

## Milk quality in Holstein dairy herds with and without antimastitis intradermal vaccination in the Bohemian-Moravian Highlands (Czech Republic)

### Kvalita mléka ve stádech dojnic holštýnského skotu s intradermální vakcinací proti mastitidě a bez vakcinace v podmínkách Českomoravské vrchoviny

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Received: December 17, 2025; accepted: April 7, 2026

#### ABSTRACT

Vaccination is considered one of the potential tools to reduce antibiotic use in dairy herds by preventing mastitis. This study compared milk yield (2024) and milk quality (June 2022–2024) in two groups of Holstein herds in the Bohemian-Moravian Highlands (the Czech Republic): eight vaccinated herds and eight unvaccinated herds. The groups were comparable in days in milk, calving interval, and altitude. A periodic intradermal autogenous vaccine targeted gram-negative pathogens. Milk yield was slightly higher in unvaccinated herds (vaccinated vs. unvaccinated: 1<sup>st</sup> lactation 10,372 ± 935 vs. 10,503 ± 2,139 kg; later lactations 11,510 ± 1,270 vs. 11,780 ± 2,491 kg). The somatic cell count (187 ± 71 vs. 178 ± 47 10<sup>3</sup>/ml;  $P < 0.001$ ) and the total count of mesophilic microorganisms (TCM; 23.1 ± 68.4 vs. 15.0 ± 41.5 10<sup>3</sup> CFU/ml;  $P < 0.01$ ) were slightly higher in vaccinated herds. The count of coliform bacteria (COLI) was 9.7 ± 24.1 vs. 17.3 ± 34.7 CFU/ml ( $P > 0.05$ ), which may indicate the vaccine's focus on gram-negative pathogens. To assess longer-term trends, bulk tank milk samples from the pre-vaccination period (2021–May 2022) were also included. Comparison of pre- and post-vaccination periods showed observable improvements over time in vaccinated herds, more notably increased fat (2.9%), lactose (0.61%), and solids-not-fat (0.56%), and reduced free fatty acids (-3.23%), TCM (-9.09%, based on geometric means), and COLI (-60%, based on geometric means). Overall, vaccination was associated with slight improvements in several milk quality indicators, which tended to move closer to those observed in unvaccinated herds.

**Keywords:** somatic cell count, bulk tank milk, autogenous vaccine, gram-negative mastitis pathogens, antibiotic reduction

#### ABSTRAKT

Vakcinace je považována za jeden z možných nástrojů ke snížení používání antibiotik v chovech dojnic prostřednictvím prevence mastitid. Studie porovnávala mléčnou užitkovost (2024) a kvalitu mléka (červen 2022–2024) u dvou skupin stád dojnic holštýnského skotu na Českomoravské vrchovině: osmi vakcinovaných a osmi nevakcinovaných stád. Obě skupiny byly srovnatelné z hlediska dnů laktace, mezidobí i nadmořské výšky. Byla použita periodická intradermální autogenní vakcína cílená na gramnegativní patogeny. Mléčná užitkovost byla mírně vyšší u nevakcinovaných

stád, a to jak v první laktaci (vakcinovaná vs. nevakcinovaná:  $10\,372 \pm 935$  vs.  $10\,503 \pm 2\,139$  kg), tak u vyšších laktací ( $11\,510 \pm 1\,270$  vs.  $11\,780 \pm 2\,491$  kg). U vakcinovaných stád byl zaznamenán mírně vyšší počet somatických buněk ( $187 \pm 71$  vs.  $178 \pm 47 \cdot 10^3/\text{ml}$ ;  $P < 0,001$ ) i celkový počet mikroorganismů (CPM;  $23,1 \pm 68,4$  vs.  $15,0 \pm 41,5 \cdot 10^3$  CFU/ml;  $P < 0,01$ ). Počet koliformních bakterií (KOLI;  $9,7 \pm 24,1$  vs.  $17,3 \pm 34,7$  CFU/ml;  $P > 0,05$ ) by mohl souviset se zaměřením vakcíny na gramnegativní patogeny. Pro posouzení dlouhodobějších trendů byly do hodnocení zahrnuty také vzorky bazénového mléka z období před zahájením vakcinace (2021–květen 2022). Porovnání období před a po vakcinaci ukázalo u vakcinovaných stád pozorovatelná zlepšení v čase, zejména zvýšení obsahu tuku (2,9 %) a laktózy (0,61 %) a tukuprosté sušiny (0,56 %) a současně snížení obsahu volných mastných kyselin (−3,23 %), CPM (−9,09 %, vypočteno z geometrických průměrů) a KOLI (−60 %, vypočteno z geometrických průměrů). Celkově byla vakcinace spojena s mírným zlepšením vybraných ukazatelů kvality mléka, které se v průběhu sledování přiblížily hodnotám u nevakcinovaných stád.

**Klíčová slova:** počet somatických buněk, bazénové mléko, autogenní vakcína, gramnegativní původci mastitid, redukce antibiotik

## INTRODUCTION

Dairy farming is generally characterized by a continuous increase in milk yield (in the Czech Republic, from 9,099 kg per lactation in 2019 to 9,741 kg in 2023; Syřůček et al., 2024), driven by genetic improvement, improved nutrition, and enhanced animal welfare.

Production disorders of the mammary gland (disorders of milk secretion, subclinical and clinical mastitis) are a constant problem in dairy farming (Pyörälä, 2003; Sumon et al., 2020; Bucek et al., 2021). Hisira et al. (2023) mentioned: “Despite the considerable efforts made by many scientists and experts in mastitis, the disease has not been eliminated on dairy farms and remains a serious problem, causing significant losses to farmers.” The plasticity of the problem contributes to its long-term persistence, which is shaped by the dynamic changes in its nature. In the past, contagious mastitis (mainly caused by *Streptococcus agalactiae* and *Staphylococcus aureus*) represented the predominant challenge. Later, environmental mastitis (e.g., due to *Streptococcus uberis*) became more prevalent. Currently, mastitis of non-bacterial origin is gaining increasing importance, particularly in association with the unicellular alga *Prototheca bovis* (Malinowski et al., 2002; Bzdil, 2013; Ely et al., 2019; Shave et al., 2021; Seydlová et al., 2022; Beinhauerová et al., 2024). In addition, nonspecific lactation disorders and related conditions remain relevant despite continuous prophylactic measures by farmers and therapeutic interventions by veterinarians.

An important factor influencing the occurrence of mastitis is the physiological and metabolic burden associated with the onset of lactation, particularly in high-producing cows. The transition to early lactation is accompanied by negative energy balance, oxidative and inflammatory stress, and reduced immune competence (Zhang et al., 2024). These processes increase the cow's susceptibility to intramammary infections, which explains why high-yielding dairy cows are more prone to mastitis during this period (Atasever and Erdem, 2009).

Mastitis has been and continues to be a significant source of economic losses in dairy farming (Stanek et al., 2024), primarily due to reduced milk production and quality, and it is also the main reason for the use of antibiotics (ATB) for both treatment and prevention. Kvapilík et al. (2014, 2015) estimated the economic impacts of mastitis on milk production. The total loss per case of mastitis was approximately CZK 9,000 (EUR 360). This still could be valid: the largest loss from reduced milk yield (37%); the second from culling of dairy cows (20%), etc. However, the total loss should be adjusted upward by about 30% due to significant inflationary effects (i.e., to approximately CZK 11,700; EUR 468). Arikan et al. (2024) mentioned that the total economic loss in subclinical mastitis, as the main cause of losses in the herd, is not only due to the loss of milk production, but also to the cost of treatment and the loss of milk from the cow in the case of ATB treatment. They estimated this volume at 41.15, 80.5 and 114.5 L of milk per treatment in

cases of mild, moderate and severe disease, respectively. Furthermore, Atasever and Erdem (2009) recorded in Holstein cows in Turkey with an average somatic cell count (SCC) for 10 years of 705  $10^3$ /ml relative losses in daily milk yield, lactation milk yield and milk yield for 305 days of lactation of 14.6 to 17.4, 11.6 and 12.8 to 15.4%, respectively.

A similar problem is the continuously increasing resistance of pathogens to ATB (Pitkälä et al., 2004; Fergestad et al., 2021; Naranjo-Lucena and Slowey, 2023). The overuse of ATB in both human medicine and agriculture accelerates the rapid emergence of antimicrobial resistance (AMR) and multidrug-resistant bacterial “superbugs” worldwide. AMR has been recognized by the World Health Organization (WHO) as one of the top ten global threats to public health (O’Neill, 2016; Roope et al., 2019; Abdelaziz et al., 2024; Maeda and Furusawa, 2024). The risks associated with AMR affect both domestic animals and human populations, where even the treatment of minor diseases can become problematic (Boireau et al., 2018; Murray et al., 2022; Laxminarayan, 2022; The Lancet, 2022; Alenezi et al., 2024). A significant source of ATB use – and consequently of ATB residues entering the environment – is the treatment of mastitis in dairy cows. Therefore, there is increasing pressure to ensure the responsible and effective use of ATB in animals, not only in pigs and poultry but also in companion animals such as dogs (Singleton et al., 2020; Nunan, 2022).

The potential to reduce ATB consumption in dairy farming is supported, among other factors, by ecological (organic) farming compared to conventional practices. The relevant studies indicated that further reduction of ATB consumption in dairy farming is still realistic (Wilhelm et al., 2009; Krogh et al., 2020). Modern methods such as vaccination (Freick et al., 2016; Sedky et al., 2020; Rainard et al., 2021) or newly tested procedures, such as acoustic pulse technology in the therapy of dairy cow mastitis (Leitner et al., 2021), are offered to reduce ATB consumption in the mastitis treatment.

Another option to reduce ATB consumption may be to switch from routine preventive treatment of mastitis with ATB during the dry period of dairy cows (all animals). Here, a more efficient way of ATB use (reducing their overuse) is to select cows for ATB treatment during the dry period. This switch was described in Finland by Niemi et al. (2022) in the period 2015–2017, where they also warned against introducing this switch too radically due to the risk of losses in milk yield and high SCC in the following lactation. Since up to 31% of dairy farms still use blanket preventive administration of ATB during the dry period, there is room for rationalization of ATB application. This is in line with the concept of socially acceptable animal husbandry (Bennewitz et al., 2021). Therefore, the general goal is to reduce ATB consumption in dairy farming.

The possibility of vaccination of dairy cows as a prevention of mastitis has already been mentioned. In the CR, antimastitis intradermal vaccination of dairy cows (AIVDC) was carried out in several dairy herds before 2021 (Mašek et al., 2022). Hypothetically, this could be considered a means to improve mammary gland health in dairy cows, enhance milk yield and quality, and reduce ATB use for both economic and environmental reasons. In general, in this variant, this vaccination method (vaccine) is effective primarily in solving problems with gram-negative pathogens (i.e., environmental pathogens - mastitis).

This work aimed to evaluate the possible impact of long-term application of AIVDC (2021–2024) in Holstein cow herds in the CR (the Bohemian-Moravian Highlands) on the quality of their milk compared to reference herds with the absence of AIVDC. The results of the impact of the above type of mastitis vaccination on milk production and quality are not well known. Similar results have not been published in the available materials (CR) so far.

## MATERIAL AND METHODS

### *Study herds, data sources, and sampling procedure*

Data from the dairy herds (8 without AIVDC and 8 with AIVDC) were extracted from the official milk quality control database (Czech Moravian Breeders' Corporation, a.s. (ČMSCH, a.s.)). For the vaccination period (June 2022–2024; 31 months), a total of 2,676 bulk tank milk samples were collected, of which 1,388 were from herds without AIVDC and 1,288 with AIVDC. For the period before vaccination (2021–May 2022; 17 months), 1,302 samples were included, 756 without AIVDC and 546 from later vaccinated herds. Milk yield (kg per lactation) in 2024 was obtained from the milk recording system (ČMSCH, a.s.). In the experimental comparison, approximately 200–440 dairy cows per herd were included in each of the 8 herds without AIVDC, and 150–260 dairy cows per herd in each of the 8 herds with AIVDC during the respective time periods. All samples were collected proportionally using automatic sampling devices once or twice a month, immediately cooled ( $\leq 6$  °C) and transported to a laboratory.

### *Environmental and nutritional conditions*

Feed production and nutrition of dairy cows may be related to altitude, especially when it comes to the quantity and quality of production of energy-rich roughage (maize and maize silage). Feed utilization may also be related to the cow breed. For this reason, all 16 dairy herds were of the Holstein breed and were located at altitudes of  $\geq 400$  m. The cows were kept in free-range barns with straw-filled box bedding and a controlled microclimate. The animals were fed by TMR (total mixed ration), mainly twice a day with roughage moving closer to the animals regularly on the feeding table. The TMR consisted of corn silage, grass silage (feed base from fermented roughage), hay, feed concentrates, and mineral and vitamin supplements. The TMR was adjusted to the needs of the dairy cows according to their milk yield and lactation group in terms of nutrient intake. The cows were milked in milking parlors (fishbone or side-by-side).

### *Intradermal vaccination protocol*

A preventive method maintaining the health of the mammary gland of cows, AIVDC (Mašek et al., 2022) focused on gram-negative mastitis pathogens, consists of a vaccine applied to dairy cows four times a year, in intradermal form. The high-pressure Agro-Jet device (Medical International Technologies Inc., MIT, Canada) powered by compressed CO<sub>2</sub>, was used for vaccine administration. A veterinary technician can vaccinate approx. 300 dairy cows per hour with this device, depending on farm conditions. It applies the vaccine approx. 2–5 mm deep into the subcutaneous tissue. The intradermally applied vaccine (Sevax manufactured by Sevaron Ltd.) acts through Langerhans cells in the skin, which transmit information to the animal's immune system. It is a gentle vaccination method, considerate of animal welfare, with a low risk of abscess formation. Sevax is an autogenous preparation, an antigen and its required concentration is obtained by taking samples on the farm and their subsequent laboratory microbiological processing. The dose of vaccine for the animal was 0.5 ml. Revitalization of vaccines (a microbiological upgrade) during revaccination of cows, according to the results of microbiological examination of milk samples, took place twice a year. Vaccines were therefore prepared individually at half-year intervals (June 2022–2024) based on previous bacteriological examinations of dairy herds. The vaccines were herd-specific in their antigenic spectrum, with a primary focus on environmental mastitis. The following agents were recorded in the spectrum of antigen origin of specific vaccines: *Escherichia coli*; *Klebsiella pneumoniae* (permanent contact with the herd is key to catching the correct serotype, most often 2, i.e. 90% of the possible 11 for effectiveness); *Serratia* species. The concentration of antigens is 20 to 25 units. Antigens may change in composition and characteristics relevant to the periodic detection of the dominance of gram-negative mastitis pathogens in the dairy herd during revaccination after 6 months. Table 1 presents a methodological decision-making scheme for approaching AIVDC in a herd based on previously observed and investigated manifestations of problems with the health of the mammary glands of dairy cows.

**Table 1.** Methodological and decision-making framework for implementing AIVDC in a dairy herd

| Acute mastitis                                    | Chronic mastitis   |   | Overall deterioration of health |                     |
|---|--|---|---------------------------------|---------------------|
|   | Local therapy  | Cultivation   |                                 | Infusion therapy    |
|   | G-   | G+  | Sterile                         |                     |
| Yeasts  |  |   |                                 |                     |
| <i>Candida albicans</i> ,<br><i>Candida kefyr</i> | Vaccination according to herd status, somatic cells, cultivation.  | Antibiotics, both general and local, infusion therapy.  |                                 | Supportive therapy. |
| NSAID, infusions, local therapy.                  | Infusion and supportive therapy<br>Antibiotics only at temperatures above 40 °C<br><i>Klebsiella pneumoniae</i> ,<br><i>Escherichia coli</i> ,<br><i>Serratia marcescens</i> . | <i>Streptococcus uberis</i> – antibiotics always, even without fever.<br><i>Streptococcus dysgalactiae</i> – intramammary preparations<br><i>Staphylococcus xylosus</i> – intramammary and submucosal therapy.<br><i>Staphylococcus aureus</i> – often does not respond to therapy. |                                 |                     |

AIVDC = antimastitis intradermal vaccination of dairy cows; G- and G+ = gram-negative and gram-positive bacteria.

Farmers' motivation for accessing herd AIVDC was twofold: - to improve the health status, milk yield and milk quality when the herd's health state was deteriorated due to mastitis; - to save on ATB costs when the herd's health state was average and better, while covering the costs of AIVDC from these savings.

#### **Analyses of bulk tank milk samples (milk quality control)**

Milk samples were analyzed according to the relevant standard operating procedures of an accredited laboratory (testing laboratory ČMSCH, a.s. 1312.2 accredited by the ČIA according to ČSN ISO/IEC 17025 (ČAS, 2018), Milk Analysis Laboratory (LRM) Brno-Tuřany) for: - component indicators, i.e. fat content (F; %), crude protein (CP; %), casein (CA; %), lactose monohydrate (LM; %), solids-not-fat (SNF; %), urea (U; mg/100 ml), free fatty acids in milk fat (FFA; mmol/100 g of fat); - milk freezing point equivalent (MFPE; in °C × -1,000 (e.g. -0.525 = 525.0)). The analyses were performed on infrared milk analyzers using the MIR-FT method (infrared spectroscopy in the mid-infrared region with a Michelson interferometer and use of Fourier transformation). CombiFoss FT+ instruments (Foss Analytical A/S, Hillerød, Denmark) were used. The values that exceeded the MFPE limit (in °C × -1,000) using the MIR-FT method were confirmed through validation measurement of MFP (milk freezing

point) using the cryoscopic method on the CryoStar automatic device (Funke-Gerber, Berlin, Germany) according to the ČSN ISO 5764 (ÚNMZ, 2010b).

SCC was determined using the flow cytometry (FC) method on a fluoro-opto-electronic particle counter CombiFoss FT+ (Foss Analytical A/S, Hillerød, Denmark). This was regularly calibrated (ČSN ISO 13366-1 (ČNI, 2008) and ČSN ISO 13366-2 (ČNI, 2007)).

The total count of mesophilic microorganisms (TCM; 10<sup>3</sup> CFU/ml) was determined using FC by direct counting of bacterial cells on the BactoScan FC device (Foss Analytical A/S, Hillerød, Denmark) with subsequent conversion using the relevant conversion equation to CFU/ml according to the reference cultivation method according to ČSN ISO 4833-1 (ÚNMZ, 2014).

Count of coliform bacteria (COLI; CFU/ml) by cultivation at 30 °C for 24 hours with pouring with agar medium (agar with crystal violet, neutral red, bile salts; Milcom Tábor) according to ČSN ISO 4832 (ÚNMZ, 2010a).

Residues of inhibitory substances (RIS; -/+ ) were checked using a microbiological procedure (*Geobacillus stearothermophilus*; a test microorganism with high sensitivity to ATB), an inhibition test (cultivation at 65 °C) with the pH indicator Eclipse 50 (ZEU-IMMUNOTEC, Spain).

### Statistical evaluation of results

The following parameters were calculated for the milk indicators: mean values (arithmetic mean ( $\bar{x}$ ), median ( $m$ )); variability in the form of standard deviation (SD) and coefficient of variation (CV in %); minimum and maximum. The significance of differences between the means of milk indicator values was tested by a classical unpaired *t*-test (MS Excel, Microsoft, Redmond, Washington, USA) at conventional confidence interval levels ( $P > 0.05$  ns;  $P \leq 0.05$  \*;  $P \leq 0.01$  \*\*;  $P \leq 0.001$  \*\*\*). Data with frequent deviation from normal frequency distribution, such as SCC, TCM and COLI, were logarithmically transformed ( $\log_{10}$ ) to approach normal frequency distribution and calculate geometric mean ( $\bar{x}_g$ ).

### RESULTS AND DISCUSSION

The results in Tables 2 and 3 summarize the basic breeding characteristics of the herds with and without AIVDC in the 1<sup>st</sup> and higher lactations. The differences were also due to the relatively lower number of herds, statistically insignificant ( $P > 0.05$ ), with the exception of F (Table 3, in the 2<sup>nd</sup> and higher lactations;  $P < 0.05$ ). F (Table 3) was recorded by 0.16% higher ( $3.93 \pm 0.1\%$ ) in the group with AIVDC, compared to the reference herds ( $3.77 \pm 0.17\%$ ). The difference in the 1<sup>st</sup> lactation was similar, 0.15% ( $3.95 \pm 0.1$  vs.  $3.8 \pm 0.18\%$ ;  $P > 0.05$ ). The other indicator differences are insignificant. The differences in CP content were 0.06% (Tables 2 and 3) in favor of herds without AIVDC ( $3.29 \pm 0.03$  vs.  $3.35 \pm 0.16\%$ ).

**Table 2.** Basic breeding characteristics of Holstein dairy herds in the vaccinated (with AIVDC) and unvaccinated (without AIVDC) groups according to summarized data from milk recording and significance of differences between them at the end of the vaccination period (2024, control year of milk recording system), first lactation

| Group         | Indicator | NL    | DIM   | MY (kg) | F (%) | F (kg) | CP (%) | CP (kg) |
|---------------|-----------|-------|-------|---------|-------|--------|--------|---------|
| With AIVDC    | n         | 8     | 8     | 8       | 8     | 8      | 8      | 8       |
|               | $\bar{x}$ | 152.3 | 294.6 | 10,372  | 3.95  | 408.3  | 3.29   | 341.3   |
|               | SD        | 73.8  | 2.7   | 935     | 0.1   | 27.9   | 0.03   | 28.7    |
|               | CV        | 13.2  | 0.9   | 9       | 2.5   | 6.8    | 0.9    | 8.4     |
|               | min       | 27    | 291   | 9,547   | 3.74  | 373    | 3.25   | 317     |
|               | max       | 239   | 298   | 12,557  | 4.07  | 469    | 3.32   | 409     |
|               | m         | 153   | 295   | 10,152  | 3.95  | 403    | 3.3    | 332     |
| Without AIVDC | n         | 8     | 8     | 8       | 8     | 8      | 8      | 8       |
|               | $\bar{x}$ | 238.5 | 297.3 | 10,503  | 3.8   | 397    | 3.35   | 353.5   |
|               | SD        | 111.8 | 2.6   | 2,139   | 0.18  | 71.9   | 0.16   | 75.9    |
|               | CV        | 46.9  | 0.9   | 20.4    | 4.8   | 18.1   | 4.9    | 21.5    |
|               | min       | 32    | 294   | 5,839   | 3.55  | 238    | 3.06   | 178     |
|               | max       | 437   | 301   | 12,524  | 4.07  | 469    | 3.64   | 418     |
|               | m         | 230   | 298   | 11,260  | 3.78  | 417    | 3.35   | 373     |
| Difference    | <i>t</i>  | 1.82  | 1.99  | 0.16    | 2.04  | 0.41   | 1.03   | 0.43    |
|               | sig       | ns    | ns    | ns      | ns    | ns     | ns     | ns      |

AIVDC = antimastitis intradermal vaccination of dairy cows; n = number of cases;  $\bar{x}$  = arithmetic mean; SD = standard deviation; CV = coefficient of variation (%); min = minimum; max = maximum; m = median; *t* = value of the test criterion of the unpaired *t*-test; sig = significance (ns =  $P > 0.05$ , not significant); NL = number of lactations; DIM = days in milk; MY = milk (milk yield); F = fat in milk; CP = crude protein in milk; everywhere standardized lactation = 305 days. According to Table 2, the comparison (2024, control year of milk recording system) included: - with AIVDC ( $152.3 \times 8$ ) 1,218 closed lactations (each lactation includes 10 measurements of each milk indicator during lactation in the milk recording system, such as ML, F and CP), it means 12,180 individual measurements; - without AIVDC ( $238.5 \times 8$ ) 1,908 closed lactations, i.e. 19,080 individual measurements.

and  $3.29 \pm 0.04$  vs.  $3.35 \pm 0.14\%$ ). Milk yield was slightly higher in herds without AIVDC (Tables 2 and 3). This may be related to the trend in F. The herd groups are very similar in terms of days in milk (Tables 2 and 3). This is important for the objectivity of the comparison. The same argument also applies to the reproductive indicator of the length of the calving period (Table 3;  $382.8 \pm 8.0$  vs.  $380.0 \pm 11.8$  days). A significant argument for a good possibility to compare herd groups is the insignificant difference between the average altitudes of the location of herd groups (Table 3;  $P > 0.05$ ; with AIVDC vs. without AIVDC,  $555.8 \pm 73.5$  vs.  $558.8 \pm 75.8$  m). This is perceived as an indicator of the comparability of the

nutritional and feeding characteristics of the cow herd groups. Although there are larger differences in the number of lactations (NL) included (Tables 2 and 3), the differences are insignificant, mainly due to their large variability among herds. If the main motivations for AIVDC technology adoption are considered – namely, the potential for reduced ATB use and related cost savings, as well as improved dairy cow health leading to increased milk yield – then the comparable milk yield per lactation between groups (with AIVDC vs. without AIVDC =  $10,372 \pm 935$  vs.  $10,503 \pm 2,139$  kg and  $11,510 \pm 1,270$  vs.  $11,780 \pm 2,491$  kg; Tables 2 and 3) provides evidence that the intended goal could be achieved.

**Table 3.** Basic breeding characteristics of Holstein dairy herds in the vaccinated (with AIVDC) and unvaccinated (without AIVDC) groups according to summarized data from milk recording and significance of differences between them at the end of the vaccination period (2024, control year of milk recording system), second and other lactations

| Group         | Indicator | A (m) | BC (day) | NL    | DIM   | MY (kg) | F (%) | F (kg) | CP (%) | CP (kg) |
|---------------|-----------|-------|----------|-------|-------|---------|-------|--------|--------|---------|
| With AIVDC    | n         | 8     | 8        | 8     | 8     | 8       | 8     | 8      | 8      | 8       |
|               | x         | 555.8 | 382.8    | 437.6 | 295   | 11,510  | 3.93  | 451.8  | 3.29   | 378.5   |
|               | SD        | 73.5  | 8        | 238   | 2.3   | 1,270   | 0.1   | 38.8   | 0.04   | 38.7    |
|               | CV        | 13.2  | 2.1      | 54.4  | 0.8   | 11      | 2.6   | 8.6    | 1.2    | 10.2    |
|               | min       | 494   | 373      | 74    | 292   | 10,427  | 3.7   | 409    | 3.25   | 347     |
|               | max       | 708   | 397      | 795   | 298   | 14,486  | 4.04  | 537    | 3.35   | 470     |
|               | m         | 523   | 382      | 440   | 296   | 11,068  | 3.96  | 442    | 3.28   | 367     |
| Without AIVDC | n         | 8     | 8        | 8     | 8     | 8       | 8     | 8      | 8      | 8       |
|               | x         | 558.8 | 380      | 635.8 | 297.5 | 11,780  | 3.77  | 440.8  | 3.35   | 396     |
|               | SD        | 75.8  | 11.8     | 286.1 | 2.7   | 2,491   | 0.17  | 82.4   | 0.14   | 85.8    |
|               | CV        | 13.6  | 3.1      | 45    | 0.9   | 21.1    | 4.6   | 18.7   | 4.3    | 21.7    |
|               | min       | 420   | 361      | 96    | 293   | 6,446   | 3.56  | 261    | 3.13   | 202     |
|               | max       | 658   | 399      | 1,082 | 301   | 13,965  | 4.05  | 520    | 3.63   | 470     |
|               | m         | 573   | 379      | 718   | 298   | 12,649  | 3.72  | 473    | 3.36   | 421     |
| Difference    | t         | 0.08  | 0.55     | 1.51  | 1.99  | 0.27    | 2.26  | 0.34   | 1.15   | 0.53    |
|               | sig       | ns    | ns       | ns    | ns    | ns      | *     | ns     | ns     | ns      |

AIVDC = antimastitis intradermal vaccination of dairy cows; n = number of cases; x = arithmetic mean; SD = standard deviation; CV = coefficient of variation (%); min = minimum; max = maximum; m = median; t = value of the test criterion of the unpaired t-test; sig = significance (ns =  $P > 0.05$ , not significant); \* =  $P < 0.05$ , significant; A = altitude; BC = between calving period; NL = number of lactations; DIM = days in milk; MY = milk (milk yield); F = fat in milk; CP = crude protein in milk; everywhere standardized lactation = 305 days. According to Table 3, the comparison (2024) included: - with AIVDC ( $437.6 \times 8$ ) 3,501 closed lactations (each lactation includes 10 measurements of each milk indicator during lactation in the milk recording system, such as MY, F and CP), it means 35,010 individual measurements; - without AIVDC ( $635.8 \times 8$ ) 5,086 closed lactations, i.e. 50,860 individual measurements. In total (Tables 2 and 3), this equals 117,130 measurements for each milk indicator separately. In this way, 11,713 dairy cows (2024, control year of milk recording system) were included in the comparison. This means 4,719 dairy cows with AIVDC and 6,994 without AIVDC.

In accordance with this reasoning Mašek et al. (2022), in a retrospective case study of a five-year profile (approx. in the middle of the period there was transition from injectable, i.e. intramuscular, vaccine application to intradermal) of one Holstein dairy herd with AIVDC stated that an autogenous vaccine with *Escherichia coli* and *Klebsiella pneumoniae* antigens, with the simultaneous use of the Valiant post-dip, brought: - a decrease in mastitis cases (clinical and severe subclinical) per year from 98 to 9; - a decrease in the % of cows culled and dead in connection with mastitis by more than 93%; - based on cultures, significantly reduced ATB consumption from 1,160 thousand CZK (2<sup>nd</sup> year) to 135 thousand CZK (5<sup>th</sup> year); - an increase in milk sales from 93.3% (3<sup>rd</sup> year) to 99.2% (5<sup>th</sup> year). Since the beginning of AIVDC, the following effects have been observed: - lower incidence of reproductive diseases (pyometra, retained placenta); - lower costs of reproduction and lower costs of mastitis treatment. At slaughter, quarters of the mammary glands affected by abscesses after injection of the vaccine were confiscated, which was eliminated by intradermal application. The characteristics of milk quality indicators before the vaccination period are summarized in Table 4. In comparison, significant differences were noted between herd groups (with AIVDC (in the future) and without AIVDC) for: F, CP, CA, LM, SNF, U, FFA, MFP, TCM, log TCM, and COLI. Overall, the milk quality, as well as general indicators of lactation metabolism of cows in the pre-vaccination period, can be assessed as slightly better for the group without AIVDC. This may (by the previous bacteriological status of the herds) indicate a reason for higher motivation for the later introduction of AIVDC. The indicators CP (+0.03%, in original units of %), CA (+0.04%), LM (+0.12%), SNF (+0.16%), U, FFA (+0.07 mmol/100 g of fat) and MFPE are slightly better. The results of TCM (-14, respectively -4 10<sup>3</sup> CFU/ml) and COLI (-22.1, respectively -5 (xg) CFU/ml) are clearly better. However, the aforementioned differences were not very significant from a practical point of view. Examination of milk samples (Table 4) for the presence of RIS showed a ratio of positive findings of 0 out of 299 in total (i.e. 299 negative) in the group with AIVDC and 0 out of 646 (646 negative) in the group without AIVDC.

The characteristics of milk quality indicators during the vaccination period are summarized in Table 5. Due to the high number of samples (2,676; with AIVDC vs. without AIVDC, 1,288 vs. 1,388) and, of course, experimental influences, most of the differences were significant. Insignificant differences were recorded for MFPE, log SCC, COLI and log COLI (Table 5). Logically, a trend difference F ( $P < 0.001$ ) between groups was recorded in Table 5 during the 31 months of the experimental period, as shown also in Tables 2 and 3. This difference was 0.16% (with AIVDC vs. without AIVDC =  $3.9 \pm 0.22$  vs.  $3.74 \pm 0.22\%$ ). This may be related to the slightly lower milk yield in the group with AIVDC, as already mentioned. However, the difference (Table 5) in the same trend as in Tables 2 and 3 for CP was lower, 0.04%, although significant ( $P < 0.001$ ;  $3.35 \pm 0.11$  vs.  $3.39 \pm 0.15\%$ ). The same trend is observed in the CA difference (Table 5):  $P < 0.001$ ;  $2.63 \pm 0.09$  vs.  $2.66 \pm 0.12\%$ . For LM, the same difference ( $P < 0.001$ ) was  $4.91 \pm 0.07$  vs.  $4.95 \pm 0.04\%$ . For solids-not-fat content, the difference ( $P < 0.001$ ) was  $8.96 \pm 0.16$  vs.  $9.05 \pm 0.17\%$ . For U, the difference ( $P < 0.001$ ) was  $23.93 \pm 4.28$  vs.  $22.82 \pm 5.69$  mg/100 ml. This difference was relatively, also practically, small, although significant. The content of FFA also differed significantly ( $P < 0.05$ ),  $0.9 \pm 0.3$  vs.  $0.86 \pm 0.19$  mmol/100 g of fat.

SCC was slightly higher ( $P < 0.001$ ) in the group with AIVDC ( $187 \pm 71 > 178 \pm 47$  10<sup>3</sup>/ml), but this is practically negligible. The above points to the possibility that herds adopting this technology to improve cow health (with a worse initial state of mammary gland health) and reduce milk losses gradually achieved their goal. The difference in the TCM (Table 5) was significant ( $P < 0.01$  and  $< 0.05$ ) and amounted to for the groups with AIVDC vs. without AIVDC  $23.1 \pm 68.4 > 15.0 \pm 41.5$  10<sup>3</sup> CFU/ml. It points to a worsening initial hygienic condition of milking in herds with AIVDC, while the incidence of COLI showed an insignificant difference ( $P > 0.05$ ; lower number of measurements)  $9.7 \pm 24.1 < 17.3 \pm 34.7$  CFU/ml. It may perhaps indicate the original targeted orientation of AIVDC on gram-negative bacteria.

**Table 4.** Statistical parameters of milk quality indicators of Holstein dairy herds in the later vaccinated (with AIVDC) and unvaccinated (without AIVDC) groups, including a test of the significance of the differences between them during the monitoring in the period before vaccination (2021–May 2022, 17 months)

| Group         | Indicator | F    | CP   | CA   | LM    | SNF   | U     | FFA  | MFPE   | SCC  | Log SCC | TCM   | Log TCM | COLI  | Log COLI |
|---------------|-----------|------|------|------|-------|-------|-------|------|--------|------|---------|-------|---------|-------|----------|
| With AIVDC    | n         | 546  | 546  | 546  | 546   | 546   | 546   | 546  | 546    | 546  | 546     | 300   | 300     | 21    | 19       |
|               | x         | 3.79 | 3.35 | 2.62 | 4.88  | 8.91  | 23.47 | 0.93 | 526.85 | 179  | 2.2322  | 26.3  | 1.0334  | 40.1  | 1.0207   |
|               | SD        | 0.29 | 0.17 | 0.1  | 0.24  | 0.27  | 4.67  | 0.41 | 2.9    | 52   | 0.141   | 61.4  | 0.467   | 59.8  | 0.825    |
|               | CV        | 7.6  | 5.1  | 3.9  | 4.9   | 1.8   | 19.9  | 44.1 | 0.6    | 29.3 |         | 233.5 |         | 149   |          |
|               | min       | 1.79 | 3.03 | 2.36 | 4.0   | 8.1   | 12    | 0    | 516    | 18   | 1.2553  | 5     | 0.699   | 0     |          |
|               | max       | 4.35 | 3.99 | 2.87 | 5.49  | 9.23  | 42    | 1.95 | 534    | 338  | 2.5289  | 599   | 2.7774  | 151   | 2.179    |
|               | m         | 3.86 | 3.35 | 2.62 | 4.96  | 8.99  | 24    | 0.97 | 526.05 | 184  | 2.2648  | 7     | 0.8451  | 2     | 0.602    |
|               | xg        |      |      |      |       |       |       |      |        |      |         |       |         |       |          |
| Without AIVDC | n         | 756  | 756  | 756  | 756   | 756   | 756   | 756  | 756    | 756  | 756     | 647   | 647     | 469   | 433      |
|               | x         | 3.76 | 3.38 | 2.66 | 5     | 9.07  | 21.74 | 0.86 | 527.98 | 177  | 2.23    | 12.3  | 0.8488  | 18    | 0.6998   |
|               | SD        | 0.21 | 0.14 | 0.12 | 0.06  | 0.15  | 5.14  | 0.39 | 4.23   | 47   | 0.137   | 49    | 0.26    | 34.6  | 0.772    |
|               | CV        | 5.5  | 4.1  | 4.7  | 1.3   | 1.7   | 23.6  | 45.7 | 0.8    | 26.6 |         | 397.9 |         | 192.2 |          |
|               | min       | 3.04 | 2.81 | 2.15 | 4.73  | 8.5   | 5     | 0    | 514    | 45   | 1.6532  | 5     | 0.699   | 0     |          |
|               | max       | 4.72 | 3.64 | 2.91 | 5.17  | 9.42  | 35    | 2.73 | 540    | 342  | 2.534   | 599   | 2.7774  | 151   | 2.179    |
|               | m         | 3.75 | 3.39 | 2.66 | 4.99  | 9.07  | 22    | 0.85 | 528    | 181  | 2.2565  | 6     | 0.7782  | 4     | 0.6021   |
|               | xg        |      |      |      |       |       |       |      |        |      |         |       |         |       |          |
| Difference    | t         | 2.19 | 3.52 | 6.17 | 13.23 | 13.62 | 6.23  | 3.12 | 5.39   | 0.59 | 0.28    | 3.76  | 7.79    | 2.76  | 1.77     |
|               | sig       | *    | ***  | ***  | ***   | ***   | ***   | **   | ***    | ns   | ns      | ***   | ***     | **    | ns       |

AIVDC = antimastitis intradermal vaccination of dairy cows; n = number of samples tested; x = arithmetic mean; SD = standard deviation; CV = coefficient of variation (%); min = minimum; max = maximum; m = median; xg = geometric mean; F = fat content (%); CP = crude protein content (%); CA = casein content (%); LM = lactose monohydrate content (%); SNF = solids-not-fat content (%); U = urea concentration (mg/100 ml); FFA = content of free fatty acids in milk fat (mmol/100 g of fat); MFPE = milk freezing point equivalent ( $^{\circ}\text{C} \times -1,000$ ); SCC = somatic cell count ( $10^3/\text{ml}$ ); log SCC =  $\log_{10}$  SCC; TCM = total count of mesophilic microorganisms ( $10^3$  CFU/ml); log TCM =  $\log_{10}$  TCM; COLI = count of coliform microorganisms (CFU/ml); log COLI =  $\log_{10}$  COLI; t = value of the test criterion of the unpaired t-test; sig = significance (ns =  $P > 0.05$ , not significant); \*, \*\* and \*\*\* = significant,  $P$  (probability of the null hypothesis)  $\leq 0.05$ ,  $\leq 0.01$  and  $\leq 0.001$ ).

**Table 5.** Statistical parameters of milk quality indicators of Holstein dairy herds in the vaccinated (with AIVDC) and unvaccinated (without AIVDC) groups, including a test of the significance of the differences between them during the monitoring of the vaccination period (June 2022–2024, 31 months)

| Group         | Indicator | F     | CP    | CA    | LM    | SNF   | U     | FFA   | MFPE   | SCC   | Log SCC | TCM   | Log TCM | COLI  | Log COLI |
|---------------|-----------|-------|-------|-------|-------|-------|-------|-------|--------|-------|---------|-------|---------|-------|----------|
| With AIVDC    | n         | 1,288 | 1,288 | 1,288 | 1,288 | 1,288 | 1,288 | 1,288 | 1,288  | 1,288 | 1,288   | 663   | 663     | 49    | 43       |
|               | x         | 3.9   | 3.35  | 2.63  | 4.91  | 8.96  | 23.93 | 0.9   | 529.91 | 187   | 2.2394  | 23.1  | 0.9898  | 9.7   | 0.5745   |
|               | SD        | 0.22  | 0.11  | 0.09  | 0.07  | 0.16  | 4.28  | 0.3   | 6.11   | 71    | 0.174   | 68.4  | 0.414   | 24.1  | 0.576    |
|               | CV        | 5.7   | 3.3   | 3.3   | 1.4   | 1.8   | 17.9  | 32.8  | 1.2    | 37.7  |         | 296.8 |         | 248.7 |          |
|               | min       | 2.88  | 2.56  | 1.85  | 3.86  | 7.16  | 8     | 0.09  | 414    | 50    | 1.699   | 5     | 0.699   | 0     |          |
|               | max       | 4.98  | 3.7   | 2.91  | 5.04  | 9.32  | 40    | 3.88  | 547    | 532   | 2.7259  | 599   | 2.7774  | 151   | 2.179    |
|               | m         | 3.93  | 3.35  | 2.63  | 4.91  | 8.98  | 24    | 0.89  | 530    | 184   | 2.2648  | 7     | 0.8451  | 2     | 0.301    |
|               | xg        |       |       |       |       |       |       |       |        |       |         | 174   |         | 10    |          |
| Without AIVDC | n         | 1,388 | 1,388 | 1,388 | 1,388 | 1,388 | 1,388 | 1,388 | 1,388  | 1,388 | 1,388   | 1,170 | 1,170   | 775   | 717      |
|               | x         | 3.74  | 3.39  | 2.66  | 4.95  | 9.05  | 22.82 | 0.86  | 529.63 | 178   | 2.2349  | 15    | 0.9541  | 17.3  | 0.6703   |
|               | SD        | 0.22  | 0.15  | 0.12  | 0.04  | 0.17  | 5.69  | 0.49  | 5.13   | 47    | 0.114   | 41.5  | 0.31    | 34.7  | 0.791    |
|               | CV        | 5.8   | 4.4   | 4.4   | 0.8   | 1.9   | 24.9  | 56.5  | 1      | 26.7  |         | 276.8 |         | 200.8 |          |
|               | min       | 2.66  | 2.85  | 2.21  | 4.69  | 8.3   | 1     | 0     | 491    | 54    | 1.7324  | 5     | 0.699   | 0     |          |
|               | max       | 4.34  | 3.8   | 2.98  | 5.13  | 9.48  | 36    | 6.24  | 542    | 664   | 2.8222  | 599   | 2.7774  | 151   | 2.179    |
|               | m         | 3.74  | 3.39  | 2.67  | 4.95  | 9.06  | 23    | 0.82  | 530    | 175   | 2.2418  | 7     | 0.8451  | 3     | 0.4771   |
|               | xg        |       |       |       |       |       |       |       |        |       |         | 172   |         | 9     |          |
| Difference    | t         | 18.75 | 7.87  | 7.51  | 19.03 | 14.11 | 5.67  | 2.55  | 1.29   | 4.07  | 0.8     | 3.13  | 2.09    | 1.5   | 0.78     |
|               | sig       | ***   | ***   | ***   | ***   | ***   | ***   | *     | ns     | ***   | ns      | **    | *       | ns    | ns       |

AIVDC = antimastitis intradermal vaccination of dairy cows; n = number of samples tested; x = arithmetic mean; SD = standard deviation; CV = coefficient of variation (%); min = minimum; max = maximum; m = median; xg = geometric mean; F = fat content (%); CP = crude protein content (%); CA = casein content (%); LM = lactose monohydrate content (%); SNF = solids-not-fat content (%); U = urea concentration (mg/100 ml); FFA = content of free fatty acids in milk fat (mmol/100 g of fat); MFPE = milk freezing point equivalent ( $^{\circ}\text{C} \times -1,000$ ); SCC = somatic cell count ( $10^3/\text{ml}$ ); log SCC =  $\log_{10}$  SCC; TCM = total count of mesophilic microorganisms ( $10^5$  CFU/ml); log TCM =  $\log_{10}$  TCM; COLI = count of coliform microorganisms (CFU/ml); log COLI =  $\log_{10}$  COLI; t = value of the test criterion of the unpaired t-test; sig = significance (ns =  $P > 0.05$ , not significant); \*, \*\* and \*\*\* = significant,  $P$  (probability of the null hypothesis)  $\leq 0.05$ ,  $\leq 0.01$  and  $\leq 0.001$ ).

The discussed papers in the field of antimastitis vaccination mainly deal with intramuscular application. Furthermore, there are not many works that comment on differences in milk quality over a longer period. Mata et al. (2023) studied the effectiveness of vaccines in the prevention of mastitis by meta-analysis: - effectiveness decreases over time; - vaccines administered after calving are not effective; - vaccines administered before calving show reduced effectiveness; - commercial vaccines are ineffective; - own production of vaccines (relevant to microbiological examinations before treatment) shows effectiveness in increasing milk and dry matter production. Therefore, vaccination can be considered a complementary tool to traditional preventive measures. The results of this work show that AIVDC technology can be one of the methods of controlling part of mastitis problems in the practice of dairy herds, with support for milk quality in time. However, for possible further control of the decrease in ATB consumption in dairy farming, further support for reduction is necessary, e.g. by selecting their preventive use during the drying-off of lactation in dairy cows. Comparison of the average results of milk indicators in consecutive observation periods (before and during vaccination) in groups of dairy cow herds with and without AIVDC is carried out in Tables 6 and 7. Significant changes in milk indicators over time in the group with AIVDC were recorded (Table 6) for: F, CA, LM, SNF, U, MFPE, SCC, COLI, and log COLI. Changes in F (2.9%, relatively), LM (0.61%), SNF (0.56%), FFA (-3.23%), TCM (xg, -9.09%) and COLI (xg, -60%) can be considered as more significant improvements. With regard to the corresponding results in the group without AIVDC (Table 7), it can be stated that a more significant improvement in milk quality over time during the vaccination period was more often observed in the group with AIVDC. Here, it is also possible to observe the reason for a certain partial motivation for the use of AIVDC health technology in dairy herds. However, it should be objectively noted that this improvement was not very significant overall. From a practical point of view, this technology needs to be further evaluated.

Increasing milk yield is a prerequisite when using antimastitis vaccination of cows. This secondary premise is conditioned by the primary premise of reducing the frequency of mastitis. Mata et al. (2023) mentioned the observed increase in milk yield in the case of in-house vaccine production. Ismail (2017) mentioned that milk production parameters significantly improved in cows vaccinated with autogenous vaccines (Freick et al., 2016). In this study, similar milk yields were found after a longer period of AIVDC application. These herds were able to catch up with the milk yield of herds originally without significant problems with mammary gland health. Tashakkori et al. (2020) compared the efficacy of two commercial vaccines (Startvac® and Mastivac®) against bovine mastitis. When administered, the incidence of clinical mastitis did not differ significantly between groups. Similarly, SCC means did not differ significantly during the 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> months of lactation. Isakova et al. (2021) determined the quality profile of milk in high-yielding dairy cows vaccinated (Startvac vaccine) against mastitis. During the test period, the detection of *Staphylococcus aureus* decreased by 19.41%. Over the four years of testing, a decreasing trend in SCC in bulk tank milk was detected. SCC in milk from vaccinated animals decreased by 286 at the milk unit and 432 10<sup>3</sup>/ml on the farm. With regard to the aforementioned literary results, in our case, a slight improvement in milk quality and production was shown through the use of the AIVDC method. This was in accordance with a little bit more significant results (Freick et al., 2016; Ismail, 2017; Isakova et al., 2021; Mašek et al., 2022; Mata et al., 2023) discussing similar and other forms of antimastitis vaccination of dairy cows. Our conclusions were also only slightly different from those of Tashakkori et al. (2020). Samples tested for RIS (Table 5) showed a positive rate of 0 out of 663 in the group with AIVDC (i.e. 663 negative) and 0 out of 1,165 in the group without AIVDC (1,165 negative). This suggests that no difference was observed between the investigated groups, with RIS frequency results of 0 and 0%.

~~Table 6. Significance of differences between the means (during vaccination - before vaccination) of milk indicators in the group of dairy herds with AIVDC~~

| Group      | Indicator | F    | CP   | CA   | LM   | SNF  | U     | FFA   | MFPE   | SCC  | SCC (xg) | TCM  | TCM (xg) | COLI | COLI (xg) |
|------------|-----------|------|------|------|------|------|-------|-------|--------|------|----------|------|----------|------|-----------|
| During     | x         | 3.9  | 3.35 | 2.63 | 4.91 | 8.96 | 23.93 | 0.9   | 529.91 | 187  | 174      | 26.3 | 10       | 40.1 | 4         |
| Before     | x         | 3.79 | 3.35 | 2.62 | 4.88 | 8.91 | 23.47 | 0.93  | 526.85 | 179  | 171      | 26.3 | 11       | 40.1 | 10        |
| Difference | d         | 0.11 | 0    | 0.01 | 0.03 | 0.05 | 0.46  | -0.03 | 3.06   | 8    | 3        | 0    | -1       | 0    | -6        |
|            | rd        | 2.9  | 0    | 0.38 | 0.61 | 0.56 | 1.96  | -3.23 | 0.58   | 4.47 | 1.75     | 0    | -9.09    | 0    | -60       |
|            | t         | 8.84 | 0    | 2.15 | 4.15 | 4.96 | 2.05  | 1.76  | 11.18  | 2.4  | 0.85     | 0.7  | 1.45     | 3.05 | 2.45      |
|            | sig       | ***  | ns   | *    | ***  | ***  | *     | ns    | ***    | *    | ns       | ns   | ns       | **   | *         |

AIVDC = antimastitis intradermal vaccination of dairy cows; F = fat content (%); CP = crude protein content (%); CA = casein content (%); LM = lactose monohydrate content (%); SNF = solids-not-fat content (%); U = urea concentration (mg/100 ml); FFA = content of free fatty acids in milk fat (mmol/100 g of fat); MFPE = milk freezing point equivalent ( $^{\circ}\text{C} \times -1,000$ ); SCC = somatic cell count ( $10^3/\text{ml}$ ); TCM = total count of mesophilic microorganisms ( $10^3 \text{ CFU/ml}$ ); COLI = count of coliform microorganisms (CFU/ml); x = arithmetic mean; xg = geometric mean; d = difference; rd = relative difference (%; 100 % = group before); t = value of the test criterion of the unpaired t-test; sig = significance (ns =  $P > 0.05$ , not significant); \*, \*\* and \*\*\* = significant, P (probability of the null hypothesis)  $\leq 0.05$ ,  $\leq 0.01$  and  $\leq 0.001$ ).

Table 7. Significance of differences between the means (during vaccination - before vaccination) of milk indicators in the group of dairy herds without AIVDC

| Group      | Indicator | F     | CP   | CA   | LM    | SNF   | U     | FFA  | MFPE   | SCC  | SCC (xg) | TCM   | TCM (xg) | COLI  | COLI (xg) |
|------------|-----------|-------|------|------|-------|-------|-------|------|--------|------|----------|-------|----------|-------|-----------|
| During     | x         | 3.74  | 3.39 | 2.66 | 4.95  | 9.05  | 22.82 | 0.86 | 529.63 | 178  | 172      | 15    | 9        | 17.3  | 5         |
| Before     | x         | 3.76  | 3.38 | 2.66 | 5     | 9.07  | 21.74 | 0.86 | 527.98 | 177  | 170      | 7     | 11       | 5     | 10        |
| Difference | d         | -0.02 | 0.01 | 0    | -0.05 | -0.02 | 1.08  | 0    | 1.65   | 1    | 2        | 8     | -2       | 12.3  | -5        |
|            | rd        | -0.53 | 0.3  | 0    | -1    | -0.22 | 4.97  | 0    | 0.31   | 0.56 | 1.18     | 114.3 | -18.2    | 246.0 | -50       |
|            | t         | 2.06  | 1.53 | 0    | 22.64 | 2.69  | 4.34  | 0    | 7.55   | 0.13 | 0.88     | 1.24  | 7.33     | 0.36  | 0.62      |
|            | sig       | *     | ns   | ns   | ***   | **    | ***   | ns   | ***    | ns   | ns       | ns    | ***      | ns    | ns        |

AIVDC = antimastitis intradermal vaccination of dairy cows; F = fat content (%); CP = crude protein content (%); CA = casein content (%); LM = lactose monohydrate content (%); SNF = solids-not-fat content (%); U = urea concentration (mg/100 ml); FFA = content of free fatty acids in milk fat (mmol/100 g of fat); MFPE = milk freezing point equivalent ( $^{\circ}\text{C} \times -1,000$ ); SCC = somatic cell count ( $10^3/\text{ml}$ ); TCM = total count of mesophilic microorganisms ( $10^3 \text{ CFU/ml}$ ); COLI = count of coliform microorganisms (CFU/ml); x = arithmetic mean; xg = geometric mean; d = difference; rd = relative difference (%; 100 % = group before); t = value of the test criterion of the unpaired t-test; sig = significance (ns =  $P > 0.05$ , not significant); \*, \*\* and \*\*\* = significant, P (probability of the null hypothesis)  $\leq 0.05$ ,  $\leq 0.01$  and  $\leq 0.001$ ).

## CONCLUSIONS

The treatment of dairy cows with AIVDC is widely recognised as a modern supportive preventive measure in dairy herd health management.

In this study, the milk quality indicators in herds with AIVDC in the Czech Republic were comparable to those in herds without AIVDC. It can be inferred that this comparable quality was gradually achieved during the observed period, as the herds with AIVDC had a slightly worse starting position. The most pronounced, though still modest, differences in the group with AIVDC between the pre- and post-vaccination periods were the increases in F, LM, and SNF, and the reduction of FFA, TCM, and COLI. This allows us to presume that AIVDC has the potential to improve milk quality.

However, further research with larger datasets is needed to confirm these tendencies.

## ACKNOWLEDGEMENTS

This work was supported by the projects of the Ministry of Agriculture, NAZV Země QK 21010123, of the Ministry of Agriculture RO 1425 and the Grant Agency of South Bohemia University in České Budějovice, GAJU 023/2025/Z.

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