

ACTUAL TRENDS IN CALF FEEDING TECHNOLOGY AND THE USE OF PROBIOTIC FEED ADDITIVES IN CALF NUTRITION

DENISA POLÍVKOVÁ¹, GABRIELA VALTOŠOVÁ¹,
NGOZI MERCY UMEZURIKE-AMAHAH¹, ANNA ŠEBKOVÁ¹,
LUBOŠ ZÁBRANSKÝ¹, KATEŘINA SEZIMOVÁ¹,
JANA VACHOUŠKOVÁ¹, VOJTĚCH BRABENEC¹

¹Department of Animal Husbandry Sciences, Faculty of Agriculture
and Technology, University of South Bohemia, Studentská 1668, 370
05 České Budějovice, Czech Republic

Corresponding email address: polivd00@fzt.jcu.cz

ABSTRACT

The main aim of this study was to evaluate the effect of diverse probiotic strains on the health of the calves during the period of colostrum and milk feeding. The blood samples were also collected to check the level of immunoglobulins in the blood plasma and for the blood count test.

Not only all groups significantly surpassed the normal BRIX value which is 8.4 % BRIX but also all calves that received *Bifidobacterium* supplementation had higher BRIX levels, indicating that *Bifidobacterium* supports effective passive immunity transfer.

In all three weighing there was found no statistical difference observed in average weight gain between groups. However, in the second and third weighing, the BEL combination still showed higher weight gain than any other experimental group.

As for the frequency of diarrheal diseases, there were no significant differences between individual groups. Nevertheless, the experimental animals receiving single-strain *Lactobacillus* had a higher incidence of diarrhea compared to the control animals, meanwhile the opposite was observed in the *Bifidobacterium* experimental group.

This study shows that supplementation with single-strain or combination probiotics did not significantly affect weight gain, BRIX levels, or the incidence of diarrheal diseases in calves. Despite a numerical increase in weight gain with combination probiotic supplementation, these differences were not statistically significant.

Keywords: calf nutrition, probiotic supplementation, feed supplements

INTRODUCTION

The maternal and fetal blood supplies are separated due to the cow's placenta, preventing the utero transmission of protective immunoglobulins. This makes the intake of colostrum after birth crucial for calves, as it contains immunoglobulins that provide passive immunity until their immune system becomes functional (Godden, 2008). The efficient transfer and absorption of antibodies are influenced by factors such as the method and volume of colostrum intake, immunoglobulin concentration, and timing of colostrum ingestion (Weaver et al., 2008). Low immunoglobulin concentration is associated with high mortality rates due to infections (Blum, 2006).

Newborn calves have poor immune capability, making them vulnerable to infections (Mukhtar et al., 2015). Calf diarrhea is a common issue that leads to productivity and economic losses for cattle producers. It is a multifactorial disease caused by infectious agents like *Cryptosporidium*, *Clostridium perfringens*, *Escherichia coli*, and

Salmonella, as well as non-infectious factors such as poor sanitation, insufficient colostrum intake, and inappropriate environmental conditions (Cho & Yoon, 2014; Caffarena et al., 2021; Muktar et al., 2015).

The gastrointestinal health of calves plays a crucial role in disease control, with the gastrointestinal microbiota being a key factor. The gastrointestinal tract (GIT) undergoes rapid changes in structure and microbiota composition during early life (Meale et al., 2017). In newborn calves, the GIT is not fully developed, with the abomasum being the main site of digestion. The development of the forestomach, influenced by the intake and quality of solid feed, can take several months after birth (Górka et al., 2018; Guilloteau et al., 2009).

The GIT of calves is believed to be sterile in utero, with colonization occurring during and after birth. This colonization is vital for the maturation of the immune system, influencing the health and performance of the young calves (Klein-Jöbstl et al., 2019; Malmuthuge et al., 2015). In pre-weaned calves, bacterial groups such as *Bifidobacterium*, *Lactobacillus*, *Fecalibacterium*, and *Enterococcus* are present in fecal samples (Malmuthuge & Guan, 2017). As the forestomach develops, the rumen's microbiome evolves to include bacteria, archaea, protozoa, and fungi, with microbial composition depending on factors such as host age, diet, season, and geographic region (Malmuthuge et al., 2015; Dill-McFarland et al., 2019).

Diarrhea is a leading cause of morbidity and mortality in calves, typically managed with oral antimicrobials. However, associated variable efficacy and rising concerns about antimicrobial resistance makes it an unsuited solution (Smith, 2015). Hence, alternatives like probiotics are being explored. Probiotics are live, nonpathogenic

microorganisms that improve gastrointestinal microbial balance in the GIT (Williams, 2010). Studies show that calves fed probiotic strains such as *Lactobacillus plantarum*, *L. casei*, *Enterococcus* spp., *Bacillus* spp., *Bifidobacterium* spp., and *Saccharomyces cerevisiae* exhibit better health, reduced neonatal diarrhea, and increased growth (Wang et al., 2022; Stefańska et al., 2021).

This study aims to evaluate the effect of selected single and combined probiotic strains on calf growth and prevention of digestive disorders in calves. This research would provide more insight into the potential benefits of probiotics in calf rearing.

MATERIAL AND METHODS

An experiment was performed with a total of 300 calves, randomly assigned to three groups. Each group was further divided into an experimental group and a control group. The first group, L, was fed with probiotics containing the single strain *Lactobacillus sporogenes*, with a total of 83 calves. The second group, B, was also fed with a single strain probiotic containing *Bifidobacterium bifidum* and a total of 70 calves, equally divided into control and experimental groups. The third group, BEL, was given probiotics containing a mixture of *Bifidobacterium bifidum*, *Enterococcus faecalis*, and *Lactobacillus sporogenes*, represented by 147 calves, equally divided into control and experimental groups.

Calves were left with their mother for no longer than two hours after birth. They were then moved to a clean, disinfected outdoor box with straw bedding and weighed. At least two hours after calving, calves received their first drink of frozen colostrum, slowly heated to 39°C with a volume of at least 2.5 liters. The quality of freshly obtained

colostrum was measured using a refractometer. Calves in the experimental group L were given a probiotic pill dissolved in milk from the first feeding with colostrum to the third day of life. Calves in the experimental group B received 3 g of *Bifidobacterium bifidum* before the first feeding with colostrum and subsequently for 21 days, always in the morning feeding. Calves in the experimental group BEL were given a probiotic mixture at the first feeding and subsequently for 5 days, in a dose of 3 g, always before the morning feeding.

Between the third and fifth days, blood was taken from the jugular vein of the calves to obtain samples for immunoglobulin level checks in blood plasma and for laboratory determination of the blood count test. Blood centrifugation was performed at 2000 RPM to obtain plasma for total protein level measurement using a digital refractometer. Blood samples were collected into tubes containing sodium EDTA and sodium fluoride for blood count test and into tubes with Heparin for biochemical analysis. Samples were mixed with anticoagulants immediately after collection, placed in a cooling box, and transported to the laboratory. Biochemical analysis was performed using an Ellipse Dialab device, and a blood count was conducted using an Exigo LABtechnik device.

To monitor weight gain, which was a crucial aspect of the experiment, calves were weighed when moved from the farrowing box to an outdoor individual box at approximately 30 days of age, weighed a second time when moved to group pens at approximately 70 days. Last weighing was at approximately 150 days of age, when calves were at the end of weaning and were completely on a plant-based diet. A two-wheeled cart with built-in tensometric scales was used.

Fresh fecal samples were collected by-hand from the rectum of animals at a depth of 5cm using clean gloves and placed in a sterilized plastic tube. Immediately after collection, samples were stored in a refrigerator at a temperature of -4°C and transported to the lab.

In describing statistics, we used relative frequencies for categorical variables. Within descriptive statistics, we use valid n , min, median, mean, max, and SD for numerical characteristics. The chi-square goodness-of-fit test was used to detect differences in categorical variables by role in the experiment or by Brix level (≤ 8.4 and ≥ 8.4). To find differences in the numerical characteristics by role in the experiment or according to the Brix level (below 8.4 and 8.4 and over), the method "Compare mean" and t-test were used. All available data were tested; only statistically significant connections are shown in the next part of the presentation. The level of significance for this type of statistic is 0.05 in both cases (< 0.001; $0.001-0.01$; $0.01-0.05$...).

RESULTS AND DISCUSSION

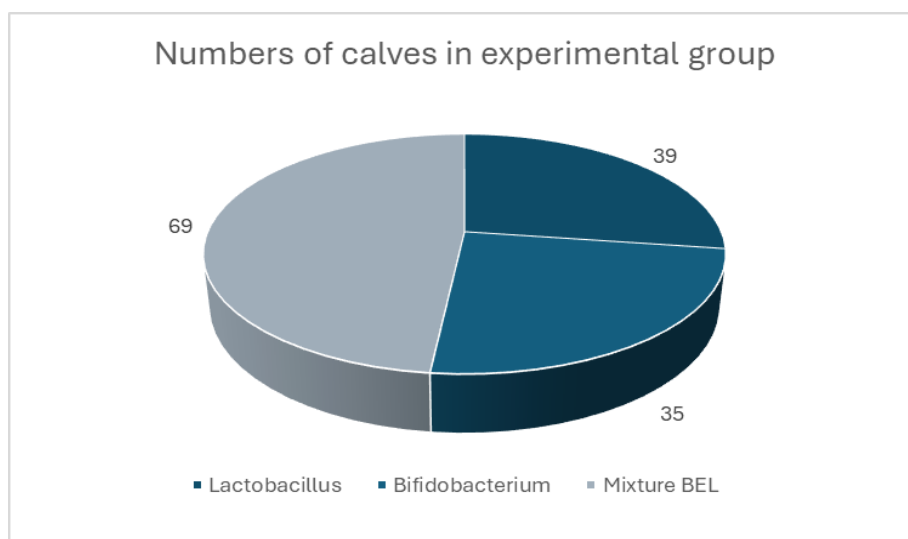


Figure 1. Summary of number of experimental calves in each group

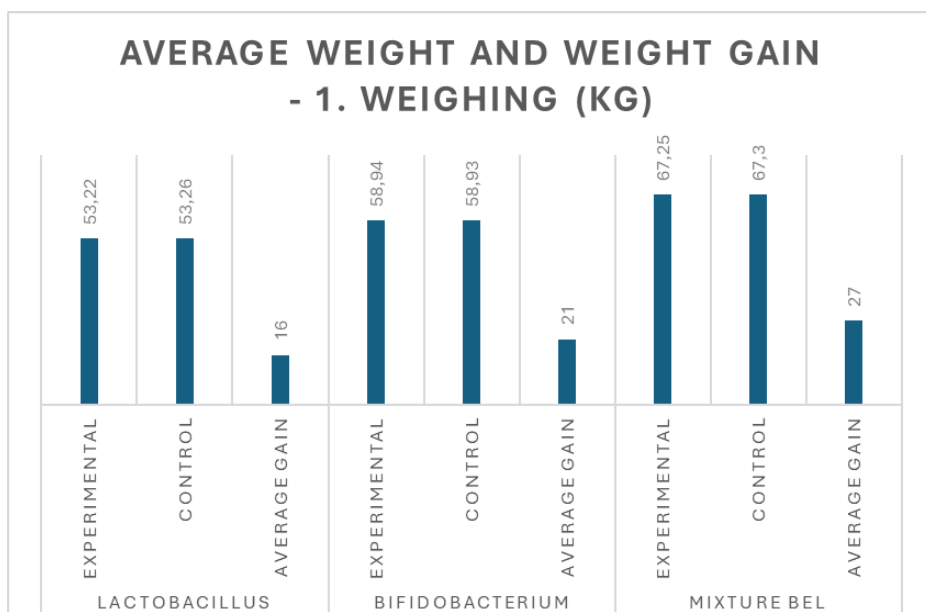


Figure 2. Average weight and weight gain at first weighing

Calves were weighed approximately 30 days at initial weighing. Average weight gain is diameter of all weight gains in each group. We found no statistical difference observed between groups (Figure 2). Both the experimental and the control animals in each group exhibited similar results, showing no significant difference between them.

At second weighing, calves were approximately 70 days of age. We found no statistical difference observed between groups with respect to average weight gain (Figure 3). Again, the experimental and control animals within each group responded similarly with no difference. The BEL combination consistently showed higher weight gain than any other experimental group which might suggest its potential efficacy in promoting growth.

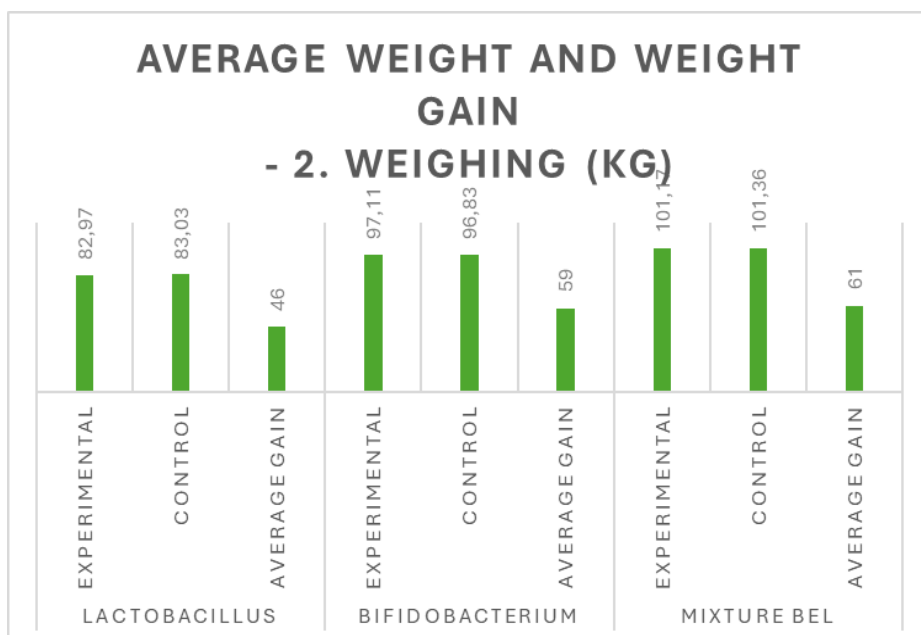


Figure 3. Average weight and weight gain at second weighing

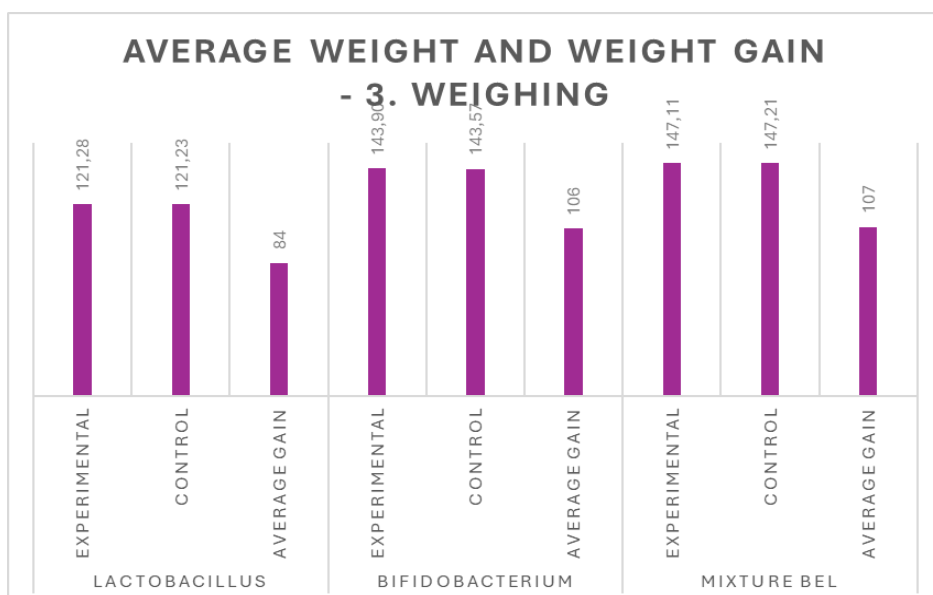


Figure 4. Average weight and weight gain at third weighing

We observed no statistically significant differences in average weight gain between the groups (Figure 4). At the final weighing, both the control and experimental animals in each group were within the same

weight range. Although numerically higher, the weight gain in the experimental animals of BEL combination was not significantly different from that of animals receiving single-strain treatments. This finding is consistent with Guo et al. (2022), who reported no significant differences in body measurement parameters of calves using a multi-strain probiotic over a 30-day period. Additionally, Stefańska et al. (2021) and Fisher et al. (2023) found no significant improvements in growth or live weight gain in their studies on probiotics and multispecies probiotic combinations for dairy calves. Compared to a study carried out by Zábranský et al. (2022) who discovered the positive effect of the combination of *Lactobacillus sporogenes*, *Enterococcus faecium* and *Bifidobacterium bifidum* on live weight and the calves had the highest live gain compared to other groups.

The physiologically normal value for immunoglobulins in blood serum is 8.4% BRIX (Deelen et al., 2014), though it can also be expressed in grams per liter depending on the methodology. The efficient transfer and absorption of antibodies are influenced by factors such as the method and volume of colostrum intake, immunoglobulin concentration, and timing of colostrum ingestion (Weaver et al., 2008). In this study, all calves that received *Bifidobacterium* supplementation had BRIX levels to and above the required threshold, indicating that *Bifidobacterium* supports effective passive immunity transfer. Gaspers et al. (2014) found a correlation between higher birth weights and improved passive transfer of immunoglobulins. On average, all groups significantly surpassed the normal BRIX value, suggesting a positive correlation.

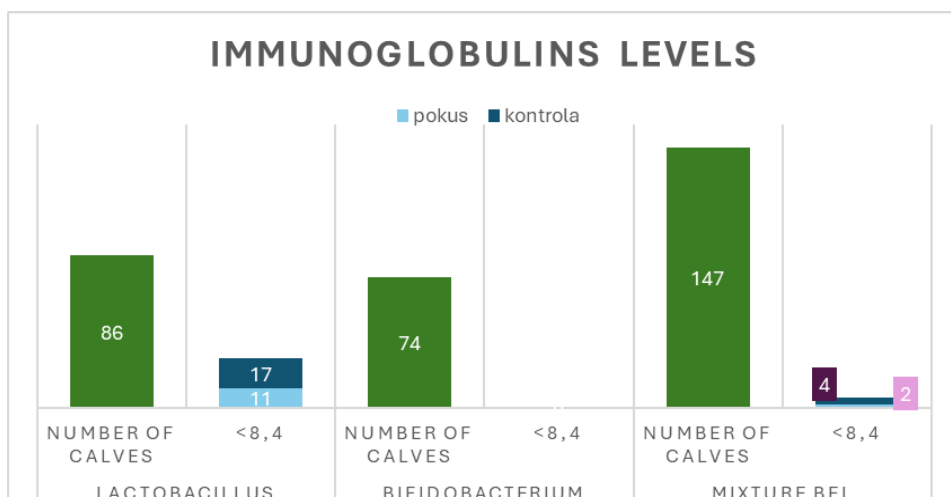


Figure 5. Evaluation of the level of total protein in the blood serum

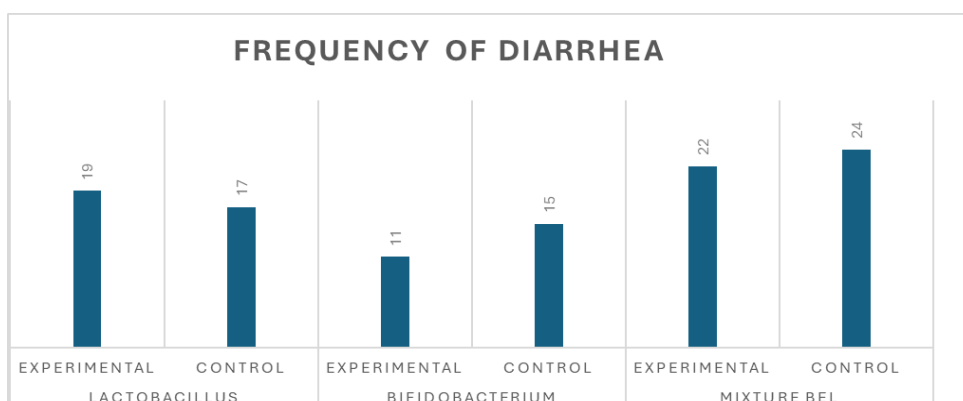


Figure 6. Frequency of diarrhea

The graph below (Figure 6) illustrates the number of diarrheal cases in each treatment group, showing no significant differences. Interestingly, the experimental animals receiving single-strain *Lactobacillus* had a higher incidence of diarrhea compared to the control animals, while the opposite was observed in the *Bifidobacterium* experimental group.

Previous research suggests that multi-strain probiotics are more effective than single strains. *Lactobacillus* inhibits pathogenic bacteria by lowering the pH in the large intestine and competitively attaching to the gut lining (Riddle et al., 2010), which generally supports a healthier gut environment and reduces harmful microbes. *Bifidobacterium*

bifidum disrupts the invasion of host epithelial cells, produces bifidocin to induce cell death in gram-positive bacteria, and synthesizes exopolysaccharides that inhibit pathogen growth (Sarkar and Mandal, 2016). Despite these theoretically beneficial mechanisms, our study found no reduction in diarrhea incidence with the BEL combination.

This report is consistent with the research conducted by Renaud et al. (2019), who investigated the administration of multispecies probiotics and yeast bolus in calves, and Fisher et al. (2023), who assessed the effects of a multispecies probiotic on the health and performance of pre-weaned dairy calves. Both studies found no significant reduction in diarrhea incidence.

CONCLUSION

Our findings show that supplementation with single-strain or combination probiotics did not significantly affect weight gain, BRIX levels, or the incidence of diarrheal diseases in calves. Despite a numerical increase in weight gain with combination probiotic supplementation, these differences were not statistically significant. Specifically, neither individual strains (*Lactobacillus sporogenes*, *Bifidobacterium bifidum*) nor combinations (*Bifidobacterium bifidum*, *Enterococcus faecalis*, and *Lactobacillus sporogenes*) showed a significant impact.

We recommend further investigation into multispecies probiotic combinations in young ruminants, with a focus on selecting strains that may have synergistic effects. Future research should aim to clarify the mechanisms behind the varying results observed in this study compared to previous studies that reported beneficial effects. Such research will enhance the development of effective supplementation strategies.

ACKNOWLEDGEMENT

The project was supported by the NAZV QK1910438

REFERENCES

- Blum, J. W. (2006). Nutritional physiology of neonatal calves. *Journal of Animal Physiology and Animal Nutrition*, 90(1-2), 1-11.
- Caffarena, R. D., Casaux, M. L., Schild, C. O., Fraga, M., Castells, M., Maya, L., Corbellini, L. G., Riet-Correa, F., & Giannitti, F. (2021). Causes of neonatal calf diarrhea and mortality in pasture-based dairy herds in Uruguay: A farm-matched case-control study. *Veterinary Microbiology*, 52, 977-988.
- Cho, Y., & Yoon, K. (2014). An overview of calf diarrhea – infectious etiology, diagnosis, and intervention. *Journal of Veterinary Science*, 15(1), 1-17.
- Dill-McFarland, K., Weimer, P. J., Breaker, J. D., & Suen, G. (2019). Diet influences early microbiota development in dairy calves without long-term impacts on milk production. *Applied and Environmental Microbiology*, 85(e02141-18).
- Gaspers, J. J., et al. (2014). Relationship between birth weight and calving ease with passive transfer of immunoglobulins in neonatal beef calves. *North Dakota Beef Report*, 37-39.
- Godden, S. (2008). Colostrum management for dairy calves. *Veterinary Clinics of North America: Food Animal Practice*, 24(1), 19-39.
- Górka, P., Kowalski, Z. M., Zabielsko, R., & Guilloteau, P. (2018). Invited review: Use of butyrate to promote gastrointestinal tract development in calves. *Journal of Dairy Science*, 101(6), 4785-4800.
- Guilloteau, P., Zabielsko, R., & Blum, J. W. (2009). Gastrointestinal tract and digestion in the young ruminant: Ontogenesis, adaptations, consequences and manipulations. *Journal of Physiology and Pharmacology*, 60(3), 37- 46.
- Klein-Jöbstl, D., Quijada, N. M., Feldbacher, B., Wagner, M., Drilich, M., Schmitz-Esser, S., & Mann, E. (2019). Microbiota of newborn calves and their mothers reveals possible transfer routes for newborn calves' gastrointestinal microbiota. *PLOS ONE*, 14(8), e0220554.
- Malmuthuge, N., Griebel, P. J., & Guan, L. L. (2015). The gut microbiome and its potential role in the development and function of newborn calf gastrointestinal tract. *Frontiers in Veterinary Science*, 2, 36.

- Malmuthuge, N., & Guan, L. L. (2017). Understanding the gut microbiome of dairy calves: Opportunities to improve early-life gut health. *Journal of Dairy Science*, 100(7), 5996-6005.
- Muktar, Y., Mamo, G., Tesfaye, B., & Belina, D. (2015). A review on major bacterial causes of calf diarrhea and its diagnostic method. *Journal of Veterinary Medicine and Animal Health*, 7(5), 173-185.
- Meale, S. J., Chaucheyras-Durand, F., Berends, H., Guan, L. L., & Steele, M. A. (2017). From pre- to postweaning: Transformation of the young calf's gastrointestinal tract. *Journal of Dairy Science*, 100(7), 5984-5995.
- Stefańska, B., Sroka, J., Katzer, F., Goliński, P., & Nowak, W. (2021). The effect of probiotics, phytobiotics and their combination as feed additives in the diet of dairy calves on performance, rumen fermentation and blood metabolites during the preweaning period. *Animal Feed Science and Technology*, 272, 114738.
- Wang, H., Yu, Z., Gao, Z., Li, Q., Qiu, X., Wu, F., Guan, T., Cao, B., & Su, H. (2022). Effects of compound probiotics on growth performance, rumen fermentation, blood parameters, and health status of neonatal Holstein calves. *Journal of Dairy Science*, 105(3), 2190-2200.
- Weaver, D. M., Tyler, J. W., VanMetre, D. C., Hostetler, D. E., & Barrington, G. M. (2008). Passive transfer of colostral immunoglobulins in calves. *Journal of Veterinary Internal Medicine*, 14(6), 569-577.
- Williams, N. T. (2010). Probiotics. *American Journal of Health-System Pharmacy*, 67(6), 449-458.
- Guo, Y., Li, Z., Deng, M., Li, Y., Liu, G., Liu, D., Liu, Q., Liu, Q., & Sun, B. (2022). Effects of a multi-strain probiotic on growth, health, and fecal bacterial flora of neonatal dairy calves. *Animal Bioscience*, 35(2), 204-216.
- Renaud, D. L., Buss, L., Wilms, J. N., Han, J. I., & Steele, M. A. (2019). Administration of a multispecies probiotic and a monospecies yeast product to dairy calves: Effects on health and growth performance. *Journal of Dairy Science*, 102(11), 10303-10312.
- Fischer, A. J., Song, Y., He, Z., Haines, D. M., Guan, L. L., & Steele, M. A. (2023). Impact of a multispecies probiotic on the health and performance of preweaned dairy calves. *Journal of Dairy Science*, 106(4), 2728-2743.
- Riddle, M. S., et al. (2010). Probiotic therapy for the prevention of travelers' diarrhea: A systematic review and meta-analysis.

American Journal of Tropical Medicine and Hygiene, 82(2), 365-373.

Sarkar, P., & Mandal, S. (2016). Bacteriocins: A review of their applications in food preservation and as therapeutic agents. *International Journal of Food Science and Technology*, 51(7), 1426-1439.

Zábranský, L., Poborská, A., Gálik, B., Šoch, M., Brož, P., Kantor, M., Kernerová, N., Řezáč, I., Rolinec, M., Hanušovský, O., Strnad, L., Havrdová, N. (2022). Influence of Probiotic Strains *Bifidobacterium*, *Lactobacillus*, and *Enterococcus* on the Health Status and Weight Gain of Calves, and the Utilization of Nitrogenous Compounds. *Antibiotics*, 11(9):1273.