EFFECT OF A HOMEOPATHIC COMPOUND ON SOMATIC CELL COUNT AND MILK PRODUCTION IN "PARDA ALPINA" GOATS

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ABSTRACT

The aim of this study was to evaluate the efficacy of a homeopathic compound in the treatment and prevention of mastitis in goats, especially by analyzing its effect on somatic cell count and milk production before, during and after its administration. Forty dairy goats of "Pardo Alpina" breed were randomly divided in a double-blind experiment into two groups. The animals were weekly evaluated for somatic cell count (SCC) and milk production. Before the homeopathic treatment, the control group had 1.42 $x10^3$ cells/mL (log-transformed value) and 1.10 liters of daily mean production, compared to 1.31 $x10^3$ cells/mL and 0.9 liters of mean production by the experimental group. During the experiment, the control group had increased LogSCC values and decreased milk production (2.11 $x10^3$ cells/mL and 1.00 L, respectively); the same was noticed for the experimental group (1.97 $x10^3$ cells/mL and 0.80 L milk, respectively). Such facts suggest that there is interference of external factors on the increased milk cellularity in the different periods, not necessarily due to the homeopathic compound. In the present study, SCC and milk production were not affected by the homeopathic compound.

Key words: goats, homeopathy, mastitis, somatic cell count.

EFEITO DE UM COMPOSTO HOMEOPÁTICO NA CONTAGEM DE CÉLULAS SOMÁTICAS E PRODUÇÃO DE LEITE EM CABRAS PARDO ALPINAS

RESUMO

O objetivo do presente estudo foi avaliar a eficácia de um composto homeopático no tratamento e prevenção da mastite em cabras, principalmente analisando seu efeito na contagem de células somáticas e produção de leite antes, durante e depois de sua administração. Em um estudo duplo cego, quarenta cabras leiteiras da raça Parda Alpina foram aleatoriamente divididas em dois grupos. Os animais foram avaliados semanalmente pela contagem de células somáticas (CCS) e produção leiteira. Antes do uso do composto contendo medicamento homeopático, o grupo controle apresentou 1.42 x 10^3 células/mL de

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leite (valor convertido em escala logarítmica) e 1.10 litros de produção média diária de leite, comparado a 1.31 x 10^3 células /mL e 0.9 litros de produção média do grupo experimental. Durante o experimento, o grupo controle teve seus valores de LogSCC aumentados e produção leiteira reduzida (2.11 x 10^3 células/mL e 1.00 litros respectivamente); o mesmo foi observado para o grupo experimental (1.97 x 10^3 células/mL e 0.80 litros de leite respectivamente). Tais resultados sugerem que fatores externos interferem na celularidade do leite, não necessariamente o composto homeopático. No presente estudo, CCS e produção leiteira não foram afetadas pelo composto utilizado.

Palavras-chave: cabras, contagem de células somáticas, homeopatia, mastite.

EFECTO DE UN COMPUESTO HOMEOPÁTICO DE CÉLULAS SOMÁTICAS Y LA PRODUCCIÓN DE LECHE EN CABRAS "PARDA ALPINA"

RESUMEN

El objetivo de este estudio fue evaluar la eficacia de un compuesto homeopático en el tratamiento y prevención de la mastitis en cabras, especialmente teniendo en cuenta su efecto sobre el recuento de células somáticas y producción de leche antes, durante y después de su administración. En un estudio doble ciego, cuarenta cabras lecheras de la raza "Parda Alpina" fueron divididas aleatoriamente en dos grupos. Los animales fueron evaluados semanalmente por el recuento de células somáticas (RCS) y la producción de leche. Antes de el compuesto homeopático, el grupo de control presento 1.42 x 10^3 células / ml de leche (valor convertido en una escala logarítmica) y 1.10 litros de producción media diaria de leche, frente a 1,31 x 10^3 células / ml y 0,9 litros de litros de producción media del grupo experimental. Durante el experimento, el grupo control tuvo sus valores de LogSCC aumentados y su producción de leche reducida (2.11 x 10^3 células / ml y 1.00 litros, respectivamente), lo mismo se observó para el grupo experimental (1,97 x 10^3 células / ml y 0.80 litros de leche respectivamente). Estos factores sugieren que factores externos influyen en la celularidad de la leche, no necesariamente el compuesto homeopático. En este estudio, RCS y producción de leche no se vieron afectados por el compuesto utilizado.

Palabras clave: cabras, recuento de células somáticas, homeopatía, mastitis

INTRODUCTION

Goat milk is important in human diet and is recommended for children, elders and frail people due to its high nutritive value. Since it is less allergenic, goat milk has been accepted by people that are intolerant to bovine milk(1). Although it is important as food, it may transmit several pathogenic microorganisms, as well as their toxins, to humans.

Mastitis is a complex multifactorial disease that represents a serious problem for dairy production (2). It is characterized as an inflammatory response of the mammary gland, resulting in great economic losses to the dairy industry of small ruminants or cattle (3). While there is a lot of information regarding mastitis in cows, few studies have been conducted with goats (4). Mastitis represents a highly relevant problem not only due to the economic losses it causes to producers, but also because of the decrease in quality and safety of dairy products provided to humans (5)

When the infection is established, white blood cells migrate to the infection site in order to combat the microorganism, inducing the inflammation and an increase in somatic cell count (SCC). In addition to SCC increase, lactose concentration is significantly lower in milk samples from infected mammary gland. Protein concentration tends to increase and milk production tends to decrease (6, 7).

The limit for SCC in goat milk bulk tanks in the USA is 1 x 10^6 cells per mL, and several farmers cannot reach such a limit. The European Union has not established its limit yet (8). Goat milk has higher SCC than cow milk (9). Even for environments free of intramammary infection (IMI), the values in the literature range from 0.27 to 2.0 x 10^6 cells/mL (10). High-quality milk has low somatic cell number and low bacterial count, and is free of pathogens and antibiotic residues (9).

Antimicrobial therapy remains the most used treatment for mastitis in goats and cattle (11). The use of antimicrobials to treat mastitis may lead to the presence of residues in the milk and contribute to increased microbial resistance (7). Several antimicrobials have presented weak or moderate activity against microorganisms isolated from goat milk (12). There are reports in the literature indicating a positive relationship between high-cellularity milk and presence of antimicrobials (9).

The development of resistance by bacterial agents to antimicrobials has become a great concern. Theoretically, resistance should not exist; however, this problem has increased (13), and such concern is not only restricted to veterinarians, but also to farmers (14).

Traditional programs to control mastitis have not been commonly applied to dairy caprinoculture, and few farmers adopt the practice of therapy at drying off (12).

The interest in homeopathic therapy has increased during the last years and received great attention in veterinary medicine especially because such compounds do not have side effects (15). Several consumers have looked for alternative products in the market, which has increased the demand for organic dairy products due to the safety of foods from traditional production. In the European Union, organic farmers still use antibiotics to treat clinical mastitis; however, they have adopted alternative treatments, especially homeopathy (11).

There are scarce data on the efficacy of homeopathic treatment in veterinary medicine. A few studies performed have failed to show the efficacy of homeopathy, including the mastitis (16, 17). Similarly to other alternative therapies, a holistic view of this disease is emphasized, and the individual therapy is important. These differences make studies involving homeopathy challengeable, and few of them are accepted scientifically when compared to conventional therapies (18).

The aim of this study was to evaluate the efficacy of a homeopathic compound in the treatment and prevention of mastitis in goats, especially by analyzing its effect on somatic cell count and milk production before, during and after its administration.

MATERIALS AND METHODS

Forty dairy goats of Alpine Parda breed, different ages, different calving number and similar lactation stage were used in this study. The animals were randomly divided in a double-blind experiment into two groups: a control group and an experimental group, both constituted of 20 animals under the same conditions. Controlled clinical trial of double-blind type is considered the most reliable method to evaluate a medical treatment (19).

During fifteen days before the treatment, the animals were weekly evaluated for SCC and mean milk production by the animal on the previous day. After this period, the animals from the experimental group were daily medicated for two months, and samples were weekly collected for the same analyses. A homeopathic formulation (20 mL) was elaborated in accordance with the methodology previously described by Nóbrega et al. (20), in a

prescription pharmacy and consisted of *Phytolacca decandra* CH 12, Lachesis CH 12, Belladona CH 12, Phosphorus CH 30, *Bryonia dióica* CH 12, *Conium maculatum* CH 12, *Apis mellifica* CH 30, *Mercurius solubilis* CH 12, and Pyrogenium CH 6. These components were properly ground with 5 kg crystal sugar (vehicle, like the sucrose present in the homeopathic medicine of human or veterinary use, sold as globules) until the formulation was homogenous. The mixture was kept cold, protected from sun, light, heat, and electric and magnetic irradiation. Daily, each animal received 10 g formulation, i.e. 200 g together with their food. The same quantity of sugar was provided for the control group, with no addition of the homeopathic hydroalcoholic solution (placebo).

After treatment, another two collections with one-week interval were done to perform the previously mentioned analyses again.

To evaluate milk cellularity, milk samples from each udder of all animals used in 12 collections were analyzed. SCC was electronically obtained by using the automated cell counter Somacount-300 (Bentley) calibrated for goats. Spreadsheets containing data on milk production before sample collection were obtained in the farm.

For all statistical tests, each udder was considered independent from the adjacent one of the same animal, except for tests involving milk production, which both udders composed just one datum to be evaluated per animal. According to some authors, intramammary infection dynamics is better understood in goats when SCC is analyzed in each udder independently (21). Furthermore, intramammary infection may affect the milk composition from a mammary gland, and in most cases the milk from the adjacent udder is not affected (3).

Statistical Analysis

The obtained SCC values were converted into a logarithmic scale (LogSCC) and expressed as mean values at 95% confidence interval. The normal distribution was checked using Shapiro-Wilk test.

The General Linear Model with repeated measures was used to evaluate the group effect on the dependent variable by considering LogSCC for the 8 weeks of treatment the dependent variable; mean LogSCC values from the first two and the last two collections as covariates; and the group as the independent variable.

The results for cellularity were grouped by animal into three means, representing the three different study periods. LogSCC was compared between groups in the three periods using t-tests. Analysis of variance (ANOVA) with repeated measures (dependent) were used in both groups to evaluate the hypothesis of an effect of period and animal lot on milk cellularity. Post-hoc tests (Tukey, REGWK, Hochberg's GT2) were developed to further evaluate the results if there was a significant difference between study periods.

For production analyses, median values were calculated per collection and per period for each animal, including percentiles 25 and 75 of such values. The General Linear Model with repeated measures was also used to evaluate the group effect on the dependent variable by considering milk production for the 8 weeks of treatment as the dependent variable; the production median values from the first two and the last two collections, the covariates; and the group, the independent variable.

Results for milk production were also grouped into the three proposed periods. Mann-Whitney non-parametric test was used for independent samples for comparison between groups. Friedman's test was used to compare one same group in different periods for milk production. If it was detected a significant difference, Wilcoxon signed ranks tests were applied comparing the same group in the three different periods using Bonferroni's correction for the P value based on the number of comparisons. All other tests were considered significant when P value (two-tailed) was lower than or equal to 0.05.

RESULTS

All values regarding somatic cell count per week and grouped according to periods for the groups are shown in Table 1.

Table 1. Mean values of somatic cell count, expressed in logarithmic scale ($x10^3$ cells/mL), per week and grouped according to periods (before, during and after homeopathic treatment), and upper (UB 95%) and lower (LB 95%) bounds at 95% confidence interval

Control				Experimental			
Week	Mean	LB 95%	UB 95%	Week	Mean	LB 95%	UB 95%
1	1.44	1.21	1.66	1	1.57	1.34	1.79
2	1.41	1.17	1.65	2	1.05	0.81	1.29
3	1.86	1.63	2.08	3	1.86	1.64	2.08
4	1.92	1.71	2.13	4	1.78	1.54	2.01
5	2.09	1.87	2.31	5	1.97	1.80	2.13
6	2.65	2.48	2.82	6	2.50	2.27	2.74
7	2.03	1.81	2.25	7	1.99	1.76	2.23
8	1.67	1.51	1.83	8	1.47	1.25	1.60
9	2.24	2.08	2.40	9	2.05	1.86	2.24
10	2.40	2.20	2.60	10	2.17	1.97	2.37
11	2.66	2.45	2.86	11	2.68	2.51	2.84
12	2.46	2.25	2.67	12	2.43	2.18	2.69
Before	1.42^{ae}	1.26	1.58	Before	1.31^{ae}	1.14	1.48
During	2.11^{bf}	2.03	2.18	During	1.97^{bf}	1.89	2.05
After	2.56^{cf}	2.42	2.71	After	2.55^{cf}	2.40	2.71

a–c Means within a row with different superscripts differ ($P < 0.05$)
e–f Values within a column with different superscripts differ ($P < 0.05$)

Considering the t-tests for independent samples, the groups did not have significant differences in the three homeopathic treatment periods in terms of SCC ($P = 0.428$, 0.102 and 0.954 for the periods before, during and after treatment, respectively).

To compare the SCC for one same group during the experiment, ANOVA with repeated measures was performed. The control group showed a significant difference ($P < 0.001$) that was caused by a higher somatic cell count during and after the treatment periods in contrast to the lower count in the period before the treatment, showed by the post-hoc tests. A similar result was observed for the experimental group, both in the ANOVA and the post-hoc tests ($P < 0.001$). Such facts suggest that there is interference of external factors on the increased milk cellularity in the different periods, not necessarily due to the homeopathic treatment, as we observed.

According to the proposed model of repeated measures, the only factor that had presented a significant contribution to the model was the milk cellularity before the beginning of homeopathic compound supply. The remaining factors such as the group did not have significant contributions to the model, which reinforces the results obtained in the parametric tests before and suggests that homeopathy had no effect on milk cellularity.

Information on milk production by the animals is available in Table 2.

Table 2. Values of median and percentiles 25 (P25) and 75 (P75) of milk production (in liters) per week and grouped according to periods (before, during and after homeopathic treatment) for the different groups

Control				Experimental			
Week	Median	P25	P75	Week	Median	P25	P75
1	1.10	0.67	1.72	1	0.82	0.52	1.61
2	1.20	0.70	1.50	2	0.95	0.72	1.55
3	1.10	0.80	1.70	3	0.95	0.72	1.40
4	0.90	0.67	1.52	4	1.00	0.70	1.30
5	1.00	0.87	1.80	5	0.85	0.65	1.47
6	1.00	0.70	1.80	6	0.90	0.60	1.52
7	1.00	0.60	1.35	7	0.80	0.55	1.50
8	0.90	0.67	1.60	8	0.75	0.35	1.57
9	0.90	0.75	1.47	9	0.80	0.52	1.27
10	0.90	0.47	1.22	10	0.75	0.32	1.37
11	0.70	0.40	0.90	11	0.50	0.25	0.70
12	0.80	0.57	1.22	12	0.55	0.32	0.90
Before	1.10^{ae}	0.70	1.50	Before	0.90^{ae}	0.61	1.57
During	1.00^{be}	0.70	1.57	During	0.80^{be}	0.60	1.40
After	0.75^{cf}	0.50	1.10	After	0.50^{cf}	0.32	0.87

a–c Values within a row with different superscripts differ ($P < 0.05$)
e–f Values within a column with different superscripts differ ($P < 0.0167$ with Bonferroni's correction)

Mann-Whitney test indicated that the groups were not different for milk production in any experimental period (*P* values for the periods before, during and after treatment were 0.419, 0.235 and 0.136, respectively). When the same group was evaluated in the three periods, Friedman's test indicated that both groups were different for production ($P < 0.001$ and 0.003 for control and experimental groups, respectively), especially due to the decreased production after treatment in comparison with the periods before and during treatment as showed in the post-hoc tests ($P < 0.001$ for both comparisons in both groups). These findings suggest that there is a possible effect of seasonality on production, inversely proportional to the increase in SCC, which was already known.

According to the proposed model of repeated measures, the only factor that significantly contributed to estimate milk production during treatment was the mean milk production after the homeopathic compound supply period, which was included as a covariate. The remaining factors such as group did not have significant contributions, which suggests that homeopathy had no effect on milk production.

DISCUSSION

The demand for alternative therapies in veterinary medicine has increased in the last years (22). To treat production animals, alternative therapies have focused especially on the organic production in order to reduce the use of chemical substances (17). Bergonier et al. (23) reported that infusion of a formulation containing amoxicillin / clavulanic acid and prednisolone in goats during lactation led to the detection of residues in the milk for up to 112h after administration.

The mastitis indicator SCC is considered less reliable for goats than for cattle. In the former, SCC is affected by several factors, including lactation number, days of lactation, occurrence of dry period and its length, besides factors such as estrus, infection by caprine arthritis-encephalitis virus (CAEV) or simply the naturally higher cellularity of goat milk compared to that of cow milk (3, 8, 10, 23-29). Furthermore, intramammary infection can be considered the main responsible for increased SCC in goat milk (6).

In the present study, SCC was not affected by the homeopathic treatment, and the influence of several external factors was evident since SCC values increased over the experiment both in control and experimental groups.

For goats with intramammary infection, milk production can be considered a mastitis indicator since it tends to be lower in affected animals compared to healthy ones (30). In the present study, milk production was clearly influenced by external factors since it decreased over the experiment for both groups, ruling out a positive or negative influence of the homeopathic treatment on this variable.

The interaction between LogSCC and milk production was visible in the present study. Before the homeopathic treatment, the control group had 1.42 $x10^3$ cells/mL milk and 1.10 liters of daily mean production (average values), compared to 1.31 $x10^3$ cells/mL and 0.9 liters of mean production by the experimental group. During the experiment, the control group had increased LogSCC values and decreased milk production (2.11 $x10^3$ cells/mL and 1.00 L, respectively); the same was noticed for the experimental group (1.97 $x10^3$ cells/mL and 0.80 L milk, respectively). After the experimental period, both groups again had increased LogSCC and decreased milk production (2.56 $x10^3$ cells/mL and 0.75 L for the control group and 2.55 $x10^3$ cells/mL and 0.50 L for the experimental group). Such results indicate the clear relationship between SCC and milk production and make evident the influence of external factors, leading to certain seasonality, to which the studied flock was subjected, as previously mentioned. Similarly, Bergonier et al. (23) noticed a correlation between increased SCC and decreased milk production in small ruminants, also reported by other authors (21, 26).

Cases of clinical mastitis were not diagnosed over the present experiment. The milk of small ruminants has the same cell types as the cows. However, the composition of somatic cells is different between goat and cow milk. Considering animals free of intramammary infection, neutrophils represent from 5 to 20% somatic cells in bovine milk, and from 45 to 74% in goat milk (31), which suggests the migration of neutrophils is faster and may contribute to a naturally higher SCC in goats than in bovines. In addition, it is probably that goats have better response to the infection (4). The incidence of cases of clinical mastitis in the present study was according to those reported by other authors, who detected low incidence of clinical mastitis in goats, suggesting that a larger number of neutrophils in goat milk may represent higher protection against pathogens (24).

There was no significant difference in the studied variables when groups were compared with the different adopted tests. These results indicate that the studied homeopathic compound was not capable of preventing new infections and healing preexisting ones. Homeopathy is based on individualized therapies. In contrast whit the classical literature, such procedure was not adopted. In individualized therapies, the symptoms of each animal are observed and the medicine chosen for the treatment is that most suitable to the symptoms manifested by the animal. The principle of individualized homeopathic therapy is considered the main obstacle to evaluate experimental clinical trials (17).

Similarly, other authors have observed that the homeopathic solution provided to animals was not capable of influencing milk cellularity, even those animals presenting significant difference in SCC, which was not attributed to the homeopathic treatment (32).

Studies under the same conditions as those of the present work (double-blind, placebo-controlled clinical trial) did not have satisfactory results compared to placebo and experimental groups subjected to homeopathic therapy to treat and prevent diarrhea in calves (33).

Arlt et al. (22) performed a randomized, double-blind clinical trial and noticed that the evaluated homeopathic treatment was not effective to prevent bovine endometritis and improve the reproductive performance or the metabolic condition.

Conversely, homeopathic treatment did not differ from conventional antibiotic therapy and placebo in studies involving bovine mastitis (17). The association between homeopathy and biotherapy to treat clinical mastitis in cows was not effective in studies conducted overseas (34).

There are different protocols used in homeopathy, which impair their utilization in the daily practice; in addition, the obtained results are different. These factors indicate the need of further studies about this subject to better define the homeopathic procedures to be implanted.

Homeopathic medicines gained certain popularity during the 19th century probably due to their lower toxicity relative to that of conventional therapies. However, at the beginning of the 20th century, the use of homeopathic therapy drastically decreased due to the lack of its competitiveness with the recent advances in medical and educational areas (16).

In the present study, the homeopathic compound was an inefficient therapy for mastitis since two of the main parameters analyzed were not influenced by this medicine. Several countries currently have cellularity limits for milk, especially for that produced by cattle, which forces producers to develop strategies to control and decrease the number of mastitis cases and improve the conditions under which the product is obtained in order to have a low-cellularity product. However, the developed therapies should not only control the incidence of new cases of mastitis, but also make the animal mammary gland a secretory source of a low-cellularity product, which did not occur in the present study. Several authors in behalf of the homeopathic therapy have stated that homeopathy does not decrease milk cellularity but prevents new infections, keeping cellularity at high levels. For goats, milk cellularity is naturally higher, which may make homeopathy not recommended to treat and control mastitis in small ruminants (20, 32). In cases of new infections (evidenced by clinical or subclinical signs of mastitis), milk cellularity should have increased or milk production should have decreased in the control group relative to the experimental group, which was not noticed.

CONCLUSION

There was no difference between groups regarding milk production and cellularity, which suggests the used homeopathic compound is not capable of reducing mastitis and prevent new infections in animals studied under the present conditions. These results support the works of other authors and indicate the need of further studies in the homeopathic field to allow the use of this therapy.

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