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Addition of a homeopathic, preventive product for mastitis in dairy cow feed: effects on etiologic agents, animal health, production, composition, and quality of milk

Adição de um produto homeopático preventivo para mastite na alimentação de vacas leiteiras: efeitos sobre os agentes etiológicos, saúde animal, produção, composição e qualidade do leite

Adición de un producto preventivo homeopático para la mastitis en la alimentación de las vacas lecheras: efectos sobre los agentes etiológicos, la salud animal, la producción, la composición y la calidad de la leche

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Resumo

O objetivo deste estudo foi determinar se a adição de um produto homeopático à ração para vacas leiteiras melhoraria a saúde e a eficiência da produção, bem como a composição e qualidade do leite. O produto testado aqui foi produzido para estimular a imunidade e indiretamente para prevenir a colonização da glândula mamária por bactérias, diminuindo assim a contagem de células somáticas e bactérias no leite de vaca. Foram utilizadas 50 vacas leiteiras, divididas em dois grupos: Controle (n = 25) e Tratada (n = 25). Por 90 dias consecutivos, 50 gramas do produto homeopático (grupo tratado) foram adicionados ao concentrado; 50 g do veículo produto (calcário) foram adicionados ao concentrado das vacas controle. Foram realizadas análises de composição e qualidade do leite (dias 1, 15, 30, 45, 60, 75 e 90), bem como coleta de sangue para realização de análises hematológicas e metabólicas (dias 1, 30, 60 e 90). Descobrimos que o teor de gordura nas vacas leiteiras que consumiam o agente homeopático era maior do que no grupo controle. Devido ao aumento do teor de gordura, houve uma tendência de maior teor de sólidos totais nas vacas tratadas. Um efeito do tratamento foi encontrado em termos de contagem bacteriana total (CBT); ou seja, houve menor CBT no leite dos animais tratados do que nos animais controle. Houve uma tendência de efeito do tratamento versus dia para contagem de células somáticas (CCS); ou seja, houve menor CCS nos animais que consumiram o homeopático nos dias 15 e 30 do experimento. Não houve diferença entre os grupos em termos de produção de leite; entretanto, houve uma correlação negativa entre a produção de leite e CBT ou CCS no leite de vacas que consumiram produtos homeopáticos. Os animais do grupo tratado apresentaram menor contagem total de leucócitos e linfócitos do que o grupo controle, bem como tendência para menor contagem de neutrófilos nesses animais. Várias bactérias foram isoladas do leite de vaca durante o período experimental, sem efeito do tratamento. Em particular, isolamos

Corynebacterium spp., Staphylococcus aureus, Staphylococcus epidermidis e *Staphylococcus hyicus* em maior número de vacas em comparação com outros agentes etiológicos. Com base nesses resultados, concluímos que o consumo do produto homeopático por vacas leiteiras teve efeitos positivos na qualidade do leite.

Palavras-chave: Imunidade; Homeopatia; Contagem de células somáticas; Contagem bacteriana total.

Abstract

The aim of this study was to determine whether the addition of a homeopathic product to dairy cow feed would improve health and production efficiency, as well as composition and quality of milk. The product tested here was produced to stimulate immunity and indirectly to prevent the colonization of the mammary gland by bacteria, thereby decreasing somatic cell counts and bacteria in cow's milk. Fifty dairy cows were used, divided into two groups: Control (n = 25) and Treated (n = 25). For 90 consecutive days, 50 grams of homeopathic product (treated group) was added to the concentrate; 50 g of the product vehicle (limestone) was added to the concentrate of the control cows. We performed composition and quality analysis on the milk (days 1, 15, 30, 45, 60, 75, and 90) as well as blood collection to carry out hematological and metabolism analyses (days 1, 30, 60, and 90). We found that fat content in the milk cows consuming the homeopathic agent was greater than that of the control group. Because of this increased fat content, there was a tendency towards higher total solids content in treated cows. A treatment effect was found in terms of total bacterial count (TBC); that is, there were lower TBCs in milk of treated animals than in control animals. There was a trend of a treatment effect versus day for somatic cell count (SCC); that is, there were lower SCC in animals that consumed the homeopathic on days 15 and 30 of the experiment. There was no difference between groups in terms of milk production; however, there was a negative correlation between milk production and TBC or SCC in the milk of cows that consumed homeopathic product. Animals in the treated group had lower total leukocyte and lymphocyte counts than did the control group, as well as a tendency toward lower neutrophil counts in these animals. Several bacteria were isolated from the cows' milk during the experimental period, with no treatment effect. In particular, we isolated *Corynebacterium spp., Staphylococcus aureus, Staphylococcus epidermidis* and *Staphylococcus hyicus* in greater numbers of cows compared to other etiologic agents. Based on these results, we conclude that consumption of the homeopathic product by dairy cows had positive effects on milk quality.

Keywords: Immunity; Homeopathy; Somatic cell count; Total bacterial count.

Resumen

El objetivo de este estudio fue determinar si la adición de un producto homeopático a la alimentación de las vacas lecheras mejoraría la salud y la eficiencia de producción, así como la composición y calidad de la leche. El producto probado aquí fue producido para estimular la inmunidad e indirectamente para prevenir la colonización de la glándula mamaria por bacterias, disminuyendo así el recuento de células somáticas y bacterias en la leche de vaca. Se utilizaron cincuenta vacas lecheras, divididas en dos grupos: Control (n = 25) y Tratadas (n = 25). Durante 90 días consecutivos, se añadieron al concentrado 50 gramos de producto homeopático (grupo tratado); Se añadieron 50 g del vehículo producto (piedra caliza) al concentrado de las vacas de control. Realizamos análisis de composición y calidad de la leche (días 1, 15, 30, 45, 60, 75 y 90) así como extracción de sangre para realizar análisis hematológicos y metabólicos (días 1, 30, 60 y 90). Encontramos que el contenido de grasa en las vacas lecheras que consumían el agente homeopático era mayor que el del grupo de control. Debido a este mayor contenido de grasa, hubo una tendencia hacia un mayor contenido de sólidos totales en las vacas tratadas. Se encontró un efecto del tratamiento en términos de recuento bacteriano total (RBT); es decir, hubo RBT más bajos en la leche de los animales tratados que en los animales de control. Hubo una tendencia de un efecto del tratamiento frente al día para el recuento de células somáticas (RCS); es decir, hubo menor RCS en los animales que consumieron el homeopático los días 15 y 30 del experimento. No hubo diferencia entre los grupos en términos de producción de leche; sin embargo, hubo una correlación negativa entre la producción de leche y RBT o RCS en la leche de las vacas que consumieron productos homeopáticos. Los animales del grupo tratado tenían recuentos de leucocitos y linfocitos totales más bajos que el grupo de control, así como una tendencia hacia recuentos de neutrófilos más bajos en estos animales. Se aislaron varias bacterias de la leche de vaca durante el período experimental, sin efecto del tratamiento. En particular, aislamos *Corynebacterium* spp., *Staphylococcus aureus*, *Staphylococcus epidermidis* y *Staphylococcus hyicus* en un mayor número de vacas en comparación con otros agentes etiológicos. Con base en estos resultados, concluimos que el consumo del producto homeopático por las vacas lecheras tuvo efectos positivos sobre la calidad de la leche.

Palabras clave: Inmunidad; Homeopatía; Recuento de células somáticas; Recuento total de bacterias.

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1. Introduction

Subclinical mastitis, one of the most common problems in dairy production, is characterized by inflammation usually an infectious character. Mastitis causes a drop in milk quality and a decrease in herd productivity (Simões and Oliveira, 2012). Despite several options for allopathic treatment of mastitis, most are costly, and many, in turn, leave residues in the milk, making it necessary to discard the milk during the treatment period (Vragovíc et al., 2011). Furthermore, the indiscriminate and inappropriate use of antibiotics may be related to the emergence of multidrug-resistant bacteria (FAO, 2015). For these reasons, newer less aggressive treatment options are sought, mainly for the production of organic milk (Busanello et al., 2017). Among these options, homeopathy has gained ground in conventional and organic systems because homeopathic products used as preventives do not require disposal of milk (Bonamin, 2019). Studies report the use of homeopathic products for the treatment of mastitis (Doehring and Sundrum, 2016; Mathie and Clausen, 2015; Werner and Sundrum, 2006); nevertheless, it remains unknown scientifically as to whether these products effectively prevent control mastitis.

Homeopathic companies have generated several products, including some indicated for mastitis control; these companies have grown economically, because rural producers have bought these products to improve production and milk quality. Most homeopathic products are intended to stimulate immunity and thereby to prevent and control subclinical mastitis, consequently improving milk quality by reducing somatic cell counts (SCC) and total bacterial count (TBC) (Santos and Fonseca, 2007). Stress and low immunity are common situations in high-yielding dairy cows (Dahl et al. 2020) that are expected to diminish with daily consumption of homeopathic product. Homeopathic product sales sector reports indicate that these products can decrease production costs, reducing cases of mastitis in herds and thereby decreasing treatment costs, discarding of milk and early disposal of the animal; furthermore, they are less costly than allopathic treatments (Neto and Zappa, 2011).

The financial growth of the homeopathic pharmaceutical industry has been enormous, with several positive reports from producers; nevertheless, scientifically, the evidence remains limited. Therefore, the objective of this study was to determine whether the addition of a homeopathic product to dairy cow feed would improve health as well as production efficiency, composition, and quality of milk.

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2. Materials and Methods

2. 1 Homeopathic product

We used a homeopathic product indicated to prevent mastitis in dairy cows, in addition to improving the quality of milk (Estimulac H®, Organic Veterinary Homeopathy, Chapecó, SC, Brazil). The formulation of this product, prepared according to the Hahnemannian method (CH), includes *Staphilococcinum* (14CH), *Colibacillinum* (30CH), *Streptococcinum* (14CH), *Bryonia alba* (14CH), *Silicea terra* (200CH), *Phytolacca* (14CH), and carbonic limestone (14CH). The product was designed for daily consumption of 50 to 100 grams per animal.

2.2 Animals

We used 50 Holstein (n = 26), Jersey (n = 10) and crossbred (n = 14) dairy cows from two private properties located in the municipalities of Coronel Freitas and Xaxim in the state of Santa Catarina, Brazil. The two farms were chosen because they agreed to participate in the study and had histories of elevated SCC in milk.

The animals used differed in terms of ages, numbers of lactations, body weight and stages of lactation (1st and 2nd third of lactation). All animals were in the lactation phase and were apparently healthy.

In one of the properties, identified as "A," 33 cows were selected; they were confined to the same compost barn shed. These animals received a diet based on silage, hay and concentrate (Supplementary Table 1) during the experimental period.

The other property, identified as “B,” adopted a semi-extensive system, with animals fed on pasture of perennial grasses, African star, and Jiggs during the day, and silage and concentrate in the feeder after morning and afternoon milking. On this property, we selected 17 lactating cows, all of whom were healthy.

During the experiment (beginning, middle, and end), we collected samples of feed consumed by the cows on both farms. The samples were frozen and analyzed separately at the end of the experiment in a specialized laboratory. The bromatological analyses (dry matter, crude protein, ether extract, crude fiber, neutral detergent fiber (NDF), and acid detergent fiber (FDA)) of food was performed using the NIRS method (FOSS DS 2500). The results of the chemical composition of the food are presented in Supplementary Table 1.

2.3 Experimental design

Two groups of 25 cows each were formed, identified as control and treated. For the formation of homogeneous groups, we distributed the cows according to the following criteria: a) by property (farm A - 16 control and 17 treated; farm B - 9 control and 8 treated); b) breed (Holstein, Jersey, and Mixed); c) lactation phase (1st or 2nd third of lactation); and d) age.

The cows in the treated group consumed a daily dose of 50 grams of powdered homeopathic product per cow mixed with the concentrate, divided twice a day (07:00 AM and 06:00 PM) individually. The control group consumed the vehicle used to formulate the homeopathic in the same amount (50 g), that is, limestone. The experiment lasted 90 days, and the treatment was carried out during this experimental period.

Table 1. Feed used in cow feeding and chemical composition of feed.

	Property A		Property B	
Feed				
Concentrate (kg MS/animal)[1]	7		5	
Forage[2]	ad libitum		ad libitum	
Chemical composition[3]				
	Fodder	**Concentrate**	**Fodder**	**concentrate**
Dry matter	37.3 (6.7)	88.6 (0.96)	26.3 (0.67)	88.7 (0.67)
Crude protein	11.1 (3.5)	19.1 (5.2)	7.78 (1.05)	17.7 (0.47)
Ethereal extract	2.34 (0.46)	4.73 (0.83)	3.35 (0.22)	4.72 (0.33)
Ash	6.76 (2.2)	6.85 (0.25)	5.69 (0.24)	7.34 (0.64)
Crude fiber	-	5.64 (0.90)	-	5.42 (0.42)
NDF	44.2 (2.15)	-	46.7 (4.17)	-
ADF	26.8 (1.28)	-	30.1 (2.52)	-

[1] The concentrate was formulated based on corn, soy bean, wheat bran and mineral, acquired commercially by producers
[2] The experiment lasted 90 days, so the forage available to cows differed during the experimental period, with emphasis on silage and hay.
[3] For analysis of the chemical composition, samples were collected at three times (beginning, middle and end); samples analyzed separately and results presented as means and standard deviations.
Source: Authors.

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2.4 Measurement of milk

Milk was measured on days 1, 15, 30, 45, 60, 75, and 90 of the experiment, using meters coupled to the milking system (model Milk Meter), in which it was possible to measure the production of animals in kg of milk/cow/day.

2.5 Sample collection

For the microbiological culture analysis, milk samples were collected on days 1, 30, 60, and 90 of the experiment into sterile bottles, after cleaning the teat, washing with running water, drying with disposable paper towels and using alcohol solution iodinated to 5%, for disinfection of the teat orifice. After the first three jets of milk were discarded, 10 mL of milk was collected for microbiological examination. The samples were transported frozen in an isothermal box with recyclable ice to the laboratory of the University of the West of Santa Catarina (UNOESC), where the analyses were carried out on an outsourced basis (NMC, 2004).

For analysis of milk composition and quality, we used a homogeneous sampling of the morning milking stored in the milk meter during the measurement of the volume of milk produced. The sample contained 50 mL of milk from each cow, allocated in specific bottles with two preservative tablets (Bronopol®). The samples were stored at 10 ºC and sent to the Centralized Laboratory for Milk Analysis of the Paraná Herd Analysis Program (PARLPR - APCBRH) in Curitiba-PR, Brazil. This laboratory is accredited by the Ministry of Agriculture, Livestock and Supply (MAPA) and is part of the Brazilian Milk Quality Network (RBQL).

Blood samples were collected on days 1, 30, 60, and 90 of the experiment, drawn from the caudal vein with needles and vacuum tubes. For hematological analysis, blood samples were collected in tubes with anticoagulants (EDTA); for biochemical analyses, we used tubes without anticoagulant. All blood samples were stored in isothermal boxes with ice, maintained at 10 ºC during transport to the laboratory where the samples from tubes without anticoagulant were subjected to centrifugation (3400 g for 10 minutes) to obtain the serum, and were stored at –20 ºC.

2.6 Laboratory analysis

2.6.1 Centesimal composition of milk

Fat, protein, lactose, total solids, and urea were measured in raw milk using the medium infrared spectrometry method (ISO 9622/IDF 141, 2013).

2.6.2 Somatic cell count and total bacterial count

The flow cytometry method was used to obtain somatic cell counts (ISO 13366-2/IDF 148-2, 2006) and total bacterial counts (ISO 16297/IDF 161, 2013; ISO 21187/IDF 196, 2004). Both analyses were accredited by the Ministry of Agriculture, Livestock and Supply (MAPA) and by the National Institute of Metrology, Quality and Technology (Inmetro).

2.6.3 Microbiological culture

The samples were cultured on blood agar supplemented with 5% defibrinated sheep blood, MacConkey agar and Sabouraud agar. A 10 µl volume of milk was inoculated into each culture medium and the plates were incubated at 37 ºC for 24–72 hours. The microorganisms that grew on the plates were identified according to the morpho-dye characteristics and methodologies described by NMC (2004) and Markey et al. (2013).

2.6.4 Hemogram

The blood count was performed within 2 hours after sample collection, with the count of erythrocytes, total leukocytes, and hemoglobin using a semiautomatic blood cell counter (model CELM CC530). Hematocrit was measured using capillary tubes, centrifuged for 1 minute at 14,000 rpm. The leukocyte differential was performed through evaluations of blood smear stained using Panotico Rapido kits.

2.6.5 Serum biochemistries

Serum levels of total protein, albumin, triglycerides, cholesterol, and glucose were measured using a semi-automatic analyzer (BioPlus 2000®) with commercial kits (Analisa®, Brazil), following the manufacturer's recommendations. Globulin levels were obtained by subtracting albumin from total protein (globulin = total protein – albumin).

2.7 Statistical analysis

Each animal was considered the experimental unit for all analyses. All dependent variables were tested for normality using the Univariate procedure of SAS (SAS Inst. Inc., Cary, NC, USA; version 9.4) and log-transformed (SCC and TBC) when needed. Then, all data were analyzed using the MIXED procedure of SAS, with the Satterthwaite approximation to determine the denominator degrees of freedom for the test of fixed effects. All variables were analyzed as repeated measures and tested for fixed effects of treatment, day, and treatment × day, using animal (treatment) and farm as random variables. All results obtained on d 1 for each variable, days in milk and number of calving of each cow were included as covariates, but were removed from the model when $P > 0.10$. The compound symmetric covariance structure was selected for milk production and milk concentration of protein; the Toeplitz covariance structure was selected for serum concentration of glucose and hematocrit, neutrophils, and eosinophils; and the first order autoregressive covariance structure was selected for all other variables. The covariance structures were selected according to the lowest Akaike information criterion. A simple Pearson correlation was evaluated among the variables using CORR procedure of SAS to determine the interrelation between these. Means were separated using PDIFF and all results were reported as LSMEANS followed by SEM. Significance was defined when $P \leq 0.05$, and tendency when $P > 0.05$ and ≤ 0.10.

3. Results

3.1 Milk production and composition

There was no effect of treatment, day and treatment versus day relationship for milk production and lactose concentration in milk ($P > 0.05$) (Table 2). A treatment effect was observed in the fat concentration ($p = 0.05$), significantly higher in the milk of cows that consumed the homeopathic product. We also found a tendency for a higher percentage of total solids in the treated cows ($p = 0.08$) (Table 2). Effect of the day was observed for protein, fat, total solids, and urea in the milk of cows in both groups (Table 2).

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Table 2. Milk production and quality of dairy cows that received homeopathic product via diet (treated group) compared to cows in the control group.

Variables[1]	**Treatments**[2]		**SEM**	***P* – values**[3]		
	Control	**Treated**		**Treat**	**Day**	**Treat × Day**
Milk Production (L/day)				0.86	0.20	0.59
d 1	17.58	17.83	0.52			
d 15	18.74	17.68	0.52			
d 30	17.39	17.49	0.52			
d 45	17.49	17.64	0.52			
d 60	17.82	17.58	0.52			
d 75	17.29	17.00	0.52			
d 90	17.00	17.37	0.52			
Average	17.55	17.47	0.42			
Protein (%)				0.41	0.01	0.58
d 1	3.22^{BC}	3.19^{B}	0.07			
d 15	3.18^{C}	3.19^{B}	0.06			
d 30	3.15^{C}	3.07^{C}	0.06			
d 45	3.21^{BC}	3.27^{A}	0.06			
d 60	3.27^{BC}	3.28^{A}	0.07			
d 75	3.40^{A}	3.31^{A}	0.07			
d 90	3.31^{AB}	3.25^{A}	0.06			
Average	3.25	3.20	0.05			
Fat (%)				0.05	0.05	0.55
d 1	4.26^{A}	4.11^{AB}	0.44			
d 15	3.34^{B}	3.92^{AB}	0.44			
d 30	3.78^{AB}	4.21^{AB}	0.44			
d 45	3.76^{AB}	3.72^{B}	0.44			
d 60	3.69^{B}	4.00^{AB}	0.44			
d 75	3.76^{AB}	4.07^{AB}	0.44			
d 90	3.99^{AB}	4.33^{A}	0.44			
Average	3.80^{x}	4.05^{y}	0.40			
Lactose (%)				0.26	0.25	0.63

d 1	4.46	4.44	0.03			
d 15	4.43	4.51	0.03			
d 30	4.42	4.51	0.03			
d 45	4.49	4.55	0.03			
d 60	4.45	4.45	0.03			
d 75	4.45	4.49	0.03			
d 90	4.44	4.43	0.03			
Average	4.45	4.48	0.02			
Total solids (%)				0.08	0.01	0.48
d 1	12.89^{AB}	12.67^{AB}	0.52			
d 15	11.84^{AB}	12.44^{B}	0.52			
d 30	12.25^{BC}	12.72^{AB}	0.52			
d 45	12.42^{AB}	12.54^{B}	0.52			
d 60	12.34^{BC}	12.59^{AB}	0.52			
d 75	12.51^{C}	12.70^{AB}	0.52			
d 90	12.65^{A}	13.08^{A}	0.52			
Average	12.41^{x}	12.68^{y}	0.48			
Urea				0.13	0.01	0.37
d 1	16.28^{BC}	16.52^{B}	0.81			
d 15	21.33^{A}	21.90^{A}	0.81			
d 30	15.64^{BC}	16.32^{B}	0.81			
d 45	15.36^{BC}	15.97^{B}	0.81			
d 60	13.45^{CD}	14.79^{B}	1.41			
d 75	12.50^{D}	15.40^{B}	0.81			
d 90	10.36^{E}	9.51^{C}	0.81			
Average	14.98	15.77	0.37			

[1]Control and Treated represents control cows and those that received homeopathic product via diet daily, respectively.
$^{x\text{-}y}$Within a row, without a common superscript differ ($P \leq 0.05$) or tends to differ ($P \leq 0.10$).
$^{A\text{-}E}$Within treatment, without a common superscript differ ($P \leq 0.05$) or tends to differ ($P \leq 0.10$).
Source: Authors.

3.2 Milk quality

There was a tendency effect in the treatment x day ratio for the variable somatic cell count in milk (p = 0.09), with a lower SCC in the milk of cows that consumed the homeopathic product on days 15 and 30 of the experiment (Table 3). For this variable, there was no effect of treatment or day (P >0.05).

A treatment effect was observed for variable TBC in milk (p = 0.05), lower in cows in the treated group than in the control group (Table 3). We also found an effect of the day for TBC in milk in both groups of cows (p = 0.01).

Table 3. Somatic cell count (SCC) and total bacterial count (TBC) values in the milk of dairy cows that received homeopathic product via diet (treated group) compared to cows in the control group.

Items[1]	**Treatments**[2]		**SEM**	***P* – values**[3]		
	Control	**Treated**		**Treat**	**Day**	**Treat × Day**
SCC (CS/mL)				0.30	0.57	0.09
d 1	424.62	372.33	109.81			
d 15	688.62^{x}	293.55^{y}	109.81			
d 30	650.71^{x}	352.51^{y}	109.81			
d 45	325.57	363.59	109.81			
d 60	397.71	442.64	109.81			
d 75	307.05	384.72	109.81			
d 90	315.40	404.59	109.81			
Average	450.37	375.28	59.90			
TBC (UFC/mL)				0.05	0.01	0.56
d 1	118.32^{A}	78.02^{AB}	28.77			
d 15	35.43^{B}	32.27^{B}	28.77			
d 30	76.40^{AB}	42.07^{B}	28.77			
d 45	49.21^{B}	42.40^{B}	28.77			
d 60	60.09^{B}	77.65^{AB}	28.77			
d 75	72.67^{AB}	33.13^{B}	29.11			
d 90	112.21^{A}	95.36^{A}	28.77			
Average	74.90^{x}	57.27^{y}	21.20			
Subclinical mastitis incidence (%)				0.07	0.18	0.15

d 1	51.58	57.12	0.06
d 15	51.58	44.08	0.06
d 30	68.97	52.77	0.06
d 45	68.97	52.77	0.06
d 60	68.97	44.08	0.06
d 75	64.62	65.82	0.06
d 90	73.31	57.12	0.06
Average	64.00^x	53.39^y	0.05

[1]Somatic cell count (SCC) and total bacterial count (TBC).
[2]Control and Treated represents control cows and cows that received homeopathic product via diet daily, respectively.
[x-y]Within a row, without a common superscript differ ($P \leq 0.05$) or tends to differ ($P \leq 0.10$).
[A-E]Within treatment, without a common superscript differ ($P \leq 0.05$) or tends to differ ($P \leq 0.10$).
Source: Authors.

3.3 Serum biochemistry

There was a trend of treatment effect x day for serum albumin levels (p = 0.09), higher in the serum of cows in the treatment group on day 30 of the experiment (Table 4). We also found a tendency toward a treatment effect for total protein levels (P = 0.10), higher in the serum of cows that consumed the homeopathic product (Table 4). There were effects of the day for both groups with respect to total protein and globulin (Table 4). However, the effects of treatment, day and the treatment x day relationship were not observed for urea, cholesterol, triglycerides, or glucose (p > 0.05) (Table 4).

Table 4. Biochemistry serum of dairy cows that received homeopathic product via diet (treated group) compared to cows in the control group.

Items	**Treatments[1]**		**SEM**	***P* – values[2]**		
	Control	**Treated**		**Treat**	**Day**	**Treat × Day**
Albumin (g/dL)				0.25	0.45	0.09
d 1	3.48	3.10	0.35			
d 30	2.65^x	3.58^y	0.32			
d 60	2.48	2.74	0.34			
d 90	2.42	3.22	0.40			
Average	2.75	3.16	0.30			
Total protein (g/dL)				0.10	0.01	0.86

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d 1	8.81^{A}	9.14^{AB}	1.12			
d 30	8.09^{AB}	9.26^{A}	1.11			
d 60	9.32^{A}	9.75^{A}	1.11			
d 90	7.41^{B}	8.11^{B}	1.11			
Average	8.40^{x}	9.07^{y}	1.00			
Globulin (g/dL)				0.14	0.01	0.38
d 1	5.33^{B}	6.04^{AB}	0.78			
d 30	5.44^{B}	5.68^{B}	0.77			
d 60	6.84^{A}	7.01^{a}	0.77			
d 90	4.99^{B}	4.89^{B}	0.76			
Average	5.65	5.91	0.71			
Urea (mg/dL)				0.55	0.20	0.42
d 1	31.08	26.36	3.84			
d 30	32.39	35.20	3.51			
d 60	35.88	36.19	3.77			
d 90	29.86	22.86	4.21			
Average	32.30	30.15	2.51			
Colesterol (mg/dL)				0.77	0.35	0.93
d 1	127.99	133.94	18.59			
d 30	124.95	136.40	16.71			
d 60	155.83	151.41	17.61			
d 90	105.45	114.11	20.68			
Average	128.55	133.96	13.47			
Triglycerides (mg/dL)				0.14	0.59	0.70
d 1	33.44	33.16	6.60			
d 30	38.76	31.23	5.92			
d 60	37.63	29.96	6.50			
d 90	49.60	36.71	7.34			
Average	39.86	32.77	4.36			
Glucose (mg/dL)				0.97	0.18	0.30
d 1	60.08	62.44	6.83			
d 30	53.51	43.15	6.20			
d 60	60.74	61.76	6.50			

d 90	47.15	55.10	7.33
Average	59.40	56.95	3.63

[1]Control and Treated represents control cows and cows that received homeopathic product via diet daily, respectively.
[x-y]Within a row, without a common superscript differ ($P \leq 0.05$) or tends to differ ($P \leq 0.10$).
[A-B]Within treatment, without a common superscript differ ($P \leq 0.05$) or tends to differ ($P \leq 0.10$).
Source: authors.

3.4 Hematological analysis

A treatment effect was observed for total leukocytes (p = 0.05) and lymphocytes (p = 0.02) during the experimental period, with both variables showing lower counts in the blood of cows that consumed homeopathic product (Table 5). We found a trend of the treatment x day effect for the number of neutrophils (p = 0.07), that is, neutrophil counts were lower in the blood of cows in the treated group than in the control group on day 90 of the experiment (Table 5). An effect of the day was observed for both groups (p < 0.05) for hemoglobin concentration, number of erythrocytes, number of total leukocytes, neutrophils, and eosinophils (Table 5).

Table 5. Values of hematological analysis of dairy cows that received homeopathic product via diet (treated group) compared to cows in the control group.

Items	**Treatments[1]**		**SEM**	***P* – values[2]**		
	Control	**Treated**		**Treat**	**Day**	**Treat × Day**
Hematocrit (%)				0.37	0.13	0.90
d 1	26.23	28.30	1.92			
d 30	24.83	24.37	1.81			
d 60	23.31	25.54	1.96			
d 90	27.89	28.77	2.12			
Average	25.56	26.75	1.04			
Hemoglobin (g/dL)				0.35	0.01	0.96
d 1	8.10^{B}	7.91^{BC}	0.25			
d 30	8.63^{A}	8.29^{AB}	0.23			
d 60	8.86^{A}	8.60^{A}	0.26			
d 90	8.03^{B}	7.72^{C}	0.29			
Average	8.40	8.13	0.18			

Erythrocytes ($x10^6$µL)				0.37	0.01	0.45
d 1	5.03^{AB}	5.14^{A}	0.24			
d 30	5.33^{B}	5.22^{A}	0.24			
d 60	4.62^{C}	4.57^{B}	0.24			
d 90	4.88^{BC}	4.53^{B}	0.24			
Average	4.96	4.87	0.20			
Leukocytes ($x10^3$ µL)				0.05	0.01	0.12
d 1	5.71^{B}	3.11^{B}	1.84			
d 30	11.91^{A}	3.93^{B}	1.62			
d 60	12.70^{A}	8.91^{A}	1.63			
d 90	13.32^{A}	10.46^{A}	1.86			
Average	10.91^{x}	6.60^{y}	1.43			
Neutrophils ($x10^3$/µL)				0.44	0.09	0.07
d 1	1.50^{B}	2.26^{A}	0.90			
d 30	4.24^{A}	3.23^{A}	0.77			
d 60	3.36^{AB}	3.24^{A}	0.83			
d 90	5.40^{Ax}	3.09^{Ay}	1.02			
Average	3.62	2.95	0.60			
Lymphocytes ($x10^3$/µL)				0.02	0.12	0.35
d 1	7.40	4.53	0.94			
d 30	7.65	4.14	0.94			
d 60	7.82	5.25	0.94			
d 90	6.79	4.59	0.94			
Average	7.42^{x}	4.63^{y}	0.86			
Monocytes ($x10^3$/µL)				0.66	0.11	0.77
d 1	0.54	0.51	0.12			
d 30	0.43	0.42	0.12			
d 60	0.35	0.37	0.12			
d 90	0.36	0.23	0.12			
Average	0.42	0.39	0.10			
Eosinophils ($x10^3$/µL)				0.82	0.01	0.87
d 1	0.13^{AB}	0.09^{B}	0.03			
d 30	0.19^{A}	0.19^{A}	0.03			

d 60	0.15^{A}	0.14^{AB}	0.03
d 90	0.08^{B}	0.09^{B}	0.03
Average	0.14	0.13	0.02

[1]Control and Treated represents control cows and cows that received homeopathic product via diet daily, respectively.
[x-y]Within a row, without a common superscript differ ($P \leq 0.05$) or tends to differ ($P \leq 0.10$).
[A-C]Within treatment, without a common superscript differ ($P \leq 0.05$) or tends to differ ($P \leq 0.10$).
Source: Authors.

3.5 Pearson correlation

The results of Pearson's correlation by treatment are shown in Table 6. In both groups, there was a negative correlation between milk production and milk composition (fat, protein, total solids); there was a positive correlation trend between milk production and urea in milk only in the treated group. Only in the milk of cows in the treated group did we see a negative correlation between milk production and milk quality (SCC and TBC), that is, the higher the milk production, the lower the levels of SCC and TBC in that group. In both groups, we found a positive correlation between SCC and TBC (Table 6).

The results of Pearson's correlation considering cows from the two treatments are presented in Table 7. There was a negative correlation between milk production and milk quality (fat, protein, total solids, TBC, and SCC) and white blood cells (leukocytes and lymphocytes); as well as positive correlation between milk production and urea levels in milk. There was a positive correlation between TBC and SCC, different from the correlation between TBC versus blood count (erythrocytes, leukocytes and eosinophils), which was negative.

Table 6. Pearson correlation coefficients among milk production versus milk quality (fat, protein, total solids, urea, TBC and SCC) and TBC versus SCC to control group and treated group, evaluated separate by group.

	Treatments			
	Control		**Treated**	
Variables[1]	**Correlation coefficients**	***P* - values**	**Correlation coefficients**	***P* – values**
Milk Production (L/day)				
× Milk fat (%)	-0.42	0.01	-0.21	0.01
× Milk protein (%)	-0.39	0.01	-0.27	0.01
× Milk total solids (%)	-0.50	0.01	-0.22	0.01
× Urea (%)	0.14	0.09	0.18	0.05
× TBC (UFC/mL)	-0.07	0.37	-0.19	0.01
× SCC (CS/mL)	-0.08	0.32	-0.21	0.01
TBC (UFC/mL)				
× SCC (CS/mL)	0.47	0.01	0.36	0.01

[1]Somatic cell count (SCC) and total bacterial count (TBC). Only show the variables that differ ($P \leq 0.05$) or tended to differ ($0.05 > P \leq 0.10$).
Source: Authors.

Table 7. Pearson correlation coefficients among milk production versus milk quality (fat, protein, total solids, urea, TBC and SCC) and white blood cells (Leukocytes and lymphocytes); TBC versus SCC; TBC versus hemogram (erythrocytes, leukocytes and eosinophils) considering data from cows from both groups.

Variables[1]	**Pearson correlation coefficients**	***P* - values**
Milk Production (L/day)		
× Milk fat (%)	-0.31	0.01
× Milk prot (%)	-0.34	0.01
× Milk total solids (%)	-0.36	0.01
× Urea (%)	0.15	0.01
× TBC (UFC/mL)	-0.14	0.01
× SCC (CS/mL)	-0.17	0.01

× Leukocytes (x10^3 µL)	-0.20	0.01
× Lymphocytes (x10^3/µL)	-0.21	0.01
TBC (UFC/mL)		
× SCC (CS/mL)	0.39	0.01
× Erythrocytes (x10^{6}µL)	-0.19	0.03
× Leukocytes (x10^3 µL)	-0.18	0.05
× Eosinophils (x10^3/µL)	-0.17	0.05

[1]Somatic cell count (SCC) and total bacterial count (TBC). Only show the variables that differ ($P \leq 0.05$) or tended to differ ($0.05 > P \leq 0.10$).
Source: Authors.

3.6 Microbiological isolation in milk

In both groups, the most isolated bacteria were *Staphylococcus* and C*orynebacterium*, followed by *Streptococcus*, with *Staphylococcus hyicus* being the most prevalent in both groups. *Corynebacterium* spp. were isolated from more animals on days 30, 60, and 90 in both groups; however, these were not isolated from any animals on day 1 (Table 8).

Table 8. Bacteria isolated from the milk of cows in the control and treated groups (homeopathic product) in four moments of collection (day 1, 30, 60 and 90).

Results/bacteria	**Day 1 (n/group)**		**Day 30 (n/group)**		**Day 60 (n/group)**		**Day 90 (n/group)**	
	CON	**TREAT**	**CON**	**TREAT**	**CON**	**TREAT**	**CON**	**TREAT**
No bacterial growth	3	5	1	4	3	2	3	3
Enterococcus saccharolyticus	4	2	2	1	-	2	-	1
Chromobacterium	2	3	-	-	-	-	-	-
Corynebacterium	-	-	5	5	2	2	7	8
Enterobacter aerogenes	3	2	-	-	1	-	-	-
Enterococcus faecalis	-	-	-	-	1	-	-	1
Staphylococcus	-	-	1	-	-	-	-	-

acidominimus								
Staphylococcus aureus	1	4	3	3	2	3	2	4
Staphylococcus epidermidis	2	3	2	2	7	6	3	1
Staphylococcus hyicus	8	6	6	4	7	9	5	4
Staphylococcus intermedius	2	1	2	1	-	1	4	2
Staphylococcus coagulase negativa	-	-	-	2	-	-	-	-
Staphylococcus	-	-	-	1	-	-	-	1
Streptococcus acidominimus	1		1	3	-	-	1	2
Streptococcus alactolyticus	2	-	-	-	1	-	-	-
Streptococcus equinus	-	1	-	1	-	-	-	-
Streptococcus uberis	-	-	1	4	1	3	5	4
Streptococcus spp.	-	-	-	-	-	-	1	1
Streptococcus agalactiae	-	-	-	-	-	1	1	1
Streptococcus dysgalactiae	1	2	-	-	1	1	-	-
Acinetobacter spp.	-	2	1	-	-	1	-	-
Alcaligenes faecalis	1	-	-	-	-	-	-	-
Aeromonas spp.	-	-	1	-	-	2	-	-
Citrobacter diversus	-	-	1	-	-	-	-	-
Trueperella spp.	-	-	-	-	2	1	-	-

Source: Authors.

4. Discussion

The effect of the homeopathic product was not significant in all the variables analyzed. However, higher fat content related to lower TBC and SCC in milk are desirable results for the industry and for the regulatory structures in force in Brazil (Brasil, 2018). The fat content in both groups was within the minimum limits recommended by IN 76 (Brasil, 2018); therefore, the increase in the fat content in cows that consumed homeopathic product is important for higher yield of dairy production. Furthermore, farmers receive bonuses from milk-collecting industries that provide milk-quality payment programs. The trend towards higher percentages of total solids is probably related to the higher fat content in milk. We believe that the desirable effects on milk quality are indirect effects of homeopathy; that is, daily consumption improved the health of the cows, and this was reflected in the composition and quality of their milk. Differently from our study, researchers found that there was a tendency to increase SCC in milk when a homeopathic combination was provided to cows with good mammary gland health (Silva et al. 2011). Like the authors, we have not been able to increase the SCC for this reason; as well as explaining the discrepancy between the results; although we have worked with different homeopathic products.

Recently, we found that a homeopathic product indicated for the control of bacterial infections stimulated immune responses and controlled experimental infections by *E. coli* in an experimental model (Jaguezeski et al., 2020). This can be explained by the reduction in levels of environmental agents and the increase in levels of contagious agents during the experiment. However, in the context of contagious mastitis, there is usually an increase in CSS, and this was not seen in our experiment. This information is important for bovine mastitis, an inflammatory pathology in the mammary gland caused mainly by bacteria, especially *Streptococcus agalactiae*, *Streptococcus uberis*, *E. coli*, and *S. aureus*, among other staphylococci, in addition to non-bacterial agents fungi and even microalgae, with *S. aureus* being the pathogenic microorganism most frequently isolated in raw milk (Zecconi and Hahn, 2000; Langoni et al., 2011). In our study, *Corynebacterium* and *Staphylococcus* were the primary isolates. These bacteria generate contagious mastitis but it is also an opportunistic agent and are transmitted between animals via handling during milking (Tomazi et al., 2014). *Corynebacterium* colonizes the teat channel of dairy cows. It has been used as a hygiene indicator at the time of milking, as its prevalence has been documented on farms where control of contagious mastitis is not correctly applied (Gonçalvez et al., 2016). In the present study, we did not verify the visual effect of homeopathic treatment; that is, in both groups, the behavior of cows contaminated by microorganisms was similar.

The use of homeopathic agents has been increasing in recent years. These agents function as immunological stimulants (Mazón-Suástegui et al., 2017), and they have direct effects on the control of diarrhea, decreasing the count of pathogenic bacteria in the feces of calves and lambs (Fortuoso et al., 2018; 2019). Phytotherapeutics and minerals are used in mixtures of homeopathic compounds because of their immunological properties (Kayne, 1992). Homeopathic drugs differ from allopathic medicines in that they have extremely low concentrations, in order to be safer and present lower risks of toxicity (Halberstein, 2005).

As already mentioned, the formulation of homeopathic products uses ingredients of specific interest, following the guidelines of the Brazilian Homeopathic Pharmacopoeia. For example, *Colibacillinum* was used to produce the homeopathic used in this study; it has antimicrobial effects that have been described in the literature, in particular, control of infections by *E. coli* (Lopes, 2006). *Silicea terra* helps treat infectious and inflammatory diseases (Ribeiro Filho, 2017). *Phytolacca* is a plant that has biological properties capable of controlling diseases of the mammary glands, including clinical and subclinical mastitis (Almeida, 2005; Mangiéri et al., 2007). Our findings suggest that, directly or indirectly, the homeopathic product reduced bacterial counts in milk, fulfilling its antimicrobial purpose when added to the formulation of the commercial product.

Almeida et al. (2005) carried out a study with three groups of animals experimentally inoculated with *S. aureus* and treated with a homeopathic and sodium cefoperazone, maintaining a group as a control. The homeopathic used by the researchers included some ingredients also present in the commercial product tested in the current study (Almeida et al. 2005); the authors found that cows with clinical mastitis and subjected to homeopathic treatment were those with the highest percentage of cured mastitis (negative CMT test) when compared to other treatments. In our study, we did not have any cows with clinical mastitis during the experimental period; and subclinical mastitis evaluated by analyzing individual CCS per animal at each collection; nevertheless, the negative correlation between milk production and SCC and TBC suggests that the intake of homeopathic had positive effects on the health of the mammary gland, reducing the number of cows with subclinical mastitis. We cannot explain why the SCC decreased in cows that consumed the homeopathic agent in the first 30 days of the experiment; nevertheless, SCC increased again in subsequent analyzes, perhaps due to the increase in levels of contagious agents.

Homeopathic agents are also important for reducing bacterial resistance to antibiotics, and for increasing their curative and/or preventive effects (Weiermayer, 2018). The mechanisms by which a homeopathic product controls mastitis may involve technical explanations described above; nevertheless, we cannot discard the principles of homeopathy. According to the literature, homeopathy encourages healing mechanisms through immune stimulation to fight viruses, bacteria, fungi, tumors, and other diseases, allowing the restoration of balance in the animal's system and encouraging organic responses that can lead to a reduction of stress (Da Costa Filho et al. 2014), as well as stimulating the proliferation of leukocytes in healthy dogs that consumed a homeopathic product daily (Marchiori et al., 2019). Homeopathic medicines can decrease microbial infection by strengthening immunity (Bonamin, 2019). We believe that the lower contamination by microorganisms of the mammary gland (lower TBC) reduced the inflammatory response, explaining the lower counts of total leukocytes, lymphocytes, and neutrophils in the blood of cows that consumed the homeopathic. The leukogram results suggest that the homeopathic product indirectly reduced the systemic inflammatory response, which is positive in farm animals, because animals spend less energy (ATP) to activate defense cells, and so this energy can be used for milk production. In the present study, we did not identify an effect of the treatment on the volume of milk produced by the cows; however, when we correlated milk production with SCC and TBC, we found that there was an effect of the homeopathic treatment on milk production, because the cows that had better milk quality (lower SCC and TBC) were the ones that produced more milk.

Despite there being lower lymphocyte counts in cows that consumed the homeopathic product, this did not negatively affect levels of total protein and globulins in the serum; by contrast, we found numerically higher levels of total protein in these animals. Importantly, the low white blood cell counts in healthy animals is expected, because there is no need to destroy pathogens (Salami, 2018; Duan et al., 2019); similarly, this is important for maintaining high levels of globulins, the animal's humoral defense system. Other studies reported an increase in the number of lymphocytes when the animals received homeopathic medicines (Leal et al. 2012; Abud et al. 2006; Burbano et al. 2009; Cesar et al. 2008), unlike what we reported here. We believe that the lower degree of bacterial contamination in the mammary gland was responsible for the reduction of lymphocytes and neutrophils, because these are the first lines of immune defense against pathogens (Cohn and Hirsch, 1960). In particular, neutrophils destroy pathogens by phagocytosis, production of reactive oxygen species, as well as secretion of antimicrobial peptides (Dale and Boxer, 2008).

We did not observe any effects of the homeopathic agent on red blood cell counts, or on systemic carbohydrate, lipid, and protein metabolism. Nevertheless, in milk, we observed a positive correlation between milk production and urea levels; this can be interpreted as a positive effect that may be related to better metabolic health of these animals.

5. Conclusion

The consumption of the homeopathic product by the cows had positive effects on the quality of the milk, particularly the reduction of TBC and higher fat content in the milk. We conclude that there is an effect of homeopathic treatment on productive efficiency and milk quality, demonstrated by the finding that the cows that consumed the homeopathic agent had higher milk production and lower SCC and TBC. Cows that consumed homeopathic agent had lower total leukocyte, lymphocyte, and neutrophil counts, and this is likely related to lower degrees of bacterial contamination in the mammary gland.

Ethics committee

This project was approved by the Committee for the Use of Animals in Research (CEUA) of the University of the State of Santa Catarina (UDESC) under protocol number 2000060919.

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