Annals of Warsaw University of Life Sciences – SGGW Animal Science No 58 (2), 2019: 105–113 (Ann. Warsaw Univ. of Life Sci. – SGGW, Anim. Sci. 58 (2), 2019) DOI 10.22630/AAS.2019.58.2.11

Garlic (*Allium sativum* L.) as an antibiotic alternative determining the hygienic quality of cow's milk from organic farms

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Abstract: Garlic (Allium sativum L.) as an antibiotic alternative determining the hygienic quality of cow's milk from organic farms. The purpose of this study was to determine the efficacy of cow's diet supplementation with a garlic as an antibiotic alternative in the prevention and treatment of subclinical mastitis. The study was conducted on an organic farm located in the Natura 2000 area in the Narew river valley. The major criterions of choosing organic farm to the experiment were as follows: possession a herd of Black and White Polish Holstein-Friesian cows (numbering more than 30 cows) with diagnosed subclinical mastitis and holding a certificate for ecological production and compliance with organic farming standards. The study involved 7 cows diagnosed with subclinical mastitis, whose diet was supplemented with a garlic in a dose of 5 g/per capita/per day. The supplement was administered every day at the same time (in the evening), for 21 days individually into each animal's feeder. The hygienic status of milk samples, measured based on the somatic cells count (SCC) and colony forming units (CFU), was investigated as an indicator of the subclinical mastitis (initial level is a diagnosis). The dietary inclusion of garlic has improved each of the above-mentioned traits during supplementation, without compromising milk production and technological quality. After 21 days of supplementation, the SCC and CFU decreased by about 60%. The study needs to be repeated, taking into account the greater number of cows.

Key words: garlic, subclinical *mastitis*, somatic cell count, colony-forming unit, organic farming, cow

INTRODUCTION

Organic farming is an alternative to the traditional farming and aims to manufacture food and products of animal origin using natural and traditional methods, excluding the use of synthetic fertilizers and plant protection agents, antibiotics, hormones, growth stimulators, and genetically modified organisms. In such an environment, all cow health interventions should be undertaken with regard to the prevention and without using allopathic medicines and antibiotics. Phytobiotics and plant extracts, homeopathic preparations, and microelements are recommended as therapeutics in morbidity prevention (Amber et al. 2018, Kuczyńska et al. 2018).

In the case of life-threatening or painrelieving symptoms, allopathic medicines (under supervision of a veterinarian) are allowed, providing the required waiting time – doubled taking specific medicines or standing for at least 48 hours, unless otherwise specified. With the exception of vaccinations, elimination of parasites, and obligatory elimination of diseases, the animals treated with the allopathic medicines twice or more times a year, will have to be included into the conversion program for at least 6 months.

Mastitis is one of the main causes of decreased milk production among metabolic and reproductive disorders. Its etiological factors include about 150 species of microorganisms, the key ones including: Streptococcus agalactiae. Str. uberis, Str. dysgalactiae, Staphylococcus aureus, Escherichia coli, Mycobacterium bovis, and Aspergillus sp. At conventional farms, mastitis is treated with allopathic medicines in about two weeks. Subclinical mastitis usually afflicts 20-70% of the herd, showing no clinical nor milk composition changes (except for the somatic cell count). At certified organic farms, herds affected by the subclinical mastitis may be subject to the alternative treatment based on the usage of phytobiotics. Phytobiotics are plant-derived preparations obtained from herbs that contain biologicallyactive secondary metabolites. The form of herb application depends on the species of animal, the production group, the management system, and, potentially, on the type and form of the food. Most studies on the use of plant-derived materials in mastitis have been conducted in developing countries and examined traditional medical practices as part of a very extensive and low input production. According to Cheng et al. (2014), alternatives to antibiotics should exhibit specific properties regarding animals (non-toxicity or no side effects, easiness of elimination from the body, stability in feed and in the gastrointestinal tract) and microorganisms (the ability to kill or inhibit the growth of pathogenic bacteria, not causing resistance to bacteria or destroying intestinal microflora), and additionally should not affect the natural environment (should be easily degradable). Phytobiotics, including garlic, are valuable therapeutic products greatly appreciated by the organic agriculture as an alternative to prohibited antibiotics due to their potential medical and therapeutic applications.

Sustainable agriculture is one of the forms of organic production having the least negative impact on the natural environment. For instance products containing capsules with garlic oil (combined with cinnamaldehyde) have been shown to modulate rumen fermentation (in vitro), and thus inhibit methane production (Blanch et al. 2016). In turn, Gülzari et al. (2018) study results showed that greenhouse gases emission fell by 3.7% by reducing SCC level from 8.0 to 0.50×10^5 ml⁻¹. Hashemzadeh-Cigari et al. (2014) have showed improved feed intake, performance, udder health, ruminal fermentation, and plasma metabolites in cows with moderate or high SCC in the milk due to their diet supplementation with a phytobiotics-rich herbal mixture. In turn, an experiment performed by Pasca et al. (2017) to test the inhibitory effect of phytobiotics obtained from several plant species has shown a stronger antimicrobial activity of the coupled use of the phytobiotics compared to the individual application of each plant-based products. In the literature available, not many researchers deal with the influence of garlic on mastitis prevention, especially at organic farms. Caribu et al. (2016) demonstrated that the supplementation of dairy cows diet with a garlic combined with organic minerals (Se, Cr, Zn) increased milk production from 12.9 to 20.1 kg and was ensured optimal milk production activity, as demonstrated SCC reduction in milk by

41.09% (from 3.48×10^5 ml⁻¹ to $2.05 \times$ \times 10⁵ ml⁻¹). A possible threat of garlic passing into milk and dairy products was the subject of research conducted by Rossi et al. (2018) study. The garliclike aroma, taste, and flavor of milk and cheese were significantly influenced by experimental treatments, in particular by the highest dose of garlic cloves. In addition, these authors found close exponential relationships between milk and cheese for garlic-like aroma ($R^2 = 0.87$) and garlic-like flavor ($R^2 = 0.79$) as a result of adding 400 g/day of garlic to the feed of lactating dairy cows. Garlic has various properties, including anti-fungal, antimicrobial, antiviral, anti-inflammatory, hepatoprotective, anti-carcinogenic, and immuno-stimulating in cow's nutrition. The above-mentioned properties of garlic are ascribed to its allicin. Allicin (diallylthiosulfinate) is a defence 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 (Borlinghaus et al. 2014). This chemical compound can also inhibit cell proliferation and induce apoptosis of tumor cells (in mammalian cell lines). Li et al. (2015) showed that a fresh garlic extract may increase the sensitivity of antibiotic-resistant strains to certain antibiotics in vitro. Similar results were obtained by Sheppard et al. (2018) and Najafi et al. (2016) where allicin-inspired pyridyl disulfides were demonstrated to be efficient agents against multidrugresistant Staphylococcus aureus (MRSA). The findings reported by Gholipour et al. (2018) have demonstrated that the addition of garlic powder the feed mixture of growing calves may be an effective substitute for commonly sued ionophore antibiotics, like e.g. monensin, and that it contributes to the increased nutrient digestibility, growth performance, and improvement of blood markers being indicative of their health status. A recent review by Mushtaq et al. (2018) addressing the plant treatment of bovine *mastitis*, has provided examples of the administration of numerous herbs, however it did not mention the use of garlic.

The aim of the current research was to determine the effects of a specially designed garlic on the hygienic quality of organic cows in the summer feeding season.

It was assumed that the dietary inclusion of garlic would improve the hygienic quality of milk of cows from organic production with diagnosed subclinical mastitis.

MATERIALS AND METHODS

Animals

The experiment was conducted at an organic farm located in the Natura 2000 area in the Narew river valley. The major criterions of choosing organic farm to the experiment were as follows: possession a herd of Black and White Polish Holstein-Friesian cows (numbering more than 30 cows) with diagnosed subclinical mastitis and holding a certificate for ecological production and compliance with organic farming standards. The study involved 7 cows diagnosed with subclinical *mastitis*.

Cows were housed in tie-stalls, bedded with softwood shavings on rubber mats

and provided free access to water. All cows grazed on the same pasture (*ad libitum*) and were fed the same concentrate (1 kg). Differences in treatment were achieved by weighing 5 g of the garlic before feeding and its administration with the feed mixture to individual cows. The chemical composition and nutritional value of feed mixtures presented Table 1.

Sciences (WULS), they were analyzed for the somatic cell count and colony forming units. Cytological quality was determined using Somacount 150. In turn, WASP equipment (Whitley Automated Spiral Plate) was used to distribute microbiological cultures on Petri dishes. The level of CFU was established using Counter mat Flash equipped with a video camera and a special application.

Treatment	Crude Ash	Crude protein	Fat ether extract	Crude fiber	NDF	ADF	UFL	PDIN	PDIE
Pasture ad libitum	8.62	17.41	3.99	26.1	48.72	30.16	0.85	102.52	83.24
Feed mixture + concentrate (1 kg) + + garlic (5 g)	2.26	13.87	2.17	6.28	38.12	24.55	1.06	74.95	97.85

TABLE 1. Chemical composition (% of DM) and nutritional value of feed mixtures (g/kg)

DM – dry matter; NDF – Neutral-detergent fiber; ADF – Acid-detergent fiber; UFL – Feed Unit for milk production, PDIN – protein digested in the small intestine when rumen-fermentable nitrogen is limiting; PDIE – protein digested in the small intestine when rumen-fermentable energy is limiting.

The cows were fed at 6 PM, and the garlic was administered in the same proportions to the individual feed ratios at 5 g/per capita/per day.

Measurements and analytical methods

The samples (50 ml) were collected during evening milking, placed in sterile plastic bottles, and transported at a temperature of 4°C. The hygienic status of milk samples collected during milking in the summer feeding season was determined based on the somatic cell count and colony forming units as indicators of the individuals with the subclinical *mastitis* phase. Once delivered to the Cattle Breeding Division Milk Testing Laboratory of Warsaw University of Life The following culture media were used for the microbiological analysis of milk samples: bacteriological agar 2%; Uri-color chromogenic medium for the selection of indole (+) *E. coli*; KESC group bacteria, *Enterococcus*, *Proteus vulgaris* and *Staph. Aureus*; TBX (Tryptone Bile X-glucuronide) medium for selective proliferation of *E. coli* beta glucuronidase (+) (Bio Corp Company).

Feeding experiment was dived into two phases: initial (lasting 7 days, with an incrementally introduced dose of garlic) and final (lasting 21 days, during which each cow received supplements mixed with the concentrate (500 g). Milk samples were collected four times during the supplementation period – on day 7, 14, and 21 of supplementation, and a control sample was collected as well (start of experience).

The obtained data were analyzed statistically using analysis of variance and Tukey post-hoc test using SPSS 23.0 software.

RESULTS AND DISSCUSION

The reported study has shown a positive effect of the dietary inclusion of the garlic on both the cytological and microbiological quality of milk from cows with subclinical *mastitis*. A significant decrease in the number of somatic cells was observed in all samples during the supplementation period. However, SCC levels varied among individual samples collected in the following days after the experiment had been completed (Table 2). The number of colony forming units showed a downtrend from the demonstrated level of over 100 thous./ml to the value of several tens of thousands (Table 3). In addition, a strong correlation ($P \le 0.01$) was observed between SCC and CFU with a correlation coefficient accounting for $R^2 = 0.763$.

The score of somatic cells reflects the status of health of the milk udder and their elevated level (over 100-200 thous./ml) indicates inflammation of this gland caused by infections. The increase in the score of somatic cells is an immune response to the presence of microbes and their metabolites. In milk from healthy udder, 75% of somatic cells are epithelial cells. In contrast, from pieces with a diseased mammary gland, their share decreases from 20 to 30% in advantage of leukocytes. In turn, the content of bacteria in milk depends on the degree of mastitis in infectious germs as well as the hygiene of milking and the conditions under which milk is stored. Pathogenic agents that cause mastitis

Times of experiment	SCC	SE	
start of experience – control samples	477.57 ^{ab}	47.94	
7th day of supplementation	418.14	84.22	
14th day of supplementation	237.43ª	92.98	
21st day of supplementation	218.00 ^b	70.61	

Means in the same rows marked with the same letters differ significantly at: a, a $P \le 0.05$; SCC – mean of somatic cell count (thous./ml); SE – standard error; N = 7 of each group.

TABLE 3. The influence of garlic supplementation on the microbiological quality of milk

Times of experiment	CFU	SE	
start of experience – control samples	174.29 ^{AB}	15.28	
7th day of supplementation	132.94 ^{cd}	45.49	
14th day of supplementation	51.57 ^{Ac}	20.43	
21st day of supplementation	41.17 ^{Bd}	10.65	

Means in the same rows marked with the same letters differ significantly at: A, A P \leq 0.01; c, c P \leq 0.05 *CFU* – mean of colony forming units (thous./ml); *SE* – standard error; *N* = 7 of each group.

can be divided into two groups: major and minor pathogenes. The first group (major pathogenes) includes Streptococcus agalactie, Streptococcus dysgalactie, Streptococcus uberis, Staphylococcus aureus. Actinomyces pyogenes. Escherichia coli and Mycoplasma bovis. The other (minor pathogenes) include coagulase negative staphylococci (CNS) and Corvnobacterium bovis. Infections caused by the first group of pathogenic bacteria, including i.a. Staphylococcus aureus, Streptococcus agalactiae, Streptococcus uberis, and Streptococcus dysgalactiae, increase the SCC. Another type of udder inflammation induced by the second group of pathogenic bacteria, including i.a. Corynebacterium bovis and coagulase-negative staphylococci, results in a slight increase of SCC. In the present study, 10 different microorganisms were isolated from milk samples and identified. The most abundant pathogens causing subclinical mastitis were the catalase-negative bacteria: Streptococcus, Enterococcus, and Clostridium, that were responsible for 75-95% of the infections. Staphylococcus aureus was isolated from 5.2% to 25.0% of the samples, however it was not found as a single species but in a mixed flora. The inflammations caused by E. coli and Klebsiella were more likely due to the summer season characterized by high outdoor temperatures reaching 40°C.

A study conducted by Mussarat et al. (2014) on a traditional medicinal plant used in the treatment of mastitis in the Indus River region has shown the efficacy of 150 g of garlic bulbs ground and mixed with butter in 7-day recovery or an alternative dose of 1–2 glasses of garlic, resulting in a 10-day recovery,

both administrated orally. A similar experiment was carried out by Amber et al. (2018) in the northwest Pakistan and has demonstrated that a dose of 100 g of a garlic mixture caused a 7-day recovery from mastitis in cows, buffalos, sheep, and goats. In turn, Gull et al. (2012) determined the inhibitory effect of Allium sativum and Zingiber officinale extracts on clinically-relevant drugresistant pathogenic bacteria, including S. typhi, Shigella, P. aeruginosa, E. coli, B. subtillus, S. aureus, S. epidermidis, and K. pneumoniae. These authors used 6 types of extracts, each prepared from 10 g of garlic powder or ginger, dissolved in 100 ml of distilled water, ethanol or methanol, separately. The highest antibacterial activity of the plant extracts, measured as a diameter (mm) of the inhibition zone, against most of the strains (except for E. coli and E. shigella), was observed for the garlic aqueous extract.

Grzesiak et al. (2018) conducted a study to determine the effect of essential oils obtained from various herbs, including *Thymus vulgaris* L., *Origanum vulgare* L., *Origanum majerana* L., *Mentha* \times *piperita* L., and *Allium ursinum* L. against *Prototheca zopfii* strains responsible for udder inflammation. Results of the determination of the minimum inhibitory concentration (MIC) have shown the *P. zopfii* strains to be resistant to wild garlic essential oil.

The garlic extract not only prevents the *mastitis*, but also improves the quality of milk. Pinilla et al. (2017) have observed that use of garlic extract in a concentration of 5% (both free and encapsulated) reduced the viable cell count of four strains of *Listeria spp*. by 4 log cycles (within 10 h). Montironi et al. (2016) have demonstrated that both M. verticillata essential oil and the addition of limonene ensured the antimicrobial efficacy against S. uberis strains. The minimum inhibitory concentration (MIC) values determined for the essential oil were in the range from 14.3 to 114.5 mg/ml and these assayed for the limonene ranged from 3.3 to 52.5 mg/ml. The minimum bactericidal concentration (MBC) was between 114.5 and 229.0 mg/ml for *M. verticillata*, and from 3.3 to 52.5 mg/ml for limonene. Additionally Kuczyńska et al. (2015) found the highest concentration of β -carotene (0.450 mg/l) in milk of cows with the highest level of SCC, and believe milk SCC varies significantly with the concentration of β -carotene in cow's milk.

CONCLUSIONS

Based on study results presented above, it can be concluded that supplementing diets of cows maintained at certificated farms with phytobiotics, including e.g. a garlic extract (Allium sativum L.), at only up to 5 g/cow/day from day 1 to day 70 since diagnosis of the subclinical mastitis may improve cows' health and hygienic quality of their milk. Study results have shown a 60% decrease in both SCC and CFU. Moreover, a tendency for the improving hygienic status of milk can be observed after discontinuation of the supplementation. The garlic extract is a completely safe phytobiological supplement and its hypothetical, accidental overdose may in the worst case cause a temporary reduction in feed intake. However, the study needs to be repeated, taking into account the greater number of cows.

Acknowledgements

Research was realized within the project "Research on novel methods of reducing the occurrence of diseases and parasites of farm animals in the conditions of organic production" according to the agreement no. HOR.re.027.6.2017 financed by the Polish Agriculture Ministry.

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Streszczenie: Czosnek (Allium sativum L.) jako atlernatywa dla antybiotykoterapii ksztaltująca jakość higieniczną mleka krowiego z gospodarstw ekologicznych. Celem pracy było sprawdzenie skuteczności działania profilaktycznego i leczniczego czosnku w przypadkach stanu subklinicznego zapalenia gruczołu mlekowego krów w chowie ekologicznym. Badania przeprowadzono w gospodarstwie ekologicznym zlokalizowanym na terenie obszaru Natura 2000 w dolinie rzeki Narew. Głównymi kryteriami wyboru gospodarstwa ekologicznego do eksperymentu były: posiadanie stada krów rasy polskiej holsztyńsko--fryzyjskiej odmiany czarno-białej (liczącej ponad 30 krów) ze zdiagnozowanym subklinicznym zapaleniem gruczołu mlekowego i posiadanie certyfikatu produkcji ekologicznej i zgodności z normami rolnictwa ekologicznego. Badaniami objeto 7 krów rasy polskiej holsztyńsko-fryzyjskiej (o wadze 600 kg) z wcześniej zdiagnozowanym stanem subklinicznym zapalenia gruczołu mlekowego. Eksperyment przeprowadzono w sezonie żywienia letniego, w trakcie wypasu pastwiskowego przez okres 21 dni. Czosnek zadawano w ilości 5 g/sztukę/dobę codziennie o tej samej porze przed dojem wieczornym. W próbkach mleka oceniano stan higieniczny mierzony jako liczbę komórek somatycznych (LKS) i ogólna liczbe bakterii (OLB) jako wskaźniki subklinicznej fazy mastitis (poziom wyjściowy tj. diagnoza). Zadawany w tym czasie czosnek w ilości 5 g/sztukę/dobę do paszy treściwej na pół godziny przed dojem przyczynił się do poprawy parametrów jakości cytologicznej i mikrobiologicznej mleka, bez uszczerbku dla produkcji mleka i jakości technologicznej. Poziom LKS i OLB uległ obniżeniu o 60% w 21. dniu suplementacji w porównaniu do poziomu wyjściowego. Badanie wymaga powtórzenia, uwzględniając większą liczebność krów.

Słowa kluczowe: czosnek, subkliniczne zapalenie, liczba komórek somatycznych, jednostki tworzące kolonie, rolnictwo ekologiczne, krowa

MS received 27.02.2019 MS accepted 19.05.2019

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