were fed only that diet during the remaining 10 wk of the trial. Feed was offered once daily at 1000 h; orts were collected and recorded once daily at about 0900 h. Feeding rate was adjusted daily to yield orts equal to about 10% of feed offered. Weekly composites of each TMR and type of orts, alfalfa silage, and high moisture ear corn were collected from daily samples of about 0.5 kg and stored at –20°C. Content of alfalfa silage and high moisture ear corn of as-fed diets was adjusted weekly based on DM determined at 60°C (48 h). Intake of DM was computed based on the (60°C) DM contents of TMR and orts. Weekly samples of the fish meal, and solvent soybean meal were also collected and stored at 20 to 25°C.

Cows were milked twice daily and individual milk yields were recorded at each milking. Milk was sampled during 1 p.m. and 1 a.m. milking midway through wk 2 of the covariate period and midway through wk 2, 4, 6, 8, and 10 of each experimental period and analyzed for fat, protein, lactose, and SNF by infrared analysis (Wisconsin DHI Cooperative, Madison). Yields of 3.5% FCM were computed (Sklan et al., 1992). Milk also was deproteinized (Shahani and Sommer, 1951) and analyzed for milk urea N using an automated diacetyl monoxime reaction (Broderick and Clayton, 1997). Blood was sampled 4 h after feeding at the end of wk 4 and 10 from the coccygeal artery or vein of each cow. Blood was heparinized and stored at –20°C; thawed whole blood was analyzed for glucose and urea N (Broderick, 1986). Efficiency of conversion of feed DM was computed for each cow over the 2-wk covariate and over the 10-wk experimental periods by dividing mean milk yield by mean DMI. Apparent efficiency of N utilization, without correction for N mobilization or retention, was computed for each cow by dividing mean milk N output (total milk protein/6.38) by mean N intake. Cows were weighed on 3 consecutive days at the start and end of the trial to determine mean BW change over the 10-wk experiment.

Composites of each dietary ingredient and the TMR were prepared for each 2-wk period over the 16 wk by mixing equal DM (60°C; 48 h) from samples of alfalfa silage and high moisture ear corn, both ground shelled corns (with and without monensin), soybean meal, and fish meal that had been ground through a 1-mm screen (Wiley mill; Arthur H. Thomas, Philadelphia, PA).

Table 1. Composition of dietary ingredients.

Item[1]	Alfalfa silage	High moisture ear corn[2]	Solvent soybean meal	Fish meal[3]	Ground shelled corn[4]
	(% of DM)				
CP	20.7	8.8	53.3	70.6	9.4
Ash	11.3	1.6	6.7	21.7	1.5
NDF	45.1	12.9	10.4	15.0	9.5
ADF	35.2	5.6	5.3	5.0	3.4
NDICP	3.1	0.9	5.3	17.0	0.1
Fat	3.7	3.7	2.0	6.3	4.2
NFC	22.3	74.0	33.0	3.4	75.5
NE_L,[5] Mcal/kg DM	1.20	1.94	2.21	2.23	2.01

[1]NDICP = Neutral detergent insoluble crude protein, NFC = nonfiber carbohydrates.

[2]High moisture ear corn was ground through a 10 mm screen.

[3]Low solubles fish meal ("Sea-Lac," Zapata Proteins, Hammond, LA).

[4]Monensin carrier.

[5]From NRC (2001) tables, assuming DM intake = 3% of BW.

These composites were analyzed for DM at 105°C, ash and OM (AOAC, 1980), total N by a combustion assay (Leco FP-2000 N Analyzer; Leco Instruments, Inc., St. Joseph, MI), and sequentially for NDF and ADF using heat stable α-amylase (Van Soest et al., 1991) and Na_2SO_3 (Hintz et al., 1995). Composites of dietary ingredients also were analyzed for NDF-CP using the combustion N assay (Leco) on residual NDF and for fat (Dairyland Laboratories, Arcadia, WI) to compute nonfiber carbohydrates. At the end of the trial, weekly samples of alfalfa silage were thawed and water extracts were prepared (Muck, 1987). Extract pH was measured, then extracts were deproteinized (Muck, 1987) and analyzed for total AA and NH_3 (Broderick and Kang, 1980) and for NPN using a combustion assay (Leco FP-2000). Proportions of dietary DM from each component and overall dietary compositions were computed from ingredient analyses plus DM contents determined at 105°C (AOAC, 1980) for dry ingredients and at 60°C for alfalfa silage and high moisture ear corn. The NE_L contents were calculated using NE_L values reported for 3× maintenance in NRC (2001) tables. Compositions of the major feed ingredients are in Table 1, and the diets are in Table 2.

Ruminal Metabolism Trial

Eight ruminally cannulated, multiparous Holstein cows with means (±SD) at the start of the trial of 604 (±47) kg of BW, parity 3.1 (±2.5), 52 (±48) DIM, and 33 (±7) kg/d of milk were blocked by DIM into 2 groups and randomly assigned to 4 treatment sequences in 2 balanced 4 × 4 Latin squares with 4-wk periods (total 16 wk). The diets were the same as those fed in the lactation trial (Table 2), and this study ran at the same time as the lactation trial. Although milk yield also was determined, DMI is the only production trait that will be reported for these cows. On d 27 of each period, samples of strained ruminal fluid were taken from each cow from the ventral sac at 0 (just prior to feeding), 1, 2, 3, 4, and 6 h after feeding; samples were prepared by straining contents through 2 layers of cheesecloth. After pH was measured, samples were preserved by adding 0.2 mL of 50% (vol/vol) H_2SO_4 to 10 mL of ruminal fluid and 5 mL of concentrated formic acid to 5 mL of ruminal fluid, then storing at –20°C. Samples acidified with H_2SO_4 were thawed, centrifuged (30,000 × *g*, 15 min, 2°C), and supernatants analyzed for NH_3 and total AA (Broderick and Kang, 1980). Samples acidified with formic acid were thawed, centrifuged (10,000 × *g*, 40 min, 2°C), and supernatants analyzed for acetate, propionate, isobutyrate, butyrate, isovalerate plus 2-methylbutyrate, valerate, and total VFA (Brotz and Schaefer, 1987) by GLC (Varian Vista 6000; Varian Instrument Group, Walnut Creek, CA).

On d 28 of each period, one sample of about 250 ml of ruminal fluid was taken from the ventral sac of each cow at 4 h after feeding for ruminal in vitro incubations. After straining through four layers of cheesecloth, 100-mL aliquots of ruminal fluid from each cow were added to flasks containing 100 mL of warm (39°C) pH 7, 0.1 m*M* potassium phosphate buffer containing 3.0 m*M* mercaptoethanol. Flasks were gassed with CO_2, mixed and capped with Bunsen valves. After ruminal fluid-buffer mixtures had been rewarmed to 39°C, they were continuously stirred, and 1.00 mL per tube was dispensed by repipet to 4 sets of 8 duplicate tubes containing: 0.50 mL of McDougall's buffer (blanks), or 0.50 mL of McDougall's buffer plus 0.50% (wt/vol) casein (no. C-5890; Sigma Chemical Co., St. Louis, MO), acid-hydrolyzed casein (Sigma no. C-9386), or enzymatically hydrolyzed casein (Sigma no. C-1026). The head space

Table 2. Composition of diets.

Item	Control	Control + Fish Meal	Monensin	Monensin + Fish Meal
	(% of DM)			
Alfalfa silage	56.4	56.4	56.4	56.4
High moisture ear corn[1]	38.8	35.9	38.8	35.9
Solvent soybean meal	2.8	2.8	2.8	2.8
Fish meal[2]	—	2.9	—	2.9
Ground shelled corn[3]	0.96	0.96	—	—
Monensin supplement[4]	—	—	0.96	
Dicalcium phosphate	0.60	0.60	0.60	0.60
Trace-mineralized salt[5]	0.30	0.30	0.30	0.30
Potassium magnesium sulfate[6]	0.06	0.06	0.06	0.06
Vitamin premix[7]	0.10	0.10	0.10	0.10
Mean composition				
CP, %	16.7	18.5	16.7	18.5
NDF, %	30.8	30.9	30.8	30.9
ADF, %	22.2	22.2	22.2	22.2
Monensin,[3,4] mg/kg DM	0.6	0.6	10.2	10.2
Nonfiber carbohydrates,[8] %	43	41	43	41
NE_L,[8] Mcal/kg DM	1.51	1.52	1.51	1.52

[1]High moisture ear corn was ground through a 10 mm screen.

[2]Low solubles fish meal ("Sea-Lac," Zapata Proteins, Hammond, LA).

[3]Control, ground shelled corn fed during the last 6-wk of the trial (weeks 11 to 16) was contaminated with Monensin. Control diets contained 0 Monensin during weeks 1 to 10 and 2.1 mg Monensin/kg DM during weeks 11 to 16. Therefore, overall mean, measured concentration in Control diets as fed was 0.6 mg Monensin/kg DM. See Table 3.

[4]Monensin was supplemented as a mixture of ground shelled corn and commercial Rumensin pre-mix, blended into diets to give 12.0 mg Monensin/kg DM. The overall mean, measured concentration in Monensin diets as fed was 10.2 mg Monensin/kg DM. See Table 3.

[5]Provided 27 mg of Mn, 27 mg of Zn, 17 mg of Fe, 7 mg of Cu, 0.40 mg of I, 0.30 mg of Se, and 0.10 mg of Co/kg of DM.

[6]Provided 66 mg of Mg, 108 mg of K, and 132 mg of S/kg of DM.

[7]Provided 3880 IU of vitamin A, 730 IU of vitamin D, and 0.73 IU of vitamin E/kg of DM.

[8]Dietary contents of nonfiber carbohydrates and NE_L were computed from ration content and composition (Table 1) of each ingredient.

of each tube was gassed with CO_2 to help exclude O_2, and tubes were capped and incubated in a water bath at 39°C. One set of 8 tubes from each cow was killed at 0, 1, 2, and 3 h by adding 0.50 mL of 20% (wt/vol) perchloric acid per tube; killed tubes were stored at –20°C. Perchloric acid was added to the 0-h tubes prior to addition of inocula. A second 100-mL aliquot of strained ruminal fluid from each cow was added to a bottle containing 1.0 mL of formalin; bottles were swirled then held on ice until returned to the laboratory (about 6 h later). Microbial pellets were harvested by centrifugation (15,000 × *g*, 30 min, 2°C), washed twice with McDougall's buffer, then recentrifuged at the same speed. Pellets were analyzed for DM (105°C, 24 h) and N (Leco N-analyzer) to estimate microbial CP content of the inocula. The 32 samples from in vitro incubations using ruminal fluid from each cow were later thawed, centrifuged (10,000 × *g*, 40 min, 2°C), and supernatants analyzed for NH_3 (Broderick and Kang, 1980). Rates of NH_3 release from casein, and from acid- and enzymatically-hydrolyzed casein, were determined from linear regression of concentrations (per unit microbial CP) on time (0 to 3 h) for the inocula from each cow during each of the four periods.

Statistical Analysis

Statistical analyses of most of the production traits from the lactation trial (DMI, yield of milk, 3.5% FCM, fat, protein, lactose, and SNF, milk content of fat, protein, lactose, and SNF, and milk yield/DMI) was done using the general linear model procedure of SAS (1989) with a model that included the covariate value for each trait, diet, block(period), and the diet × period interaction. No diet × period interactions were significant (P = 0.11 for fat yield and $P \geq 0.25$ for the other traits). The diet × period interaction term was removed from the model when $P > 0.25$. A single mean value was computed for each cow in the lactation trial for BW change, apparent milk N/N intake and for blood urea and glucose concentrations. Statistical analysis of these 4 traits was done using the same model except without a covariate value. Again, no diet × period interactions were significant ($P \geq 0.11$). Statistical analyses of milk urea

Table 3. Monensin concentrations on a DM basis measured in Control and Monensin supplements and in diets.

Week	Sample[1]	Monensin, mg/kg		Sample[1]	Monensin, mg/kg	
		Supplement	Diet[2]		Supplement	Diet[2]
		(DM basis)			(DM basis)	
1	C1	0	0	M1	1156	11.1
2	C2	0	0	M2	958	9.2
3	C3	0	0	M3	1152	11.1
4	C4	0	0	M4	1218	11.7
5	C5	0	0	M5	1100	10.6
6	C6	0	0	M6	1006	9.7
7	C7	0	0	M7	1166	11.2
8	C8	0	0	M8	1073	10.3
9	C9	0	0	M9	1011	9.7
10	C10	0	0	M10	991	9.5
11	C11	246	2.4	M11	1138	10.9
12	C12	221	2.1	M12	1086	10.4
13	C13	206	2.0	M13	1104	10.6
14	C14	246	2.4	M14	998	9.6
15	C15	203	1.9	M15	839	8.1
16	C16	198	1.9	M16	981	9.4
Wk 1-10	Mean	0	0		1083	10.4
(Period 1)	(SD)				(89)	(0.9)
Wk 7-16	Mean	132	1.3		1039	10.0
(Period 2)	(SD)	(115)	(1.1)		(95)	(0.9)
Overall	Mean	66	0.6		1061	10.2

[1]Samples C1 to C16 were Control supplements and M1 to M16 were Monensin supplements prepared using ground shelled corn.

[2]Diets averaged 0.96% of DM from the Control or Monensin supplements.

data (5 biweekly observations/cow) were done using the mixed procedure of SAS (Littell et al., 1996) with a model including diet, block, block(period), cow (period), week, and week × diet and period × diet interactions. All terms were considered fixed, except for cow (period) and residual error, which were considered random. Statistical analyses of DMI, ruminal concentrations of microbial CP, and in vitro NH_3 production rates from the 3 N substrates in the ruminal metabolism trial were done as a 4 × 4 Latin square, replicated twice, using the general linear models procedure of SAS (1989). The model included diet, cow, period, and period × diet interaction. Statistical analyses of ruminal pH (analyzed as H^+ concentration and converted back to pH; Murphy, 1982) and concentrations of N metabolites, and VFA were done with repeated measures on time using the mixed procedure of SAS (Littell et al.,1996) with a model including diet, period, cow, time, and time × diet and interaction. All terms were considered fixed, except for cow and residual error, which were considered random. Dietary treatment effects were evaluated for all traits in both trials using 3 orthogonal contrasts: 1) monensin addition, 2) fish meal addition, and 3) monensin × fish meal interaction. Significance was declared at $P \leq 0.05$, and trends were identified at $P \leq 0.10$.

RESULTS AND DISCUSSION

Lactation Trial

Alfalfa silage fed in this trial contained 43% DM and 20.7% CP (DM basis) and extract pH averaged 4.6 (computed from mean [H^+]), indicating it was of good quality. The NH_3 and NPN contents were typical (Luchini et al., 1997), accounting for 9.7 and 52% of the total N. Thus, by difference, free AA plus peptide N accounted for about 42% of the total CP equivalent in the alfalfa silage. Analysis of total AA in the NPN fraction indicated that 35% of alfalfa silage N, about 25 and 22% of the total N in the diets containing, respectively, 16.7 and 18.5% CP, was fed as free AA. Therefore, these rations should have been appropriate for testing effectiveness of monensin for reducing ruminal catabolism of free AA (Yang and Russell, 1993).

Results from feeding these diets on the production of lactating dairy cows are in Table 4. Although there was no effect of fish meal or monensin feeding on DMI, several other production traits were influenced by diet. Body weight gain and yield of milk, protein, and SNF were increased, and there was a trend for greater lactose yield, with fish meal supplementation. Production of milk and milk components has been improved by supplementing fish meal to dairy cows fed forage as

Table 4. Effect of fish meal and Monensin supplementation on DMI, BW gain, milk production, milk urea, and blood urea and glucose.[1]

Item[2]	Control	Control + 3% FM	Monensin	Monensin + 3% FM	SE	Contrast $P < F$[3] FM	Mon	FM × Mon
Monensin, mg/d	15	16	241	255	—	—	—	—
Fish meal CP, kg/d	0	0.51	0	0.50	—	—	—	—
DMI, kg/d	25.0	25.2	23.7	25.3	0.5	0.11	0.22	0.19
BW gain, kg/d	0.57	0.52	0.33	0.68	0.06	0.02	0.52	<0.01
Milk, kg/d	37.1	39.4	36.4	37.8	1.0	0.05	0.26	0.63
3.5% FCM, kg/d	34.4	36.1	32.2	33.8	1.1	0.13	0.04	0.90
Milk composition, %								
Fat	3.15	3.01	2.86	2.77	0.12	0.32	0.03	0.82
Protein	3.09	3.08	3.02	3.00	0.04	0.72	0.03	0.98
Lactose	4.79	4.83	4.81	4.77	0.04	0.95	0.61	0.28
SNF	8.59	8.62	8.53	8.48	0.06	0.86	0.08	0.48
Yield, kg/d								
Fat	1.14	1.17	1.04	1.07	0.05	0.42	0.03	0.98
Protein	1.13	1.22	1.08	1.14	0.03	0.02	0.05	0.65
Lactose	1.77	1.91	1.73	1.81	0.06	0.06	0.27	0.60
SNF	3.16	3.40	3.06	3.21	0.10	0.04	0.14	0.61
Apparent efficiency								
Milk/DMI	1.46	1.56	1.55	1.52	0.04	0.37	0.50	0.14
FCM/DMI	1.37	1.43	1.38	1.32	0.04	0.96	0.24	0.14
Milk N/N-intake	0.258	0.255	0.274	0.234	0.007	<0.01	0.01	<0.01
MUN, mg N/dl	10.6	13.9	11.1	14.7	0.5	<0.01	0.25	0.83
BUN, mg N/dl	13.1	16.7	13.0	16.9	0.5	<0.01	0.91	0.79
Blood Glucose, mg/dl	52.8	49.9	55.3	52.6	1.3	0.04	0.06	0.94

[1]BUN = Blood urea N, FM = fish meal, Mon = Monensin, MUN = milk urea N.

[2]Least square means (n = 12 for all values except MUN for which n = 60).

[3]Probabilities of significance for orthogonal contrasts.

alfalfa silage (Broderick, 1992) or grass silage (Keady et al., 1998; Heikkila et al., 1998). These consistently positive responses occur because ruminant-grade fish meal contains relatively high proportions of RUP (NRC, 2001) and Met and Lys status is improved (Santos et al., 1998). Apparent N efficiency (milk N/N intake) was lower, although milk protein increased about 80 g/d, because CP intake was about 600 g/d greater when fish meal was fed. It should be noted that this estimate of N efficiency did not involve total collections and ignored possible N mobilization or retention. Increased milk and blood urea concentration resulted because both parallel dietary CP content (Broderick and Clayton, 1997). Blood glucose concentration was reduced about 3 mg/dL with fish meal; however, reduced blood glucose was not observed in several earlier trials when fish meal was added to the diet (Broderick, 1992, 1995a). Increased milk and component yield with fish meal feeding indicated that metabolizable protein supply limited production on the unsupplemented diets in the present trial. Moreover, computations made using the NRC (2001) model indicated that the 2 diets containing fish meal supplied sufficient metabolizable protein to support 36.5 kg/d of milk, which was 7.2 kg/d more milk than the yield computed for the 2 diets without supplemental fish meal.

Addition of monensin to the diet resulted in reduced yield of 3.5% FCM; this occurred because monensin depressed milk fat content and yield (Table 4). An additional finding was that monensin reduced milk protein content and yield; depressed protein concentration was related to a trend for reduced SNF content in milk. Reduced milk fat and protein contents also were reported in studies where monensin feeding increased milk yields (Phipps et al., 2000; Ruiz et al., 2001). It was anticipated that monensin might improve N utilization through reduced ruminal AA deamination (Yang and Russell, 1993) or via increased glucose synthesis from elevated ruminal propionate (Van Maanan et al., 1978). Increased propionate supply also might have reduced the demand for gluconeogenesis from AA. There was a trend ($P = 0.06$) for increased blood glucose with monensin feeding. Prepartum supplementation of dairy cows with a slow-release form of monensin increased serum glucose, reduced serum ketones (Duffield et al., 1998), and reduced incidence of clinical ketosis (Duffield et al., 2002). Vallimont et al. (2001) reported that periparturient supplementation of monensin improved glucose status of cows in the subsequent lactation. A fish meal × monensin interaction was noted for BW gain—average daily gain was about equal on the control with or without fish meal but was about double on monensin plus

fish meal versus monensin alone. Moreover, a significant effect of monensin on milk N/N intake was related to a fish meal × monensin interaction. Apparent N efficiency was greater on monensin versus control without added fish meal but declined when fish meal was fed with monensin. Milk N/N intake, uncorrected for N mobilization or retention, averaged 0.254 and 0.257 with and without monensin. In grazing cows, monensin supplementation has been reported to improve milk yield (van der Werf et al., 1998) and to reduce milk urea N from 22 to 19 mg/dL, an indication of improved N efficiency (van der Merwe et al., 2001). Moreover, significant increases in apparent CP digestibility also have been observed with monensin feeding (Plaizier et al., 2000).

The second batch of ground shelled corn fed in the control diets during the last 6-wk of period 2 of the lactation trial was contaminated with monensin (Table 3). Thus, the average differential in monensin intake between cows fed monensin and control diets was greater during period 1 (257 versus 0 mg/d for first 24 cows) than during period 2 (243 versus 31 mg/d, or 212 mg/d, for the second 24 cows). However, probabilities for period × diet interactions were not significant for any production variable, ranging from 0.11 for fat yield to 0.90 for milk yield; probabilities for period × diet interactions for yield of FCM, protein, lactose, and SNF were, respectively, 0.25, 0.68, 0.76, and 0.81. This indicated that responses to supplements did not differ between the 2 periods and that contamination of the control diets with the small amount of monensin during period 2 did not alter experimental results. Monensin intake in the current trial was lower than that fed in a number of other lactation studies. Monensin was fed at 300 mg/d in several trials (van der Werf et al., 1998; van der Merwe et al., 2001; Vallimont et al., 2001; Ruiz et al., 2001). Phipps et al. (2000) supplemented monensin at 150, 300, and 450 mg/d in one lactation study and obtained increased milk yields at all 3 levels. Monensin was fed for longer than 10-wk periods in all of these studies. For example, Phipps et al. (2000) supplemented monensin for 19-wk in one trial and from 3 and 8 wk prepartum through 32 wk after calving in 2 consecutive lactations in a second trial. It should be noted, however, that the effects of monensin on ruminal microorganisms appear to be mediated very rapidly (see below).

Ruminal Metabolism Trial

Four-week periods were used in the Latin square with ruminally cannulated cows because16-wk was the total length of the production trial and because there was evidence in the literature that adaptation to monensin is rapid. The potassium depletion effect of monensin on mixed ruminal bacteria occurred within less than 15 min in vitro and within 4 d in vivo (Lana and Russell, 1996). Yang and Russell (1993) reported that decreased ruminal NH_3 occurred within 3 to 5 d of adding monensin to the diet. Moreover, observations in the present study were made after a 26-d adaptation period. Table 5 reports the results of feeding the same diets as in the lactation trial on ruminal metabolite concentrations and on catabolism of casein and casein hydrolysates to NH_3 and total AA. There were no effects of dietary supplementation on ruminal pH or on concentrations of NH_3-N, microbial CP, and total VFA. However, feeding fish meal depressed ruminal total AA, despite the fact that fish meal supplied additional RDP. Fish meal appeared to reduce propionate on control and to increase butyrate on both control and monensin diets. On average, monensin supplementation decreased acetate, butyrate, and acetate: propionate ratio, and increased propionate. These effects all have been commonly observed with monensin (Chalupa, 1980), but the changes found in the present study were of small magnitude. This may be due to the relatively low amounts of monensin fed. For example, Prange et al. (1978) reported that adding 33 mg of monensin/kg DM to a diet with 70% alfalfa-grass hay in steers increased molar proportion of propionate from 19.1 to 24.7%, and decreased molar proportions of acetate from 71.0 to 66.8% and butyrate from 9.9 to 8.5% of total VFA. Ruiz et al. (2001) observed changes in VFA pattern of similar magnitude, plus reduced ruminal NH_3, with feeding 350 mg/d of monensin to lactating cows. Several significant fish meal × monensin interactions also were detected in the current trial. Without monensin, feeding fish meal increased acetate, reduced propionate, and increased acetate:propionate ratio, while the reverse was true in the presence of dietary monensin. Concentrations of branched-chain VFA increased slightly (but nonsignificantly) with fish meal addition to control, but were numerically lower when fish meal was fed with monensin. Greater RDP from fish meal would be expected to increase branched-chain VFA in the rumen. Reduced concentrations of branched-chain VFA when monensin was fed with fish meal would have occurred if AA catabolism were depressed (Yang and Russell, 1993).

In vitro ruminal degradation of casein to NH_3 and NH_3 release from enzymatically hydrolyzed casein (a mixture of free AA and small peptides) were not influenced by feeding either fish meal or monensin (Table 5). However, NH_3 release from acid-hydrolyzed casein (a mixture of free AA) was increased 12% by fish meal ($P = 0.04$) and 11% by monensin ($P = 0.06$). Increased ruminal catabolism of free AA with fish meal feeding

Table 5. Effect of fish meal and Monensin supplementation on DMI, ruminal pH, NH_3, total free AA, microbial CP, VFA, and rates of in vitro NH_3 production.[1]

Item[2]	Control	Control + 3% FM	Monensin	Monensin + 3% FM	SE	Contrast $P < F$[3] FM	Mon	FM × Mon
Monensin intake, mg/d	15	13	229	229	—	—	—	—
Fish meal CP, kg/d	0	0.43	0	0.45	—	—	—	—
DMI, kg/d	22.3	21.4	22.5	22.5	1.0	0.64	0.50	0.62
Ruminal concentrations								
$[H^+]$, μM	0.98	0.82	0.74	0.72	0.12	0.38	0.12	0.47
pH[4]	6.01	6.09	6.13	6.14	—	—	—	—
NH_3-N, mg/dl	21.1	21.0	21.2	20.4	1.2	0.70	0.82	0.72
Total AA, mM	3.59	2.36	3.41	2.48	0.44	0.01	0.95	0.70
Microbial CP, mg/ml	8.1	7.6	7.5	8.0	0.4	0.93	0.85	0.36
Total VFA, mM	135	131.4	130.7	135.4	3.6	0.82	0.95	0.11
Individual VFA, mol/100 mol								
Acetate	63.2	64.4	63.5	62.6	0.3	0.67	0.05	0.01
Propionate	20.4	18.1	20.1	20.4	0.4	0.02	0.02	0.01
Ac:Pr	3.16	3.65	3.21	3.12	0.02	0.08	0.04	0.02
Butyrate	11.2	12.0	10.9	11.5	0.1	<0.01	<0.01	0.69
Isobutyrate	1.25	1.32	1.34	1.30	0.003	0.60	0.23	0.04
Isovalerate + 2-methylbutyrate	1.98	2.20	2.16	2.10	0.01	0.17	0.53	0.02
BC-VFA	3.22	3.53	3.50	3.40	0.02	0.20	0.34	0.01
Valerate	1.95	2.00	2.01	2.05	0.01	0.37	0.34	0.87
NH_3 production, nmol/(hr*mg protein), from								
Casein	86.0	90.9	86.0	82.7	7.1	0.92	0.61	0.63
CHE	117.8	130.8	130.4	137.6	6.7	0.19	0.21	0.72
CHA	107.7	118.7	117.3	133.3	5.2	0.04	0.06	0.69

[1]Ac:Pr = Acetate:propionate ratio, BC-VFA = total branched-chain VFA (isobutyrate plus isovalerate + 2-methylbutyrate), CHA = acid-hydrolyzed casein, CHE = enzymatically-hydrolyzed casein, FM = fish meal, Mon = Monensin.

[2]Least square means (n = 48 for all values except microbial CP and NH_3 production rates, for which n = 8).

[3]Probabilities of significance for orthogonal contrasts.

[4]Computed from the mean $[H^+]$ (Murphy, 1982).

may account for the depression of total AA observed in vivo (Table 5). Feeding true protein has been observed to stimulate microbial AA deamination (Allison, 1970). Overall, there was no evidence from the current study that monensin fed to dairy cows at 210 to 250 mg/d decreased AA and peptide catabolism in the rumen. Although significant, the small alterations in ruminal acetate, propionate and butyrate suggested that the amount of monensin fed also may have been insufficient to greatly alter ruminal catabolism of free AA and peptides. In their summary of a number of feeding trials, Goodrich et al. (1984) reported that increasing dietary monensin from 11 to 28 mg/kg DM significantly improved feed: gain ratio in feedlot animals. Yang and Russell (1993) fed 52 mg of monensin/kg DM when they observed a 30% reduction in NH_3 concentration with the addition of protein hydrolysates to the in vivo rumen. Monensin appeared to reduce NH_3 production by suppressing most if not all of the species of NH_3-hyperproducing bacteria that have been isolated from the rumen (Attwood et al., 1998; Russell and Rychlik, 2000). However, at least one important species of NH_3-hyperproducing bacteria was not eliminated from the rumen at 350 mg/d of monensin in the diet (Russell and Rychlik, 2000). Further experimentation will be required to determine whether monensin must be supplemented at greater than 10 to 12 mg/kg DM to suppress ruminal AA deamination in dairy cows fed diets based on alfalfa silage.

SUMMARY AND CONCLUSIONS

Supplementing monensin at 10 mg/kg DM (~250 mg/d) reduced yields of fat, protein, and FCM in cows fed a diet containing 56% alfalfa silage DM. However, supplementation with fish meal increased yields of milk, protein, and SNF, and increased BW gain, indicating that the basal diet was limiting in metabolizable protein. Apparent N efficiency was reduced, and milk urea increased, by fish meal feeding, suggesting that the requirement for RUP was exceeded. There was a significant monensin × fish meal interaction on apparent N efficiency (milk N/N intake), which was highest on monensin but lowest on monensin plus fish meal. Feeding fish meal reduced ruminal total free AA but monensin supplementation did not alter ruminal NH_3 or total free AA. Monensin increased blood glucose and ruminal propionate, and decreased ruminal acetate, butyrate

and acetate:propionate ratio. However, these changes were much smaller than has been observed previously, suggesting that monensin may have been fed at too low a level. Both fish meal and monensin increased the rate of NH_3 formation from casein free AA. There was no evidence from this trial that monensin fed at 10 mg/kg DM improved N utilization by altering ruminal AA catabolism. Additional trials are required with rations based on alfalfa silage to identify a possible optimal level of dietary monensin and to confirm that feeding monensin has positive effects on ruminal N metabolism and milk production in lactating dairy cows.

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