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Evaluation of the use of probiotics in the compost bedding for feedlot dairy cows

Abstract

The feedlot of dairy cows is widely used when you want to intensify production with animals with superior genotypes. Feedlot systems like compost barn or free stall can be harmful due to the high humidity of the bedding and the presence of microorganisms that cause mastitis. These factors can compromise the health of the Dairy cows and then, the quality and quantity of milk produced. The objetctive of this research was to evaulated the effect of probiotics on the temperature, relative hymidity, total bacteria Count (TBC) and microbial culture in compost bed for feedlot dairy cows. The research was carried out in four Dairy farms, located in South of Brazil. Three farms uses the compost barn system and one used the free stall system. It was registered the relative humidity data, environment temperature, bed temperature and bed TBC, during six week. It was used a completely randomized design, with two treatments (without and with probiotics) and four replications by treatment, with repeated measures over time. The application of probiotic in bed of Dairy cows did not change TBC, temperature or humidity, with averages of 38,042 x 1,000 CFU/g, 26.9º C and 61,2 %, respectively. The use of probiotics reduced the count of some microorganisms such as *Escherichia coli*, *Penicillium* and *S. dysgalatiae* and increased the count of anothers as *klebsiella* and *trichoderma*.

Keywords: compost barn, *Escherichia coli*, Free stall, microrganisms, total bacterial count, yeast

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Eduarda Carneiro Araújo,[1] Renan Jesse Ribeiro,[2] Rubia Fernandes Soares[2], Victor Breno Pedrosa,[3] Adriana de Souza Martins[4]

[1]Post-graduate Program in Animal Science, Deparment of Animal Science, Ponta Grossa State University, Brazil
[2]Graduate in Animal Science, Deparment of Animal Science, Ponta Grossa State University, Brazil
[3]Colaborator professor of Post-graduate Program in Animal Science, Deparment of Animal Science, Ponta Grossa State University, Brazil
[4]Professor of Post-graduate Program in Animal Science, Deparment of Animal Science, Ponta Grossa State University, Brazil

Correspondence: Adriana de Souza Martins. Professor of Ponta Grossa State University - UEPG, Av. Gal. Carlos Cavalcante, no 4748, Ponta Grossa- Paraná State, Brazil. Tel +55(42) 99915-3403 Email dri261@yahoo.com.br

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Introduction

In the face of economic factors related to milk yield, it's made necessary an investigation to improve the comfort and welfare of animals to obtain high productive indexes. In this sense, the main objective of feedlot systems is to provide the animals with comfort, especially in terms of bedding, room temperature and flooring. In feedlot, animals tend to spend less energy on body maintenance and convert it efficiently into milk production.[1]

In free stall feedlot, the cows are housed in individual beds with free acess. Materials commonly used as bedding are sand, rice husk, sawdust, among others.[2] This feedlot system has several benefits, as well as better productive indexes due to the animal's reduced movement.[1]

The compost barn system consists of a covered area for the rest of the dairy cows, being composed, in its majority, of sawdust and manure. Cows circulate, urinate and defecate freely, what makes the aeration and management of beds necessary. In this system, the floor is covered with bedding that is dispersed within all over the collective resting area.[3]

When the bedding is not composted properly, the Whole system can be compromised, meaning that cases of clinical mastitis as well as somatic cell counts can increase. On the Other hand, When the bedding is well composted, mastitis is reduced. As a result, milk quality tends to increased.[4]

In composted, the organic constituents of the waste are transformed into a stable product by microbial fermentation, witch takes place in na aerobic environment and generates heat through the action of thermophilic bacteria. In addition, in this process, part of the organic carbon is converted into humic substances and part is converted in carbon dioxide, methane and others volatile componds, reducing the carbon contente.[5]

The main advantages of composted are the reduction of pathogenic microrganismo and obtaining a product with excelente fertilizing characteristics that can be used in plant production.[6] In view of this, it's extremely important to monitor variables such as the temperature (of the bedding and the environment) and the moisture content of the bedding. These factors are fundamental to providing a dry compost bed with less contamination.[5]

According to,[6] when manure is fermented with microorganisms considered efficient, the maturation period is reduced. These microorganisms contribute to an increase in the concentration of nutrients and with the absence of odor in the compost, allowing fermentation to take place more quickly and efficiently.

The use of commercial probiotics is an alternative for speeding up the decomposition of organic matter and promoting a balance of microorganisms in the compost bedding. These probiotics are made up of beneficial, efficient and non-pathogenic microorganisms, such as yeasts and lactic acid bacteria. They act by breaking down the organic compounds in the bedding, such as minerals, sugars, proteins and fats, promoting the rapid decomposition of organic matter and the release of nutrients.

Due to challenges presented by the use of Free Stall systems, and especially Compost barn bedding, it is necessary to evaluate strategies that make this system viable, reducing the negative impacts of bedding moisture on the performance of feedlot animals. The aim of this study was to evaluate the effect of a probiotic on temperature, moisture content, total bacterial count (TBC) and microbiological culture in Compost barn and Free Stall beds.

Material and methods

The experiment was carried out in partnership between the State University of Ponta Grossa and Frísia Cooperativa Agroindustrial, located in the Carambeí city, state of Paraná, Brazil. The aim was to verify the action and effectiveness of a probiotic in composting beds for dairy cattle in Compost barn and Free Stall systems. The study was carried out on four farms, three of which used the compost barn system and only one farm the free stall system.

The predominant climate in the region is temperate, with no defined dry season and a mild summer. In the coldest months, the average temperature is below 18ºC, and in the hottest months, the average temperature is below 22ºC. The average annual rainfall in the municipality is 17ºC to 18ºC and 1,800 to 2,000 mm, respectively. The farms are located between the coordinates 24º47'02.0"S, 50º12'30.5W.[7]

The experiment lasted a total of six weeks, starting on september, 3rd, 2022 (winter) and ending on october, 21th, 2022 (Spring).

To analyze the effect of the probiotic on the compost beds, the treatments were divided into:

1) Compost bedding without the addition of the probiotic (control).

2) Composting bedding with the addition of the probiotic.

Description of breeding systems

Compost barn system: On the first farm there were 72 lactating cows of the Holstein, Jersey and Holstein-Jersey breeds. The housing had no fans and the bedding was aerated twice a day (morning and afternoon) with a scarifier. The density per animal in the housing was 15 m²/cow. On the second farm there were 141 lactating Holstein cows. The housing had fans and the bedding was turned twice a day. The density used was 14 m²/cow. The third farm had 30 lactating cows of the Jersey, Holstein and Holstein-Jersey breeds. The housing had no fans. The bedding was aerated twice a day with a rotary hoe. The animal density was 13 m²/cow.

The material used as bedding on these three farms was sawdust. During the experiment, it was replaced when the humidity was above 60% and depending on the availability of sawdust on the market. The housing on the three farms were divided into two areas of the same size: one without the probiotic and one with the probiotic applied to the compost beds. The cows circulated in both areas.

Free Stall system: On this farm had 25 Jersey cows in lactation. During the experiment, the beds were cleaned daily, and sawdust was replenished when necessary. The housing contained 25 beds, each measuring 1.5 m long x 1 m wide and 15 cm deep. The housing had fans and the dimensions were 10 m wide by 15 m long.

Probiotic description and date collection: The commercial product tested was made up of probiotics*: Lactobacillus casei var. ramnosus* – 4,7 x 10^4 CFU/mL; *Lactobacillus acidophilus* – 3,2 x 10^4 CFU/mL, *Saccharomyces cerevisae* -1,5 10^4 CFU/mL), with a pH of 3.5. It was applied at a rate of 1 liter of the product per 300 m² of bedding. To be used, the product had to be activated one week before application. Activation consisted of diluting the probiotic in water and sugarcane molasses, which served as a substrate for the microorganisms. The following proportions were used for activation: 90 liters of water, 5 kg of cane molasses and 5 kg of the probiotic. On the days of application to the compost bedding, the product was diluted again, directly in the following proportion: 1 liter of the product and 19 liters of water. The product was applied to the surface of the bed using a Kawashima model PEM-P20 manual electric backpack sprayer with a capacity of 20 liters.

In the Free Stall system, the product was more diluted, following the manufacturer's recommendation: 200 ml of the product diluted in 1.8 liters of water. As a result, the amount of probiotic applied was proportional to the area of bedding applied in the Free Stall. In this system, 10 beds were randomly selected. The product was applied to five beds and the probiotic was not applied to the other five.

The environment temperature and relative humidity were measuared three times a week with a datalogger (Table 1). In the first week, data was recorded before the start of the experiment. The experimental period occurred from 2nd to 6th week.

1st week: before the start of the experiment. 2nd to 6th week: experimental period.

A digital skewer thermometer with a 15-centimeter stem was used to measure the environment temperature. In the treated and untreated areas (in the Compost barn systems) seven different points were chosen at random for the evaluation, in order to obtain 14 reading points. In the Free Stall beds, 10 reading points were analyzed. In both systems, the depth of the thermometer used to record the temperature was 15 cm from the surface.

Sub-samples were collected from the composting beds, and in the Compost barn system seven sub-samples were collected from the area treated with the product and seven sub-samples from the untreated area. The sub-samples were homogenized in plastic bags with a capacity of 2 kg and then placed in sterilized plastic bags for later laboratory analysis. The samples were divided into four parts, two samples from the treated area and two samples from the untreated area, from each property.

The samples were identified by farm, date and treatment (with or without the probiotic). The samples were sent to the laboratory for microbiological analysis on the same day they were taken. The other two samples from each farm were frozen at -10º C for later analysis of moisture content.

The samples were all collected in the morning, starting at eight o'clock and finishing at ten o'clock. Around 21 days after the end of the experiment, samples were taken again from all the properties to check the residual effect of the product.

Application schedule of probiotic: During the first experimental week, the product was not applied, but samples were taken (time zero), and the relative humidity and environment temperature were recorded three times a week. From the second week onwards, the product was applied to the compost bedding. Fifteen applications were made to the compost bedding during the experimental period, three applications a week for five weeks. In addition to the procedures carried out in the first week, it was necessary to dilute the product in water and then distribute it over the surface of the bed in the area that would receive the treatment.

The practices carried out during the third, fourth, fifth and sixth weeks of the experiment were the same. Therefore, there were 15 applications of the probiotic in the compost bedding during the experimental period, three applications a week for five weeks.

Laboratory analyses: The microbiological analyses were carried out at the LabVet Animal Pathology laboratory, located in Carambeí-PR. Eight samples were sent to the laboratory every week to identify

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the microorganisms and determine the total bacterial count. The methodology used for the analysis was the standard plate count. The samples were processed and analyzed to dry matter at the Animal Nutrition Laboratory of the State University of Ponta Grossa. For the dry matter content, the samples were dried in a forced ventilation oven at 55º C for 72 hours. The samples were ground on sieves of 5mm meshes. The dry matter contente was determined according to.[8] The bed mousture was calculated by difference.

The relative Frequency of microrganisms in bed was obtained by the number of times the microorganism was present in the sample in each collection. The concentration of each microrganism in the bed samples was also obtained (CFU/g).

Statistical design: The experimental design was completely randomized with two treatments and four replications (dairy farms), with repeated measures over time (three times a week for five weeks). The F test was used to compare the means of each variable at a 5% significance level. For the statistical analysis of CBT data, the values were transformed to the logarithmic scale (log10). The SAS Statistical Program (version 9.4) was used.

Results

The average environment temperature during the experiment was 16.9º C, with minimum of 13º C and maximum of 22º C. The average relative humidity (RH) was 80.3%, ranging from 51% (1 record) to 100% (1 record). In the variance analisys of the temperature and moisture content of bed, TBC was not influenced by the treatments (bed treated or not with the probiotic). In addition, the application period of the probiotic caused a significant difference ($P<0.05$) in CBT levels.

The average temperature and humidity levels of the bed (Table 2) recorded in the housing were similar between the untreated and treated bed ($P>0,05$). P values below 5%, differ by the Tukey test. Table 2

The use of the probiotic in the cows' bed did not cause any changes in the CBT values, with average values of 42,145,000 CFU/g and 33,940,000 CFU/g being observed, respectively, for the bedding treated and not treated with the probiotic. It can therefore be seen that, although there was no statistical difference, there was a numerical reduction in CBT in the bed that received the product.

With regard to the periods when the probiotic was applied to the bed, Figure 1 shows that from the 1st to the 6th application (2nd and 3rd week, respectively), there was an increase in the bed's TBC. In the last application (6th week), there was a significant reduction in TBC, both in the treated and not treated bed with the probiotic.(Figure 1)

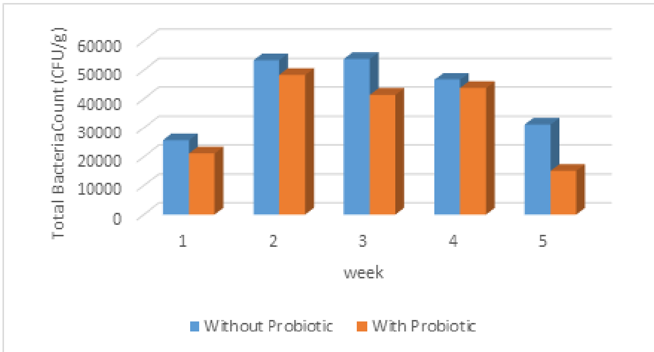

Figure 1: Levels of total bacterial count (TBC - CFU/g) of compost bedding according on whether or not the probiotic application.

Although there was no effect of using the probiotic on the cows' bedding, the total bacterial count varied ($p<0.05$) with the application period (Table 3). These results show that other factors can affect the microbial action on the compost bedding.

[1]September,13, 14 and 16,2022; [2]September, 20, 21 and 23, 2022; [3]September, 27, 28 and 30, 2022; [4]October, 4, 5 and 7, 2022; [5]October, 11, 12 and 14, 2022; [6]October, 18, 19 and 21, 2022. Means followed by different letters in the same column differ by the Tukey test ($P<0.05$). Figure 2

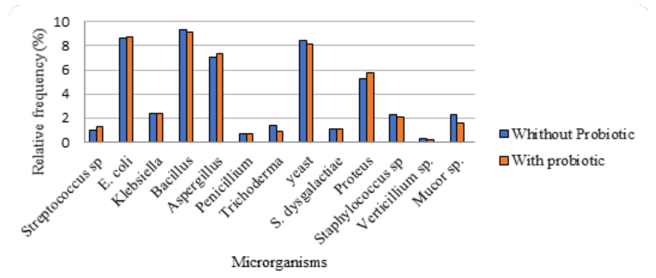

Figure 2: Shows the microorganisms and the relative frequency found in the compost bed treated or not with the probiotic. Relative frequency (%) of microrganisms on bed without or with probiotic application.

The microorganisms with the highest relative frequency in the bed samples were *E.coli, Bacillus, Aspergillus,* Yeasts e proteus.

When evaluating the Count (FCU/g) of each microorganism present in the bedding, it was observed reduction in some these, specially *E. coli*, *Penicillium*, *Leveduras*, *S. dysgalactiae*, *Proteu and Staphylococcus sp.* (Figure 3) with the probiotic application. (Figure 3)

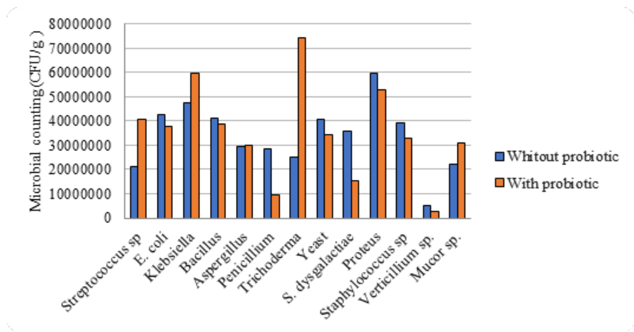

Figure 3: Microbial counting of microorganisms on compost bedding with or without probiotic.

On the Other hand, the count of *Streptococcus*, *Klebsiella*, *Tricoderma* and Mucor was increased with probiotic application.

Discussion

Probiotics such as yeasts and lactic acid bacteria are used to improve the physicochemical and microbiological conditions of the compost bedding material, as they help speed up the decomposition process of the organic waste present in the bed. This makes it possible to reduce the humidity of the material by producing more heat inside the compost.[9] The composted bedding process is influenced by various factors that can interfere with microbiological activity, including ambient temperature and relative humidity. During most of the experiment, the environment temperature was relatively low, with rain on some of the evaluation days (Table 1). Therefore, under cold and humid conditions, such as those recorded in this study, the temperature may have limited the action of the probiotic on the litter.

Table 1: Mean values of environment temperature and realative humidity in Dairy farms during experiment

Item	Week 1st	2nd	3rd	4th	5th	6th
Environment temperature (°C)	16.5±1.8	15.6±1.4	15.1±1.4	17±1.9	18.8±1.5	18.8±2.5
Relative Humidity (%)	80±11.0	78±16.1	86±5.4	73±11.7	81±6.3	82±6.8

According to Piovesan,[10] for cow comfort, the ideal relative humidity range is between 40 and 70%, while the optimum environment temperature range is between 5 and 25º C. According to Damasceno,[11] high relative humidity associated with low temperatures makes it difficult to manage the bedding and also to reduce humidity in Compost barn systems. However, very low relative humidity values can promote mucosal dryness and respiratory problems in dairy cattle. When the temperature and humidity of the bedding are not adequate, the development of microorganisms is altered, affecting their growth time and, consequently, their activity in the compost bedding.

For the bed to compost properly, it needs to be between 40 and 60% moist. [12] Therefore, bedding moisture is one of the main factors responsible for microbiological activity during the composting process, since the structure of microorganisms is made up of 90% water. In cases of new cell production, composting is able to provide water and all the nutrients required for the process.[10]

On the other hand, excessive bed moisture can inhibit the activity of aerobic bacteria due to greater compaction (lower oxygen availability), compromising the composting process. Therefore, the high bed moisture obtained in this study (Table 2) may have compromised microbiological action.

Table 2: Average values for temperature, moisture content and total bacterial count (TBC) in the compost bedding of dairy cows according to treatments

Item	Treatment: Without Probiotic	With probiotic	P - value
Bed Temperature (°C)	26.8±1.00	26.9±0.86	0.979
Bed humidity (%)	61.3±7.29	61.2±6.71	0.844
Bed TBC (x 1.000 CFU/g)	42,145±13.133	33,940±14,793	0.452

According to Leso,[13] an indicator for the proper functioning of the composting process is the achievement of high temperatures, as they optimize the evaporation of moisture, eliminating pathogenic microorganisms that cause mastitis. Situations of low bedding temperature, excess moisture and organic matter indicate that it is necessary to add bedding material to renew the carbon source that has been consumed by the degradation process by the microorganisms. [14]

[5]mentioned that the composting process is divided into two phases: the active degradation phase and the maturation phase. The first involves the action of thermophilic microorganisms, which are active at temperatures between 45 and 65ºC. In this phase, easily degradable organic matter, such as carbohydrates, is broken down and the biomass is stabilized. In the maturation phase, mesophilic microorganisms are active at temperatures between 20 and 45ºC. However, at this stage, it is ideal for the temperature to remain between 30 and 45º C. At the end of the ripening phase, temperatures between 25 and 30º C are common.

In general, the ideal temperature range for the composting process is 45ºC to 55ºC. When temperatures are below 45ºC, the process takes place slowly and there is no elimination of pathogenic microorganisms, which may be present in the bedding material. However, temperatures above 65ºC can inhibit the process. It is important to note that, in general, in Brazil, the average temperatures of compost bedding are not high enough to eliminate pathogenic microorganisms.[11]

In this study, the average bed temperature (26.8ºC) was within the range mentioned for the final stage of maturation (Table 2). However, for most of the experiment, temperatures were not high enough to identify a fermentation process, even with the use of probiotics. Therefore, the low temperature of the beds may have limited the action of the probiotic.

The action of probiotics in compost bedding occurs efficiently through the fermentation process. The action of the probiotic and the bedding microorganisms were not sufficient to raise the temperature and favor the composting process. In addition, the average surface temperature of the bed was low (10.5º C) and remained close to the environment temperature on all collection days.

On the Compost barn systems, it was possible to observe high bedding compaction, despite the fact that the bedding was turned daily. Another factor observed was that the bedding showed greater compaction on the sides, next to the wall of the housing, probably due to the greater agglomeration of the animals in these places, promoting a greater concentration of feces and urine.[10] On the farms that didn't use fans, this situation was more intense.

According to,[4] bedding that has a dry and comfortable surface is adequately composted, as the microorganisms produce CO_2 and consume water during fermentation, leaving the bedding with a lower moisture content and consequently drier. On the other hand, when the humidity is high, the bedding tends to adhere more easily to the

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animals' coats, making it necessary to add dry material to reduce the humidity, as this exposes the animals to greater contamination of the mammary quarters and the incidence of mastitis.

Although the probiotic had no direct effect on the composting process, the results on the relative frequency of the microorganisms and their quantification (Figures 1 and 2) indicated a competitive exclusion between the microorganisms, causing some to predominate over others. Among the microorganisms with increased counts, *Trichoderma* stood out (Figure 3). Studies have shown the fungi action in the biological control of diseases in agriculture cultures, acting as macroscopic parasites and rotting organic matter, considered environmental opportunists. It has rapid growth, and highly capable of parasitizing or preying on other fungi.[15]

The main function of probiotics is to facilitate the process of decomposition of organic matter, which is important for speeding up fermentation and, consequently, improving the composting process. The combination of natural microorganisms such as yeasts and *Lactobacillus* can promote several benefits for the microbial flora in compost bedding, such as beneficial antioxidant fermentation. It was therefore hoped that by using the probiotic, there would be a synergism between the microorganisms in the bed and the microorganisms in the probiotic and, consequently, the characteristics of the compost bedding would be improved.

The variation in TBC during the experiment (Table 3) was mainly due to climatic conditions. In the composting process, various chemical and biological transformations are carried out by a wide variety of microorganisms, such as fungi, bacteria and actinomycetes, which use carbon and other nutrients for their survival. However, the number of microorganisms changes depending on the conditions for their development, which occurs throughout the fermentation process. In addition, the predominance of microorganisms varies according to the temperature of the litter, moisture content, oxygen availability, C/N ratio and pH of the litter.[5]

Table 3: Total bacterial count values of the compost bedding without or with probiotic addition

Number of applications	Without Probiotic	With probiotic	P
Without application[1]	16,942,000[e]	20,617,000[e]	0.712
1st to 3rd[2]	26,944,000[f]	24,179,000[d]	0.784
4th a 6th[3]	65,591,000[a]	56,816,000[a]	0.523
7th a 9th[4]	53,841,000[b]	41,375,000[c]	0.883
10th a 12th[5]	46,666,666[c]	43,700,000[b]	0.929
13th a 15th[6]	31,166,666[d]	15,141,000[f]	0.241

The microorganisms present in animal feces produce the intracellular and extracellular enzyme urease, which hydrolyzes urea (CH_4NO2) and breaks it down into NH_4^+ and CO2. Saprophytic bacteria and various species of fungi are responsible for decomposing the organic materials present in the bedding. In the anoxic (anaerobic) zone, gases such as H2S, CO2 and CH4 are formed, which can negatively affect the microbiological activity of the litter. Microorganisms that oxidize NH3 to NO2- can have their enzymes blocked by the presence and action of H2S gas.[11]

The microorganisms from the probiotic used (*yeasts* and *Lactobacillus*) are facultative aerobes, i.e. their action can occur in both aerobic and anaerobic environments, allowing them to act in both aerobic and anaerobic conditions during the composting process.

There are different types of microorganisms in the composting bed, but the main ones are those that carry out fermentation (aerobic or anaerobic) and pathogenic microorganisms that, when not eliminated, cause mastitis in confined cows. The main microorganisms that develop in compost beds and cause mastitis are: *Escherichia coli*, *Streptococcus* spp, *Staphylococcus,* and *Bacillus* spp.[16]

In this study, it was found that the pathogenic microorganisms that cause mastitis (*Staphylococcus, E. coli* and *S. dysgalactiae*) had their concentrations reduced (Figure 3) in the beds treated with the probiotic. Therefore, even without the action of the probiotic on the humidity and temperature levels during the composting process, the profile of pathogenic bacteria was reduced in the beds treated with the probiotic.

In Compost barn systems the litter is mostly organic. This fact raises concern regarding greater exposure to environmental pathogens that cause mastitis. For clinical mastitis, the main causative agents are coliforms such as Klebsiella spp and environmental streptococci (*S. uberis and S. dysgalactiae*), being the most frequent cause of mastitis clinic caused by *E. coli* followed by staphylococcus.[11]

Conclusion

The application of the probiotic to compost and free stall bedding for dairy cows had no effect on the humidity, temperature and total bacterial count of the bedding. Factors such as low environment temperature and rainfall directly affected the composition of the bedding. On the other hand, in the bedding treated with the probiotic, there was a reduction in the microbial load of some environmental microorganisms such as *Escherichia coli* and *S. dysgalactiae*, which cause clinical mastitis. Due to the variation in the moisture content, temperature and TBC of the bedding depending on the environmental conditions, further studies with the use of probiotics over a longer period are suggested.

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Conflicts of interest

The authors declare that there are no conflicts of interest.

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