agriculture

MDPI

Communication

Sensor-Generated Data for Evaluation of Subclinical Mastitis Treatment Effectiveness with Garlic Extract (Allicin) in Dairy Cattle

Ramūnas Antanaitis [1,*], Lina Anskienė [2], Karina Džermeikaitė [1], Dovilė Bačėninaitė [1], Aloyzas Januškauskas [3], Kęstutis Sincevičius [3], Walter Baumgartner [4] and Anton Klein [5]

1 Large Animal Clinic, Veterinary Academy, Lithuanian University of Health Sciences, Tilžės Str. 18, LT-47181 Kaunas, Lithuania; karina.dzermeikaite@lsmu.lt (K.D.); dovile.baceninaite@lsmu.lt (D.B.)
2 Department of Animal Breeding, Veterinary Academy, Lithuanian University of Health Sciences, Tilžės Str. 18, LT-47181 Kaunas, Lithuania; lina.anskiene@lsmuni.lt
3 Vetmarket, Nemuno Str. 4, LT-53458 Kaunas, Lithuania; aloyzas.januskauskas@vetmarket.lt (A.J.)
4 University Clinic for Ruminants, University of Veterinary Medicine, Veterinaerplatz 1, A-1210 Vienna, Austria
5 Carton BV, Julekesweg 7, 7451 PB Holten, The Netherlands
* Correspondence: ramunas.antanaitis@lsmuni.lt; Tel.: +370-67349064

Abstract: The aim of this study was to determine the impact of subclinical mastitis treatment in dairy cattle on biomarkers registered with in-line sensors such as milk yield (MY), electric milk conductivity (EC), rumination time (RT), and somatic cell count (SCC). At the start of the experiment, all cows according to SCC level were divided into two groups: healthy cows (n = 30, with SCCs less than 200,000 per mL and without the growth of bacteria in the milk samples) and cows with subclinical mastitis (n = 32), with SCC levels greater than 200,000 per mL and with growth of bacteria. *Streptococcus* spp. was found in 15 samples, and *Strep. uberis* was found in 17 samples. *Streptococcus* spp. and *Strep. uberis* were sensitive to amoxicillin and calvulanic acid. According to these results, 32 cows with subclinical mastitis were treated with two treatment protocols: one 1 (n = 16) and two (n = 16). In the first protocol, we used SCC boluses and nonsteroidal anti-inflammatory drugs (SCCB and NSAID). The second protocol consists of intramammary antibiotics and anti-inflammatory medications (Synulox LC and NSAIDs). All parameters (MY, EC, RT, and SCC) were recorded with Lely Astronaut® A3 milking robots on the day of mastitis diagnosis (0 day) and 14 days after treatment began. All animal experimental procedures were approved by the ethical committee; the approval number is PK01696. On the basis of our findings, we may infer that SCC boluses and NSAIDs are effective in treating subclinical mastitis. After 14 days of treatment, the electrical conductivity of milk in cows treated with AB and NSAID was also higher in all quarters of the udder compared to cows treated with SCCB + NSAID. The RT of cows on disease diagnosis day of cows treated with AB and NSAID was 11.41% lower compared to cows treated with SCCB and NSAID, while the RT of cows after 14 days treated with AB and NSAID was 7.01% lower compared to cows treated with SCCB and NSAID. On the practical side, for treatment of subclinical mastitis, we recommend using a feed supplement SCC bolus (one per os) with a composition containing Meloxicam 20 mg with a single subcutaneous injection at a dosage of 2.5 mL per 100 kg body weight.

Keywords: subclinical mastitis; dairy cattle; treatment; garlic extract

Citation: Antanaitis, R.; Anskienė, L.; Džermeikaitė, K.; Bačėninaitė, D.; Januškauskas, A.; Sincevičius, K.; Baumgartner, W.; Klein, A. Sensor-Generated Data for Evaluation of Subclinical Mastitis Treatment Effectiveness with Garlic Extract (Allicin) in Dairy Cattle. *Agriculture* **2023**, *13*, 972. https://doi.org/10.3390/agriculture13050972

Academic Editors: Hao Li, Xiaoshuai Wang, Qianying Yi, Kai Liu and Tomas Norton

Received: 12 March 2023
Revised: 17 April 2023
Accepted: 25 April 2023
Published: 27 April 2023

1. Introduction

Subclinical mastitis (SCM) has a negative effect on farm economies worldwide because of the decrease of milk production and milk quality, increased treatment costs, and culling rate [1,2]. SCM's economic cost was determined by parameters such as lower milk yield, higher veterinarian, labor, and treatment expenditures, decreased milk output, and premium payments received, among others [3]. One of major outcomes of this disease is

a decrease in milk production and farm revenues [4]. Even after complete recovery from SCM, milk output does not totally return. Milk from an inflamed udder is altered in a variety of ways, including changes in composition [5]. An increased number of somatic cell count has been found to be associated with the decrease in milk lactose percentage due to changes in the homeostasis of mammary glands during SCM infection [6]. Several studies have shown that the electrical milk conductivity (EC) of milk from cows with clinical and subclinical mastitis is greater than the EC of milk from healthy cows [7]. Nevertheless, previous studies have shown that the use of only EC in different detection algorithms was unable to achieve mastitis detection [4]. With using a new system during the peripartum period, differences in RT between healthy and sick cows have been found. Mainly a shorter RT during the first 10 days in milk (DIM) was observed in cows with health disorders as compared to healthy cows [8].

The most popular biomarkers used for subclinical mastitis detection and control of treatment effect are milk yield (MY), SCC, EC, and RT [9]. The quarter is deemed subclinical when quarterly SCC is equal to or greater than 200,000 cells/mL and when bacteria are found in the absence of clinical changes [1].

Antibiotic-based mastitis therapy leaves much to be desired, including antibiotic resistance, necessitating research to find adjuncts or alternatives to lessen the devastating impacts of this costly disease [10]. A range of therapeutic treatments has been used to treat bovine and bubaline clinical and subclinical mastitis, either as replacements or as adjuncts to antibiotics [10]. Non-antibiotic antibacterial treatments are now preferred to treat not just intramammary infections but also infections of other systems [11].

Sustainable agriculture is one of the organic production systems with the least environmental impact. Garlic oil capsules (for example, combined with cinnamaldehyde) have been shown to change rumen fermentation (in vitro) and hence lower methane generation. [12]. Greenhouse gases emission fell by 3.7% by reducing the SCC level from 8.0 to 0.50 $\times$ 10^5 mL^{-1} [13]. Improvements have been made in feed intake, performance, udder health, ruminal fermentation, and plasma levels. Metabolites in milk from cows with moderate or high SCC as a result of a phytobiotic-rich herbal supplementation [14]. The inhibitory impact of phytobiotics derived from various species of plants has revealed that the combined usage of phytobiotics has more antibacterial activity than the solitary use of each plant-based product [15]. So far, little research has looked at the benefits of garlic on mastitis prevention, particularly on organic farms. In cow nutrition, garlic contains antifungal, antimicrobial, antiviral, anti-inflammatory, hepatoprotective, anti-carcinogenic, and immuno-stimulating activities. Garlic's above-mentioned qualities are attributed to its active ingredient, allicin. Allicin (diallylthiosulfinate) is a defense molecule from garlic (*Allium sativum* L.) with a broad range of biological activities. Allicin is produced upon tissue damage from the non-proteinogenic amino acid alliin (S-allylcysteine sulfoxide) in a reaction that is catalyzed by the enzyme alliinase [16].

According to information from the literature, our hypothesis is that we could use in-line sensors to evaluate the effectiveness of subclinical mastitis treatment in dairy cattle.

The aim of this study was to determine the effectiveness of subclinical mastitis treatment with garlic extract (Allicin) with the evaluation of biomarkers registered with in-line sensors such as milk yield (MY), electric milk conductivity (EC), RT, and somatic cell count (SCC) in dairy cattle.

2. Materials and Methods

2.1. Location, Animals

For this experiment, we had certain criteria for farm selection: it must be a large farm (more than 1000 milking cows), with high productivity (>10,000 kg milk per lactation) and with no fewer than 500 cows milked by milking robots, which are registering rumination time, milk yield, the number of somatic cells in the milk, and the electrical conductivity of the milk from all parts of the udder. The experiment was performed at one Lithuanian dairy farm with 1500 milking cows (location: 54.97378759003201, 23.76954146935687). From

the 1500 cows, 640 were milked with 8 Lely astronaut A4 (Lely Campus, Cornelis van der Lely an 1, 3147 PB, Maassluis, The Netherlands) milking robots. The milking robots used were free-traffic Lely Astronaut® A4 milking robots. Cows were fed two times per day (at 07:00 a.m., and 07:00 p.m.) with a balanced feed ration. The composition of the feeding ration for dairy cows is shown in Table 1. All animal experimental procedures were approved by the ethical committee. The approval number is PK01696.

Table 1. Composition of feed ration.

Parameters	Units	Quantity
Dry matter	%	45.0
Dry matter intake (DM)	kg DM/d	27.3
Net energy lactation	MJ/kg DM	6.42
Crude protein	g/kg DM	173
Crude Fat	g/kg DM	45
Fatty acids	g/kg DM	37
Protein balance in rumen	g/kg DM	23
Neutral detergent fiber	g/kg DM	285
Starch	g/kg DM	206

2.2. Detection and Treatment of Subclinical Mastitis

The veterinarians of the herds chose cows at random from the herds. Groups were formed according to the principles of analogues according to breed, number of lactations conducted, days of lactation, productivity. The selection criteria were identified from a farm with 640 Lithuanian black and white dairy cows. We paid special attention to this because mastitis is usually diagnosed in conjunction with another disease. Among all selected cows, we discovered 10 with mastitis and other diseases (ketosis, metritis) during clinical examination. Cows having these characteristics were not included in the study.

We used the most often used SCC diagnostic, the California Mastitis Test (CMT), for this investigation, and multiple CMT score cut-off points were used to identify a positive CMT reaction [17]. The gold standard for calculating diagnostic test characteristics was the single milk bacteriological culture result [18]. The California Mastitis Test was done on all cows' udder quarters. CMT results were graded as negative (0+) or positive 1+ (traces), 2+ (gel), or 3+ (clumps) [18]. Milk samples were taken aseptically from CMT >1+ quarters and sent to the state company Pieno Tyrimai for somatic cell counting (SCC) and bacteriological testing in milk (using Somascope, CA-3A4, Delta Instruments, Drachten, The Netherlands). The number of somatic cells in milk samples that tested negative for CMT were counted in the Pieno Tyrimai laboratory.

From 640 cows, 62 were selected with an average of 2.8 (± 0.34) lactations and 60 (2.6) days in milk that fulfilled the inclusion criteria; 32 cows had indications of subclinical mastitis, and 30 were clinical healthy. All 62 cows' milk samples were collected for microbiological investigation. Before the study, samples of each cow's milk were taken in the morning for a microbiological test. All microbiological tests on milk were done at the Lithuanian University of Health Sciences, the Veterinary Academy, and the Microbiology Laboratories according to the rules of the National Mastitis Council [19]. No bacteria were found in any of the 30 cow samples. In 32 samples, we found growth of bacteria. *Streptococcus* spp. was found in 15 samples, and *Strep. uberis* was found in 17 samples. *Streptococcus* spp. and *Strep. uberis* were sensitive to amoxicillin and calvulanic acid.

All cows according to SCC level were divided into two groups [1]: healthy cows (n = 30, with SCCs less than 200,000 per mL and without the growth of bacteria in the milk samples), and cows with subclinical mastitis (n = 32), with SCC levels greater than 200,000 per mL and with growth of bacteria. *Streptococcus* spp. was found in 15 samples,

and *Strep. uberis* was found in 17 samples. According to these results, the second group (cows with subclinical mastitis) was treated with two treatment protocols: one (n = 16) with an average of 2.7 (±0.33) lactations, 55 (±2.6) days in milk, and SCC average of 849,000 (±50) per mL and two (n = 16) with an average of 2.9 (±0.33) lactations, 65 (±2.9) days in milk, and SCC average of 792,000 (±47) per mL. Cows with subclinical mastitis and a positive CMT test but no isolated pathogen were ruled out of the study. In terms of reproductive status, healthy cows were comparable to those with mastitis.

Protocol 1 (n = 16—SCCB + NSAID—SCC bolus and nonsteroidal anti-inflammatory drugs (SCCB + NSAID). Protocol 2 (n-16)- AB + NSAID—treatment with intramammary antibiotics and anti-inflammatory drugs (Synulox LC + NSAID).

Composition of a 90 g SCC bolus: Garlic extract, microcrystalline cellulose, lactose, ethylcellulose, maltodextrin, magnesium strearate. Cows were treated with one orally administered bolus once at the day of mastitis diagnosis. Anti-inflammatory drugs used: Melovem® (meloxicam 30 mg/mL). Cows were treated with single subcutaneous injection at a dosage of 2.5 mL/100 kg body weight. According to CMT results, milk from all udder quarters in this group was 2+ (gel), or 3+ (clumps).

Antimicrobials used: according to CMT results, milk from all udder quarters was 2+ (gel), or 3+ (clumps). Synulox LC (for lactating cows), each 3 g syringe containing 200 mg amoxicillin (as amoxicillin trihydrate), 50 mg clavulanic acid (as potassium clavulanate), and 10 mg prednisolone) was given intramammarily in all udder quarters soon after milking and at 12 h intervals for three consecutive milkings.

Following the final milking, antibiotic infusions were administered as follows: trained staff wearing clean disposable gloves cleansed the teat ends for at least 5 s with 70% isopropyl alcohol-soaked cotton swabs before the antibiotic treatment was infused into the mammary gland and again before ITS was infused into the teat cistern [1]. The treatment was performed by a local veterinarian.

2.3. Measurements and Duration of Measurements

Each time the cows were milked, Lely Astronaut® A4 milking robots recorded parameters such as rumination RT, MY, SCC, and EC of all quarters of the udders (front left (FL), front right (FR), rear left (RL), and rear right (RR). All parameters were recorded on the day of mastitis diagnosis (0 day) and 14 days after treatment began.

2.4. Data Analysis and Statistics

According to the SCC, subclinical mastitis cows were grouped in classes: to SCC classes (1) 200,000–600,000/mL; (2) 600,000–1.000,000/mL; (3) ≥1,000,000/mL. The Kolmogorov–Smirnov test was used to examine normal distributions using descriptive statistics. The outcomes were presented as the mean standard error of the mean (M ± SE). To find significant differences between comparison groups, Student's *t*-test was performed. When the normality and equal variance assumptions were not fulfilled, the non-parametric approach (Kruskal–Wallis one-way ANOVA) was utilized to evaluate significance. At $p < 0.05$, data were judged statistically significant. A binary logistic regression method was used a treatment group (Group 1—SCCB + NSAID; group 2—AB + NSAID) as a dependent variable to project the relationship between milk yield and RT and SCC in cows' milk. Estimates were used to calculate odds ratios (OR).

The distribution of the cows with different SCC classes according to two protocol groups was Protocol 1—31.25% in class 1, 50% in class 2, and 18.75% in class 3; Protocol 2—22.22% in class 1, 55.55% in class 2, and 22.22% in class 3.

3. Results

On a subclinical mastitis diagnosis day, we found 17.85% higher milk yield in healthy cows, compared to cows with subclinical mastitis (OR = 1.267, $p = 0.021$). Following 14 days of initiation of treatment, the milk yield of clinical healthy cows was 16.54% higher

compared to cows with subclinical mastitis (OR = 1.015, $p = 0.038$). Data from binary logistic regression analysis (is) are presented in Table 2.

Table 2. Relationship of milk yield with groups.

Source		B	SEM	Wald χ^2	df	*p*	OR
MY on diagnosis day	Group	−0.062	0.027	5.355	1	0.021	1.267
	Constant	1.887	0.966	3.816	1	0.051	
MY following 14 days of diagnosis day	Group	−0.054	0.026	4.311	1	0.038	1.015
	Constant	1.555	0.916	2.885	1	0.089	

B—the unstandardized regression milk yield in kg, S.E.—standard error for B, Wald χ^2—the Wald chi-square statistic, df—degrees of freedom, OR—odds ratio. Groups: 1 healthy cows; 2—subclinical mastitis. MY—milk yield.

A significant relationship between treatment group of cows and milk yield has been found ($p < 0.05$). After 14 days of treatment, the milk yield of cows treated with SCCB and NSAID was 20.57% higher compared to cows treated with AB and NSAID, (OR = 1.361, $p = 0.011$) (Figure 1). Data from binary logistic regression analysis are presented in Table 3.

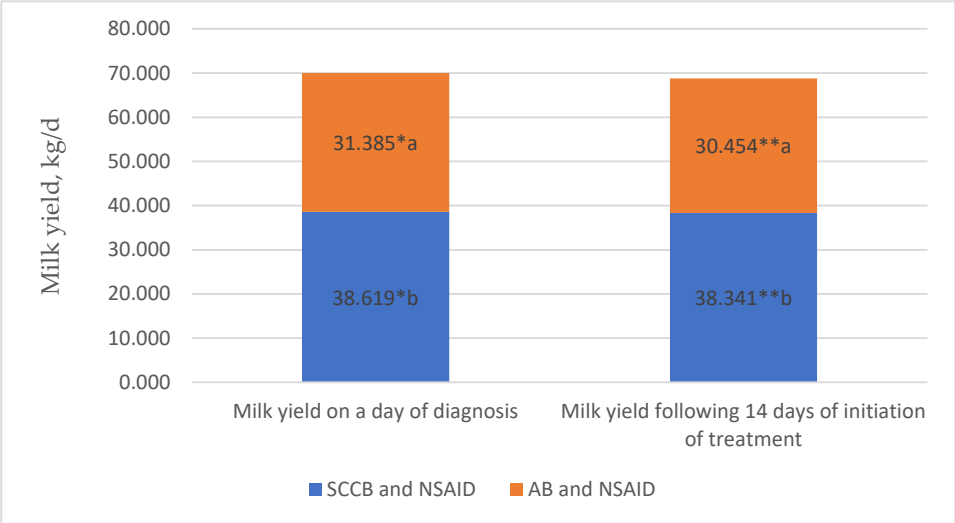


Figure 1. Changes milk yield on the day of diagnosis and after 14 days of treatment of subclinical mastitis. a, b—values indicate significant differences; * $p < 0.05$., ** $p < 0.01$.

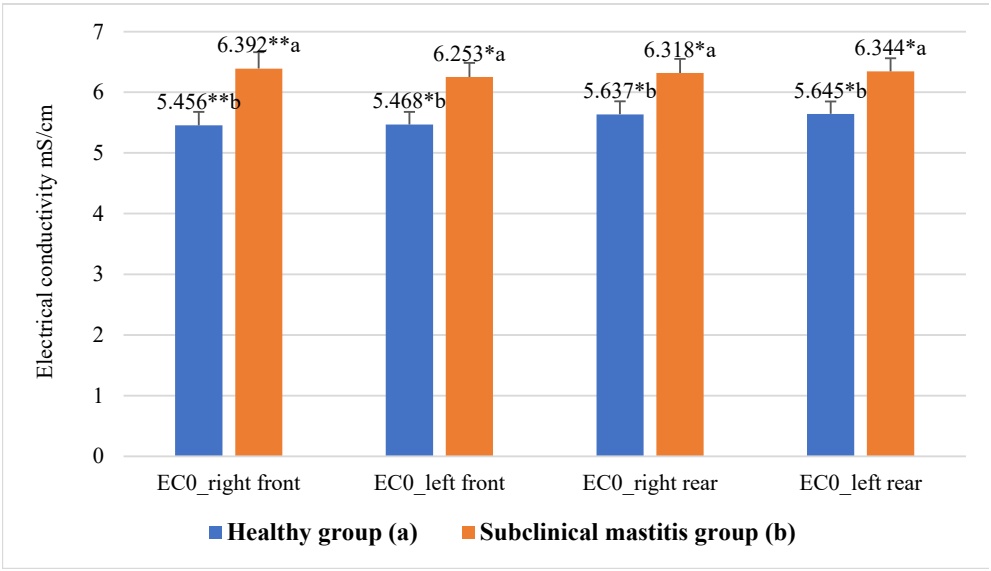
Table 3. Relationship of milk yield with groups of treatment.

Source		B	S.E.	Wald χ^2	df	*p*	OR
MY on diagnosis day	Treatment group	−0.066	0.028	5.793	1	0.016	1.289
	Constant	1.949	0.979	3.958	1	0.047	
MY after 14 days of diagnosis day	Treatment group	−0.072	0.028	6.520	1	0.011	1.361
	Constant	2.081	0.971	4.595	1	0.032	

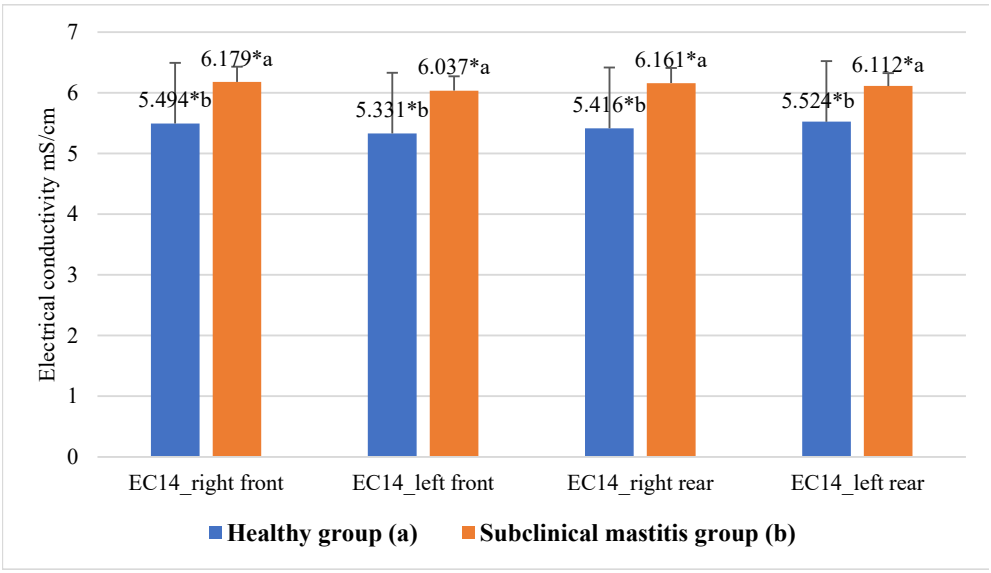
B—the unstandardized regression milk yield in kg, S.E.—standard error for B, Wald χ^2—the Wald chi-square statistic, df—degrees of freedom, OR—odds ratio. Groups: protocol 1—SCCB + NSAID; protocol 2—AB + NSAID. MY—milk yield.

The electrical conductivity of milk on disease diagnosis day in subclinical mastitis group was higher in all quarters of the udder compared to healthy cows (Figure 2A), from 10.78% (right rear quarter), ($p < 0.05$) to 14.64% (right front quarter), ($p < 0.01$). The differences between groups in EC means in all udder quarters were significant ($p < 0.05$–0.01). The electrical conductivity of milk after 14 days of treatment in subclinical mastitis group

was also higher in all quarters of the udder compared to cows in healthy group (Figure 2B), from 9.62% (left rear quarter), ($p < 0.05$) to 12.09% (right rear quarter), ($p < 0.05$).



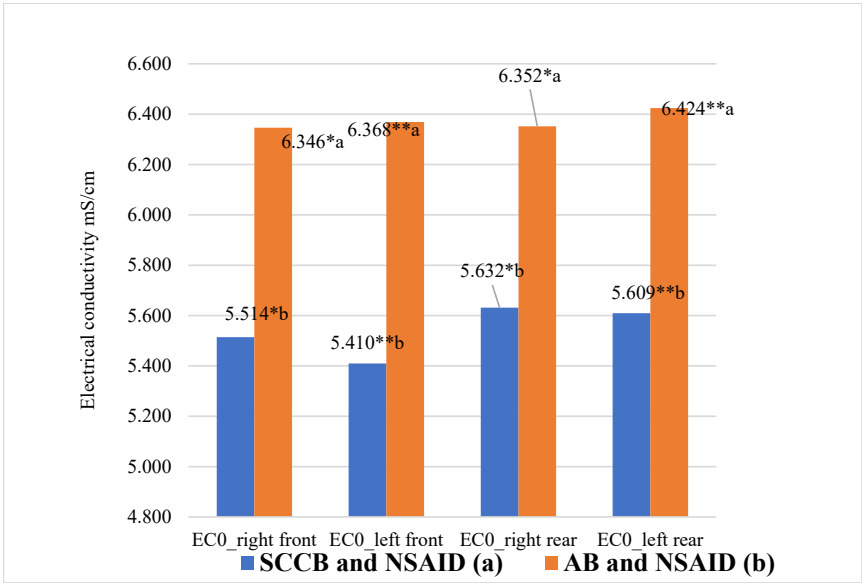
(A)



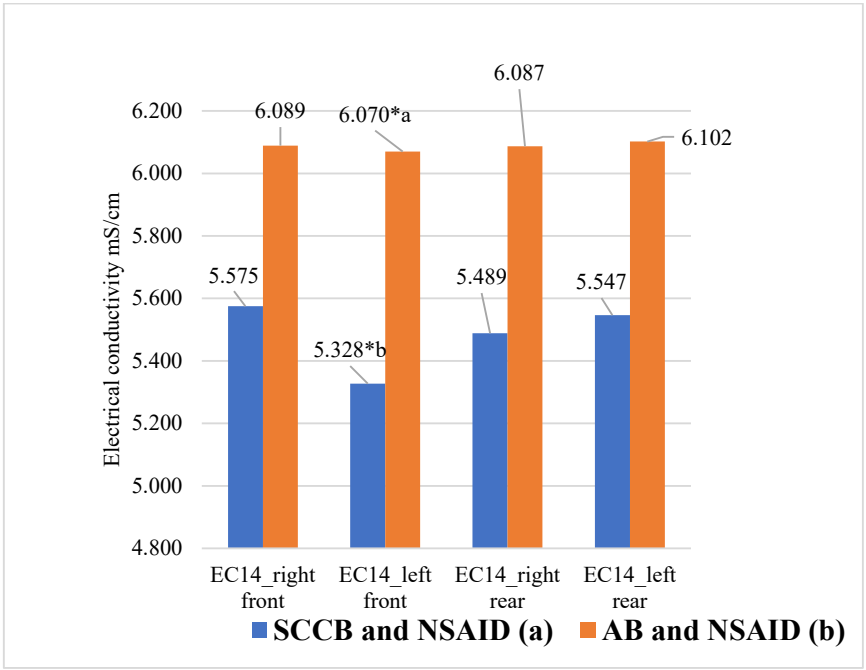
(B)

Figure 2. (**A**) Electrical conductivity of cows' milk at the level of udder quarters: a—on a day of diagnosis; (**B**) Electrical conductivity of cows' milk at the level of udder quarters after 14 days of treatment. a, b—values indicate statistically significant differences; * $p < 0.05$, ** $p < 0.01$.

Analysis of electrical conductivity according to the treatment groups showed that the EC of milk on disease diagnosis day in subclinical mastitis cows was higher in all quarters of the udder compared to healthy cows (Figure 3A), ranging from 12.78% (right rear quarter) to 17.72% (left front quarter; $p < 0.05$), the differences between groups in EC means in all udder quarters were significant ($p < 0.05$). The electrical conductivity of milk with subclinical mastitis after 14 days of treatment in cows treated with AB and NSAID was higher in all quarters of the udder compared to cows treated with SCCB and NSAID (Figure 3B), from 9.22% (right front quarter), to 13.94% (left front quarter; $p < 0.05$).



(**A**)



(**B**)

Figure 3. (**A**) Electrical conductivity of cows' milk at the level of udder quarters: on a day of diagnosis; (**B**) Electrical conductivity of cows' milk at the level of udder quarters after 14 days of treatment. a, b—values indicate statistically significant differences; * $p < 0.05$, ** $p < 0.01$.

A significant relationship between milk yield of cows and classes of SCC in milk has been estimated ($p < 0.05$). In class 1 ((1) <600,000/mL SCC), we found 19.69% higher milk yield compared to cows of group 3 (≥1,000,000/mL SCC; $p < 0.05$), while after 14 days of treatment the milk yield of cows of class 1 was 20.56% higher compared to cows of class 3, ($p < 0.05$; Figure 4).

The electrical conductivity of milk on subclinical mastitis diagnosis day in class 3 was higher in all quarters of the udder compared to cow's milk of SCC class 1 (Figure 5A), from 16.35% (left front quarter), ($p < 0.05$) to 20.47% (right front quarter) compared to cows of SCC class 3, ($p < 0.01$), while fewer differences were estimated comparing SCC class 3 with SCC class 2; the range was from 3.81% (left front quarter) to 7.60% (right front

quarter), but the differences between the average of these SCC classes of EC quarters were not significant.

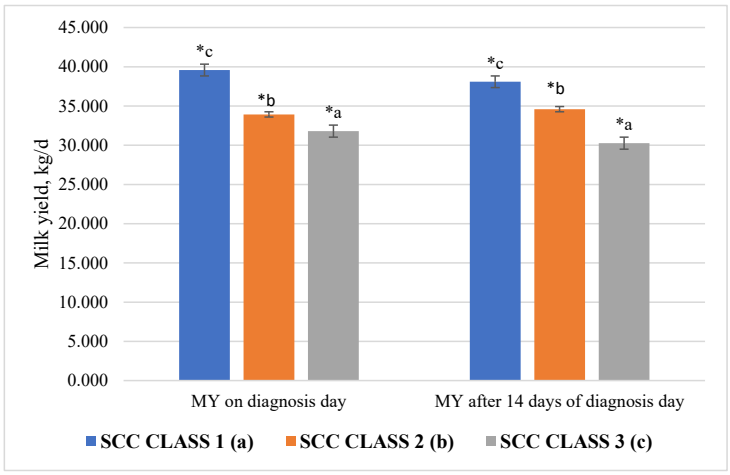
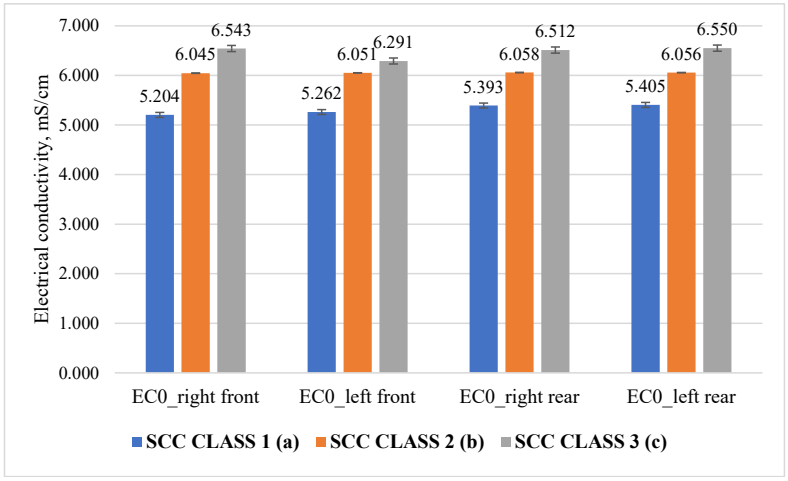
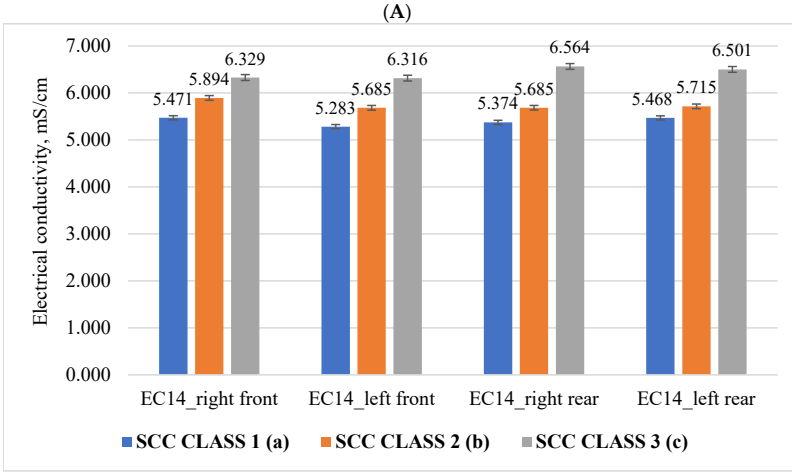


Figure 4. Milk yield of cows according to SCC classes ((1) <600 thousands/mL; (2) 600–1000 thousands/mL; (3) ≥1000 thousands/mL). a–c—values indicate statistically significant differences; * $p < 0.05$.



(**A**)



(**B**)

Figure 5. (**A**,**B**). Electrical conductivity of cows' milk at the level of udder quarters and SCC classes. SCC—somatic cell count.

The same tendency was estimated after 14 days of subclinical mastitis treatment data, where the electrical conductivity of milk in class 3 was higher in all quarters of the udder

compared to cow's milk of SCC class 1 (Figure 5B), from 13.55% (right front quarter), ($p < 0.05$) to 18.14% (right rear quarter) compared to cows of SCC class 3, ($p < 0.01$), while fewer differences were estimated comparing SCC class 3 with SCC class 2; the range was from 6.87% (right front quarter) to 13.39% (right rear quarter), but the differences between the average of these SCC classes of EC quarters were not significant.

Rumination time of clinical healthy cows was 10.29% higher compared to cows with subclinical mastitis during diagnosis day ($p < 0.05$), while RT of cows after 14 days of treatment was longer in both groups of cows, but still it was by 11.21% higher of clinical healthy cows compared to cows with subclinical mastitis, indicating that in cows with higher SCC, RT is lower (OR = 4.582, $p = 0.01$). The average RT of cows treated with AB + NSAID 14 days later was 7.01% lower than cows treated with SCCB + NSAID, ($p < 0.05$) showing the tendency that RT is decreasing with the treatment (Figure 6).

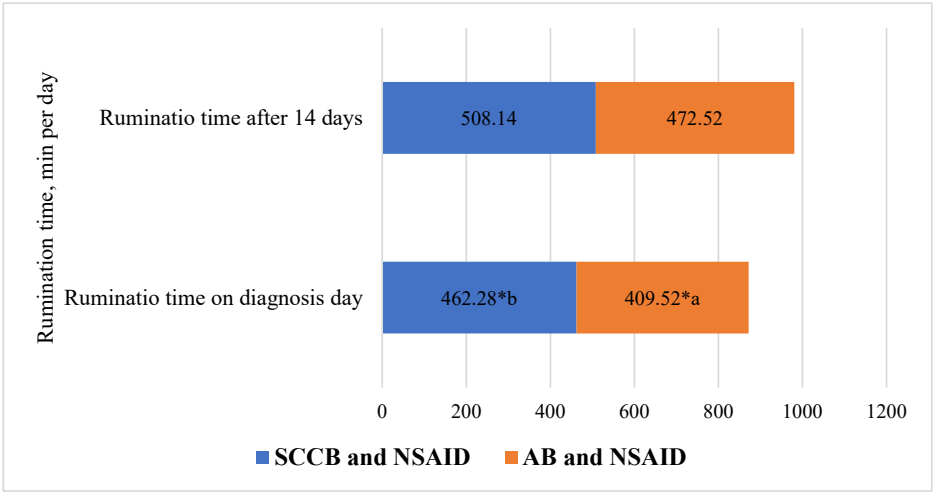


Figure 6. Changes in rumination time on the day of diagnosis and after 14 days of treatment of subclinical mastitis. a, b—values indicate statistically significant differences, * $p < 0.05$.

SCC of clinical heathy cows was 47.65% lower compared to cows with subclinical mastitis form ($p < 0.001$), (OR = 2.435), while SCC of cows after 14 days of treatment was lower in both groups of cows and was by 54.30% lower in clinical healthy cows compared to cows with subclinical mastitis (OR = 2.977, $p = 0.000$; Figure 7).

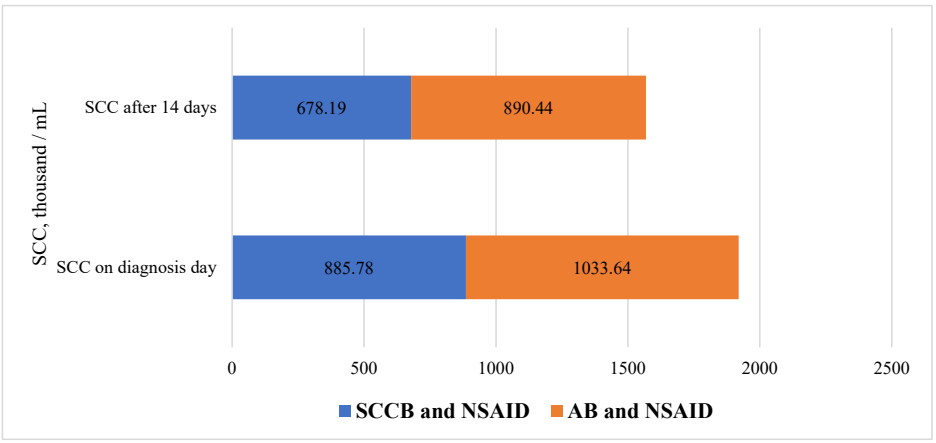


Figure 7. Changes in SCC on the day of diagnosis and after 14 days of treatment of subclinical mastitis.

SCC of clinical healthy cows was 16.69% lower compared to cows with subclinical mastitis form, while the SCC of cows after 14 days of treatment was lower in both groups of cows. It was 31.30% lower in milk of cows with clinical healthy cows compared to cows

with subclinical mastitis form, but the differences between the average of the SCC and group of treatment were not significant.

4. Discussion

Due to the reduction in inspection time necessary to identify cows with mastitis requiring veterinary intervention, interest in and acceptance of automatic (robotic) milking systems (AMS) have created a need for reliable automatic detection of mastitis [19]. Efficient mastitis identification provides the chance to execute early and adequate treatment protocols and minimize excessive use of antibiotics, so preserving animal health and welfare by minimizing pain and discomfort, boosting the recovery rate, and maximizing economic returns to farmers [9].

The current study aimed to evaluate the effectiveness of subclinical mastitis treatment with garlic extract (Allicin) with the evaluation of biomarkers registered with in-line sensors such as MY, EC, RT, and SCC in dairy cattle. We found that in cows treated with AB + NSAID, electrical conductivity of milk was higher in all quarters of the udder compared to cows treated with SCCB + NSAID. Additionally, the RT of cows treated with AB + NSAID after 14 days was 7.01% lower compared to cows treated with SCCB + NSAID.

Cows with subclinical mastitis produce less milk, have a higher composite SCC and a higher probability of developing clinical mastitis (CM), and are culled earlier than their healthy herd mates [20]. Milk losses for treated cases of mastitis were estimated by Adriaens et al. [3] to be extremely varied among cases, with a median of 101 kg per case. This information can be used to improve udder health management. Bar et al. [2] found that parity and lactation stage affect mastitis' influence on milk losses, and their estimates were similar to this study's although with a lower data granularity. In addition, Shim et al. [21] established that the real milk losses rely on the therapies that are used, whereas Wilson et al. [22] discovered an effect of simultaneous health problems other than mastitis having an impact on the situation.

Although the antimicrobial mechanism of action of various non-antibiotic drugs is unknown, it is possible that they fulfill such tasks via altering cell permeability, bacterial efflux pumps, and ion transporters and via disrupting the operation of essential enzymes [23]. Because DNA polymerase may be an emerging antimicrobial site involved in DNA replication, NSAIDs work by interfering with bacterial DNA replication and repair by eavesdropping on DNA polymerase [24]. Histopathological examination is a valuable method for assessing tissue damage caused by bacterial invasion. However, drug candidates discovered using in vitro screening procedures may have distinct therapeutic effects when tested in vivo due to differences in drug membrane permeability, metabolism, and host immune system engagement [25]. NSAIDs decrease *Staphylococcus* virulence via decreasing agrA-regulated virulence, preventing hemolysis, suppressing staphyloxanthin synthesis, and downregulating the expression of fnbA and icaA genes, which are required for biofilm formation [26]. NSAIDs are medications that are regularly administered in conjunction with antibiotics to treat pain and fever caused by bacterial infections [27]. Meloxicam inhibit the expression of the icaA gene, which encodes a main constituent of the polysaccharides from the extracellular matrix, polysaccharide intercellular adhesion, which is a crucial component of the *Staphylococcus* EPS matrix [28]. Non-steroidal anti-inflammatory medications are increasingly being used in cattle to control the inflammation, pain, and endotoxin-induced symptoms such as fever that accompany opportunistic bacterial infections [10,29].

Garlic extract concentration was found to be highly significant in inhibiting the growth of mastitis-causing bacteria [30]. Supplementing the diet of dairy cows with garlic increased milk production from 12.9 to 20.1 kg and ensured optimal milk production activity, as demonstrated by a 41.09% reduction in SCC in milk (from 3.48×10^5 mL^{-1} to 2.05×10^5 mL^{-1}) [31]. Allicin is synthesized from the non-proteinogenic amino acid alliin (S-allylcysteine sulfoxide) during tissue injury via a process catalyzed by the enzyme alliinase [16]. This chemical molecule can also limit cell proliferation and promote tumor cell death (in mammalian cell lines). According to Li et al. [32], fresh garlic extract can

boost the sensitivity of antibiotic-resistant bacteria to particular drugs in vitro. Sheppard et al. [33] and Najafi et al. [34] produced similar results, demonstrating that allicin-inspired pyridyl disulfides are effective against multidrug-resistant *Staphylococcus aureus.* Gholipour et al. [35] discovered that adding garlic powder to the feed mixture of growing calves may be an effective substitute for commonly used ionophore antibiotics, such as monensin, and that it contributes to increased nutrient digestibility, growth performance, and improvement of blood markers indicative of their health status. Mushtaq et al. [36] offered examples of the administration of several herbs in a recent review addressing the plant therapy of bovine mastitis; however, it did not mention the use of garlic. Montironi et al. [37] found that the essential oil of *M. verticillata*, as well as the addition of limonene, ensured antibacterial effectiveness against *S. uberis* strains. Garlic in the diet has improved each of the SCC throughout supplementation without impacting milk output or technological quality [31]. However, the garlic aqueous extract showed the best antibacterial activity of the plant extracts, as evaluated by the diameter (mm) of the inhibitory zone, against most of the organisms (except for *E. coli* and *E. shigella*) [38].

In this study, we found an impact of SCCB + NSAID on the electrical conductivity of milk. After 14 days of treatment, the electrical conductivity of milk in cows treated with AB + NSAID was higher in all quarters of the udder compared to cows treated with SCCB + NSAID. The electrical conductivity of milk after 14 days of treatment in subclinical mastitis group also was higher in all quarters of the udder compared to clinical healthy group. During mastitis, the EC of the udder goes up because of changes in the ionic makeup of the milk, such as higher levels of Na^+ and Cl^- and lower levels of other minerals. During the milking process, modern businesses have begun implementing computerized herd management systems. These systems make it possible for factors such as milk yield, flow rate, and EC to be automatically recorded. Then, based on EC, the mastitis status of each individual cow was analyzed, and an alert was activated to signify mastitis. Although these alerts are important for CM and SCM detection, they may be misinterpreted if they are heard at the wrong moment [39]. Most of the time, the in-line sensors that are used to find mastitis in AMS are the ones that measure electrical conductivity. During milk collection, these sensors can continuously measure the amount of ions in the milk, but the results can be different [13]. With the goal of replacing the traditional CMT method, Ribeiro et al. [40] used EC to detect subclinical mastitis in raw milk samples. Previous research has demonstrated that the composition of milk after alveolar ejection differs from its composition prior to ejection, with decreased sensitivity for mastitis markers such as EC and SCC [41]. Therefore, by eliminating and not measuring strict foremilk, AMS may be overlooking arguably the most useful milk in terms of mastitis detection [42]. In AMS, in-line sensors that measure electrical conductivity (EC) are most frequently employed to diagnose mastitis. During the process of milk harvesting, these sensors are able to do continuous measurements of the concentration of ions in the milk, but with varying results [42].

We found that RT of cows on disease diagnosis day of cows treated with AB + NSAID was 11.41% lower compared to cows treated with SCCB + NSAID, while the RT of cows after 14 days treated with AB + NSAID was 7.01% lower compared to cows treated with SCCB + NSAID. Rumination time is a key measure for evaluating the health of dairy cows, and it has been demonstrated that RT decreases with the beginning of numerous health conditions, including mastitis [43]. Rumination is essential in the digestive physiology of ruminants. It can be defined as a process characterized by regurgitation, mastication, and re-swallowing of ingesta [44]. Liboreiro et al. [43] reported the days relative to calving, stillbirth, subclinical hypocalcemia, and retained fetal membranes as the most important factors associated with the daily RT during the prepartum period. Rumination monitoring can detect clinical and subclinical disease early, allowing producers to start remedial medicines sooner, reducing costs and production losses [44]. An increase in rumen contractions (as measured by ocular inspection) when meloxicam was administered intravenously 4 h after endotoxin infusion. NSAIDs, such as flunixin meglumine [45], flurbiprofen [45],

carprofen, and ketoprofen, had a favorable effect on ruminating after artificially generating mastitis [46]. Fitzpatrick et al. [47] found that meloxicam relieved udder pain and reduced udder edema and body temperature in the hours following infusion, but it had no effect on rumination time, or SCC.

5. Conclusions

On the basis of these findings, we may infer that we can evaluate the effectiveness of subclinical mastitis treatment with garlic extract (Allicin) with the evaluation of biomarkers registered with in-line sensors such as MY, EC, RT, and SCC in dairy cattle. For this evaluation we used EC, because after 14 days of treatment, the electrical conductivity of milk in cows treated with AB + NSAID was also higher in all quarters of the udder compared to cows treated with SCCB + NSAID. Rumination time of cows on disease diagnosis day of cows treated with AB + NSAID was 11.41% lower compared to cows treated with SCCB + NSAID, while the RT of cows treated with AB + NSAID after 14 days was 7.01% lower compared to cows treated with SCCB + NSAID.

On the practical side, for treatment of subclinical mastitis infected By *Streptococcus* spp. and *Strep. uberis*, we recommend using a feed supplement SCC bolus (one per os) with a composition of Meloxicam 20 mg with a single subcutaneous injection at a dosage of 2.5 mL/100 kg body weight.

Author Contributions: Design of the study: R.A., A.J., K.S. and A.K. Conduction of the samples and data: K.D. and D.B. Statistical analysis: L.A., R.A. and W.B. wrote the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Vetmarket, Nemuno Str. 4, Virbališkiai, LT-53458, Kaunas, Lithuania.

Institutional Review Board Statement: This study was conducted according to the guidelines of The Declaration of Helsinki and approved by the Ethics Committee (the study approval number is PK016965, 6 June 2017).

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. National Research Council. *Preparing for an Aging World: The Case for Cross-National Research*; National Academies Press: Washington, DC, USA, 2001; ISBN 978-0-309-07421-6.
2. Bar, D.; Gröhn, Y.T.; Bennett, G.; González, R.N.; Hertl, J.A.; Schulte, H.F.; Tauer, L.W.; Welcome, F.L.; Schukken, Y.H. Effects of Repeated Episodes of Generic Clinical Mastitis on Mortality and Culling in Dairy Cows. *J. Dairy Sci.* **2008**, *91*, 2196–2204. [CrossRef]
3. Adriaens, I.; Van Den Brulle, I.; Geerinckx, K.; D'Anvers, L.; De Vliegher, S.; Aernouts, B. Milk Losses Linked to Mastitis Treatments at Dairy Farms with Automatic Milking Systems. *Prev. Vet. Med.* **2021**, *194*, 105420. [CrossRef] [PubMed]
4. van den Borne, B.H.P.; Vernooij, J.C.M.; Lupindu, A.M.; van Schaik, G.; Frankena, K.; Lam, T.J.G.M.; Nielen, M. Relationship between Somatic Cell Count Status and Subsequent Clinical Mastitis in Dutch Dairy Cows. *Prev. Vet. Med.* **2011**, *102*, 265–273. [CrossRef] [PubMed]
5. Krömker, V.; Leimbach, S. Mastitis Treatment—Reduction in Antibiotic Usage in Dairy Cows. *Reprod. Domest. Anim.* **2017**, *52*, 21–29. [CrossRef] [PubMed]
6. Jamali, H.; Barkema, H.W.; Jacques, M.; Lavallée-Bourget, E.-M.; Malouin, F.; Saini, V.; Stryhn, H.; Dufour, S. Invited Review: Incidence, Risk Factors, and Effects of Clinical Mastitis Recurrence in Dairy Cows. *J. Dairy Sci.* **2018**, *101*, 4729–4746. [CrossRef] [PubMed]
7. Mollenhorst, H.; van der Tol, P.P.J.; Hogeveen, H. Somatic Cell Count Assessment at the Quarter or Cow Milking Level. *J. Dairy Sci.* **2010**, *93*, 3358–3364. [CrossRef] [PubMed]
8. Soriani, N.; Trevisi, E.; Calamari, L. Relationships between Rumination Time, Metabolic Conditions, and Health Status in Dairy Cows during the Transition Period. *J. Anim. Sci.* **2012**, *90*, 4544–4554. [CrossRef]
9. Royster, E.; Wagner, S. Treatment of Mastitis in Cattle. *Vet. Clin. Food Anim. Pract.* **2015**, *31*, 17–46. [CrossRef] [PubMed]
10. Ijaz, M. An Economical Non-Antibiotic Alternative to Antibiotic Therapy for Subclinical Mastitis in Cows. *Pak. Vet. J.* **2021**, *41*, 475–480. [CrossRef]

11. Yousaf, A.; Sarfaraz, I.; Zafar, M.; Abbas, R.; Hussain, A.; Manzoor, D. Effect of Treatment with Tri-Sodium Citrate Alone and in Combination with Levamisole HCl on Total Milk Bacterial Count in Dairy Buffalo Suffering from Sub-Clinical Mastitis. *Rev. Vet.* **2010**, *21*, 187–189.
12. Hashemzadeh-Cigari, F.; Khorvash, M.; Ghorbani, G.R.; Kadivar, M.; Riasi, A.; Zebeli, Q. Effects of Supplementation with a Phytobiotics-Rich Herbal Mixture on Performance, Udder Health, and Metabolic Status of Holstein Cows with Various Levels of Milk Somatic Cell Counts. *J. Dairy Sci.* **2014**, *97*, 7487–7497. [CrossRef]
13. Özkan Gülzari, Ş.; Vosough Ahmadi, B.; Stott, A.W. Impact of Subclinical Mastitis on Greenhouse Gas Emissions Intensity and Profitability of Dairy Cows in Norway. *Prev. Vet. Med.* **2018**, *150*, 19–29. [CrossRef] [PubMed]
14. Bertoni, G.; Trevisi, E.; Han, X.; Bionaz, M. Effects of Inflammatory Conditions on Liver Activity in Puerperium Period and Consequences for Performance in Dairy Cows. *J. Dairy Sci.* **2008**, *91*, 3300–3310. [CrossRef] [PubMed]
15. Blanch, M.; Carro, M.D.; Ranilla, M.J.; Viso, A.; Vázquez-Añón, M.; Bach, A. Influence of a Mixture of Cinnamaldehyde and Garlic Oil on Rumen Fermentation, Feeding Behavior and Performance of Lactating Dairy Cows. *Anim. Feed Sci. Technol.* **2016**, *219*, 313–323. [CrossRef]
16. Borlinghaus, J.; Albrecht, F.; Gruhlke, M.C.H.; Nwachukwu, I.D.; Slusarenko, A.J. Allicin: Chemistry and Biological Properties. *Molecules* **2014**, *19*, 12591–12618. [CrossRef] [PubMed]
17. Dingwell, R.T.; Leslie, K.E.; Schukken, Y.H.; Sargeant, J.M.; Timms, L.L. Evaluation of the California Mastitis Test to Detect an Intramammary Infection with a Major Pathogen in Early Lactation Dairy Cows. *Can. Vet. J.* **2003**, *44*, 413–416. [PubMed]
18. Oliver, S.P.; Gonzalez, R.N.; Hogan, J.S.; Jayarao, B.M.; Owens, W.E. *Microbiological Procedures for the Diagnosis of Bovine Udder Infection and Determination of Milk Quality*; National Mastitis Council: Verona, WI, USA, 2004; 47p.
19. Council: Laboratory and Field Handbook on Bovine Mastitis—Google Scholar. Available online: https://scholar.google.com/scholar_lookup?title=Laboratory+Handbook+on+Bovine+Mastitis&author=National+Mastitis+Council&publication_year=1999 (accessed on 18 January 2023).
20. Halasa, T.; Kirkeby, C. Differential Somatic Cell Count: Value for Udder Health Management. *Front. Vet. Sci.* **2020**, *7*, 609055. [CrossRef] [PubMed]
21. Shim, E.H.; Shanks, R.D.; Morin, D.E. Milk Loss and Treatment Costs Associated with Two Treatment Protocols for Clinical Mastitis in Dairy Cows. *J. Dairy Sci.* **2004**, *87*, 2702–2708. [CrossRef] [PubMed]
22. Wilson, D.J.; González, R.N.; Hertl, J.; Schulte, H.F.; Bennett, G.J.; Schukken, Y.H.; Gröhn, Y.T. Effect of Clinical Mastitis on the Lactation Curve: A Mixed Model Estimation Using Daily Milk Weights. *J. Dairy Sci.* **2004**, *87*, 2073–2084. [CrossRef]
23. Tyski, S. Non-Antibiotics—Drugs with Additional Antimicrobial Activity. *Acta Pol. Pharm.* **2003**, *60*, 401–404.
24. Lee, S.S.; Tranchina, D.; Ohta, Y.; Flajnik, M.F.; Hsu, E. Hypermutation in Shark Immunoglobulin Light Chain Genes Results in Contiguous Substitutions. *Immunity* **2002**, *16*, 571–582. [CrossRef] [PubMed]
25. Muzammil, I.; Ijaz, M.; Saleem, M.H.; Ali, M.M. Drug Repurposing Strategy: An Emerging Approach to Identify Potential Therapeutics for Treatment of Bovine Mastitis. *Microb. Pathog.* **2022**, *171*, 105691. [CrossRef] [PubMed]
26. Abbas, H.A.; Atallah, H.; El-Sayed, M.A.; El-Ganiny, A.M. Diclofenac Mitigates Virulence of Multidrug-Resistant *Staphylococcus aureus*. *Arch. Microbiol.* **2020**, *202*, 2751–2760. [CrossRef] [PubMed]
27. Davies, N.M.; Reynolds, J.K.; Undeberg, M.R.; Gates, B.J.; Ohgami, Y.; Vega-Villa, K.R. Minimizing Risks of NSAIDs: Cardiovascular, Gastrointestinal and Renal. *Expert Rev. Neurother.* **2006**, *6*, 1643–1655. [CrossRef]
28. Ahmed, E.F.; El-Baky, R.M.A.; Ahmed, A.B.F.; Fawzy, N.G.; Aziz, N.A.; Gad, G.F.M. Evaluation of Antibacterial Activity of Some Non-Steroidal Anti-Inflammatory Drugs against *Escherichia coli* Causing Urinary Tract Infection. *Afr. J. Microbiol. Res.* **2016**, *10*, 1408–1416. [CrossRef]
29. Yin, Z.; Wang, Y.; Whittell, L.R.; Jergic, S.; Liu, M.; Harry, E.; Dixon, N.E.; Kelso, M.J.; Beck, J.L.; Oakley, A.J. DNA Replication Is the Target for the Antibacterial Effects of Nonsteroidal Anti-Inflammatory Drugs. *Chem. Biol.* **2014**, *21*, 481–487. [CrossRef]
30. Syamsi, A.N.; Pratiwi, M.; Nugroho, A.P. Inhibition Activity of Garlic (*Allium sativum*) Skin Aqueous Extract on Mastitis Causing Microorganisms. *Anim. Prod.* **2020**, *21*, 38–42. [CrossRef]
31. Bochenek, A.; Kuczyńska, B. Garlic (*Allium sativum* L.) as an Antibiotic Alternative Determining the Hygienic Quality of Cow's Milk from Organic Farms. *Ann. Wars. Univ. Life Sci.-SGGW Anim. Sci.* **2019**, *58*, 105–113. [CrossRef]
32. Li, G.; Ma, X.; Deng, L.; Zhao, X.; Wei, Y.; Gao, Z.; Jia, J.; Xu, J.; Sun, C. Fresh Garlic Extract Enhances the Antimicrobial Activities of Antibiotics on Resistant Strains in Vitro. *Jundishapur J. Microbiol.* **2015**, *8*, e14814. [CrossRef] [PubMed]
33. Sheppard, J.G.; McAleer, J.P.; Saralkar, P.; Geldenhuys, W.J.; Long, T.E. Allicin-Inspired Pyridyl Disulfides as Antimicrobial Agents for Multidrug-Resistant *Staphylococcus aureus*. *Eur. J. Med. Chem.* **2018**, *143*, 1185–1195. [CrossRef]
34. Najafi, F.; Zangeneh, M.M.; Tahvilian, R.; Zangeneh, A.; Amiri, H.; Amiri, N.; Moradi, R. In Vitro Antibacterial Efficacy of Essential Oil of *Allium sativum* against *Staphylococcus aureus*. *Int. J. Pharmacogn. Phytochem. Res.* **2016**, *8*, 2039–2043.
35. Gholipour, A.; Foroozandeh Shahraki, A.D.; Tabeidian, S.A.; Nasrollahi, S.M.; Yang, W.Z. The Effects of Increasing Garlic Powder and Monensin Supplementation on Feed Intake, Nutrient Digestibility, Growth Performance and Blood Parameters of Growing Calves. *J. Anim. Physiol. Anim. Nutr.* **2016**, *100*, 623–628. [CrossRef] [PubMed]
36. Mushtaq, S.; Shah, A.M.; Shah, A.; Lone, S.A.; Hussain, A.; Hassan, Q.P.; Ali, M.N. Bovine Mastitis: An Appraisal of Its Alternative Herbal Cure. *Microb. Pathog.* **2018**, *114*, 357–361. [CrossRef]
37. Montironi, I.D.; Cariddi, L.N.; Reinoso, E.B. Evaluation of the Antimicrobial Efficacy of *Minthostachys verticillata* Essential Oil and Limonene against *Streptococcus uberis* Strains Isolated from Bovine Mastitis. *Rev. Argent. Microbiol.* **2016**, *48*, 210–216. [CrossRef]

38. Pașca, C.; Mărghitaș, L.; Dezmirean, D.; Bobiș, O.; Bonta, V.; Chirilă, F.; Matei, I.; Fiț, N. Medicinal Plants Based Products Tested on Pathogens Isolated from Mastitis Milk. *Molecules* **2017**, *22*, 1473. [CrossRef]
39. Lien, C.-C.; Wan, Y.-N.; Ting, C.-H. Online Detection of Dairy Cow Subclinical Mastitis Using Electrical Conductivity Indices of Milk. *Eng. Agric. Environ. Food* **2016**, *9*, 201–207. [CrossRef]
40. Ribeiro, E.S.; Lima, F.S.; Greco, L.F.; Bisinotto, R.S.; Monteiro, A.P.A.; Favoreto, M.; Ayres, H.; Marsola, R.S.; Martinez, N.; Thatcher, W.W.; et al. Prevalence of Periparturient Diseases and Effects on Fertility of Seasonally Calving Grazing Dairy Cows Supplemented with Concentrates. *J. Dairy Sci.* **2013**, *96*, 5682–5697. [CrossRef]
41. Kamphuis, C.; Mollenhorst, H.; Heesterbeek, J.a.P.; Hogeveen, H. *Data Mining to Detect Clinical Mastitis with Automatic Milking*; VetLearn: Wellington, New Zealand, 2010; pp. 568–572.
42. Halasa, T.; Huijps, K.; Østerås, O.; Hogeveen, H. Economic Effects of Bovine Mastitis and Mastitis Management: A Review. *Vet. Q.* **2007**, *29*, 18–31. [CrossRef]
43. Liboreiro, D.N.; Machado, K.S.; Silva, P.R.B.; Maturana, M.M.; Nishimura, T.K.; Brandão, A.P.; Endres, M.I.; Chebel, R.C. Characterization of Peripartum Rumination and Activity of Cows Diagnosed with Metabolic and Uterine Diseases. *J. Dairy Sci.* **2015**, *98*, 6812–6827. [CrossRef]
44. Paudyal, S. Rumination, Activity, Milk yield and Milk Components Analysis for Disease Detection during the Transition Period of Dairy Cows. Master's Thesis, West Texas A&M University, Canyon, TX, USA, 2016.
45. Lohuis, J.A.; Van Leeuwen, W.; Verheijden, J.H.; Brand, A.; Van Miert, A.S. Flunixin Meglumine and Flurbiprofen in Cows with Experimental *Escherichia coli* Mastitis. *Vet. Rec.* **1989**, *124*, 305–308. [CrossRef]
46. Banting: Efficacy of Meloxicam in Lactating Cows … —Google Scholar. Available online: https://scholar.google.com/scholar_lookup?title=Efficacy%20of%20meloxicam%20in%20lactating%20cows%20with%20E.%20coli%20endotoxin-induced%20acute%20mastitis&publication_year=2000&author=A.%20Banting&author=H.%20Schmidt&author=S.%20Banting (accessed on 5 February 2023).
47. Fitzpatrick, C.E.; Chapinal, N.; Petersson-Wolfe, C.S.; DeVries, T.J.; Kelton, D.F.; Duffield, T.F.; Leslie, K.E. The Effect of Meloxicam on Pain Sensitivity, Rumination Time, and Clinical Signs in Dairy Cows with Endotoxin-Induced Clinical Mastitis. *J. Dairy Sci.* **2013**, *96*, 2847–2856. [CrossRef] [PubMed]