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Nutrients balances and milk fatty acid profile of mid lactation dairy cows supplemented with monensin

Balanço de nutrientes e perfil de ácidos graxos do leite de vacas leiteiras no terço médio e lactação suplementadas como monensina

GANDRA, Jefferson Rodrigues[1*]; RENNÓ, Francisco Palma[1]; FREITAS JÚNIOR, José Esler[1]; MATURANA FILHO, Milton[1]; BARLETTA, Rafael Villela[1]

[1]Universidade de São Paulo, Faculdade de Medicina Veterinária e Zootecnia, Departamento de Nutrição e Produção Animal, Pirassununga, São Paulo, Brasil.
*Endereço para correspondência: jeffersongandra@usp.br

SUMMARY

The aim of this study was to evaluate the nutrients balance and milk fatty acids profile of dairy cows supplemented with monensin. Twelve Brazilian Holstein dairy cows were distributed into four balanced 3x3 Latin squares, and fed with the following diets: control (C), basal diet without addition of monensin, monensin 24 (M24), addition of 24mg/kg DM of monensin, and monensin 48 (M48), addition of 48mg/kg DM. The experimental diets influenced the efficiency of net energy of lactation utilization. A quadratic effect was observed for the energy balance. It was observed effect of diets on nitrogen balance. It was observed effect of monensin in the milk yield, composition and in the milk fatty acids profile. Monensin in diets of dairy cows in mid lactation, using corn silage, improved the nutrients balance and milk fatty acid profile with 24mg/kg DM.

Keywords: energy and nitrogen balance, ionophore, metabolism, milk fat.

RESUMO

Objetivou-se avaliar o balanço de nutrientes e o perfil de ácidos graxos do leite de vacas leiteiras suplementadas com monensina. Doze vacas leiteiras foram distribuídos em 4 quadrados latinos, balanceados 3x3 e alimentados com as três dietas seguintes: controle (C), dieta basal sem adição de monensina; monensina 24 (M24), a adição de 24mg/kg MS de monensina, e monensina 48 (M48), a adição de 48mg/kg de MS. As dietas experimentais influenciaram a eficiência de utilização da energia líquida de lactação. Foi observado efeito quadrático para o balanço energia e nitrogênio. Também foi observado efeito de monensina sobre a produção e composição do leite e no perfil de ácidos graxos do leite. Constatou-se que monensina na dieta de vacas leiteiras, no terço médio de lactação, que usam silagem de milho, melhora o balanço de nutrientes e o perfil de ácidos graxos do leite na dose de 24mg/kg de MS.

Palavras chave: balanço energia e nitrogênio, gordura do leite, ionóforo, metabolismo.

INTRODUCTION

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 & HOEHN, 1967), that alters the flux of monovalent ions by the membrane of gram-negative bacteria, changing its normal function (DUFFIELD & BAGG, 2000).

The mode of action of ionophores results in alteration of ruminal bacterial populations, with several impacts on ruminants metabolism, including the improvement of energy and protein metabolism. The increment of gram-negative bacteria participation in the rumen alters the final products of fermentation, increasing of propionate proportion and reducing acetate and butyrate proportions (DUFFIELD et al., 2008b).

The utilization of ionophores in lactating animals influences rumen fermentation, and this has affected the productive performance of lactating cows. The increase of propionate production and the decrease in acetate, butyrate and methane production, increase the supply of glucose for milk synthesis, directly influencing milk yield due to the higher number of precursors for lactose synthesis.

Researches that involve ionophores for lactating cows have produced divergent results, indicating interaction among diet and physiological effects involved (IPHARRAGUERRE & CLARK, 2003). Review by Ipharraguerre & Clark (2003) suggested that the reduction in intake seems to happen more often when cows are in mid and late lactation. Similarly, according to these authors, based on the potential of monensin to increase the supply of gluconeogenic precursors, such as propionate, its administration for lactating cows can increase hepatic glucose synthesis, and therefore, improve the energy balance, with consequent increase of milk yield.

The use of sodium monensin as a supplement for dairy cows has been indicated for any phase of lactation and for several categories of animals in dairy cattle systems, being important to know the right amount for each category, stage of lactation and type of forage utilization.

The objectives of this study was to evaluate the effects of monensin in mid lactation dairy cows in energy and nitrogen balance, milk yield and composition, milk fatty acids profile.

MATERIAL AND METHODS

This experiment was conducted at the School of Veterinary Medicine and Animal Science, University of São Paulo, Pirassununga-SP, Brazil. Twelve Holstein cows with average live weight of 580kg, 157 to 214 days of lactation, and average milk yield of 23.0kg/cow/day were used. The animals were grouped in four balanced 3x3 Latin squares, with an experimental period of 21 days, being 14 days of adaptation and 7 days of sample collection.

The following diets were used, formulated according to NRC (2001): Control (C), basal diet without addition of monensin; Monensin 24 (M24), basal diet with inclusion of 24mg/kg DM of sodium monensin (Bobiovet 10 Premix®, Indukern do Brasil Química Ltda), and Monensin 48 (M48), basal diet with inclusion of 48mg/kg DM of sodium monensin. The monensin was added to the concentrate. The respective diets, water and mineral salt were supplied *ad libitum* during the whole experimental period. The forage used during the experiment was corn silage.

For better control of sodium monensin administration, a premix with the sodium monensin was formulated and supplied to the animals during the experimental period, together with the concentrate, in doses equivalent to 24 or 48mg/kg DM of sodium monensin,

except in the control treatment, in which the animals received placebo.

The quantities of corn silage and concentrate supplied, and the orts of each diet were weighed daily to estimate individual intake. The animals were fed according to the dry matter intake of the previous day, to keep 5-10% of orts for not have restriction of consumption. Samples of the supplied ingredients were collected and stored at -20ºC for later chemical analysis.

The supplied ingredients and orts samples were analyzed for dry matter (DM), organic matter (OM), ash, ether extract (EE), crude protein (CP), neutral detergent insoluble nitrogen (NDIN), acid detergent insoluble nitrogen (ADIN) and lignin content according to the methodologies described AOAC (2006). The crude protein (CP) content was obtained by multiplying the total nitrogen ratio by 6.25. The total carbohydrates (TC), non-fiber carbohydrates contents (NFC) and total digestible nutrients were calculated according to (AOAC, 2006).

The neutral detergent fiber (NDF), neutral detergent fiber corrected for ash and protein (NDFap) and acid detergent fiber (ADF) contents were obtained according to method described by AOAC, (2006) in Ankon® System (Tables 1 and 2).

The feces were collected at the 13th to 18th day of each experimental period, always before the morning and afternoon milking, being stored in plastic bags and kept at –20^{o}C. At the end of the collection period, samples were composed by each animal based in dry matter. For the determination of total apparent digestibility of dry matter and nutrients, the total quantity of fecal dry matter excreted was estimated by the concentration of indigestible acid detergent fiber (iADF).

To evaluate the indigestible components contents, the processed samples were packed in bags of none-woven textile (NWT-100g/m2), with dimensions of 4 x 5cm. The aliquots were packed in all bags, according to the ratio of 20 mg of dry matter by square centimeter of surface (CASALI et al., 2008).

Before samples incubation, two Holstein cows were adapted during 7 days with a diet based on soybean meal and ground corn, and receiving corn silage as forage. After the adaptation period, the samples were incubated in the rumen for 240 hours, according to technique described by (CASALI et al., 2008). After removal from the rumen, the bags were washed in flowing water until total clearing, and immediately placed in forced ventilation oven (60º/72 hours).

Table 1. Proportion of the ingredients of the concentrate and the diet expressed in dry matter (% DM)

Ingredients	Concentrate	Diet[1]
Corn silage	-	58.00
Corn ground	52.14	21.90
Soybean meal	39.10	16.42
Urea	1.74	0.73
Ammonium sulfate	0.12	0.05
Sodium bicarbonate	1.48	0.62
Magnesium oxide	0.05	0.02
Mineral mix [2]	4.67	1.96
Limestone	0.24	0.10
Sodium Chloride	0.48	0.20

[1]C=control; M24=24 mg/kg DM of sodium monensin diet; M48=48mg/kg DM of sodium monensin diet. [2]Composition for mineral mix: Ca180g; P 90g; Mg 20g; S 20g; Na 100g; Zn 3g; Cu 1g; Mn 1,25g; Fe 2g ; Co 0.2g; I 0.09g; Se 0.036g; F(máx.)0.9g.

Table 2. Chemical composition of ingredients concentrate, corn silage and experimental diets

Nutrients (%)	Corn ground	Soybean meal	Concentrate	Corn Silage	Diet[1]
DM	88.5	89.8	89.4	29.0	54.4
OM	97.5	93.6	90.0	94.5	92.6
CP	8.20	49.2	27.7	8.9	16.8
ADIN	7.2	3.5	4.7	13.5	9.9
NDIN	14.4	10.6	13.2	19.8	17.0
Fat	4.9	1.1	2.9	2.9	2.9
Ash	2.50	6.40	10.0	5.5	7.4
NDF	10.6	12.6	9.9	53.2	35.0
ADF	6.2	10.0	7.9	43.7	28.7
iADF	1.3	0.9	1.0	14.4	7.9
Lignin	1.0	1.6	1.1	5.4	3.6
NFC	77.9	39.5	56.0	29.5	40.6
TC	83.4	47.3	61.3	82.7	72.9
TDN	88.5	74.9	82.5	62.7	71.0
NE_L (Mcal/kgDM)	2.2	2.5	2.1	1.4	1.6
GE(cal/g/DM)	4489	4684	4181	4312	4249

[1]C=control; M24=24 mg/kg DM of sodium monensin diet; M48=48mg/ kg DM of sodium monensin diet. DM = dry matter, OM = organic matter, CP = crude protein, ADNI = acid detergent insoluble nitrogen, NDIN = neutral detergent insoluble nitrogen, NDF = neutral detergent fiber, ADF = indigestible acid detergent fiber, iADF = indigestible acid detergent fiber, NFC = non-fiber carbohydrate, TC = total carbohydrate, TDN = total digestible nutrients.

Afterwards, the bags were submitted to treatment with acid detergent Mertens (2002) for one hour, in the Ankon® fiber analyzer. After this period, the bags were washed with hot water and acetone, being dried and weighted according to the previous procedure. At the end of this treatment, iADF was obtained.

To obtain the gross energy intake and calculate efficiency of use of intake energy, samples of silage, ingredients and concentrates were analyzed for its gross energy content in calorimetric bomb, according to (HARVATINE & ALLEN 2006). The digestible energy intake (DEI) was obtained by the digestibility coefficient of the experimental diets and the gross energy intake, according to the energy values obtained for the ingredients and corn silage (HARVATINE & ALLEN, 2006). The net energy intake (NEI), the values of net energy of production (NEp), net energy of gain (NEg), and the empty body weight change (EBWC) were calculated according to the following NRC (2001) equations: NEI (Mcal/day) = 0.703 × ME (intake) – 0.19 + {[(0.097 × ME (intake) + 0.19)/97] × [EE − 3]}; ME (intake) = 1.01 × (DE (intake) – 0.45] + 0.0046 × (EE − 3) where: ME= metabolizable energy; EE= ether extract; DE= digestible energy. The metabolizable energy values (ME) were obtained by the following formula: ME (Mcal/kg) = [1.01 * {(%NFC/100) *4.2+ (%NDF/100) *4.2 + (%CP/100) *5.6 + (%FA/100) * 9.4 - 03)}-0.45] + 0.0046 * (EE-3.0). The values of net energy of production (NE_p) were calculate following the formula: NE_p (Mcal/day) = milk yield (kg) × (0.0929 × F% + 0.0563 ×TP% + 0.0395 × lactose %) where: F%= milk fat content; TP= milk

true protein content.

The empty body weight change (EBWC) was calculated from the body weight (BW) where: EBW = 0.817* BW. The net energy of gain was calculated through the formula: NE_g = 1.42 ME – 0.174*ME + 0.0122*ME*1.65. The efficiency of energy utilization was calculated according to Harvatine and Allen (2006) as follows: Milk yield efficiency = NE (intake) − NE (BW gain) − NE (milk); Lactation Efficiency = (NE milk yield + NE of BW gain)/DE Intake.

For the calculation of nitrogen balance was performed the determination of the creatinine concentration in urine according to methodology described by (RENNÓ et al., 2008). Spot samples of 50 ml of urine were obtained from all cows at the 20th day of each experimental period, four hours after the morning feeding, during urination stimulated by massage of the vulva. A sample of pure urine was stored for determination of total nitrogen compounds, urea and creatinine. Creatinine concentrations were determined using commercial kits (Laborlab®), using kinetic calorimetric enzymatic reaction in equipment SBA-200 CELM®.

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, according to (CHIZZOTTI et al., 2007). Daily creatinine urinary excretion was estimated from the proposition of 24.05mg/kg of body weight (GONZÁLEZ-RONQUILLO et al., 2003). So, with the average daily creatinine excretion and the creatinine concentration (mg/dl) in the spot urine sample, the total urine volume was estimated, in liters per cow per day, for calculating nitrogen balance.

The analysis of the urea concentration in milk deproteinized were performed using commercial kits (Laborlab® and CELM®).The concentration of milk urea nitrogen (MUN) was indirectly determined using the following formula: MUN = urea (mg/dl)/2.14.

Cows were mechanically milked twice a day, at 06:30 and at 15:30, being the milk yield recorded daily throughout the experimental period. Milk yield was corrected for 3.5% of fat (FCM) according to formula, where FCM = (0.432 + 0.1625 * milk fat content) * kg of milk. Samples used for analysis of milk composition were obtained at the 13th and 16th day of each experimental period, each sample coming from the two daily milkings. The contents of fat, protein and lactose were determined for infrared spectroscopy.

The milk samples used for evaluating fatty acids profile were obtained at the 16th day of each experimental period, being each sample coming from the two daily milkings. Initially, the samples were centrifuged at 17.800 × *g* for 30 minutes at 4^{o}C and next for 19.300 × *g* for 20 minutes at 4^{o}C, according to (FENG et al., 2004). The separated fat (300 to 400 mg) was extracted and converted to methyl esters according to (KRAMER et al., 1997). Two internal C18:0 and C19:0 standards were used for correcting for losses during the process of methylation.

The fatty acids were quantified by gas chromatography (Shimatzu GC 2010 with automatic injection), using capillary column SP-2560 (100m × 0.25mm i.d. with 0.02mm of film thickness, Supelco®, Bellefonte, PA). The start temperature was 70ºC for 4 minutes 13ºC/minute until it reached 175ºC maintaining for 27 minutes. After, a new increase of 4^{o}C/minute was initiated until 215^{o}C, maintaining for 31 minutes. Hydrogen (H_2) was used as

carrier gas with flux of 40 cm/s. During the process of identifying four patterns were used, standard C4-C24 fatty acids (Supelco® TM 37), vacenic acid C18:1 *trans*-11 (V038-1G, Sigma®), CLA C18:2 *trans*-10, *cis*-12 (UC-61M 100MG), and CLA C18:2 *cis*-9, *trans*-11 (UC-60M 100MG), (NU-CHEK-PREP, INC. P.O. BOX 595 ELYSIAN, MN 56028 USA®).

Data were analyzed using the PROC MIXED procedure Version 9.1.3 (SAS INSTITUTE, 2004) according to the following statistical model:

$Yijky = \mu + Qi + Aj + Py + Tk + eijyk$

where: Yijyk = dependent variable, μ = overall mean, Qi = fixed effect of square (i = 1 to 4), Aj = animal effect (j = 1 to 12), Py = period effect (y = 1 to 3), Tk = fixed effect of treatment (k =1 to 3), and eijk = error. Random effect used was Aj and Py = animal and period.

The obtained data were submitted to simple polynomial regression and 5% as level of significance. Regression was chosen (for the answer linear or quadratic), according to the P value of the equations, taking into account all the components generated, and was accepted as significant (P<0.05).

RESULTS AND DISCUSSION

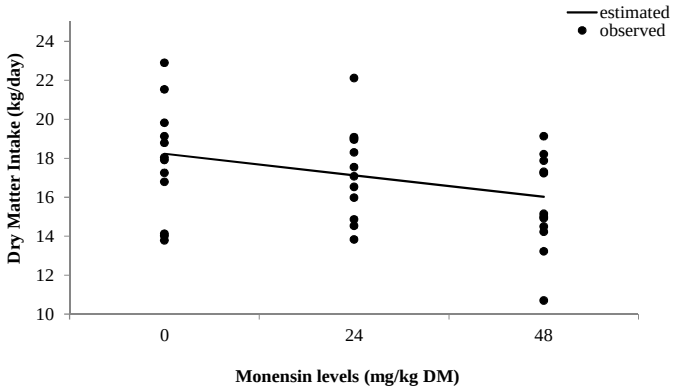
It was observed decreasing linear effect (P <0.05) for dry matter intake (Table 3 and Figure 1). It was observed a reduction of 12.40% when compared to control diet for the M48. It was observed a mean daily intake of monensin 420mg/day diet for M24 and 758mg/day diet for M48, only decrease in performance was observed for M48 diet, no was observed signs of toxicity, since the dose where begins to observe signs of intoxication would be six times the intake presented by diet M48.Quadratic effect was observed (P<0.05) for milk yield and FCM (kg/day). The optimal level of monensin was 21.75 and 24.30mg/kg DM respectively for milk yield and FCM.

Table 3. Intake, milk yield and composition for mid lactation dairy cows

Item	Treatments[1]			SEM[2]	*P*[3]	
	C	M24	M48		L	Q
Yield kg/d						
Intake	18.03	17.50	15.79	0.16	<0.001	0.126
Milk	23.92	24.58	22.63	0.75	0.003	0.002*
FCM 3.5%	22.53	23.41	20.71	1.17	0.410	0.037
Fat	0.71	0.80	0.69	0.03	0.655	0.125
Crude Protein	0.70	0.72	0.66	0.02	0.167	0.583
Lactose	1.08	1.11	1.01	0.03	0.346	0.451
Milk Composition %						
Fat	2.97	3.15	3.16	0.07	0.113	0.393
Crude Protein	2.95	2.93	2.88	0.05	0.134	0.737
Lactose	4.50	4.53	4.53	0.03	0.247	0.438
Total solids	11.78	11.67	11.54	0.14	0.070	0.936
MUN (mg/dL)	12.81	13.70	14.01	0.40	0.128	0.662

[1]C=control; M24=24 mg/kg DM of sodium monensin; M48=48 mg/ kg DM of sodium monensin;[2]SEM = standard error of the mean;[3]L e Q=probability for linear e quadratic effect, respectively;*Model analyzed.

1a



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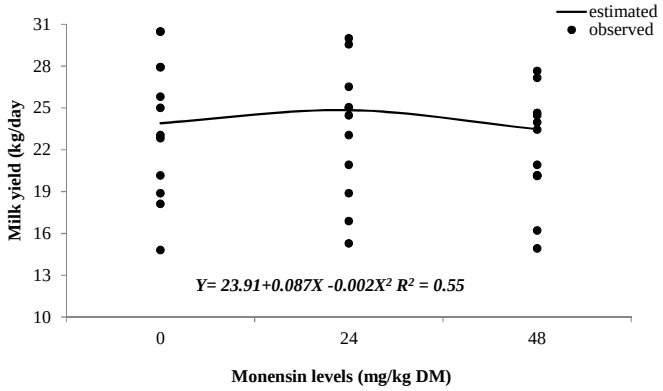

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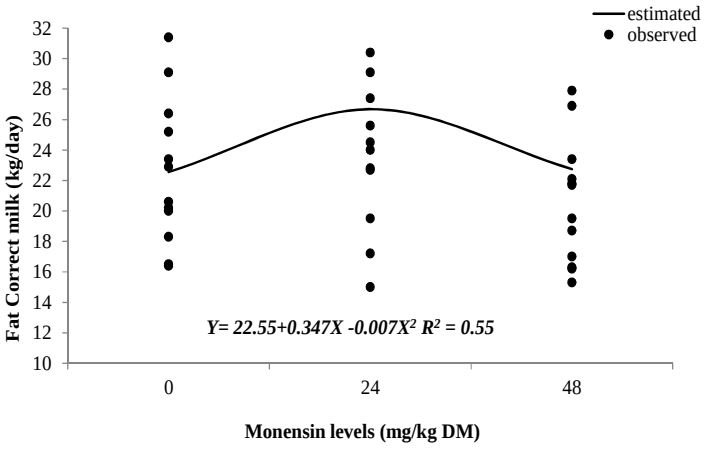

Figure 1. Regression equations of dry matter intake (kg/day) - (1a), milk yield (kg/day) – (1b), fat correct milk (kg/day) – (1c)

Diet M24 had higher production compared to control diet at 2.7% and 8.0% compared to M48 for milk yield. For FCM was observed 3.8% higher than the control and 11.5% higher than M48 (Table 3 and Figure 1).

There was no linear or quadratic effect (P>0.05) for fat, protein, lactose, and total solids (%) and (kg/day), as well as the concentration of MUN and protein (kg/day).

Physiologically, monensin acts in the reduction of dry matter intake due to its effect on rumen fermentation, increasing the molar concentration of propionic acid, and reducing the acetate/propionate ratio. This metabolic effect of the intake regulation becomes concrete with the increase of propionate hepatic flux, responsible by the increase of the glucose concentration in mammary gland (IPHARRAGUERE AND CLARK, 2003).

The milk yield and milk composition were influenced by monensin, because the differences between the control and M48 diet, with higher milk yield for the control diet. However a better metabolic and nutritional balance was observed for the diet M24, justified by the action of monensin in changing the profile of short chain fatty acids in the rumen environment, providing a major input of hepatic propionate and therefore contributing to milk yield and productive efficiency. The concentrations of acetate, propionate and butyrate observed in this trial was (56.44; 56,71 and 50,76mmol/L); (15,97; 22,01 and 19,34mmol/L) and (7,54; 6,74 and 5,85mmol/L), respectively for diets C, M24 and M48, results that justified the, dry matter intake and milk yield.

Duffield et al. (2008b) concluded that monensin addition in diets of dairy cows has the potential to increase milk yield at 0.70 kg/day, also increasing the efficiency of milk yield at 2.5%, similar data as obtained in the present study.

According to the review of Ipharraguerre & Clark (2003), lactating cows that received monensin had, on average, an increase of 1.3kg/day, or 5% higher than not supplemented cows. The most of studies with monensin in concentrations around 24mg/kg DM added in diet resulted in better productive performance of mid- and late-lactation dairy cows in relation to the control diet. Similarly, studies that used sodium monensin concentrations around 12 to 20mg/kg DM, often did not report favorable results on productive performance, the same occurring with doses above 35mg/kg DM, as in the present study (48mg/kg DM). There are great variation in the results found in the literature according to the dose of supplemented monensin, lactation phase, production level, mode of monensin supplementation, and type of basal diet (especially forage), in comparison to not supplemented animals, especially in corn silage based diets.

Zahra et al. (2006), reported reduction in milk fat content, and no change in protein content, when supplementing lactating cows with monensin. However, Silva et al. (2007) observed that addition of monensin did not cause any effect on milk fat and protein contents. Milk fat and protein contents decreased when monensin was supplemented in lactating cows in the studies by (BRODERICK, 2004; PETERSSON-WOLFE et al., 2007; ODONGO et al., 2007). In studies which monensin reduced milk fat and protein contents, there was a parallel expressive increase in milk yield, suggesting that the dilution effect was, in part, responsible for the changes in milk composition (GANDRA et al., 2009).

In this study there was no effect (P>0.05) of using different levels of sodium monensin addition to the diets on the contents of milk non-protein nitrogen, true protein, casein, casein/true protein ratio, whey protein, and on all these fractions expressed as percentage of crude protein as reported by Gandra et al. (2010). Even with the increase in milk yield when the animals received the M24 diet, or with the decrease in yield of the animals receiving the M48 diet, the proportion of milk fractions, expressed at percentage of milk (%) and milk protein (%CP), were not altered (GANDRA et al. 2010).

There was no linear or quadratic effect (P>0.05) for the concentration of short, medium or long chain fatty acids in milk. However when measuring the production of fatty acids in milk quadratic effect was observed (P<0.05) for fatty acids <C16 and C16. The optimal level of monensin was 27.38 and 21.90mg/kg DM respectively for <C16 and C16. Diet M24 had higher production compared to control diet at 20.10% and 23.95% compared to M48 for <C16. For C16 was observed 17.30% higher than the control and 22.80% higher than M48 (Table 4 and Figure 2).

Table 4. Milk fatty acid profile for mid lactation dairy cows

Item	Treatments[1]			SEM[2]	*P*[3]	
	C	M24	M48		L	Q
Fatty acids (g/100 fatty acids)						
C10:0	1.456	1.520	1.393	0.07	0.640	0.421
C12:0	1.765	1.905	1.691	0.09	0.665	0.240
C14:0	6.486	7.052	6.284	0.30	0.727	0.191
C15:0	0.652	0.728	0.630	0.02	0.671	0.062
C16:0	19.96	20.97	19.29	0.63	0.655	0.304
C16:1, cis	1.656	1.559	1.608	0.08	0.825	0.697
C17:0	0.416	0.427	0.400	0.01	0.626	0.500
C18:0	6.671	7.078	6.880	0.25	0.650	0.072
C18:1,trans 9	0.088	0.068	0.063	0.02	0.582	0.840
C18:1,cis 9	16.74	15.26	17.12	0.83	0.863	0.080
C18:1,trans 11	1.235	1.133	0.893	0.12	0.250	0.785
C18:2,trans 6	0.094	0.082	0.100	0.01	0.849	0.590
C18:2,cis 6	1.401	1.201	1.358	0.08	0.851	0.372
cis-9,trans-11	0.421	0.405	0.362	0.02	0.328	0.797
C18:3	0.151	0.128	0.147	0.07	0.822	0.226
C20:0	0.059	0.059	0.064	0.05	0.717	0.863
Total	63.88	64.40	62.87	1.82	0.827	0.797
<C16	14.29	15.38	13.83	0.61	0.697	0.205
C16	21.62	22.53	20.90	0.68	0.657	0.366
>C16	57.51	54.25	57.68	2.33	0.979	0.539
C18	26.81	25.36	26.93	1.10	0.968	0.553
insat/sat C18	3.040	2.693	3.023	0.16	0.965	0.303
Fatty acids (grams)						
<C16	99.93	125.05	95.09	7.17	0.638	0.005
C16	140.32	169.65	131.00	8.33	0.518	0.011
>C16	419.18	433.20	389.50	27.85	0.626	0.584
C18	195.56	202.61	182.00	13.15	0.635	0.577

[1]C=control; M24 = 24mg/kg DM of sodium monensin; M48 = 48mg/kg DM of sodium monensin; [2]SEM = standard error of the mean;[3]L e Q = probability for linear e quadratic effect, respectively.

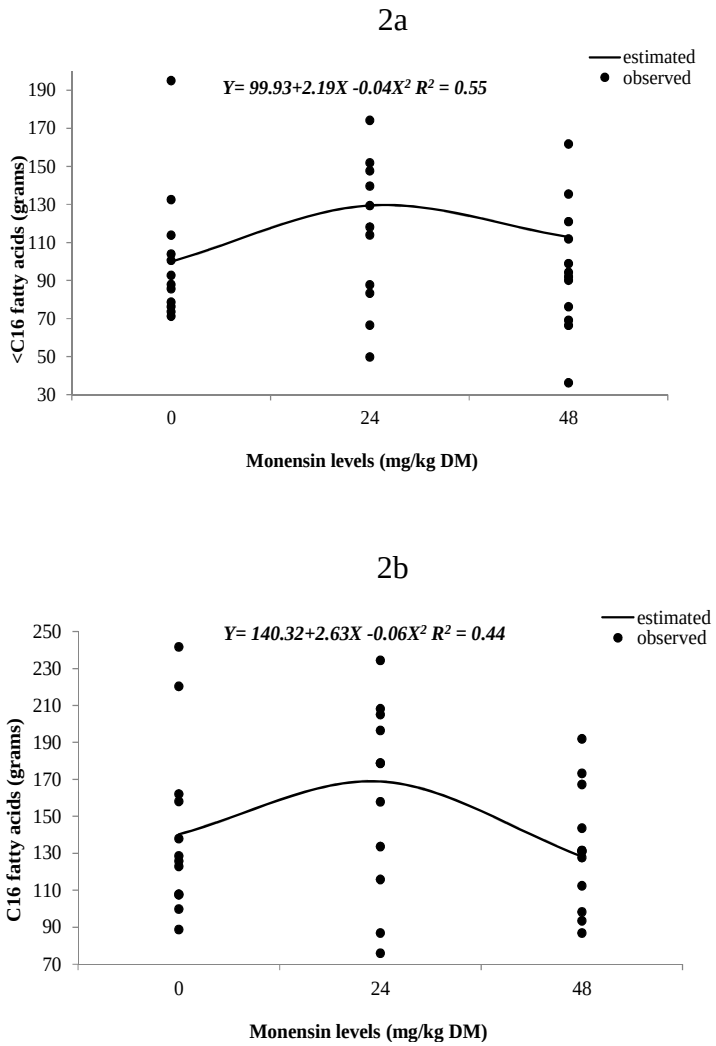

Figure 2. Regression equations of fatty acids > C16 grams (2a), C16 grams (2b).

Monensin supplemented diets providing greater stability in ruminal pH, ie able to maintain the higher pH especially in high concentrate diets as in the case of diets of lactating cows. The pH stability is directly related to the lipolysis of fatty acids in ruminal level, a fact that is predominant for formation of fatty acid intermediates, which are directly linked to reduced milk and also the profile of fatty acids in milk. Diets that provide low pH tend to increase the concentration of saturated fatty acids in milk due to high activity level on ruminal biohydrogenation. Monensin supplemented diets tend to increase the concentration of polyunsaturated fatty acids in the milk.

Martineau et al. (2007), Odongo et al. (2007) and Al-Zahal et al. (2008) measured the profile of milk fatty acids in diets supplemented with sodium monensin, in levels that varied from 22 to 24mg/kg DM. The results obtained by these authors are similar to the ones found in this study, except by the absence of the CLA *trans*-10, *cis*-12 in the present study. The results obtained in the present study, for the profile of milk fatty acids, could be related to the

lactation period of the animals. Trials involving the relationship of monensin and fatty acid profile of milk are scarce and mostly inconclusive. Due to this series of events further studies should be performed in order to clarify this possible relationship. These results suggest that monensin was at least partly effective in inhibiting the biohydrogenation of unsaturated fatty acids in the rumen and consequently increased the percentage of polyunsaturated fatty acids total in milk, thus enhancing the nutritional properties of the milk in terms of human health.

Decreasing linear effect was observed (P<0.05) for intake of GE, DE, ME and NE_L. It was observed reduction of 13.47; 15.40; 13.55 and 12.65% when M48 was compared to the control diet (Table 5 and Figure 3). It was also observed decreasing linear effect (P<0.05) for the NE_g and efficiency (NE_L milk/ DE intake). For the NE_g was observed a reduction of 42.74% when M48 was compared to the control diet.

Quadratic effect was observed for Milk NE_L and for energy balance. The optimal level of monensin inclusion for Milk NE_L was 21.05mg/kg DM and the energy balance was 25.0mg/kg DM. Diet M24 had higher production compared to control diet at 4.90% and 9.52% compared to M48 for Milk NE_L. For energy balance was observed 12.35% higher than the control and 11.30% higher than M48 (Table 5).

Table 5. Energy balance and efficiency for mid lactation dairy cows

Item	Treatments[1]			SEM[2]	*P*[3]	
	C	M24	M48		L	Q
Intake (Mcal/d)						
GE[4]	76.64	73.65	66.31	1.81	<0.001	0.224
DE[5]	55.27	53.38	46.76	1.39	<0.001	0.133
ME[6]	50.79	45.93	43.91	1.29	0.004	0.488
NE_L[7]	32.25	30.97	28.17	0.78	<0.001	0.296
Production (Mcal/d)						
Milk NE_L[8]	14.98	15.75	14.25	0.50	0.044	0.010*
Empty BW change	1.37	1.21	1.11	0.23	0.620	0.938
NE_g[9] Empty BW /d	8.12	5.79	4.65	0.52	<0.001	0.345
BCS change, 21d	1.68	1.49	1.36	0.28	0.617	0.961
Balance (Mcal/d)						
NE_L Avail Maint[10]	9.15	10.44	9.26	0.12	0.356	0.034
Efficiency						
NE_LProd/DE intake[11]	0.41	0.41	0.41	0.008	0.970	0.970
NE_LMilk/DE intake[12]	0.27	0.30	0.31	0.008	0.008	0.392

[1]C = control; M24 = 24mg/kg DM of sodium monensin; M48 = 48mg/kg DM of sodium monensin; [2]SEM = standard error of the mean; [3]L e Q= probability for linear e quadratic effect, respectively; [4]Obtained by calorimetry bomb;[5]Digestible $Energy_{(intake)}$ (DE) = ((NFC_{dig} X NFC/100)x4.2) + ((NDF_{dig} X NDF/100) X 4.2) + ((CP_{dig} X CP/100) X 5.56) + ((EE_{dig} x EE/100) X 9.4) – 0.3 (NRC.2001); [6]Energia $Metabolizable_{(intake)}$ (ME) = [1.01 x (DE) – 0.45] + 0.00046 X (EE- 3)(NRC.2001); [7]Net Energy $Lactaton_{(intake)}$ (NE_L) = 0.703 X (ME) - 0.19 + ([(0.097 X (ME)+ 0.19)/97] X [EE - 3]) (NRC.2001); [8]Net Energy $Lactation_{(milk)}$ = Milk yield (kg) × (0.0929 × fat% + 0.0563 × true protein% + 0.0395 × lactose%) (NRC, 2001); [9]Net Energy gain $_{(empty\ body\ weight\ change)}$ calculated according to NRC (2001); [10]NE_L Available for Mantence = NE_L(intake) − NE_L(BW Gain) − NE_L(milk); [11](NE_L Milk yield + NE_L BW gain)/DE intake;[12](NE_L Milk yield)/DE intake.*Model analyzed.

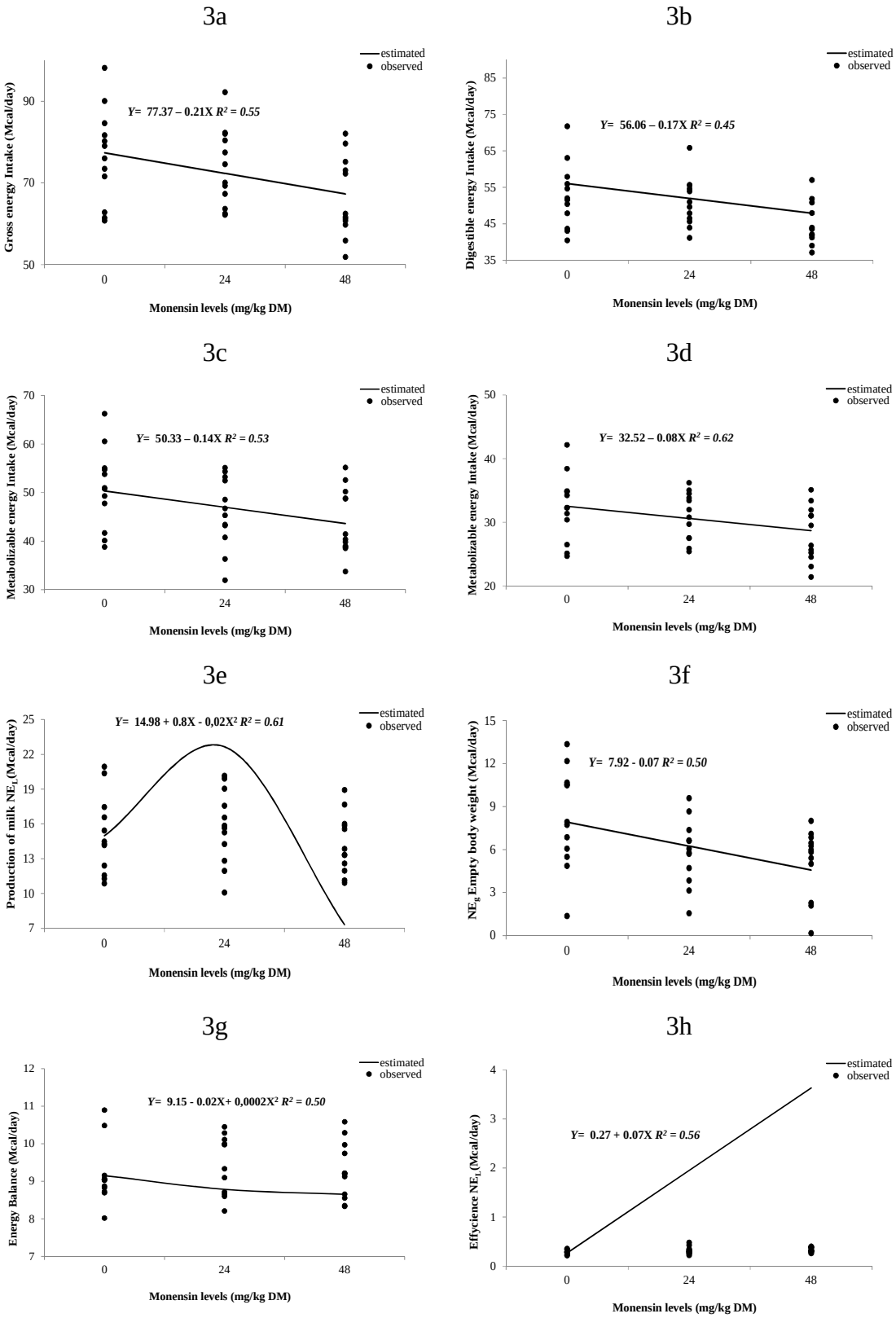

Figure 3. Regression equation of intake of GE (3a), DE (3b), ME (3c), NE_L (3d), production of milk NE_L (3e), NE_g (3f), energy balance NE_L (3g) and efficiency NE_L (3h)

The highest concentration of Milk NE_L for diet M24 may be related to the increased fat yield d presented by this diet associated with the dry matter intake, milk yield and improved energy balance observed. These results are due to the action of monensin on ruminal fermentation in relation of pH stability, decreasing lipid biohydrogenation, fact that affects the fat content of milk and increasing the concentration of propionate, decreasing the dry matter intake and increasing milk yield.

Decreasing linear effect was observed ($P<0.05$) for the intake of nitrogen and nitrogen excretion in feces (g/day). There was a reduction of 14.0 and 17.20%. When compared to M48 diet was the control diet, respectively for the intake of nitrogen and nitrogen excretion in feces (Table 6 and Figure 4).

Quadratic effect was observed ($P<0.05$) for the excretion of nitrogen in milk and nitrogen balance (g/day). The optimal level for inclusion of monensin in milk nitrogen excretion was 13.0mg/kg DM and the nitrogen balance was 23.75mg/kg DM. Diet M24 had higher production compared to control diet at 0.4% and 9.6% compared to M48 for excretion of nitrogen in milk. For nitrogen balance was observed 47.30% higher than the control and 67.90% higher than M48 (Table 6 and Figure 4). The higher nitrogen concentration observed for the M24 diet is related to the concentration of MUN (mg/dL) and crude protein content of milk observed for M24 and also by lower nitrogen excretion in feces, indicating a higher uptake of nitrogen in small intestine

The improvement in energetic efficiency as consequence of the monensin supplementation is directly related to the increase of hepatic propionate supply due to changes in the rumen microbes. The efficiency of use of net energy of lactation was also influenced by the decrease in dry matter intake and alterations in levels of milk yield due to the increase in sodium monensin levels on experimental diets.

Table 6. Nitrogen balance and efficiency for mid lactation dairy cows

Item	Treatments[1]			SEM[2]	*P*[3]	
	C	M24	M48		L	Q
Nitrogen (g/day)						
Intake	475.50	458.69	408.89	11.89	<0,001	0.151
Feces	146.86	125.78	121.65	5.56	0.021	0.342
Urine	197.60	183.93	166.46	5.56	0.140	0.915
Milk	115.60	116.03	104.82	3.36	0,002	0.011*
Balance	22.91	43.50	13.97	8.85	0.607	0.037
Nitrogen (%)						
Feces	31.89	28.15	30.62	1.06	0.536	0.092
Urine	41.52	39.96	40.78	1.76	0.860	0.743
Milk	24.34	23.37	25.74	0.70	0.410	0.263
Balance	2.25	8.52	2.86	1.91	0.895	0.309
Efficiency[4]	0.24	0.25	0.26	0.004	0.012	0.862

[1]C=control; M24=24 mg/kg DM of sodium monensin; M48=48mg/kg DM of sodium monensin. [2]SEM = standard error of the mean.[3]L e Q = probability for linear e quadratic effect, respectively.[4]Milk nitrogen (kg)/nitrogen intake (kg).*Model analyzed

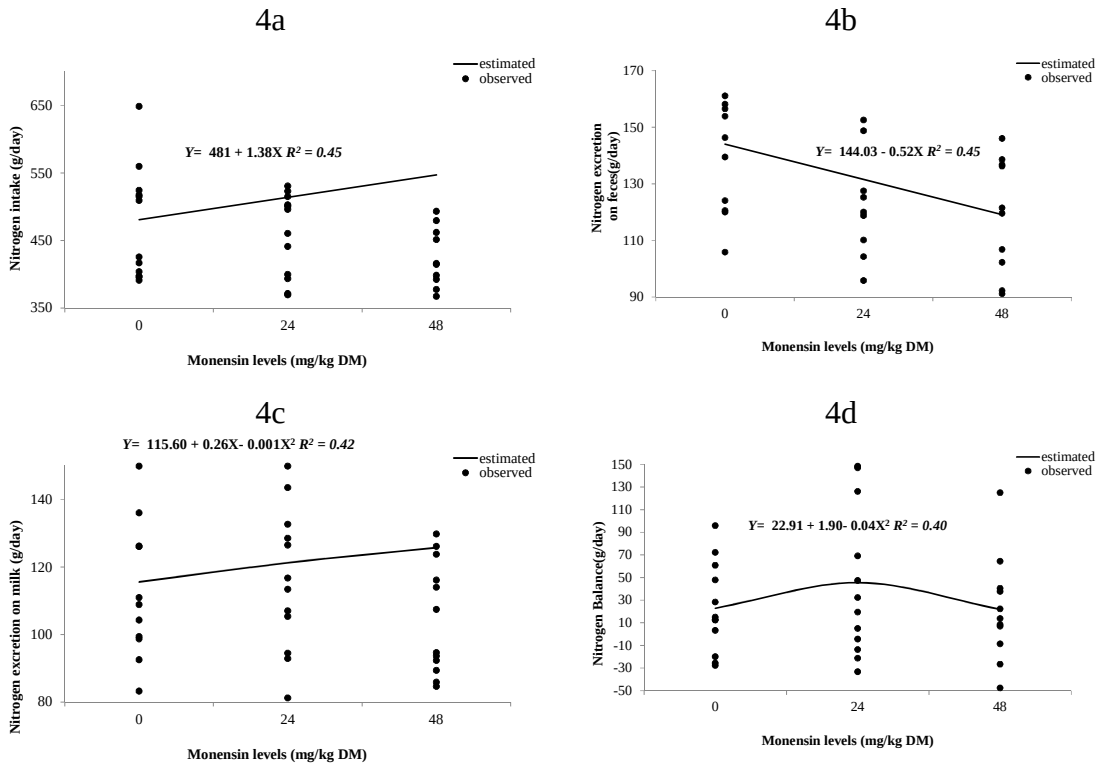

Figure 4. Regression equation of intake of nitrogen (4a), nitrogen excretion on feces (4b), nitrogen excretion on milk (4c), and nitrogen balance (4d)

For nitrogen balance, the best results were obtained in diets supplemented with monensin, possibly due to the better nitrogen utilization as available amino acids in the intestine, resulting from changes in rumen fermentation caused by monensin (DUFFIELD et al., 2008b).

Monensin has the ability to select bacteria that degrade in the rumen environment amino acids thus deamination occurs a decrease in the rumen. Increased flow protein to the small intestine will up-regulate the amino acid uptake capacity of the small intestine, resulting in a greater extraction of amino acids from the intestinal lumen. Based on these results, it appeared that monensin altered the pattern of excretion nitrogen, so that the monensin may increase the proportion of amino acids absorbed positively by increasing the quality and quantity of nitrogen absorbed.

Benchaar et al. (2006) did not find effect on nitrogen balance when cows were supplemented with 16mg/kg DM of monensin in early lactation. However, the authors reported the value of 35g/day of retained nitrogen, similar result as observed in the present study. The observed differences between the two studies are due to the lactation period of the animals. Gehman et al. (2008) did not observed effect on nitrogen balance when cows were supplemented in mid lactation with 16mg/kg DM of monensin and, found a value of 0.30 for efficiency of nitrogen utilization, similar results to this study.

The nitrogen balance and the efficiency of nitrogen use can be improved with supplementation of sodium monensin. However, these effects are likely dependent of the lactation period, dose

of monensin, as well as the level of animal production. Therefore, the implications and recommendation should be careful, taking into consideration the factors described above.

The utilization of sodium monensin in corn silage based diets for mid-lactation dairy cows improved the productive performance of the animals. Based in the results of productive performance, the dose of 24mg/kg DM of sodium monensin is indicated for cows in mid-lactation. The dose of 48mg/kg DM should not be indicated, because of the strong decline in dry matter intake, together with the reduction of productive performance.

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