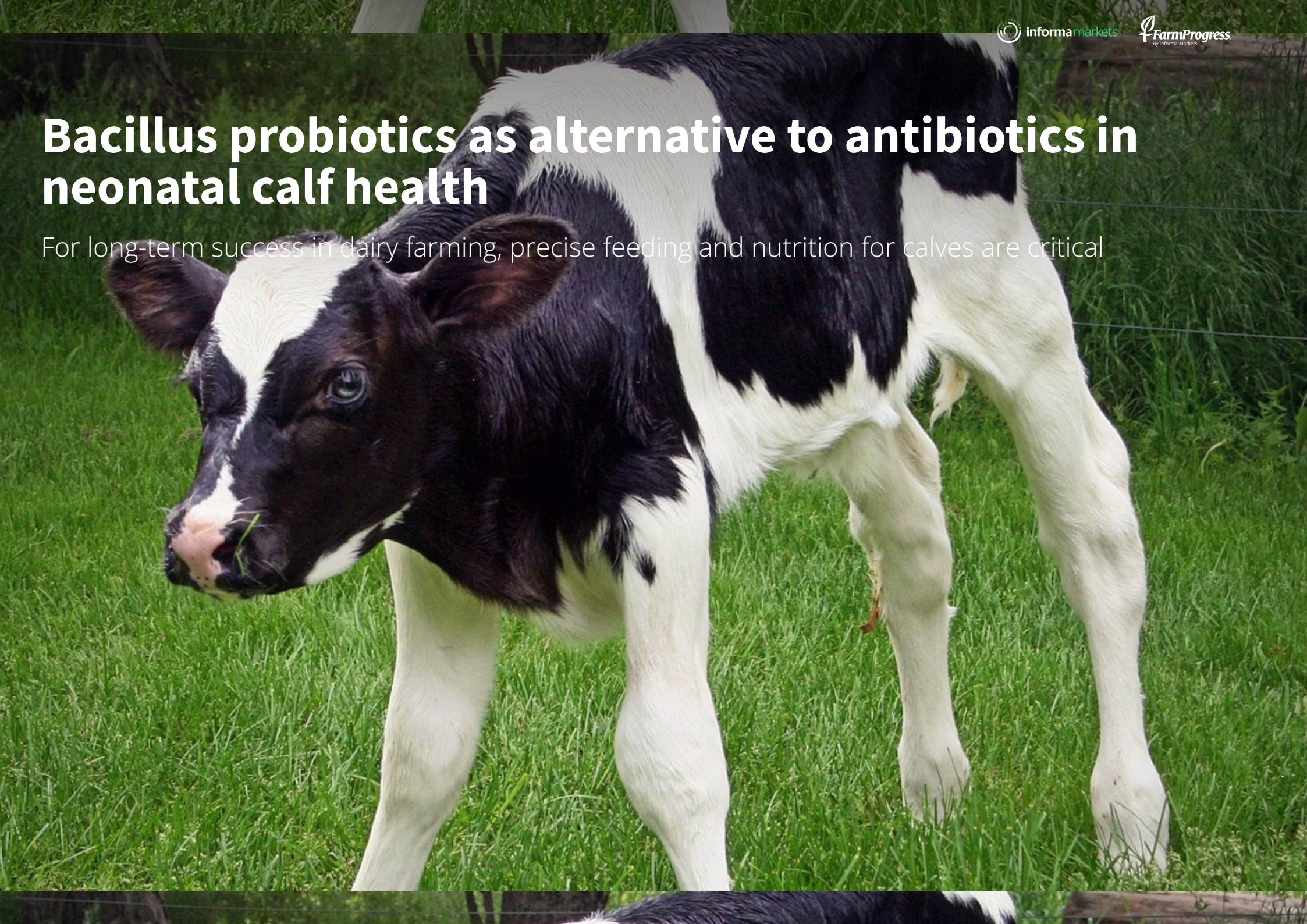


Bacillus probiotics as alternative to antibiotics in neonatal calf health

For long-term success in dairy farming, precise feeding and nutrition for calves are critical

By Alvaro Garcia

The early stages of a calf's life are crucial for farms but come with risks like diseases and stress. Separation from their dam, insufficient first milk intake, and the stress of movement can make them sick, causing issues like diarrhea and respiratory problems, impacting farms significantly. The bacteria in their gut play a big role in their health, but stress can disrupt this balance, making them more vulnerable to harmful bacteria. While antibiotics can help, they raise concerns about resistance and residues in meat and milk products. Probiotics, formulated with beneficial bacteria are being explored as an alternative to antibiotics. They can help maintain a healthy balance of good and bad bacteria in their stomachs, improve digestion, and promote growth. Some studies even suggest they strengthen the animals' immune systems, potentially reducing illness. Using probiotics could help ensure a strong population of good gut bacteria in calves, minimizing stomach issues.

For long-term success in dairy farming, precise feeding and nutrition for calves are critical. Adding probiotics to their diet has proven beneficial, aiding growth and gut health. There's growing interest in probiotics due to concerns about antibiotic downsides. Initially, scientists focused on specific types of bacteria, but now they're looking at others, particularly Bacillus subtilis and licheniformis, which seem effective in balancing gut bacteria and promoting growth. Some research indicates that B. subtilis might strengthen the immune system and contribute to essential vitamin production.

Bacillus subtilis: the research

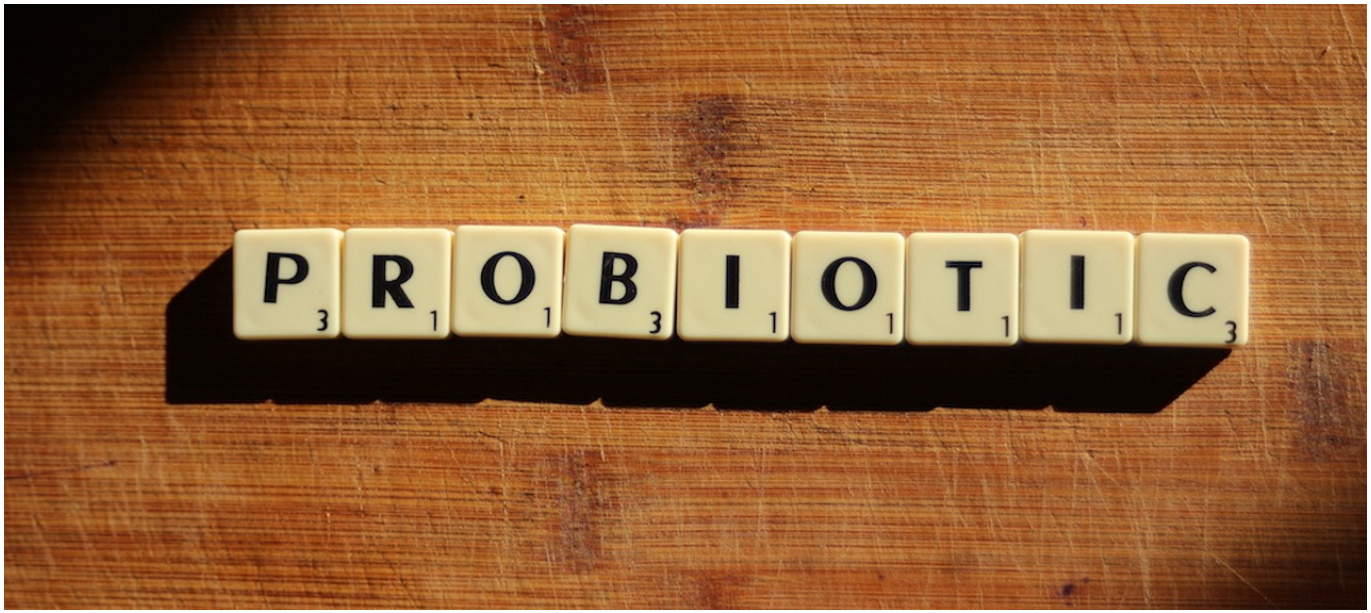
A study conducted at the research farm of the Chinese Academy of Agricultural Sciences in Beijing, China (Sun et al. 2010), examined the impact of a Bacillus subtilis probiotic strain on the performance and immune responses of dairy calves. The focus was on pre-weaning performance, weaning age, and immune function in Holstein dairy calves. The calves were divided into two groups with and without probiotic supplementation.

To administer the treatment the probiotic culture was combined with fresh cow milk at a ratio of 1:1 (vol/vol) and directly given to the calves. The experimental group received a daily dose of at least 1×10^{10} colony-forming units (cfu) of B. subtilis in a 10 mL culture during morning feedings, while the control group received no additive. Regular bacterial concentration checks ensured a supplementation level of 10^9 cfu of viable bacteria/mL. The experiment concluded at weaning, where blood samples were collected from all calves.

The supplementation of B. subtilis positively impacted calf growth without

affecting milk or starter intake. It accelerated weaning, and supported calf growth. Quite important, the probiotic did not trigger allergic reactions and increased serum IgG levels while influencing Th1 cytokine concentrations, particularly IFN-γ (Interferon-gamma). Cytokines are small proteins that act as signaling molecules, regulating the body's response to infection, inflammation, and trauma. Among these, IFN-γ, produced by immune cells like T cells and natural killer cells, plays a key role in activating immune cells to eliminate pathogens modulating immune responses and inflammation.

These findings underscore the positive effects of B. subtilis on calf growth and immune function. Direct administration of this probiotic enhanced growth performance and immune responses without adverse effects, indicating its promising potential for enhancing calf health.



Mode of action

Macrophages, part of the innate immune system, play a role in engulfing and eliminating pathogens. When infected with microorganisms, macrophages activate machinery that produces substances like nitric oxide and reactive oxygen species, contributing to pathogen degradation. These cells can also generate extracellular traps (ETs), DNA-based structures that trap and neutralize microorganisms to prevent their spread.

A recent experiment (Romo-Barrera et al. 2021) aimed to assess whether B. licheniformis and B. subtilis could prompt macrophages to create extracellular traps and if these traps could combat pathogens like Staphylococcus aureus. The study highlighted that both Bacillus species

infected macrophages but were subsequently rapidly eliminated. While B. licheniformis was cleared within 24 hours, over 80% of B. subtilis was eradicated in the same timeframe. However, this interaction affected macrophage viability, leading to about 50% cell death after 24 hours. Infected macrophages exhibited ETosis, forming extracellular traps akin to those induced by zymosan, an established ETs inducer. ETosis involves changes in cell structure where infected macrophages undergo chromatin de-condensation, resulting in the release of traps outside the cell to capture and neutralize pathogens, aiding in their destruction.

Further exploration into the induction of macrophage extracellular traps by these probiotics revealed specific components - histone citrullination and myeloperoxidase - distributed within the cellular cytoplasm and extensions, signifying the initiation of macrophage extracellular traps formation. Histone citrullination refers to a biochemical process where the amino acid arginine in histone proteins undergoes a modification into citrulline. Histones are proteins around which DNA is wound, forming a structure called chromatin. These modifications can alter chromatin structure, impacting gene expression and various cellular processes. Citrullination of histones, regulated by enzymes known as peptidyl arginine deiminases, can affect chromatin compaction and gene regulation, influencing cellular functions like immune responses.

Myeloperoxidase is an enzyme found in immune cells, particularly in neutrophils and some types of macrophages. It plays a crucial role in the immune system's defense mechanisms against pathogens. Myeloperoxidase generates reactive oxygen species by catalyzing the reaction between chloride ions and hydrogen peroxide. These reactive oxygen species serve as powerful antimicrobial agents, aiding in the destruction of invading microorganisms by neutrophils. Additionally, myeloperoxidase is involved in the formation of neutrophil extracellular traps, which are web-like structures composed of DNA, histones, and antimicrobial proteins. These traps help immobilize and kill pathogens, enhancing the immune response against infections.

Bacillus subtilis and licheniformis a true symbiotic relationship?

Bacillus subtilis and Bacillus licheniformis can potentially interact in symbiosis, although their relationship isn't often described as classical symbiosis. These bacteria are known to coexist and sometimes complement each other's functions in maintaining gut health and improving animal performance. While they might not exhibit a classical symbiotic relationship like some organisms do, their combined presence in the gut microbiota can contribute positively to overall gut health

and animal well-being.

This experiment demonstrated that infected macrophages exhibited a significant production of reactive oxygen species, implicating their role in eliminating Bacillus species. Notably, B. licheniformis triggered a higher release of DNA and increased reactive oxygen species production, potentially contributing to its more effective clearance in comparison to B. subtilis. This study offers valuable insights into the intriguing capability of these probiotics to prompt macrophage extracellular traps, as well as the symbiotic association between both bacterium, which could significantly impact their therapeutic efficacy and safety profile.

In conclusion, Bacillus probiotics, particularly Bacillus subtilis and licheniformis, showcase promising potential as alternatives to antibiotics in boosting neonatal calf health. Research indicates their positive influence on calf growth, immune system modulation, and their capacity to induce macrophage extracellular traps, potentially aiding in pathogen elimination. The observed advancements in calf growth without adverse effects and the enhancement of immune responses highlight the viability of these probiotics in mitigating early morbidity and improving farm economics. As we deal with concerns of antimicrobial resistance and residues in animal products, Bacillus probiotics offer an exciting alternative for sustainable and effective interventions in calf health management. Further exploration into their mechanisms and broader application in livestock farming could signify significant progress in promoting healthier, resilient animal populations while simultaneously addressing global concerns surrounding antibiotic usage.

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