

Supplementation of dairy calves with digestive enzymes and fermentation products of *Aspergillus oryzae* and *Aspergillus niger*^{1,2}

Melissa A. Vaz-Ramírez³, Jaime E. Curbelo-Rodríguez⁴
and Guillermo Ortiz-Colón^{5*}

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ABSTRACT

The effect of supplementation with *Aspergillus oryzae* and *A. niger* fermentation products and digestive enzymes on weight gain, growth, and health of Holstein (n=11) and Jersey (n=9) calves was evaluated. All calves, individually housed, were fed unpasteurized waste milk at 0600 (2 L) and 1800 (2 L) for 49 days. All calves were fed *ad libitum* a calf starter that contained 18% CP; 2.5% fat; 8% CF; 14% ADF; 1.5% Ca; 0.5% P; 0.20 mg/kg Se; 2,273 IU/kg vitamin A; and 66 g/Tm of Lasalocid. Treated animals (five Holsteins and four Jerseys) were fed 2 g of commercial mixture of *A. oryzae* and *A. niger* fermentation products and the digestive enzymes α amylase, pectinase, endoglucanase, β glucanase, xylanase, and mannanase. Weight and height were recorded weekly using a Nasco® measuring tape and measuring stick, respectively. There was no interaction of treatment by breed by period (P=0.9636). Treatment did not affect weight gain across periods (P \geq 0.7215). As expected, Holsteins were heavier than Jerseys (P=0.0284) and both breeds increased in weight over time (P<0.0001). Treatment did not affect the height at the hip (P=0.7971) or withers (P=0.4248). Holstein's height at the hip for the treatment group (HT) was 80.8 cm \pm 2.31 at 49 days and 80.5 cm \pm 3.25 for the control group (HC). Jersey treatment group (JT) ended with 85 cm \pm 1.98 whereas the control group (JC) ended with 82.1 cm \pm 2.17 at the hip. Height at the withers for HT measured 75.3 cm \pm 2.62 compared to 80.5 cm for HC. Moreover, calves in JT at the end of the experiment measured 79.9 cm \pm 1.23 compared to JC 76.7 cm \pm 2.59. Health status did not benefit from the supplementation of *A. oryzae* and *A. niger* in combination with enzymes (P=0.1444). Supplementation with *A. oryzae* and *A. niger* fermentation products and digestive enzymes did not increase weight gain, growth, or health status in Holstein and Jersey calves fed waste

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³Ex-Graduate student, Department of Animal Science, University of Puerto Rico-Mayagüez Campus, Mayagüez, Puerto Rico 00680.

⁴Associate Professor, Department of Animal Science, University of Puerto Rico-Mayagüez Campus.

⁵Associate Professor, Molecular Biology Laboratory, Department of Animal Science, University of Puerto Rico-Mayagüez Campus. Alfonso Valdez Blvd. Jesús T. Piñero Bldg. P-014, Mayagüez, P.R. 00680. *Corresponding author, Email: guillermo.ortiz@upr.edu

milk under the tropical conditions in Puerto Rico.

Key words: prebiotics, digestive enzymes, dairy calves, waste milk

RESUMEN

Suplementación con enzimas digestivas y productos de fermentación de *Aspergillus oryzae* y *Aspergillus niger* en becerras lecheras

Se evaluó el efecto de suplementar becerras Holstein y Jersey con productos de la fermentación de *Aspergillus oryzae* y *A. niger*, más enzimas digestivas, sobre el aumento de peso, altura a la cruz, y estado de salud. Veinte becerras (11 Holstein; nueve Jersey), albergadas individualmente, fueron alimentadas con 2 L de leche de descarte sin pasteurizar a las 0600 y a las 1800, y alimento iniciador *ad libitum* durante 49 días. El alimento iniciador contenía 18% PC, 2.5% grasa, 8% FC, 14% ADF; 1.5 Ca; 0.5 P; 0.20 mg/kg Se; 2,273 IU/kg vitamina A y 66 g/Tm Lasalocid. A los animales bajo tratamiento (cinco Holstein y cuatro Jersey) se les añadió 2 g de un producto comercial que contenía una mezcla de los productos de fermentación de *A. oryzae* y *A. niger*; y las enzimas digestivas α amilasa, pectinasa, endoglucanasa, β glucanasa, xilanasasa y mananasa. El peso y altura a la cruz de las becerras se evaluó semanalmente utilizando una cinta calibrada y una regla, respectivamente, ambas de la marca Nasco®. No hubo interacción del tratamiento por raza por periodo ($P=0.9636$). No hubo efecto del tratamiento sobre la ganancia de peso durante el periodo ($P\geq 0.7215$). Como era de esperarse, las becerras Holstein eran más pesadas que las Jersey ($P=0.0284$) y ambas razas ganaron peso durante el experimento ($P < 0.0001$). El tratamiento no afectó la altura de la cadera ($P=0.7971$) ni la altura a la cruz ($P=0.4248$). Las medidas de altura de cadera de las becerras Holstein del grupo tratado (HT) fueron $80.8 \text{ cm} \pm 2.31$ y de $80.5 \text{ cm} \pm 3.25$ en el grupo control (HC). Las becerras Jersey del grupo tratado (JT) obtuvieron una altura en la cadera de $85 \text{ cm} \pm 1.98$ y el grupo control (JC) obtuvo $82.1 \text{ cm} \pm 2.17$. Referente a la altura de la cruz, HT midió 75.3 ± 2.62 vs. HC, 80.5 ± 3.25 . Al finalizar el experimento, JT midió 79.9 ± 1.23 vs. JC, 76.7 ± 2.59 . El estado de salud no se benefició de la suplementación con *A. oryzae* y *A. niger* en combinación con enzimas digestivas ($P=0.1444$). La suplementación con los productos fermentados de *A. oryzae* y *A. niger* en combinación con enzimas digestivas no aumentó el crecimiento ni mejoró la salud de las becerras Holstein y Jersey alimentadas con leche de descarte bajo las condiciones tropicales en Puerto Rico.

Palabras clave: prebióticos, enzimas digestivas, becerras lecheras, leche de descarte

INTRODUCTION

Dairy calves must be raised properly and in optimum health to enhance their future milk production (Kertz et al., 2017). They tend to be very susceptible to infections in their gastrointestinal tract (GIT), especially during the first few weeks of life (Hulbert and Moisés, 2016). Moreover, GIT problems can ultimately result in malnourishment and increase susceptibility to other diseases, which ultimately compromise general welfare and reduce weight gain (Lorenz and Fagan, 2011).

More research is needed on preventive strategies that promote calves' health and ultimately improve growth and development during the first stages of the calf's life.

Direct-fed microbials (DFM) are feed supplements that include viable microbial cultures, microbial culture extracts and microbial enzyme isolates (Yoon and Stern, 1995). Direct-fed microbials could positively affect the host animal by improving its intestinal microbial balance, and consequently, might help calves through their first stages of life (Fuller, 1989). Several fungal products are available as DFM. For example, supplementation with fermentation extracts of the mold *Aspergillus oryzae* (AO) has been proven to increase ruminal microbial activity in calves and also positively influences the metabolism of ruminal microorganisms (Beharka and Nagaraja, 1998). Moreover, Van Horn et al. (1979) reported a 29% increase in apparent digestion of organic matter in the rumen with the addition of an AO fermentation product to dairy cattle diets. *Aspergillus niger* (AN), a filamentous fungus, is widely used in biotechnology to produce food ingredients, pharmaceuticals, and industrial enzymes; but it also has been used as a DFM (Meyer et al., 2011). *Aspergillus niger* secretes substantial amounts of a wide variety of enzymes needed to release nutrients from biopolymers (Meyer et al., 2011).

When used as feed additives, digestive enzymes can break down organic compounds into substances animals and microbes can use as nutrient sources, increasing the efficiency of nutrient utilization (Kung, 2001). Enzymes utilized in feed additives are extracted from complex microbial fermentation mixtures of fungi like AO and AN, and bacteria (Meyer et al., 2011). Enzyme supplementation will ultimately be increasing the amount of microbial protein and energy available for the growing ruminant (Kung, 2001). Some of the enzymes that have supplemented the diet of dairy cattle to improve performance are alpha amylase, pectinase, endo-glucanase, beta-glucanase, xylanase and mannanase (Kung, 2001).

The objective of this study was to evaluate the effects of supplementing dairy calves' diet with AO and AN fermentation products and the enzymes alpha amylase, pectinase, endo-glucanase, beta-glucanase, xylanase and mannanase, on weight gain, skeletal growth and the general health of Holstein and Jersey calves fed waste milk under tropical farm conditions during their first 49 days.

MATERIALS AND METHODS

Experimental facilities and conditions. The experimental protocol was approved by the *Institutional Animal Care and Use Commit-*

tee of the University of Puerto Rico, Mayagüez Campus. The experiment was carried out on *Tai South Farm*, a private dairy farm located in southwest Puerto Rico (18°01'53" N, 67°05'43" W). Average temperature during the experiment was 30° C with 75% humidity (<https://www.accuweather.com>). The calf nursery area had a concrete roof without walls, providing appropriate air circulation for the calves. The calves were housed in individual pens with walls made of cast iron, and iron floors covered with a plastic cover to provide better comfort for the calves. The pens measured 53.34 cm wide x 121.92 cm long (0.65 m²). The experimental period lasted from birth until 49 days of life.

Animal selection. Twenty female calves (11 Holstein and nine Jersey) were randomly assigned to treatments. During the first two days after birth the calves remained with their dams before being moved to their pens, according to the standard operating procedure of this commercial dairy farm. Calves were placed either into a control group (CON; five Holstein, five Jersey) or the treatment group (TRT; six Holstein, four Jersey). Treatment consisted of a daily dose of 2 g of a prebiotic proprietary commercial mixture containing *A. oryzae* and *A. niger* fermentation products and the enzymes alpha amylase, pectinase, endo-glucanase, beta-glucanase, xylanase, and mannanase. The TRT product was mixed with 2 L of milk during the morning feeding. All calves were fed 2 L of unpasteurized waste milk at 0600 and at 1800, and also fed, ad libitum, a commercial calf starter that contained 18% crude protein; 2.5% fat; 8% CF; 14% ADF; 1.5% Ca; 0.5% P; 0.20 mg/kg Se; 2,273 IU/kg vitamin A; and 66 g/Tm of Lasalocid. Water was offered ad libitum to all calves.

Growth evaluation. Weight gain and height were determined weekly. Weight was measured using a Nasco[®] Weight by Breed Dairy Management Tape (Fort Atkinson, WI, USA) around the calf's barrel. Height was measured with a Nasco[®] Measuring Stick for Beef or Dairy Cattle and determined from the floor to the animal's hip and withers.

Health evaluation. A calf health scoring chart, developed by the University of Wisconsin School of Veterinary Medicine, Madison, WI, was used to evaluate the calves (McGuirk, 2020). The scoring criteria included: rectal temperature, cough incidence, nasal discharge, eye secretions, ear position and movement, and fecal scores (diarrhea incidence). For each criterion the evaluator assigned a value between zero (0=normal) and three (3=morbid). The evaluator performed a general

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addition of the points for each criterion; higher health scores were indicative of higher morbidity. All calves were systematically evaluated once a week.

Statistical analysis. A complete randomized design was used to analyze results. Within each breed, a weekly average of body weight, wither height, hip height, and health score were analyzed with the Proc Mixed procedure of SAS (SAS University Edition, 2018, SAS Institute Inc., Cary, NC, USA). The experimental model included treatment, week and their interactions as fixed effects. Week was used as a repeated measurement with calf as the experimental unit.

RESULTS

Weight gain. No interaction was detected between treatment by breed by week ($P=0.9636$) when considering weight gain. Treatment did not affect weight gain across weeks ($P=0.7215$). As expected, Holstein calves were heavier than Jerseys ($P=0.0284$), and both breeds gained weight over time ($P<0.0001$). Holstein calves from the control (HC) group started the trial with an average of 43.5 kg and weighed 70 kg at the end of the trial, for a total weight gain of 26.5 kg over 49 days (0.541 kg/day). The initial weight of treated Holstein calves (HT) was 34.7 kg, and the final weight was 53.8 kg, for a total weight gain of 19 kg over 49 days (0.388 kg/day) (Figure 1A). Jersey calves in the control (JC) had an initial weight of 30.6 kg and a final weight of 58.9 kg, for a total weight gain of 28.3 kg over 49 days (0.578 kg/day). Jersey calves in the treated (JT) group started with 33.4 kg and had a final weight of 67.8 kg, for a total weight gain of 34.4 kg over 49 days (0.702 kg/day) (Figure 1B).

Structural growth. There was no interaction between treatment by breed by week ($P=0.9974$) in terms of hip height. Moreover, no interactions were detected between week by breed ($P=0.9848$), week by treatment ($P=0.9978$) or treatment by breed ($P=0.2385$). Changes in hip height were not different between treatments ($P=0.3342$). As expected, hip height increased over time in both breeds ($P=0.0173$). Calves in the HC group had an initial hip height of 78.2 ± 4.75 cm and a final height at the hip of 85.5 ± 3.5 cm, growing a total of 7.3 cm in 49 days. In HT calves, initial hip height was 75.2 ± 1.75 cm and final height was 80.8 ± 2.31 cm with growth totaling 5.6 cm over 49 days (Figure 2A). Calves in the JC group had an initial hip height of 71.9 ± 2.02 cm and ended with 82.1 ± 2.17 cm for a total increase in hip height of 10.2 cm (Figure 2B). The JT group started with 74.8 ± 1.23 cm and had a final hip height of 85.0 ± 1.98 cm with a total increase in height of 10.3 cm at the end of 49 days (Figure 2B).

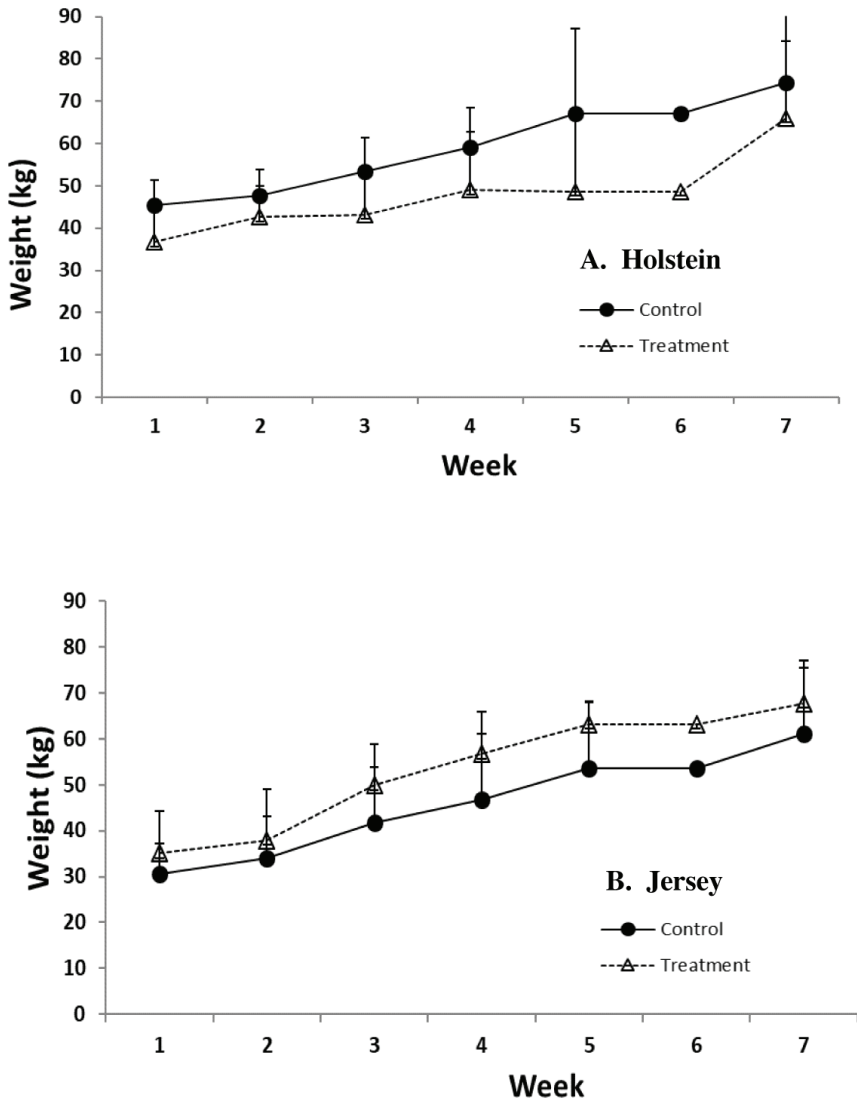


FIGURE 1. Calves' weekly weight during a period of 49 days. Weight was recorded using a Nasco® Weight by Breed Dairy Management tape (Fort Atkinson, WI) around the calf's barrel. Treatment group (TRT) received 2 g of a prebiotic commercial mixture in the a.m. feeding containing *A. oryzae* and *A. niger* fermentation products, and the enzymes alpha amylase, pectinase, endo-glucanase, beta-glucanase, xylanase, and mannanase. The control group (CON) did not receive any product. A) Holstein calves: TRT, n=6; CON, n=5. B) Jersey calves: TRT, n=4; CON, n=5. Treatment did not affect weight ($P=0.7215$).

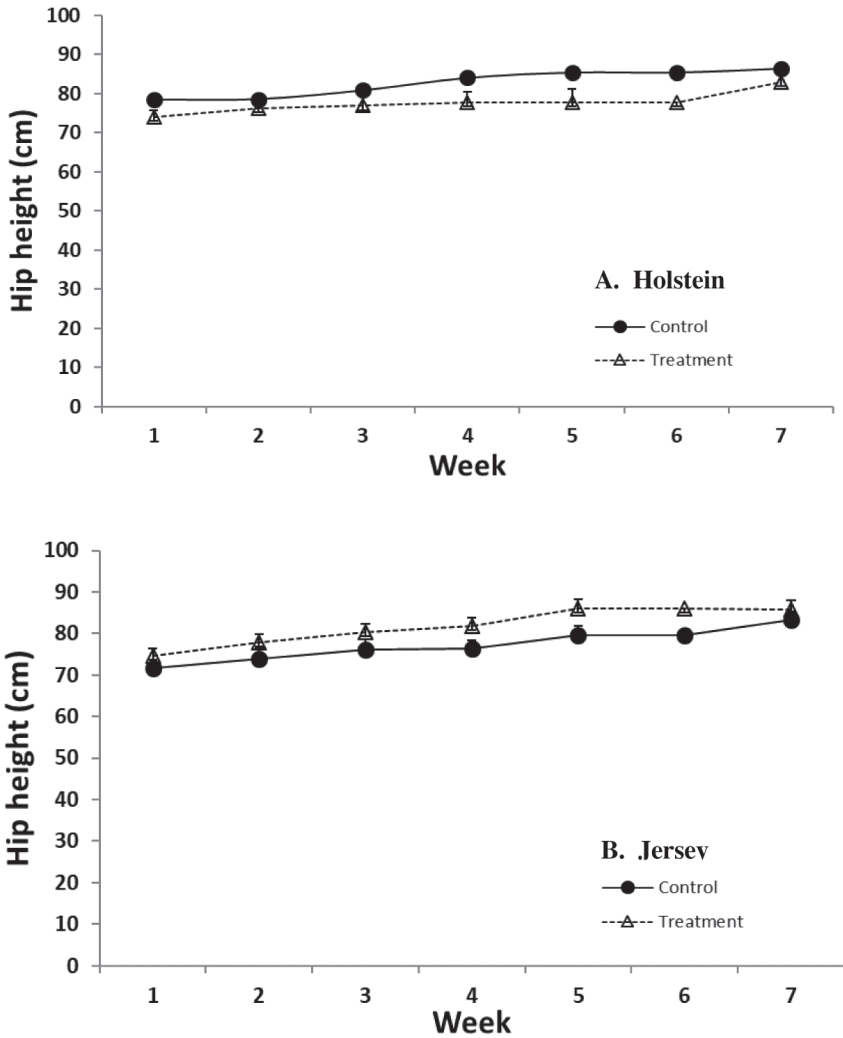


FIGURE 2. Calves' weekly hip height during a period of 49 days. Skeletal growth was measured with Nasco® Measuring Stick for Beef or Dairy Cattle, from the floor to the animal's hip. Treatment group (TRT) received 2 g of a prebiotic commercial mixture in the a.m. feeding containing *A. oryzae* and *A. niger* fermentation products, and the enzymes alpha amylase, pectinase, endo-glucanase, beta-glucanase, xylanase, and mannanase. The control group (CON) did not receive any product. A) Holstein calves: TRT, n=6; CON, n=5. B) Jersey calves: TRT, n=4; CON, n=5. Changes in hip height were not different between treatments (P=0.3342).

Regarding wither height, no interactions were detected between treatment by breed by week ($P=0.7448$), week by breed ($P=0.7690$), week by treatment ($P=0.9695$), or treatment by breed ($P=0.6397$). Wither height was not affected by treatment ($P > 0.05$). As expected, wither height increased over time in both breeds ($P=0.0173$). Initially, calves in the HC group measured 73.9 ± 2.89 cm at withers, and by the end of the trial, 49 days later, they measured 80.5 ± 3.25 cm for a total increase of 6.8 cm. In the HT group, initial wither height was 71.3 ± 0.24 cm, while the final height was 75.3 ± 2.62 cm, for a total growth of 4 cm in 49 days (Figure 3A). The JC group started with 70.2 ± 0.48 cm of height at withers and ended with 76.7 ± 2.59 cm for a total increase of 6.5 cm. The JT group started with 72.3 ± 1.10 cm in wither height and had a final height of 79.9 ± 1.23 cm for a total growth of 7.6 cm over 49 days (Figure 3B).

Health score. In terms of health status, there was no interaction of treatment by breed by week ($P=0.6567$), between week by breed ($P=0.5369$), week by treatment ($P=0.9604$), or treatment by breed ($P=0.6026$). Treatment did not affect health status in Holstein ($P=0.1444$) or Jersey calves ($P=0.1055$) (Figure 4). There are six (6) different health criterion, each assigned 0 to 3 points, with the maximum of 3 to indicate morbidity; hence a seriously sick calf could receive an overall score of 18 points. However, calf morbidity was low throughout the experiment. Calves in the HC group had an average health score of 2.0 ± 0.53 (Figure 4A). In the HT group, calves averaged a health score of 2.4 ± 0.53 . In the JC group, calves had an average health score of 1.9 ± 0.53 ; JT calves scored an average of 2.4 ± 0.53 (Figure 4B).

DISCUSSION

The objective of this research was to determine if supplementation treatment with *A. oryzae* (AO) and *A. niger* (AN) fermentation products, and the enzymes alpha amylase, pectinase, endoglucanase, beta-glucanase, xylanase, and mannanase could improve weight gain, skeletal development, and decrease morbidity in dairy calves. Although the treatment was expected to improve the growth and health of dairy calves, we found no evidence that the supplementation evaluated had a positive impact on the calves. However, in the literature, results of the use of AO and/or AN supplements for raising calves have been variable, possibly due to the different levels of fungal culture inclusion, the source of the fungal culture, and mixture with other microorganisms and components like enzymes. Moreover, management conditions affecting the calves can also influence the effects of AO and AN supplementation.

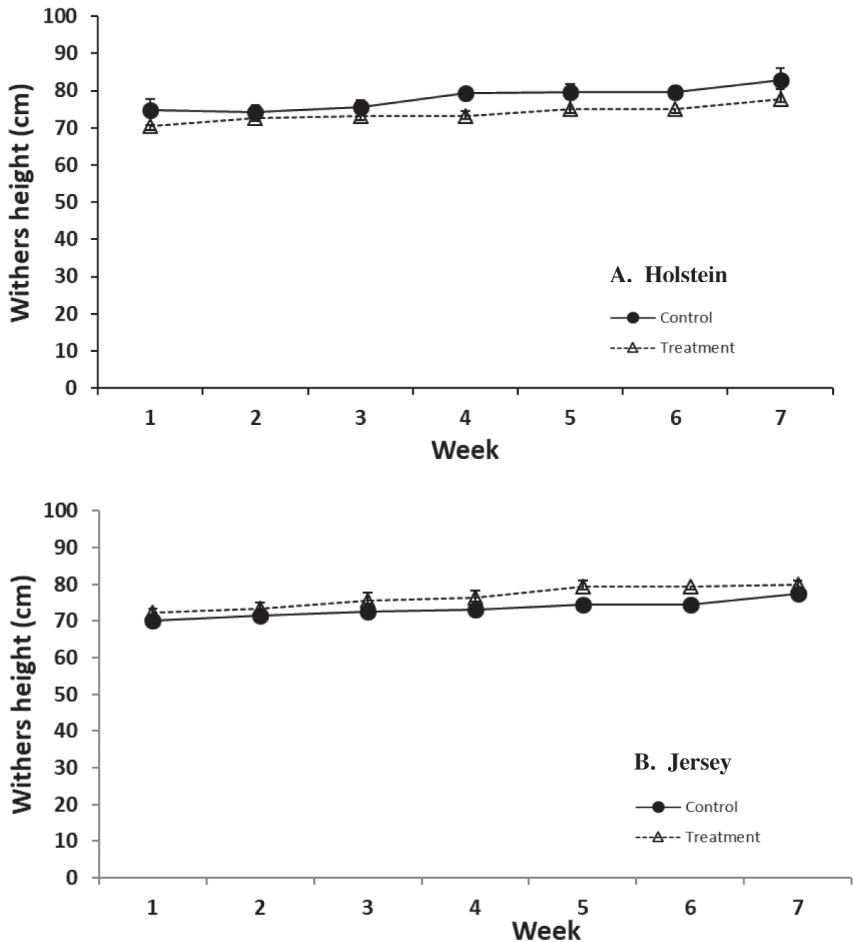


FIGURE 3. Calves' weekly measuring of withers height during a period of 49 days. Skeletal growth was measured with Nasco® Measuring Stick for Beef or Dairy Cattle, from the floor to the animal's withers. Treatment group (TRT) received 2 g of a prebiotic commercial mixture in the a.m. feeding containing *A. oryzae* and *A. niger* fermentation products, and the enzymes alpha amylase, pectinase, endo-glucoanase, beta-glucoanase, xylanase, and mannanase. The control group (CON) did not receive any product. A) Holstein calves: TRT, n=6; CON, n=5. B) Jersey calves: TRT, n=4; CON, n=5. Treatment did not affect withers height ($P \geq 0.05$).

Allison and McCraw (1989) and Wiedmeier (1989), for example, reported higher average daily weight gain of calves supplemented with AO. Furthermore, Beharka et al. (1991) concluded that AO supplemented calves were weaned one week earlier than the control group. Conversely, in our study, calves were not affected by the addition of

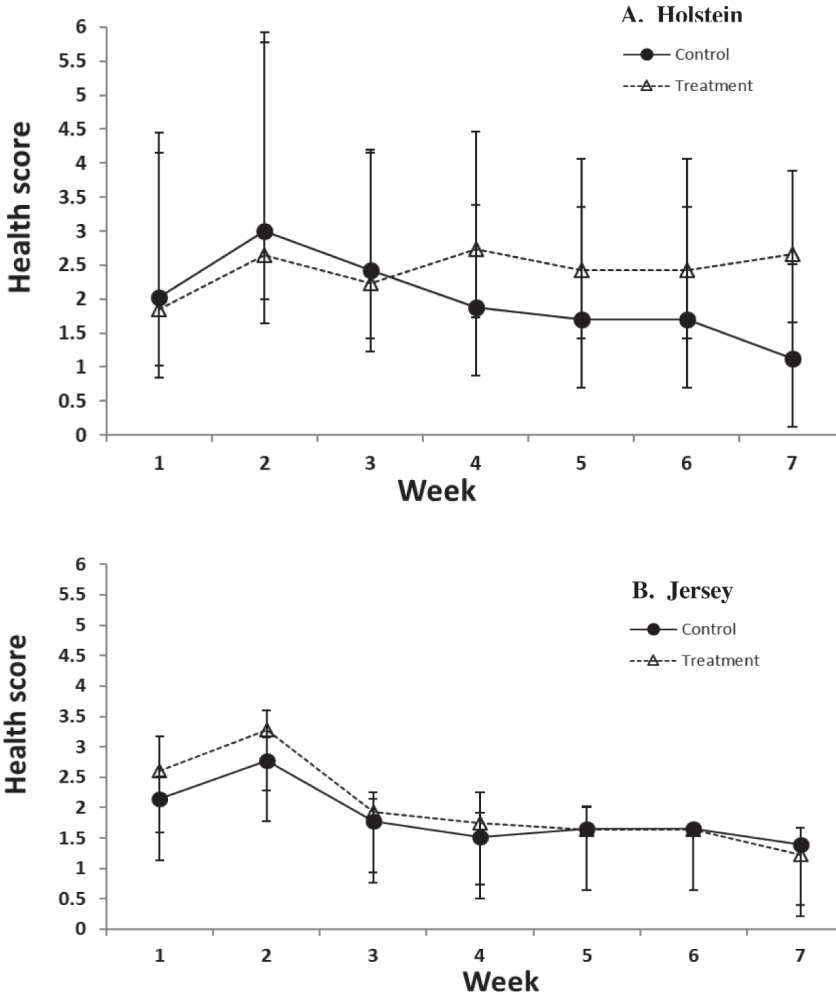


FIGURE 4. Calves' Health Scoring for 49 days. A calf health scoring criteria table from the University of Wisconsin was used (<https://www.vetmed.wisc.edu/dms/fapm/apps/chs.htm>). Treatment group (TRT) received 2 g of a prebiotic commercial mixture in the a.m. feeding containing *A. oryzae* and *A. niger* fermentation products, and the enzymes alpha amylase, pectinase, endo-glucanase, beta-glucanase, xylanase, and mannanase. The control group (CON) did not receive any product. A) Holstein calves: TRT, n=6; CON, n=5. B) Jersey calves: TRT, n=4; CON, n=5. Treatment did not affect health status ($P \geq 0.1055$).

AO and AN fermentation products in combination with enzymes, as both groups maintained the same health score and gained weight similarly. Likewise, Rush et al. (1990) concluded that performance of British crossbred steers did not improve when adding supplements of AO

along with vitamins and minerals. Moreover, adding AO to the diet of lambs had no effect on growth and carcass weight (Herring et al., 1989).

There are many conceivable explanations for the ineffectiveness of AO and AN inclusion on the performance of dairy calves in our study. For example, in some studies with positive results supplementation with AO and AN has been combined with other microorganisms. Di Francia et al. (2007) fed water buffalo (*Bubalus bubalis*) calves with a milk replacer supplemented with a mixture of AO and *Saccharomyces cerevisiae* (SC), and obtained improvements in consistency of fecal scores compared to the control group. In that particular study, the positive effects could have been caused by SC and not AO.

Indeed, many studies have documented the positive effects of supplementing dairy calves' diet with SC. A study by Broadway et al. (2015) evaluated the effect of a supplementation with SC on the immune response of calves infected with pathogens *Citrobacter ferundii* and *Salmonella enterica* and revealed that calves treated with SC had a lower neutrophil-to-lymphocyte ratio and lower fecal scores, suggesting a better gut health. Indeed, Galvão et al. (2005) reported that calves fed SC in milk replacer had fewer days of diarrhea during the pre-weaning period, and Magalhães et al. (2008) found that feeding an SC culture in grain decreases the incidence of diarrhea and the mortality rate in calves within the first 70 days of life.

However, the effect of using SC as a direct-fed microbial (DFM) in dairy calves has been variable. Wagner et al. (1990) reported no effect on the growth of young Holstein calves when an SC culture was added to a high concentrate diet. Also, Quigley et al. (1992) observed no difference in feed intake or body weight gain of calves fed a starter feed supplemented with a SC culture product. In a more recent study, He et al. (2017) found no difference between weight gain and final body weight when *Saccharomyces boulardii* (SB) was added to a milk replacer. In our study, the treatment was mixed with unpasteurized waste milk, which might have compromised its plausible positive effect. Moreover, since we evaluated only one concentration of the treatment, it is possible that positive effects could be achieved at concentrations higher than 2 g per head per day of the prebiotic proprietary commercial mixture containing *A. oryzae* and *A. niger* fermentation products and the enzymes alpha amylase, pectinase, endo-glucanase, beta-glucanase, xylanase, and mannanase.

In addition, the time when calves are fed colostrum after birth might affect the effectiveness of DFM supplementation because colostrum has a profound effect on the functional development of the GIT. Passive immunity acquired through maternal immunoglobulins (IgG)

in colostrum provides newborns with early protection against pathogens (Indart et al., 2012). Failure of passive transfer of immunity due to poor colostrum management leads to high incidence of diarrhea (Lorenz and Fagan, 2011). In our study calves stayed with the dam during their first two days of life, as is the common practice in Puerto Rico (De León-Torres et al., 2016). Thus, in our calves, the time of colostrum ingestion after birth, as well as the volume and the quality of the colostrum are unknown.

Another reason for the ineffectiveness of AO and AN inclusion on the performance of dairy calves in our study might be that they were fed unpasteurized waste milk. Intestinal integrity cannot be optimized with the use of waste milk because feeding untreated mastitic milk can facilitate the transmission of infectious pathogens and provoke disease in calves (Abb-Schwedler et al., 2014). A few studies focus on how feeding waste milk influences the intestinal microflora of calves. Wray et al. (1990) evaluated the antibiotic resistance of fecal *Escherichia coli* of calves fed either waste milk or milk replacer and found that the inhibitory concentration of streptomycin was higher in *E. coli* isolated from calves fed milk containing antibiotics. On the other hand, Langford et al. (2003) were able to demonstrate a dose-dependent relationship between the penicillin concentration in the milk feed and the level of antibiotic resistance in gut bacteria isolated from calves. The European Food Safety Authority (EFSA) concluded that the practice of feeding calves waste milk containing antibiotic residues increases the fecal shedding of antibiotic resistant bacteria by calves (Pereira et al., 2018). Therefore, by providing a constant source of pathogens, feeding unpasteurized waste milk might neutralize the potential beneficial effects of feeding AO and AN fermentation products, because the beneficial bacteria that could benefit from the AO/AN treatment would have to compete to colonize the lumen of the calves' GIT with the pathogens provided daily with the waste milk.

Another possible explanation for no differences between our treatment groups might be stress exposure. When a calf is born, it goes through a stressful period, starting with the abrupt separation of the calf from its dam and relocation to individual pens (De León-Torres et al., 2016). In dairy production, calves go through a variety of stresses that can alter microorganism populations in the developing rumen and lower GIT, resulting in decreased performance and increased morbidity and mortality (Krehbiel et al., 2003). In our study the space of the pens where the calves spent their first 49 days of life was limited (0.65 m²). Hulbert and Moisés (2016) observed the benefits of increased space allowance for calves during the first six weeks of life and after weaning. Calves with more space allowance (three times the space of a conven-

tional hutch of 1.23 m²) had better neutrophil response and less basal cortisol secretion after five weeks of age (Hulbert and Moisé, 2016). In addition, body weight and starter consumption were enhanced with increased space allowance (Hulbert and Moisé, 2016). Hurnik and Lewis (1991) suggested that the minimal space allowance requirement was 60% of the calf's body surface area; this space allowance formula allows the calf to be able to stand and rest in sternal and lateral recumbency with its legs extended. Actually, the stressful conditions experienced by calves in commercial dairy operations can negatively influence the composition and symbiotic interactions of gut microbiota (Indart et al., 2012). Therefore, the administration of DFM to repopulate the gut might reduce the changes in the microbial population caused by stress (Krehbiel et al., 2003), and potentially improve performance and health in calves. However, in our study, where calves were provided only 0.65 m² of living space, presumably a stressful living condition, a DFM supplementation did not show differences in growth, weight gain and health when compared with the control group.

Because in milk fed calves the rumen is developing, it is plausible that enzyme supplementation could help calves digest dry feeds and accelerate the establishment of a healthy rumen microbial population. In our study, however, supplementing calves with a proprietary mixture of the enzymes alpha amylase, pectinase, endoglucanase, beta-glucanase, xylanase, and mannanase did not result in improvements in weight gain of the calves. Similarly, many different studies have found no effect of supplementing animal feeds with exogenous enzymes (Bowman et al., 2002; Beauchemin et al., 2000; Rode et al., 1999).

Variable results of feeding DFM products on weight gain and health may relate to the type of microorganism used, strain of microorganism, form (live vs. culture products), amount fed and delivery method (in milk vs. in starter). Moreover, nutrition management, pathogen load and overall stress present could affect the calves' responses to DFM supplementation. Conceivably, dairy calves that are fed waste milk are exposed to high counts of pathogenic bacteria, which may neutralize the potential beneficial effects of DFM. Indeed, future evaluations to determine if DFM could benefit calves' growth and health under tropical conditions should be performed using best management practices, such as the use of pasteurized milk or milk substitutes, and appropriate housing space.

CONCLUSION

Supplementing Holstein and Jersey dairy calves with a commercial direct-fed microbial (DFM) containing *A. oryzae* and *A. niger* fermenta-

tion products along with the enzymes alpha amylase, pectinase, endoglucanase, beta-glucanase, xylanase, and mannanase, did not result in improved growth and health status during their first 49 days of life.

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