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Development of a Methane Emission Reduction Technique for Dairy Cattle Using Soil Microorganisms: A Small-Scale Pilot Trial

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Abstract

Reducing greenhouse gases, particularly methane (CH_4), a significant contributor to global warming, has become an urgent international priority. Ruminants, particularly dairy cattle, emit substantial amounts of methane, necessitating the development of effective mitigation strategies. Methane is primarily produced by microorganisms in the rumen and expelled into the environment through eructation. In this small-scale pilot trial, we investigated the impact of a feed additive composed of mass-cultured, inactivated soil bacteria on methane emissions in dairy cattle. The additive was administered via drinking water to Holstein heifers, functioning as an exploratory case-based approach. Within three days, a significant reduction in methane emissions and eructation frequency was observed. When dairy cattle were provided with free access to drinking water containing 10 g/L of BX-1, the number of eructations was reduced by approximately 77%, and the concentration of methane gas in exhaled air decreased by about 80%. No adverse clinical symptoms or abnormalities in blood test results were detected in cattle. These findings suggest that utilizing soil bacterial formulations as feed additives could serve as a sustainable method for reducing greenhouse gas emissions in the livestock industry, potentially contributing to global warming mitigation. Further larger-scale trials are required to confirm these preliminary results.

Keywords

Methane Emissions, Dairy Cattle, Soil Microorganisms, Feed Additive

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1. Introduction

Among greenhouse gases contributing to global warming, methane (CH_4) is second only to carbon dioxide (CO_2) [1]-[3]. Methane has a global warming potential that is approximately 28 times that of CO_2, making even small amounts highly impactful. Ruminants such as dairy cattle and sheep produce large quantities of methane during digestion, accounting for approximately 5% of total greenhouse gas emissions [4]-[7].

The rumen of dairy cattle harbors a diverse microbial community that facilitates feed decomposition [8] [9]. During this process, hydrogen (H_2) and carbon dioxide (CO_2) are produced as byproducts, which methanogenic archaea use to generate methane. This methane is released into the atmosphere through eructation, contributing to global warming [10]. Furthermore, it has been reported that this process results in an energy loss of approximately 2% - 15% of digestible feed [4].

Recent research has identified a novel bacterial strain in the rumen of dairy cows that is expected to help reduce methane emissions, suggesting that the rumen microbiome still holds untapped potential for methane mitigation. Moreover, previous studies have demonstrated that certain feed additives, such as garlic and seaweed, can effectively reduce methane emissions [11] [12], making them promising candidates for practical application. In addition, genetic selection and innovative feeding strategies have been explored to further decrease methane emissions in ruminants [4] [6] [13].

Livestock harbors a wide array of microorganisms, and we have focused on artificially maintaining a balanced microbial community. To date, we have administered various soil-derived bacterial formulations to livestock in an effort to enhance feeding efficiency and strengthen disease prevention systems. BX-1 is a feed additive produced by culturing a cocktail of multiple soil bacteria on a large scale, then inactivating (killing) them and converting them into powder (Kawashima Co., Ltd., Japan). A key advantage is that, as long as the original live bacteria are available, BX-1 can be mass-produced in virtually limitless quantities anywhere—even close to cattle facilities. In other words, BX-1 can be rapidly manufactured exactly where and when it is needed. This feature sets it apart from other additives, such as garlic or seaweed. Furthermore, because the soil bacteria that make up BX-1 are fully inactivated and powdered, it is easy to transport, resistant to both heat and cold, and can be stored long-term (about 2 years). It also does not proliferate in the environment or in cattle, ensuring safe use. In this study, we developed and tested BX-1 for its effectiveness and feasibility in mitigating methane emissions in dairy cattle.

2. Materials and Methods

2.1. Preparation and Administration of BX-1

BX-1 is a powdered feed additive composed of mass-cultured and inactivated soil bacteria that are commercially available as livestock feed supplements (Kawashima Co., Ltd., Japan). Bacterial species belonging to BX-1 are listed in Table 1.

Table 1. Micro-organisms in BX-1.

Taxon name	Proportion (%)
Staphylococcus saprophyticus group	59.5524
Staphylococcus kloosii	32.5953
Weissella paramesenteroides group	2.0747
Staphylococcus aureus group	1.3063
Kocuria koreensis	1.1814
Staphylococcus pettenkoferi group	0.7108
Bacillus cereus group	0.5283
Leuconostoc pseudomesenteroides group	0.3218
Pediococcus acidilactici group	0.1153
Kocuria kristinae	0.0912
Staphylococcus sciuri group	0.0720
Corynebacterium variabile group	0.0672
Enterococcus italicus group	0.0672
Brachybacterium faecium group	0.0528
Brevibacterium DL489154_s	0.0480
Leuconostoc mesenteroides group	0.0384
Corynebacterium nuruki group	0.0336
Citricoccus muralis group	0.0240
Corynebacterium flavescens	0.0192
Corynebacterium minutissimum group	0.0192
Enterococcus faecium group	0.0192
Bacillus megaterium group	0.0144
Lactobacillus acidipiscis	0.0144
Lactococcus lactis group	0.0144
Actinomyces slackii	0.0096
Arthrobacter agilis group	0.0096
Arthrobacter echini	0.0096
Blautia wexlerae	0.0096
Clavibacter michiganensis group	0.0096
Kocuria rhizophila group	0.0096
Neomicrococcus lactis	0.0096
Ruminococcus faecis	0.0096

Continued

Actinomyces provencensis group	0.0048
Anaerostipes hadrus group	0.0048
Bacillus carboniphilus group	0.0048
Bacillus smithii	0.0048
Bifidobacterium adolescentis group	0.0048
Blautia faecis	0.0048
Brevibacterium iodinum group	0.0048
Caenibacillus caldisaponilyticus	0.0048
Caldibacillus debilis	0.0048
Corynebacterium tuberculostearicum	0.0048
Corynebacterium xerosis group	0.0048
Dorea formicigenerans	0.0048
Enterococcus saccharolyticus group	0.0048
Fusicatenibacter saccharivorans	0.0048
Geobacillus stearothermophilus group	0.0048
Geobacillus thermoleovorans group	0.0048
Geobacillus toebii group	0.0048
Kurthia zopfii group	0.0048
Lactobacillus dextrinicus	0.0048
Lactobacillus sakei group	0.0048
Lactococcus taiwanensis	0.0048
Listeria grayi	0.0048
Dorea PAC000479_s	0.0048
Sporobacter PAC001162_s	0.0048
Sporobacter PAC001306_s	0.0048
PAC001201_g PAC002029_s	0.0048
Romboutsia timonensis	0.0048
Rummeliibacillus pycnus	0.0048
Staphylococcus succinus group	0.0048
Weissella ghanensis group	0.0048

2.2. Effect of BX-1 on Eructation Suppression and Methane Emissions in Dairy Cattle

Five 12-month-old Holstein heifers were provided with drinking water containing either 1 g or 10 g of BX-1 per liter over three days. Eructation frequency was recorded for 40 min, starting at four hours post-feeding. The bar graphs represent the mean values of five cattle, with * indicating a significant reduction in comparison to the control group (Student's *t*-test).

Additionally, the methane concentration in the exhaled breath was measured five times at four-minute intervals just from four hours post-feeding, and the average values were calculated. To measure methane concentrations, a methane gas sensor probe was positioned at approximately 10 cm from the cow's mouth. The bar graphs represent the mean values of five cattle, with * indicating a significant reduction in comparison to the control group (Student's *t*-test).

3. Results

Cattle typically burp once every 1 - 2 min. The number of burps in cattle measured in our study showed no significant difference from reports from other research groups [14]. However, we found that the oral administration of BX-1 drastically reduced this frequency.

The administration of BX-1 resulted in a dramatic reduction in both eructation frequency and methane concentrations in exhaled breath. Specifically, eructation frequency decreased markedly (**Figure 1**), whereas the methane concentration underwent an even more dramatic reduction (**Figure 2**). When dairy cattle were provided with free access to drinking water containing 10 g/L of BX-1, the number of eructations was reduced by approximately 77%, and the concentration of methane gas in exhaled air decreased by about 80%. Furthermore, veterinary examinations revealed no clinical symptoms. These results indicate that BX-1 is an effective methane reduction technology for dairy cattle in this small-scale pilot study.

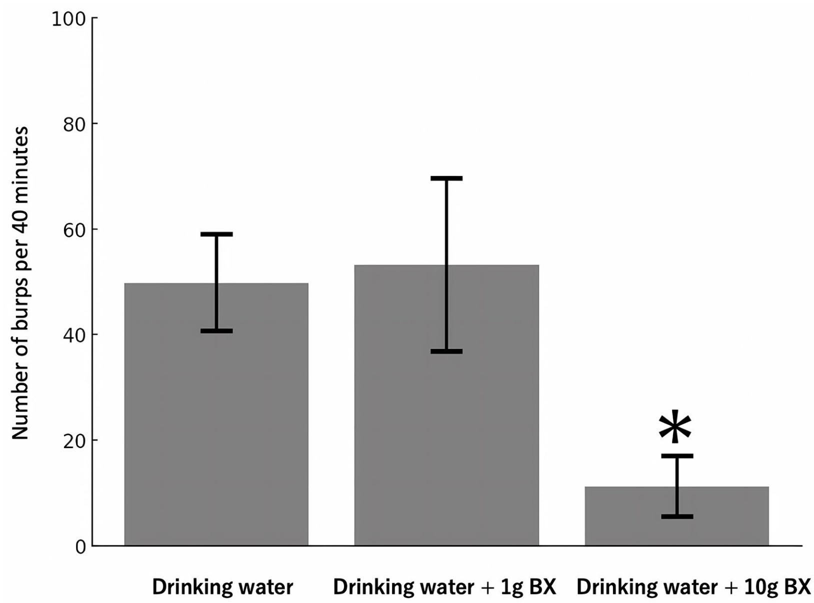

Figure 1. Suppression of eructation in dairy cattle by oral administration of BX-1. Five 12-month-old Holstein heifers were given ad libitum access to drinking water containing BX-1 powder (1 g or 10 g per liter) for three days. The number of eructation was recorded for 40 minutes starting four hours after feeding. Each bar in the graph shows the mean numbers of eructation from the five cattle. Asterisks indicate a significant decrease ($P < 0.01$, Student's *t*-test) relative to the control group which received water alone.

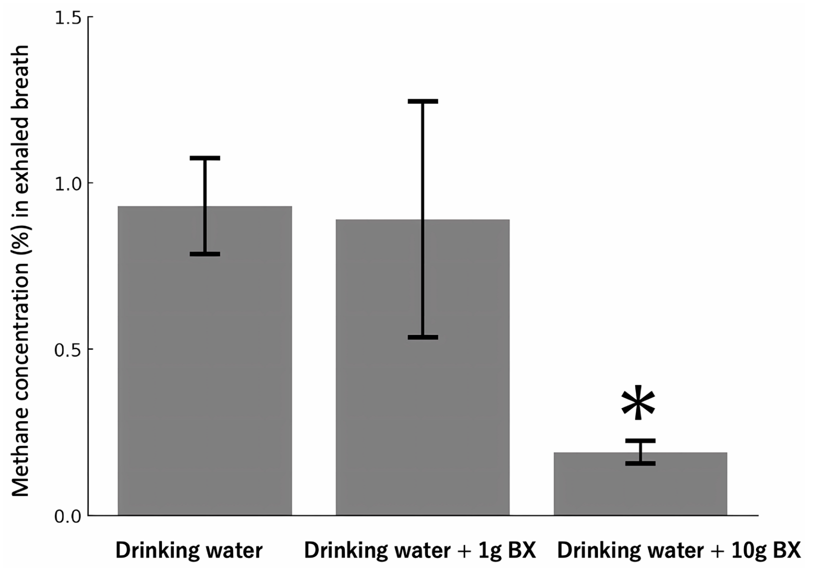

Figure 2. Reduction of methane emissions in dairy cattle by oral administration of BX-1. Five 12-month-old Holstein heifers were given ad libitum access to drinking water containing 1 g or 10 g of BX-1 per liter for three days. Starting four hours after feeding, methane concentrations in eructed gas were measured 5 times at four-minute intervals for each heifer, and the average value was calculated. Each bar in the graph shows the mean methane concentration of the five cattle. Asterisks indicate a significant decrease ($P < 0.01$, Student's *t*-test) in comparison to the control group which received water alone.

4. Discussion

This study demonstrated that soil bacteria-based feed additive BX-1 effectively suppressed methane emissions and eructation in dairy cattle over a short period. The rumen of ruminants hosts a diverse microbial community, including methanogenic archaea responsible for methane production [8]-[10]. BX-1 is composed of inactivated bacterial cells that do not proliferate in the digestive tract. These results suggest that BX-1 may have altered the microbial balance in the rumen, potentially suppressing the metabolic activity of methanogens or exerting direct inhibitory effects on methanogenesis through bacterial enzymes or other components. Additionally, previous research has reported the efficacy of BX-1 for controlling Salmonella infection in hatched chickens and laying hens, as well as Newcastle disease in poultry [14]-[16]. These findings imply that BX-1 may have broader applicability beyond methane mitigation, potentially improving the health of various livestock. In this small-scale trial, a remarkable reduction in methane concentration was observed, which is a highly significant effect; however, validating these findings in more diverse and larger-scale settings is essential (at least 20 cows for each dosage of BX-1). We also plan to test BX-1 under various housing conditions, including indoor barns and open pastures. Future research should clarify the metabolic pathways through which BX-1 influences methanogens, identify any limitations or conditions under which its effect might diminish, and consider the impact of prolonged administration on rumen function, animal health, and feed efficiency. Although we used young dairy heifers in this study, we plan to administer BX-1 to lactating cows in future research.

We will assess clinical signs, changes in body weight, and milk production to further evaluate the broader safety of BX-1. Cost-effectiveness and risk analysis, including safety for end consumers and environmental impact of large-scale production, will be crucial for real-world application. Moreover, dairy farming practices differ worldwide, so confirming the reproducibility of these results under various feed compositions, water availability, and housing systems is critical. It will also be necessary to investigate how BX-1 can be integrated into different feeding regimens—whether mixed in total mixed rations or provided via drinking water—and to determine optimal dosage levels and treatment durations for sustained methane reductions. Since BX-1 is a bacterial-based product, it can potentially be mass-produced in many regions, reducing logistical barriers and highlighting its high potential for global scalability. In conclusion, while this small-scale pilot trial provides encouraging data on the efficacy and safety of BX-1 in reducing methane emissions in dairy cattle, further investigations that address the mechanisms of action, limitations, cost-effectiveness, and broader applicability are needed to establish a robust evidence base for its integration into global livestock practices aimed at mitigating methane emissions.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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