Effect of Monensin Supplement during Transition Phase on Rumen Fermentation and Microbial Efficiency

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Abstract

The effect of monensin (M) supplementation of transition dairy cow diets on fermentation and microbial efficiency (g N/kg OM truly digested; MOEFF) was evaluated in 2 experiments using a continuous culture system. In experiment 1, the treatments were arranged as a completely randomized design (n=24) with 2 treatments, prepartum diet without M (Control) and with M (24 mg/kg DM). Fermentors were set at 6%/h dilution rate, incubated at 39°C, and allowed to equilibrate for 3 days followed by a 3 day collection phase. Ammonia, VFA and pH were measured at 2 h after feeding. Data were analyzed using the GLM procedure of SAS. Supplemental M increased ($P<0.05$) propionic acid (14.8 vs 12.4 mM) and decreased ($P<0.05$) acetic acid to propionic acid (A:P) ratio (3.6 vs 4.2) when compared to the Control. Ammonia, total VFA, acetic acid, butyric acid, branch chain (BC) VFA, pH and MOEFF were not altered by M ($P>0.05$). In experiment 2, the treatments were arranged as 2 x 2 factorial and analyzed using the GLM procedures of SAS that included M supplement (Control, no monensin, and M, monensin added at 24 mg/kg DM) during the prepartum phase (PP) and during the lactation phase (LP) with PP and LP used as main effects. Fermentors were set as in experiment 1 and allowed to equilibrate for 3 d followed by 3 d of dry cow diet and 3 d fed lactation cow diet. Supplemental M fed with dry cow diet significantly increased ($P=0.03$; 2.85 vs 4.42 mM) whereas supplemental M during LP had no effect ($P>0.05$) on ammonia concentration observed at 2 h post feeding. However, there was no interaction between M treatment during PP and LP on ammonia concentration ($P>0.05$) 2 h post feeding. Supplemental M during PP decreased pH ($P<0.05$), increased total VFA (123.9 vs 110.1 mM), acetic acid (81.74 vs 73.06 mM), BCVFA (8.14 vs 5.40 mM) and decreased A:P ratio ($p<0.05$; 3.73 vs 3.88). Supplemental M during PP increased bacteria dry matter flow (6.13 vs. 5.27 g), bacteria organic dry matter flow (5.33 vs. 4.58 g), bacteria N flow (0.54 vs. 0.46 g), organic matter truly digested (OMTD; 31.52 vs 30.31 g), decreased A:P ratio (3.73 vs. 3.88) but did not alter ($P>0.05$) MOEFF (16.61 vs 14.93). Supplemental M during LP increased ($P<0.05$) OMTD (31.32 vs. 30.50 g), decreased isobutyric acid ($P<0.05$; 0.69 vs. 0.84 mM), A:P ratio ($P=0.06$; 3.46 vs. 4.14), but did not alter ($P>0.05$) MOEFF (15.59 vs 15.96). Propionic acid tended to increase ($P=0.06$) when M was supplemented during PP and LP. There were no interactive effects of M supplement during PP and LP on fermentation products and MOEFF. In summary, the results of these experiments show that the addition of M during both phases of transition increased OMTD and propionic acid which would increase energy available to transition dairy cows.

Keywords: monensin, transition phase, microbial efficiency, rumen fermentation

1. Introduction

The anticipated advantage of monensin administration to lactating dairy cattle is improved energy metabolism via increased ruminal production of propionate. Higher propionate leads to an enhancement of glucose precursor flow in the circulation, and subsequently increases the rate of gluconeogensis which leads to an increase in lactose synthesis and consequently increases milk production. An indirect advantage of monensin is the sparing of amino acids for gluconeogenesis which could influence milk production and composition [1].

Microbial protein is the best available source of protein for milk synthesis [2] and contributes 40 to 90 % of the amino acid supply entering the small intestine [3]. Research data concerning the use of monensin on microbial efficiency have been variable *in vivo* and *in vitro*. The influence of monensin on efficiency of microbial growth *in vivo* and *in vitro* has not been consistent [4, 5, 6]. However, there are limited data concerning the effects of monensin on microbial fermentation and efficiency during the transition phase both *in vitro* and *in vivo*.

The objective of this study is to determine the effects of monensin supplement during prepartum and lactation phases on rumen fermentation and microbial efficiency.

2. Materials and Methods

2.1 Experiment 1

Incubation System and Experimental Design. A single-flow effluent continuous culture apparatus [7] was used to determine the effects of monensin (M) supplementation of a prepartum diet on ruminal growth, efficiency and fermentation. The study was arranged as a completely randomized design. The treatments were prepartum diet without M (Control) and with M (24 mg/kg DM). Each treatment consisted of 12 independent fermentors having side effluent outlet which allowed a liquid volume of 1460 ml within a fermentor before the contents overflowed. A buffer solution of McDougall's artificial saliva was introduced into the fermentors by peristaltic pumps from buffer reservoirs at the dilution rate of 6% per hour. Carbon dioxide was continually flushed into the fermentors to maintain an anaerobic environment inside the fermentors. Rumen fluid was obtained from a non-lactating fistulated Holstein cow fed a dry cow diet formulated to meet NRC recommendations [1]. Rumen fluid was strained through four layers of cheese cloth and diluted 1:3 with McDougall's buffer and 1,460 ml were innoculated into each fermentor. Fermentors were incubated in a water bath maintained at 39^{o}C.

Table 1. Ingredient and chemical composition of pregnant non-lactating cow (Dry cow) and lactating cow diets (Lactation).

Item	Dry cow	Lactation
	------- % of DM--------	
Alfalfa ha	-	15.19
Alfalfa silage	-	7.26
Brewer grain	-	7.77
Corn silage	30.64	15.96
Dry corn	-	19.77
Grass hay	14.90	-
High moisture corn	-	6.22
Soybean hull	27.25	5.49
Soybean meal 48%	8.05	4.66
Soy plus®	-	3.89
Whole cotton seed	-	10.33
Premix	19.16 [a]	3.46 [b]
Nutrient Analysis	Dry cow	Lactation
	-------- % of DM--------	
DM, %	57.98	57.41

Table 1. Ingredient and chemical composition of pregnant non-lactating cow (Dry cow) and lactating cow diets (Lactation) (Continued).

Nutrient Analysis	Dry cow	Lactation
	-------- % of DM--------	
OM	94.55	93.49
CP	11.19	17.69
NDF	45.16	31.44
ADF	28.47	22.08

[a] Premix 1kg: ground corn 94.25%, iodine salt 1.6%, Vit A 64,000 iu, Vit D 12,800 iu, Vit E 489.2 iu, Cu 0.6 mg, Mn 3 mg, Zn 3 mg, Co 0.015 mg, Se 0.045 mg.
[b] Premix 1kg: ground corn 45.5%, dicalcium 15%, dynamite 10%, Biotin 10%, iodine salt 10%, lime stone 3%, Vit A100,000 iu, Vit D 20,000 iu, Vit E 330 iu, Cu 300 mg, Mn 1500 mg, Zn 1500 mg, Co 7.5 mg, Se 22.5 mg.

The diet used in this study was formulated to meet NRC recommendations for a pregnant, non-lactating dairy cow (Table 1). The diet was ground frozen with dry ice to pass through a 5 mm screen and stored at 20^{o}C to keep fresh and stable. The diet was thawed at room temperature prior to feeding and was fed to each fermentor during the adaptation period (day 1 to day 3) and collecting period (day 4 to day 6) manually at a rate of 40g DM/d in three equal portions at 0600, 1400 and 2200 h. Monensin at 50 mg was dissolved with 150 ml ethanol. A 1 ml aliquot of the solution was added to assigned fermentors (ethanol without monensin in equal volume was added to control fermentors) at each feeding time during collection period to provide 24 mg/kg DM as recommended by [8].

Sampling Procedures and Analyses. The fermentor contents were allowed to turn over four times for the adaptation period [7]. The adaptation phase was followed with a three day sampling period. During the sampling period, 5 ml samples of fermentor contents were collected daily at 2 h post feeding (0800 h) and preserved with 50 µl of 6 N HCl/ml and stored frozen (-20^{o}C) until analyzed for VFA and ammonia N. The pH of fermentor contents was measured daily during collection using a pH meter (Digi-Sense: Cole-Palmer Instrument Co., Chicago, IL/Model 107). The VFA and ammonia concentrations were analysed as described by [9].

Fermentor effluent was collected and sub-sampled during three day sampling period. Fermentors were the stopped and sampled at the end of the third day of the sampling period. Diet, fermentor and effluent samples were analyzed for DM, OM [10], N (LECO FP-428, Leco Co., St. Joseph, MI). The effluent and fermentor samples were analyzed for RNA content according to the procedure of [11]. Microbial N of effluent residues was calculated using the RNA:N ratio of isolated microorganisms in conjunction with purine content of the effluent residues (4 replicates per treatment). True digestibility of diet DM and OM (4 replicates per treatment) was calculated as differences of DM and OM between diets fed to fermentors and effluent residues corrected for microbial contribution. Microbial efficiency (MOEFF) was expressed as grams of microbial N/kg OM truly digested.

Statistical Analyses. Data on fermentation end products at 2 h post feeding, digestibility and MOEFF were analyzed as a completely randomized design using SAS [12]. Differences between treatment means were assessed by Least Squares mean separation when the F test was significant ($P<0.05$). Significance was declared at $P<0.05$.

2.2 Experiment 2

Incubation System and Experimental Design. This study consisted of two phases defined as the prepartum phase (day 4 to 6; PP) and lactation phase (day 7 to 9;

LP). The study was conducted, using the incubation system as previously described in experiment 1. The experiment was a completely randomized design as the treatments were arranged as 2x2 factorial with two levels of M (without or with M, 24 mg/kg DM) supplemented during the PP when fermentors were fed a dry cow diet and LP when fermentors were fed a lactation cow diet. Four fermentors per treatment were used and were operated at a 6 %/h dilution rate.

The dry cow diet used in PP was formulated to meet NRC recommendations for a pregnant, non-lactating dairy cow (Table 1). During LP, the lactation cow diet used was formulated to meet NRC recommendations for a lactating dairy cow (Table 1). The diets were ground and stored as described in experiment 1. The non lactating cow diet was fed manually to each fermentor during the adaptation period (day 1 to day 3) and PP at a rate of 40 g DM/d. The lactating cow diet was fed during the LP at a rate of 60 g DM/d in three equal portions at 0600, 1400 and 2200 h. Monensin (50 mg) was dissolved with 150 ml of 100 % ethanol. The monensin solution at 1, and 1.5 ml was added to assigned fermentors (100 % ethanol without monensin at equal volume was used as control) at each feeding time during PP and LP, respectively, to provide 24 mg monensin/kg DM as recommended by [8]. The increase of DMI from 40 to 60 g/d was based on a review by [13] who reported that DMI increased approximately 60% from 10 d prepartum to 10 d postpartum.

Sampling Procedures and Analyses. Sampling procedures of fermentor contents and fermentor effluents during the 3 day collection phase (day 7-9) and fermentors at the termination of this experiment were done as previously described in experiment 1. Samples of diets, effluent and fermentor were analyzed for DM and OM. The effluent and fermentor samples were analyzed for RNA content as previously described in experiment 1. Ammonia and VFA of fermentor contents were analyzed, whereas microbial N of effluent residues, true digestibility of DM and OM of diets and MOEFF were also calculated as previously described in experiment 1.

Statistical Analysis. Data on fermentation end products at 2 h post feeding, digestibility and MOEFF were factorially analyzed using SAS [12]. Each fermentor was an experimental unit. Sources of variation included phase (PP and LP), and the interaction between PP and LP with the inclusion/exclusion of monensin. Differences between treatment means in the main effects and interaction means between main effects were assessed by Least Squares mean separation when the F test was significant ($P<0.05$). Significance was declared at $P<0.05$.

3. Results

3.1 Experiment 1

Data on fermentor pH 2 h post feeding during the prepartum phase are reported in Table 2. When monensin was added to the fermentors at 24 mg/kg DM, fermentor pH (6.73) was not different ($P>0.05$) compared to the Control (6.74). Monensin supplementation at 24 mg/kg DM did not affect ammonia concentration ($P>0.05$) at 2 h post feeding. At this sampling time, concentrations of ammonia in fermentor content were equal for both treatments at 0.07 mM (Table 2).

Total VFA concentration including acetic acid, propionic acid, butyric acid, and BCVFA concentrations are reported in Table 2. Total concentrations of VFA were not different between treatments and ranged from 75.71 to 77.01 mM. Propionic acid concentration was higher ($P<0.05$) in fermentor contents supplemented with monensin (14.76 mM) compared to Control (12.39 mM). However, acetic acid, butyric acid and BCVFA including isobutyric acid,

valeric acid, isovaleric acid concentrations were not different due to the treatment. Acetic acid to propionic acid ratio (A:P) was higher (P<0.05) in control fermentors (4.64) compared to fermentors supplemented with monensin (3.62).

Data on digestibility and microbial efficiency during the prepartum phase were reported in Table 3. There were no treatment effects (P>0.05) on dry matter flow, organic matter flow, bacterial dry matter flow, bacterial organic matter flow, bacterial N flow, and percentage of bacteria N. Dry matter flow ranged from 25.67 to 26.77 g with organic matter flow ranging from 23.46 to 24.37 g. Bacterial dry matter flow ranged from 2.28 to 2.76 g while bacterial organic matter flow ranged from 1.97 to 2.40 g. Bacteria dry matter flow and bacterial organic matter flow tended to be lower (P=0.09) in fermentors supplemented with monensin (2.28 and 1.97 g, respectively) compared to Control (2.76 and 2.4 g, respectively). Bacteria N flow ranged from 0.19 to 0.23 g while percentage of bacteria N ranged from 8.21 to 8.53. As reported in Table 3, bacterial purine concentration was not different (P > 0.05) in bacteria isolated from the fermentor supplemented with monensin (107.44 mg/g DM) compared to Control (114.66 mg/g DM). Organic matter apparent digestibility, organic matter true digestibility and MOEFF were not different (P>0.05) due to the supplementation of monensin (Table 3).

3.2 Experiment 2

Effect of monensin supplementation during PP and LP on fermentation end products 2 hours post feeding during the lactation phase are reported in Table 4. Monensin supplementation during PP caused lower (P<0.05) pH (6.33) compared to the Control (6.43). Ammonia concentration of fermentors fed diet with monensin supplementation during PP (4.42 mM) was higher (P<0.05) than the Control (2.85 mM). Supplementation of monensin to diet fed fermentors during PP had higher (P<0.05) total VFA, acetic acid, isobutyric acid, valeric acid, isovaleric acid, and BCVFA concentrations but lower acetic acid to propionic acid ratio than the Control (Table 4). Propionic acid concentration also was higher (P=0.06) in fermentors fed diet supplemented with monensin during PP (22.90 mM) compared to the Control (19.19 mM). However, butyric acid was not different between treatments (P>0.05) ranging from 11.10 mM (M) to 12.05 mM (Control).

However, there was no effect of monensin supplementation during LP on fermentation end products measured at 2 h post feeding except for isobutyric, propionic acid and A:P ratio. Isobutyric acid was higher (P<0.05) when monensin was supplemented during PP but was lower (P<0.05) when monensin was supplemented during LP compared to the Control. When monensin was supplemented during LP, propionic acid tended to higher (P=0.06; 22.90 mM) while there was a trend for A:P ratio to be lower (P=0.06; 3.46) compared to the Control (19.19 mM and 4.14). There were no interactions between PP and LP for any of the fermentation end products.

Data on digestibility and microbial efficiency are reported in Table 5. There were no effects of monensin supplementation during PP and LP on dry matter flow and organic matter flow (P>0.05). Dry matter flow and organic matter flow were 31.61 to 32.50 g and 28.89 to 29.66 g, respectively, for fermentors fed diet supplemented with monensin and without monensin during PP. Monensin supplementation during PP supported higher (P<0.05) bacteria dry matter flow (6.13 vs 5.27), bacteria organic matter flow (5.33 vs 4.58 g) and bacteria N flow (0.54 vs 0.46 g) compared to no monensin (Table 5). However, there was no effect of monensin supplementation during LP on these parameters. Supplementation of monensin in fermentors during PP and LP had no

effect on percentage of bacteria N, concentration of purine in bacteria dry matter, and organic matter apparent digestibility. However, grams of organic matter true digestibility (OMTD) increased (Control = 30.31 and 30.50 vs. M = 31.52 and 31.32) due to monensin feeding during both phases (Table 5). MOEFF was not different due to the treatments. There were no interaction between monensin supplementation during PP and LP observed for any of the parameters reported in Table 5.

4. Discussion

Monensin has been reported to increase rumen pH in vitro [14].In vivo [15] has significantly higher rumen pH values observed postcalving in monensin-treated cows. In the current study, there was no effect of monensin supplementation on fermentor pH in experiment 1. The lack of response may be due to the dietary conditions used, given the diet used was high in fiber, which agrees with [16] who reported no effect of monensin on ruminal pH when the diet used in the study supported low ruminal lactate production.

Fermentor pH 2 h post feeding in experiment 2 was lower when the diet contained monensin during PP, concurrent with the higher total concentration of VFA. These data suggest pH declined as a function of VFA concentration.

Ammonia concentration and protein degradation decrease with monensin supplementation indicating a protein sparing effect as dietary protein escapes rumen digestion [17]. As reported by [18], a decrease in ruminal ammonia occurred within 3 to 5 days of monensin addition to the diet. [19] suggested ionophores are more effective in reducing rumen protein degradation in diets containing rapidly degradable concentrate sources versus hay- and silage-based diets because the soluble protein in hays and silages have a high concentration of NPN compared to concentrate feeds. Therefore, the lack of monensin effect on ammonia concentration might be because of the shorter period of monensin administration (1-3 days) together with the dietary conditions which decreased the effectiveness of monensin in the present study of experiment 1. However, monensin treatment in experiment 2 found monensin supplementation during LP and combined both phases had no effect on ammonia concentration, indicating no effect of monensin on ammonia concentration when supplemented to fermentors for 6 days.

Higher ammonia concentration concurrent with higher BCVFA in experiment 2 indicates that the deamination of amino acids by bacteria was increased by monensin supplementation during PP. The predominant species of proteolytic bacterium found in the rumen of most animals is *P. ruminicola* and can comprise more than 60 % of the flora when dairy cows are fed grass silage-based diets [20]. *P. ruminicola* has shown resistance to monensin [21]. [22] compared ammonia production by individual strains (*S. ruminantium*, *M. elsdenii*, and *B. fibrisolvens*) of rumen bacteria and found that *P. ruminicola* was the only species that consistently produced ammonia from amino acid. [23] reported hyper-ammonia producing (HAP) bacteria were not eliminated from the rumen when fed 350 mg of monensin per day. Therefore, the increase in ammonia concentration found in experiment 2 when monensin was added during PP might be due to the effect of monensin on the activity of *P. ruminicola* with change in diet from low to high protein. The increase of *P. ruminicola* activity provides peptide and amino acid for HAP and other ammonia producing bacteria species to produce ammonia by deamination.

Several studies reported changes in rumen fermentation, as demonstrated by changes in VFA pattern in dairy cows [24,25]. [26] reported monensin selected

against H_2 and formate producer, e.g. *R. albus*, *R. flavefaciens* and *B. fibriosolves*, which could lead to a depression of methane production in the rumen. The increase in propionic acid concentration implies a shift in flow of electrons from formate and methane to succinate or propionate. In experiment 1, monensin increased propionic acid concentration, indicating monensin shifted fermentation, resulting in an increase in propionic acid in the diet when it was high in fiber and low in energy and protein.

No effect of monensin on acetic acid concentration in the present study indicates that monensin at 0.46 μg/ml (monensin 24 mg/kg DM; diet fed fermentor 40 g DM to 1460 ml culture at 6%/h dilution rate) during PP and 0.68 μg/ml (monensin 24 mg/kg DM; diet fed fermentor 60 g DM to 1460 ml culture at 6%/h dilution rate) during LP did not inhibit acetate producing bacteria. However, [26] reported that monensin selected against Ruminococcus and Bytyrivibrio, acetate producing organisms. *Ruminococcus albus*, *Ruminococcus flavefaciens* and *Butyrivibrio fibrisolvens* were inhibited by 2.5 μg of monensin. It is important to note the level of ionophore used by Chen and Wolin was dramatically higher than used in the current experiment. Monensin increased acetic acid in experiment 2 when added during PP, implying an increase in cellulolytic bacteria associated with monensin and the increase in energy fed when diets were changed. Butyric acid was not changed as acetic acid concentration increased, indicating that the increase in acetic acid concentration is not related to the substrate for butyric acid producing bacteria.

The decrease in acetic acid to propionic acid ratio in the present study in experiment 2 agrees with results reported by [27] who administered CRC monensin to cows during 50-70 day before expected parturition. These researchers reported a numerical decrease in acetic acid to propionic acid ratio.

Monensin treatment improved the energy status of the cows. [28] reported that cows receiving monensin CRC treatment 30 days before expected calving date through 60 days after calving had reduced loss of condition score and increased milk production and milk protein yield. The authors also found a decrease in milk fat content without an effect on milk fat yield due to the treatment. The increase of total VFA concentration in experiment 2 due to monensin supplementation during LP in the present study and the increase of OMTD in both phases suggest monensin improves energy status of the cows and the increase in energy would be available for the maintenance and milk production by the cows.

It is reasonable to expect that the beneficial effects of monensin on transition dairy cow performance is at least partly due to its action on rumen metabolism that does not affect microbial growth or efficiency. The present study found that monensin did not alter MOEFF. Even in experiment 2, there was an increase in daily bacteria N flow. The lack of differences for MOEFF between treatments would be expected due to monensin treatment having significantly higher OMTD. The fact that there was no effect of monensin on percentage of N in bacteria and concentration of purine in bacteria DM in both experiments suggests that monensin does not change microbial composition.

The effect of monensin on fiber digestion is unclear. The inhibition of cellulose digestion in vitro was also reported by [29]. However, [4] reported no effect of monensin on fiber digestion when evaluated using nylon bags suspended in the rumen. In the current study, there was an increase ing OMTD concurrent with an increase in the concentration of total VFA and acetic acid due to monensin treatment during PP and LP. These changes indicate

monensin increased fiber digestion when the diet was switched from dry cow diet to lactation diet. [30] studied monensin CRC which was administered 3 wk before calving date in transition dairy cows, and reported monensin increased fiber digestion. The authors found that NDF and ADF digestibility were increased by 9.3 and 8.0 %, respectively, by monensin precalving. Post calving monensin did not affect NDF and ADF digestibility.

Table 2. The effects of monensin fed during prepartum phase on fermentation end products at 2 hours post feeding during prepartum phase (experiment 1).

Item	Treatment [1]		SE	P-value
	Control	Monensin		
pH	6.74	6.73	0.02	0.78
Ammonia, mM	0.07	0.07	0.02	0.97
Total VFA, mM	77.01	75.71	4.33	0.66
Acetic acid, mM	52.35	52.80	3.09	0.92
Propionic acid, mM	12.39[b]	14.76[a]	0.66	0.02
Butyric acid, mM	6.72	6.49	0.75	0.83
Isobutyric acid, mM	0.56	0.48	0.04	0.18
Valeric acid, mM	1.07	1.28	0.09	0.12
Isovaleric acid, mM	1.93	1.91	0.27	0.96
BCVFA[2], mM	3.55	3.66	0.38	0.84
A:P ratio[3]	4.64[a]	3.62[b]	0.20	0.04

[a, b] Superscripts with different letters within row differed (P<0.05).
[1] Treatment represents diets supplemented without (Control) or with monensin (Monensin) at 24 mg/kg DM.
[2] Branch chain VFA includes isobutyric acid, valeric acid, and isovaleric acid concentrations.
[3] Acetic acid: propionic acid ratio.

Table 3. The effects of monensin fed during prepartum phase on digestibility and microbial efficiency during prepartum phase (experiment 1).

Item	Treatment [1]		SE	P-value
	Control	Monensin		
Dry matter flow (g)	26.77	25.67	1.00	0.47
Organic matter flow (g)	24.37	23.46	0.93	0.51
Bacteria DM Flow (g)	2.76	2.28	0.17	0.09
Bacteria organic matter flow (g)	2.40	1.97	0.15	0.09
Bacteria N flow (g)	0.23	0.19	0.01	0.13
Bacteria N (%)	8.21	8.53	0.18	0.26
Bacteria purine (mg/g DM)	114.66	107.44	5.60	0.40
Organic matter apparent digestibility (%)	35.56	37.96	2.46	0.51
Organic matter apparent digestibility (g)	13.45	14.36	0.93	0.51
Organic matter true digestibility (%)	41.91	43.17	2.40	0.72
Organic matter true digestibility (g)	15.85	16.33	0.91	0.72

Table 3. The effects of monensin fed during prepartum phase on digestibility and microbial efficiency during prepartum phase (experiment 1) (Continued).

Item	Treatment [1]		SE	P-value
	Control	Monensin		
MOEFF	14.41	11.96	1.06	0.16

[1] Treatment represents diets supplemented without (Control) or with monensin (Monensin) at 24 mg/kg DM.

Table 4. The effects of monensin supplementation during prepartum (PP) and lactation phase (LP) on fermentation end product 2 hours post feeding during lactation phase (experiment 2).

Item	PP		LP		P-value			
	Control[1]	M[2]	Control	M	SE	PP[3]	LP[4]	PPxLP[5]
Fermentor pH	6.43	6.33	6.40	6.35	0.03	0.03	0.26	0.51
Ammonia, mM	2.85	4.42	3.82	3.28	0.41	0.03	0.37	0.27
Total VFA, mM	110.10	123.88	115.15	118.83	2.68	0.003	0.35	0.10
Acetic acid	73.60	81.74	77.15	77.65	1.52	0.002	0.82	0.11
Propionic acid	19.19	22.90	19.19	22.90	1.25	0.06	0.06	0.44
Butyric acid	12.05	11.10	12.06	11.49	1.18	0.43	0.74	0.72
Isobutyric acid	0.70	0.83	0.84	0.69	0.03	0.005	0.002	0.10
Valeric acid	2.26	3.13	2.56	2.83	0.25	0.03	0.45	0.67
Isovaleric acid	2.44	4.19	3.36	3.27	0.49	0.03	0.89	0.16
BCVFA	5.40	8.14	6.76	6.79	0.49	0.002	0.97	0.16
A:P ratio	3.88	3.73	4.14	3.46	0.20	0.04	0.06	0.68

[1] Contained monensin 0 mg/kg DM in diet.
[2] Contained monensin 24 mg/kg DM in diet.
[3] Effect of monensin supplementation during prepartum phase, 0 vs 24 mg/kg DM.
[4] Effect of monensin supplementation during lactation phase, 0 vs 24 mg/kg DM.
[5] Interactive effect of monensin supplementation during prepartum and lactation phase.

Table 5. The effects of monensin supplementation during prepartum (PP) and lactation phase (LP) on digestibility and microbial efficiency during lactation phase (experiment 2).

Item	PP		LP		P-value			
	Control[1]	M[2]	Control	M	SE	PP[3]	LP[4]	PPxLP[5]
Dry matter flow (g)	32.50	31.61	32.44	31.67	0.89	0.49	0.56	0.59
Organic matter flow (g)	29.66	28.89	29.55	28.98	0.79	0.50	0.62	0.66
Bacteria Dry matter flow (g)	5.27	6.13	5.66	5.74	0.25	0.03	0.83	0.56

Table 5. The effects of monensin supplementation during prepartum (PP) and lactation phase (LP) on digestibility and microbial efficiency during lactation phase (experiment 2) (Continued).

Item	PP		LP		P-value			
	Control[1]	M[2]	Control	M	SE	PP[3]	LP[4]	PPxLP[5]
Bacteria organic matter flow (g)	4.58	5.33	4.91	5.00	0.20	0.02	0.78	0.47
Bacteria N flow (g)	0.46	0.54	0.50	0.50	0.19	0.01	0.90	0.55
Bacteria N (%)	8.76	8.79	8.87	8.68	0.11	0.84	0.26	0.88
Bacteria purine (mg/g DM)	115.98	109.50	116.56	108.93	2.93	0.14	0.90	0.86
Organic matter apparent digestibility (%)	47.13	48.51	47.31	48.33	1.40	0.50	0.62	0.66
Organic matter apparent digestibility (g)	26.44	27.21	26.54	27.11	0.79	0.50	0.62	0.66
Organic matter true digestibility (%)	55.30	58.01	56.07	57.24	1.27	0.16	0.53	0.49
Organic matter true digestibility (g)	30.31	31.52	30.50	31.32	0.15	<0.0001	0.002	0.14
MOEFF	14.93	16.61	15.96	15.59	0.75	0.14	0.74	0.88

[1] Contained monensin 0 mg/kg DM in diet.
[2] Contained monensin 24 mg/kg DM in diet.
[3] Effect of monensin supplementation during prepartum phase, 0 vs 24 mg/kg DM.
[4] Effect of monensin supplementation during lactation phase, 0 vs 24 mg/kg DM.
[5] Interactive effect of monensin supplementation during prepartum and lactation phase.

5. Conclusion

The results of these experiments show that monensin did not increase pH or decrease ammonia concentration during 3 dayd of PP and LP phases.

In experiment 1, monensin supplementation increased propionic acid concentration and decreased acetic acid to propionic acid ratio during PP. However, supplementation of monensin tended to decrease bacteria DM flow and bacteria organic matter flow without change in digestibility and MOEFF.

In experiment 2, monensin supplementation during PP increased concentration of all VFAs except butyric acid and decreased A:P ratio. Monensin supplementation during PP also increased bacteria dry matter flow, bacteria organic matter flow, bacteria N flow and OMTD without changing MOEFF. Monensin supplementation during LP increased concentrations of propionic acid and isobutyric acid, and decreased A:P ratio. Monensin supplementation during LP also increased OMTD without change in bacteria flow and MOEFF. The addition of monensin during both phases of transition increased OMTD and propionic acid concentration which would increase energy available to the transition dairy cow.

6. Implications

Monensin improves feed efficiency in ruminants via its effects on rumen microbial population which shifts fermentation. The effect of monensin, that would increase energy available to the transition dairy cow, is the increase of OMTD and propionic acid. This needs further investigation. However, the effect of monensin on ammonia concentration has not been shown to decrease during a period of 3 days as used in both phases of Experiment 2. The long term effect of monensin during PP and LP *in vitro* on this parameter might needs further investigation. The effect of monensin on this parameter *in vivo* in transition dairy cows needs to be investigated.

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