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High Levels of Monensin to Mid Lactating Dairy Cows: Nutrient Digestibility, Ruminal Fermentation and Microbial Protein Synthesis

Research Article

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ABSTRACT

The aim of this study was to evaluate the nutrient digestibility, ruminal fermentation and microbial protein synthesis of mid-lactating cows fed high dietary levels of monensin. Twelve Holstein cows were distributed into four 3 × 3 latin squares and assigned to the following treatments: control (CON), monensin 24 (M24, addition of 24 mg monensin/kg diet DM) and monensin 48 (M48, addition of 48 mg monensin/kg diet DM). Dietary levels of monensin linearly decreased dry matter intake without altering the nutrient total apparent digestibility. Monensin linearly increased ruminal fluid pH and acetate concentration. Monensin quadratically affected ruminal total short chain fatty acids, propionate concentration, acetate and propionate production and acetate to propionate ratio. Furthermore, monensin linearly increased the efficiency of microbial protein synthesis.

KEY WORDS additive, antimicrobial, indigestible acid detergent fiber, ionophore, purine derivatives.

INTRODUCTION

Sodium monensin is an ionophore approved for use in dairy cows in several countries, including Australia, Argentina, Canada, Brazil, New Zealand, South Africa and United States. The monensin is a carboxylic polyether, produced from the fungus *Streptomyces cinnamonensis* (Haney and Hoehn, 1967), which alters the flux of monovalent ions through the membrane of gram-negative bacteria, changing cellular normal function (Duffield and Bagg, 2000). Ionophores influence ruminal fermentation by increasing propionate and decreasing acetate, butyrate and methane production, and thus increasing gluconeogenesis and milk yield. Studies related to monensin supply in lactating cows has produced divergent results, indicating an interaction effect between diet and physiological processes (Ipharraguerre and Clark, 2003). Reviews by McGuffey *et al.* (2001) and Ipharraguerre and Clark (2003) suggested that the reduction in feed intake seems to happen often when monensin is supplied to mid and late lactating cows. According to the latter authors, monensin may increase hepatic glucose synthesis, based on the potential of monensin increase availability of gluconeogenic precursors (i.e. propionate), improving energy balance and milk yield. The objective of this study was to evaluate the effects of different dietary levels of sodium monensin on nutrient total apparent digestibility, ruminal fermentation and microbial protein synthesis in mid-lactating dairy cows.

MATERIALS AND METHODS

This experiment was conducted at the School of Veterinary Medicine and Animal Science, University of Sao Paulo, Pirassununga, SP, Brazil. Twelve multiparous Holstein cows (average live weight of 580±23.5 kg, 157 to 214 days in milk and average milk yield of 23.0±3.2 kg/d) were distributed into four 3 × 3 Latin squares balanced according to days in milk and milk production. Experimental periods consisted of 14 days of adaptation to treatments and 7 days of sampling. Animals were assigned to the following treatments: control (CON), monensin 24 (M24, dietary inclusion of 24 mg sodium monensin/kg diet DM) and Monensin 48 (M48, dietary inclusion of 24 mg sodium monensin/kg diet DM). The sodium monensin (Bobiovet 10 Premix®, Indukern do Brasil Quimica Ltda, Osasco, Brazil) was previously included in a premix and supplied mixed into the concentrate. Diet was formulated according to (NRC, 2001), (Tables 1 and 2) and was fed *ad libitum*. Animals were allocated throughout the experiment in individual pens, with sand beds, individual feed bunks and forced ventilation. Cows were mechanically milked daily at 06:00 h and 16:00 h. Amounts of feed offered and orts were weighed on a daily basis, to determine individual feed intake. Samples (0.5 kg) of silage were collected weekly and other diet ingredients were collected during the preparation of concentrate.

Fecal samples (0.5 kg on wet basis) of all cows were collected directly from rectum on days 13 and 16 of each period before each milking and stored at -20 ˚C. The feed, ort and fecal samples were partially dried in a forced-air oven at 55 ˚C for 72 h and ground in a knives mill to pass through a 1 mm screen (Wiley Mill, A.H. Thomas, Philadelphia, PA, USA). These samples were analyzed for dry matter (AOAC 950.15), ash (AOAC, 942.05), ether extract (EE, AOAC 920.39), crude protein (CP = N × 6.25; AOAC, 984.13), and lignin (AOAC, 973.18) according to the methods described by AOAC (2000). Neutral detergent fiber (NDF) was analyzed using alpha-amylase without addition of sodium sulfite to the detergent (TE-149 fiber analyzer, Tecnal Equipment for Laboratory Inc., Piracicaba, Brazil). The acid detergent fiber (ADF) was determined as described by Van Soest *et al.* (1991). Non-fiber carbohydrates (NFC) content were estimated according to Hall (2000) where: NFC= 100 - [(% CP-% CP from urea+% UREA) + % EE + % ASH + % NDF].

Total digestible nutrient was calculated according to NRC (2001). Indigestible acid detergent fiber (iADF) was used as an internal marker to estimate daily fecal DM excretion of cows (Nocek, 1998). Samples of feed, orts and feces were dried at 55 ˚C in a forced-air oven for 72 h and ground in a knives mill to pass through a 2 mm screen (Wiley Mill, A.H. Thomas).

Samples were placed in 4 × 5 cm non-woven textile bags (20 mg DM/cm^2 of surface) as described by Nocek (1998) and then bags were incubated during 288 h in the rumen of two fistulated dry cows adapted to the control diet of the current experiment. After 288 h, bags were removed from the rumen and washed in running tap-water, dried at 55 ˚C in a forced-air oven for 72 h and submitted to treatment with acid detergent (Van Soest *et al.* 1991) in a fiber analyzer (TE-149 fiber analyzer, TecnalEquipment for Laboratory Inc.) to determine iADF concentrations. Urine samples (50 mL) were collected from all cows by vulva massage stimulation at the same times of feces and composite samples were formed. Daily urinary volume was estimated on creatinine concentrations in urine. Creatinine concentrations were determined using commercial kits (Laborlab®, Sao Paulo, Brazil), using kinetic colorimetric enzymatic reaction in automatic biochemistry analyzer (SBA- 200 CELM®, Barueri, Brazil). Total daily urinary volume was estimated dividing daily creatinine urinary excretion by the observed values of the creatinine concentration in urine of the spot samples, as described by Chizzotti *et al.* (2007). Daily creatinine urinary excretion was estimated from the proposition of 24.05 mg/kg of body weight (González-Ronquillo *et al.* 2003). Body weights were measured using an electronic livestock scale for large animals (DeLaval, Tumba, Sweden), after milking and before feeding on days 7 and 21 of each experimental period. Ruminal microbial protein synthesis was determined according to purine derivatives methodology of Chen and Gomes (1992). Concentration of allantoin and uric acid in urine and allantoin in milk were analyzed by colorimetric method (Chen and Gomes, 1992). Milk samples were collected on the same days which urine samples were collected. Total excretion of purine derivatives (PD), in mmol/day, was calculated as the sum of quantities of allantoin and uric acid excreted in urine and milk (Orellana Boero, 2001). Absorbed purine derivatives (PD_{abs}, mmol/d) were calculated as follows:

$$PD_{abs} = (PD - 0.385 \times BW^{0.75}) / 0.84$$

Body weight (BW) and 0.84 represents the recovery of PD_{abs} as PD and 0.385 × $BW^{0.75}$ the endogenous excretion of PD (Chen and Gomes, 1992). Ruminal synthesis of nitrogen compounds (N_{mic} g of N/d) was calculated based in absorbed purine derivatives, using the equation (Chen and Gomes, 1992):

$$N_{mic} = (70 \times PD_{abs}) / (0.83 \times 0.134 \times 1000)$$

Considering 70 as the N purine derivative content (mg N/mol); 0.134 the ratio N purine derivatives/N microbial (Valadares *et al.* 1999) and 0.83 the intestinal digestibility of microbial purines.

Table 1 Ingredients (g/kg) of the experimental concentrate and basal diet

Item	Concentrate	Diet
Corn silage	-	580.0
Ground corn	521.4	219.0
Soybean meal	391.0	164.2
Urea	17.4	7.3
Ammonium sulfate	1.20	0.5
Sodium bicarbonate	14.8	6.2
Magnesium oxide	0.5	0.2
Mineral mix	46.7	9.6
Limestone	2.40	1.0
Sodium chloride	4.8	2.0

Composition of mineral mix per kilogram of product: Ca: 180 g; P: 90 g; Mg: 20 g; S: 20 g; Na: 100 g; Zn: 3 g; Cu: 1 g; Mn: 1.25 g; Fe: 2 g; Co: 0.2 g; I: 0.09 g; Se: 0.036 g and F(max.) 0.9 g.

Table 2 Chemical composition of ingredients in concentrate, corn silage and basal diet ((g/kg DM, otherwise stated)

Chemical composition	Ground corn	Soybean meal	Concentrate	Corn silage	Diet
Dry matter (g/kg wet basis)	885.0	898.0	894.2	289.6	543.6
Organic matter	974.8	935.7	899.8	944.7	925.8
Crude protein	82.2	491.7	277.3	88.2	167.7
ADIN	71.7	34.8	47.3	135.3	98.3
NDIN	144.4	106.0	131.9	197.9	170.2
Ether extract	48.8	10.8	29.0	29.1	29.1
Ash	25.2	64.3	100.2	55.3	74.2
Neutral detergent fiber	106.1	125.9	98.9	532.0	350.1
Acid detergent fiber	62.0	100.5	79.4	436.9	286.8
iADF	12.8	9.2	9.7	143.8	79.3
Lignin	10.3	16.3	10.7	54.4	36.1
Non-fiber carbohydrate*	778.8	395.4	559.6	295.4	406.4
Total digestible nutrient*	884.9	749.1	824.8	627.3	710.2

ADIN: acid detergent insoluble nitrogen; NDIN: neutral detergent insoluble nitrogen and iADF: indigestible acid detergent fiber.
* Non-fiber carbohydrate estimated according to Hall (2000) and total digestible nutrient calculated according to NRC (2001).

Ruminal fluid samples were collected from all cows before (0 h) and 3 hours after the morning feeding using an oesophageal gavage, wherein the initial suctioned volume (250 mL) was discarded to avoid saliva contamination. Rumen fluid pH values were determined immediately after collection using a potentiometer (MB-10, Marte, Sapucai, Brazil).

Short-chain fatty acids were determined according to (Erwin *et al.* 1961), using a gas chromatograph (GC-2014, Shimadzu, Tokyo, Japan) equipped with a capillary column (Stabilwax, Restek, Bellefonte, EUA).

The gases used were helium (8.01 mL/min flow) as the carrier gas, hydrogen (pressure of 60 kPa) as the fuel gas and synthetic air (pressure of 40 kPa) as the oxidizer gas. The steamer temperature was set at 220 ˚C, the ionization detector flames at 250 ˚C and the separation column at 145 ˚C for 3 min, which was then, raised 10 ˚C/min up to 200 ˚C.

Statistical analysis

Data were analyzed using the MIXED procedure of SAS (SAS, 2004) according to the statistical model:

$$Y_{ijkl} = \mu + S_i + P_j + T_k + A_l(Q_i) + e_{ijkl}$$

Where:
Y_{ijkl}: dependent variable.
μ: overall mean.
S_i: fixed effect of square (i=1 to 4).
P_j: fixed effect of period (y=1 to 3).
T_k: fixed effect of treatment (k=1 to 3).
$A_l(S_i)$: random effect of animal within square.
e_{ijkl}: residual.

The obtained data were submitted to simple polynomial regression and significance level was set at 0.05.

RESULTS AND DISCUSSION

Nutrient intake and total apparent digestibility

Monensin linearly decreased (P<0.001) DM intake, but did not influence nutrient total apparent digestibility (Table 3). Monensin decreases DM intake due to its effect on ruminal fermentation, increasing the propionic acid concentration and reducing the acetate to propionate ratio.

Table 3 Effects of high dietary levels of monensin on dry matter intake and nutrient total apparent digestibility of mid-lactating dairy cows

Item	Treatment			SEM	P-value	
	CON	M24	M48		Linear	Quadratic
Dry matter intake (kg/d)	18.03	17.50	15.79	0.16	< 0.001	0.126
Total apparent digestibility (g/kg)						
Dry matter	686.6	693.3	664.8	0.74	0.176	0.206
Organic matter	698.3	709.0	685.3	0.76	0.388	0.196
Crude protein	681.0	716.5	693.8	1.05	0.540	0.116
Ether extract	825.4	827.3	819.6	0.91	0.819	0.826
Neutral detergent fiber*	566.6	557.1	515.2	1.27	0.100	0.541
Non-fiber carbohydrate*	862.0	877.9	877.9	0.95	0.446	0.661
Total digestible nutrient	663.8	671.8	651.0	0.74	0.361	0.238

CON: control; M24: dietary inclusion of 24 mg/kg DM of sodium monensin and M28: dietary inclusion of 28 mg sodium monensin/kg diet DM.
SEM: standard error of the means.
* Non-fiber carbohydrate estimated according to Hall (2000) and total digestible nutrient calculate according to NRC (2001).
Regression equation of dry matter intake: Y= 18.2 – 0.046X (R^2=0.56).

The increase of propionate hepatic flux is responsible for the increase in glucose availability to mammary gland and may improve milk production of cows (Ipharraguerre and Clark, 2003).

Duffield *et al.* (2008) reported that the average decrease of DM intake was 0.30 kg/d when monensin was supplied to lactating cows. Despite the decrease in DM intake, total apparent digestibility was not affected by monensin. Although the total apparent digestibility of nutrients was not affected by treatments, cows fed M24 exhibited numerically higher values of nutrient digestibility compared to the other treatments.

Sodium monensin may influence the total apparent digestibility of DM and nutrients due to changes in rumimal fermentation, in which fiber and protein are the main components influenced. Oelker *et al.* (2009) supplied 17 mg/kg of sodium monensin to lactating cows fed corn-silage based diets and did not report differences in total apparent digestibility of nutrients, and an average DM intake of 21.30 kg/d.

Silva *et al.* (2007) and Gehman *et al.* (2008) supplementing mid-lactating cows with 20 and 16 mg/kg DM of sodium monensin, respectively, also did not observe effect of monensin supplementation on total apparent digestibility of dry matter and nutrients, emphasizing the influence of the lactation period in the total apparent digestibility of diets supplemented with monensin.

Ruminal fermentation and microbial protein synthesis

Increasing dietary doses of monensin linearly increased ruminal pH and propionate production of cows (Table 4). In addition, monensin linearly decreased ruminal acetate and butyrate concentrations and butyrate production. Moreover, monensin quadratically affected ruminal total short chain fatty acids and propionate concentrations, and acetate and propionate productions.

The increase in ruminal pH with monensin supplementation can occur due to decrease of bacterial populations which produce lactate and maintenance of populations that use lactate as substrate to fermentation. Other studies (Gehman *et al.* 2009; Oelker *et al.* 2009) also observed an increase of ruminal pH when animals were fed monensin; however, the pH values reported in the current study were higher than observed in the previous cited studies, and this result is probably related to diet forage content and to milk yield of animals. The ruminal ammonia nitrogen concentration of the current study is similar to frequently reported in literature when mid-lactating cows are fed diets containing sodium monensin (Ipharraguerre and Clark, 2003). Indeed, monensin supplementation has not altered ruminal ammonia nitrogen concentration in several studies (Ramanzin *et al.* 1997; Eifert *et al.* 2006; Gehman *et al.* 2008; Oelker *et al.* 2009).

The results of short chain fatty acids observed in this study agree with other experiments (Broderick, 2004; Eifert *et al.* 2006; Benchaar *et al.* 2006; Martineau *et al.* 2007; Gehman *et al.* 2008; Oelker *et al.* 2009) which report the capacity of sodium monensin shift fermentation towards a more energetically efficient process, increasing propionate ruminal concentrations and reducing acetate to propionate ratio with high dietary doses of monensin.

Eifert *et al.* (2006) supplemented early lactating cows with sodium monensin (16 mg/kg diet DM) and reported values of 67.90, 25.50 and 6.60% of acetic, propionic and butyric acid ruminal concentrations, respectively. These authors also reported value of 2.70 for the acetate to propionate ratio, similarly found in the current study. However, Oelker *et al.* (2009) evaluated the inclusion of 17 mg monensin/kg diet DM of mid-lactating cows and found lower ruminal acetate and propionate concentrations, and acetate to propionate ratio (59.60%, 26.30% and 2.30, respectively) than reported in the current study.

Table 4 Effects of high dietary levels of monensin on ruminal fermentation parameters of mid-lactating dairy cows

Ruminal fermentation parameters	Treatment			SEM	P-value		Regression	
	CON	M24	M48		Linear	Quadratic	Equation	R^2
pH	6.45	6.62	6.83	0.03	0.034	0.209	Y= 6.44 + 0.007X	0.61
NH_3-N (mg/ dL)	18.55	20.00	19.52	1.35	0.687	0.616	Y= 19.36	
Total SCFA (mmol/L)	79.97	85.47	75.96	2.23	0.308	0.050	$Y= 67.92 + 1.032X – 0.022X^2$	0.53
Acetate (mmol/L)	56.44	56.71	50.76	1.34	0.014	0.204	Y= 57.47 – 0.1183X	0.42
Propionate (mmol/L)	15.97	22.01	19.34	0.82	0.036	0.003	$Y= 19.00 + 0.33X – 0.0056X^2$	0.34
Butyrate (mmol/L)	7.54	6.74	5.85	0.30	0.006	0.893	Y= 8.64 – 0.0044X	0.45
Acetate (g/kg)	709.6	669.8	671.0	0.46	< 0.001	0.004	$Y= 717.4 –2.7X + 0.036X^2$	0.32
Propionate (g/kg)	198.0	251.4	253.3	0.46	< 0.001	< 0.001	$Y= 205.5 – 3.1X + 0.04X^2$	0.47
Butyrate (g/kg)	92.2	78.6	75.5	0.23	0.001	0.230	Y= 90.7 – 0.34X	0.56
Acetate: propionate	3.53	2.57	2.62	0.07	< 0.001	< 0.001	$Y= 34.6 – 0.50X + 0.07X^2$	0.55

CON: control; M24: dietary inclusion of 24 mg sodium monensin/kg diet DM and M28: dietary inclusion of 28 mg sodium monensin/kg diet DM.
SEM: standard error of the means.
SCFA: short chain fatty acids.

Despite no differences in purine derivatives excretion in urine and in milk, microbial protein synthesis linearly increased (P=0.0008) with monensin supplementation (Table 5). Data of the influence of monensin on microbial protein synthesis lack in literature. Changes in efficiency microbial protein synthesis may occur due to decrease of DM intake and increase of productive efficiency when supplementing monensin to cows. Gehman *et al*. (2008) supplemented dairy cows during mid lactation with 16 mg monensin/kg diet DM and also did not observe effect of monensin on total purines derivatives production and microbial protein synthesis. A greater efficiency of microbial protein synthesis is related to the energy and protein ruminal availability and considering the mechanisms of monensin action, this ionophore has the capacity to select microorganisms that are more efficient to synthesize protein. The metabolic equilibrium between energy and protein, in the rumen environment, with monensin supplementation, is likely the preponderant fact explaining the greater efficiency of microbial protein production.

Table 5 Effects of high dietary levels of monensin on microbial protein synthesis of mid-lactating dairy cows

Item	Treatment			SEM	P-value	
	CON	M24	M48		Linear	Quadratic
Urinary allantoin (mmol/L)	290.60	287.48	293.03	4.50	0.778	0.563
Milk allantoin (mmol/L)	3.80	3.77	3.67	0.16	0.577	0.860
Uric acid (mmol/L)	25.09	21.90	24.66	1.34	0.864	0.180
Total purines derivatives (mmol/L)	323.55	313.06	321.26	5.26	0.805	0.253
Allantoin (% purines derivatives)	92.29	92.98	92.43	0.33	0.842	0.303
Absorbable purines (mmol/L)	353.04	339.59	350.00	6.14	0.675	0.345
Microbialnitrogen (g/d)	222.19	213.73	220.28	3.86	0.783	0.176
Microbial crude protein (g/d)	1388.75	1335.87	1376.78	24.16	0.795	0.226
Efficiency*	111.11	119.03	130.53	4.14	0.008	0.079

CON: control; M24: dietary inclusion of 24 mg sodium monensin/kg diet DM and M28: dietary inclusion of 28 mg sodium monensin/kg diet DM.
SEM: standard error of the means.
* Efficiency: microbial protein synthesis (grams of crude protein) per kilogram of total digestible nutrient intake.
Regression equation efficiency: $Y= 107.52 – 0.40X$ (R^2=0.63).

CONCLUSION

Increasing dietary levels of monensin to mid-lactating dairy cows fed corn silage based diet improved digestive metabolism of the animals. Based on the results of the current study, the dose of 24 mg sodium monensin/kg diet DM is recommended for cows in mid-lactation. The dietary dose of 48 mg monensin/kg diet DM should not be used, because of the large decline effect on feed intake.

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