REGULAR ARTICLES

Effects of *Saccharomyces cerevisiae* and monensin on digestion, ruminal parameters, and balance of nitrogenous compounds of beef cattle fed diets with different starch concentrations

João Paulo Ismério dos Santos Monnerat ·
Pedro Veiga Rodrigues Paulino · Edenio Detmann ·
Sebastião Campos Valadares Filho ·
Rilene Diniz Ferreira Valadares · Márcio Souza Duarte

Abstract This study was carried out aiming to evaluate the effects of yeast or monensin supplementation on dry matter intake, nutrients digestibility, ruminal volatile fatty acids profile, ruminal pH and ammonia concentration, microbial protein synthesis, and the balance of nitrogen compounds of cattle fed high concentrate diet (80 % dry matter (DM) basis) with two different levels of starch. Eight crossbred beef steers fitted with rumen cannula were assigned to two simultaneous 4×4 Latin squares arranged in a 4×2 factorial design. Two different starch levels (23 and 38 % of DM) were assigned to each Latin square, independently. Within each Latin square, four treatments were randomly assigned to the experimental animals (control; monensin; 1-g yeast [1 g/100 kg body weight (BW)/day] treatment; and 2.5-g yeast [2.5 g/100 kg BW/day] treatment). Feed additives did not influence ruminal pH ($P>0.05$). Total ruminal volatile fatty acids (VFA) concentration was greater ($P<0.05$) in the diet with the lowest starch level. Similarly, monensin and 1-g yeast treatments resulted in greater ($P<0.05$) VFA concentration in the rumen. Monensin inclusion in the diet with the highest starch level led to a decrease ($P<0.05$) in lactate concentration in the rumen. However, acetate levels were increased ($P<0.05$) by the inclusion of 1 g of yeast in the diet with lowest starch level. Ruminal concentrations of propionate and butyrate, and ammonia-N were not influenced ($P>0.05$) by none of the additives evaluated. However, propionate concentration was greater ($P<0.05$) in the low-starch diets. Low-starch diets resulted in lower ruminal ammonia-N concentration and greater neutral detergent fiber digestibility ($P<0.05$). The excretion of urinary nitrogenous compounds, purine derivatives, synthesis of microbial protein, microbial efficiency, and balance of nitrogenous compounds were not affected by treatments evaluated ($P>0.05$). Monensin or yeast inclusion in high concentrate beef cattle diets in tropical regions as in Brazil is not justified by do not alter nutrient digestibility, nitrogen balance, and main ruminal parameters.

Keywords Ionophore · Feed additives · Feedlot · Yeast

Introduction

Beef cattle production in tropical regions such as Brazil relies almost exclusively on pastures. However, more recently, intensive beef production systems have gained increased interest by some beef producers. The focus is to produce a differentiated product in a vertically integrated manner to target both domestic, but particularly international markets. In this context, finishing cattle coming from pasture in feedlot grain-based diet is emerging as an interesting alternative.

However, excessive intake of fermentable carbohydrates decreases ruminal pH, which can predispose cattle to

J. P. I. S. Monnerat (✉) · P. V. R. Paulino · E. Detmann ·
S. C. Valadares Filho · R. D. F. Valadares · M. S. Duarte
Departament of Animal Science, Universidade Federal de Viçosa,
Viçosa, Minas Gerais 36570-000, Brazil
e-mail: joaopaulomonnerat@hotmail.com

acidosis (Vasconcelos and Galyean 2008). Thus, recent efforts have been made in order to evaluate feed additives with potential to reduce digestive disturbances such as ruminal acidosis.

Monensin is a feed additive that improves feed efficiency, reduces feed intake variation, and increases ruminal pH of cattle fed high-concentrate diets (Nagaraja et al. 1981). However, some markets from beef produced in tropical regions in South America are signaling a future ban on beef coming from animals fed monensin. Thus, the use of other alternatives, like yeast, has been increased due to its capacity to optimize ruminal digestion and allow beef labeling as natural.

Several mechanisms have been proposed to explain the benefits of live yeasts utilization in animal nutrition. The mode of action of yeasts is typically related to lactate-utilizing bacteria in the rumen, increase in fiber digestion, and flow of microbial protein from the rumen which may be beneficial for feedlot cattle fed high-concentrate diets (Beauchemin et al. 2006). Additionally, yeasts may also supply stimulatory factors to ruminal bacteria in the rumen (Martin and Nisbet 1992). Among those factors, malic acid, can contribute to enhance growth and activity of lactate-utilizing bacteria than prevent abrupt changes in ruminal pH.

In this context, the current study was developed aiming to evaluate the effects of monensin or yeast supplementation on intake, nutrients digestibility, ruminal fermentation, and nitrogen balance of cattle fed high concentrate diets.

Material and methods

This experiment was carried out at the Universidade Federal de Viçosa, Brazil. Eight rumen-cannulated crossbred steers with an average body weight (BW) of 499 ± 50 kg were used. Initially, all animals submitted to an adaptation period to the diets prior to the beginning of the experiment. The adaptation period consisted of a 28-day step-up period, when concentrate was progressively increased.

The experimental design used was two simultaneous 4×4 Latin square. The experiment was divided into four periods of 17 days each, with 14 days for adaptation and 3 day for sampling.

The animals were allotted in a 4×2 factorial arrangement being divided in two groups of four animals and each group was fed diets containing one of the two levels of starch [23 and 38 % of dry matter (DM)]. Within each group, four treatments were assigned to the experimental animals. Treatments consisted of a control diet (no feed additives); inclusion of monensin (25 mg/kg of DM); and inclusion of

yeast (*Saccharomyces cerevisae* strain 1026) at levels of 1.0 (yeast, 1.0 g) or 2.5 g/100 kg of BW (yeast, 2.5 g).

The diets were fed ad libitum, three times a day, allowing up to 10 % of orts. From days 15 to 17, the diet and orts from each animal were sampled separately. At the end of each period, diet and ort samples were homogenized to obtain a composite sample per animal for each period. Total feces collection was performed from days 15 to 17 of each experimental period in order to determine digestibility coefficients of the diets.

On day 16, blood samples were collected from all animals, 4 h after feeding, into tubes containing separator gel and heparin. All samples were immediately centrifuged to obtain blood serum for further analyses of serum urea nitrogen (SUN).

On day 17, total urine was collected. For each animal, a 10-mL urine sample was diluted with 40 mL of sulfuric acid (0.036 N) to prevent bacterial degradation of purine derivatives and to allow uric acid precipitation.

In order to determine pH and ammonia nitrogen ($N-NH_3$) concentration, ruminal fluid were sampled on days 15 and 16 of each experimental period every 2 h after feeding. The evaluation of $N-NH_3$ concentration as described by Chaney and Marbach (1962).

Another sample of ruminal fluid was obtained just before feeding (0 h) and at 2, 4, 6, and 8 h after the first feeding for volatile fatty acids (VFA) analysis. Composite samples of diet, orts, and feces were assayed for DM, organic matter, crude protein (CP), and ether extract (EE), as described by AOAC (1990). Neutral detergent fiber (NDF) according Mertens (2002) and nitrogen (Licitra et al. 1996). Nonfiber carbohydrates were calculated according to Detmann and Valadares Filho (2010). Starch concentration was determined as described by Bach Knudsen (1997).

Urine samples were analyzed for allantoin and uric acid by a colorimetric method (Fujihara et al. 1987). The amount of absorbed microbial purines was calculated using the equations described by Orellana-Boero et al. (2001). The intestinal flow of microbial nitrogenous compounds was calculated according to the equation proposed by Chen and Gomes (1992).

Serum and urine samples were assayed for urea content using the modified diacetil method. Urine N content was determined using the Kjeldahl method.

The experiment was analyzed according to 4×4 Latin square design, with two simultaneous squares, in a factorial arrangement. Within each square, each additive was applied (control, monensin, yeast [1.0 or 2.5 g]). Diets with different starch levels were independently applied to each Latin square. Mean comparison were conducted using Fisher's least significant difference with 0.05 being considered the critical probability level (Table 1).

Table 1 Ingredients and chemical composition of experimental diets

Ingredients	Diets	
	Low starch	High starch
Feed composition (g/kg DM)		
Corn silage	202.5	203.6
Ground corn	290.6	551.8
Soybean meal	48.9	73.7
Whole cottonseed	130.6	131.4
Soybean hulls	281.6	–
Urea	4.5	7.1
Mineral supplement	32.2	32.4
Total	1,000.00	1,000.00
Chemical composition (g/kg DM)		
Dry matter	768.0	763.0
Organic matter	933.4	940.8
Crude protein	141.6	148.5
Ether extract	44.0	46.3
NDF[a]	365.5	214.0
Nonfibrous carbohydrates	364.1	543.9
Starch	232.6	381.8
Minerals	66.6	59.2

[a] Corrected for ash and protein

Results

No interactions of starch level and feed additives ($P>0.05$) were observed for any of the variables evaluated. Therefore, the main effects of feed additives and starch levels were discussed independently.

Yeast 2.5 g treatment had higher ruminal pH value when compared to yeast 1.0 g treatment, but did not differ ($P>0.05$) from control and monensin and ruminal pH values (Table 2).

Monensin and yeast 1.0 g increased ($P<0.05$) ruminal concentration of total VFA compared to control. However, supplementation with yeast 2.5 g resulted in lower ($P<0.05$) concentration of VFA when compared to supplementation with 1.0-g yeast monensin, but similar when compared to the control treatment (Table 2).

Lactic acid concentration did not differ ($P>0.05$) among treatments with yeast and control regardless the dose of yeast used. However, lactic acid concentration was negatively affected ($P<0.05$) by monensin supplementation when compared to the control and to the yeast-added treatments (Table 2).

Total VFA and lactic acid concentrations were lower ($P<0.05$) in the high-starch diet when compared to the low-starch diet (Table 2). Conversely, A/P was higher in the high starch diet ($P<0.05$).

Proportions of acetate and propionate did not differ ($P>0.05$) among feed additives treatments and neither did A/P. Surprisingly, ruminal molar proportion of acetate was higher and that of propionate was lower in the high-starch diet ($P<0.05$) than on the low starch diet (Table 2). Ruminal molar proportion of butyrate differed ($P<0.05$) between monensin and 2.5-g yeast treatments, where supplementation with monensin resulted in greater value (Table 2). However, both of them did not differ ($P>0.05$) from the value observed in the control treatment.

There was no effect ($P>0.05$) of feed additives on N-NH$_3$ ruminal concentration. However, differences were found ($P<0.05$) between the starch levels, where animals fed high-starch diet had the highest N-NH$_3$ ruminal concentration (Table 2). No effect was observed ($P>0.05$) to intake by feed additives (Table 3).

NDFap digestibility in the monensin and 2.5-g yeast treatments was greater ($P<0.05$; Table 4) than 1-g yeast treatments. However, the control treatment had intermediate NDFD value, which was similar ($P>0.05$) to those observed for treatments with feed additives.

Similarly, EE digestibility was affected ($P<0.05$) by the inclusion of feed additives (Table 4), being greater for the diet containing 2.5 g yeast than followed for diets with monensin and 1.0 g yeast, being the lowest EE digestibility value observed in the control treatment. No differences were found ($P>0.05$) for SUN among any of the treatments evaluated (Table 5).

Starch level did not affect ($P>0.05$) daily urinary excretion of N (Table 5). Similarly, no differences were found ($P>0.05$) among feed additive treatments for N urinary excretion (Table 5). However, feed additives inclusion affected ($P<0.05$) the urinary N-urea loses, where treatment with monensin had greater N-urea excretion than 1 g yeast.

Additionally, microbial N, microbial efficiency, and N balance also were also not affected ($P>0.05$) by any of starch levels and additives used (Table 5).

Discussion

The increase in ruminal pH as a result of monensin utilization occurred possibly due to a decrease on lactate producing bacteria, which could proliferate when monensin was not added to the diet (Russell and Strobel 1989). Additionally, monensin would modulate ruminal pH by controlling feed intake into more and smaller meals. On the other hand, yeast utilization on a higher dose would have stimulated the growth of lactate-utilizing bacteria, resulting in higher pH values.

The relatively high inclusion of concentrate in diets of this study would potentially reduce ruminal pH due to the fast degradation of starch leading to an increase in VFA and lactate production. However, the high-starch diets could have resulted in an increased passage rate, reducing the

Table 2 Ruminal parameters of steers fed different additives and starch levels in the diet

Item	Starch level[a]		Feed additive				SEM
	Low	High	Control	Monensin	1 g Yeast[b]	2.5 g Yeast[b]	
pH	6.44 a	6.52 a	6.48 ab	6.51 ab	6.40 b	6.53 a	0.06
Lactic acid, mmol/dL	3.67 a	3.17 b	3.52 a	3.15 b	3.46 a	3.55 a	0.11
Total VFA, mmol/dL	18.20 a	16.68 b	16.78 ab	18.17 a	18.19 a	16.56 b	0.69
Acetic acid, %	45.16 b	47.58 a	46.63 a	44.70 a	46.80 a	43.36 a	1.57
Propionic acid, %	40.93 a	38.31 b	39.51 a	40.00 a	39.05 a	39.91 a	1.29
Butyric acid, %	13.91 a	14.12 a	13.86 ab	15.30 a	14.15 ab	12.73 b	0.95
A/P[c]	1.24 b	1.51 a	1.39 a	1.31 a	1.40 a	1.41 a	0.12
Ammonia-N, mg/dL	14.91 b	18.08 a	15.88 a	16.64 a	17.05 a	16.42 a	1.48

Means in the same row with different superscripts differ ($P<0.05$)

Total VFA total volatile fatty acids

[a] Starch levels were 23 (low) and 38 % (high)

[b] Yeast fed at rates of 1.0 and 2.5 g/100 kg BW

[c] Acetato to propionate ratio

exposure of starch to ruminal microorganisms. This observation is supported by data presented in Table 2, which shows that low-starch and high-starch diets had no difference to concentration of total VFA. According to Britton and Stock (1987), the total amount of organic acids produced in the rumen could be responsible for some acidosis. This suggests that high VFA concentration can contribute to similar behavior on pH. The pH values observed in this study are close to the maximum values reported by Owens et al. (1997) as normal for high concentrate diets, which would be 5.5–6.5. This may have happened due to the step-up adaptation period established at the beginning of the experiment. In addition, the corn used in this study was coarsely ground, which could have avoided fast starch degradation by ruminal microorganisms, and thus avoiding ruminal disturbances.

The increase in ruminal VFA molar concentration due to monensin and 1.0 yeast inclusion may have occurred due to

an improvement in the ruminal environment either directly or indirectly, selecting desirable strains of rumen bacteria and reducing or eliminating the population of undesirable strains. These feed additives might have improved the ruminal microorganism's population resulting in a better fermentation of carbohydrates into VFA.

A higher concentration of total VFA and lactic acid and greater A/P in the low-starch diet seems contradictory. Additionally, this could also be related to the low degree of processing of the corn used in this experiment, which could have resulted in a higher passage of starch to the small intestine, which could result in decreased lactate and propionate production in the rumen. Owens (2007) suggested that low-processed corn could results in up to 50 % reduction in starch ruminal digestion when compared to more intensive processing methods due the physical barrier imposed by the protein matrix that impedes access to starch by microorganisms.

Table 3 Intake of steers fed different additives and starch levels in the diet

Item[a]	Starch level[b]		Feed additive				SEM
	Low	High	Controle	Monensin	1 g Yeast[c]	2.5 g Yeast[c]	
Dry matter	7.76 a	7.91 a	8.06 a	8.14 a	7.47 a	7.67 a	0.55
Organic matter	7.25 a	7.47 a	7.57 a	7.64 a	7.02 a	7.21 a	0.55
Crude protein	1.09 a	1.15 a	1.16 a	1.18 a	1.05 a	1.10 a	0.08
Ether extract	0.34 a	0.38 a	0.38 a	0.37 a	0.35 a	0.34 a	0.03
NDF[d]	2.85 a	1.56 b	2.27 a	2.41 a	2.17 a	2.17 a	0.15
NFC	2.78 b	4.01 a	3.61 a	3.53 a	3.07 a	3.37 a	0.30
Starch	1.62 b	2.77 a	2.33 a	2.28 ab	2.01 b	2.14 ab	0.17
TDN	5.50 a	5.56 a	5.63 a	5.86 a	5.15 a	5.50 a	0.50
Dry matter, g/kg BW	16.2 a	15.6 a	17.2 a	16.6 a	14.9 a	14.9 a	1.21

Means in the same row with different superscripts differ ($P<0.05$)

NFC nonfiber carbohydrates

[a] Expressed in kilograms

[b] Starch levels were 23 (low) and 38 % (high)

[c] Yeast fed at rates of 1.0 and 2.5 g/100 kg BW

[d] Corrected for ash and protein

Table 4 Total apparent digestibility coefficients on steers fed different additives and starch levels in the diet

Item[a]	Starch level[b]		Feed additive				SEM
	Low	High	Controle	Monensin	1 g Yeast[c]	2.5 g Yeast[c]	
Dry matter	0.728 a	0.720 a	0.706 a	0.728 a	0.728 a	0.734 a	0.027
Organic matter	0.741 a	0.725 a	0.720 a	0.739 a	0.738 a	0.744 a	0.028
Crude protein	0.696 a	0.696 a	0.680 a	0.706 a	0.694 a	0.704 a	0.028
Ether extract	0.781 a	0.775 a	0.699 c	0.781 b	0.779 b	0.851 a	0.029
NDF[d]	0.703 a	0.564 b	0.624 ab	0.658 a	0.585 b	0.666 a	0.029
NFC	0.773 a	0.785 a	0.772 a	0.775 a	0.801 a	0.767 a	0.036
Starch	0.819 a	0.852 a	0.846 a	0.837 a	0.816 a	0.841 a	0.045
TDN, g/kg DM	709.2 a	699.4 a	696.5 a	718.6 a	687.4 a	714.6 a	30.64

Means in the same row with different superscripts differ (*P*<0.05)

NFC nonfiber carbohydrates

[a] Expressed in grams per gram

[b] Starch levels were 23 (low) and 38 % (high)

[c] Yeast fed at rates of 1.0 and 2.5 g/100 kg BW

[d] Corrected for ash and protein

The reduction in lactic acid concentration in the rumen due to the inclusion of monensin in the diet might have being the result of ionophore inhibition of lactate producing bacteria (Russell and Strobel 1989). However, ruminal lactate concentrations observed in this study were much lower than those reported by Sutton el al. (2003) in studies with high-concentrate diets.

The lack of response to yeast inclusion on lactate concentration may be explained by the fact that this additive acts stimulating the growth of lactate-utilizing microorganisms which are resistant to low pH. Most lactate-utilizing bacteria are sensitive to low pH (Owens et al. 1997), which

suggests that in conditions where ruminal pH is not critically low, yeasts may not be as effective in stimulating those microorganisms. Therefore, the response of decreased lactate in the rumen as a result of yeast supplementation may be more substantial under lower pH conditions.

A possible reason for the lack of difference in the molar ratio of propionate among additives may be due to the fact that starch would have increased propionate in the rumen independently of the feed additives. According to Lana and Russell (1997), the increase in propionate concentrations are very low in high-concentrate diets. In the literature, it is commonly reported that monensin increases the molar

Table 5 Serum and urinary N, microbial synthesis, and N balance of steers fed different additives and starch levels in the diet

Item	Starch level[a]		Feed additive[b]				SEM
	Low	High	Control	Monensin	1 g Yeast[b]	2.5 g Yeast[b]	
Serum urea-N, mg/dL	14.74 a	15.97 a	15.81 a	16.27 a	14.53 a	14.79 a	1.19
Urinary urea-N[c]	75.23 a	71.79 a	69.04 b	85.94 a	62.41 b	76.64 ab	7.86
Urinary N[c]	89.21 a	85.62 a	82.82 a	91.21 a	86.38 a	89.42 a	8.66
Balance of N[c]	32.09 a	43.54 a	43.80 a	41.62 a	30.42 a	34.52 a	11.45
EfN[d]	0.179 a	0.233 a	0.232 a	0.222 a	0.185 a	0.186 a	0.058
Microbial N[c]	95.62 a	103.89 a	96.35 a	101.29 a	100.25 a	101.13 a	10.77
Microbial efficiency[e]	110.05 a	118.69 a	108.16 a	108.03 a	123.9 a	117.38 a	9.66

Means in the same row with different superscripts differ (*P*<0.05)

[a] Starch levels were 23 (low) and 38 % (high)

[b] Yeast at rates of 1.0 and 2.5 g/100 kg BW

[c] Expressed in grams per day

[e] Grams of MCP per kilogram DTN

[d] Retained N/intake of N

proportion of propionate. According to Russell and Strobel (1989), the resistance of propionate-producing bacteria to ionophore is related to the presence of an external membrane in gram-negative bacteria, which acts as a protecting layer to the access of ionophores to the cellular membrane.

Felix et al. (2012) in order to evaluate inclusion of momensin in feedlot diets found no effects on the rumen fermentation pattern. Likewise, Chung et al. (2011) evaluating the risk of subacute acidosis in dairy cows fed with diet supplemented with strains of *S. cerevisiae* reported that the commercial Yeast strain had no major effects on none of the ruminal fermentation characteristics evaluated in their study. However, no commercial yeast strain increased the propionate concentration and decreased A/P. The use of commercial yeast strain may justify no improvement in ruminal fermentation characteristics in this study.

Although the production of butyrate is rarely manipulated (Owens et al. 1997), changes in the molar proportion of the others VFA can alter butyrate molar proportion as well. Monensin could have reduced the ammonia-N concentration in the ruminal fluid by decreasing the peptide and amino acid degradation as monensin inhibits growth of hyperammonia producing bacteria. However, as described previously, some gram-positive bacteria may be resistant to monensin which was also observed by Krause and Russell (1996) who found no effects of monensin on *Clostridium aminphilum* which is a gram-positive bacterium that contributes to amino acid degradation in the rumen.

Arambel and Kent (1990) suggested that yeast products might be more effective under stress (diet transition period), rather than in normal conditions as in the current study in which the steers were adapted to high-grain diets.

The effect on EE and NDF digestibility to additives supplementation was not low enough to reduce the amount of digestible nutrients available to the animals from those treatments. Similarly, Chung et al. (2011) did not observe effect of yeast supplementation for apparent total-tract digestibility of nutrient, except to CP digestibility which was lower in cows yeast supplemented.

N concentration in the diets, CP intake, and CPD were similar among treatments no influence was observed on SUN. Santos et al. (2001) suggested that greater protein degradation in the rumen increases ruminal ammonia and, consequently, SUN concentration and N excretion in the urine. Ruminal ammonia concentration did not differ among treatments with or without feed additives and consequently no differences were observed for urinary N losses.

The analyses of a Brazilian data set suggested that under tropical conditions, 120 g/kg of TDN would be more reliable (Valadares Filho et al. 2006). The average value obtained in the current study for microbial protein yield (114.37 g/kg TDN) was close to this data.

Conclusions

Monensin and yeast supplementation is not recommended to improve performance of beef cattle fed high concentrate diets as no significant changes in metabolic and digestive parameters was observed.

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