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Effect of *Saccharomyces cerevisiae* and Mannan-Oligosaccharides on Daily Weight Gain and Health of Pre-Weaned Holstein Calves in Chile

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Abstract: The objective was to compare the effect of *S. cerevisiae* and mannan-oligosaccharides on Average Daily Gain (ADG), live weight and health of dairy calves in Chile. Calves were weighed at birth and randomly assigned to 3 groups: (i) Yeast (YS): 5 g/calf/day of a live yeast (n = 25); (ii) Mannan (MN): 3 g/calf/day of mannan-oligosaccharides (n = 25); (iii) Control (CON): no additives (n = 25). Products were offered from the second day of life in the milk replacer during the morning. A blood sample was collected between 2 to 8 days of age to monitor passive immune transfer. Calves were fed a milk replacer (21% crude protein, 17% fat, 14.3% dilution), concentrate (21% crude protein, 2.5% fat) and water free choice until weaning. Diarrhea and pneumonia events were recorded. ADG were analyzed by ANOVA, live weight at 0, 30 and 60 days old by ANOVA for repeated measures and disease incidence by chi-squared fisher exact test. ADG were statistically different between YS (0.63 kg/d) and CON (0.56 kg/d) ($p \leq 0.05$). No differences were found between MN and CON ($p > 0.05$). The incidence of digestive disorders was 22.7, 23.5 and 14.2% for CON, MN and YS, respectively. This difference was statistically significant ($p \leq 0.05$) between YS vs. MN and YS vs. CON, respectively. It is concluded the supplementation with lived yeast improved ADG until weaning and decreased digestive disorders in dairy calves under Chilean conditions.

Keywords: Dairy Calves, Live Yeast, Mannan-Oligosaccharides, *Saccharomyces cerevisiae*

Introduction

Saccharomyces cerevisiae is a yeast that have been used as an additive in adult and young cattle demonstrating a positive effect on Dry Matter Intake (DMI), rumen pH and nutrient digestibility (Cole *et al*., 1992; Dann *et al*., 2000). Some studies have shown that yeasts stimulate growth of rumen cellulolytic bacteria (Callaway and Martin, 1997), which might be beneficial for carbohydrate digestion and rumen development in newborn calves. Other studies have demonstrated improvements in calf growth when yeasts were incorporated into the diet (Lesmeister *et al.*, 2004; Galvão *et al*., 2005). Furthermore, yeast culture has increased Feed Efficiency (FE), rumen pH, total ruminal volatile fatty acid concentration and ruminal butyrate and acetate production when included in calf diets (Quigley *et al.*, 1992).

The cell wall of *S. cerevisiae* contains oligosaccharides in the form of glucan and mannan which have been shown to modulate the immune system and to impact host-pathogen interactions in the digestive tract (Magalhães *et al.*, 2008). This may benefit young calves, as they are usually challenged by several pathogens such as bacteria, viruses and protozoa. Indeed, *S. cerevisiae* fed with concentrate reduced the number of days with diarrhea (Galvão *et al.*, 2005) and reduced the incidence of fever and the number of antibiotic treatments from birth to 46 days old (Seymour *et al.*, 1995). In addition, yeast culture tested *in vitro* has been shown to inhibit bacteria growth (Jensen *et al.*, 2008)

and modulate the immune system (Jensen *et al.*, 2007). The use of yeast culture based on *S. cerevisiae* and products based on glucan and mannan have not been studied under Chilean conditions; consequently the objective of this field trial was to compare the effect of *S. cerevisiae* and β-glucan-mannan based product on daily weight gain, live weight and health of young calves in a Chilean dairy farm.

Materials and Methods

Study Farm

The study was conducted in a dairy farm located at the central area of Chile (33°28' S; 70°56' W) which is characterized by Mediterranean climate (390 mm of rainfall) and a temperature-humidity index of 57 to 70 (Arias and Mader, 2010).

The farm consisted of 1,100 lactating cows, milked three times a day and producing 10,000 kg of milk per lactation (Mature Equivalent 305 days). Cows were fed 3 times per day a total mixed ration based on alfalfa hay, corn silage and concentrate to meet or exceed nutritional requirements of NRC (2001).

Cows were dried-off at 7 month of gestation and moved to a prepartum lot 30 days before expected parturition. Near parturition or at any sign of calving cows were moved to an individual maternity lot bedded with wheat straw. During calving, cows were monitored by farm personnel and assistance was provided as needed. At birth, calves were immediately removed from the dam, placed in a transition hutch and fed 3 liters of high quality thawed natural colostrum (IgG>70 g/L) within the first 2 h of life and another 2 liters within the next 2 h of life (IgG>50 g/L). Colostrum was collected individually from each dam within 3 h after parturition and evaluated through the use of a colostrometer (Biogenics, Florence, OR, USA). Any colostrum visually normal with an IgG content > 50 g/L was individually stored, labeled and frozen at -20°C in 2 liters plastic containers, within 2 h after collection. The colostrum was not pasteurized. Each calf was fed colostrum from a different dam. Calves were provided with colostrum by bottle fed. If they did not voluntarily consume, they were fed by a plastic esophageal tube. After colostrum was fed calves were moved to a permanent individual metal hutches bedded with wheat straw.

Experimental Design

In order to determine a difference of 35 grams on daily weight gain per calf per day from birth to weaning (600 Vs. 635 g/day) (SD = 49 g) between a control and a treatment group, with a 95% of confidence and 80% of power, a sample size of 25 female calves per group was calculated (SAS, 2003).

Calves were weighed at birth using an electronic scale and were randomly assigned into one out of 3 treatment groups. Experimental groups were: (i) Yeast group (YS): Receiving 5 g/calf/day of a live yeast culture of *Saccharomyces cerevisiae* (10×10^9 live cells) diluted in the milk replacer during the morning feeding (Procreatin-7 ®, Lesaffre feed additives, France); (ii) MannaOligo group (MN): Receiving 3 g/calf/day of manna-oligosaccharides (concentrate of yeast hulls) diluted in the milk replacer during the morning feeding (Safmannan ®, Lesaffre feed additives, France) and (iii) Control group (CON): Receiving no additives. Milk replacer was prepared consistently with water at 50°C and fed at 38-39°C in plastic containers of 5 liters of capacity.

Each calf was identified with an ear tag and processed according to the farm standard protocol. Any calf that showed any anatomic or behavioral abnormality or was born from a severe dystocia was removed from the assignment. A blood sample was collected once a week between 1 and 8 days of age (day 0: day of birth) (Godden, 2008) to follow the normal routine of the farm and serum total proteins were measured by a clinical refractometer (Master-SUR/Nα; ATAGO USA, Inc., Bellevue, Washington) to monitor passive immune transfer. Calves were fed a milk replacer immediately the following day after birth (21% crude protein, 17% fat; Fokkamel plus ®, Olen, Belgium) diluted at a concentration of 14.3% (1 kg of milk replacer diluted with 7 liters of water), 2 liters, twice a day (8:00 am and 5:00 pm) until 15 days old and then, 3 liters twice a day until 49 days old. From that age, calves were fed only 3 liters of milk replacer during the morning until 56 days old. Consequently animals were handled homogeneously during the first 24 h of life and then calves were assigned randomly to one of the 3 groups, receiving the experimental product only in the milk replacer from day 1 until weaning, except the control group, which received no additives. After 56 days old, calves received no milk and were fed only concentrate (starter) and water free choice until a minimum of 2 kg of starter was consumed. Starter was pelleted (21% crude protein, 2.5% fat, 11% crude fiber, Suralim ®, IANSAGRO, Chile) and was offered with water free choice from 3 days old until weaning.

Calves were weighed on an electronic scale at 0, 30 and 60 days old before feeding and daily weight gains were calculated. During the entire study diarrhea and pneumonia events, their duration, severity and treatment were recorded. Animals were evaluated visually in a daily basis. If the calf showed any abnormal behavior a more detailed physical examination was conducted (rectal temperature, respiratory auscultation and heart auscultation). Diarrhea was defined as loose manure but enough consistency to remain on bedding or watery feces with or without presence of blood, with dehydration > 4% and/or depressed attitude (McGuirk, 2008). Respiratory disease was defined as any calf with depressed appetite, dull attitude, high respiratory rate,

nasal and ocular discharges, coughing, abnormal respiratory sounds at auscultation and fever (rectal temperature > 39.8°C) (McGuirk, 2008).

Statistical Analysis

Daily weight gain from birth to weaning was analyzed by ANOVA constructing a mixed model considering the calf as random effect nested within treatment group. Explanatory variables were effect of treatment, parity number of the dam and serum total protein concentration of calves.

The model was defined as follow:

$$Y_{ijklmn} = \mu + Ti + Par_j + Calf_k\ (T_i) + TP_1 + BWB_m + ei_{jklmn}$$

Where:

Y_{ijklm}	=	Dependent variable (average daily gain)
μ	=	Overall mean
T_i	=	Fixed effect of treatment (YS, MN, CON)
Par_j	=	Randon effect of dam's parity number (1, 2, 3 or more)
$Calf_k\ (T_i)$	=	Random effect of calf nested within treatment
TP_1	=	Random effect of calf serum total proteins (g/dl)
BWB_m	=	Body weight at birth
e_{ijklmn}	=	Error term

Body weight at birth, 30 and 60 days was analyzed by ANOVA for repeated measures, constructing a mixed model considering the calf as random effect nested within treatment group. Explanatory variables were effect of treatment, day of weight, parity number of the dam, serum total protein concentrations and the interaction of treatment by day.

For all models, the best goodness of fit was specified according to the Schwarz's Bayesian Criterion (Littell *et al*., 1998). Least squares means ± SEM were reported. Significant effects were considered when P was ≤ 0.05. A tendency was considered with a P value between 0.05 and 0.1.

Diarrhea, respiratory and total disease incidence was analyzed through a chi-squared fisher exact test. Statistical analyses were conducted using the corresponding procedures (PROC MIXED and PROC FREQUENCY) of SAS 9.1 for windows (2003)

Results

Calves from CON, MN and YS reached a consumption of 2 kg starter and were moved to a collective group at 63.5±0.8, 62.3±0.7 and 62.8±0.8 days olds, respectively (p>0.05). Weight at birth was 44.8, 45.2 and 45.5 kg for CON, MN and YS group, respectively. Birth weights were not statistically different (p>0.05), indicating appropriate randomization of animals by treatment group. Live weights at 0, 30 and 60 days of age by treatment group are shown in Fig. 1. Daily weight gain by treatment group until 60 days of age are shown in Fig. 2. Average daily gain at 60 days of age were statistically different between the YS and CON groups (p≤0.05); however, no differences were determined between the MN and CON groups (p>0.05). The incidence of total disease and respiratory events until 60 days of life (Fig. 3) were similar for the 3 groups (p>0.05); however, the incidence of digestive disorders were statistically different between YS and CON groups (p≤0.05) and YS and MN group (p≤0.05), but not between MN and CON groups (p>0.05). Data from duration of health events were inconsistently recorded; therefore this information was not considered for the analysis.

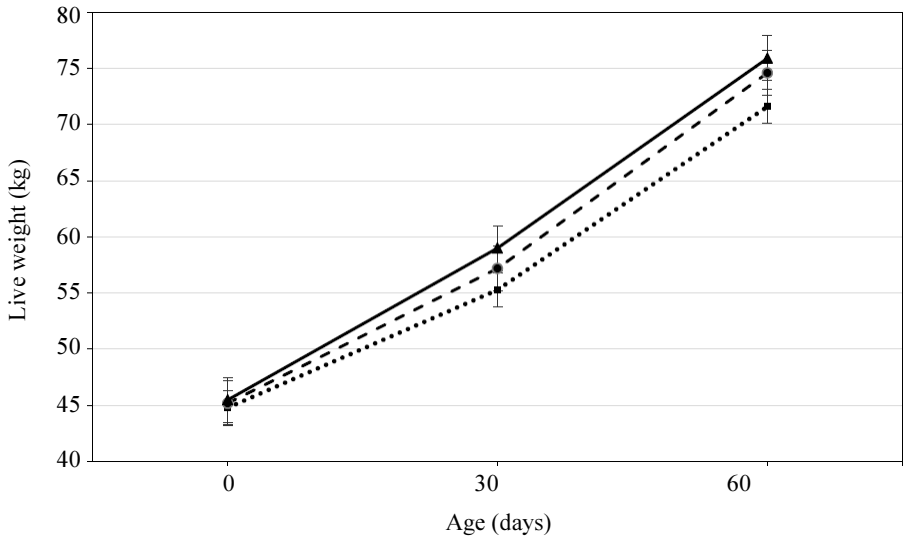

Fig. 1: Least squared means (± SEM) for live weight from birth to 60 days old (kg) CON group (n = 25, ⋯), MN group (n = 25, ---), YS group (n = 25, ─). Interaction treatment by day (p≤0.05). YS Vs. CON (p≤0.05), YS vs. MN (p>0.05), MN vs. CON (p>0.05)

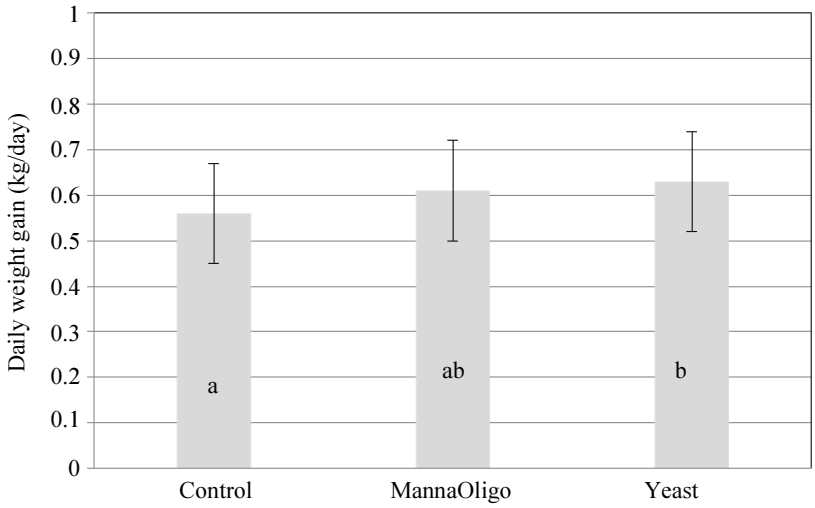

Fig. 2: Least squared means (± SEM) average daily weight gain from birth to 60 days old (kg/day). CON group (n = 25), MN group (n = 25), YS (n = 25). Different letters between bars indicate statistical differences (p≤0.05)

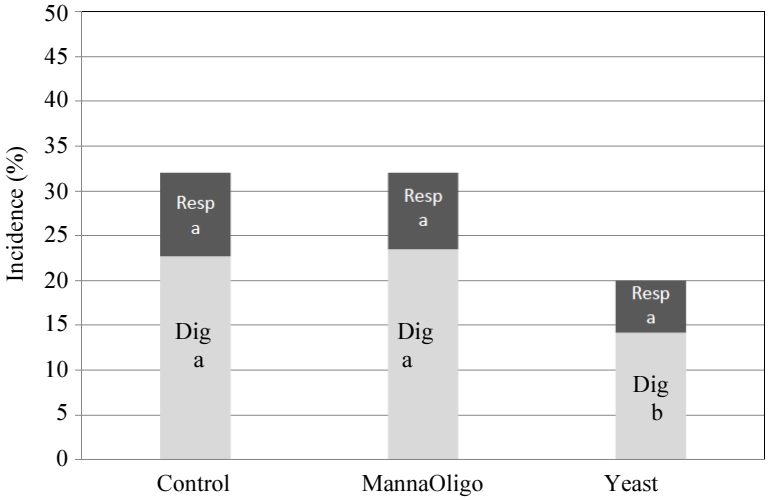

Fig. 3: Incidence of diarrhea and pneumonia (%) from birth to weaning. CON group (n = 25), MN group (n = 25), YS group (n = 25). Different letters between bars within health condition statistical differences (p≤0.05). Resp: respiratory; Dig: digestive

Discussion

The hypothesis of this field trial was that the addition of yeast based probiotics has a positive effect on average daily gain and health of pre-weaned calves. In part this hypothesis was demonstrated as calves supplemented with a product based on live yeast had better average daily gains than the control group. However, the group based on mannan-oligosaccharides did not improve the daily gain compared to the control group. These results are similar to a study where *Saccharomyces cerevisiae* improved the average daily gains and concentrate intake relative to a control group (Lesmeister *et al*., 2004). However, in another study, the addition of live yeast or mannan-oligosaccharides neither improved feed consumption, nor average daily gain and feed efficiency in both Holstein and Jersey calves (Hill *et al*., 2009). In another report, using a large number of calves per group, the average daily gain was not different between a group receiving yeast and a control group, but the incidence of diseases was lower and the score of feces improved in the treatment than the control group. Unfortunately, this improvement in health was not reflected in greater weaning weights (Magalhães *et al*., 2008). In a recent study, live yeast improved the average daily gain and decreased the negative effects of calves experimentally infected with *Salmonella spp.* (Brewer *et al*., 2014). Also, in a previous study (Galvão *et al*., 2005), it was observed that yeast improved the average daily gain, concentrate intake and feed efficiency, but only in calves with low total protein levels at birth (failure transfer of passive immunity). In our study, the statistical model for daily gain corrected by the level of serum total protein (colostrum management) and the age of the calf's dam,

therefore the effect of live yeast on daily gain may have been a pure effect on calf growth (better feed and digestive efficiency) and/or through an improvement in the health status, because the YS group had a lower incidence of digestive disorders than the control and MN groups, respectively.

Contrary to our results, the weaning weight of calves receiving an additive based on mannan-oligosaccharides exceeded about 6.5% weight of the control group ($p \leq 0.05$) (Curiquén and Gonzales, 2006). These differences may have been due to increased consumption of concentrate. Indeed, in a recent study, *Saccharomyces cerevisiae* supplemented in the milk replacer improved starter intake and fecal consistency in calves challenged with *Salmonella enterica* (Harris *et al*., 2017); however, the supplementation of fresh bovine colostrum with mannan oligosaccharide affected negatively the efficiency of immunoglobulins absorption during the first 24 h of life of calves. This study neither report average daily gains nor incidence of diseases (Brady *et al*., 2015). Unfortunately, in our study was not feasible to assess feed intake. It is desirable that weaning weights of calves and their development is as high as possible, uniform and consistent, as calves with higher weights and development at weaning can become more fertile and profitable in the future (Donovan *et al*., 1998; Godden, 2008).

Even though the sample size was small, differences in digestive disorders incidence between YS vs. CON and YS vs. MN group were statistically significant. Other studies have shown that the addition of mannan-oligosaccharides in milk replacer decreased the incidence of diseases (Heinrichs *et al*., 2003; Brewer *et al*., 2014). However, in the current study, only live yeast demonstrated to reduce the incidence of digestive disorders. This might be explained because *S. cerevisiae* can modulate the immune system and impact host-pathogen interactions in the digestive tract (Jensen *et al*., 2007; Magalhães *et al*., 2008). Indeed, *S. cerevisiae* fed with concentrate reduced the number of days with diarrhea (Galvão *et al*., 2005) and reduced the incidence of fever and the number of antibiotic treatments from birth to 46 days old (Seymour *et al*., 1995).

Conclusion

The supplementation with lived yeast improved the average daily gain between birth and weaning and decrease the incidence of digestive disorders when compared to a control group. These benefits were not observed in the group receiving derived from yeast cell walls (oligomannans) in dairy calves managed under Chilean conditions.

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Conflict of Interest

The authors declare that in this study there are no conflicts of interest

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Author's Contributions

Pedro Melendez: Experimental, design, statistical analysis and writing of the manuscript.

Angelica Arias: Execution of the experiment sampling and records.

Pablo Pinedo: Experimental design and statistical analysis.

Maria Paz Marin: Experimental design.

Ethics

The authors declare there are no ethical issues related to this observational study

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