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Original Article

No effects of homeopathy on somatic cell count, bovine milk yield and composition

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ABSTRACT

This study aimed to evaluate the effect of a homeopathic complex, on the reduction of milk somatic cell count, production of milk, fat percentage and protein percentage of milk in lactating Holstein cows, over a period of three months. Twenty cows, in second parity, were randomly assigned in blocks with two treatments, after an initial period of adaptation in the experimental area for 15 days. The cows were distributed into a control group (that did not receive the homeopathic complex) and a treated (that received the homeopathic complex), evaluated for six test-day records. Laboratory analysis of fat and protein were made via the infrared absorption and somatic cell count by flow cytometry, wherein this latest has been transformed into logarithmic scale for statistical analysis. Analysis of variance to test the effects of treatment, test-day record and the interaction between these factors, was performed through repeated measures over time. For all traits, there was no significant interaction between treatment and test-day record ($p > 0.05$) and there was no statistical difference ($p > 0.05$) between the control and treated groups. The use of the homeopathic complex was not effective in reducing somatic cell count of dairy cows, for the trial period of three months. The use of homeopathy did not interfere significantly with milk yield or the protein percentage in Holstein cows.

INTRODUCTION

Mastitis is the most prevalent disease in dairy cattle, especially in its subclinical form and is responsible for major losses in the milk industry (TOMAZI et al., 2015). The consequent inflammation of the mammary gland can result in a reduction in milk production and solids, caused by changes in secretory epithelial cells and vascular permeability of secreting alveoli during infection (ZHAO; LACASSE, 2008). The intensity of the inflammatory process can be influenced by several factors such as severity of the infection, type of the pathogen causing mastitis, the animal's age, time of year, nutritional status, erroneous management and genetic predisposition. With parturition stages progresses, there is an increase in somatic cell count (SCC) due to the amplified cellular response to current infections and residual lesions from previous infections (CUNHA et al., 2008). Moreover, mastitis is correlated with milk quality since it reduces lactose, fat and casein and elevates sodium and chloride levels (MELLO et al., 2012).

Despite the availability of various antimicrobial agents for mastitis treatment, the resistance of microorganisms has increased, due to indiscriminate and improper use of antibiotics, particularly in Brazil (COSTA et al., 2013). Treatment failure can also be related to intracellular survival ability of some bacteria as well as the pathologic changes induced by certain infections which prevent access of the drug to the infected cells (MOTA et al., 2005).

An effective program of mastitis control should be based primarily on preventative measures. In view of this, the

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use of homeopathic therapy can be an alternate method of prevention which assists the animal's specific and non-specific defenses in eliminating the invading microorganisms (ORJALES et al., 2015). Some authors mentioned that homeopathic practice can be considered an alternative to allopathic medicine, due to its lower cost and easy administration. So, the animals are not subjected to restraint and to the trauma caused by injectable medications. In addition, homeopathic products are administered via food and water and therefore would not require changes in management, thereby minimizing stress (LEVIONNOIS; MORMÈDE, 2014).

Furthermore, there is a growing interest in the livestock sector in alternative treatments to antibiotics, since preventive homeopathic treatment produces no known adverse effects on the environment (MARTINS et al., 2007). However, there are many different homeopathic protocols without specific recommendations for dairy cow producers, suggesting the need for studies to define the dosages and lengths of treatment time for these methods to be effective (Werner et al., 2010). Therefore, the objective of this study was to evaluate the effect of a homeopathic complex, over a period of three months, on the milk somatic cell count (SCC), milk yield (MY), fat percentage (%FAT) and protein percentage (%PROT) of lactating Holstein cows.

MATERIAL AND METHODS

This study was approved and performed under the guidelines of Ethics Committee in Animal Use (CEUA) of the Ponta Grossa State University (UEPG), according to statement nº 014/2014, in accordance to the regulations of the Brazilian Collegiate of Animal Experimentation (COBEA). The experiment was conducted in the municipality of Castro, Paraná State, whose geographic coordinates are: 24º51'30.23" S, 49º56'27.13" W. The farm is located at an average altitude of 1007 m above sea level. According to the research report by Embrapa (2002), the weather is considered wavy and is under the influence of climate type Cfb of Köeppen (humid, subtropical, mesothermal climate), with an average annual temperature of 18 ºC and average annual rainfall of 1500 mm.

Twenty Holstein cows with 31.8 kg of milk yield average and moderate individual SCCs (305,000 of arithmetic average and ranging between 250,000 - 400,000 cells), all in second parity, were distributed in a completely randomized block design, according to level production (high or low). The treatments were: a control group did not receive the homeopathic complex (CG, n = 10), and treatment group received the homeopathic complex (TG, n = 10). Each treatment were distributed in ten blocks, containing two animals of both level productions, high (≥ 15 liters/day) and low (< 15 liters/day) levels.

The mathematical model used to estimate the statistical results was:

$$\Delta Y = \mu + Q_j + M_m + Q \times M + B_k + D_l + e$$

Where: ΔY is the difference in each evaluated variable (milk yield, fat, protein and SCC) between CG and TG; μ is the overall mean difference; Q_j is the number of the test-day record (j = 1 to 6); M_m is the presence or absence of homeopathic therapy (m = 1 to 2; CG and TG); Q×M is the interaction between the fixed effects, B_k is the number of blocks (k = 1 to 10), D_l is days in milk (l = 94 to 282) as a covariable and e is the random error term.

The animals were housed in a semi-confined system, fed twice a day, and provided mineral supplementation *ad libitum*. In the experiment period, the animals had free access to grazing oats, ryegrass and Tifton 85. The total mixed ration contained corn silage, pre-dried rye grass, concentrate with 18% crude protein, mineral salt, yeast and corn bran. According to the total mixed ration, the average total dry matter intake/day (DMI/d) was 11.37 kg DMI/d and 10.51 kg DMI/d for high and low production cows, respectively.

The experiment lasted 90 days, between the months of September and December of 2014, and comprised six test-day records (fortnightly). Prior to the experiment, the animals were allowed to have 15-days to adapt to the experimental area, as recommended by Brown et al. (2006). After the 15-day period, the TG received the homeopathic complex mixed into their diet. The homeopathic complex offered for low and high yielding cows contained, respectively, 0.00005 g and 0.00007 g of *Bryonia alba*, 0.00005 and 0.00007 g *Mercurius solubilis*, 0.0001 and 0.00014 g *Phosphorus albus*, 0.0001 and 0.00014 g *Pulsatilla nigricans*, 0.0001 and 0.00014 g of *Staphylococcinum* and 0.0001 and 0.00014 g of *Streptococcinum* offered in a mineral carrier in total amounts of 10 g/animal/day of the homeopathic complex for low producing cows and 14 g/animal/day for high producing cows.

The groups of medicines used in the homeopathic preparation attended the pathogenetic activity, as mentioned by Naresh et al. (2005) and Martins et al. (2007), being *Mercurius solubilis, Staphylococcinum and Streptococcinum* with action under subclinical mastitis, *Bryonia alba, Pulsatilla nigricans* nd *Phosphorus albus* with anti-inflammatory action. To obtain the potencies, the hahnemannian method was used in a decimal scale of the derived pharmaceutical forms, whose technique is described in the Farmacopéia Homeopática Brasileira (2011). After preparation of the desired potencies, all the medicaments contained were mixed and impregnated in 10% calcium carbonate, according to the technique of powder impregnation, also described in the Farmacopéia Homeopática Brasileira (2011).

The response variables analyzed were SCC, milk production in kg (MY), fat percentage (%FAT) and protein percentage (%PROT). Laboratory analysis of fat and protein were made via the infrared absorption and SCC by flow cytometry using a high-capacity somatic cell counter (Somacount300®, Bentley Instruments Inc., Chaska, MN, USA), wherein this latest has been transformed into logarithmic scale score of somatic cells (ECS) based on the procedure developed by Shook (1982), wherein ECS = $\log_2$ (SCC/100) + 3. For statistical analysis, the effects tested were CG and TG treatments, test-day record (1st, 2nd, 3rd, 4th, 5th and 6th), as well as the interaction between homeopathic treatment and test-day record. For this, we used the PROC MIXED of statistical software SAS (2009) for analysis of variance by repeated measurements over time, considering a 5% level of significance by the F-test.

RESULTS AND DISCUSSION

According to the results, there was no interaction between the treatments and test-day record ($p>0.05$) for all traits (Table 1). Therefore, it was clear that the different test-day record did not interfere with the different treatments adopted in the study. These results are in agreement with Silva et al. (2011) who evaluated the interaction between different homeopathic treatments and the week of sample collection and showed no significant effect at the 5% level of significance.

Table 1 – Somatic cell count (SCC), milk production in kg (MY), fat percentage (%FAT) and protein percentage (%PROT) in Holstein cows supplemented (treated group) or not (control group) with homeopathic product.

Variables	Mean (± D.P.)		p-value		
	CG	TG	Treatments	Test-day record	Interaction
SCC (x1000 mL-1) (before the experiment)	286.09 (± 217.33)[1]	285.46 (± 208.78)[1]	-	-	-
SCC (x1000 mL-1) (during the experiment)	287.45 (± 268.28)[1]	322.26 (± 226.82)[1]	0.495[2]	0.035[2]	0.904[2]
MY (kg)	32.36 (± 9.43)	31.41 (± 6.20)	0.756	<0.0001	0.246
%FAT	3.54 (± 0.92)	3.58 (± 0.71)	0.895	<0.0001	0.802
%PROT	3.30 (± 0.42)	3.40 (± 0.33)	0.501	<0.0001	0.547

[1] Original values of SCC (Arithmetic average)
[2] Statistic results based on values of SCC transformed in score (log10).

Similarly, our results of milk quality parameters did not differ significantly between CG and TG ($p > 0.05$) for the characteristics evaluated in this study (Table 1). This indicated that the homeopathic treatment was not effective for the control of mastitis, when offered for a 90 days period. One possible explanation for this fact is that homeopathic treatment produces changes in homeostasis after a certain period of time, usually after six months (MACLEOD, 2012). Thus, depending on the disease being treated, a medium to long-term treatment period may be necessary to achieve satisfactory results with homeopathy (MARTINS et al., 2007). In addition, feedlot and even semi-feedlot, are not considered as original breeding grounds for cattle and, therefore, for a homeopathic view of health, these animals will never be considered healthy and will require being medicated most of times.

Nóbrega et al. (2009) evaluated homeopathic supplementation in 50 animals over a two month period and also found no significant difference in SCC. According to those authors, homeopathic treatment aims to produce a host response capable of eliminating microorganisms causing mastitis, which consequently will increase the cellularity in milk, raising the SCC levels in treated animals. These authors suggest, therefore, that the SCC levels tend to be highest early in the treatment, decreasing to lower levels after longer periods of treatment. In this case, it is possible to believe that the short period of experiment (two months) was not long enough to result in an effective action against the microorganisms that causes mastitis. In addition, it is known that one of the characteristics of the homeopathic medicine is that it was not made to remove any living organism. Its action in the animal body is to strengthen the defense mechanisms in order to achieve a healthy balance, seeking to minimize the occurrence of harmful processes.

Healthy animals supplemented with 150 g of a homeopathic complex in a 63-day trial period presented an increase in SCC which might be associate to overdose (SILVA et al., 2011). Compared to the present study, it seems that the dose administered may be considered elevated for healthy animals. Furthermore, Silva et al. (2011) concluded that there may be an increase in SCC in

animals supplemented with a homeopathic complex, even if they have good mammary gland health. This fact could also be related to an increased milk cellularity, due to an increased capacity of animals to eliminate pathogenic microorganisms, because of the stimulation of neutrophils to effectively act in the infection site. It is important to mention that at the beginning of homeopathic treatment a strong eliminatory action of toxins occurs, which may compromise the results (ie, for reasons of dose dependence).

Another important aspect, as Bueno et al. (2005) mentioned, is that several factors influence the variation in SCC in lactating cows, such as age, parturition order, lactation phase, season, time of treatment, and especially the severity of infection. Thus, there is no surprise to not see a significant reduction of the SCC with short-term (less than one month) use of a homeopathic complex, as homeopathic treatments should be primarily administered long-term (more than six months) and should be used with preventive, instead of curative, aims (LANGE-CONSIGLIO et al., 2014). According to the results obtained in this study, as well as the literary support, it is evident that the homeopathic use in order to reduce SCC has no effect when used for a shorter period than three months. However, it is important to emphasize that homeopathy differs from allopathic medication, since the homeopathic process does not allow a forced reaction of the organism, working together with the physiology of the treated individual. This factor makes the homeopathic treatment more susceptible to management failures, feeding and other environmental factors.

There was no difference of milk yield, %FAT and %PROT between TG and CG (Table 1). Similarly, in a study by Mitidiero (2002), there was no significant difference in MY in 28 animals with homeopathic supplements. In that study, the homeopathic product was offered for a long period of time (eleven months), and therefore could have potentially influenced the animals' physiology and consequently milk productivity, since it is expected that the action of the homeopathic complex is effective when used over a long period of time. However, this effectiveness was not observed in the mentioned research.

Relating mastitis with milk yield, Machado; Cassoli (2003) point out that high average levels of SCC limit potential increases in production. According to these authors, an animal with mastitis may fail to produce 0.5 to 3 L per day due to high levels of SCC. However, Tomazi et al. (2015) did not show any influence of mastitis caused by coagulase-negative staphylococci on production and milk composition, which suggests that productivity may be affected by the severity of inflammation and even the causative agent. Comparing the results of both mentioned studies, it seems that milk yield is only affected when average levels of SCC are high enough to result in a severe inflammatory process that interferes with the proper physiological functioning of the animal. In the present study, we believe that the SCC levels were not high enough to interfere with MY and composition, and we showed that the SCC levels were relatively controlled throughout the experiment for both TG and CG.

The results of the comparison between TG and CG for %FAT ($p > 0.05$) was consistent with the study by Salvador et al. (2008), which did not demonstrate a difference in the fat levels between animals that received organic versus inorganic sources of supplementation. According to the authors of that study, only the utilization of acidogenic diets resulted in increased fat levels, and thus, not having the interference of homeopathic factors to increase fat content in milk.

The results obtained in our study for %PROT ($p > 0.05$) were analogous to the findings of Martini et al. (2000), who evaluated 50 cows for 17 months and showed no change in the milk protein content due to the use of homeopathic products, even for a long trial period. Also, according to Fernandez et al. (2009), higher rates of SCC, characterized by the presence of mastitis, can increase the total protein concentration in milk, but with a significant reduction in casein, which would not be desirable. It is noteworthy that a decrease in casein would cause losses to the dairy industry since this is the most important protein for the production of dairy products and therefore, is directly related to the product quality (Comin et al., 2008). Another important aspect to be considered is that the %PROT may increase in animals presenting mastitis, but not due to the growth of milk quality, but due to the decreased on milk yield resulting from the inflammatory process in the mammary gland system.

Statistical difference ($p = 0.035$) was observed for SCC in test-day record effect, showing significant fluctuations between the six test-day records performed in this experiment (Figure 1). In addition, also for test-day effect, there was difference ($p < 0.0001$) for MY, %FAT and %PROT, as shown in Figures 2, 3 and 4. In the first three evaluations of milk components, the average SCC, both in TG and in CG, tended to decrease, with a more intense fall in TG. This fact could be related both to the action of the homeopathic product as well as an environmental interference. However, from the 4° test-day record the SCC average increased again, supporting an argument that the drop in SCC in the first months of the experiment may be more related to environmental factors than influence of the homeopathic complex, even utilizing the block design trying to control the environmental effects. Another important factor that corroborated with increasing SCC from 4° test-day record was the weather transition, as there were rising temperatures in the second half of the experiment (early summer). Given this increase in temperature, it was

expected that the TG would demonstrate greater control over the increase in SCC between the second and third month of the experiment, however, this did not occur.

Figure 1 – Means of somatic cell count (SCC) for TG (treated group) and CG (control group) on six test-day records.

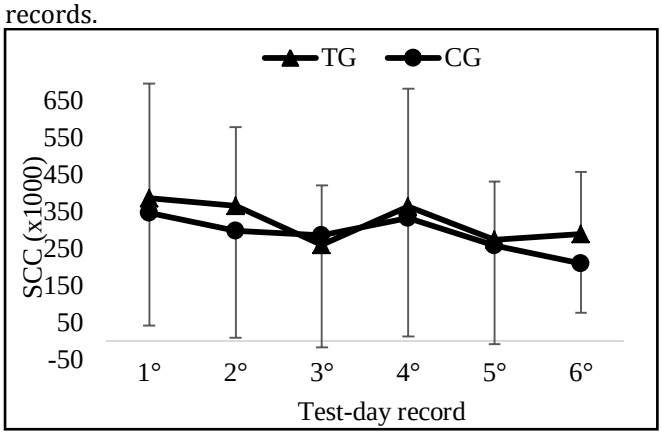


Figure 2 – Means of milk yield for TG (treated group) and CG (control group) on six test-day records.

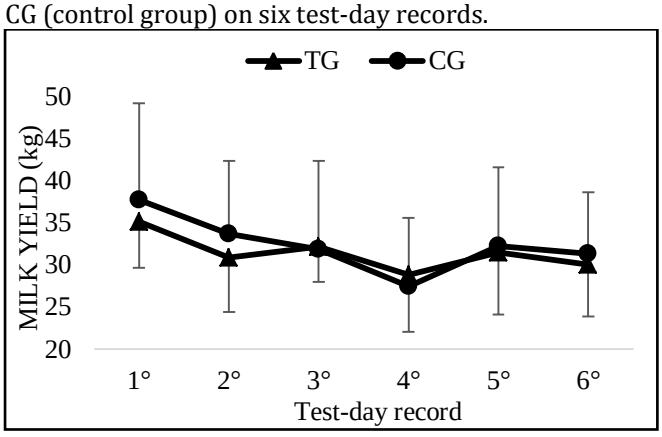


Figure 3 – Means of fat percentage for TG (treated group) and CG (control group) on six test-day records.

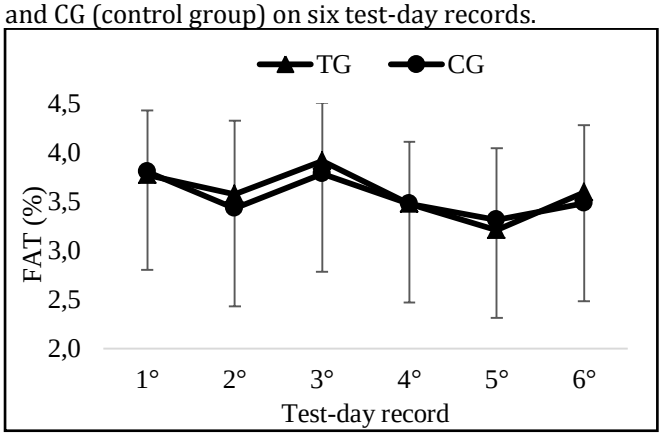
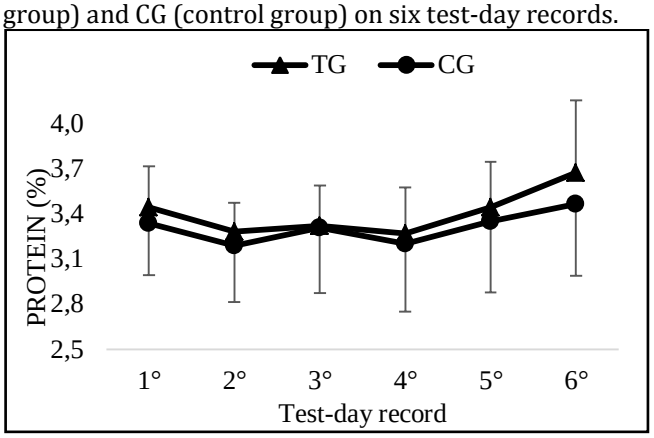


Figure 4 – Means of protein percentage for TG (treated group) and CG (control group) on six test-day records.



CONCLUSIONS

The animals treated with the homeopathic complex showed similar performance to the untreated group for the SCC trait, in an experimental period of three months. In addition, there was no difference between control and treatment group in the production of milk and solids, demonstrating that the use of the homeopathic complex did not interfere in the productivity of Holstein cows.

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