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The Effect of Intrauterine Administration of Zeolite on the Puerperal Period in Dairy Cows

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ABSTRACT

Background: The development of new protocols has become a priority today in the context of the adverse effects of conventional treatments. Zeolite falls into this category due to its numerous applications reported in the specialized literature. In this study, the main expected objectives were to demonstrate that intrauterine administration of zeolite can contribute to improving the indicators underlying the development of pathologies during the postpartum period.

Methods: To determine the dosage and potential toxic effects on the uterine mucosa, we conducted a study on rats, to which we administered different doses on the second day after delivery. Ultrasound measurements of the uterus, collection of bacteriological samples and uterine tissue were performed at 7, 14 and 21 days. The results were satisfactory in terms of tissue effects, with the 20% dosage being the most suitable. Following the completion of the preliminary study, we grouped the animals into two equal groups (n=32). Intrauterine administration of zeolite was performed on the second day after delivery, and monitoring of uterine involution was carried out on days 7, 14 and 21 of the puerperium. At 14 days, we collected samples to determine the number of bacteria and uterine tissue.

Result: The data obtained indicate that the group receiving intrauterine zeolite (n=16) experienced a decrease in the number of bacteria, significantly faster uterine involution on days 14 and 28, a more favourable uterine score and a significantly shorter service period. However, no significance was established regarding the number of doses of seminal material used.

Key words: Puerperal period, Uterine environment, Zeolite.

INTRODUCTION

The right herd reproduction organization is one of the most important tasks in dairy farming. Effective cattle management is impossible without the implementation of a complex of organizational and zoo veterinary measures (Semenov *et al.,* 2020). The duration of the postpartum period has an important influence on reproductive performance. So, the next pregnancy depends on the completion of uterine involution and the cyclic function of ovaries (Sharma *et al.,* 2018). Therefore any reproductive or metabolic disorder around calving time will have a depressing effect on uterine involution and other reproductive measures, such as days to first heat, conception to first service, and services per conception (Call *et al*., 1989). Significant interest has evolved for the genital tract microbiota in recent years within the scientific community such that the genital microbiota is thought to be associated with infertility or uterine diseases (Adnane *et al.,* 2022). Indeed, 80-100% of animals have bacteria in their uterine lumen within the first 2 weeks after calving. Although immune responses progressively eliminate the microbes, up to 40% of animals still have a bacterial infection 3 weeks after calving (Sheldon *et al.,* 2008) However, the longevity of these bacteria in the uterine environment can cause uterine diseases (Sheldon *et al.,* 2008; Grunert *et al.,* 2005; Bicalho *et al.,* 2012).

Unique and outstanding physical and chemical properties of zeolite materials make them extremely useful in a variety of applications including agronomy, ecology,

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manufacturing, industrial processes, veterinary and human medicine (Pavelic *et al.,* 2018). An interesting characteristic is the antibacterial property of zeolite, as a mechanism, antimicrobial activity usually involves the slow release of antimicrobial metal ions (such as Cu 2+, Zn 2+, or Ag +) from the zeolite network, and the inactivation of some pathogens has been attributed to the metal ion held by the aluminosilicate network (Ferreira *et al.,* 2012; Hrenovic *et al.,* 2013; Durcic *et al.,* 2020).

The use of zeolite in veterinary medicine has yielded results that can be considered in the symptomatic treatment of undifferentiated diarrhoea in calves (Cerbu *et al*., 2020). Additionally, a natural zeolite's influence on *E. coli* has been documented in another study involving broilers *in vivo* (Pvelic *et al.,* 2018; Wu *et al.,* 2013). Furthermore, dietary zeolite supplementation has shown better effects on growth performance, intestinal oxidative status, and gut integrity in broilers compared to antibiotic supplementation (Qu *et al.,* 2019).

Interest in postpartum diseases is high among both farmers and veterinarians due to significant losses, amounting to $622 per cow (Kim *et al*., 2019). Currently, widely used medications include oxytocin, natural and synthetic analogues of PGF2α, aimed at improving uterine contractility (Rizzo *et al.,* 2018). Antibiotics are also used directly to treat infections, but they come with withdrawal periods and the emergence of antibiotic resistance. To reduce the side effects of traditional treatments, a new approach is needed. As a preventive measure in early postpartum periods, we propose the intrauterine administration of zeolite.

MATERIALS AND METHODS

The research was carried out between 10.07.2022 - 01.08.2023, including testing on rats and subsequent application on dairy cows.

Zeolite was extracted from the Rupea area in Brasov County. It has a particle size of 20 microns and is chemically defined as a natural aluminium silicate containing alkali and alkaline earth metals. It is crystalline and hydrated, belonging to the tectosilicate group. The clinoptilolite content is approximately 87-90%, and its empirical chemical formula is $(Ca, K_2, Na_2, Mg)_4 Al_8 Si_{40}O_{96}.24H_2O$. Zeolite powder was prepared in physiological saline at various concentrations (10%, 20%, 30%).

Preliminary study

Before administering intrauterine zeolite to cattle, a preliminary study was conducted on Rattus norvegicus rats. In this study, 24 female rats were selected and divided into three groups, to which intrauterine zeolite was administered on the second day after parturition. Each group consisted of 8 females further divided into 4 subgroups based on the concentration of zeolite administered, *i.e*., 10%, 20%, 40%, and a control group to which physiological saline was administered. The three groups were sacrificed at different time intervals, specifically at 7, 14 and 21 days after inoculation. Bacteriological and histopathological examinations were conducted at these time points. Ultrasound was performed to assess uterine involution before administration, after inoculation and before the scheduled sacrifice for each group.

For the microbiological examination, a 10 µL sample of uterine horn content was collected from each rat and solubilized in 990 µL of sterile physiological saline. The obtained solution was used to flood Mueller-Hinton agar plates and streak Petri dishes with blood agar, MacConkey agar and Sabouraud agar. After incubation at 37°C for 24 hours for bacteria and at 28°C for 48 hours for yeasts, a qualitative and quantitative evaluation of the results was performed.

Following the tissue sample collection for the microbiological examination, the uterine tissue samples were fixed in 10% formalin. Subsequently, they were dehydrated through a gradual immersion in ethanol solutions, cleared in acetone, embedded in paraffin, sectioned to a thickness of 5-6 µm and stained with hematoxylin and eosin. Finally, the prepared slides were examined under a microscope.

The evaluation of the biopsies was conducted according to a simplified scale described by Chapwanya *et al.* (2009), which was also utilized by Madoz *et al.* (2014). The scale included the following categories: 0 = absence of inflammatory infiltrate in the uterus, 1 = minimal inflammation characterized by a low level of lymphocytic infiltration and polymorphonuclear leukocytes (PMNL), 2 = moderate inflammatory changes characterized by lymphocytic infiltration and prominent PMNL and 3 = severe inflammation characterized by a high level of monocyte and PMNL infiltration. A cow was considered healthy only if it received a uterine score of 0.

Cattle study

A total of 32 cattle were included in the study and randomly divided into two groups. The treatment group received intrauterine zeolite in a 20% concentration on the second day after calving, while the control group received physiological saline. Intrauterine administration was performed by cervical fixation and the introduction of a catheter attached to a 100 ml syringe through the vulvar commissure. The total administered dose was 200 ml.

Uterine involution was monitored through ultrasonography (using an Easy Scan - IMV Imaging USA, equipped with a linear probe of 3-7.5 MHz) and transrectal palpation on postpartum days 7, 14, 21 and 28. The size of the uterus was measured according to the Grunert method (Grunert *et al.,* 1979). The uterus could be retracted and the uterine horn $\leq$ 2 cm (score 1), the uterus could be retracted and the uterine horn was 3-5 cm (score 2), the uterus could be retracted and the uterine horn was 6-8 cm (score 3), margins of the uterus can be delimited by the hand and the uterine horn was 9-20 cm (score 4), part of the uterus was not palpable or incompletely palpable (score 5), or margins of the uterus cannot be delineated by hand (score 6). The measurements were performed only on the gravid uterine horn, with the probe positioned approximately 2 cm cranial to the bifurcation.

Vaginal secretion collection was carried out at 14 days postpartum using a plastic probe guided by a seeding rod, protected by a covering layer. The probe was inserted up to the level of the uterine horns, from where uterine secretions were aspirated. The microbiological sample processing procedure was identical to that used for the uterine secretions obtained from rats.

Uterine biopsy collection was performed using a 63 cm long stainless steel biopsy rod equipped with a 5 mm slot and a sharp edge sliding over it. Once the rod that crossed the cervix was fixed on the uterine wall and with the help of the sharp edges the slit containing the uterine tissue sample was covered. After extracting the rod, the tissue sample was stored in a 50 ml tube containing 10% formaldehyde buffered solution. The interpretation of the results was conducted according to the scale proposed by Chapwanya *et al*. (2009), as described previously.

Throughout the study, the animals were clinically examined and data related to the development of postpartum pathologies were recorded. Monitoring of the cattle continued until the confirmation of a new pregnancy.

The data collected were analyzed using the IBM SPSS Statistics 22 software and the research method applied was ANOVA (Analysis of Variance). ANOVA is a statistical technique used to analyze and compare the means of multiple groups to determine if there are significant differences between them. This analysis can help identify any significant effects of the treatment with zeolite compared to the control group in your study.

RESULTS AND DISCUSSION

Uterine involution

The concentration with the most significant positive impact on uterine involution was the 20% zeolite concentration, according to Table 2. Indeed, when compared to the 10% and 40% concentrations, presented in Table 2 and Table 3, the differences were not very significant in some subgroups. However, the most considerable difference was observed in the control group, where uterine involution was slower compared to the subgroups treated with zeolite.

Uterine bacterial population

As shown in Fig 1, the control group exhibited a higher bacterial population compared to the groups that received intrauterine zeolite. When comparing the administered concentrations, there is a trend of lower bacterial counts favouring the 20% zeolite concentration. Concerning the distribution of microorganism species encountered in all animals in the study, a higher prevalence of *Escherichia*

Table 1: The difference in ultrasound measurements performed in group A (7 days after inoculation).

Group	Subgroup	Uterus			Left uterine body		Left uterine body	
		Diameter	Vol	Area	Diametru	Vol	Diameter	Vol
A	A1	0.55	0.03	0.035	0.19	0	0.16	0
7 days	A2	1.96	0.065	0.085	0.475	0.01	0.425	0.015
	A3	1.875	0.05	0.075	0.415	0.01	0.39	0.01
	A4	0.495	0.02	0.03	0.12	0	0.145	0

Subgroups: A1: 10% Concentration; A2: Concentration 20%; A3: 40% Concentration; A4: Control group.

Table 2: The difference in ultrasound measurements performed in group B (14 days after inoculation).

Group	Subgroup	Uterus			Left uterine body		Left uterine body	
		Diameter	Vol	Area	Diameter	Vol	Diameter	Vol
B	B1	0.825	0.075	0.12	0.675	0.015	0.505	0.01
14 days	B2	0.98	0.09	0.16	1.21	0.025	0.82	0.02
	B3	0.575	0.08	0.05	0.675	0.02	0.46	0.015
	B4	0.365	0.07	0.035	0.42	0.01	0.3	0.01

Subgroups: B1: 10% Concentration; B2: 20% Concentration; B3: 40% Concentration; B4: Control group.

Table 3: The difference in ultrasound measurements performed in group C (21 days after inoculation).

Group	Subgroup	Uterus			Left uterine body		Left uterine body	
		Diameter	Vol	Area	Diameter	Vol	Diameter	Vol
C	C1	2.165	0.105	0.11	1.67	0.035	0.905	0.015
21 days	C2	2.435	0.115	0.16	1.955	0.05	1.055	0.02
	C3	1.7	0.06	0.14	0.71	0.015	0.97	0.015
	C4	0.455	0.03	0.07	0.8	0.01	0.41	0.025

Subgroups: C1: 10% Concentration; C2: 20% Concentration; C3: 40% Concentration; C4: Control group.

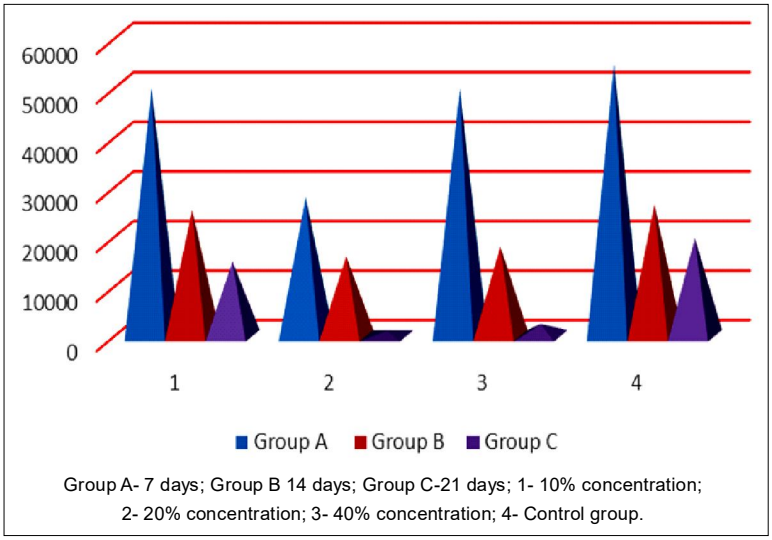

Fig 1: Determination of the number of bacteria after administration.

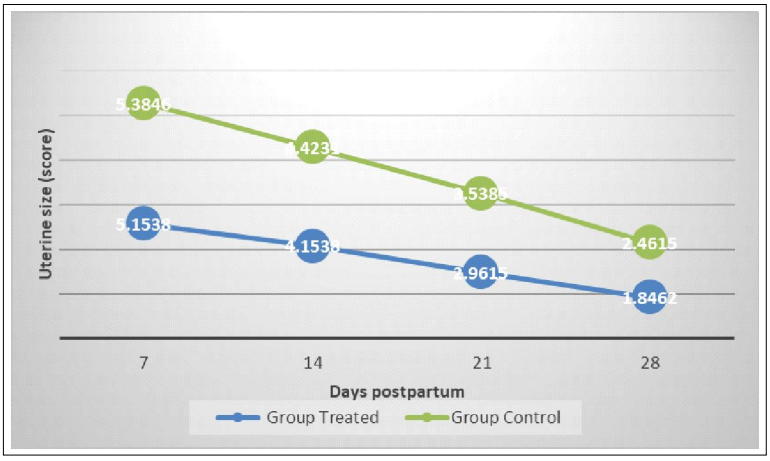

Fig 2: Uterine involution score for the studied groups.

coli was observed in the animals that received zeolite, while in the control group, the most common species were *Bacillus* spp., *Staphylococcus* spp. and *Streptococcus* spp.

Histological evaluation of uterine tissue

The most common histological lesions were observed in Group A, evaluated at 7 days postadministration. Among these lesions were minimal apoptosis (endometrium), minimal amyloidosis, moderate focal granulomatous inflammation (myometrium), focal granulomatous inflammatory focus with central amyloidlike material, focal fibrinoid hyaline degeneration, and a moderate number of pigmentladen cells. Comparatively, Groups B (14 days) and C (21 days) had a much lower frequency of lesions, with the exception being observed in Group C, Subgroup 1 (10%), where there was the presence of a granulomatous focus with presumptive birefringent foreign material, likely zeolite.

The uterine score was calculated as the average score for each subgroup, regardless of the collection period. The average uterine score for Subgroup 1 (10%) and Subgroup 4 was 1. Subgroup 2 (20%) had a uterine score of 0.33 and Subgroup 3 (40%) had a uterine score of 0.66.

Cattle study

Uterine involution

The graphical representation of the uterine involution score (Fig 2), obtained through ultrasound and transrectal palpation, indicates a difference between the groups regarding endometrial thickness, uterine horn size and diameter. Specifically, throughout the entire study period, uterine involution was more accelerated in the group that received intrauterine zeolite, even though the results were satisfactory in terms of the average score obtained. Statistically, significance was observed only on days 21 and 28 of monitoring ($p<0.05$).

From the calculation of the percentage difference between the two groups, an increasing difference over time can be observed. For day 7, it was 4.29%, for day 14, it increased to 6.09%, on day 21, it was 16.31%, and on day 28, there was a 25% difference. This suggests a continuous

improvement in uterine involution in the group that received intrauterine zeolite as time passed.

Uterine bacterial population

The uterine secretions collected at 14 days and subsequently processed in the laboratory indicated an average of 15,890.38 CFU (Colony Forming Units) for the treated group (n=26) and 17,661.53 CFU for the control group (n=26), with a statistically significant difference ($p<0.05$). Among the most common bacterial species were *Escherichia coli*, *Bacillus* spp. and *Streptococcus* spp.

Out of all the animals studied, three were diagnosed with acute endometritis, with the distribution as follows: 2 in the control group and 1 in the treated group. Additionally, one cow in the control group was diagnosed with puerperal metritis.

Histological evaluation of uterine tissue

From a histological perspective, the uterine score evaluation proposed by Chapwanya *et al*. (2009) resulted in an average score of 0.4231 for the treated group (n=26) and an average score of 0.7308 for the control group (n=26), with a statistically significant difference ($p<0.05$).

In terms of the highlighted lesions, they can be characterized as follows: mild hyperplasia of uterine glands, superficial lamina propria showing mild, diffuse infiltration with mononuclear cells and multifocal hyaline degeneration of uterine arteries.

Total puerperium evaluation

Upon the completion of the puerperium, there is a need for the conception of a new calf to ensure the economic profitability of dairy cows. Therefore, the research concluded at the moment when the subsequent pregnancy was diagnosed.

For this purpose, the average service period was recorded as 59.73 days for the treated group (n=26), while the average for the control group (n=26) was 65.03 days, with a statistically significant difference ($p<0.05$).

The number of doses of semen for the new pregnancy ranged from 1 to 3, with an average of 1.23 for the treated group and 1.46 for the control group. From a statistical perspective, no significance was found regarding the number of doses of semen used.

Zeolites are microporous minerals commonly used as adsorbents in industry and agriculture due to their ability to bind cations and some anions (Mumpton *et al.,* 1999). The use of zeolites in animal science has been proposed for a long time (Mumpton *et al*., 1977). In this context, our study aims to enrich the use of zeolite. To the best of our knowledge, zeolite has not been administered intrauterinely to cattle to improve the main pathologies encountered during the puerperal period.

To address questions about what concentration to administer and whether zeolite has adverse effects on the reproductive tract, we conducted a preliminary study on rats. The conclusions indicated that there were no adverse effects on uterine tissue and a concentration of 20% yielded the best results.

Numerous studies have shown that orally administered zeolite has positive effects on improving metabolic and reproductive indicators (Crookenden *et al*., 2020; Garzón *et al*., 2017; Franklin *et al*., 2021; Đurièić *et al*., 2020; Khachlouf *et al*., 2018). Furthermore, the antibacterial effects of zeolite have been demonstrated in calf diarrhoea cases (Wu *et al*., 2013). Zeolite is also capable of partially absorbing and inactivating *E. coli* enterotoxins, and agar diffusion tests have shown zones of activity against *E. coli* and *S. aureus* (Khachlouf *et al*., 2018; Taaca *et al*., 2017). Therefore, the results presented earlier demonstrate a significant reduction in the number of bacteria, which has benefited local immunity, maintaining a balance within physiological limits.

After establishing the average uterine score obtained from transrectal and ultrasound examinations, we found that both groups had lower values compared to those obtained by Braga *et al*. (2019), for healthy animals. In the case of the difference observed between the two groups for the first two intervals studied, no significance could be determined. However, in the subsequent intervals, there was a noteworthy trend that brought to our attention the longterm effects on uterine involution.

Starting from the assertion made by Madoz *et al*. (2014), which suggested an association between uterine biopsy and the health of the uterus in dairy cows, we observed a significant improvement in uterine tissue in cows treated with zeolite, thereby complementing the previously mentioned indicators.

Therefore, the results obtained open new perspectives for improving the main pathologies encountered during the puerperal period in cattle. Furthermore, due to its ecological nature, zeolite brings an additional benefit by reducing antibiotic resistance. Indeed, this study demonstrates a positive implication of zeolite throughout the entire puerperal period, but future research should focus on specific target pathologies.

CONCLUSION

The study revealed that intrauterine zeolite administration had a positive impact on uterine involution, with the 20% zeolite concentration showing the most significant effect. This effect became more pronounced over time, with statistically significant differences observed at 21 and 28 days. The control group exhibited a higher uterine bacterial population compared to the groups treated with zeolite. Escherichia coli was more prevalent in the treated groups, while *Bacillus* spp., *Staphylococcus* spp. and *Streptococcus* spp. were common in the control group.

The histological analysis revealed that the treated group had a lower average uterine score compared to the control group. The most common histological lesions included minimal apoptosis, minimal amyloidosis, and moderate

granulomatous inflammation. These differences were statistically significant. The service period for achieving a subsequent pregnancy was significantly shorter in the group treated with zeolite compared to the control group. There was no statistically significant difference in the number of semen doses used for the new pregnancy.

Overall, the findings prove that intrauterine zeolite administration positively influences uterine involution, reduces bacterial populations, and improves uterine health in cattle during the postpartum period, leading to shorter service periods for subsequent pregnancies.

Conflict of interest

All authors declare that they have no conflicts of interest.

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