

## Forticept® innovative products' effectiveness for complex udder hygiene

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### ABSTRACT

The quality of cow's milk and the spread of mastitis greatly depend on the level of udder hygiene. Our author's research was conducted to evaluate the effects of Forticept® Udder Wash and Forticept® Udder Forte, used before and after milking, on the treatment and prevention of subclinical mastitis and hyperkeratosis of cows' udders. A total of 6880 milk samples were taken from 430 cows, 3-4 years old, 470-490 kg weight, at 60 ± 15 days of lactation. The therapeutic efficacy coefficient (EC) of Forticept® polymers based on benzetonium and benzalkonium chloride, against the subclinical form of mastitis was 73.8% compared to iodine-containing agents; preventive EC - 32.4%. Dipping hygiene of the udder using Forticept® on the 30<sup>th</sup> day reduced the number of 1-3 stage teat hyperkeratosis cases from 78.8% to 41.3%. Also, during this period, a significant (P<0.05) improvement in the chemical parameters of the milk was observed - an increase in fat (up to 3.94 ± 0.08%), protein (3.35 ± 0.07%), casein (3.15 ± 0.09%) and dry matter (14.91 ± 0.28%). After using Forticept®, the total bacteria count (TBC) of the milk decreased to 3×10<sup>5</sup> CFU/cm<sup>3</sup>, and the somatic cell count (SCC) - to 130 thousand/cm<sup>3</sup> (P<0.05). Test day milk yield (TDMY) increased by 1.15% due to efficient dipping with Forticept® (P<0.05).

**Key words:** Forticept®; hyperkeratosis; subclinical mastitis; hygiene; milk

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## Introduction

Milk quality is a multi-factor indicator. It is greatly dependent on the level of breeding work with the herd, the composition and nutritional value of rations, the hygiene of housing, and the status of cows' health (YURCHENKO and BIHUN, 2012). Any form of mammary gland inflammation adversely affects the composition and physical, chemical and biological properties of the milk (SRI BALAJI et al., 2016). A case of clinical mastitis incurred causes financial loss from 100 to 1000 euros (LITWIŃCZUK et al., 2015).

In the case of subclinical mastitis, the milk most often has no visible changes. However, after filtering such milk, tiny particles of coagulated protein can be detected in it (MURPHY et al., 2016). With the development of the inflammatory process, the somatic cell count (SCC) always increases in the mammary gland secretion (SOUZA et al., 2016; KASYANCHUK et al., 2013). The amount of chlorides and alkalinity increases in milk from affected udder quarters (pH 6.83-7.19). The fat content and density of this milk are reduced. There is a decrease in the solids content due to lower amounts of lactose, casein and calcium (JAGADEESH et al., 2016; NEKRASOV et al., 2018; BOGATKO et al., 2019). Reduced resistance to heat leads to spontaneous coagulation of mastitis milk during its heat treatment (PYZ-ŁUKASIK et al., 2015).

Another problem with mastitis on a farm are the inhibitors in the milk during and after the treatment of sick cows. These substances are mainly antibiotics, sulphonamides and nitrofurans (BAUMBERGER et al., 2016).

In order to ensure production of high-quality milk, it is necessary to follow the udder hygiene and regularly maintain milking machines (SACHUK et al., 2016). The functional load on the mammary gland tissue during milking creates ultimate pressure on the organ tissue (PAULRUD et al., 2005). Prone to drying, often the weathered skin of the teats is covered with cracks, around which inflammation develops, which contributes to the penetration of microorganisms into the mammary gland (NEIJENHUIS, 2004; ELESIN, 2010; GRAFF et al., 2007). Bacteria (*Streptococcus* spp.,

*Staphylococcus* spp., *Trueperella pyogenes*, *E. coli*, *Klebsiella* spp., etc.) are the main etiological factor of mastitis (TEGEGNE and TESFAYE, 2017). Thus, the organization of effective udder hygiene has a leading role in obtaining high quality milk (SCHROEDER, 2012).

Most preparations for mammary gland hygiene (dipping and pre-dipping) are based on chlorhexidine, iodine and lactic acid (PRASANTHI et al., 2012; BELKIN et al., 2015). However, classic antiseptics rarely meet all contemporary requirements. These hygienic solutions should provide reliable protection against pathogenic microflora, have a beneficial effect on the skin, and be have no effect on the composition of milk (HORBATOVA and HUN'KOVA, 2010; IZQUIERDO et al., 2017).

An innovative hygienic antiseptic foaming agent for treating teats before milking, Forticept® Udder Wash, contains benzalkonium chloride (BZK, 0.13 mg/mL) as an active substance (AS). Forticept® Udder Forte is a disinfecting film-forming preparation with a prolonged restorative effect for treating udders after milking, with AS - benzethonium chloride (BZT - 0.1 mg/mL) and thymol (0.063 mg/mL). These preparations do not contain antibiotics, hormones and similar synthetic substances. Both preparations contain a complex of chamomile extracts (*Matricaria recutita* extract), yarrow (*Achillea millefolium* extract), and emollients (lanolin, shea butter).

The aim of the studies was to determine the effect of Forticept® Udder Wash and Forticept® Udder Forte preparations on the treatment and prevention of subclinical forms of mastitis and hyperkeratosis of cows' udders, taking into account the physicochemical and bacteriological parameters of the milk.

## Materials and methods

*Study population.* The studies were conducted in 2018 on a private dairy farm located in the territory of Western Podolia of Ukraine (Khmelnitsky region). 430 cows were involved and 6880 milk samples were analyzed. All cows were black-pocked breed, 3-4 years old, 470-490 kg in weight, at 60 ± 15 days of lactation. The management and feeding conditions were identical. Daily milk yield

Table 1. The distribution of cows into groups according to research objectives

Group	Number of sample (n)	Clinical status	Treatment type	Research objectives
Experimental A	Cows - n = 104; Milk samples - n = 416; Udder teats - n = 416	SMF, hyperkeratosis of the teats	Forticept® Udder Wash Forticept® Udder Forte	Treatment of SMF and hyperkeratosis of the teats
Experimental B	Cows - n = 104; Milk samples - n = 416; Udder teats - n = 416	SMF, hyperkeratosis of the teats	Iodine-containing preparations	
Experimental C	Cows - n = 111; Milk samples - n = 444	Healthy	Forticept® Udder Wash Forticept® Udder Forte	Prevention of SMF and hyperkeratosis of the teats
Experimental D	Cows - n = 111; Milk samples - n = 444	Healthy	Iodine-containing preparations	

SMF - subclinical mastitis form

was recorded daily using the Del Pro Farm Manager computer system (DeLaval, Tumba, Sweden). The milking procedure was carried out using linear milking machines of the type AID-1-01 (Damilk, Ukraine), twice a day. On the control days of the experiment (1<sup>st</sup>, 10<sup>th</sup>, 20<sup>th</sup>, and 30<sup>th</sup>), milk samples were taken individually from each cow according to DSTU (State Standard of Ukraine) ISO 707:2002 (ISO 707:1997, IDT) and visual assessment performed of changes in the condition of the mammary gland teat skin.

*Drugs and treatments.* Forticept® Udder Wash (Lidan Inc., USA, Marietta) was dissolved in water (1:4) according to the instructions, before use, and the resulting solution was applied to the teat skin before milking using foaming cups, with an exposure time of 30 s. After milking, the teats of the cows in groups A and C (Table 1) were dipped for 1-3 s in disposable glasses with Forticept® Udder Forte solution. As a result, a protective film was formed on the teats, which prevented the penetration of pathogenic microflora into the milk channel of the cow's udder before the next milking.

Standard disinfectants were used for cows in groups B and D: before milking, a solution containing 0.1% crystalline iodine was applied to the udder teats by wetting. After milking, the teats were immersed in dipping cups with a composite solution based on 0.5% useful iodine, 5% glycerol and sorbitol.

The efficiency coefficient (EC) of Forticept® products was evaluated according to the formula proposed by BRIKO (2001):

$$EC (\%) = \frac{\alpha \times 100}{A} - \frac{\beta \times 100}{B}$$

where:

$\alpha$  - the number of healthy animals in the experimental group;

$\beta$  - the same in the control group;

A - the number of animals for experimental drug testing in the experimental group;

B - the number of animals for comparative drug testing in the control group.

*Laboratory research.* Mammary gland examinations were performed by examination, palpation, and analysis of milk samples from each quarter of the cow's udder. The subclinical mastitis form (SMF) was determined by the "California mastitis test" with Profilac Reagent N, Westfalia. The reaction was evaluated by changes in the milk's consistency in the hole of the control plate directly next to the animal. Milk from animals with questionable results of the Californian test (+ and ++) was rechecked by the SMF detector, «MAS-D-TEC» (USA). The exact number of SC in cows' milk with SMF was measured in laboratory conditions using the device «Somacaunt-150» according to DSTU (State Standard of Ukraine) ISO 13366-2/IDF 148-2:2014 (ISO 13366-2:2006/IDF 148-2:2006, IDT).

Milk samples for chemical and bacteriological analysis were taken individually in 100 mL sterile bottles, and transported on ice to the laboratory. Physical and chemical indicators of the milk (freezing point, fat, protein, casein, dry defatted milk residue (DDMR), and lactose) were determined by the analyzer Milkotester Master Classic LM3 (Bulgaria) according to DSTU (State Standard of Ukraine) ISO 9622:2013 (ISO 9622:1999, IDT) to control milk quality indicators. Samples for bacteriological analysis were prepared according to DSTU (State Standard of Ukraine) IDF 122C:2003 (IDF 122C:1996, IDT).

Total bacteria count (TBC) in the milk was determined according to DSTU (State Standard of Ukraine) ISO 4833:2006 (ISO 4833:2003, IDT); DSTU (State Standard of Ukraine) IDF 100B:2003 (IDF 100B:1991, IDT); DSTU (State Standard of Ukraine) 7357:2013.

As normative indicators for high-quality milk, we used the standard DSTU (State Standard of Ukraine) 3662-97 and Regulation (EC) No 853/2004.

*Statistical analysis.* Mathematical analysis of the data was carried out using STATISTICA 12 for Windows (StatSoft, Inc., USA). The mean values ( $\bar{x}$ ) and standard deviations (SD) were calculated. The significance of differences in the average values in the studied groups was determined using the one-way analysis of variance method using the Fisher criterion. A value of  $P < 0.05$  was considered significant.

## Results

During the experiments, no deterioration in clinical condition, or development of side effects in cows under the influence of the tested drugs were observed. In the experiment (group A), udder dipping with Forticept® Udder Wash and Forticept® Udder Forte cured SMF in 59.6% of cases already on the 20<sup>th</sup> day of use (Fig. 1), therapeutic EC = 73.8% compared to the iodine-containing agents in the control. After 30 days of Forticept® administration to healthy cows (group C), 96.4% of milk samples showed no signs of SMF, with prophylactic EC = 32.4%.

In cows with SMF (groups A and B), teat hyperkeratosis was observed. The dipping hygiene of the udder on the 30<sup>th</sup> day using Forticept® reduced the number of teats affected by hyperkeratosis of varying severity (stages 1-3) from the initial 78.8 to 41.3% (Fig. 2). In many cows, previously problematic teats with sphincter corpus callosum and skin cracks, became smooth, pink, elastic, without seals or damage. The number of damaged teats after using iodine-containing products for the same period decreased from 79.8 to 58.7%.

The recorded improvement of the teats, regardless of the type of antiseptic hygiene used, mainly concerned cases of uncomplicated hyperkeratosis (stage 1).

Over 30 days, Forticept® preparations use tripled (from 45.2 to 15.5%) and reduced the number of teats affected by stage 1 hyperkeratosis, completely normalizing their physiological state (stage 0). Treatment of the udder with iodine-based agents reduced the number of teats with stage 1 hyperkeratosis from 49.0 to 29.8%.

The number of teats with complicated hyperkeratosis (stages 2-3) in group B cows remained almost stable (-2.0%). The presence of rough calloused rings, cracks, inflammation of the tissues and gaping of the teat canal, in addition to the use of hygiene products during milking, requires the use of specific therapeutic drugs. However, in group A, on the 30<sup>th</sup> day after use of Forticept® preparations, 7.7% of the teats with stage 3 hyperkeratosis had improved to the milder stage 2. That is, the presence of radial cracks with edema of the teat apex and the opening of the channel was replaced by the formation of coarse callosity rings with small cracks.

Improvement in the physical condition of udders after using Forticept® products led to positive changes in the composition of the milk (Table 2).

Thirty days of intensive udder hygiene in both groups and, therefore, regardless of the selected preparations' composition, led to a slight increase in milk fat, protein and lactose. However, in group A, after using Forticept® products, these indicators increased more intensively. Also, in group A, we observed an increase in casein (max by 19.77%,  $P < 0.05$ ) and dry matter (max by 22.72%,  $P < 0.05$ ) in the milk, which did not occur under the influence of iodine-containing hygienic preparations.

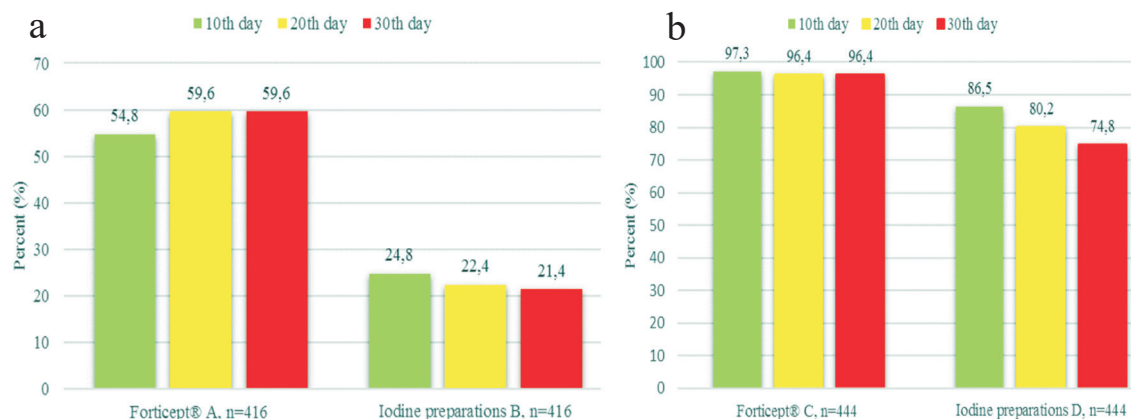


Fig. 1. Anti-mastitis effectiveness of antiseptic hygiene products, % milk samples (n) without signs of SMF: a -treatment of cows with mastitis in groups A and B; b -prevention of SMF in healthy cows in groups C and D

Table 2. Effects of antiseptic hygiene products on the chemical composition and physical properties of milk (x ± SD)

Experiment day	Milk indicators						
	Fat (%)	Protein (%)	Casein (%)	Lactose (%)	Dry matter (%)	Dry defatted milk residue (%)	Freezing temperature (°C)
<b>Forticept® A (n = 104)</b>							
1 <sup>st</sup> day	3.28 ± 0.11	3.02 ± 0.08	2.63 ± 0.07	4.64 ± 0.08	12.15 ± 0.19	8.18 ± 0.06	-0.55 ± 0.01
10 <sup>th</sup> day	3.44 ± 0.09	3.19 ± 0.07	2.82 ± 0.05	4.74 ± 0.09	12.84 ± 0.24	8.33 ± 0.12	-0.55 ± 0.01
20 <sup>th</sup> day	3.61 ± 0.09	3.26 ± 0.09	2.99 ± 0.06	4.84 ± 0.05	13.70 ± 0.30	8.60 ± 0.13	-0.55 ± 0.01
30 <sup>th</sup> day	3.94 ± 0.08	3.35 ± 0.07	3.15 ± 0.09	4.93 ± 0.03	14.91 ± 0.28	8.92 ± 0.08	-0.56 ± 0.01
<b>Iodine preparations B (n = 104)</b>							
1 <sup>st</sup> day	3.32 ± 0.06	2.97 ± 0.07	2.59 ± 0.14	4.69 ± 0.07	12.24 ± 0.29	8.26 ± 0.07	-0.56 ± 0.01
10 <sup>th</sup> day	3.48 ± 0.07	2.99 ± 0.07	2.63 ± 0.09	4.76 ± 0.08	12.38 ± 0.23	8.28 ± 0.09	-0.56 ± 0.01
20 <sup>th</sup> day	3.65 ± 0.09	3.12 ± 0.08	2.65 ± 0.10	4.77 ± 0.06	12.44 ± 0.21	8.33 ± 0.09	-0.57 ± 0.01
30 <sup>th</sup> day	3.83 ± 0.13	3.18 ± 0.09	2.61 ± 0.08	4.84 ± 0.06	12.41 ± 0.23	8.42 ± 0.07	-0.57 ± 0.01

Table 3. Biological milk safety indicators after antiseptic hygiene products using (x ± SD)

Experimentday	SCC (cell/cm <sup>3</sup> )	TBC (CFU/cm <sup>3</sup> )	Inhibitors
<b>Forticept® A (n = 104)</b>			
1 <sup>st</sup> day	507.36 ± 5.28	5×10 <sup>5</sup>	absent
10 <sup>th</sup> day	352.17 ± 4.15	4×10 <sup>5</sup>	absent
20 <sup>th</sup> day	220.44 ± 3.67	3×10 <sup>5</sup>	absent
30 <sup>th</sup> day	129.76 ± 2.18	3×10 <sup>5</sup>	absent
<b>Iodine preparations B (n = 104)</b>			
1 <sup>st</sup> day	537.36 ± 4.14	5×10 <sup>5</sup>	absent
10 <sup>th</sup> day	461.55 ± 7.23	4×10 <sup>5</sup>	absent
20 <sup>th</sup> day	346.87 ± 5.19	4×10 <sup>5</sup>	absent
30 <sup>th</sup> day	305.1 ± 3.04	4×10 <sup>5</sup>	absent

SCC -somatic cell count; TBC - total bacteria count



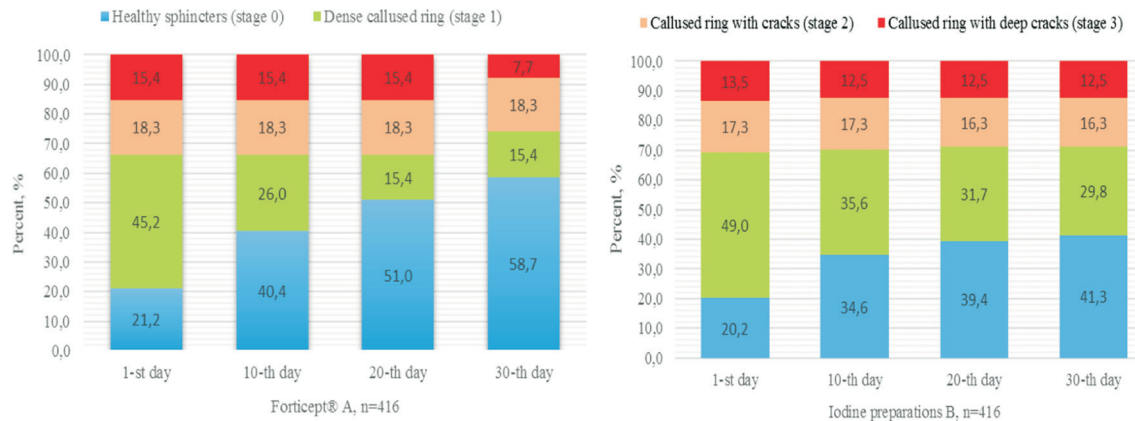


Fig. 2. Effects of antiseptic hygiene products for the condition of the teats (n) with different stages of hyperkeratosis

The applied dipping procedures during the month of the experiment in both groups significantly ( $P < 0.05$ ) improved hygienic quality of milk (Table 3). But only in experimental group A, after the use of Forticept® Udder Wash and Forticept® Udder Forte preparations, did the bacteriological indicators of milk comply with the highest national standards according to DSTU 3662-97 (State Standard of Ukraine).

The safety of testing Forticept® products was confirmed by the absence of inhibitors in milk samples in the experimental group.

In group A, due to effective dipping with Forticept®, an increase in test day milk yield (TDMY) was observed from  $29.17 \pm 0.02$  kg on the 1<sup>st</sup> day, to  $29.51 \pm 0.02$  kg on the 30<sup>th</sup> day, *i.e.* by 1.15% ( $P < 0.05$ ). The dynamics of TDMY in group A during the experiment period showed an upward trend (Fig. 3).

In group B, a downward trend in TDMY was observed over the same period. The decrease in the indicator at the control points (1<sup>st</sup> and 30<sup>th</sup> days) was 0.6% ( $P < 0.05$ ): from  $29.08 \pm 0.02$  kg to  $28.9 \pm 0.02$  kg.

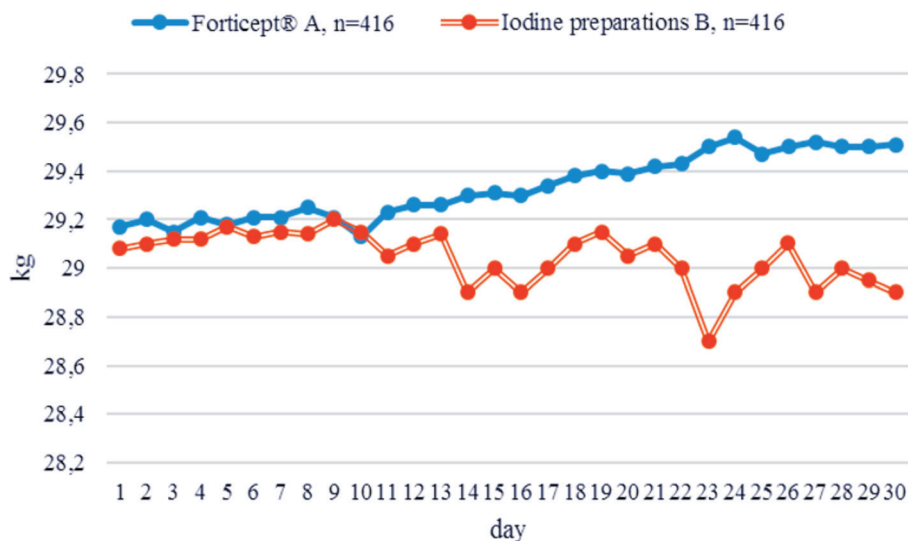


Fig. 3. Changes in test day milk yield under the influence of antiseptic hygiene products

## Discussion

Dairy farming in Ukraine, as in other countries (ABATE and WOLDE, 2015; AZEVEDO et al., 2016; ABEBE et al., 2016; KOUŘIMSKÁ et al., 2014), encounters the main problem of intensive exploitation of dairy cows: the vulnerability of highly productive animals to the influence of external negative factors. This primarily manifests itself in the form of mammary gland inflammation, mainly in SMF.

The physical, chemical and biological indicators of milk significantly worsen even in the early stages of mastitis. In the absence of clinical symptoms of the disease, pathological and inflammatory processes are already developing in the body. Many researchers have studied the quality of milk in SMF and the prospects for its processing (SILANIKOVE et al., 2014; HOQUE et al., 2015; BOGATKO et al., 2019). In fact, our results confirmed that low fat (~ 3.3%), protein (~ 3.0%), casein (~ 2.6%) and dry matter content (~ 12.2%) are observed in the milk of cows with SMF. These data explain the figures of official national statistics, according to which in Ukraine 50% of milk satisfies the criteria for extra and high class (POPKO, 2020). It should be noted that the highest class of dairy raw materials in Ukraine, according to EU standards, is assessed as only "acceptable". Thus, studies aimed at increasing the productivity of dairy enterprises in Ukraine are extremely relevant for the national economy.

The usual hygiene procedures (massage, washing the udder) do not provide reliable protection for cows from SMF. According to the latest data, the main reason of this is the open teat canal after milking, the gateway to pathogenic microflora, which is often resistant to most classical antiseptics (NETO et al., 2015; NAQVI et al., 2018). In this regard, the development of new generation germicides is a trend in veterinary pharmacology worldwide (SEMACAN et al., 2012; KNIGHT-JONES et al., 2016).

In this experiment, dipping with Forticept® preparations showed high therapeutic and prophylactic anti-mastitis efficacy (EC 73.8 and 32.4%, respectively). This result was achieved by intensive sanitation of the udder before milking

with Forticept® Udder Wash solution, and regular use of film-forming Forticept® Udder Forte after milking, which prevented microbial colonization of the teat canal and further contamination of the milk.

Hyperkeratosis of the teats is often a favourable factor in the development of inflammation of the udder. Significant lesions in the udder epidermis (for example, deep cracks) create the conditions for the penetration of intramammary infection pathogens into the mammary gland (NEIJENHUIS, 2004; ELESIN, 2010; GRAFF et al., 2007). Thus, in addition to antibacterial components, an ideal product for hygienic treatments of the udder before milking should contain udder skin care substances. To this end, Forticept® formulas successfully combine AS and additional components. The patented composition enhances the synergy of a cleansing and antibacterial effect, and contributes to the improvement of epidermis and the neutralization of bacteria on the teats. Our own experimental data indicate that Forticept® 30-day hygiene procedures helps to treat cows with uncomplicated stage 1 hyperkeratosis, and to facilitate the course of stage 3 advanced hyperkeratosis.

Researchers often overlook the fact that the degree of teat hyperkeratosis development correlates with an increase in SCC and total bacterial contamination of raw milk (RODRIGUES et al., 2009). At the same time, the close correlation between the SCC in milk and the TDMY has been proven (PINEDO et al., 2009).

After applying Forticept® products and improving the condition of the skin and udder parenchyma (Fig. 2), a significant (up to 4 times,  $P < 0.05$ ) decrease in SCC was observed in the cows' milk (Table 3). This was additional evidence of the beneficial effect on the mammary gland physiology. There was also a significant ( $P < 0.05$ ) increase in the milk components - fat ( $3.94 \pm 0.08\%$ ), protein ( $3.35 \pm 0.07$ ), casein ( $3.15 \pm 0.09\%$ ), and dry matter ( $14.91 \pm 0.28\%$ ), which increased the technological value of the milk for further processing.

Therefore, Forticept® Udder Wash as a means for hygienic treatment of cows' udders before milking, and Forticept® Udder Forte after it, may be considered effective and safe products

for the prevention and control of SMF and teats' hyperkeratosis in a specialized livestock complex. In addition, the regular use of these products leads to an improvement in the physical and chemical parameters of cows' milk. Also, the use of Forticept® products results in a stable milk yield, with the tendency of growth. We recommend using these products for rotation with classic hygienic disinfectants (including iodine-containing) in order to reduce the incidence of SMF and the udder skin lesions in cows for machine milking.

### Conclusions

Regular 30-days use of the products Forticept® Udder Wash before milking and Forticept® Udder Forte after milking, prevents (96.4% of cases) subclinical mastitis and heals already affected udder quarters (59.6%). The patented composition of the active substances positively affects the condition of the mammary gland skin, and can be used to prevent and facilitate the teats' hyperkeratosis. The use of these antiseptics leads to an improvement in the chemical composition of the milk (in the experiment, an increase in the percentage of fat, protein, lactose, casein and dry matter was observed,  $P < 0.05$ ) and a significant reduction in its bacterial contamination (according to the standards of highest grade milk according to DSTU 3662-97, State Standard of Ukraine). Thus, the use of innovative Forticept® products in integrated cow udder care scheme leads to an increase in the amount of high-class milk produced.

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#### **SAŽETAK**

Kvaliteta kravljeg mlijeka i prisutnost mastitisa uvelike ovise o razini higijene vimena. Ovo je istraživanje provedeno kako bi se ustanovila učinkovitost proizvoda Forticept® Udder Wash i Forticept® Udder Forte, upotrijebljenih prije i poslije mužnje, na liječenje i prevenciju supkliničkog mastitisa i hiperkeratoze vimena u krava. Ukupno 6880 uzoraka mlijeka uzeto je od 430 krava, dobi 3 – 4 godine, tjelesne mase 470 – 490 kg, 60. ± 15. dan laktacije. Terapeutski koeficijent učinkovitosti (EC) polimera Forticepta®, temeljen na benzetonijevu i benzalkonijevu kloridu, na supklinički oblik mastitisa bio je 73,8 % u usporedbi s tvarima koje sadržavaju jod a preventivni koeficijent učinkovitosti (EC) 32,4 %. Higijena vimena uranjanjem u Forticept® 30. dan smanjila je broj slučajeva hiperkeratoze bradavica 1. – 3. stadija sa 78,8 % na 41,3 %. Također, u tom je razdoblju uočen znakovit napredak ( $P < 0,05$ ) u kemijskim pokazateljima mlijeka – porast sadržaja masnoće (do  $3,94 \pm 0,08$  %), proteina ( $3,35 \pm 0,07$  %), kazeina ( $3,15 \pm 0,09$  %) i suhe tvari ( $14,91 \pm 0,28$  %). Nakon upotrebe Forticepta® ukupan broj bakterija (TBC) u mlijeku smanjen je na  $3 \times 10^5$  CFU/cm<sup>3</sup>, a broj somatskih stanica (SCC) na 130 000/cm<sup>3</sup> ( $P < 0,05$ ). Dnevni prinos mlijeka (TDMY) porastao je za 1,15 % zahvaljujući učinkovitoj primjeni Forticepta® ( $P < 0,05$ ).

**Ključne riječi:** Forticept®; hiperkeratoza; supklinički mastitis; higijena; mlijeko

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