Northern New York Agricultural Development Program

2021-2022 Project Report

Effects of Kefir Supplementation During the First 21 Days of Life on Growth, Diarrhea Incidence, and Antibiotic Use in Holstein Calves

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Background

Diarrhea is the leading cause of death in calves less than 1 month of age (McGuirk, 2008); 56.4% of preweaned heifer mortality on US dairy farms was attributable to diarrhea or other digestive issues (USDA, 2018). Early stressors to the developing gastrointestinal tract due to diarrhea or the antimicrobials used to treat it can negatively impact the diversity and stability of the intestinal microbial population, increase disease susceptibility (Tao et al., 2020), and negatively affect absorption of nutrients (Araujo et al., 2015). Other gastrointestinal disturbances due to early-life infection can result in poor development, decreased performance in first lactation, and concerns regarding animal welfare (Cho and Yoon, 2014).

Public health and consumer concerns over increasing antimicrobial resistance have resulted in a push to reduce antimicrobials used on farms, especially in situations where they are not effective or necessary. A 2020 NNYADP-funded study titled "Determining the Enteropathogens Causing Neonatal Diarrhea and Associating it with Antibiotic Usage on NNY

Dairy Farms" found that of 72 calves treated with antimicrobials for diarrhea, only approximately 33% of these calves had bacterial diarrhea that would potentially respond to the treatment. Many options exist within the research sector to explore and evaluate preventative care strategies for the mitigation of enteric disease outbreaks, including supplementation with probiotics. While the supplementation of probiotics in liquid diets to improve health and growth of calves is well-documented (Timmerman et al., 2005; Renaud et al., 2019; Liang et al., 2020, Roodposhti and Dabiri, 2012; Casper et al., 2021), more data is necessary to further determine the effects, strains, and proper dosages of probiotics for use as a preventative strategy against neonatal calf diarrhea. Additionally, supplements need to be cost effective and easily implemented on-farm.

Kefir is a fermented milk beverage traditionally made from cows' milk that contains substantial amounts of probiotic bacteria, many of which have been identified as possessing antimicrobial properties. Lactic acid bacteria, such as *Lactobacillus* spp., are widely valued as enhancers of normal gut microflora against opportunistic pathogens. Kefir is relatively easy and inexpensive to prepare, and could serve as an efficient way to provide probiotic supplementation to calves on-farm. To date, only two other studies have examined supplementation of whole milk with kefir as a means of growth promotion and diarrhea reduction in individually-housed Holstein calves up to 70 days of age (Fouladgar et al., 2016; Satik and Günal, 2017). This NNYADP-funded research provides an opportunity to explore the potential benefits of probiotic use in calves under different environmental and housing conditions in use on regional farms, as well as further contribute to antibiotic stewardship efforts by farmers in Northern New York.

Methods

Research was conducted at three farms in Northern New York, each utilizing individual calf feeding and housing strategies, between April and September of 2022. All experimental procedures involving calves were approved by the Animal Care and Use Committee of the William H. Miner Agricultural Research Institute (2022AUR02).

- Farm A housed calves in individual pens bedded with sawdust and straw in a mechanically ventilated barn, with calf jackets used from April-May 2022.
- Farm B housed calves outdoors in hutches bedded with sand.
- Farm C housed calves outdoors in hutches bedded with sawdust.

A total of 140 Holstein heifers (n= 60 Farm A, n= 40 Farm B, n= 40 Farm C) were enrolled in the study, with the following feeding strategies:

- Farm A fed a commercial milk replacer (24% protein, 20% fat; Opti-Milk 4-Seasons, Poulin Grain Inc., Newport, VT) reconstituted to 13.6% solids at a maximum feeding level of 10 quarts per day.
- Farm B fed whole salable milk at a maximum rate of 12 quarts per day.
- Farm C fed pasteurized whole salable milk at a maximum rate of 12 quarts per day.

Farms A and B fed commercial calf starter (Alphaline Start Calf Starter, Poulin Grain Inc.) and Farm C fed a custom-milled grain mix ("Calf Crack"; Feed Commodities International, Malone, NY). Intake of milk replacer and starter was recorded daily on Farm A, but not on Farms B or C. Water was provided for ad libitum intake during the study on each farm, but intake was not measured. As per normal management, electrolytes (Bovine GoldLyte; BioVet, Barneveld, WI) were provided to every calf daily up to 14 d of age on Farm C. Electrolytes (Land O' Lakes Electrolyte System; Arden Hills, MN) were administered to calves showing signs of dehydration or diarrhea on Farm A.

After initial colostrum and transition milk feedings, calves on each farm were randomized to one of two treatments: a control (no supplementation) or 1/4 cup (60 mL) of kefir once daily at the morning feeding for the first 21 d of life. To maintain nutritional balance, 1/4 cup of salable whole milk was used as a control on Farm A. Farms were responsible for kefir fermentation and feeding. Kefir fermentation was performed by adding kefir grain to saleable whole milk in a glass jar, sealed, and allowed to ferment for 24 h at room temperature (68-78 °F). The kefir was strained into a clean plastic jug for feeding, and the grains were either placed into fresh whole milk to ferment another batch or stored refrigerated (39°F) in whole milk until next use.

Measurements

Feed intake and refusals. Intake (feed offered and feed refused) of milk replacer and starter were recorded for each calf daily through weaning on Farm A. Milk replacer was mixed and dispensed using a 150 L MilkTaxi (Holm & Laue, Westerrönfeld, Germany).

Growth. Body weight and stature measurements (hip height, wither height, heart girth, and body length) were measured within the first week of life (day (d) 2 on Farm A, 1-7 d on Farms B and C), weekly through weaning at Farm A, and at 4 and 8 weeks after first observation (4 and 8 weeks of age) at Farms B and C. Body weight was measured using digital platform scales, and average daily gain (ADG) was calculated at 4 and 8 weeks of age.

Health. To evaluate signs of diarrhea, dehydration, and respiratory disease, health scores (McGuirk, 2015) were recorded daily for each calf at Farm A and once weekly at Farms B and C. Fecal scores were assigned on a 1-4 scale 1 = normal and well formed, 2 = soft but still holds form, 3 = loose without form, and 4 = loose and soaks through bedding. Respiratory scores were assigned on a 1-5 scale: 1 = normal breathing, 2 = open mouth breathing, 3 = open mouth breathing with nasal discharge, 4 = dry cough, 5 = wet cough. Hydration scores were assigned on a 1-4 scale: 1 = normal, 2 = mild; skin tent <3 sec, 3 = moderate, skin tent 3-10 sec, eyes slightly recessed, 4 = severe, skin tent >10 sec, eyes recessed.

Blood. Blood samples were collected via jugular venipuncture during the first week of life (24-48 hours after birth on Farm A, 1-7 d at Farms B and C) and on d 21 for all three farms using 10-mL evacuated serum separation tubes (Becton Dickinson, Franklin Lakes, NJ). Blood was allowed to clot for 30 minutes (min) before centrifugation at 1,300 × *g* for 20 min to separate serum. Initial serum protein was measured from the sample collected during the first week of life with a handheld refractometer (Vet 360; Leica Microsystems Inc., Buffalo, NY).

Feed analysis. Samples of milk or milk replacer, calf starter, and kefir were collected weekly and composited by month for further analysis for the respective farms. Milk replacer was analyzed for DM, CP, fat, and ash by standard wet chemistry methods (Dairy One, Ithaca, NY). Starter was analyzed using wet chemistry techniques for DM, CP, lignin, ash, starch, NFC, and ME (Dairy One). Milk samples collected from all three farms were analyzed for fat, true protein, and solids by mid-infrared procedures (CombiScope FTIR 300 Hp; Delta Instruments, Drachten, The Netherlands).

Antibiotic Usage. All medical treatments and electrolytes given to each calf were recorded at each farm, as well as the reason for treatment.

Statistical Analysis. Due to differences in housing and feeding strategies, each farm was analyzed separately. Data was analyzed as a randomized, complete block design using the Statistical Analysis System (SAS 9.4; SAS Institute Inc., Cary, NC). Feed and milk analysis were summarized using the MEANS procedure of SAS and reported as descriptive statistics (mean ± standard deviation). Data for intake and growth were analyzed with mixed linear models using the MIXED procedure of SAS, with the fixed effects of treatment, time, and their interaction and block as a random effect. Calf within treatment was the subject for repeated measurements. Models for occurrence of health events and achievement of target weight at weaning were evaluated by logistic regression using a binomial distribution in SAS using the GLIMMIX procedure. The model for likelihood of diarrhea treatment was evaluated for logistic regression using a binomial distribution in SAS, and results reported as an odds ratio. Significance was declared at $P \leq 0.05$ and tendencies reported at $0.05 < P \leq 0.10$.

Results and Discussion

Nutrient Composition and Intake

Nutrient analysis of whole milk, milk replacer (MR), and calf starter are reported in Table 1. The milk replacer fed on Farm A was within the range of solids content of the whole milk fed at the other two farms (range 12.92-13.76%). Milk replacer dry matter intake (DMI) did not differ between treatment groups on Farm A ($P > 0.10$, Table 2). Interestingly, starter DMI in calves fed kefir increased beginning at week six and was greater at week 8 ($P < 0.01$) compared to calves in the control group (Figure 1). Consequently, these calves tended to have greater total DMI ($P = 0.06$, Figure 2), and significantly greater metabolizable energy (ME) and crude protein (CP) intakes at week 8 ($P = 0.05$).

While treatment did not affect cumulative preweaning starter intake ($P > 0.10$), calves in the kefir treatment group consumed nearly six pounds more starter than calves in the control group during the preweaning period. Kefir was only fed for the first 21 days of life, yet there may be evidence of positive residual effects of supplementation that merit further exploration. Considerations include reduced weaning stress and accelerated development of the small intestine to accommodate increased starter intake. Carbohydrate intake is critical to the development of the calf's gastrointestinal tract (Quigley, 2019) and the volatile fatty acid butyrate produced from carbohydrate fermentation is essential to rumen development (Laarman et al., 2012). Growth and stature measurements were not recorded post-weaning, so any effects of increased starter intake were not captured, but should be in future study.

Growth

Treatment did not affect body weight (Figure 5) or ADG (Figure 6) on Farms A, B, or C. Industry standard for calf weight gain is to double birth weight by 56 d of age (Dairy Calf and Heifer Gold Standards), which was achieved on both Farms A and C irrespective of treatment ($P > 0.10$). Calves fed kefir on Farm B were more likely to meet or surpass their target weight at eight weeks of age ($P = 0.02$) than calves fed a control. Wither height, heart girth, and body length were also not different between treatments ($P > 0.10$).

While hip heights were not different at Farms A and C, calves fed kefir at Farm B tended to have greater hip height ($P = 0.06$; Figure 7).

Feed Efficiency

Feed efficiency was calculated as gain:feed (lb/lb) for calves at Farm A (Figures 3 and 4). Gain per lb of MR DMI did not differ between treatments ($P > 0.10$), nor did gain per lb of total DMI (MR + starter). The increase in starter DMI in kefir-supplemented calves did not translate to improved feed efficiency.

Health

Average initial total protein on Farms A and B was above the recommended cutoff of 5.5 g/dL, indicating successful passive transfer of antibodies. Farms A and B averaged 6.2 ± 0.8 g/dL. An equipment malfunction with the handheld refractometer was reported by Farm C, so initial total protein was not summarized. As this study intended to study potential health effects, calves with initial total protein below 5.5 g/dL were not excluded. No differences in fecal, respiratory, or hydration scores were observed between treatment groups at each of the three farms, and average fecal scores were not elevated (Table 3).

Cumulative observations of diarrhea in calves at each farm did not differ between treatments, nor did likelihood of medical intervention for scours ($P > 0.10$). Because each farm is unique in terms of environment and disease challenges, it is critical to recognize the relationship between management practices and calf health (Wells et al., 1996) and that challenges to the calf's immune system may cause energy required for maintenance and growth to be partitioned elsewhere to fight off a health event.

A bout of diarrhea is classified as a fecal score >2 for 3 or more consecutive days. At Farm A, diarrhea bouts occurred most frequently around 15 days of age, which is a common infection period for the diarrhea-causing agents *E. coli*, *Cryptosporidium,* rotavirus, coronavirus, and *Clostridium* (Heller and Chigerwe, 2017). At Farms B and C, a similar pattern was observed, with diarrhea bouts occurring on or after day 10 of age. This observation, coupled with the lack of differences in fecal scores between treatments at each farm, indicates that supplementation with kefir did not improve diarrhea incidence during the first 21 days of life.

Antibiotic Usage

The need for antibiotic use was not different between treatments on all three farms ($P > 0.10$).

Conclusions and Outcomes

While supplementation of liquid diet with 1/4 cup (60 mL) kefir had limited improvement on growth, diarrhea incidence, or need for antibiotic use during the first 21 d of life, the increased starter DMI observed in calves receiving kefir on Farm A suggests a potential metabolic or developmental benefit to the calf that could be explored with future research. As questions still remain regarding optimal dosage, duration, and mode of action of probiotic supplementation, as well as the potential of kefir as a cost-effective option, there is opportunity to continue this research on farms in support of antibiotic stewardship efforts. At least one farm is continuing to use kefir as part of their feeding strategy, and each farm found implementing the task of preparing and feeding kefir into their daily routine to be simple.

Education and Outreach

Project leaders have encountered great interest in the outcomes of this project-

- Results from this study were shared with producers in the six-county NNY region at both Miner Institute and Cornell Cooperative Extension Dairy Days. A recording of the presentation will be made available on YouTube in the near future.
- Project results will also be presented at the 2023 American Dairy Science Association annual meeting (Ottawa, Ontario, Canada) and the 2023 Smart Calf Rearing Conference (Kaiserslautern, Germany).
- A manuscript will be submitted to a peer-reviewed journal for publication.
- Results will be summarized in a Miner Institute Farm Report and will be posted on the NNYADP website for public access.

Acknowledgements

We would like to thank the three farms that collaborated on this study, as well as Casey Havekes for her time, support, and enthusiasm for the project. We also thank the dairy producers of Northern New York for their interest in and dedication to improving animal health, and the Northern New York Agricultural Development Program for funding this research.

For More Information

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APPENDIX

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Effects of Kefir Supplementation During the First 21 Days of Life on Growth, Diarrhea incidence, and Antibiotic use in Holstein Calves

Table 1. Nutrient analysis (mean ± standard deviation) on a dry matter (DM) basis of whole milk, calf starter, and milk replacer collected during the preweaning period on three dairy farms feeding either a control (CON; 60 mL salable whole milk for Farm A or nothing for Farm B and C) or 60 mL kefir (KEF) once daily in whole milk or milk replacer for the first 21 days of life. Whole milk was used to ferment kefir on all three farms, and fed to calves on Farms B and C. NNYADP Effects of Kefir Supplementation During the First 21 Days of Life on Growth, Diarrhea Incidence, and Antibiotic Use in Holstein Calves project, 2022.

	Farm A	Farm B	Farm C
Whole Milk			
Fat, %	4.12 ± 0.23	3.82 ± 0.13	4.69 ± 0.23
True Protein, %	2.99 ± 0.04	3.08 ± 0.05	3.33 ± 0.03
Solids, %	12.92 ± 0.19	12.66 ± 0.15	13.76 ± 0.15
Somatic Cell Count	95.53 ± 71.84	129.54 ± 55.21	80.50 ± 20.51
Calf Starter			
DM, %	87.30 ± 0.54	87.23 ± 0.17	87.97 ± 0.90
Fat, % DM	5.06 ± 0.18	4.93 ± 0.17	6.90 ± 0.10
Crude Protein, % DM	26.58 ± 0.16	25.03 ± 1.38	22.13 ± 1.13
Lignin, % DM	2.84 ± 0.38	2.38 ± 0.52	2.60 ± 0.36
Starch, % DM	16.34 ± 1.00	18.40 ± 2.60	26.03 ± 1.36
Ash, % DM	8.18 ± 0.25	7.53 ± 0.26	5.93 ± 0.45
Non-fiber Carbohydrates, % DM	39.16 ± 0.83	45.85 ± 2.66	42.87 ± 1.94
Milk Replacer			
DM, %	96.78 ± 0.15	--	--
Fat, % DM	23.02 ± 1.17	--	--
Crude Protein, % DM	24.12 ± 0.25	--	--
Ash, % DM	7.34 ± 0.17	--	--

Table 2. Intake on a dry matter basis recorded during the preweaning period for dairy calves on Farm A receiving either a control (CON; 60 mL salable whole milk) or 60 mL kefir (KEF) once daily in milk replacer for the first 21 days of life. NNYADP Effects of Kefir Supplementation During the First 21 Days of Life on Growth, Diarrhea Incidence, and Antibiotic Use in Holstein Calves project, 2022.

	Treatment			*P*-values		
Variable	CON	KEF	SEM[1]	Treatment	Time	Treatment × Time
Milk replacer dry matter intake (DMI), lb/d	2.16	2.16	0.01	0.51	<0.01	0.98
Starter DMI, lb/d	0.61	0.70	0.04	0.15	<0.01	<0.01
Preweaning cumulative starter intake, lb	37.70	43.44	2.60	0.12	--	--
Total DMI, lb/d	2.78	2.87	0.02	0.13	<0.01	0.06
Total metabolizable energy, kcal/d	4145	4287	70	0.14	<0.01	0.05
Total crude protein, g/d	310	321	5	0.13	<0.01	0.05

[1] Standard error of the mean.

Table 3. Health summary during the preweaning period for dairy calves at three farms receiving either a control (CON; 60 mL salable whole milk for Farm A or nothing for Farm B and C) or 60 mL kefir (KEF) once daily in whole milk or milk replacer for the first 21 days of life. NNYADP Effects of Kefir Supplementation During the First 21 Days of Life on Growth, Diarrhea Incidence, and Antibiotic Use in Holstein Calves project, 2022.

	Farm A					Farm B					Farm C				
	CON	KEF	SEM[1]	CI[2]	*P*-value	CON	KEF	SEM[1]	CI[2]	*P*-value	CON	KEF	SEM[1]	CI[2]	*P*-value
Average Fecal Score[3]	1.56	1.57	0.04	--	0.77	1.69	1.66	0.06	--	0.71	1.60	1.59	0.07	--	0.86
Average Respiratory Score[3]	1.00	1.00	<0.01	--	0.60	1.00	1.00	0.01	--	1.00	1.00	1.00	<0.01	--	0.31
Average Hydration Score[3]	1.01	1.02	<0.01	--	0.43	1.04	1.05	0.02	--	0.86	1.06	1.02	0.02	--	0.13
% calves with diarrhea	7.35	7.05	0.07	--	0.62	8.52	8.20	0.21	--	0.80	9.57	8.95	0.22	--	0.75
Cumulative days with diarrhea[4]	7.93	7.61	--	6.18-9.38	0.77	1.21	1.21	0.19	0.82-1.92	0.82	1.36	1.53	--	0.93-2.20	0.66
Likelihood of diarrhea treatment[5]	--	0.45	--	0.04-4.92	0.50	--	0.54	--	0.10-3.05	0.49	--	1.11	--	0.34-3.65	0.86

[1]Standard error of the mean
[2]Confidence interval
[3]Rated on a 1-4 scale (McGuirk, 2015): 1=normal, 4=severe.
[4]Observations conducted daily on Farm A and weekly on Farms B and C.
[5]Reported as odds ratio with control as the reference.

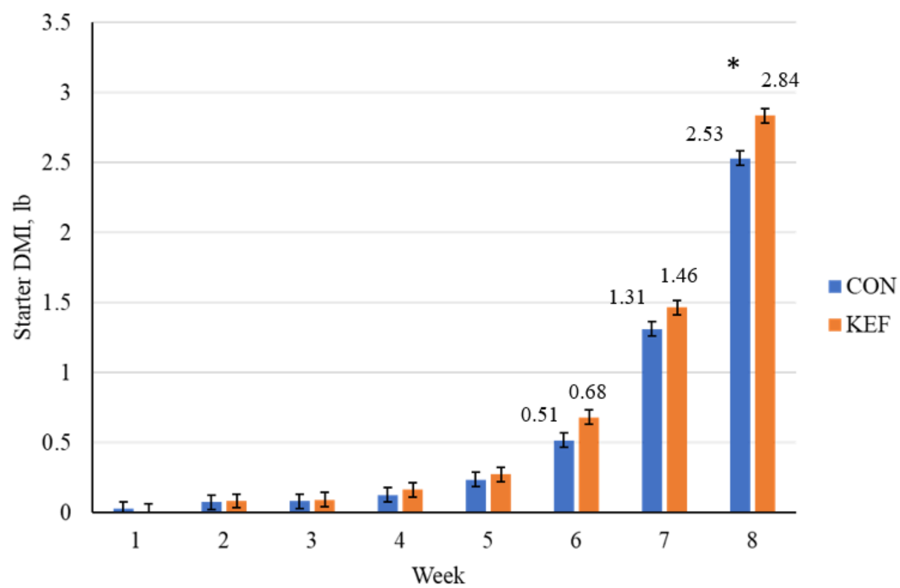

Figure 1. Calf starter dry matter intake (DMI) during the preweaning period recorded for dairy calves at Farm A receiving either a control (CON; 60 mL salable whole milk) or 60 mL kefir (KEF) once daily in milk replacer for the first 21 days of life. Asterisk indicates significant differences in starter DMI at $P \leq 0.05$ observed at week 8. NNYADP Effects of Kefir Supplementation During the First 21 Days of Life on Growth, Diarrhea Incidence, and Antibiotic Use in Holstein Calves project, 2022.

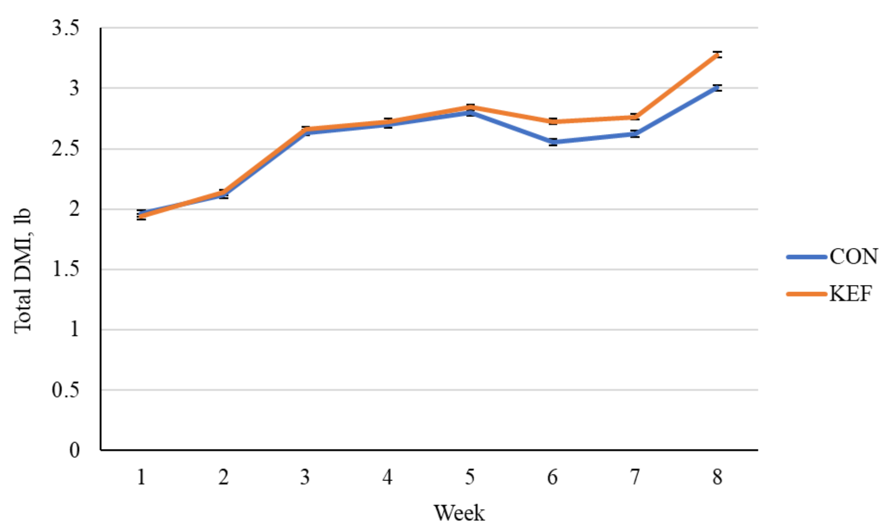

Figure 2. Total dry matter intake (DMI; milk replacer and starter) during the preweaning period recorded for dairy calves at Farm A receiving either a control (CON; 60 mL salable whole milk) or 60 mL kefir (KEF) once daily in milk replacer for the first 21 days of life. NNYADP Effects of Kefir Supplementation During the First 21 Days of Life on Growth, Diarrhea Incidence, and Antibiotic Use in Holstein Calves project, 2022.

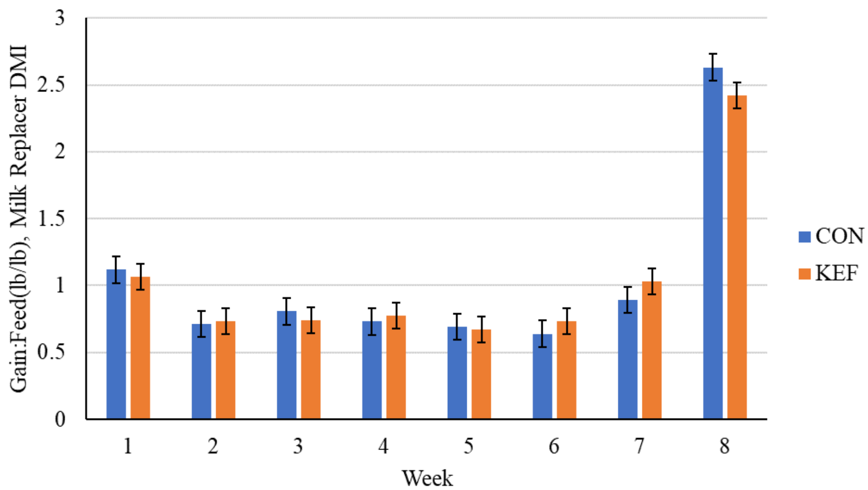

Figure 3. Feed efficiency of milk replacer dry matter intake (DMI) expressed as a ratio of gain:feed (lb of gain/lb of DMI) during the preweaning period for dairy calves at Farm A receiving either a control (CON; 60 mL salable whole milk) or 60 mL kefir (KEF) once daily in milk replacer for the first 21 days of life. NNYADP Effects of Kefir Supplementation During the First 21 Days of Life on Growth, Diarrhea Incidence, and Antibiotic Use in Holstein Calves project, 2022.

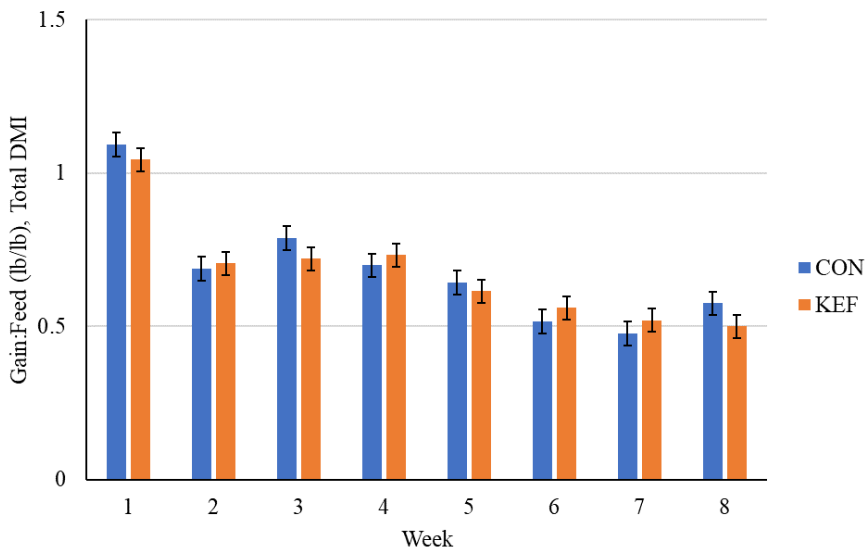

Figure 4. Feed efficiency of total dry matter intake (DMI; milk replacer + starter) expressed as a ratio of gain:feed (lb of gain/lb of DMI) during the preweaning period for dairy calves at Farm A receiving either a (CON; 60 mL salable whole milk) or 60 mL kefir (KEF) once daily in milk replacer for the first 21 days of life. NNYADP Effects of Kefir Supplementation During the First 21 Days of Life on Growth, Diarrhea Incidence, and Antibiotic Use in Holstein Calves project, 2022.

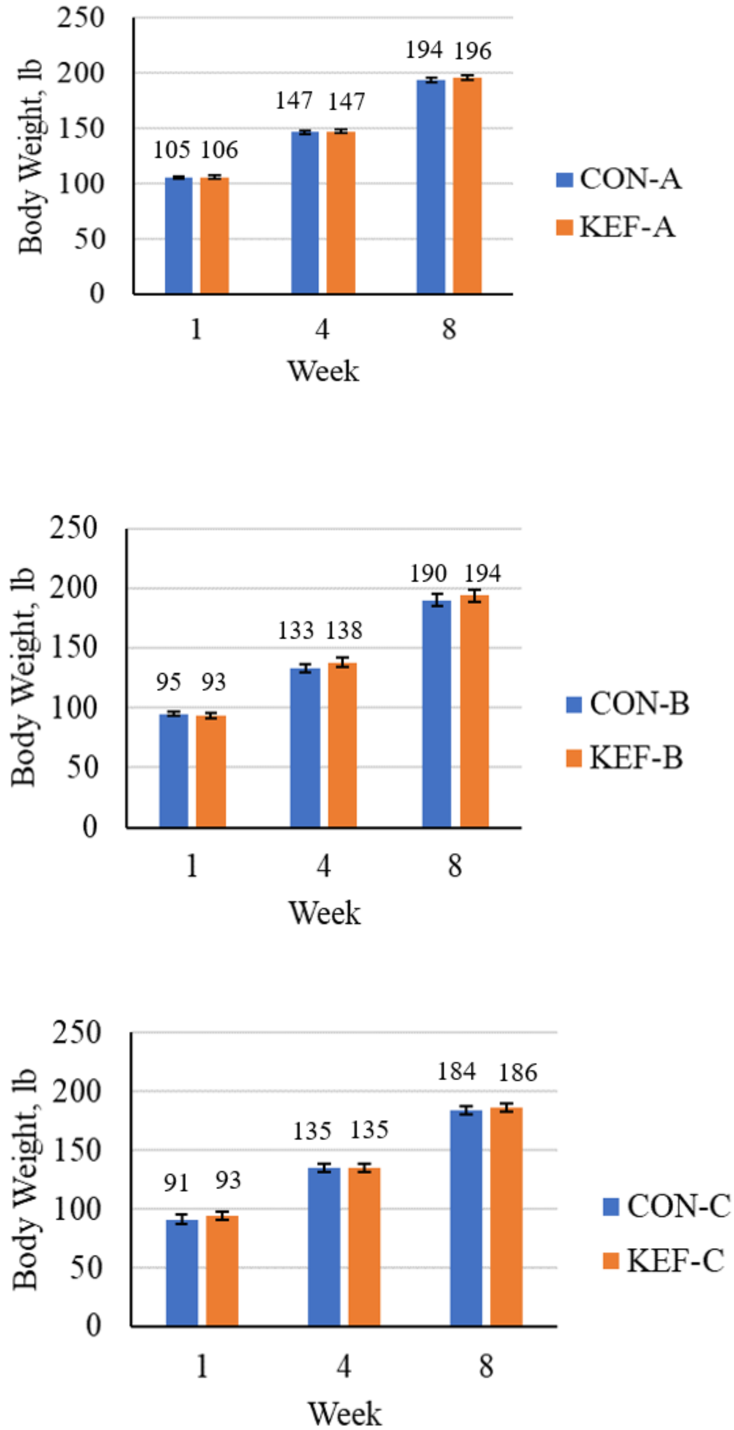

Figure 5. Body weights (lb) during the preweaning period of dairy calves at three farms (A, B, C) receiving either a control of 60 mL whole milk (Farm A) or no supplementation (Farms B and C; CON) or 60 mL kefir (KEF) once daily in whole milk or milk replacer for the first 21 days of life. NNYADP Effects of Kefir Supplementation During the First 21 Days of Life on Growth, Diarrhea Incidence, and Antibiotic Use in Holstein Calves project, 2022.

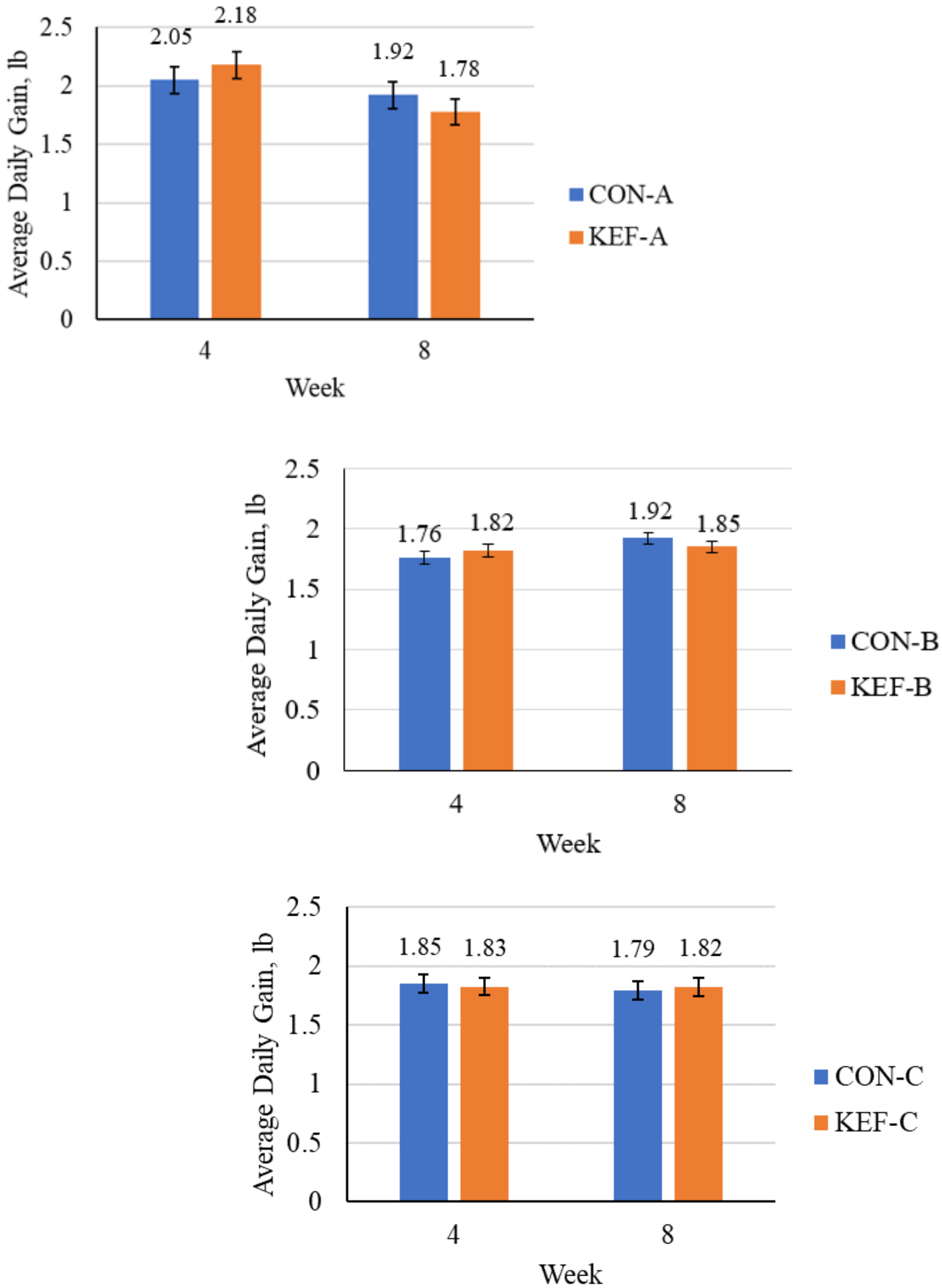

Figure 6. Average daily gain (lb) during the preweaning period of calves at three farms (A, B, C) receiving either a control of 60 mL whole milk (Farm A) or no supplementation (Farms B and C; CON) or 60 mL kefir (KEF) once daily in whole milk or milk replacer for the first 21 days of life. Average daily gain was calculated from initial measurement to week 4, and week 4 to week 8. NNYADP Effects of Kefir Supplementation During the First 21 Days of Life on Growth, Diarrhea Incidence, and Antibiotic Use in Holstein Calves project, 2022.

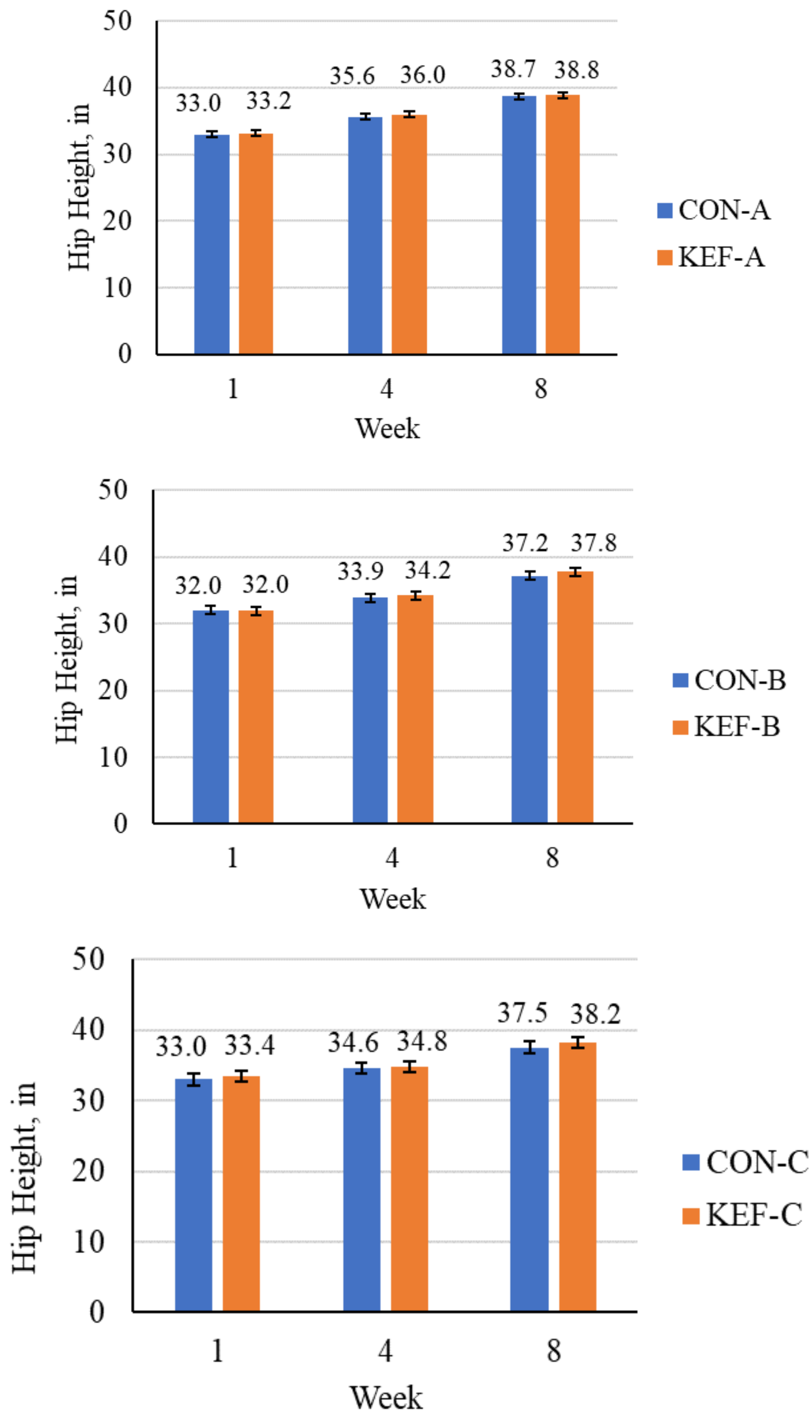

Figure 7. Hip heights (in) during the preweaning period of dairy calves at three farms (A, B, C) receiving either a control of 60 mL whole milk (Farm A) or no supplementation (Farms B and C; CON) or 60 mL kefir (KEF) once daily in whole milk or milk replacer for the first 21 days of life. NNYADP Effects of Kefir Supplementation During the First 21 Days of Life on Growth, Diarrhea Incidence, and Antibiotic Use in Holstein Calves project, 2022.

PHOTOS:

Left: Preparing kefir supplement for calves in the NNYADP-funded research evaluating kefir as a probiotic to enhance calf health. Right: Miner Institute personnel weigh a calf in the NNYADP-funded "kefir as a probiotic supplement" research project. Photos: Miner Institute