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Effect of including probiotic corn in the diet of Girolando cows on milk production and composition

Efeito da inclusão de milho probiotado na dieta de vacas Girolando sobre a produção e composição do leite

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Abstract: The objective was to evaluate the influence of the use of probiotic corn in the diet of dairy cows. Twelve Girolanda cows were used, with an average weight of 549 ± 66 kg, average production of 22.8 ± 4.8 kg of milk/day, which were distributed in three Latin squares (3x3). The experiment consisted of three treatments, three experimental periods, lasting 15 days each. The animals received a control diet with corn silage, 22% crude protein concentrate, access to tropical forage paddocks, and for the other treatments, 0.5 kg and 1.0 kg of probiotic corn were added to the control diet on a dry matter basis. Data on milk production and composition, and ingestive behavior were subjected to analysis of variance adopting a significance level of 10%, evaluated in a Latin square design, using regression to evaluate means. The addition of probiotic corn to the diet provided linear growth in milk production. Live weight and body condition score did not show significant differences with the inclusion of probiotic corn. Total solids, protein and fat contents of milk increased linearly. Probiotic corn decreased idle time and increased rumination time when added to the diet. There was no yeast growth during the incubation period of probiotic corn. Regarding the economic analysis, the addition of 0.5 kg of probiotic corn to the diet showed a positive balance. It is concluded that the use of probiotic corn proved to be technically and economically viable with 0.5 kg animal/day.

Key-words: additives; dairy cattle; nutrition; yeast.

Resumo: O objetivo deste estudo foi avaliar a influência da utilização do milho probiotado na dieta de vacas leiteiras. Foram utilizadas doze vacas Girolandas, com peso médio de 549 ± 66 kg, produção média de 22,8 ± 4,8 kg de leite/dia, as quais foram distribuídas em três quadrados latinos (3x3). O experimento foi composto de três tratamentos, três períodos experimentais, com duração de quinze dias cada. Os animais receberam dieta controle com silagem de milho, concentrado de 22% de proteína bruta, acesso a piquetes de forrageiras tropicais, e, para os demais tratamentos, foi adicionado à dieta controle 0,5 kg e 1,0 kg de milho probiotado com base em matéria seca. Os dados de produção e composição do leite e comportamento ingestivo foram submetidos à análise de variância, adotando-se nível de significância de 10%, avaliados em delineamento em quadrado latino utilizando regressão para avaliação de médias. A adição de milho probiotado à dieta proporcionou crescimento linear na produção de leite. O peso vivo e escore de condição corporal não apresentaram diferença significativa com a inclusão do milho probiotado. Os teores de sólidos totais, proteína e gordura do leite aumentaram linearmente. O milho probiotado diminuiu o tempo em ócio e aumentou o tempo de ruminação quando adicionado à dieta. Não houve crescimento de leveduras no período de incubação do milho probiotado. Em relação à análise econômica, a adição de 0,5 kg de milho probiotado à dieta mostrou um saldo positivo. Conclui-se que a utilização do milho probiotado demonstrou-se viável técnica e economicamente com 0,5 kg animal/dia.

Palavras-chave: aditivos; bovinos de leite; leveduras; nutrição.

1. Introduction

Achieving high performance in dairy cows requires a diet rich in energy and metabolizable protein (MP). To meet these demands, diets composed of significant amounts of grains and legumes are often used(1). Thus, food processing, combined with the use of additives, has been evaluated in search of productive and economic efficiencies(2). In Brazil, approximately 96.5% of the corn used for cattle feed is of the Flint (hard) type, which requires processing to increase the availability of starch in the rumen. The main objectives of processing are to improve digestibility by increasing the contact surface, to break down the protein matrix and to use the acids generated during fermentation(3,1). Among the processing techniques, the following stand out: ensiling, milling and rehydration of grains.

The use of feed additives is another strategy to minimize undesirable substances, since, in addition to enhancing beneficial effects for the animal, these additives improve nutritional efficiency and reduce production losses(4,5). In this context, supplementation with Saccharomyces cerevisiae yeast strains has gained prominence due to its ability to improve the ruminal environment, as well as the health and performance of animals(6). Yeasts act to modify ruminal fermentation in order to improve nutrient digestion and stabilize ruminal pH, reducing the risk of metabolic disorders(7,8). Furthermore, they promote the growth of ruminal bacteria, especially cellulolytic bacteria such as Ruminococcus albus, Ruminococcus flavefaciens and Fibrobacter succinogenes, resulting in more efficient degradation of plant cell wall compounds, especially cellulose and hemicellulose(9, 10).

To optimize the utilization of corn(11), a laboratory study investigated the effect of inoculating corn with yeast, called probiotic corn by EMATER MG, and the aerobic fermentation time over the viability of yeasts in relation to the control (without yeast inoculation). The fermentation time that allowed the greatest yeast population was between 240 hours and 360 hours, and this time provided an increase in the starch in situ digestibility of up to 40%, being a promising technology in ruminant nutrition. In the production of probiotic corn, the grain is broken down into smaller particles, rehydrated and the cornmeal is aerobically fermented with live Saccharomyces cerevisiae yeasts, aiming for a source of probiotics and improved corn digestibility. However, there is still no scientific basis to prove the effectiveness of the product, and research is needed to provide better information.

In this context, the objective of this study is to analyze the impact of including probiotic corn in dairy cow diets, evaluating aspects such as milk production and composition, body condition, live weight, ingestive behavior, financial balance and yeast viability.

2. Material and methods

OThe experiment was conducted in the dairy cattle farming sector of the Instituto Federal do Sudeste de Minas Gerais-IF Sudeste MG during the months of October to December 2022. The municipality is located in the Zona da Mata Mineira region, under the geographic coordinates 21°16'29'' S latitude, 43°10'45'' W longitude, with an average rainfall of 132.31 mm of rain and an average minimum temperature of 19°C and a maximum of 26°C. All procedures performed were approved by the Ethics and Animal Use Committee (CEUA) of IF Sudeste MG, Campus-Rio Pomba, with protocol no. 10/2022.

Twelve lactating Girolando cows were used, with an average weight of 549 ± 66 kg, average milk production of 22.8 ± 4.8 kg per day and average LD of 112 days at the beginning of the experiment. The animals were distributed in three Latin squares (3x3), balanced according to the lactation period and milk production. Three treatments were evaluated, consisting of the control diet (without the addition of probiotic corn), and the addition of 0.5 kg or 1.0 kg of probiotic corn in the diet based on dry matter (DM). Each experimental period lasted fifteen days, with the first thirteen days for adaptation of the animals to the treatments and last two days for data collection.

The animals received a diet containing corn silage produced in the cattle breeding sector of the Department of Animal Science, commercial concentrate with 22% crude protein, maintaining the ratio of 3 liters of milk/kg of concentrate, according to the manufacturer's recommendations. The concentrate was composed of ground whole corn, soybean meal, ground whole sorghum, wheat bran, livestock urea, calcium salts of fatty acids, calcitic limestone, sodium bicarbonate, sodium chloride, chromium yeast, dicalcium phosphate, ventilated sulfur, calcium iodate, copper sulfate, magnesium oxide, sodium selenite, cobalt sulfate, manganese sulfate, zinc sulfate, vitamin A, vitamin D3, vitamin E, flavoring additive, antioxidant additive, and growth promoter additive (sodium monensin).

The animals also had access to tropical pastures with forage species of the genera Urochloa decumbens (brachiaria decumbens), Panicum maximum (cv. Mombaça) and Cynodon spp. (cv. Tifiton 85), being considered the control treatment. In the other treatments, the control diet was provided, plus 0.5 kg or 1.0 kg of probiotic corn on a dry matter (DM) basis, respectively. The diet was estimated at a bulky feed:concentrate ratio of 67:33. To evaluate the quality of the pasture, the simulated grazing method was used, according to the Johnson methodology[12].

The bromatological composition of the ingredients used in the animals' diets is described in Table 1. The bromatological analyses followed the analysis specifications according to AOAC International[13]; Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF) analyses were determined according to the methods of Van Soest *et al*.[14]. Dry matter (INCT-CA G-003/1) and mineral matter (INCT-CA M-001/2) analyses were performed by the method of Detmann *et al.*[15], and crude protein (CP) analysis was determined by the Kjeldahl method (AOAC)[16]. The analysis of ether extract was performed with the ANKON® EXTRACTUR equipment. Total carbohydrates were calculated using the equation proposed by Snifen *et al*.[17]:

$$CHO = 100 - (\%\mathrm{CP} + \%EE + \%\,Ashes)$$

in which CH0 = total carbohydrates; CP = crude protein; EE = ether extract.

Table 1. Bromatological composition of the control diet and probiotic corn fed to the animals.

Variables (%)	Commercial Concentrate 22%	Corn Silage	Forage (*B. decumbens*, Mombaça and Tifton 85)	Probiotic Corn
Dry Matter	87.0	30	22.54	36.00
Crude Protein	22.0	4.25	18.18	8.58
Ether Extract	2.0	5.02	7.85	3.27
Fiber in Neutral Detergent	-	48.40	47.09	9.44
Fiber in Acid Detergent	11.0	28.80	23.48	2.11
Mineral Matter	9.0	3.25	10.20	0.94
Total Carbohydrates	67.0	87.48	63.77	87.21

The probiotic corn was produced according to the Louzada methodology[11], using the yeast strain Saccharomyces cerevisiae (UFLA CA-11), dechlorinated water and brown sugar. The product underwent a five-day incubation period before being supplied, and baits of this probiotic corn were used in the other preparations of the product during the experiment.

The animals were kept in paddocks with forages of the Urochloa, Panicum and Cynodom genera. They were also provided with a drinker in a collective lot with permanent water for 24 hours. Furthermore, they received part of the diet in the trough: half after the morning milking, around 8 am, and the other half after the afternoon milking, at 2 pm. The cows were individually restrained, and the probiotic corn was provided on top of the concentrate and bulky feed before the afternoon milking.

The cows were mechanically milked twice a day, and milk production was recorded weekly to monitor the effect of probiotic corn on production. Milk samples were collected on the 14th day of each experimental period, stored in plastic bottles with preservatives (Bronopol®), kept between 2 and 6 °C. The samples were sent to the Milk Clinic Laboratory in Piracicaba, SP, for analyses of crude protein, fat, lactose, total dry extract, urea nitrogen and somatic cell count. On the 14th day of the experimental period, the animals were individually weighed to assess weight variation and body condition; the body condition score (BCS) was assessed according to the methodology described by Edmonson *et al*.[18]. This method is based on visual and tactile assessments of body reserves in some parts of the animal's body, such as transverse processes, sacral ligament, coxolateral joint, ileum and ischia. Thus, the cows were classified on a scale ranging from 1 (thin) to 5 (extremely fat), with increments of a quarter of a point (0.25).

On the 15th day of the experimental period, the cows were subjected to visual observation to assess their ingestive behavior. The evaluation was performed using the methodology described by Silva *et al.*[19] in the Scan sampling model, in which the animals were observed individually every 10 minutes over a 12-hour period, from 6:00 a.m. to 6:00 p.m. The aspects observed were idle time, rumination, grazing, consumption at the trough, water intake, mineral salt intake and movement (in the milking parlor and pasture).

To assess the financial balance of probiotic corn, the price per kg of cornmeal, commercial concentrate with 22% crude protein, labor, yeast cost, bulky feed cost and milk price were considered. Prices were obtained through surveys in agricultural stores and feed mills in the Rio Pomba region - Minas Gerais during the months of October to December 2022.

To calculate the labor cost, the minimum wage of R$1,320.00 and the time spent on handling probiotic corn (20 minutes per day) were used as a basis, spending R$1.50/day on labor. The cost of bulky feed was estimated based on the pasture rent of R$40.00/head/month and considering that half of the bulky feed in the diet came from pasture and the other half from corn silage with an average price of R$0.42/kg.

The average price of commercial concentrate was R$96.00 per 40 kg bag, while cornmeal was R$60.00 per 50 kg bag (cost of R$1.36/kg of DM). Saccharomyces cerevisiae yeast cost R$39.90 per 50-gram package (cost of R$0.13/day/animal). The price per liter of milk paid to producers by dairy commerce in the Rio Pomba region was R$2.50. All prices were estimated in July 2023.

Every 1st and 5th day of incubation, samples of probiotic corn were taken from the drums to perform yeast analyses. For the analyses, 10 ml of probiotic corn diluted in 40 ml of deionized water were used. The samples were analyzed under an optical microscope, using the Neubauer Chamber, and the yeast count was performed manually using a 40x magnifying lens, according to ASBC[20].

Data on milk production and composition, live weight, body condition score and ingestive behavior were subjected to analysis of variance using a significance level of 10%. The evaluations were performed in a Latin square design and the means were compared by regression using the Sisvar statistical program[21].

3. Results and Discussion

According to the metabolic energy requirement of the cows, estimated at 64.42 Mcal/day, the diets of the three treatments met the needs of the animals (Table 2). However, the addition of 0.5 and 1.0 kg of probiotic corn to the diet resulted in an estimated increase of 13.3% in the starch content and 6.87% in the total digestible nutrients (TDN).

Table 2. Estimation of the composition and energy fraction of the diet provided to animals with the requirements calculated according to a spreadsheet supplied with data from the NRC [22].

Variables (DM)	Control	Probiotic corn (DM)	
		0.5 kg	1.0 kg
Concentrate consumption 22% CP (kg)	6.0	6.0	6.0
Corn silage consumption (kg)	6.0	6.0	6.0
Estimated pasture consumption (kg)	6.0	6.0	6.0
Dietary starch (%)	18.92	20.22	21.45
Dietary TDN (%)	67.85	68.33	68.78

The cows responded positively to the consumption of probiotic corn in amounts of 0.5 kg and 1.0 kg, except for only two cows that rejected it. Although attempts to stimulate consumption by mixing it into the diet were unsuccessful, it is possible to consider that probiotic corn has high palatability, since the different amounts used in the treatments did not significantly affect its consumption.

The addition of 0.5 kg and 1.0 kg of probiotic corn to the cows' diet provided greater milk production (P < 0.10) (Table 3).

Table 3. Milk production, live weight and body condition score of dairy cows receiving or not probiotic corn.

Variables	Control	Probiotic corn (MS)		CV	P-value
		0.5 kg	1.0 kg		
Milk production (liters/day)	21.15	22.36	22.5	6.3	0.0826 [1]
Live weight (kg)	569.5	572.92	572.92	1.1	0.3557 [2]
Variation in live weight (kg)	0.45	1.06	0.75	125.87	0.3360 [3]
Body condition score (1-5)	2.92	2.9	2.92	3.51	0.8493 [4]
Body condition score variation (%)	-0.0096	-0.0041	-0.0098	-121.09	0.3084 [5]

CV - Coefficient of Variation; P- Probability; Equations = / [1] Y=20.65 + 0.6792 X (R^2=83.17 %); [2] Y= 571.78; [3] Y=0.75; [4] Y= 2.91; [5] Y= -0.008

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An increase of 0.679 kg of milk was observed per kg of probiotic corn added to the diet. This increase in milk production can be attributed to the increase in energy provided by the inclusion of carbohydrates in the diet, as well as to the use of rehydrated corn, which increases the availability of nutrients and the digestibility of starch. Therefore, to produce probiotic corn, it is necessary to leave the corn in water under aerobic fermentation conditions[(23)].

The linear increase in milk production can also be attributed to the presence of yeast. According to a review carried out by Sniffen *et al.*[(24)] on supplementation of dairy cows fed diets based on corn silage and citrus pulp using Saccharomyces cerevisiae, the authors observed an increase of 1.45 L in milk production.

Jiang *et al.*[(25)] observed an increase in the concentration of amylolytic and cellulolytic bacteria in the rumen with the use of active yeasts, such as Saccharomyces cerevisiae. This effect resulted in improvements in the apparent digestibility of fibers, such as neutral detergent fiber (NDF) and acid detergent fiber (ADF), in addition to dry matter, contributing to the stabilization of ruminal pH and stimulating the development of gram-negative bacteria. These benefits promoted greater feed efficiency and, consequently, an increase in milk production.

The inclusion of probiotic corn in the diet of Girolando cows linearly increased, up to the levels tested (P < 0.10), the fat, protein and total solids contents (Table 4).

Table 4. Composition of milk from dairy cows with the inclusion of probiotic corn.

Variables	Control	Probiotic corn (DM)		CV	P- value
		0.5 kg	1.0 kg		
Fat (%)	3.3	3.23	3.68	10.14	0.0265 [1]
Protein (%)	2.99	2.9	3.01	3.2	0.0379 [2]
Lactose (%)	4.67	4.69	4.59	2.28	0.1228 [3]
Total Solids (%)	11.91	11.72	12.15	3.46	0.0955 [4]
Defatted Dry Extract (%)	8.6	8.49	8.47	2.04	0.1776 [5]
Somatic Cell Count(thousand cells/mL)	240	280	125.5	123.82	0.3809[6]
Urea Nitrogen (mg/dL)	11.73	11.94	12.79	18.29	0.4952[7]

CV - Coefficient of Variation; P - Probability; Equations= / [1] Y= 3.0275 + 0.1892X (R^2= 60.91%); [2] Y= 2.9550 + 0.0079X (R^2= 1.64%); [3] Y= 4.65; [4] Y=11.69 + 0.11X (R^2= 31.50%); [5] Y= 8.52; [6] Y=215.38; [7] Y= 12.15.

The treatment with the 1.0 kg level of probiotic corn showed a higher fat content in the milk. It was observed that each kg of probiotic corn added to the animals' diet resulted in an increase in the fat content of 0.18%. This effect can be attributed to the presence of the yeast Saccharomyces cerevisiae in the ruminal environment. Yeast reduces the oxygen concentration in the rumen, promoting the growth of cellulolytic bacteria such as Ruminococcus and F. succinogenes. These bacteria are responsible for the degradation of fiber, increasing the production of acetate, a volatile fatty acid responsible for increasing fat in milk[25, 26].

According to Wallace[27] and Vohra *et al.*[28], the inclusion of live yeast promotes an increase in the total number of bacteria in the rumen, mainly fibrolytic bacteria, being the main mechanism by which supplementation improves fiber digestion. Furthermore, Erasmus *et al.* [29], in a study on the effect of yeast culture and monensin on ruminal fermentation and production in multiparous Holstein cows, highlighted that increased fiber digestion could enhance fat production in milk. This effect was corroborated in some studies carried out with lactating cows[30, 31].

Regarding milk protein content, an increase of 0.0079% was observed for each kg of probiotic corn added to the diet. This increase can be attributed to the role of probiotic corn starch as an energy source for the ruminal microbiota, in addition to the presence of live yeast that maintains ideal conditions in the rumen. This favors the growth of ruminal bacteria, increasing the flow of microbial protein to the intestine and, consequently, contributing to the increase in protein concentration in milk.

This microbial protein of high biological value and better amino acid profile for animals results in an increase in milk protein[25]. Microbial growth can be stimulated by dietary yeast[32, 33], which in turn could increase protein flux to the intestine[34]. However, it is important to note that this data needs further elucidation, since the coefficient determination of the linear equation for milk protein was low, indicating a limited fit of the model.

A significant increase (P<0.10) in total milk solids content was observed. This increase was mainly due to changes in fat content, the fraction with the greatest variation. With the provision of probiotic corn to the cows, there was an increase in both fat content and total milk solids.

In the study conducted by Arcari *et al.*[35], replacing dry corn with rehydrated and ensiled corn demonstrated an increase in the consumption and digestibility of nutrients, which may contribute to the increase in the production of milk components. Regarding the ingestive behavior of the animals, there was a significant difference in the variables time in rumination and idleness (P < 0.10) (Table 5).

Table 5. Evaluation of the ingestive behavior of dairy cows receiving probiotic corn in a 12-hour/day evaluation.

Variables	Control	Probiotic corn (MS) 0.5 kg	Probiotic corn (MS) 1.0 kg	CV	P- value
Idle time (min)	243.75	205	233.75	11.92	0.0203 [1]
Ruminating (min)	181.25	203.75	187.5	7.75	0.0152 [2]
Grazing (min)	93.75	111.25	112.5	24.4	0.2004 [3]
Trough consumption (min)	168.75	168.75	152.5	12.81	0.1361 [4]
Water (min)	8.75	7.5	8.75	62.45	0.7990 [5]
Mineral salt (min)	3.75	0	1.25	173.21	0.0350 [6]
Moving (min)	20	23.75	23.75	30.09	0.3429 [7]

MIN - Minutes; CV - Coefficient of Variation; P - Probability; Equations = [1] Y= 237.50 – 5.00 X (R^2=6.18%); [2] Y= 184.58 + 3.1250 X (R^2=7.24%); [3] Y=105.83; [4] Y=163.33; [5] Y= 8.33; [6] Y=1.66; [7]Y=16.66..

It was found that rumination time increased (P<0.10) linearly by 3.12 minutes per kg of probiotic corn added to the cows' diet (Table 5). Idle time reduced linearly (P<0.10), around 5 minutes per kg of probiotic corn added to the diet (Table 5).

Although the coefficients of determination of the linear equations indicate a low adjustment of the linear model, it is suggested that the idleness and rumination variables may have been influenced by the dry matter intake of the animals. This occurred due to the addition of 0.5 kg and 1.0 kg of dry matter to the diet through probiotic corn. As a result, the animals may have increased their intake rate, reducing idle time and increasing the time spent on rumination.

According to Lashkari *et al.*[36], the supply of organic chromium to animals through yeast is a factor that can influence the modulation of dry matter intake and the increase in rumination time. This can take a supporting role in increasing the time dedicated to rumination, especially with the increase in the concentration of probiotic corn and yeast in the diet of dairy cows.

In the economic analysis of milk production and feed costs (Table 6), it was found that the most economically viable treatment was the use of 0.5 kg of probiotic corn in the animals' diet.

Table 6. Daily costs of feeding cows supplemented with probiotic corn.

Variables	Control	Probiotic corn (DM)	
		0.5 kg/day	1.0 kg/day
Concentrate consumption (kg/day)	6	6	6
Commercial concentrate price 22% PB (R$/kg)	2.4	2.4	2.4
Concentrate cost (R$/day)	14.4	14.4	14.4
Consumption of probiotic corn (kg/day)	-	0.5	1.0
Price of probiotic corn (cornmeal + yeast + labor)	-	1.68	3.36
Corn silage consumption (kg/day)	20	20	20
Corn silage price (R$/kg)	0.42	0.42	0.42
Cost of corn silage (R$/day)	8.40	8.40	8.40
Pasture cost (R$/day)	0.66	0.66	0.66
Total expenditure on bulky feed (R$/day)	9.06	9.06	9.06
Total expenditure on diet (concentrate + probiotic corn + bulky feed)	23.46	25.14	26.82
Daily milk production (liters/day)	21.15	22.36	22.5
Price of milk (R$/liters)	2.5	2.5	2.5
Recipe with milk (R$/day)	52.88	55.9	56.25
Daily financial balance (R$/day)	29.42	30.76	29.43
Relative financial balance (%)	100	104.6	100.05

The use of 0.5 kg of probiotic corn added R$1.68 to the animal's daily rate. With the increase in production of 1.21 liters of milk, this resulted in an increase of R$3.02 in revenue and had a positive impact of 4.6% on the financial balance. In this situation, the inclusion of probiotic corn not only covered the production cost but also generated profit.

The treatment with 1.0 kg of probiotic corn presented a higher total daily cost compared to the control treatment and the treatment with 0.5 kg of probiotic corn. The inclusion of 1.0 kg of probiotic corn increased the daily cost by R$ 3.36 per animal. To cover this additional cost, the production of 1.35 liters of milk more than the control treatment only equaled the nutritional investment, and the calculations did not consider the milk quality items that could increase the revenue.

There was no significant difference (P> 0.05) for the yeast concentration over a 5-day incubation period (Figure 1).

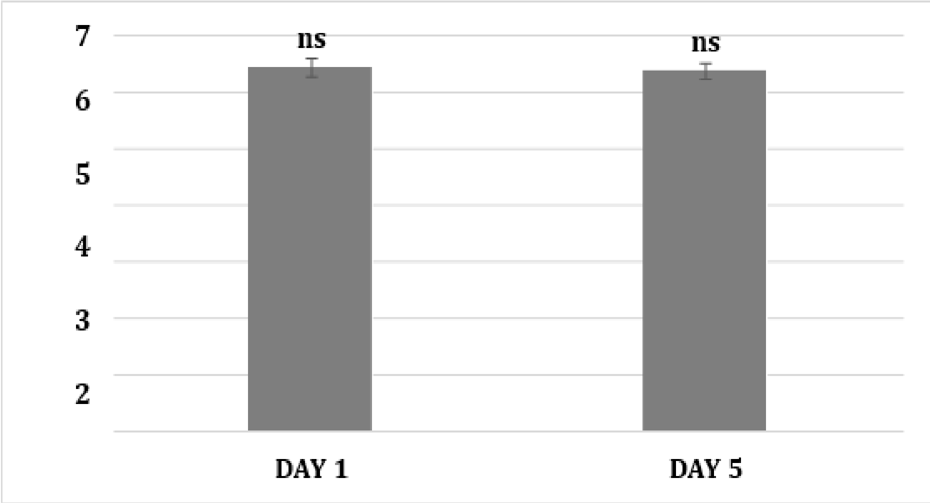

Figure 1. Viability of Saccharomyces cerevisiae yeasts on the 1st and 5th day of incubation of probiotic corn.
NS = no sig

On the 1st day of incubation, the yeast concentration in the probiotic corn was 6.42 Log Cell mL-1 and, on the 5th day, the concentration was 6.35 Log Cell mL-1 (Figure 1). The probiotic corn acts both as a food source and as a vehicle to provide live yeast to cattle. However, no differences in yeast concentration were observed when comparing the 1st day with the 5th day of incubation, which suggests that it is not necessary to wait the full five days of incubation to introduce probiotic corn to cattle. In any case, technology still requires further studies, especially with longer incubation times given the possibility of increasing the number of live yeasts.

4. Conclusion

It is concluded that the use of probiotic corn has proven to be a promising technique due to the significant increase in milk production and composition. Therefore, the use of 0.5 kg/DM of the product is recommended as it is more economically viable.

Conflicts of interest statement

The authors declare that there is no conflict of interest.

Data Availability Statement

Data will be provided upon request by the corresponding author.

Author contributions

Conceptualization: Teixeira, R. M. A. Formal analysis: Teixeira, R. M. A. Investigation: Silva, L. A. S. V. Investigation: Silva, L. A. S. V. Original Draft: Silva, L. A. S. V., Silva, M. I. M. Review and Editing: Silva, L. A. S. V., Silva, M. I. M.

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