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Effects of Probiotic Supplementation on Growth and Health of Pre-Weaned Dairy Calves

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ABSTRACT

Dairy calf immune development leaves calves susceptible to illness during the pre-weaned stage of life. Previous studies have shown that the use of probiotics during this time may result in increased growth rates and decreased incidence of illness, particularly diarrhea and scours. We hypothesized that by providing probiotics during the time from birth until weaning, calves on study would experience similar growth and health benefits. Neonatal calves (N=32) were provided milk replacer three times daily, with half of the calves receiving Probios® supplements mixed in with their first morning feeding. Growth and health measurements were collected weekly, and data was analyzed using SAS statistical software suite. Probiotic administration significantly increased both the hip height (P=0.0297) and the wither height (P=0.0350). There were no significant differences found between probiotic treatment and weight or heart girth (P>0.05). There were also no significant findings related to health data, however less calves who received probiotics experienced diarrhea when compared to the control group, showing a possible correlation between supplementation and health benefits.

LITERATURE REVIEW

Introduction

Dairy calves are particularly susceptible to diarrhea and upper respiratory illness in the time between birth and weaning, due to the delayed nature of ruminant immune development and the inevitable exposure to pathogens within their environment. This has the potential to lead to decreased growth rates, resulting in significant economic losses for the operation (Mokhber-Dezfouli et al. 2007). Previous studies have shown that the use of probiotics may be able to counteract these factors and reduce the incidence of disease in young calves while also promoting growth (Zhang et al. 2019). The objective of this study was to determine if the

supplementation of a multistrain probiotic during the period between birth and weaning would lead to increased growth and decreased incidence of illness in dairy calves.

Role of Probiotics in Disease Prevention

Calf diarrhea, also known as scours, is a disease common to calves during early life and can be caused by a number of pathogens obtained through various routes. The disease is characterized by "frequent removal of soft feces" which may contain other abnormalities such as blood or mucus, and can prove to be fatal if not diagnosed and treated (Signorini et al. 2011). In turn, symptoms of calf diarrhea can require medical intervention, leading to financial losses through the cost associated with medications or in the case of fatalities (Brockmann 2014). The effectiveness of probiotics in preventing diarrhea in pre-weaned calves has been demonstrated through multiple studies (Mokhber-Dezfouli et al. 2007, Gorgulu et al. 2003, Zhang et al. 2019).

In Mokhber-Dezfouli's study (2007), 120 female calves were split in two groups, with half being provided multistrain probiotics (treatment group) and half remaining in the control group. Both groups were not challenged with any type of pathogen, but were held indoors in concrete pens. While scours occurred to some degree in both the treatment and control groups, results showed a decreased incidence of diarrhea in the calves provided probiotics. 11 calves in the treated group experienced diarrhea compared to 35 calves in the untreated group. Additionally, there was found to be a reduction in the severity of scours in the treated calves who experienced diarrhea when compared to the untreated calves. 7% of the affected calves in the control group were classified as having "severe fluid" stool, while none of the treated calves were classified at this degree of severity (Mokhber-Dezfouli et al. 2007).

Similar results were found in research performed by Gorgulu and colleagues (2003), utilizing multistrain probiotics. In this study of 24 male calves, 8 out of the 12 calves in the

control group experienced symptoms of diarrhea, compared to 3 out of the 12 calves who received probiotics. Of all the calves who experienced diarrhea, three from the control group and one from the probiotic group died. However, of the remaining calves who recovered, the medication cost was found to be significantly lower for the probiotic group (Gorgulu et al. 2003). Based on these results, this demonstrates that the calves in the probiotic group required less medical intervention for their symptoms, or experienced less severe symptoms than their counterparts in the control group. Regardless, calves who received probiotics in this study who had symptoms of diarrhea experienced a higher rate of recovery compared to calves who did not receive the probiotic supplementation.

Following a similar protocol, Zhang (2019) examined the effect of the monostrain bacteria *Lactobacillus rhamnosus* GG on the health and growth of Holstein calves. Within this study of 24 calves, 12 calves were assigned to the control group and 12 to the treatment group. Findings were consistent with other similar research; probiotic supplementation was associated with lower fecal scores, or reduced diarrhea (Zhang et al. 2019).

While diarrhea leads to the most severe complications in calves during their first 30 days of life, upper respiratory illnesses are also characteristic of pre-weaned calves at 30 days and older (McGuirk 2008). Bovine respiratory disease (BRD), caused in part by *Mannheimia haemolytica*, poses a large threat to young calves with the potential to develop into pneumonia and severe lung damage when colonized in the upper respiratory tract. In research done by Amat and colleagues (2017) utilizing multiple strains of *Lactobacillus*, adhesion and colonization of the bovine respiratory pathogen *Mannheimia haemolytica* was examined (Amat et al. 2017). Results of this study demonstrated the ability of probiotics to adhere to bovine bronchial cells, inhibiting the growth of pathogenic bacteria. Additionally, none of the calves included in the

study portrayed symptoms commonly associated with upper respiratory infection, such as nasal discharge, cough, or increased respiratory rate. Results were therefore consistent with the theory that probiotics may protect against infection of the respiratory tract (Amat et al. 2017).

The beneficial effects of multistrain probiotics were also demonstrated by Timmerman and others (2005) during their study looking at the overall health and growth of veal calves being fed two different types of probiotics. One probiotic preparation contained commercially available strains, while the other was formulated based on the specific microbiota found in calf stomach contents and feces. In the study, both probiotics were found to reduce the incidence of respiratory disease when provided in their milk replacer for the first 15 days following birth. There were also notable decreases in the incidence of diarrhea corresponding with both treatments when administered shortly after parturition with milk replacer (Timmerman et al. 2005).

Significance of Disease Prevention

There are many benefits to the application of probiotics, especially for the purpose of disease prevention. Economic benefits can be connected to probiotic use due to both the decreased medical costs associated with disease treatment and the lower mortality rates. The treatment costs were specifically discussed in research done by Gorgulu and others, in which they found that there was a 275% proportional cost of medication required for pre-weaned calves in the control group, compared to 100% for calves in the group provided with probiotics (Gorgulu et al. 2003).

MATERIALS AND METHODS

Probiotic Supplementation and Animals

Upon comparison of the aforementioned studies examining the effectiveness against scours and upper respiratory illness, data suggested a decreased incidence of disease and respiratory symptoms associated with the use of multistrain probiotic supplements as opposed to monostrain (Timmerman et al. 2004). Due to these results, this study focused on the effects of supplementation of the multistrain probiotic, Probios® Dispersible Powder. The live microorganisms contained within the product were lactic acid bacteria, specifically *Enterococcus faecium*, *Lactobacillus acidophilus*, *Lactobacillus casei*, and *Lactobacillus plantarum*, in amounts greater than 10 million colony forming units per gram (Vets-Plus Inc. 2021). This product was specifically chosen due to its microbial makeup, in addition to its widespread commercial availability as a low cost, easy to use product.

The Probios® supplement was administered according to manufacturer instructions, with each calf in the probiotic group receiving 1 rounded teaspoon (approximately 5 grams, measured with the included scoop) per day, combined with their allotted milk replacer during the morning feeding. This was given starting with the first milk replacer feeding and continued once daily until the calf was weaned. Each calf was fed Bovine Innovations Group (B.I.G.) Milk Replacer three times daily, at a rate of 2 quarts of warm water with 10 ounces of milk replacer powder. The amount of liquid milk replacer provided per feeding was dependent on breed and increased weekly, as described in Table 1. Calves in the control group received equivalent amounts of milk replacer each week, without the addition of probiotic supplement.

In addition to receiving milk replacer, the calves were provided with medicated calf starter feed, ad libitum. Monensin was the active drug found in the starter feed (labeled as

Rumensin® by Elaco), with the feed containing 42.6 grams of Monensin per ton of pellets. Monensin, an ionophore, has been indicated for "improved feed efficiency, for increased rate of weight gain, and for the prevention and control of coccidiosis caused by *Eimeria bovis* and *Eimeria zuernii*" (Elanco 2020). While the impact of medicated feed was not examined directly in this study, it was provided to all calves on study for the entire duration.

The experiment took place in the Frances E. Osborne Kellogg Dairy Center at the University of Connecticut. The protocols for animal use within the experiment were approved by the Institutional Animal Care and Use Committee (IACUC) prior to the start. The study included 32 female Holstein and Jersey calves. Each calf was randomly assigned by birth date to one of two treatment groups; the untreated control group or the group which received probiotic treatment. The number of calves from each breed was not controlled within the experiment, resulting in 20 Holstein calves on study with 9 in the probiotic group, and 12 Jersey calves on study with 7 in the probiotic group.

Data Collection

The effectiveness of Probios® supplementation on the health of pre-weaned calves was quantified by a series of weekly observations. Each calf was examined for signs of a cough, nasal discharge, eye discharge, or ear discharge. These observations were rated on a numerical scale ranging from zero to three, based on the intensity of the symptoms. The quantity assigned weekly for each category was based upon the "Calf Health Scoring Criteria" seen in Figure 1, as suggested by the School of Veterinary Medicine at University of Wisconsin-Madison (2008).

Calf growth was monitored throughout the study using a series of measurements performed on each calf. As per research done by Zhang and colleagues, growth measurements were recorded on a weekly basis (Zhang et al. 2019). Body weight and heart girth measurements

were found using a Nasco weight-by-breed dairy cow tape. Both wither and hip heights were measured in inches using a standard horse measuring stick, placed at the highest point of the withers and hip. This data was then used to determine gains in physical measurement throughout the study period.

Statistical Analysis

All experimental data was analyzed within the SAS statistical software suite. Standard error of the mean (SEM) and P-values were calculated for each measurement based on both the treatment and breed of calf. The relationship between treatment and breed for each measurement was examined as well.

HYPOTHESIS

The main objective of this experiment was to determine if the supplementation of a multistrain probiotic would benefit pre-weaned dairy calves, by increasing growth and decreasing the incidence of scours or respiratory illness. It was hypothesized that the calves who received probiotics would experience increases in weight gain and height when compared to calves who did not receive the treatment. Additionally, we hypothesized a decreased incidence of disease in treated calves, characterized by normally formed fecal and a lack of both ocular and nasal discharge.

RESULTS

Body Structure Measurements

The results of body structure measurements can be found in Table 2. Probiotic administration significantly increased both the hip height (P=0.0297) and the wither height (P=0.0350). There were no significant differences found between probiotic treatment and weight

or heart girth (P>0.05). However, there were significant differences in growth between the Jersey and Holstein calves on study, with Jersey calves increasing in weight at a faster rate than Holstein calves (P=0.005).

Health Measurements

Calf health, as measured weekly by abnormalities in eye discharge, nasal discharge, and fecal abnormalities, was compared to the scoring criteria depicted in Figure 1. The results of these health measurements can be found in Table 3. There were 12 calves who exhibited notable eye discharge, with four calves in the control group having a mean health score of 1 and eight calves in the probiotic group having a mean health score of 1. There were 3 calves who exhibited nasal discharge, with two calves in the control group having a mean health score of 1 and one calf in the probiotic group having a mean score of 2. There were 8 calves who exhibited fecal abnormalities, with six calves in the control group having a mean health score of 1.16, and two calves in the probiotic group having a mean health score of 1.5.

Of the 14 remaining calves, seven calves received probiotics and seven calves did not. These results demonstrated that both treated and untreated calves experienced illness over the course of the study, and any improvement in the qualitative health measurements as a result of probiotic use was not statistically significant.

DISCUSSION

Growth Data

According to the data collected, it was discovered that probiotics had a positive impact on growth when quantified in terms of hip and wither heights, which were both measured using a standard horse measuring stick. In contrast, the measurements obtained through the use of a

weight tape were found to demonstrate no significant differences between the probiotic and control groups. This may be in part due to lack of precision throughout the study, as a result of inconsistencies in the positioning of the weigh tape during the weekly collections. While all students collecting data were trained prior to the start of the study on proper measurement methods, the involvement of nine individuals had the potential to lead to varying results. Specifically, the weigh tape may have been wrapped more tightly or loosely around the body depending on the day and individual measuring. The location of the weight tape on the body may have also varied on a weekly basis, since this was particularly challenging to control. These problems were less apparent when measuring the hip and wither heights, since placement on the body of the calf was more consistent.

While breed was not controlled within the study, results demonstrated that the Jersey calves included in the study grew at a significantly faster rate when compared to the Holstein calves. It is possible that this is related to normal size differences between the breeds; Jerseys are typically smaller with their 50th percentile body weight at two months of age being 122 lbs, while Holsteins are larger with their 50th percentile body weight at two months of age being 161 lbs (Penn State Extension, 2017). Regardless, results were consistent with similar data in the field, such as that collected by Handcock and colleagues, who found the highest growth rates in young Jersey heifers when compared with larger breeds such as Holstein-Friesian crossbred heifers (Handcock et al. 2018).

Health Data

Out of the calves who experienced illness over the course of the study, those who received probiotics were found to have decreased incidence of abnormal feces. While calves in both the control and probiotic group experienced other symptoms such as ocular discharge or a

combination of illnesses, the only calves who experienced diarrhea did not receive probiotics. This may confirm the hypothesis that probiotics reduce diarrhea in pre-weaned calves, however as a result of the small sample size due to the onset of the COVID-19 pandemic, no statistical significance was found.

While we did find evidence of disease through these measurements, the use of the aforementioned scale to quantify data (as seen in Figure 1) was relatively subjective. Since multiple individuals were observing the calves weekly for any symptoms, this left room for interpretation of the severity of the diarrhea, coughing, nasal discharge, or other symptoms. Additionally, the use of Rumensin® medicated feed may have reduced the incidence of diarrhea and respiratory symptoms in the calves. Further research would be required to examine the effects of preventative antibiotic use when paired with probiotics.

Future Studies

While the study confirmed the findings of previous research related to probiotic use, growth and decreased incidence of disease, further studies should seek to resolve minor faults in the procedure and further examine breed interactions. For example, by utilizing a body weight scale rather than the weigh tape, this would eliminate human error in measurements and allow for increased accuracy in determination of growth rates. Additionally, by using calves of only one breed or ensuring an equal number of Holsteins and Jerseys in each group, we would be better able to see the impact of breed on other measures. Similarly, the housing of calves (hutches versus indoors) was not controlled for by breed or group and was not recorded throughout the study. While the impacts of housing were not considered in this study, it could be controlled for or examined in future research.

Feed intake was initially measured by weighing the feed provided and subtracting what was left, in hopes of being able to determine daily grain intake. This data was later eliminated, as a result of inconsistent feed weight measurements for a number of reasons. It was found that each time the food was to be weighed and replenished, significant amounts of grain had ended up on the floor as a result of the calf playing with the feed bottle. By transitioning particularly difficult calves to buckets we were able to minimize some waste, but still felt that the measurements taken were not accurate. Additionally, there were inconsistencies in feed weight measurements as a result of numerous individuals participating in the data collection process. This resulted in quantities of "full" or "empty" being recorded, rather than specific weights in kilograms. While this allowed us to estimate the amount of grain consumed, we ultimately determined that feed intake could be eliminated without sacrificing the goals of the study. However, being able to better measure feed intake in future studies may prove beneficial in examining the rate of growth related to consumption between calves.

The COVID-19 pandemic played a major role in the progress of data collection, and the effects should be considered if future research was to be conducted. While we had initially aimed to include 100 calves in the study and hold the study over a longer period of time, no calves were added to the study after March 2020 due to facility shutdowns. We collected partial data from seven calves who were born prior to this, however these calves were not included in the analysis due to incomplete data sets. This limited us to 32 calves over a span of about 7 months. If similar studies were to be conducted in the future, it would be beneficial to include a larger population in order to improve accuracy in the data collected and increase the likelihood of statistical significance.

CONCLUSION

In conclusion, multistrain probiotic supplementation in pre-weaned dairy calves significantly influenced both hip height (P=0.0297) and wither height (P=0.0350) but did not have any effect on body weight or heart girth. This may have been related to error in data collection methods, but future research would be required to determine these implications. Significant differences in growth rates between the Jersey and Holstein calves in both the control and probiotic groups suggested that breed may play a role in these results. Probiotic use was also associated with decreased incidence of diarrhea, with few instances of diarrhea occurring in calves who received probiotics. However, further research using increased sample sizes would be required in order to see the full effects. The results of this study suggest that pre-weaned calves may benefit from Probios® supplementation. Future studies should consider using body weight scales and more secure feeding technology, to increase the accuracy of measurements overall and examine the effects of probiotic supplementation on feed intake and efficiency. Additionally, the sample size and duration of the study should be increased to allow for more concrete results, since this was not possible due to the effects of the pandemic. By exploring the impacts of probiotic supplementation on the growth and health of pre-weaned dairy calves, this may drive agricultural facilities to include probiotics in their management practices to increase growth and prevent disease.

FIGURES AND TABLES

Table 1: Milk Replacer Feeding Schedule (quarts/feeding): Milk replacer was provided three times daily to all calves from birth through Week 8. The amount of milk replacer (quarts per feeding) varied based on age and breed, as depicted below. Weaning began during Week 9, at which point all calves received 1 quart of milk replacer in the morning and warm water only at the afternoon and nightly feedings. At Week 10 the calves were all completely weaned, and their diet consisted of hay, grain, and water.

Breed	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
Holstein	1.5	1.5	2	2	2	2	1.5	1
Jersey	1	1	1.5	1.5	1.5	1.5	1	1

Table 2: Least square means of body structure growth measurements (cm)

	Treatment			Breed			P-Value		
Item	CON	PRO	SEM	Jersey	Holstein	SEM	Treatment	Breed	Treatment x Breed
Weight	1.0426	0.9817	0.07359	1.1233	0.9010	0.07359	0.4149	0.0055	0.1353
Heart girth	0.2573	0.2533	0.01860	0.2732	0.2374	0.01860	0.8325	0.0647	0.2445
Hip height	0.1581	0.2253	0.02921	0.1882	0.1952	0.02921	0.0297	0.8127	0.1617
Wither height	0.1543	0.1992	0.02019	0.1502	0.2033	0.02019	0.0350	0.0140	0.9977

Table 3: Health measurements by treatment group: Health measurements were collected weekly, and scores were given based on the visual aids provided in Figure 1. While other symptoms were analyzed weekly, only eye discharge, nasal discharge, and fecal abnormalities were observed over the course of the study.

Symptom	CON		PRO	
	Number of affected calves	Mean Score	Number of affected calves	Mean Score
Eye Discharge	4	1	8	1
Nasal Discharge	2	1	1	2
Fecal Abnormality	6	1.16	2	1.5

Figure 1: Calf Health Scoring Criteria: Calf health data for cough, nasal discharge, eye scores, and fecal scores were quantified over the duration of the study using the numerical system described in this graphic. Rectal temperature was not monitored in this study.

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Calf Health Scoring Criteria			
0	**1**	**2**	**3**
Rectal temperature			
100-100.9	101-101.9	102-102.9	≥103
Cough			
None	Induce single cough	Induced repeated coughs or occasional spontaneous cough	Repeated spontaneous coughs
Nasal discharge			
Normal serous discharge	Small amount of unilateral cloudy discharge	Bilateral, cloudy or excessive mucus discharge	Copious bilateral mucopurulent discharge
			
Eye scores			
Normal	Small amount of ocular discharge	Moderate amount of bilateral discharge	Heavy ocular discharge
			
Ear scores			
Normal	Ear flick or head shake	Slight unilateral droop	Head tilt or bilateral droop
			
Fecal scores			
Normal	Semi-formed, pasty	Loose, but stays on top of bedding	Watery, sifts through bedding
			

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