Part 1. Veterinary medicine

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EFFICACY OF UDDER HYGIENE PRODUCTS OF 'FORTICEPT' LINE IN THE PREVENTION OF SUBCLINICAL MASTITIS IN COWS

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Summary. The aim of the research was to test experimental samples of udder hygiene products 'Forticept Udder Wash' and 'Forticept Udder Forte' in the prevention of subclinical mastitis in cows. To study the effect of experimental products for udder treatment before and after milking of the line 'Forticept', two groups of cows (n = 48) were formed in PE 'Demetra-2010' (Kamianets-Podilskyi District, Khmelnytskyi Region). Animals of the experimental group were treated with experimental samples of drug 'Forticept Udder Wash' before milking and 'Forticept Udder Forte' after milking. Cows of control group were treated with the drug for udder hygiene based on iodine — 'Uberaseptic SB'. The criteria for selection of animals into groups were the somatic cells count (SCC) in the milk of each quarter of the udder and the quantity of mesophilic aerobic and facultative anaerobic microorganisms (QMAFAnM) in milk. All animals of the experimental and control groups were diagnosed for the presence of a subclinical form of mastitis using the reagent 'Profilac Reagent N' (Westfalia). Examination of cattle for the presence of subclinical mastitis, just on the 10th day of drug application, revealed a 2.0-fold decrease in the percentage of sick animals in the group where the 'Forticept' complex was used in comparison with control animals. At the end of the experiment, this indicator among the animals of the control group treated with the water-containing drug 'Uberaseptic SB' was 2.9 times higher than in the experimental group. On the 30th day of the survey, the prevalence of subclinical mastitis increased to 18.3% in the group of animals treated with iodine and decreased to 59.0% when treated with 'Forticept Udder Wash' and 'Forticept Udder Forte', which was 2.4 times lower. Application of 'Forticept Udder Wash' and 'Forticept Udder Forte' improved the quality of milk, in particular, there was an increase in fat content by 16.4%, digestible protein content by 10.4% ($p < 0.05$) and a decrease in protein content by 26.5% ($p < 0.05$), dry matter content by 16.5% ($p < 0,01$), and 3.3 times decrease of SCC due to a decrease of QMAFAnM by 9.5% and total bacteria count to > 100 CFU/cm^3, and relevant inflammatory products. The use of 'Forticept' complex allows to obtain stable milk yields with a tendency to increase: gross yield in the experimental group increased by 1.3% during 30 days (with a decrease in the control group by 3.7%)

Keywords: 'Forticept Udder Wash', 'Forticept Udder Forte', prevention, milk, hyperkeratosis, subclinical mastitis

Introduction. The exclusive relationships of three separate biosystems — environment–macroorganism–infectious agent (pathogen) — play a role in the emergency of mastitis. The main route of penetration into the udder is through the teat canal, which remains open for 30 min after milking, and its complete closure, in some animals, lasts up to 2 h (Skliar and Skliar, 2015). It is necessary during this period to create an artificial barrier to the penetration of pathogens. According to scientists (Smoliar, 2014; Borodina and Nosevych, 2017; Paliy, 2017), treatment of teats with special drugs after each milking, reduces the risk of mastitis by 50–70%.

Therefore, one of the elements of the mastitis prevention program on the farm is the application of udder treatment products, before and after milking.

Hygiene products used after the act of milking are subject to quite high requirements: the drugs must provide reliable protection of the teat canal from pathogenic microflora, have a stable and prolonged effect and have an emollient effect on the skin, must dry quickly, simply, easily and completely to be removed, and also be convenient and economic at use.

Today, the market of udder hygiene products is dominated by drugs based on three main active substances: chlorhexidine, iodine, and lactic acid.

Chlorhexidine-based preparations are relatively cheap and affordable, have a rapid biocidal effect, but have a somewhat narrow spectrum of action, dry the skin, and long-term use forms the resistance of pathogenic microflora.

Among the advantages over the previous group of iodine-based drugs is a wider range of antimicrobial action, the short duration of which does not cause addiction to pathogenic flora, and anti-inflammatory

properties. However, there are some disadvantages: iodine is reactive, it is difficult to combine with other substances, it harms the skin, overdrying it. Iodine preparations preferably do not have prolonged bactericidal, fungicidal, and sporicidal action (Prasanthi, Murty and Nirmal, 2012).

As for drugs based on lactic acid, they have good cosmetic properties: they have a preservative effect, moisturize the skin. However, their disadvantage is the weak antimicrobial action in skin-safe concentrations. In addition, many yeasts and molds can include lactic acid in their metabolism, which can cause fungal skin lesions (Tkachenko et al., 2017).

Currently, the line of hygienic products for udder treatment includes novelties: 'Forticept Udder Wash', which contains benzalkonium chloride and cosmetic components for skin care (chamomile and yarrow extracts) and 'Forticept Udder Forte', which includes active components of artificial (benzethonium chloride) and natural origin (thyme oil, lanolin, chamomile extracts and yarrow).

However, these drugs have not been tested in modern dairy farming in Ukraine, so the **aim of our research** was to test experimental samples of udder hygiene products 'Forticept Udder Wash' and 'Forticept Udder Forte' in the prevention of subclinical mastitis in cows.

Materials and methods. The work was carried out at PE 'Demetra-2010' (Boryshkivtsi, Kamianets-Podilskyi District, Khmelnytskyi Region) specializing in breeding Red-spotted, Black-spotted and Simmental cows.

To study the effect of experimental products for udder treatment before and after milking of the line 'Forticept' in PE 'Demetra-2010', two groups of cows were formed (n = 48). Animals of the experimental group were treated with experimental samples of drug 'Forticept Udder Wash' before milking and 'Forticept Udder Forte' after milking. Cows of control group were treated with the drug for udder hygiene based on iodine — 'Uberaseptic SB' produced by SE 'Sumy Biological Factory'.

Foaming and detergent product for hygienic treatment of the udder before milking 'Forticept Udder Wash' contains benzethonium chloride, thymol, and plant extracts. It is a product for cleaning, protection and preparation of udder teats for the act of milking. Stable foam of the drug provides long-lasting biocidal and cleansing effect.

The product was diluted before use according to the instructions, teats and udder base were treated just before milking with an exposure of 15 s, followed by thorough drying of the udder with a paper towel.

'Forticept Udder Forte' is designed for hygienic treatment of udder nipples after milking. It contains benzalkonium chloride, plant extracts of chamomile and yarrow. The prophylactic effect of the drug is to create a film barrier that accelerates the closure of the teat canal after milking, protects the nipples from adverse environmental factors and the penetration of pathogenic microflora. The drug helps to heal wounds and cracks of teats, softens the skin of teats, relieves itching and inflammation after insect bites.

The drug was applied at the end of milking, immediately after removing the milking cups, by immersing the nipples of the udder for 1–3 s in a dipper (glass for processing).

All animals of the experimental and control groups were diagnosed for the presence of a subclinical form of mastitis, using reagent 'Profilac Reagent N' (Westfalia) (which takes into account two indicators when adding the reagent: color change when the pH shifts and changes in milk consistency in the wells of the control plate).

For 30 days of the experiment, the condition of the skin of the udder teats was observed.

Qualitative and quantitative characteristics of milk were determined by control milking in both groups, before the experiment and on the 30th day of the experiment.

Qualitative indicators of milk were determined in the laboratory of PJSC 'Ternopil Dairy'.

A sampling of raw milk and its delivery to the laboratory was carried out according to DSTU 4834:2007 (DSSU, 2007) and DSTU IDF 122C:2003 (DSSU, 2003b).

Determination of the content of fat, protein, and dry matter in milk was performed on the device 'Lactan' according to DSTU 7057:2009 (DSSU, 2009).

Protein was determined by the Kjeldahl method according to DSTU 8063:2015 (UAS, 2015).

A number of ten-fold dilutions were prepared from the selected samples according to DSTU IDF 122C:2003 (DSSU, 2003b). The quantity of mesophilic aerobic and facultative anaerobic microorganisms (QMAFAnM) and the coliform count (CC) were determined according to DSTU 7357:2013 (MEDTU, 2013) and DSTU IDF 100B:2003 (DSSU, 2003a).

For determine the somatic cells count (SCC) in raw milk we used rapid tests using reagent 'Profilac Reagent N'. The reaction was recorded by controlling the formation of gel and discoloration of the milk sample due to changes in pH.

The study was performed on milk control plates directly near the animals. To do this, in each plate recess we milked 1 cm^3 of milk to the control line from the appropriate udder quarter and added 1 cm^3 of a solution of reagent 'Profilac Reagent N' from a bottle with an automatic pipette. The milk with the reagent was stirred with a glass rod for 10–15 s. The color of the mixture and the formation of a jelly-like clot were taken into account during the reaction.

The test results were evaluated according to the following criteria:

'–' — < 100,000 cells/cm^3. No coagulation of milk, liquid consistency. The samples are easily poured into small portions;

'+' — 100,000–300,000 cells/cm^3. There is a slight coagulation. Samples can be poured in portions;

'++' — 300,000–500,000 cells/cm^3. There is coagulation of milk with the formation of a small amount of gel. It is difficult to pour samples in portions;

'+++' — 500,000–1.5 million cells/cm^3. Coagulation with the formation of a gel from almost the entire sample. It is becoming increasingly difficult to pour samples into portions;

'++++' — > 1.5 million cells/cm^3. The sample was completely collapsed to form a gel. Pouring in portions is not possible.

Different ranges of SCC are classified by visible changes in the color and consistency of raw milk samples. The test responds to 100,000 somatic cells.

As for the color change, it is known that fresh milk from healthy cows has a slightly acidic reaction (pH 6.5–6.7 with fluctuations 6.3–6.9). In the case of inflammation of the udder, the reaction of milk in most cases becomes neutral or slightly alkaline (pH 7.0 and higher). However, due to the fact that the active acidity of milk in subclinical mastitis changes infrequently or insignificantly, this indicator was not considered reliable enough to detect them.

Determination of inhibitors in milk was performed using Brilliant Black Reduction Test and Rapid One Step Assay Milk Test according to DSTU ISO 13969:2005 (DSSU, 2005).

Statistical processing of the results was performed by methods of variation statistics using the program Statistica 6.0 (StatSoft Inc., USA). Nonparametric research methods were used (Wilcoxon, Mann-Whitney criteria). We determined the arithmetic mean (×), the standard error of the mean (SE). The difference between the two means was considered statistically significant at * — $p < 0.05$, ** — $p < 0.01$, *** — $p < 0.001$.

Results and discussion. Examination of cattle for the presence of subclinical mastitis, just on the 10th day of drugs application, revealed a decrease in the percentage of sick animals in the group where the complex 'Forticept' had been used. The indicators differed twice, compared to the initial mean value and the same indicator in the control group of animals.

The use of udder hygiene complex 'Forticept' significantly reduced the number of cows with a subclinical form of mastitis. It has been experimentally shown that the application of the hygiene products during one month decreased by half the number of cows with signs of the disease. At the end of the experiment, this figure among animals of the control group, which were treated with iodine-containing drug 'Uberaseptic SB' was 2.9 times higher than in the experimental group (Fig. 1).

Thus, during the survey, the average value of latent mastitis increased to 18.3% in the group of animals treated with iodine and decreased to 59.0% in animals treated with 'Forticept Udder Wash' and 'Forticept Udder Forte', which was 2.4 times lower (Fig. 2).

Among the animals of both groups there was a significant initial manifestation of the hyperkeratosis of the teat canal, which is one of the causes of mastitis. As can be seen from Fig. 3, the reduction of the disease manifestation after the application of iodine-containing agent in the control group for 30 days was only 3.1%, while in the experimental group it decreased by 20.6% with a tendency to further reduce the manifestation of hyperkeratosis.

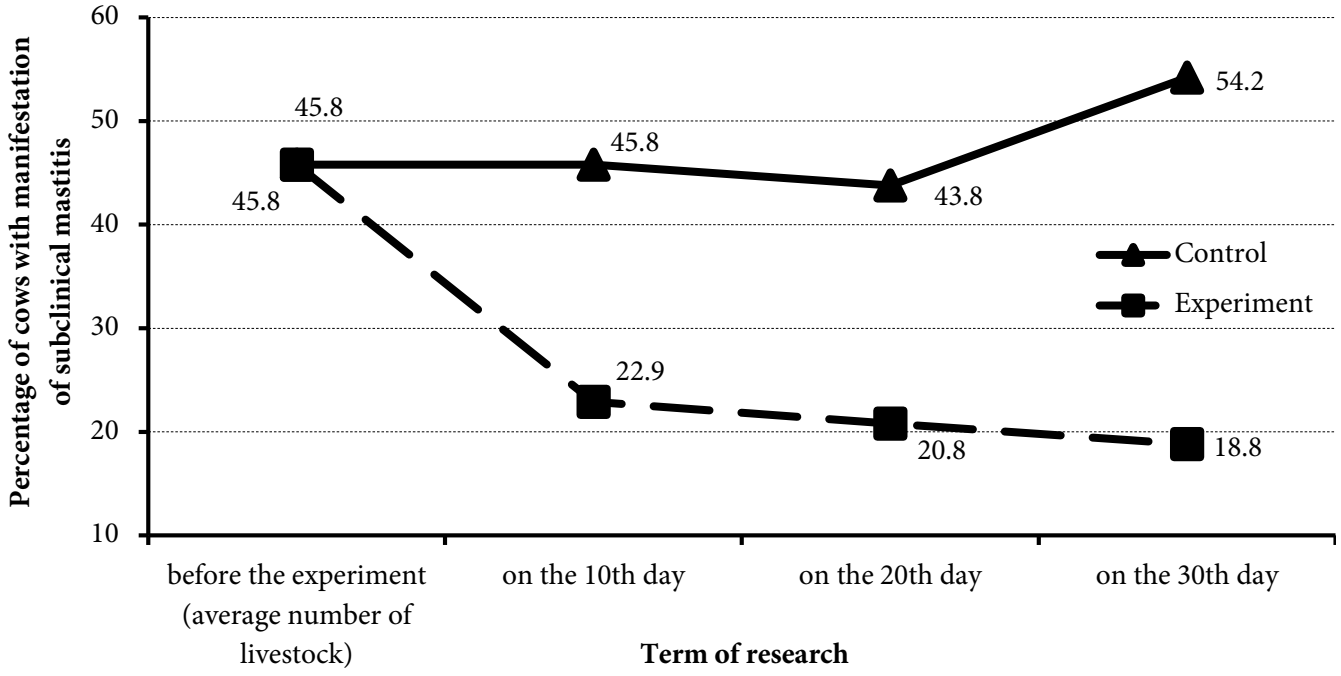

Figure 1. Dynamics of prevalence of subclinical mastitis among cows (M ± m, n = 48)

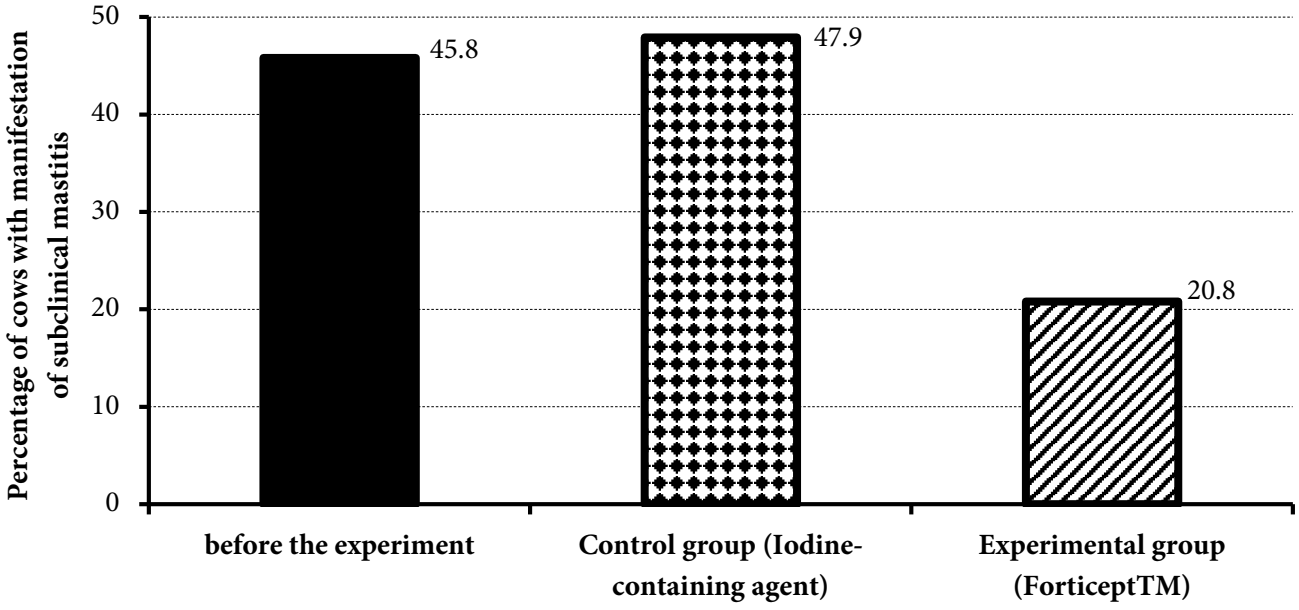

Figure 2. The average value of sick cows with subclinical mastitis for 30 days (M ± m, n = 48)

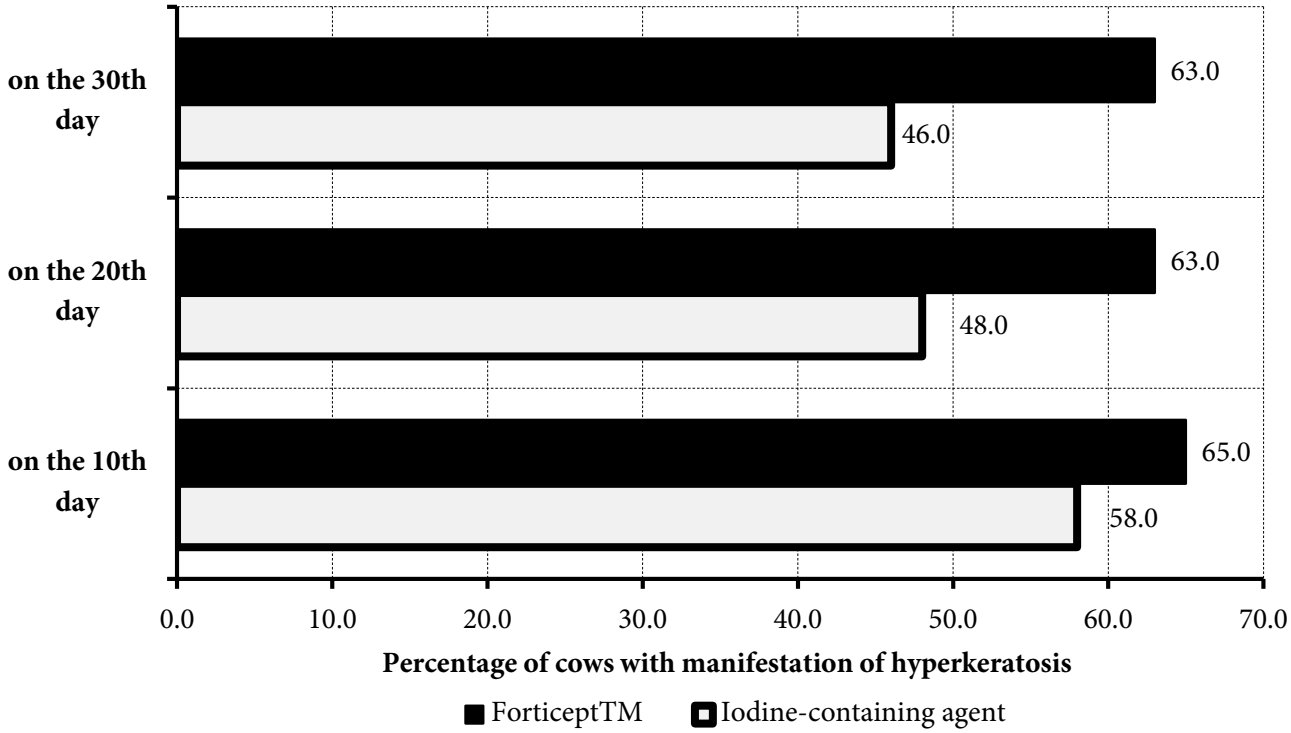

Figure 3. Dynamics of the condition of teats while using udder hygiene products in cows (M ± m, n = 48)

At the same time, on the 30th day of the experiment, there were 27.0% more animals with this pathology in the control group than in the experimental group. Laboratory studies showed that the content of fat and protein in the milk of cows of the experimental group on the 30th day after the use of drugs 'Forticept' increased by 16.4% and 10.4% ($p < 0.05$) relative to the data before the experiment, and content of protein and dry matter decreased by 26.5% and 16.5% ($p < 0.01$), respectively, whereas in the control group of animals after treatment of udder teats with iodine-containing drug reliable changes in content of fat, protein, and dry matter were not observed.

In addition to determining milk quality indicators, the antimastitis program in farms mainly depends on the control of SCC in milk and the total bacteria count (TBC) (Borodina and Nosevych, 2017; Shevchenko, Stravskyi and Sachuk, 2019). In our experiment, a significant difference in SCC in the samples of whole milk from lactating cows of the experimental and control groups was found. Thus, on the 30th day after the start of hygienic

treatments, SCC in the milk of animals treated with hygienic products 'Forticept' was 3.3 times lower, when treated with iodine-containing drug — 2.1 times.

The study of QMAFAnM in the milk of cows also revealed a reliable decrease by 9.5% ($p < 0.05$) for treatment with drugs of the series "Forticept', before treatment, this figure was $2.3 \pm 0.03 \times 10^5$ CFU/cm^3, after treatment — $2.1 \pm 0.03 \times 10^5$ CFU/cm^3, and in the control group after treatment with iodine-containing agent, this indicator increased by 31.8%. TBC in milk samples from cows of both groups before treatment was > 150 CFU/cm^3, and after treatment — > 100 CFU/cm^3. In this case, inhibitory substances in the milk samples of both groups were not detected (Table).

Table — Milk quality indicators (M ± m, n = 7)

Indicator	Experimental group		Control group	
	output data	on the 30th day	output data	on the 30th day
Fat, %	2.62 ± 0.10	3.05 ± 0.14*	2.96 ± 0.15	2.93 ± 0.13
Albumen, %	5.97 ± 0.40	4.39 ± 0.53*	3.14 ± 0.27	4.39 ± 0.61
Lactose, %	4.81 ± 0.03	4.89 ± 0.06	4.89 ± 0.04	4.79 ± 0.11
Dry matter, %	14.36 ± 0.42	11.99 ± 0.22**	13.27 ± 0.50	12.91 ± 0.56
Protein, %	2.89 ± 0.10	3.19 ± 0.14*	3.26 ± 0.13	3.18 ± 0.10
SCC, ×10^3 cells/cm^3	327.83 ± 95.38	100.42 ± 37.64*	276.50 ± 85.16	131.33 ± 38.60*
QMAFAnM, ×10^5 CFU/cm^3	2.3 ± 0.03	2.1 ± 0.03	2.2 ± 0.02	2.9 ± 0.02
TBC, CFU/cm^3	> 150	> 100	> 150	> 100
Presence of inhibitors	lack	lack	lack	lack

Notes: * — $p < 0.05$; ** — $p < 0.01$; ** — $p < 0.001$ relative to the output data.

Thus, in our opinion, the quality of milk after the action of 'Forticept Udder Wash' and 'Forticept Udder Forte' improved by increasing the content of fat and digestible protein, along with reducing the content of protein, dry matter and SCC, possibly by reducing QMAFAnM and inflammatory products.

An important factor influencing the economic indicators of the dairy industry is the amount of milk yield. Many scientists have proven a close correlation between SCC and milk yield (Pinedo et al., 2009). Even a small increase of SCC can be considered the most sensitive indicator of reduced productivity of cows. Thus, control milking showed somewhat different dynamics of volumes of milk obtained from cows in groups.

It was found that from the experimental group before the start of the experiment 1,200 l of milk was milked, and on the 30th day after the start of the use of hygienic products 'Forticept' milk production increased by 1.3% and amounted to 1,215 l.

At the same time, in the control group milk yields decreased by 3.7% during this period from 1,396 to 1,345 l (Fig. 4).

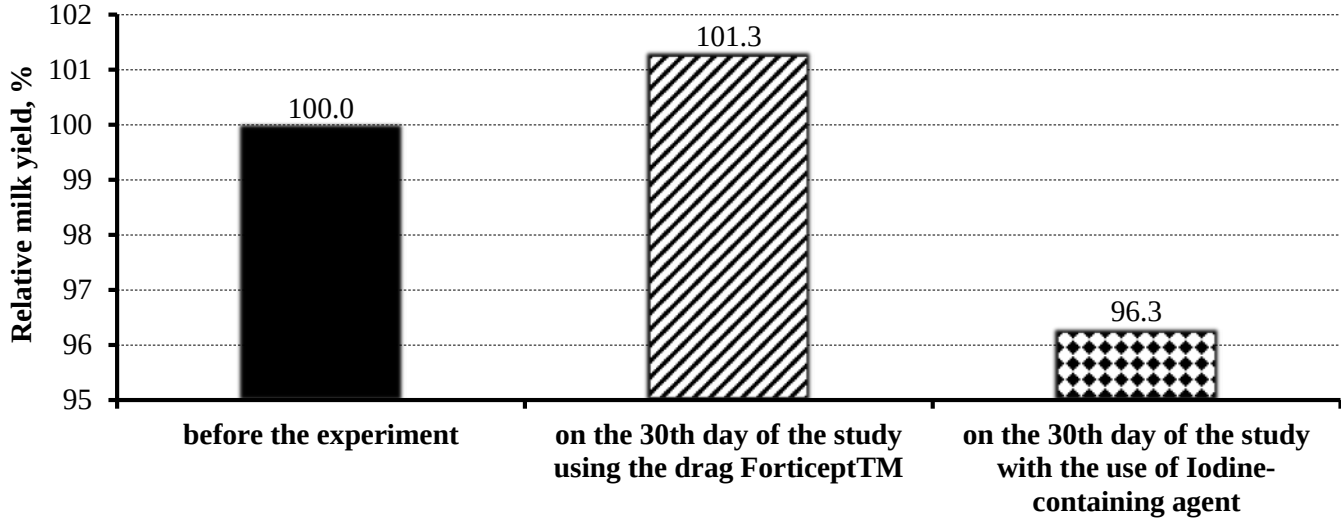

Figure 4. Relative milk yield after the application of udder hygiene products (M ± m, n = 48)

Conclusions. 1. The application of udder hygiene products 'Forticept Udder Wash' and 'Forticept Udder Forte' on the 30th day after treatment reduced the number of cows with subclinical mastitis by 59.0% and cases of hyperkeratosis by 20.6%.

2. There was an improvement in milk quality after the action of 'Forticept Udder Wash' and 'Forticept Udder Forte', including an increase in fat content by 16.4%, digestible protein by 10.4% ($p < 0.05$) and a decrease in protein by 26.5% ($p < 0.05$), dry matter by 16.5% ($p < 0.01$) and 3.3 times decrease of SCC, possibly due to a decrease of QMAFAnM by 9.5% and TBC to > 100 CFU/cm^3, the corresponding products of inflammation.

3. The use of drugs of the 'Forticept' complex allows to obtain stable milk yields with a tendency to increase: the gross milk yield in the experimental group for the 30 days increased by 1.3% (with a decrease in the control group by 3.7%).

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