



Effects of increasing monensin doses on performance of mid-lactating Holstein cows

Mayara Celpf Bailoni Santos, Ana Paula Chaves Araújo, Beatriz Conti Venturelli, Jose Esler Freitas Jr, Rafael Villela Barletta, Jefferson Rodrigues Gandra, Pablo Gomes de Paiva, Tiago Sabela Acedo & Francisco Palma Rennó

To cite this article: Mayara Celpf Bailoni Santos, Ana Paula Chaves Araújo, Beatriz Conti Venturelli, Jose Esler Freitas Jr, Rafael Villela Barletta, Jefferson Rodrigues Gandra, Pablo Gomes de Paiva, Tiago Sabela Acedo & Francisco Palma Rennó (2019) Effects of increasing monensin doses on performance of mid-lactating Holstein cows, Journal of Applied Animal Research, 47:1, 297-302, DOI: [10.1080/09712119.2019.1629298](https://doi.org/10.1080/09712119.2019.1629298)

To link to this article: <https://doi.org/10.1080/09712119.2019.1629298>



© 2019 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group



Published online: 17 Jun 2019.



Submit your article to this journal [↗](#)




Article views: 74



View Crossmark data [↗](#)

Effects of increasing monensin doses on performance of mid-lactating Holstein cows

Mayara Celpf Bailoni Santos^a, Ana Paula Chaves Araújo^a, Beatriz Conti Venturrelli^a, Jose Esler Freitas Jr ^a, Rafael Villela Barletta^a, Jefferson Rodrigues Gandra^a, Pablo Gomes de Paiva^{b,c,d}, Tiago Sabela Acedo^e and Francisco Palma Rennó^{a,c}

^aDepartment of Animal Nutrition and Production, School of Veterinary Medicine and Animal Science, University of São Paulo (USP), Pirassununga, Brazil; ^bDepartement of Animal Science, UNESP – Universidade Estadual Paulista “Júlio de Mesquita Filho” /Campus Jaboticabal, Jaboticabal, Brazil; ^cDepartment of Animal Science, Federal University of Bahia, Salvador, Brazil; ^dDepartment of Animal Science, Universidade Federal da Grande Dourados, Dourados, Brazil; ^eDSM Produtos Nutricionais Brasil S.A., São Paulo, Brazil

ABSTRACT

The objective of this study was to determine the effect of increasing monensin doses on intake, nutrient digestion, ruminal fermentation, nitrogen balance, blood metabolites, milk yield and composition of lactating dairy cows. Twelve Holstein cows (135 ± DIM; 580 ± kg of BW) were assigned to three 4 × 4 Latin square design, with 21-d periods. Cows were randomly assigned within each square to receive one of the following treatments: Control (C): 0 mg kg⁻¹ DM of monensin; Monensin 12 (M12): 12 mg kg⁻¹ DM of monensin; Monensin 24 (M24): 24 mg kg⁻¹ DM of monensin and Monensin 48 (M48): 48 mg kg⁻¹ DM of monensin. Monensin had quadratic effect on intake of dry matter. Apparent total-tract digestibility of DM were similar between treatments; however, digestibility of CP increased. Monensin had no effect on ruminal pH, NH₃ concentration, but propionate increased by monensin. Blood urea nitrogen was increased linearly by monensin. There was a quadratic effect of monensin on N intake. Monensin had a quadratic effect on milk yield, whereas 3.5% FCM was decreased linearly. These results suggest that monensin improves performance of mid-lactating dairy cows fed corn silage-based diet, and monensin can be added up to 24 mg/kg of DM diets.

ARTICLE HISTORY

Received 9 November 2017
Accepted 17 May 2019

KEYWORDS

Additive; ionophore; nitrogen utilization; ruminal fermentation

1. Introduction

Several countries such as Brazil, Australia, New Zealand, Canada and the United States liberated the use of monensin in diets of dairy cows (Plazier et al. 2000; NRC 2001). With another perspective and based on the Precautionary Principle, the European Union (EU) in 1999 banned the use of antibiotics as growth promoters and in 2006 banned the use of deionophores, based on the ‘preventive stance’ of the authorities. However, mainly results of productive efficiency make possible the use of monensin by the main milk producing countries. Sodic monensin is produced mainly by the bacteria *Streptomyces cinnamonensis* that are highly lipophilic and toxic to many microorganisms that are defined as antibiotics (Haney and Hoehn 1967).





Monensin has been extensively used to manipulate ruminal fermentation, improve performance and efficiency of the use of energy diet (Ipharraguerre and Clark 2003; Duffield et al. 2008). However, studies involving the use of monensin in diets of dairy cows have produced conflicting results, indicating that the interactions between diet and factors of the physiological processes are involved (Ipharraguerre and Clark 2003). Furthermore, there are few studies with monensin in dairy cows using corn silage as a single forage source of the diet (Oelker et al. 2009; Gandra et al. 2010), which is often found on Brazilian

farms. However, none of the cited studies was performed using high yielding (30.95 ± 1.95 kg/d) dairy cows and most of Brazilian studies used daily doses of monensin per cow (Campos Neto et al. 1995; Possatti et al. 2015), instead of doses according to dry matter intake (DMI). Duffield et al. (2008) summarized the dietary composition of 34 trials on monensin and starch content mean was 22.8% and non-fibre carbohydrate content was 38.4%. On the other hand, Brazilian diets often have more than 26% of starch content and more than 40% of non-fibre carbohydrates.

Therefore, the present study was undertaken to determine the effects of increasing doses of monensin on intake, nutrient digestion, ruminal fermentation, nitrogen utilization, blood metabolites, milk yield and composition of mid-lactating dairy cows fed corn silage as a forage source. Our hypothesis was that monensin would increase milk yield due to improvements in ruminal fermentation and the efficiency of energy use.

2. Material and methods

This study was approved by the Bioethics Committee of the School of Veterinary Medicine and Animal Science of University of São Paulo (protocol number 1879/2010).

CONTACT Pablo Gomes de Paiva  bocazoo@hotmail.com  Departement of Animal Science, UNESP – Universidade Estadual Paulista “Júlio de Mesquita Filho” /Campus Jaboticabal, Rod. Prof. Paulo Donato Castellane km 5, Rural, Jaboticabal, SP 14884-900, Brazil; Department of Animal Science, Federal University of Bahia, Salvador, BA 40170-110, Brazil; Department of Animal Science, Universidade Federal da Grande Dourados, Dourados, MS 79804-970, Brazil; Francisco Palma Rennó  francisco.renno@usp.br  Department of Animal Nutrition and Production, School of Veterinary Medicine and Animal Science, University of São Paulo (USP), Av. Duque de Caxias Norte, 225-Campus da USP, Pirassununga, SP 13635-900, Brazil; Department of Animal Science, Federal University of Bahia, Salvador, BA 40170-110, Brazil

© 2019 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

2.1. Animals and experimental treatments

Twelve multiparous Holstein cows with average 135 ± 35 days in milk (DIM), 580 ± 54 kg of body weight (BW) and 30.0 ± 5.3 kg day⁻¹ of initial milk yield were used in three 4×4 Latin square design, balanced according to DIM and initial milk yield. Each experimental period had 21 d, with 14 d of adaptation to treatment and the last seven days for data collection. Throughout the experiment, cows were housed in a free-stall barn with individual pens of 17.5 m² containing sand-beds, forced ventilation and had free access to drink water.

The cows were randomly assigned within each square to receive one of the following treatments: Control (C): 0 mg kg⁻¹ of monensin on diet DM basis; Monensin 12 (M12): 12 mg kg⁻¹ of monensin on diet DM basis; Monensin 24 (M24): 24 mg kg⁻¹ of monensin on diet DM basis; and Monensin 48 (M48): 48 mg kg⁻¹ of monensin on diet DM basis (Monensin® Tortuga, DSM Produtos Nutricionais Brasil S.A., Brazil). The control-based diet was formulated according to NRC (2001; Table 1), and monensin doses were provided to cows as 'top dress' above the diet before feeding. Cows were fed a total mixed ration twice daily at 0800 and 1300 h to supply 105–110% of expected intake.

2.2. Sample collection and chemical analysis

Feed intake was recorded daily as difference between feed offered and refused. Samples of diet ingredients (0.3 kg) and orts (0.3 kg) from each cow were collected daily during the sampling period and then combined into one sample and stored at -20°C until analysis. On 17–19 d of each period, faecal samples (0.5 kg) were collected directly from the rectum twice daily (before milking), comprising a composite sample per cow.

Table 1. Ingredient and chemical composition of control-based diet.

Ingredient (g/kg)	Diet
Corn silage	501.5
Ground corn	228.0
Soybean meal	155.0
Whole raw soybean	80.1
Urea	1.9
Ammonium sulfate	1.0
Sodium bicarbonate	8.0
Magnesium oxide	0.9
Limestone	1.4
Salt	2.4
Mineral mix ^a	19.8
<i>Chemical composition (g/kg of DM)*</i>	
Dry matter (g/kg as fed)	636.1
Organic matter	911.9
Crude Protein	180.8
Ether extract	51.5
Neutral detergent fibre	340.9
Acid detergent fibre	213.8
Non-fibre carbohydrate ^b	519.3
Total digestible nutrient ^c	720.8
Net energy lactation ^c (Mcal/kg DM)	1.60

^aContaining per kilogram: 190 g of Ca; 73 g of P; 44 g of Mg; 30 g of S; 62 g of Na; 1.350 mg of Zn; 340 mg of Cu; 940 mg of Mn; 1.064 mg of Fe; 3 mg of Co; 16 mg of I; 10 mg of Se; 200,000 UI of Vitamin A; 50,000 UI of Vitamin D; UI; 6,000 UI of Vitamin E.

^bNFC = $1000 - [(CP - CP \text{ of urea} + \text{urea}) + \text{NDF} + \text{EE} + \text{Ash}]$ by Hall (1998).

^cEstimated using NRC (2001) model.

*Unless otherwise indicated.

Samples of ingredients, orts and faces were dried in a 55°C forced-air oven 72 h, ground to pass through a 1 mm screen (Wiley Mill, Arthur H. Thomas, Philadelphia, PA, USA) and then analysed for dry matter (DM, AOAC 950.15), ash (AOAC 942.05), ether extract (EE, AOAC 920.39), crude protein (CP, $N \times 6.25$; AOAC 984.13), according to AOAC (2000). Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were obtained according to the method described by Van Soest et al. (1991). The NDF analysis was determined with heat-stable alpha-amylase without the addition of sodium sulfite to the detergent using an Ankom fibre analyser (Ankom Tech. Corp., Fairport, NY).

Total faecal excretion for each animal was determined using indigestible acid detergent fiber (iADF) as an internal marker. Samples of ingredients, orts and faeces were dried at 55°C forced-air oven for 72 h, ground to pass through a 2 mm screen (Wiley Mill, Arthur H. Thomas, Philadelphia, PA, USA), and then these samples were placed in bags of non-woven textile (100 g m⁻²) and incubated for 288 h in the rumen of two cows fed the same diet used in these trials (Casali et al. 2008). After removal from rumen, the bags were washed in running tap water, dried at 55°C in a forced-air oven and then analysed for ADF concentration as previously described. Digestibility was calculated using the ratio of iADF in feed (corrected for orts) and faeces.

On 7 and 21 d of each period, body weights were measured using a livestock scale for large animals (Brete, ME 2.8, Coimma – Dracena, Brazil), after milking and before feeding. A body condition score of cows was obtained according to Wildman et al. (1982).

2.3. Nitrogen balance and microbial protein synthesis

On 16 d of each period, spot urine samples were collected from all cows 4 h after morning feeding. Urine sample was filtered and 10 mL aliquots were diluted immediately with 40 mL of sulfuric acid (0.036 N) and stored at -20°C for analysis of uric acid and allantoin. A pure urine sample was stored for analysis of total nitrogen and creatinine. Uric acid and creatinine concentrations were analysed using commercial kits (Laborlab, Guarulhos, Brazil) in a semi-automatic spectrophotometer (SBA 200, São Caetano do Sul, Brazil). Allantoin in the urine and milk were determined by colorimetric method (Chen and Gomes 1992). Daily urine volume was estimated from daily creatinine excretion as 24.05 mg kg^{-1} of BW (Chizzotti et al. 2008). Uric acid and allantoin were considered to be the total excretion of purine derivatives and microbial protein synthesis estimated according to Chen and Gomes (1992). For calculation of microbial efficiency = grams of microbial protein/kg of TDN intake. Total nitrogen in the urine and the milk was determined (984.13; AOAC 2000) and nitrogen balance was performed according to NRC (2001) model. For calculated nitrogen efficiency = $N \text{ milk (g/day)} / N \text{ intake (g/day)}$.

2.4. Blood profile

On 19 d of each experimental period, blood samples were collected before morning feeding from all cows by puncture of the coccygeal vein using evacuated tube. Immediately after collection, blood samples were centrifuged at 2000g for 15 min and

the supernatant was separated and stored at -20°C until analysis. Glucose, urea, aspartate aminotransferase (AST) and gamma-glutamyl transferase (GGT) concentrations were analysed using commercial kits (CELM[®], São Caetano do Sul – Brazil), determined in a semi-automatic spectrophotometer (SBA-200, São Caetano do Sul – Brazil).

2.5. Ruminal fermentation parameters

On 20 d of each period, rumen fluid samples were collected using an oesophageal gavage 3 h after morning feeding. Immediately after collection, rumen pH values were determined using a digital pH meter (MB-10, Marte – Sapucaí, Brazil). Samples were centrifuged at 7000g and one aliquot mixed with metaphosphoric acid (0.25 Mol/L HPO_3) and stored at -20°C for analyses of volatile fatty acids (VFA). Another aliquot sample (2 mL) was mixed with 1 mL of sulfuric acid (0.5 Mol/L H_2SO_4) and stored 20°C for determination of ammonia nitrogen ($\text{NH}_3\text{-N}$) by a colorimetric phenol-hypochlorite method (Broderick and Kang 1980). Ruminal VFA were measured with a gas chromatograph (GC-2014, Shimadzu, Tokyo, Japan) with split injector and dual flame ionization detector temperature at 250°C and equipped with a capillary column (Stabilwax, Restek, Bellefonte, PA, USA) at 145°C , according to the method described by Erwin et al. (1961) and adapted by Getachew et al. (2002).

2.6. Milk yield and composition

Cows were mechanically milked twice daily at 0600 and 1600 h and milk production was recorded electronically by an automatic milk meter (Alpro[®], DeLaval – Tumba, Sweden). On 16–18 d of each period, milk samples proportional to two daily milkings were collected and freshly analysed for fat, protein and lactose (Milkoscan; Foss Electric, Hillerod, Denmark). 3.5% Fat corrected milk (FCM, 3.5%) was calculated according to Sklan et al. (1992). Milk efficiency was calculated as milk yield (kg/day)/dry matter intake (kg/day)

2.7. Statistical analyses

Data were subjected to SAS (version 9.1.3, SAS Institute, Cary, NC, USA 2004), verifying the normality of residuals and

homogeneity of variances using PROC UNIVARIATE, and then analysed with PROC MIXED according to the following model:

$$Y_{ijkl} = \mu + S_i + A_j + P_k + T_l + e_{ijkl}$$

in which Y_{ijkl} is the dependent variable, μ is the overall mean, S_i is the fixed effect of square ($i = 1-3$), A_j is the random effect of animal ($j = 1-12$), P_k is the fixed effect of period ($k = 1-4$), T_l is the fixed effect of treatment ($l = 1-4$) and e_{ijkl} is the residual error. The degrees of freedom were defined according to the method of Satterthwaite (ddfm = satterth). Response to monensin doses were tested with linear and quadratic contrast, which were declared significant at $P \leq .05$. The differences among treatments were determined using the adjust TUKEY test, with a significance level set at $P \leq .05$.

3. Results

There was a quadratic effect on DM (dry matter), CP (crude protein), NDF, NFC (non-fibre carbohydrate) and TDN (total digestible nutrient) intake when monensin was added to diet ($P < .05$; Table 2), but the decreased is observed only in M48 because there is no differences between C, M12 and M24. Apparent total-tract digestibility of DM and OM (organic matter) were similar between treatments ($P > .05$). However, total-tract digestibility of CP increased linearly ($P < .05$), whereas NDF digestibility decreased linearly when monensin inclusion reached 48 mg kg^{-1} on DM basis.

Ruminal pH, NH_3 concentration, total VFA and acetate concentration were not affected by treatments ($P > .05$; Table 3). However, propionate was increased linearly ($P < .05$), whereas butyrate concentration and acetate: propionate (C2:C3) ratio were decreased by M48 compared to the others ($P < .05$). Microbial nitrogen synthesis and efficiency were similar between treatments ($P > .05$).

There was no effect of monensin on blood glucose, AST and GGT concentrations ($P > .05$; Table 4). However, blood urea nitrogen concentration was increased ($P < .05$), only in diet containing 48 mg kg^{-1} of monensin. Monensin had a quadratic effect on N intake ($P < .05$; Table 4). Excretion of N faecal, N urine and N milk were linearly decreased ($P < .05$), especially when monensin addition reached mg kg^{-1} on DM basis. However, N balance and efficiency of nitrogen utilization was not affected by monensin ($P > .05$).

Table 2. Nutrient intake and total-tract digestibility of lactating dairy cows fed doses of monensin.

Item	Treatment ¹				SEM ²	P-value ³	
	C	M12	M24	M48		LIN	QUA
<i>Intake (kg/day)</i>							
Dry matter	20.34 ^a	19.95 ^a	19.86 ^a	16.76 ^b	0.43	<.01	<.01
Crude protein	3.88 ^a	3.80 ^a	3.77 ^a	3.18 ^b	0.08	<.01	<.01
Neutral detergent fibre	6.51 ^a	6.37 ^a	6.34 ^a	5.44 ^b	0.15	<.01	<.01
Non-fibre carbohydrate	8.24 ^a	8.01 ^a	7.94 ^a	6.78 ^b	0.21	<.01	.01
Total digestible nutrient	14.20 ^a	13.84 ^a	13.73 ^a	11.77 ^b	0.35	<.01	<.01
<i>Coefficient of total-tract digestibility</i>							
Dry matter	0.674	0.677	0.674	0.672	0.003	.78	.69
Organic matter	0.690	0.704	0.703	0.698	0.003	.82	.17
Crude protein	0.697 ^a	0.727 ^{ab}	0.733 ^{ab}	0.745 ^b	0.006	<.01	.42
Neutral detergent fibre	0.551 ^a	0.525 ^{ab}	0.525 ^{ab}	0.502 ^b	0.008	.01	.86

^{a,b}Least squares means within a row with different superscripts differ by Tukey test ($P < .05$).

¹C: control; M12, M24 and M48: inclusion of 12, 24 and 48 mg kg^{-1} of monensin on diet DM basis.

²Standard error of the mean.

³Probability of linear or quadratic effect.

Table 3. Ruminal fermentation of lactating dairy cows fed doses of monensin.

Item	Treatment ¹				SEM ²	P-value ³	
	C	M12	M24	M48		LIN	QUA
pH	6.54	6.61	6.60	6.48	0.04	.60	.24
NH ₃ (mg/dL)	17.24	18.36	18.27	18.72	0.86	.47	.80
Total VFA (mM)	103.91	98.74	101.13	100.68	2.37	.70	.58
Acetate (mM)	68.99	64.34	63.76	62.29	1.63	.11	.58
Propionate (mM)	24.08 ^b	24.27 ^b	26.97 ^{ab}	29.57 ^a	0.98	<.01	.38
Butyrate (mM)	10.83 ^a	10.13 ^a	10.40 ^a	8.81 ^b	0.37	.04	.32
C2:C3	3.00 ^a	2.75 ^a	2.55 ^a	2.14 ^b	0.10	<.01	.57
Microbial nitrogen (g/day)	315.39	278.91	270.81	237.63	19.84	.12	.96
Microbial efficiency ⁴	151.90	127.72	133.63	114.54	9.89	.17	0.88

^{a,b}Least squares means within a row with different superscripts differ by Tukey test ($P < .05$).

¹C: control; M12, M24 and M48: inclusion of 12, 24 and 48 mg kg⁻¹ of monensin on diet DM basis.

²Standard error of the mean.

³Probability of linear or quadratic effect.

⁴Microbial efficiency: grams of microbial protein/kg of TDN intake.

Table 4. Blood metabolites and nitrogen balance of lactating dairy cows fed doses of monensin.

Item	Treatment ¹				SEM ²	P-value ³	
	C	M12	M24	M48		LIN	QUA
<i>Blood metabolites</i>							
Glucose (mg/dL)	62.61	57.73	60.99	59.08	1.72	.63	.66
BUN ⁴ (mg/dL)	44.12 ^b	47.67 ^{ab}	45.60 ^{ab}	51.62 ^a	1.29	.01	.51
AST ⁵ (U/L)	75.66	80.71	78.25	93.81	4.40	.14	.50
GGT ⁶ (U/L)	7.47	8.47	8.12	8.26	0.27	.25	.28
<i>Nitrogen balance (g/day)</i>							
N intake	625.47 ^a	614.13 ^a	610.77 ^a	502.58 ^b	14.69	<.01	<.01
N Faeces	195.24 ^a	173.10 ^a	173.74 ^a	130.64 ^b	6.93	<.01	.09
N Urine	169.68	166.03	173.81	134.62	9.46	.01	.28
N Milk	145.42 ^a	144.96 ^a	141.44 ^a	125.60 ^b	3.65	<.01	.03
N balance	115.12	130.03	121.79	111.73	9.86	.80	.46
Nitrogen efficiency ⁷	0.233	0.236	0.234	0.232	0.01	.08	.25

^{a,b}Least squares means within a row with different superscripts differ by Tukey test ($P < .05$).

¹C: control; M12, M24 and M48: inclusion of 12, 24 and 48 mg kg⁻¹ of monensin on diet DM basis.

²Standard error of the mean.

³Probability of linear or quadratic effect.

⁴Blood urea nitrogen.

⁵AST: aspartate aminotransferase.

⁶GGT: gamma-glutamyl transferase.

⁷Nitrogen efficiency = N milk (g/day) / N intake (g/day).

There was a quadratic effect of monensin on milk yield ($P < .05$; Table 5). The 3.5% FCM and protein production were decreased linearly ($P < .05$), whereas MUN was increased linearly ($P < .05$). However, all these effects were observed especially when

monensin was added up to 48 mg kg⁻¹ g of the diet. Fat and protein proportions were similar between treatments ($P > .05$). Efficiency was increased linearly in monensin ($P < 0.05$), mainly in the diet containing 48 mg kg⁻¹ of monensin on DM basis.

Table 5. Performance of lactating dairy cows fed doses of monensin.

Item	Treatment ¹				SEM ²	P-value ³	
	C	M12	M24	M48		LIN	QUA
Milk yield (kg/day)	31.47 ^a	31.93 ^a	31.62 ^a	28.78 ^b	0.84	<.01	<.01
3.5% FCM (kg/day)	27.76 ^a	26.97 ^{ab}	27.00 ^{ab}	23.28 ^b	0.78	.01	.19
Fat (kg/day)	0.86	0.81	0.83	0.67	0.03	.04	.44
Protein (kg/day)	0.92 ^a	0.94 ^a	0.91 ^a	0.83 ^b	0.02	<.01	.02
<i>Milk composition</i>							
Protein (%)	2.96	2.97	2.93	2.90	0.03	.33	.67
Fat (%)	2.81	2.60	2.65	2.34	0.10	.13	.80
MUN (mg/dL)	9.75	9.70	9.89	10.74	0.28	.03	.18
Body weight (kg)	547.21	547.73	550.60	545.80	6.59	.91	.34
Body condition score	2.62	2.62	2.61	2.66	0.02	.25	.27
Productive efficiency ⁴	1.55 ^b	1.60 ^b	1.59 ^b	1.72 ^a	0.01	<.01	<.01

^{a,b}Least squares means within a row with different superscripts differ by Tukey test ($P < .05$).

¹C: control; M12, M24 and M48: inclusion of 12, 24 and 48 mg kg⁻¹ of monensin on diet DM basis.

²Standard error of the mean.

³Probability of linear or quadratic effect.

⁴Productive efficiency = Milk yield (kg/day)/dry matter intake (kg/day).

4. Discussion

High level of monensin inclusion in lactating dairy cow diets led to a large decrease in DMI. The treatment containing 48 mg kg⁻¹ of monensin decreased DMI 18% or 3.58 kg d⁻¹ when compared to control diet. Other previous studies reported variable effects of monensin on DMI. According to Ipharraguerre and Clark (2003), monensin decreased DMI on 1.5% or 0.3 kg/d; whereas Duffield et al. (2008) reported 2% or 0.3 kg d⁻¹ less DMI of lactating dairy cows supplemented with monensin. However, in early lactating dairy cows, monensin has increased DMI on 5% or 1.1 kg d⁻¹ (McCarthy et al. 2015). Ipharraguerre and Clark (2003) suggested that variable response to monensin supplementation in the DMI could be due to many factors, such as the stage of lactation, length of time data were collected and number of animals in the study. On the other hand, reduced DMI in higher monensin levels may be related to their effect on ruminal fermentation, which promotes an increase in propionate (Duffield et al. 2008) that occurred in the present study, leading to decreasing in meal size and reduced DMI (Allen et al. 2009). Moreover, the largest decrease in DMI in dairy cows supplemented with high monensin level (48 mg kg⁻¹ of DM) was probably related to a monensin level that exceeded the effective concentration of monensin in dairy cow diets (Ipharraguerre and Clark 2003; Duffield et al. 2008).

Apparent total-tract digestibility of CP was increased, whereas NDF was decreased with monensin supplementation, and this result was similar to that observed in other previous studies (Benchaar et al. 2006; Martineau et al. 2007). The increase in protein digestibility is due to the effect of monensin on ruminal microorganisms, especially those promote proteolysis and deamination of amino acids. This result could be related to the higher ratio of dietary to microbial CP entering the small intestine because dietary can be more digestible than microbial CP (Spears 1990), and amino acids uptake by the small intestine may be increased when monensin are supplemented (McGuffey et al. 2001; Ruiz et al. 2001). On the other hand, decreasing NDF digestibility can be explained by the reduction of the gram-positive bacterial population fibre digesters on the rumen with monensin supplementation (Oelker et al. 2009), especially in 48 mg kg⁻¹ of monensin on DM basis.

The ruminal pH and NH₃ were not affected by monensin supplementation. Similarly, Oelker et al. (2009) report no effect of monensin in lactating dairy cows fed diets based on corn silage or alfalfa hay with the addition of molasses. In the present study, monensin supplementation did not affect total VFA but did increase propionate and decrease butyrate and C2:C3 ratio. Monensin has been shown to increase ruminal propionate, which results in reduced acetate: propionate ratio (Yang and Russell 1993; Ipharraguerre and Clark 2003; Duffield et al. 2008). In contrast, in other previous studies, monensin did not change propionate production and acetate: propionate ratio in lactating dairy cows (Martineau et al. 2007; Oelker et al. 2009). However, the divergence between these studies can be related to the difference in monensin levels inclusion and interaction between diet composition and monensin. Moreover, these effects of monensin on VFA proportion is probably associated with the effects of

monensin on ruminal microorganism and digestive processes that lead to improving feed efficiency in dairy cow diets (Ipharraguerre and Clark 2003; Duffield et al. 2008), similar to results observed in this study.

In the present study, monensin supplementation had increased BUN, reduced N faecal and N milk excretion without effect on N balance. Similarly, Martineau et al. (2007) reported an increase in BUN in dairy cows supplemented with monensin, which was associated with ruminal NH₃ concentration. Moreover, these findings may be related to an increase in protein digestibility with associated hepatic metabolism of nitrogen compounds and reduced N faecal excretion (Jonker et al. 1998; Spek et al. 2013), leading to urea accumulation in the blood. Reduced N faecal excretion with monensin supplementation is likely due to better utilization of nitrogen in amino acids available in the small intestine, resulting from a change in ruminal fermentation caused by monensin. Although digestibility of N microbial is high (Van Soest 1994), when protein from feed is more digestible than that of microbial protein, amino acids uptake by small intestine can be increased (McGuffey et al. 2001; Ruiz et al. 2001) with monensin supplementation. Furthermore, ammonia concentrations could represent a balance between feed protein degradation and ammonia uptake for microbial protein synthesis (Makkar et al. 1998), which was not affected in the present study.

High monensin levels (48 mg kg⁻¹ of DM) decreased milk yield, whereas intermediate doses of monensin did not affect milk production. Likewise, Gandra et al. (2010) reported lower milk yield in dairy cows supplemented with high monensin level. Normally, previous studies report no difference (Martineau et al. 2007; Oelker et al. 2009) or increase in milk yield (Gallardo et al. 2005; Duffield et al. 2008) when dairy cows received monensin. However, lactating dairy cows that received monensin had increased milk between 0.7 and 1.3 k/d or approximately 5% when compared to dairy cows fed control diet (Ipharraguerre and Clark 2003; Duffield et al. 2008), which was associated with better efficiency in dairy cows supplemented with monensin, similar to the present study. Thus, our results suggest that high monensin levels used in this study (48 mg kg⁻¹ of DM) exceed the optimal doses of the monensin in dairy cow diets.

In general, the observed fat content is below the average of Holstein cows, which may be related to specific conditions of the herd and also to DIM. However, the behaviour of the FCM is in agreement with the milk yield, where a large reduction of the FCM for the diet M48 was observed. The biological effect of the reduction of fat content caused by the high levels of monensin, justified by the concentration of propionate (Table 3) are closely related to the results obtained for FCM and are in agreement with (Gandra et al. 2010).

5. Conclusion

High monensin level in dairy cow diets improves ruminal fermentation and CP digestibility, but DM intake and performance were impaired. Thus, our result suggests that to improve the performance of mid-lactating dairy cows fed corn silage-based diets, monensin can be added up to 24 mg kg⁻¹ of DM.

Acknowledgments

Thanks to the University of São Paulo (USP) and Dairy Cattle Research Laboratory for providing staffs and infrastructure for this study, and also DSM Produtos Nutricionais Brasil S.A.

Disclosure statement

No potential conflict of interest was reported by the authors.

ORCID

Jose Esler Freitas  <http://orcid.org/0000-0003-1559-0149>

References

- Allen MS, Bradford BJ, Oba M. 2009. Board-invited review: The hepatic oxidation theory of the control of feed intake and its application to ruminants. *J Anim Sci.* 87:3317–3334.
- [AOAC] Association of Official Analytical Chemistry. 2000. Official methods of analysis. 17th ed. Arlington (VA): AOAC International.
- Benchaar C, Petit HV, Berthiaume R, Whyte TD, Chouinard PY. 2006. Effects of addition of essential oils and monensin premix on digestion, ruminal fermentation, milk production, and milk composition in dairy cows. *J Dairy Sci.* 89:4352–4364.
- Broderick GA, Kang JH. 1980. Automated simultaneous determination of ammonia and total amino-acids in ruminal fluid and *in vitro* media. *J Dairy Sci.* 63:64–75.
- Campos Neto O, Ramos AA, Escobar MJ, Dalanesi JA, De Bem CHW. 1995. Evaluation of sodium monensin in dairy cows. *Sci Agric.* 52:268–273.
- Casali AO, Detmann E, Valadares Filho SC, Pereira JC, Henriques LT, Freitas SG, Paulino MF. 2008. Influence of incubation time and particles size on indigestible compounds contents in cattle feeds and feces obtained by *in situ* procedures. *Rev Brasil Zootec.* 37:335–342.
- Chen XB, Gomes MJ. 1992. Estimation of microbial protein supply to sheep and cattle based on urinary excretion of purine derivatives – an overview of technical details. Aberdeen: Rowett Research Institute. 21 p.
- Chizzotti ML, Valadares Filho SC, Valadares FFD, Chizzotti FHM, Tedeschi LO. 2008. Determination of creatinine excretion and evaluation of spot urine sampling in Holstein cattle. *Livest Sci.* 113:218–225.
- Duffield RF, Rabiee AR, Lean IJ. 2008. A meta-analysis of the impact of monensin in lactating dairy cattle. Part2. Production effects. *J Dairy Sci.* 91:1347–1360.
- Erwin ES, Marco GJ, Emery EM. 1961. Volatile fatty acid analysis of blood and rumen fluid by gas chromatography. *J Dairy Sci.* 44:1768–1771.
- Gallardo MR, Castillo AR, Bargo F, Abdala AA, Maciel MG, Perez-Monti H, Castro HC, Castelli ME. 2005. Monensin for lactating dairy cows grazing mixed-alfalfa pasture and supplemented with partial mixed ration. *J Dairy Sci.* 88:644–652.
- Gandra JR, Rennó FP, Freitas Júnior JE, Santos MV, Silva LFP, Araújo APC. 2010. Productive performance and milk protein fraction composition of dairy cows supplemented with sodium monensin. *Rev Brasil Zootec.* 39:1810–1817.
- Getachew G, Makkar HPS, Becker K. 2002. Tropical browses: contents of phenolic compounds, *in vitro* gas production and stoichiometric relationship between short chain fatty acid and *in vitro* gas production. *J Agric Sci.* 139:341–352.
- Hall MB. 1998. Making nutritional sense of nonstructural carbohydrate. In: Proceedings of Annual Florida Ruminant Nutrition Symposium, 9. Gainesville, FL, EUA. Gainesville (FL): Florida University Press; p. 108–121.
- Haney ME Jr, Hoehn MM. 1967. Monensin a new biologically active compound. I. Discovery and isolation. *Antimicrob Agents Chemother.* 7:349–352.
- Ipharraguerre IR, Clark JH. 2003. Usefulness of ionophores for lactating cows: a review. *Anim Feed Sci Technol.* 106:39–57.
- Jonker JS, Kohn RA, Erdman RA. 1998. Using milk urea nitrogen to predict nitrogen excretion and utilization efficiency in lactating dairy cows. *J Dairy Sci.* 81:2681–2692.
- Makkar HPS, Sen S, Blümmel M, Becker K. 1998. Effects of fractions containing saponins from *Yucca schidigera*, *Quillaja saponaria* and *Acacia auriculiformis* on rumen fermentation. *J Agric and Food Chem.* 46:4324–4328.
- Martineau R, Benchaar C, Petit HV, Lapierre H, Ouellet DR, Pellerin D, Berthiaume R. 2007. Effects of lasalosis or monensin supplementation on digestion, ruminal fermentation, blood metabolites, and milk production of lactating dairy cows. *J Dairy Sci.* 90:5714–5725.
- McCarthy MM, Yasui T, Ryan CM, Mechor GD, Overton TR. 2015. Performance of early-lactation dairy cows as affected by dietary starch and monensin supplementation. *J Dairy Sci.* 98:3335–3350.
- McGuffey RK, Richardson LF, Wilkinson JID. 2001. Ionophores for dairy cattle: current status and future outlook. *J Dairy Sci.* 84:ES:E194–E203.
- [NRC] National Research Council. 2001. Nutrient requirements of dairy cattle. 7th ed. Washington (DC): Academic Press.
- Oelker ER, Reveneau C, Firkins JL. 2009. Interaction of molasses and monensin in alfalfa hay- or corn silage-based diets on rumen fermentation, total tract digestibility, and milk production by Holstein cows. *J Dairy Sci.* 92:270–285.
- Plazier JC, Martin A, Duffield T, Bagg R, Dick P, McBride BW. 2000. Effect of a prepartum administration of monensin in a controlled-release capsule on apparent digestibilities and nitrogen utilization in transition dairy cows. *J Dairy Sci.* 83:2918–2925.
- Possatti CD, Haddade IR, Kill JL, Haese D, Neto AC, Simon CP, Rocha IA, Nascimento JVM, Garcia WA. 2015. Sodium monensin on early lactation cows: milk production and live weight. *Ciênc Rural.* 45:92–97.
- Ruiz R, Albrecht GL, Tedeschi LO, Jarvis G, Russell JB, Fox DG. 2001. Effect of monensin on the performance and nitrogen utilization of lactating dairy cows consuming forage. *J Dairy Sci.* 84:1717–1727.
- Sklan DR, Ashkenazi R, Braun A, Devorin A, Tabori K. 1992. Fatty acids, calcium soaps of fatty acids, and cotton seeds fed to high yielding cows. *J Dairy Sci.* 75:2463–2472.
- Spears JW. 1990. Ionophores and nutrient digestion and absorption in ruminants. *J Nutr.* 120:632–638.
- Spek JW, Dijkstra J, Van Duinkerken G, Hendriks WH, Bannink A. 2013. Prediction of urinary nitrogen and urinary urea nitrogen excretion by lactating dairy cattle in northwestern Europe and North America: a meta-analysis. *J Dairy Sci.* 96:4310–4322.
- Van Soest PJ. 1994. Nutritional ecology of the ruminant. 2th ed. Ithaca (NY): Cornell University Press. 476p.
- Van Soest PJ, Robertson JB, Lewis BA. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J Dairy Sci.* 74:3583–3597.
- Wildman EE, Jones GM, Wagner PEA, Boman RL, Troutt Junior HF, Lesch TN. 1982. A dairy cow body condition scoring system and its relationship to selected production characteristics. *J Dairy Sci.* 65:495–501.
- Yang CM, Russell JB. 1993. The effect of monensin supplementation on ruminal ammonia accumulation *in vivo* and numbers of amino acid-fermenting bacteria. *J Anim Sci.* 71:3470–3476.