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Evaluation of mannan-oligosaccharides offered in milk replacers or calf starters and their effect on performance and rumen development of dairy calves

Jackeline Thaís da Silva[1], Carla Maris Machado Bittar[2], Lucas Silveira Ferreira[1]

[1] Programa de Pós-Graduação em Ciência Animal e Pastagens. Departamento de Zootecnia - Universidade de São Paulo - USP/ESALQ.
[2] Departamento de Zootecnia - Universidade de São Paulo - USP/ESALQ, Avenida Pádua Dias, 11 - 13418-900. Piracicaba - SP, Brazil.

ABSTRACT - The objective of this study was to evaluate the route of administration of mannan-oligosaccharides in the diet of dairy calves and their effects on performance and plasma parameters indicative of rumen development. Following birth, twenty-four male Holstein calves were used in a completely randomized design and assigned to the following treatments: Control; 4 g/d Bio-Mos® (Alltech Biotech.) added to starter concentrate; and 4 g/d Bio-Mos® mixed into milk replacer. Animals were housed in individual hutches with free access to water, and fed 4L/d of milk replacer until weaning at six weeks. Calves also received 23g/kg crude protein of starter concentrate *ad libitum*. Fecal scores were evaluated daily. Body weights, growth measurements and blood samples for glucose, urea-N and β-hidroxibutyrate analyses were taken weekly until 8 weeks of age. There were no significant effects of treatment or treatment × age interactions for mean starter concentrate intake, weight gain or body growth. However, there was a significant age effect for all parameters. Fecal scores were not affected by treatments. Also, plasma concentration of glucose, urea-N or β-hidroxibutyrate were not affected by treatment or the treatment × age interaction. However, urea-N and β-hidroxibutyrate concentrations significantly increased with age, suggesting adequate rumen development. Under the conditions of this study, there were no calf performance benefits when mannan-oligosaccharides were incorporated into milk replacer or calf starter concentrate.

Key Words: additives, blood parameters, early weaning, fecal score

Introduction

Diarrhea is the main disease affecting dairy calves during the pre-weaning period, and is responsible for low levels of animal performance and increases in the final cost of the weaned calf. Thus, the use of antibiotics in the diet of calves, in order to improve feed efficiency and prevent diseases, especially diarrhea, has become a common practice. However, overuse of antibiotics may lead to the emergence of resistant bacteria. In spite of that, the European Union banned the subtherapeutic use of antibiotics in animal production systems (1831/2003 CES). Since then, various additives that may improve the efficiency of animal production have been proposed as alternatives to antibiotic growth promoters.

Mannan-oligosaccharides may be an alternative additive, consisting of non-fermentable carbohydrates, mainly D-mannose, not fermentable by some groups of bacteria, acting by blocking the site of adherence of enteric bacteria (Heinrichs et al., 2003). Mannan-oligosaccharides are obtained by centrifugation of fragments of *Saccharomyces cerevisiae* (Hill et al., 2009), which are then washed and spray-dried (Spring et al., 2000).

Mannan-oligosaccharides have been used as additives in animal production, particularly poultry and pigs, and have improved animal performance and fecal scores (Spring et al., 2000; Castillo et al., 2008). However, incorporating Mannan-oligosaccharides into milk replacers fed to dairy calves has shown conflicting results. Dildey et al. (1997) observed good results in performance, fecal score and reduction in mortality rate of dairy calves fed mannan-oligosaccharides. In contrast, Hill et al. (2009), in a recent study, concluded that Holstein calves do not respond to their inclusion in the milk replacer in regard to body growth, starter concentrate intake, rumen development and health parameters.

Even though the inclusion of mannan-oligosaccharides in the diet for some animal species has shown positive performance effects, the effects on the influence on dairy calves performance is inconsistent and not well characterized. The primary route of administration in calves described in the literature is based solely on a milk replacer.

Thus, the objective of this study was to evaluate the effects of mannan-oligosaccharides on the performance and blood parameters indicative of rumen development,

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Corresponding author: carlabittar@usp.br

and the route of administration (milk replacer or starter concentrate) for dairy calves.

Material and Methods

Twenty-four Holstein bull calves from commercial farms were utilized in a completely randomized experimental design. The trial was conducted at the Department of Animal Science of Escola Superior de Agricultura "Luiz de Queiroz" - USP / ESALQ, Piracicaba, SP, Brazil. After birth, calves were separated from their respective dams and received colostrum according to the management of the farm of origin.

After being transported to the University, calves were housed in individual hutches and immediately fed 4L/d milk replacer (Nattimilk®, Auster Animal Nutrition, 225.0 g/kg crude protein - CP, 185.0 g/kg ether extract - EE) split into two equal feedings (07h00 and 16h00); starter feed and water *ad libitum*. Calves were assigned to the following treatments: Control, 4 g/d Bio-Mos® (Alltech Biotech.) top dressed onto the starter concentrate; and 4 g/d Bio-Mos® mixed into the milk replacer at every feeding.

A commercial pelleted starter, formulated to meet the requirements of these animals was offered daily in individual buckets, after removal of orts, which were weighted daily.

Weaning was performed abruptly at the sixth week of age and after weaning, coast cross hay (*Cynodon dactylon* (L.) pers.) was offered *ad libitum*, in individual buckets. Samples of starting feed, milk replacer and hay were taken during the experimental period for determination of DM and EE, according to Campos et al. (2002); crude protein (CP), according to Dumas combustion using a nitrogen analyzer model FP-528 (Leco Corporation, St. Joseph, MI, USA); and neutral detergent fiber (NDF), by the method described by Van Soest et al. (1991). The values of total digestible nutrients (TDN) were calculated according to the equations proposed by Kearl (1982) for each type of feed (Table 1).

Calves were weighed weekly prior to morning feeding, until 8 weeks of age at the conclusion of the study. Weekly hip widths and withers heights were also documented with a ruler graduated in centimeters, and hearth girth with a flexible tape, also graduated in centimeters.

Fecal score was visually monitored daily using the method described by Larson et al. (1977), regarding the fluidity of feces. Feces were classified as normal (1) soft (2) loose (3), watery (4) or liquid consistency (5). Blood samples were collected weekly from the second week of life, always two hours after morning feeding, via jugular venipuncture using vacuum tubes containing sodium fluoride and potassium ethylenediamine tetraacetic acid (EDTA) for determination of plasma glucose through direct reading on biochemical YSI 2700 autoanalyzer - (Biochemistry Analyzer, Yellow Spring, OH, USA); β-hydroxybutyrate (BHBA) using biochemical assays by testing Ranbut (Randox Laboratories Ltd.) and urea nitrogen using the method described by Chaney & Marbach (1962), adapted for reading on Microplate Reader (BIO-RAD, Hercules, CA, USA) using filter absorbance of 550 nm.

The data on intake, average daily gain, measures of body growth and blood parameters were analyzed as repeated measures using the PROC MIXED of the SAS, by the model:

$Y_{ijk} = \mu + T_i + A_{ij} + I_k + TI_{ik} + E_{ijk}$,

where: Y_{ijk} = dependent variable; μ = general constant; T_i = treatment effect; A_{ij}= random animal effect (error term to treatment); I_k = age effect; TI_{ik} = treatment × age interaction effect; E_{ijk} = random error.

The best covariance structure was identified from different covariance structures by comparing the AICC statistic (Akaike Information Criteria Corrected). Because ARMA1.1 presented the best convergence information criteria, it was chosen as the covariance structure to analyze all data. Differences were considered significant at $P<0.05$ unless otherwise stated.

Fecal scores were analyzed by the nonparametric test using the Proc PAR1WAY of SAS software (Statistical Analysis System, version 5). Treatment comparisons were done by the Wilcoxon score and Kruskal-Wallis tests.

Table 1 - Chemical composition of starter concentrate, milk replacer, mannan-oligosaccharides (MOS) and coast-cross hay

	Starter	Milk replacer	MOS	Hay
Dry matter, g/kg	899.9	942.9	938.1	901.1
Crude protein, g/kg	232.5	225.5	387.8	141.6
Ether extract, g/kg	72.3	194.8	0.8	13.3
Crude fiber, g/kg	96.9	5.4	5.6	299.0
Neutral detergent fiber, g/kg	216.2	3.3	..	667.3
Total digestible nutrients, g/kg	789.1	850.5	808.2	528.5
Gross energy, Mcal/kg	3932.2	4579.1	3771.8	3658.2

Results and Discussion

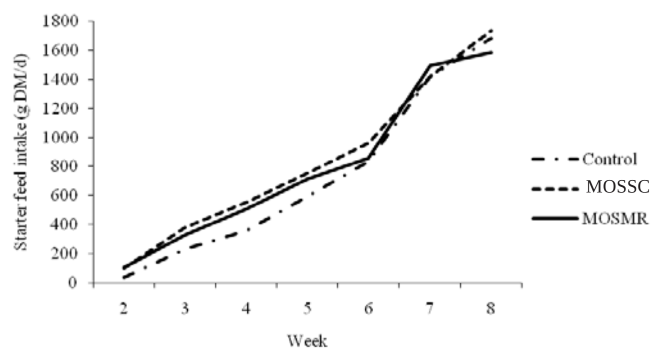
Starter diet intake was not affected by treatments (P>0.05) and no interaction of treatment and age was detected (P>0.05) (Table 2). However, significant effects of age (P<0.01) were observed, with increases in the starter intake with advancing age.

The absence of significant effects for the addition of mannan-oligosaccharides in the diet of calves during the pre-weaning period was also observed in other studies (Sandi & Mülbach, 2001; Hill et al., 2009). However, these authors observed values numerically higher for starter intake for the calves supplemented with mannan-oligosaccharides, likewise in the present study. Heinrichs et al. (2003) observed a significant effect (P<0.05) in the sixth week of age, with higher starter intake regarding the initial group of animals that received the additive, when compared with the control diet.

In other studies, the provisions of mannan-oligosaccharides during the pre-weaning period was done only with the inclusion of this additive in milk replacer. However, in this study, it was observed that the administration of mannan-oligosaccharides added to starter showed results similar to the administration by milk replacer, indicating the feasibility of this route of delivery. Although no significant difference was observed for the average of total the period of starter intake, the mannan-oligosaccharides added to starter group was higher than regarding other treatments, especially when compared with the control group (Figure 1). After weaning, the similarity observed can be explained by the limited supply of original starter (2 kg/d).

The average starter intake at weaning was higher than recommended in early weaning systems that adopt the minimum consumption. In this system, it is recommended to complete the weaning of the animals when consumption reaches 680 to 700 g/d for 3 consecutive days, to allow good animal performance (Quigley, 1996). Thus, according to this system, weaning could have been performed on the fifth week of age (Figure 1) without jeopardizing the performance, and in order to reduce the final cost of weaned calves. It was also observed that animals fed starters containing mannan-oligosaccharides, regardless of route of delivery, showed consumption of the starter suitable for weaning at younger age than animals fed control starter concentrate.

For the parameters live weight (kg) and average daily gain (g/d) there were no significant differences among treatments or treatment × age interaction. However, significant (P<0.05) age effect (weeks) was observed. The results presented in the literature are controversial; Heinrichs et al. (2003) found no significant difference concerning treatments for these two parameters, while Sandi & Mülbach (2001) observed differences. Dildey et al. (1997) observed higher final live weight (8 weeks) for animals supplemented with mannan-oligosaccharides, and a significant difference concerning treatments for daily gain in animals fed mannan-oligosaccharides mixed into milk replacer.



MOSSC - MOS added to starter concentrate; MOSMR - MOS mixed into milk replacer.

Figure 1 - Starter concentrate intake (g), according to week of age, by calves receiving mannan-oligosaccharides added to starter concentrate or mixed into milk replacer.

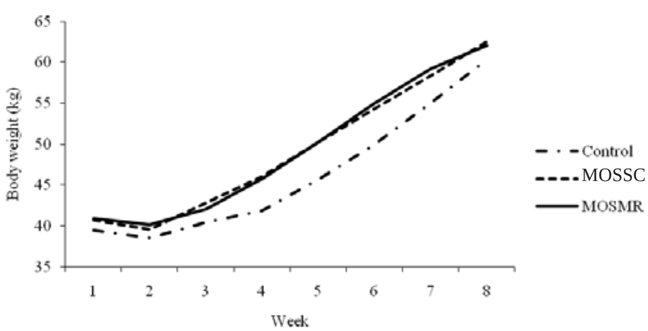
Table 2 - Least-square means of starter intake, live weight, and average daily gain by calves receiving mannan-oligosaccharides (MOS) added to starter concentrate or mixed into milk replacer

	Treatment			SEM	P Value		
	Control	MOSSC	MOSMR		T	A	T × A
Starter intake, g/d							
At weaning	835.97	961.98	856.57	92.96	..	..	..
Average	737.41	842.61	798.64	45.12	0.34	<0.01	0.94
Live weight, kg							
Initial	39.51	40.68	40.89	1.74	..	..	..
Final	60.35	62.58	62.07	1.71	..	..	..
Average	46.43	49.33	49.41	0.92	0.21	<0.01	0.91
Average daily gain, g/d	352.2	411.3	409.6	42.82	0.67	<0.01	0.22

MOSSC - MOS added to starter concentrate; MOSMR - MOS mixed into milk replacer; SEM - standard error of the mean; T - treatment effect; A - age (week) effect; T × A - treatment and age interaction effect.

The final weight values observed in the study of Dildey et al. (1997) are similar to values observed in the final weight between control and Mannan-oligosaccharides mixed milk replacer treatments, however, with greater average daily gain in the current study. Data on the performance of calves receiving mannan-oligosaccharides added to starter diet were not found in the literature. However, in studies regarding pigs, supplementation with mannan-oligosaccharides results in higher average daily gain (Rozeboom et al., 2005; Davis et al., 2002).

In general, rates of weight gain observed in this study are consistent with literature data (Hoffman, 1997;Heinrichs & Losinger, 1998; Kertz & Chester-Jones, 2004), and are the result of an adequate starter intake and milk replacer with high quality. The live weight of calves, regardless of the treatment, performed steadily increasing with advancing age (Figure 2). However, between the first and second weeks of age, a slight decline can be observed in the values of live weight in all treatments. This weight loss occurred in the period when the calves showed a higher frequency of diarrhea, and is common in most production systems.



MOSSC - MOS added to starter concentrate; MOSMR - MOS mixed into milk replacer.

Figure 2 - Body weight (kg), according to week of age, regarding calves receiving mannan-oligosaccharides (MOS) added to starter concentrate or mixed into milk replacer.

There were no significant differences (P>0.05) between treatments and treatment × age interactions with parameters withers height, heart girth and hip width (Table 3). Although the average of total period for the heart girth has not presented differences between treatments, was observed tendency (P = 0.09) of control group animals to present lower value for this parameter. As shown by Heinrichs et al. (1992), the hearth girth has a high ratio with a live weight. The lowest hearth girth accompanies the lowest value of average live weight of animals in the control group. However, as expected for growing calves, a significant effect of age (P<0.05) was observed, with increases in body measurements over the weeks.

Wither height gain/week during the study was below the recommended by Hoffman (1997), who indicated 1.2 to 1.4 cm/week, as average for Holstein calves. Heinrichs et al. (2003) showed gain/week values for wither height higher than those observed in this study for calves of similar age and supplemented with mannan-oligosaccharides. Similarly, Lesmeister et al. (2004) observed a higher gain per week in calves supplemented with various additives, in spite of the lower values for heart girth and hip width. Hill et al. (2009) also found values for wither height similar to those observed in this study for testing mannan-oligosaccharides in milk replacer. Differences between experiments may be the result of selection or variation of the animal type, so that, even from the same breed, there are differences in adult weight and hence the earnings of skeletal growth.

After weaning, the growth rate of the control group for the withers height decreased, with similar values in the following weeks at weaning. Moreover, calves supplemented with mannan-oligosaccharides had growth pace similar to the pre-weaning period. There was also a decrease in this parameter in the first two weeks that were associated with the most critical phase for animals in the pre-weaning period in which animals have diarrhea, and consequently lose weight.

Table 3 - Least-square means of withers height, heart girth and hip width of calves receiving mannan-oligosaccharides (MOS) added to starter concentrate or mixed into milk replacer

	Treatment			SEM	P Value		
	Control	MOSSC	MOSMR		T	A	T × A
Withers height, cm							
Average	78.74	79.23	79.24	0.25	0.32	<0.01	0.59
Gain, cm/wk	0.75	1.04	1.13	0.19	0.37	0.27	0.12
Heart girth, cm							
Average	82.62	83.55	83.76	0.37	0.09	<0.01	1.00
Gain, cm/wk	1.63	1.74	1.75	0.23	0.92	<0.01	0.42
Hip width, cm							
Average	22.63	22.00	22.30	0.57	0.74	<0.01	0.61
Gain, cm/wk	0.43	0.40	0.36	0.11	0.92	0.30	0.93

MOSSC - MOS added to starter concentrate; MOSMR - MOS mixed into milk replacer; SEM - standard error of the mean; T - treatment effect; A - age (week) effect; T × A - treatment and age interaction effect.

The literature considers that the calf has diarrhea when fecal score is above 3 (Larson et al., 1977). Most of the fecal score data observed throughout the experimental period, of approximately 80%, was below 2, confirming good management during the experimental period (Table 4).

Significant difference (P<0.05) between treatments were found only on week 1, with higher values for animals of the control group, followed by mannan-oligosaccharides added to starter concentrate and mannan-oligosaccharides mixed into milk replacer. However, this type of analysis of nonparametric data does not identify differences between the three treatments. Dildey et al. (1997) also observed differences between treatments, although not significant, for calves supplemented with mannan-oligosaccharides. Similarly, Heinrichs et al. (2003) observed a higher probability of normal feces in calves supplemented with mannan-oligosaccharides or antibiotics, than in calves without these additives. However, several studies on the effect of mannan-oligosaccharides in calves did not confirm improvements in fecal score for animals supplemented with mannan-oligosaccharides (Newman et al., 1993; Donovan et al., 2002). No results were found in the literature about fecal score of calves fed mannan-oligosaccharides added to the starter concentrate.

Table 4 - Fecal score of calves receiving mannan-oligosaccharides (MOS) added to starter concentrate or mixed into milk replacer

Weeks	Treatments			P Value[1]
	Control	MOSSC	MOSMR	
1	1.97	1.41	1.02	0.04
2	3.40	3.34	2.91	0.62
3	1.55	1.76	1.73	0.84
4	1.36	1.23	1.01	0.36
5	1.24	1.19	1.07	0.47
6	1.00	1.00	1.00	1.00
7	1.04	1.00	1.00	0.33
8	1.16	1.00	1.02	0.37

MOSSC - MOS added to starter concentrate; MOSMR - MOS mixed into milk replacer.
[1]P<0.05.

In this study, scores indicating diarrhea were observed only on the second week of life in all treatments. According to Lucci (1989) occurrence of diarrhea is expected during the first weeks of life of the calf, especially diarrhea caused by bacteria such as *Salmonella* spp. and *Escherichia coli*. However, it was also observed that pigs fed mannan-oligosaccharides mixed into milk replacer had lower fecal score, although this effect was not significantly different. According to Spring et al. (2000), enterobacteria are adsorbed by mannan-oligosaccharides in the digestive tract, thus preventing the colonization of the gut, and consequently decreasing the incidence of diarrhea. Thus, the difference between the fecal scores observed in this study, though only numerically, can be associated with the treatment.

The quantity of additive supplied was the same for both treatments with addition of mannan-oligosaccharides. However, calves in the mannan-oligosaccharides mixed into milk replacer treatment received 4 g/day from the first delivery of milk replacer, while the animals fed mannan-oligosaccharides added to starter concentrate had additive dependent on starter diet intake, which, on the first week, as expected, is low. Furthermore, it was observed that after weaning the calves remained with the score close to 1, with an indication of health in calves. According Radostits (1975), diarrhea is the main clinical sign of dysfunction of the digestive tract.

The inclusion of mannan-oligosaccharides in the diet did not significantly affect (P>0.05) the plasma glucose concentrations in calves until the eighth week of life (Table 5). Similarly, there was no significant effect (P>0.05) for the treatment × age interaction. However, significant effect was observed (P<0.0001) with aging.

Data of plasma glucose concentrations in calves fed a diet with the addition of mannan-oligosaccharides were not found in the literature. Studies with other additives provided data for this parameter similar to that observed in this study (Quigley et al., 1994). Nevertheless, other authors had lower values of plasma glucose concentration and decreasing values during the first weeks (Nussio et al., 2003;

Table 5 - Least-square means of plasma concentrations of glucose, urea-N (PUN) and β-hydroxybutyrate (BHBA) of calves receiving mannan-oligosaccharides (MOS) added to starter concentrate or mixed into milk replacer

	Treatment			SEM	P Value		
	Control	MOSSC	MOSMR		T	A	T × A
Glucose, mg/dL	81.46	82.69	86.67	2.45	0.30	<0.01	0.14
PUN, mg/dL	13.74	14.59	14.00	0.63	0.51	0.01	0.64
BHBA mmol/L	0.203	0.177	0.163	0.16	0.18	0.01	0.25

MOSSC - MOS added to starter concentrate; MOSMR - MOS mixed into milk replacer; SEM - standard error of mean; T - treatment effect; A - age (week) effect; T × A - treatment and age interaction effect.

Klotz & Heitmann et al., 2006). However, the averages (Figure 3) are within the recommended rates in the literature and indicate plasma concentrations between 90 and 100 mg/dL until six weeks of life in calves during the pre-weaning period (Huber, 1969).

Plasma glucose concentrations increased until the 5th week of the study then decreased (Figure 3). Quigley & Bernard (1992) observed plasma glucose concentrations similar to this study in calves receiving other additives. According to Huber (1969), glucose concentration increases during the first few weeks of life by the consumption of a diet containing lactose and rapid declines after 4 weeks due to reduced secretion of lactase in the intestine. This decline in plasma glucose concentration during the pre-weaning period may also be indicative of transition from non-ruminant to ruminant functional (Attebery & Colvin Jr., 1963).

Plasma concentrations of urea nitrogen did not differ in relation to treatment (P>0.05) in pre or post-weaning periods. The interaction treatment × age also showed no significant

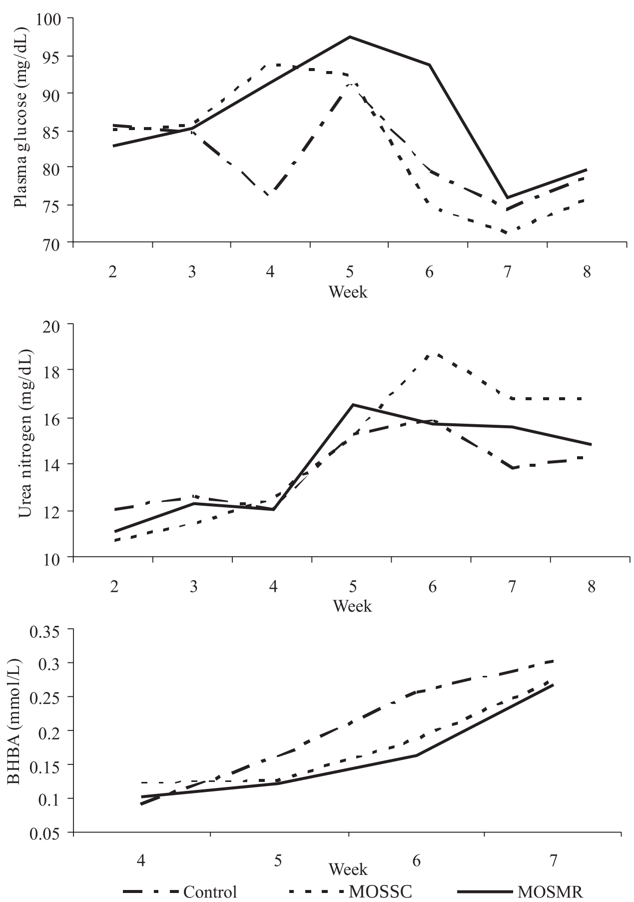


Figure 3 - Plasma glucose, urea nitrogen, and b-hydroxybutyrate (BHBA) concentration, according to week of age, by calves receiving mannan-oligosaccharides added to to starter concentrate (MOSSC) or mixed into milk replacer (MOSMR).

difference (P>0.05). However, significant effects (P<0.05) for aging were observed, with increasing concentrations throughout the experimental period (Figure 3). Heinrichs et al. (2003) observed values of plasma urea nitrogen lower than those observed in this study in calves supplemented with mannan-oligosaccharides during the pre-weaning period. Similarly, studies with different additives showed lower urea nitrogen (Nussio et al, 2003; Quigley & Bernard, 1992). Several studies have shown increasing values of urea nitrogen over the weeks (Quigley & Bernard, 1992, Quigley et al., 1994). Plasma concentrations of urea nitrogen are strongly related to the consumption of starting feed and the beginning of fermentation in the rumen, indicating extensive ruminal degradation of protein and carbohydrates of the diet (Quigley & Bernard, 1992). Thus, the increase in the concentration of urea nitrogen observed over the weeks is indicative of rumen development (Figure 3).

Analysis of plasma β-hydroxybutyrate was performed only after the 4^{th} week of age, as it is around this time that the values begin to be altered in response to the development of the rumen due to the consumption of starter concentrate. According to Quigley et al. (1991) concentrations of β-hydroxybutyrate have high relationship with the intake of the starting feed. Plasma concentrations of β-hydroxybutyrate had no effect of treatment (P>0.05) on pre or post-weaning periods. However, increases in plasma concentrations with age (P<0.0001) were observed, regardless of the treatment (Figure 3). The averages of the experimental period are shown above the average observed in other calf studies with similar age (Coverdale et al., 2004; Greenwood et al., 1997). Magalhães et al. (2008) observed increase in β-hydroxybutyrate concentrations in calves during weeks receiving a yeast culture. Similarly, other studies showed concentrations of β-hydroxybutyrate increased in calves receiving different diets in the pre-weaning period (Quigley & Bernard, 1992; Greenwood et al. 1997; Coverdale et al., 2004). Nevertheless, there are no data on dairy calves receiving mannan-oligosaccharides.

Conclusions

There are no beneficial effects of incorporating Mannan-oligosaccharides in a calf starter concentrate or milk replacer on performance of dairy calves or rumen development.

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