Veterinary and Animal Science 23 (2024) 100333

Contents lists available at ScienceDirect

Veterinary and Animal Science

journal homepage: www.elsevier.com/locate/vas

The effect of oral administration of zeolite on the energy metabolism and reproductive health of Romanian spotted breed in advanced gestation and post partum period

O.V. Giurgiu[a,*,1], D.I. Berean[a,1], A. Ionescu[b], M.S. Ciupe[a], C.R. Cimpean[d], C.I. Radu[c], D.G. Bitica[a], S. Bogdan[e], M.L. Bogdan[a]

[a] Department of Reproduction, Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Calea Manastur 3-5, Cluj-Napoca 400372, Romania

[b] Oncology Department The Royal (Dick) School of Veterinary Studies, University of Edinburgh Easter Bush Campus, Midlothian, Edinburgh, Scotland EH25 9RG, United Kingdom

[c] Department of Infectious Diseases, Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, Mănăștur Str. 3-5, Cluj-Napoca 400372, Romania

[d] Department of Animal Breeding and Food Safety, Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Calea Manastur 3-5, Cluj-Napoca 400372, Romania

[e] Department of Anesthetics and Surgical Propaedeutics, Veterinary orthopedics, Experimental University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Calea Manastur 3-5, Cluj-Napoca 400372, Romania

ARTICLE INFO

Keywords:
Reproductiv parameters
Zeolite
Puerperal period
Cow

ABSTRACT

The dairy cow experiences the most significant impact from negative energy balance during this period, which adversely affects reproductive health. Consequently, most pathologies affect dairy cows during this time frame. Thus, with the primary objective of reducing the incidence of these pathologies on dairy farms, we questioned whether supplemental zeolite administration in cattle feed would affect metabolism and reproductive health.

Therefore, we proposed introducing an antepartum and postpartum supplementation of 400 g of zeolite in the basal diet. The control group received only the basal diet without zeolite supplementation. Monitoring the results stemmed from the consideration that reproductive health can only be present based on an unaltered energy metabolism. Hence, we deemed it necessary to analyze several metabolic markers in light of the expected outcomes concerning reproductive health.

Cows treated with zeolite exhibited a calving to first service interval 12.78 days earlier than those in the control group. Moreover, the average number of services per conception used for future gestation was 0.44 lower in the zeolite-treated group compared to the control group ($p<0.05$). Additionally, the treatment group showed a lower presence of pathogens in the uterus and displayed a more favorable average uterine score.

Observations following the completion of the research point towards an improvement in the health of transition dairy cows, opening a new path for dairy farms in terms of preventing postpartum pathologies. Indeed, the benefits from this study primarily impact the animals rather than directly influencing milk production. Therefore, further research is necessary in this regard.

1. Introduction

The postpartum (PP) period is an important period in the reproductive life of dairy cows due to its enormous influence on subsequent fertility (Elmetwally, 2018). Properly managing cattle necessitates the application of both zootechnical and veterinary practices; these combined approaches are essential for successful cattle care and oversight (Semenov et al., 2020).

The Romanian Spotted cattle (RS, national name Bălțată Românească) belongs to the Simmental strain, being a dual-purpose breed, with a current census of 376,000 cows, representing 36 % of the breed structure in Romania . The RS originates in the 18th century,

* Corresponding author.
E-mail address: ovidiu.giurgiu@usamvcluj.ro (O.V. Giurgiu).

[1] These authors contributed equally.

https://doi.org/10.1016/j.vas.2023.100333

Available online 30 December 2023

being the result of non-systematic crossbreeding between Simmental bulls imported from Austria and Switzerland and local unimproved Podolic Grey cattle . The average milk yield for the RS breed ranges between 5000 and 5700 kg of milk/lactation, adult body weight in cows is 600 to 620 kg and average daily gain in fattening young bulls is 1000 to 1200 g. The selection index for the RS breed is focused on milk yield (50 %), growth rates and carcass attributes (20 %) and fitness related traits (30 %) (Ilie et al., 2023).

Zeolites are natural or synthetic materials that have been shown to be effective in a wide range of applications, including in biotechnologies and medicine. These applications are related to the adsorption and ion exchange properties of zeolites such as decontamination of the living environment from harmful elements, molecules and radioactive contaminants; detoxification of the organisms, improvement of the nutritional status and immunity of animals, separation of biomolecules and different types of cells, the construction of biosensors and the detection of biomarkers, the transport of drugs, nucleic acids and other bioactive molecules, and the scavenging of oxygen and other radicals (Bacakova et., 2018). The diversity application of zeolites is a consequence of their unique porous structure.

Thus, the pores form negatively charged channels and cavities occupied by positively charged alkalis; monovalent alkaline earth (ie $Na+$, $K+$); and divalent ions (ie, $Ca2+$), OH groups or H2O. Molecules can exchange them effortlessly with other nearby molecules and Cations (Cerbu et al., 2020; Jurkić et al., 2013; Kustova et al., 2007; Mumpton, 1999; Pavelić et al., 2018)

The zeolite in animal feed has been shown to be able to act as a detoxifying agent (including for mycotoxins and heavy metals), antioxidant, hemostatic, antidiarrheal, growth stimulatory, antiviral, antibacterial and immunostimulatory (Đuričić et al., 2020; Pavelić et al., 2001; Valpotic et al., 2016).

Reversible ion exchange and adsorption capacity, related to the elimination of toxic agents, can be used to restore homeostasis and energy balance within an animal's body; for example, by eliminating the excessive production of ammonia and other gaseous products in rumen, including CO2 and H2S, which are due to unbalanced digestion or various pathologies (Maity et al., 2021; Mastinu et al., 2019; Pavelić et al., 2018).

Karatzia et al. (2013), obtained favorable results by zeolite supplementation, which significantly increased the body score condition, serum glucose concentration and significantly decreased the blood serum concentration of ketone bodies. Clinoptilolite also significantly improved the evaluated reproductive health and significantly increased milk production (Karatzia et al., 2013).

Of all the diseases that affect cattle, approximately 75 % appear in the first 30 days after parturition (LeBlanc et al., 2006; Vergara et al., 2014), and the share of animals affected by some diseases around the calving range between 30 and 50 % (LeBlanc, 2010; Vergara et al., 2014). Diseases that appear during this period result in significant economic losses for farmers, because unhealthy cows are less productive (Calderón-Amor et al., 2021; Wittrock et al., 2011).

The main pathologies are milk fever, ketosis, and displacement of the abomasum, placental retention, uterine infections, and lameness, pathologies overlapped when there is a negative energy balance. (Ingvartsen et al., 2003; Nordlund & Cook, 2004; Vergara et al., 2014).

Energy balance is the difference between energy consumed and energy used for both maintenance and production (milk, meat, reproduction, etc.) and consequently enters a state of negative energy balance (NEB) when energy expenditure exceeds intake. Energy availability, or more specifically a lack of available energy, frequently limits milk or milk component synthesis, reduce reproductive performance, and prevents body condition replacement in cows. When NEB is severe and persists for long time, it also alters global gene expression and immune responses in the uterus of postpartum dairy cows (Holger., 2023).

The progressive genetic improvement of dairy cattle has led to continuous increases in milk production capacity. This high milk production potential can only be achieved if the diet that cows are able to consume supplies sufficient energy and nutrients, primarily through the inclusion of concentrates (Bach et al., 2023;). Unfortunately, this emphasis on concentrates has led to several issues, including a decrease in ruminal pH, resulting in ruminal acidosis and negative implications for the energy balance (Khachlouf et al., 2018).

Various strategies have been explored to manage rumen pH levels. These include supplementing rations with neutralizing agents, yeast cultures (Bach et al., 2023;), sodium bicarbonate, and zeolite. Using zeolite at a maximum quantity of 400 g/day/cow has proven to be an effective buffering agent for enhancing productivity in dairy cow herds (Khachlouf et al., 2018). The addition of zeolite resulted in the highest rumen molar percentage of acetate and reduced propionate. This also resulted in a higher acetate:propionate ratio. Increases in acetate:propionate are frequently associated with increaseD pH rumen (Dschaak et al., 2010; Sulzberger et al., 2016) and digestion and due to a more favorable environment for the rumen microbial fermentation. Other author sassociated the increase in molar percentage of acetate and the decrease in molar percentage of propionate in the rumen with the increased rumen liquid dilution rate with the buffer additive. Such alterations on the rumen volatile fatty acid proportions could affect the energy status of the animals and cause changes to the milk yield and composition of dairy cattle (Khachlouf et al., 2018).

The global trend is continuously growing for organic products that meet the demands of safe food, animal welfare and environmental impact (Shandilya et al., 2020; Sundrum, 2001; Weller & Cooper, 1996). In the context of antibiotic resistance, mineral based therapies against bacterial infections have become a topic of interest (Cerbu et al., 2020).

Since the hardest period of the reproductive life of a dairy cow is located during the puerperium, there is a need for intervention through methods that minimize the negative effects. The Romanian Spotted Cows are no exception in encountering issues during this period. Therefore, due to the focus on developing the breed's milk line and the fact that this breed is predominant in Romania, we have deemed it necessary to implement measures to improve issues during the puerperal period for this breed.

The postpartum administration was justified based on existing studies in the literature. However, the dilemma revolved around the timing of the onset of administration. To stabilize ruminal pH before the critical puerperal period, we opted for late gestation administration while monitoring indicators to ascertain if administration during this period was necessary.

Building upon the assertion that a low ruminal pH generates a negative energy balance impacting metabolism and reproductive health, we hypothesized that zeolite, through an indirect action, would help maintain balance within physiological bounds during this critical period for dairy cows.

2. Materials and methods

The zeolite was extracted from Rupea Brașov county, it is chemically defined as a natural aluminium silicate, alkaline and alkaline earth metals, crystalline and hydrated, tectosilicate, with a size of 200 μm, and the clinoptilolite content is 87–90 %, having the empirical chemical formula $(Ca,K_2,Na_2,Mg)_4Al_8Si_{40}O_{96}.24H_2O$. Its color is greygreen, odourless, and the porosity is between 32 and 44 % with an effective pore diameter of 0.4 - 0.6 nm (4 - 6 Angstrom). The hardness on the Mohs scale is 3.5–4, the ability for water absorption is 34–36 %, and has a pH value of 8.75 – 9.2 and a density of 2.377 ± 0.002 kg/m^3.

According to the technical sheet provided by the manufacturer, zeolite is not toxic, this being reinforced by numerous studies conducted (Bacakova et al., 2018; Cerbu et al., 2020), it is also not soluble in water and it is resistant to acids and bases. Chemical composition and ion exchange capacity can be found in Tables 1 and 2.

In this study, were included 82 cows of the Romanian Spotted breed from a farm in

Table 1
Chemical composition of zeolite.

Compound Percent%	Compound Percent%
SiO_2 - 68.75 %–71.30 %	Fe_2O_3 - 1.90 %–2.10 %
Al_2O_3 - 11.35 %–13.10 %	MgO - 1.18 % –1.20 %
CaO - 2.86 %–5.2 %	Na_2O - 0.82 % %–1.30 %
K_2O - 3.17 % %–3.40 %	L.i.(Lithium) 8.73 %–8.86 %

Table 2
Ion exchange capacity of zeolite.

Total exchange capacity	237.5 meq / 100 g
Cation exchange capacity for Ca^{2+}	160.4 meq / 100 g
Cation exchange capacity for Mg^{2+}	38.4 meq / 100 g
Cation exchange capacity for K^{+}	39 meq / 100 g
Cation exchange capacity for Na^{+}	27.5 meq / 100 g

Satu-Mare county, Romania, the animals were in advanced gestation (14±3 days antepartum). Animals were housed in two separate pens until the study's completion, one corresponding to each group. Feed was administered only in the morning for the entire day. It's worth noting that the treated group's feed incorporated zeolite entirely. The ration's structure and chemical composition are detailed in Table No. 3.

The number of parturients varied from the first parturition to the fifth, being equally distributed in both groups as the number of pregnancies. For the various parturitions, the distribution among the groups was as follows:

- For the first parturition, there were 11 cows in each group.
- In the second parturition, 15 cows were in the treated group, while 14 were in the control group.
- For the third parturition, 14 cows were evenly divided between both groups.
- In the fourth parturition, 5 cows were in the treated group, and 6 were in the control group.
- during the fifth calving, there were 3 cows in each group.

The cattle selected for the study were randomly and equally divided into two groups: the treated group (T) and the control group (C). For the T group, a dosage of 400 gs of zeolite/day/cow was chosen, as per Khachlouf et al., 2018, considering it as the maximum quantity without negative effects on production. The days 14±3 antepartum (AP) for incorporating into the diet were established to prepare dairy cows to optimize the ruminal environment. This was continued after birth until 28±3 days postpartum to extend beyond the critical 30 postpartum days when the energy balance is highly fragile. No Zeolite administration was performed for the C group.

The impact of zeolite clinoptilolite on the reproductive health was realized by monitoring the bacterial population in the uterine secretions, the number of polymorphonuclear leukocyte (PMNL) identified after the uterine biopsy, the clinical involution of uterus, the days open interval, the number of seminal material doses used for one pregnancy and the levels of the energy metabolism indicators (glucose and BHB).

Table 3
Chemical composition of the administered feed.

Nutrient	U.m	Content	Nutrient	U.m	Content
Dry matter	g	19,896	Calcium	g	113,932
Energy required for milk	MJ	118,01	Phosphorus	g	102,711
Crude protein	g	2692,99	Sodium	g	31,751
Crude fat	g	1012,76	Magnesium	g	63,779
Starch	g	4416,48	Vitamin A	IE	100,000
Crude fiber	g	3679,29	Vitamin D	IE	10,000
Crude fiber / Dry matter		18,49	Vitamin E	mg	800

2.1. Bacterial population monitoring

To carry out the bacteriological analyses, in order to quantify the bacterial population, uterine secretions were collected on day 21±2 postpartum in an amount of more than 1 ml/sample. Prior to collection, asepsis and antisepsis measures of the vulvar region were performed. A plastic probe guided by the insemination rod, protected by a protective film, was inserted up to the level of the uterine horns and uterine secretion was aspirated. After the samples colection, the total number of germs (NTG) and the total number of Enterobacteriaceae (NTE) were determinated at the laboratory.

The determination of the total number of germs was carried out by extracting 1 ml from the testing sample and adding it to 9 ml dilution medium (MRD), later homoge-nised by vortexing, therefore, obtaining a 10^{-1} dilution. Decimal dilution is carried out by aspirating 1 ml of the previous suspension with a lower value and passing it into the dilution medium in an amount of 9 ml, the procedure is repeated until the 10^{-4} dilu-tion is obtained.

The next stage includes the inoculation of the plates with specific medium plate count agar (PCA) by embedding; from the initial suspension obtained in the case of each 10^{-1}, respectively 10^{-2}, 10^{-3}, 10^{-4} dilution, 1 ml is extracted, dividing into two sterile plates for each dilution where 15–20 ml medium will be added later PCA.

The examination of the sample to determine the number of Enterobacteria (NTE/ml) was carried out using the same technique used in the case of NTG, the differ-ence being the inoculation of the plates with specific medium violet bile red glucose (VRBG) by embedding.

2.2. Uterine biopsy

After collecting the uterine secretion, a uterine biopsy procedure was performed. A stainless steel biopsy rod measuring 63 cm in length, equipped with a 5 mm slit and a sharp edge, was inserted into the uterus after the uterine tube was removed. The rod was fixed on the uterine wall once it crossed the cervix, and the slit containing the uterine tissue sample was covered using the sharp edges. Subsequently, the rod was removed, and the tissue sample was preserved in a 50 ml tube containing a 10 % formalhyde buffered solution.

To prepare the tissue samples for analysis, they were fixed in formalin and dehydrated using a series of ethanol solutions. The samples were then cleaned in acetone, embedded in paraffin, and sectioned at a thickness ranging between 5 and 6 μm. Hematoxylin and eosin staining was applied to the sections, and they were subsequently 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.

2.3. Determination of the next pregnancy

During the puerperium period, the data were collected through frequent visits to the farm and a good collaboration with the attending veterinarian, from the start of the research until the new pregnancy was obtained. The moment of parturition was recorded and noted regarding its evolution, weekly transrectal examinations were performed throughout the puerperium (days 7,14,21,28), and any deviations from normal development were reported.

After the completion of the puerperal period, the moment of artificial insemination and the number of doses of semen required for each cow for a new pregnancy were followed and compared between groups. The pregnancy was confirmed at an interval of 2–3 months after the last insemination..

2.4. Energy metabolism analysis

The impact of the energy balance on the evolution of the puerperium was realised by measuring the beta-hydroxybutyrate (BHB) and the concentration of glucose in the blood, both parameters were measured weekly starting from 14±3 days antepartum to 28±3 days post-partum.

A portable BHB and Glucose check machine (Pharmadoc GmbH) were used after the collection of blood by venipuncture at the level of the coccygeal vein.

The BHB is the most common ketone body used to diagnose hyperketonemia because it is the predominant and more stable circulating ketone body in cow fluids (Benedet et al., 2019). Sailer et al. (2018) validate blood BHB control with the BHB check portable device against a previously validated portable device, thus achieving a sensitivity of 91 % and a specificity of 93 % (Sailer et al., 2018). In another paper, the sensitivity was 90 % and the specificity was 89 % (Jansen et al., 2021).

2.5. Statistical analysis

The data were analyzed using the IBM SPSS Statistics 22 application, applying one simple T test Anova a research method, using parametric data. The statistical datas were directed to compare the groups, to identify the statistical differences between the treated and the non treated group. The statistical model used was based on the hypothesis that oral zeolite administration stabilizes ruminal pH, leading to the normalization of the energy balance in the early postpartum days and beneficial effects on uterine health. The variables utilized for both groups were categorized into metabolism, including BHB and glucose, and uterine health, encompassing variables such as uterine microorganism count, uterine score, days open, and the number of doses of seminal material used. Correlating these variables stemmed from the hypothesis that there exists a connection between them, a hypothesis that was confirmed.

The protocol was ethically reviewed and approved by the Ethics Committee of the Veterinary Medicine Department at the University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, under number 325/20.06.2022. Additionally, the study adhered to the ARRIVE guidelines for animal research.

3. Results

3.1. Calving to first service and service per conception

Days open for both groups recorded values in our study between 48 and 110 days with an average of 71.43 days ($n = 82$). The comparison of the average service period between the groups revealed noteworthy differences. The treated group ($n = 41$) required fewer days, approximately 65.04 open days, to achieve a new pregnancy compared to the control group ($n = 41$), which needed around 77.82 days for the next gestation. Statistical analysis indicated a significant difference ($p<0.05$).

Additionally, the average number of services per conception differed between the groups. The T-group had an average of 1.36 doses of seminal material per conception, whereas the C group had an average of 1.80 doses per conception ($p<0.05$). These findings underscore the impact of zeolite administration, highlighting reduced open days and fewer doses of seminal material needed for conception in the treated group compared to the control group.

3.2. BHB and glucose blood levels

Regarding the records made about the level of BHB antepartum (days −7 and −14) there were no considerable differences between the groups, a fact that does not influence the ketosis prediction ($p>0.05$). On the other hand the level of BHB recorded postpartum (days 7, 14, 21, 28) revealed differences between the two groups, ($p<0.05$) values (mmol/l) that are reproduced numerically in Table 4.

The recordings made against the blood glucose level antepartum do not report significant differences between the two groups or on the day of parturition ($p>0.05$). Significant differences in favor of the T group were observed PP starting from day 7 (2.07 mg/dL), day 14 (3.17 mg/dL), day 21 (5.34 mg/dL), and day 28 (4.36 mg/dL), with a decrease in the case of both groups after parturition.. However, the glucose value remained within the normoglycemia value range (40–60 mg/dL).

3.3. Uterine score and uterine bacterian population

The evaluation of the degree of inflammatory infiltrate at the level of the uterus brings a favorable average for the animals treated with zeolite, the treatment group had an average uterine score equal to 0.5610 and the control group's average was 0.7805. However, a significant difference was not established between the two groups ($p>0.05$).

Infections of the reproductive tract during the puerperal period continue to cause harm in the reproductive life of dairy cows, thus to quantify the impact of zeolite administration we resorted to establishing the number of bacteria found intrauterine. Based on the 10^{-1} dilution, it was recorded lawnlike bacterial colony development in all samples for both total germ count and Enterobacteriaceae, and no colonies were present at the 10^{-4} dilution, the results of the intermediate dilutions reported for the two groups are present in Table 5.

3.4. Correlations between monitored parameters

Table 6 contains the recorded correlations between the monitored parameters for both groups studied.

4. Discussion

According to Temesgen et al. (2022), each extra day comes with losses for the farmer ranging from 0.25 to 0.71 US (45). The existing literature contains numerous accounts that highlight the variability in the duration of service period. For instance, Dhami et al. (2017) reported a value of 85.22 ± 7.17 days in their studied treatment groups, compared to 100.67±5.60 days. Another study (Braga Paiano et al., 2019) reported a range between 124.14±7.67 and 164.82±8.66 days. Taking into account that cows administered with zeolite had a 12-day shorter service period, and considering the previously mentioned financial value, there's a cost reduction through zeolite administration. Additionally, reducing the number of services also contributes to cost reduction.

Considering the observed average value in the T group being lower than what was reported in the literature (Koeck et al., 2014; Santschi et al., 2016), a significant comparison was established when compared to the C group. From a technical standpoint, the mathematical difference indicates an improvement in the prevalence of ketosis development, which subsequently has implications on the reproductive health. Similar results were obtained by Maity et al. (2021) and Masoumi Pour et al. (2022) by lowering blood BHB levels after zeolite supplementation in dairy cow diets. Benedet et al. (2019) reported a level between 1.2 and 2.9 mmol/l as subclinical ketosis, with ketosis affecting predominantly in the first two weeks. Conforming to the obtained results, the group that received zeolite supplementation had a lower level of 1.2 mmol/L of BHB, indicating that subclinical ketosis was not present in this group. This confirms the tested hypothesis that zeolite reduces the incidence of ketosis by normalizing ruminal pH.

Moreover, the irreversible loss of glucose during lactation can affect the endocrine and metabolic state postpartum, impacting uterine health, estrous cyclicity, and subsequent pregnancy establishment (Lucy et al.,

Table 4
Levels of beta hydroxybutyrate for both groups.

		Antepartum		parturition	Postpartum			
Group/period		−14	−7	0	7	14	21	28
Treated group	**Mean**	0.039 mmol/l	0.0854 mmol/l	0.1317 mmol/l	0.3463 mmol/l	0.6902 mmol/l	0.8268 mmol/l	0.7585 mmol/l
Control group	**Mean**	0.0659 mmol/l (p>0.05)	0.1488 mmol/l	0.2561 mmol/l (p<0.05)	0.9951 mmol/l	1.478 mmol/l	1.7756 mmol/l	1.5902 mmol/l

Table 5
Comparasion of the total number of germs and Enterobacteria between the groups studied ($p < 0.05$).

Group		NTG10^{-2}	NTG10^{-3}	NTE10^{-2}	NTE10^{-3}
Treated group	Mean	62.70	13.29	39.95	10.65
	N	41	41	41	41
	Std. Deviation	17.21227	7.49081	15.49670	6.39769
Control group	Mean	80.7023	29.80	50.17	17.9512
	N	41	41	41	41
	Std. Deviation	15.18921	8.09080	11.96642	5.67429

2014). In relation to glucose levels, the postpartum administration of zeolite resulted in higher levels, mitigating the negative effects of decreased glucose mentioned earlier.

Zeolite seems to have limited effects on the immune response in the uterus, aligning with findings similar to Crookenden et al. (2020), who studied its impact on neutrophil gene expression and function. However, it's hypothesized that the reduced inflammation in cows treated with zeolite might indirectly result from changes in mineral availability.

Moreover, based on this study, it's suggested that increased glucose levels and the reduction in ketosis incidence have positive effects on uterine health. Although the detailed mechanism of zeolite's action on reproductive health isn't fully understood, there are apparent benefits in reducing uterine pathology (Masoumi et al., 2022).Therefore, by numerically establishing the bacterial population for the two groups, it can be noted that for the T group the bacteria was present in a smaller number in the uterin secretions. A plausible explanation could indeed involve a more robust local immune response within the organism. This hypothesis gains support from the post-uterine biopsy results, indicating a strengthened local immunity. Additionally, the correlation between the total number of germs and the required doses for achieving one gestation, along with the days open interval, further strengthens this

Table 6
Correlation of parameters for all animals included in the study.

		Service per conception	BHB AP	BHB PP	Glucose AP	Glucose PP	NTG 10^{-2}	NTG 10^{-3}	NTE 10^{-2}	NTE 10^{-2}	Service period
Service per conception	Pearson Correlation	1	.648**	.816**	−0.501**	−0.711**	.817**	.650**	.732**	.581**	.785**
	Sig. (2-tailed)		.000	.000	.000	.000	.000	.000	.000	.000	.000
	N	82	82	82	82	82	82	82	82	82	82
BHB AP	Pearson Correlation	.648**	1	.609**	−0.554**	−0.639**	.610**	.522**	.596**	.435**	.061
	Sig. (2-tailed)	.000		.000	.000	.000	.000	.000	.000	.000	.584
	N	82	82	82	82	82	82	82	82	82	82
BHB PP	Pearson Correlation	.816**	.609**	1	−0.566**	−0.852**	.910**	.844**	.817**	.696**	−0.551**
	Sig. (2-tailed)	.000	.000		.000	.000	.000	.000	.000	.000	.000
	N	82	82	82	82	82	82	82	82	82	82
Glucose AP	Pearson Correlation	−0.501**	−0.554**	−0.566**	1	.611**	−0.581**	−0.421**	−0.594**	−0.481**	−0.502**
	Sig. (2-tailed)	.000	.000	.000		.000	.000	.000	.000	.000	.000
	N	82	82	82	82	82	82	82	82	82	82
Glucose PP	Pearson Correlation	−0.711**	−0.639**	−0.852**	.611**	1	−0.807**	−0.765**	−0.772**	−0.719**	−0.716**
	Sig. (2-tailed)	.000	.000	.000	.000		.000	.000	.000	.000	.000
	N	82	82	82	82	82	82	82	82	82	82
NTG10^{-2}	Pearson Correlation	.817**	.610**	.910**	−0.581**	−0.807**	1	.853**	.910**	.719**	.905**
	Sig. (2-tailed)	.000	.000	.000	.000	.000		.000	.000	.000	.000
	N	82	82	82	82	82	82	82	82	82	82
NTG10–3	Pearson Correlation	.650**	.522**	.844**	−0.421**	−0.765**	.853**	1	.726**	.772**	.772**
	Sig. (2-tailed)	.000	.000	.000	.000	.000	.000		.000	.000	.000
	N	82	82	82	82	82	82	82	82	82	82
NTE10–2	Pearson Correlation	.732**	.596**	.817**	−0.594**	−0.772**	.910**	.726**	1	.737**	.792**
	Sig. (2-tailed)	.000	.000	.000	.000	.000	.000	.000		.000	.000
	N	82	82	82	82	82	82	82	82	82	82
NTE10^{-3}	Pearson Correlation	.581**	.435**	.696**	−0.481**	−0.719**	.719**	.772**	.737**	1	.603**
	Sig. (2-tailed)	.000	.000	.000	.000	.000	.000	.000	.000		.000
	N	82	82	82	82	82	82	82	82	82	82
Service period	Pearson Correlation	.785**	−0.061	−0.551**	−0.502**	−0.716**	.905**	.772**	.792**	.603**	1
	Sig. (2-tailed)	.000	.584	.000	.000	.000	.000	.000	.000	.000	
	N	82	82	82	82	82	82	82	82	82	82

** Correlation is significant at the 0.01 level (2-tailed).

hypothesis ($p < 0.05$).

The potential link observed between local immunity, microbial presence, and reproductive outcomes aligns with prior research. Williams et al. (2011) displayed the association between recognized pathogens and ovarian dysfunction during the postpartum period. This, coupled with findings from various authors (Cerbu et al., 2020; Dunislawska et al., 2022; Ivkovic et al., 2004), suggesting an immunomodulatory effect and enhanced immune response following clinoptilolite zeolite administration, implies promising results in terms of prophylaxis against reproductive tract infections in cattle. This indicates a potential avenue for enhancing reproductive health by bolstering immunity and mitigating the impact of pathogens in the reproductive system.

Furthermore, by administering zeolite, antimicrobial treatments are avoided, thus contributing to the phenomenon of reducing antibiotic residues in animal products and decreasing antimicrobial resistance.

In the present study, positive correlations ($p < 0.05$) were estabilished between the service period interval, the BHB level in the blood, the total count of germs and Enterobacteriaceae. Negative correlations ($p < 0.05$) were established with the glucose level.

The correlation of BHB in the blood recorded PP with the bacterial population at the level of the bovine uterus was a positive one ($p < 0.05$). The correlation mentioned pertains to the impact of altered metabolism on immune defense, specifically in relation to increased levels of BHB and decreased glucose levels. This correlation aligns with the findings of a recent study (Swartz et al., 2021), which demonstrated the involvement of ketosis in disrupting local defense mechanisms in the mammary gland. This disruption may also have implications for immune function in the uterus.

The level of glucose both AP and PP registered an inverse correlation ($p < 0.05$) with the bacterial population present, agreeing with the findings of Nazifi et al., 2003 which showed that cows diagnosed with uterine infection suffer metabolic disorders energy up to 4 weeks postpartum and delays in the physiological adaptations necessary to meet their energy needs.

Regarding the correlation between the two parameters of BHB metabolism and glucose, it is identified as negative ($p < 0.05$), both AP and PP, a fact confirmed by other authors (Benedet et al., 2019; Mair et al., 2016; Sakha et al., 2006).

The number of services per conception and the service period correlated with all parameters except for the service period and BHB AP level. Thus, we can deduce that the BHB AP level does not influence the duration of open days

Based on the presented data, it is evident that there is a notable correlation among the indicators. This observation highlights the interconnectedness of multiple factors that contribute to the physiological or pathological functioning of the reproductive system in dairy cows. It emphasizes the importance of considering a holistic perspective when assessing the overall wellbeing of these animals.

5. Conclusions

This study introduces a novel approach to enhancing reproductive health by incorporating zeolite administration and natural supplements into the cows' diet. Our results demonstrated that cows supplemented with zeolite experienced fewer open days and a lower service per conception. Additionally, treated cows showed an improvement in uterine score and a decrease in bacterial count, indicating an enhancement in reproductive health within this group.

Regarding the impact of zeolite on metabolism, it was shown that by increasing glucose levels and decreasing BHB, there is a potential to maintain the energy balance within physiological bounds. Thus, through a promising nutritional strategy, we may prevent the onset of pathologies during the most challenging period for dairy cows from race Romanian Spotted.

Ethical statement

Hereby, I Giurgiu Viorel Ovidiu consciously assure that for the manuscript The effect of oral administration of zeolite on the energy metabolism and reproductive health of Romanian Spotted Breed in advanced gestation and post partum period the following is fulfilled:

(1) This material is the authors' own original work, which has not been previously published elsewhere.
(2) The paper is not currently being considered for publication elsewhere.
(3) The paper reflects the authors' own research and analysis in a truthful and complete manner.
(4) The paper properly credits the meaningful contributions of co-authors and co-researchers.
(5) The results are appropriately placed in the context of prior and existing research.
(6) All sources used are properly disclosed (correct citation). Literally copying of text must be indicated as such by using quotation marks and giving proper reference.
(7) All authors have been personally and actively involved in substantial work leading to the paper, and will take public responsibility for its content.

The protocol was ethically reviewed and approved by the Ethics Committee of the Veterinary Medicine Department at the University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, under number 325/20.06.2022. Additionally, the study adhered to the ARRIVE guidelines for animal research Date: 03.10.2023 Corresponding author's signature: Giurgiu Viorel Ovidiu

CRediT authorship contribution statement

O.V. Giurgiu: Conceptualization, Formal analysis, Funding acquisition, Methodology, Project administration, Software, Writing – original draft, Writing – review & editing. **D.I. Berean:** Conceptualization, Formal analysis, Supervision, Writing – review & editing. **A. Ionescu:** Investigation, Resources, Software, Writing – original draft. **M.S. Ciupe:** Conceptualization, Resources, Software, Writing – original draft. **C.R. Cimpean:** Investigation, Visualization. **C.I. Radu:** Formal analysis, Investigation, Resources. **D.G. Bitica:** Formal analysis, Investigation, Supervision. **S. Bogdan:** Data curation, Supervision, Visualization, Writing – original draft. **M.L. Bogdan:** Conceptualization, Project administration, Software, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors would like to thank Mr. Sfâra Ciprian, Mr. Claudiu Andor and Mr. Daniel Agapie for their support through for completion of this paper.

References

Bacakova, L., Vandrovcova, M., Kopova, I., & Jirka, I. (2018). Applications of zeolites in biotechnology and medicine A review. *Biomaterials Science, 6*, 974–989.

Bach, A., Baudon, M., Elcoso, G., Viejo, J., & Courillon, A. (2023). Effects on rumen pH and feed intake of a dietary concentrate challenge in cows fed rations containing pH modulators with different neutralizing capacity. *Journal of Dairy Science, 106*(7), 4580–4598.

Benedet, A., Manuelian, C. L., Zidi, A., Penasa, M., & De Marchi, M. (2019). Invited review: Β-hydroxybutyrate concentration in blood and milk and its associations with cow performance. *Animal, 13*, 1676–1689. an international journal of animal bioscience.

Braga Paiano, R., Becker Birgel, D., & Harry Birgel, E. (2019). Uterine involution and reproductive performance in dairy cows with metabolic diseases. *Animals, 9*, 93.

Calderón-Amor, J., Hernández-Gotelli, C., Strappini, A., Wittwer, C., & Sepúlveda-Varas, P. (2021). Prepartum factors associated with postpartum diseases in pasture-based dairy cows. *Preventive Veterinary Medicine, 196*, Article 105475.

Cerbu, C., Ilaş, V. A., Czopowicz, M., Potârniche, A. V., Bodart-Nieva, E. P., Mureşan, E. A., et al. (2020). The use of activated micronized zeolite clinoptilolite as a possible alternative to antibiotics and chestnut extract for the control of undifferentiated calf diarrhea: An *in vitro* and *in vivo* Study. *Animals, 10*, 2284.

Chapwanya, A., Meade, K. G., Doherty, M. L., Callanan, J. J., Mee, J. F., & O'Farrelly, C. (2009). Histopathological and molecular evaluation of Holstein-Friesian cows postpartum: Toward an improved understanding of uterine innate immunity. *Theriogenology, 71*, 1396–1407.

Crookenden, M. A., Phyn, C. V. C., Turner, S. A., Loor, J. J., Smith, A. I., Lopreiato, V., et al. (2020). Feeding synthetic zeolite to transition dairy cows alters neutrophil gene expression. *Journal of Dairy Science, 103*, 723–736.

Dhami, A. J., Theodore, V. K., Panchal, M. T., Hadiya, K. K., Lunagariya, P. M., & Sarvaiya, N. P. (2017). Effect of peripartum nu-tritional supplementation on postpartum fertility and blood biochemical and steroid hormone profile in crossbred cows. *Indian Journal of Animal Research, 51*(5), 821–826.

Dschaak, C. M., Eun, J. S., Young, A. J., Stott, R. D.&, & Peterson, S. (2010). Effects of supplementation of natural zeolite on intake, digestion, ruminal fermentation, and lactational performance of dairy cows. *The Professional Animal Scientist, 26*, 647–654.

Dunislawska, A., Biesek, J., Banaszak, M., Siwek, M., & Adamski, M. (2022). Effect of zeolite supplementation on gene ex-pression in the intestinal mucosa in the context of immunosafety support in poultry. *Genes, 13*, 732.

Đuričić, D., Sukalić, T., Marković, F., Kočila, P., Žura Žaja, I., Menčik, S., et al. (2020). Effects of dietary vibroactivated clinoptilolite supplementation on the intramammary microbiological findings in dairy cows. *Animals, 10*, 202.

Elmetwally, M. A. (2018). Uterine involution and ovarian activity in postpartum holstein dairy cows. A review. *Journal of Veterinary Healthcare, 1*(4), 29–40.

Holger, M. (2023). Invited review: Increasing milk yield and negative energy balance: A gordian knot for dairy cows? *Animals, 13*(19), 3097.

Ilie, D. E., Gavojdian, D., Kusza, S., Neamţ, R. I., Mizeranschi, A. E., Mihali, C. V., et al. (2023). Kompetitive allele specific pcr genotyping of 89 SNPs in romanian spotted and romanian brown cattle breeds and their association with clinical mastitis. *Animals, 13*, 1484.

Ingvartsen, K. L., Dewhurst, R. J., & Friggens, N. C. (2003). On the relationship between lactational performance and health: Is it yield or metabolic imbalance that cause production diseases in dairy cattle A position paper. *Livestock Production Science*, 83.

Ivkovic, S., Deutsch, U., Silberbach, A., Walraph, E., & Mannel, M. (2004). Dietary supplementation with the tribomechanical-ly activated zeolite clinoptilolite in immunodeficiency: Effects on the immune system. *Advances in Therapy, 21*, 135–147.

Jansen, H. M., Zschiesche, M., Albers, D., Wemheuer, W., Sharifi, A. R.&, & Hummel, J. (2021). Accuracy of subclinical ketosis detection with rapid test methods for BHBA in blood in commercial dairy farms. *Dairy,, 2*, 671–683.

Jurkić, L. M., Cepanec, I., Pavelić, S. K., & Pavelić, K. (2013). Biological and therapeutic effects of ortho-silicic acid and some ortho-silicic acid-releasing compounds: New perspectives for therapy. *Nutrition. Metabolism., 10*, 2.

Karatzia, M. A., Katsoulos, P. D.&, & Karatzias, H. (2013). Diet supplementation with clinoptilolite improves energy status, reproductive efficiency and increases milk yield in dairy heifers. *Animal Production Science, 53*, 234–239.

Khachlouf, Khouloud, Hamed, Houda, Gdoura, Radhouane, & Gargouri, Ahmed (2018). Effects of zeolite supplementation on dairy cow production and ruminal parameters – A review. *Annals of Animal Science, 18*(4), 857–877.

Koeck, A., Jamrozik, J., Schenkel, F. S., Moore, R. K., Lefebvre, D. M., Kelton, D. F.&, et al. (2014). Genetic analysis of milk β-hydroxybutyrate and its association with fat-to-protein ratio, body condition score, clinical ketosis, and displaced abomasum in early first lactation of. *Canadian Holsteins Journal of Dairy Science, 97*, 7286–7292.

Kustova, M., Egeblad, K., Christensen, C. H., & Kustov, A. L. (2007). From zeolites to porous MOF materials-The 40th anniversary of international zeolite conference. In *Proceedings of the 15th international zeolite conference, Bejing, China* (p. 170). Elsevier Science.

LeBlanc, S. (2010). Monitoring metabolic health of dairy cattle in the transition period. *Journal of Reproduction and Development, 56*, S29–S35. Suppl.

LeBlanc, S. J., Lissemore, K. D., Kelton, D. F., Duffield, T. F.&, & Leslie, K. E. (2006). Major advances in disease prevention in dairy cattle. *Journal of Dairy Science, 89*, 1267–1279.

Lucy, M. C., Butler, S. T., & Garverick, H. A. (2014). Endocrine and metabolic mechanisms linking postpartum glucose with early embryonic and foetal development in dairy cows. *Animal, 8*(1), 82–90. : an international journal of animal bioscienceSuppl.

Madoz, L. V., Giuliodori, M. J., Migliorisi, A. L., Jaureguiberry, M., & Sota, R. L. (2014). Endometrial cytology, biopsy, and bacteri-ology for the diagnosis of subclinical endometritis in grazing dairy cows. *Journal of Dairy Science, 97*, 195–201.

Mair, B., Drillich, M., Klein-Jöbstl, D., Kanz, P., Borchardt, S., Meyer, L., et al. (2016). Glucose concentration in capillary blood of dairy cows obtained by a minimally invasive lancet technique and determined with three different hand-held devices. *BMC Veterinary Research, 12*, 24.

Maity, S., Rubić, I., Kuleš, J., Horvatić, A., Đuričić, D., Samardžija, M., et al. (2021). Integrated metabolomics and proteomics dynamics of serum samples reveals dietary zeolite clinoptilolite supplementation restores energy balance in high yielding dairy cows. *Metabolites, 11*, 842.

Masoumi Pour, M. M., Foroudi, F., Karimi, N., Abedini, M. R., & Karimi, K. (2022). Effect of anionic and zeolite supplements and oral calcium bolus in prepartum diets on feed intake, milk yield and milk compositions, plasma Ca concentration, blood metabolites and the prevalence of some reproductive disorders in fresh dairy cows. *Animals, 12*, 3059.

Mastinu, A., Kumar, A., Maccarinelli, G., Bonini, S. A., Premoli, M., Aria, F., et al. (2019). Zeolite clinoptilolite: Therapeutic virtues of an ancient mineral. *Molecules, 24*, 1517 (Basel, Switzerland).

Mumpton, F. A. (1999). La roca magica: Uses of natural zeolites in agriculture and industry. In *, 96. Proceedings of the national academy of sciences* (pp. 3463–3470).

Nazifi, S., Saeb, M., Rowghani, E.., & Kaveh, K. (2003). The influences of thermal stress on serum biochemical parameters of Iranian fat-tailed sheep and their correlation with tri-iodothryonine (T3), thyroxine (T4) and cortisol concentrations. *Comparative Clinical Pathology, 12*, 135–139.

Nordlund, K. V., & Cook, N. B. (2004). Using herd records to monitor transition cow survival, productivity and health. *Veterinary Clinics of North America: Food Animal Practice, 20*, 627–649.

Pavelić, K., Hadžija, M., Bedrica, L., Pavelić, J., Dikić, I., Katić, M., et al. (2001). Natural zeolite clinoptilolite new adjuvant in anticancer therapy. *Journal of Molecular Medicine, 78*, 708–720.

Pavelić, S. K., Medica, J. S., Gumbarević, D., Filošević, A., Pržulj, N., & Pavelić, K. (2018). Critical review on zeolit clinoptilolit safety and medical applications *in vivo*. *Frontiers in Pharmacology, 9*, 1350.

Sailer, K. J., Pralle, R. S., Oliveira, R. C., Erb, S. J., Oetzel, G. R.&, & White, H. M. (2018). Technical note: Validation of the BHB-Check blood β-hydroxybutyrate meter as a diagnostic tool for hyperketonemia in dairy cows. *Journal of Dairy Science, 101*, 1524–1529.

Sakha, M., Ameri, M., & Rohbakhsh, A. (2006). Changes in blood β-hydroxybutyrate and glucose concentrations during dry and lactation periods in Iranian Holstein cows. *Comparative Clinical Pathology, 15*, 221–226.

Santschi, D. E., Lacroix, R., Durocher, J., Duplessis, M., Moore, R. K.&, & Lefebvre Valacta, D. M. (2016). Prevalence of elevated milk þ-hydroxybutyrate concentrations in Holstein cows measured by Fourier-transform infrared analysis in dairy herd improvement milk samples and association with milk yield and components. *Journal of Dairy Science, 99*, 9263–9270.

Semenov, V. G., Tyurin, V. G., Smirnov, A. M., Kuznetsov, A. F., Larionov, G. A., Mudarisov, R. M., et al. (2020). Prevention of postpartum complications and management of reproductive qualities of cows with the use of Prevention-N-B-S bio-preparation. In *, 604. Proceedings of the IOP conference series: earth and environmental science*, Article 012017.

Shandilya, S. K., Singh, A. P.;., Ojha, B. K., Mishra, A., Jaiswal, M., Jaiswal, S. K., et al. (2020). Effect of organic production system on productive and reproductive performance of cattle. *Indian Journal of Animal Research, 54*, 384–387.

Sulzberger, S., Kalebich, C. C., Melnichenko, S., & Cardoso, F. C. (2016). Effects of clay after a grain challenge on milk composition, and on ruminal, blood and faecal pH in Holstein cows. *Journal of Dairy Science, 99*, 8028–8040.

Sundrum, A. (2001). Organic livestock farming- A critical review. *Livestock Production Science, 67*, 207–215.

Swartz, T. H., Bradford, B. J.&, & Mamedova, L. K. (2021). Connecting metabolism to mastitis: Hyperketonemia impaired mam-mary gland defenses during a streptococcus uberis challenge in dairy cattle. *Frontiers in Immunology, 12*, Article 700278.

Temesgen, M. Y., Assen, A. A., Gizaw, T. T., Minalu, B. A., & Mersha, A. Y. (2022). Factors affecting calving to conception inter-val (days open) in dairy cows located at Dessie and Kombolcha towns. *Ethiopia PLoS One, 17*, Article e0264029.

Valpotic, H., Terzic, S., Vince, S., Samardzija, M., Turk, R., Lackovic, G., et al. (2016). In-feed supplementation of a clinoptilolite favorably modulates intestinal and systemic immunity and some production parameters in weaned pigs. *Veterinarni Medicina, 61*, 317–327.

Vergara, C. F., Döpfer, D., Cook, N. B., Nordlund, K. V., McArt, J. A. A., Nydam, D. V., et al. (2014). Risk factors for postpartum problems in dairy cows: Explanatory and predictive modeling. *Journal of Dairy Science, 97*, 4127–4140.

Weller, R. F., & Cooper, A. (1996). Health status of dairy herds converting from conventional to organic dairy farming. *The Veterinary Record, 139*, 141–142.

Wittrock, J. M., Proudfoot, K. L., Weary, D. M., & Keyserlingk, M. A. G. (2011). Short communication: Metritis affects milk production and cull rate of Holstein multiparous and primiparous dairy cows differently. *Journal of Dairy Science, 94*, 2408–2412.