A Study of Stability and Antimicrobial Efficacy of a New Model Teat Dip Solution Containing Lactic Acid

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Abstract. *The aim of this study was to make a new teat dip formula containing lactic acid and evaluate its stability and antibacterial activity by physico-chemical and microbiological tests.*

*After selecting the concentrations of the thickener xanthan gum (0.67%) and lactic acid (6.3%), a teat dip solution was composed, which contains teat skin saving components: glycerol, sorbitol, oat and calendula extracts. According to the recipe, a teat dip solution of orange colour, homogeneous appearance, and a specific lactic acid smell was composed, the viscosity of which was 930–940 mPa*s., pH 2.7. Product physical, chemical properties and stability for 24 months were confirmed by real-time and accelerated stability studies. The teat dip solution remains homogenous, orange colour with a typical lactic acid smell, and did not become cloudy or flaky after 24 months following production. Viscosity reduction was determined after 6 months 33 mPa*s, i.e. it was 3.4%, while after 24 months, it was 12.7%. The measured pH value remains stable between 2.6 and 2.8 ($P > 0.05$). An antimicrobial study was performed according to EN 1656 by 80%, 50% and 10% concentrations with reference Staphylococcus aureus, Streptococcus uberis and Escherichia coli strains. The teat dip solution showed bactericidal efficiency (log $R > 5$) against S. uberis and E. coli reference strains. An antimicrobial effect to S. aureus was insufficient and the product was not effective against this bacteria. Teat dip at 10% did not show an antimicrobial effect to all tested strains.*

According to these data, the new model teat dip solution containing lactic acid, teat skin saving substance and thickener xanthan gum retains unchanged physical parameters and shows bactericidal activity against the reference E. coli and S. uberis strains.

Introduction

The bacterial colonization on teat skin is an important source for intramammary infection (Hassan et al., 2016). Good sanitation practices can reduce the number of bacteria on teat skin and improve the milk quality, especially the pre-milking and post-milking teat dip (Kučević et al., 2013). Teat disinfection is an important step in the control of mastitis within a dairy herd (Breen, 2019; Fitzpatrik et al., 2021). Depending on the hygiene and sanitary condition of the farm and the method of udder preparation, bacterial contamination of raw milk can decrease by 90%, and the cases of mastitis by 50–75%. Many studies have shown that well-performed teat hygiene reduces the spread of microorganisms that cause inflammation of the mammary gland, and the quality of milk improves. Various sprays, cleaners and teat dip solutions are used today for this purpose. The most widely used procedure is teat dipping. Post-milking dip solutions must have antimicrobial activity, cover the teat surface well and stay on it, moisturize without skin irritation (Oliver & Murinda, 2012; Mišeikienė et al., 2015). Therefore, products intended for cow udder and teat antiseptic – biocides – must contain a bactericidal, disinfecting substance, skin-protecting components and a thickener – a viscosity-forming substance. The following active substances can be found in veterinary biocides: chlorhexidine, iodine and its compounds, lactic acid, peracetic acid, hydrogen peroxide, and natural polymers guar or xanthan gum are used as thickening components (Nickerson, 2001; Fitzpatrick & Garvey, 2019; Chotigarpa & Lampang, 2019). There are certain requirements for teat dip solutions which are intended for the disinfection of cow udders. One of the most important is the viscosity. After applying this product to the nipple skin, a continuous film should be formed; its purpose is the nipple canal protection from the entry of infectious microorganisms (Garvey & Curran, 2016). The viscosity of solutions can be adjusted by selecting different types of thickeners, so natural thickeners are more common in veterinary medicine pharmacy as well as food industry (Alves, 2020).

Teat dip solutions must contain a disinfectant that does not harm the animal or veterinary and husbandry staff. Lactic acid is widely used in veterinary medicine because it is active against gram-positive and gram-negative bacteria and is not a toxic or dangerous substance. Teat dip solutions based on lactic acid are less harmful to the animal's skin. According to some studies, teat dip solutions which contain 5% lactic acid have noticeable antimicrobial activity against *E. coli*. The previous results have shown that the lactic acid inhibited the growth of other Gram-negative

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bacteria such as *Shigella* sp., *Salmonella* Enteritidis, and *Listeria monocytogenes* at 0.5% (In et al., 2013; Wang et al., 2015). Fitzpatrick et al. (2021) also say that, for streptococcal isolates, the product (5% w/w lactic acid) resulted in the numerically largest bacterial reduction of 70.1%.

An important feature of veterinary biocides is that the stability of the solution formula should stay throughout the product's shelf life (European chemicals agency, Guidance on the Biocidal Products Regulation). It means that the teat dip solution should retain stability of physical, chemical properties and show no changes of active substance. Lack of stability leads to diminishing antimicrobial properties.

The aim of this study was to create a new formula teat dip and antimicrobial efficacy and stability evaluation of the new model teat dip solution by physico-chemical and microbiological tests.

Materials and methods

The investigation was carried out in the Institute of Microbiology and Virology (LUHS) and Good Laboratory Practice laboratory (Vimodrone, Italy). A solution formula was developed by selecting and changing the concentration of xanthan gum and dyes so that teat dip properly covered the teat skin, persisted on the teat a required time and properly coloured the teat skin after dipping. The teat dip solution with lactic acid was produced using pharmacological substances (see Table 1 for composition) according to the technology where dry plant extracts and dyes are dissolved in a portion of purified water and filtered. The filtrate is mixed with sorbitol and glycerine compound and stirred until the solution becomes clear completely. Xanthan gum with the rest of the purified water is added with intensive mixing, and the suspension is homogenized to uniform consistency by a homogenizer (IKA T25 Ultra Turrax). Lactic acid (active substance) is added, and the solution is mixed again with the help of a laboratory mixer (IKA Eurostar 20 digital) to a homogeneous mass. This product is left in a sealed container and, after 24 hours, physico-chemical parameters are determined. The stability testing was carried out according to the guidelines on stability testing of cosmetic products (Colipa, 2004) and guidelines for assessing shelf life using real-time and accelerated stability tests (Magari, 2003). In the real-time study, the product was stored under recommended conditions, and changes of product properties were observed during time intervals (after 24 hours, 6 months, 12 months, and 24 months). When testing the teat dip in an accelerated way, it was placed in stressful conditions:

Table 1. The new model teat dip solution composition (100 g of the final product)

Pharmacological composition/ Chemical compound	Producer	CAS Number	Molecular mass	Compound, g	Notes
L-(+)-lactic acid $C_3H_6O_3$ Also indicated as l-(+)-2-hydroxypropanoic acid	Purac Biochem, The Netherlands	79-33-4	M = 90 g/mol	6.3	pH adjustment and disinfectant
Xanthan gum $C_{35}H_{49}O_{29}$ (monomer)	CP Kelco France, France	11138-66-2	M = 181.21 g/mol	0.67	Thickener
Dye CI 15985 Disodium 6-Hydroxy-5-[(4-Sulphonatophenyl)Azo]Naphthalene-2-Sulphonate	Neelikon Food Dyes & Chemicals Ltd, India	2783-94-0	M = 452.37 g/mol	0.03	Dye
Calendula officinalis dry extract (Extract Ratio 5:1)	Gonmisol, Spain	84776-23-8	The extract is the mixture of different substances (no molecular mass)	0.03	Antioxidant and anti-inflammatory effect
Avena sativa dry extract (Extract Ratio 4:1)	nVH Italia srl, Italy	84012-26-0	The extract is the mixture of different substances (no molecular mass)	0.02	Antioxidant, moisturizing effect
Glycerol 1,2,3-propantriol, $C_3H_5(OH)_3$	Aarhus Karshams Sweden AB, Sweden	56- 81-5	M = 92 g/mol Purity – 100%	9.00	Skin emollient
Sorbitol $H(CHOH)_6H$	Cargill Deutschland GmbH, Germany	50-70-4	M = 182.17 g/mol	1.00	Moisturizer
Water, H_2O		7732-18-5	M = 18.01 g/mol	Up to 100 g final product	Diluent

higher temperature, more intense freezing-heating cycle. The accelerated stability test program was carried out after 24 hours of manufacture, and after 2 months. Tests evaluate chemical, physical stability and sensory properties: colour, smell, homogeneity.

Samples of the developed teat dip containing lactic acid were used for further stability studies. Samples were selected and taken in accordance with LST EN ISO 9001 (Quality Management System) and LST EN ISO 227716 (Good Manufacturing Practices for Cosmetic Products) standards. Viscosity was measured at 20°C with a rotary (NDJ-1, COMECTA SA) viscometer. The pH measurement was carried out by a standard potentiometric test method with a laboratory pH-meter (InoLab pH 7310). Homogeneity, smell, and colour were determined by a visual method in the Light Cabinet (Byko-Spectra basic).

The microbiological examination was carried out according to MP-S_SVP-6:2020 (Edition I) and the European Pharmacopoeia (7th edition, 2.6.12). Bacteria colonies were counted on Mueller-Hinton Agar II, (BBL, Cockeysville, USA) after incubation at 35°C for 24 hours in aerobic conditions. The total number of aerobic microorganisms CFU/g in the test sample was calculated according to the formula and the final result was given as the number of microorganisms (CFU)/g: $N = \sum C/V \times 1.1 \times d$, kr: $\sum$C is the sum of the colonies counted in the two evaluated plates from two serial (one by one) dilutions.

Bactericidal activity of the teat dip containing lactic acid was determined according to the standard EN: 1656/2010/AC: 2011 at Good Laboratory Practice laboratory (Vimodrone, Italy). Briefly: 80%, 50%, and 10% concentration teat dip was tested on *S. aureus* ATCC 6538, *S. uberis* ATCC 19436, *E. coli* ATCC 10536. Test conditions: temperature 30 ± 1°C. Exposure time: 5 min. The nutrient medium Tryptone Soya Agar (TSA) was used in these tests. The indicator of biocide activity against the microorganisms used in the study is expressed as a logarithm. A teat dip solution is considered as bactericidal active if the logarithm value obtained during the test exceeds 5, i.e. log R > 5.

The final product was tested for contamination of *S. aureus, P. aeruginosa* and *C. albicans*.

Statistical analysis. Obtained research results were statistically processed using statistical data analysis packages: SPSS 19.0, Microsoft Office Excel 2007. Statistically reliable differences in arithmetic means when $P < 0.05$ between two groups were determined.

Results

The manufacturer's specification for xanthan gum as a thickener has a wide concentration range from 0.5% to 2.5%. According to the limits set by the supplier, the tests were started from the lowest concentration, and it was increased until the required viscosity value was reached and the minimum amount of the thickener was used.

Thus, the determination of the xanthan gum composition started from the lowest amounts, and during the tests, the amount of xanthan gum was increased to 0.67%. We found that this concentration of a thickener was sufficient for the product to meet the viscosity requirements. After laboratory tests, the final teat dip solution viscosity was set to 930–940 mPa*s. After a series of tests, according to the recipes (Table 1), a teat dip solution of orange colour, homogeneous appearance, specific lactic acid smell and pH 2.7 was created. Quality assurance tests were made for possible variation of pH, viscosity, sensory properties (colour, odour and homogeneity) and microbiological parameters.

The tested teat dip solution remained homogeneous throughout the whole stability testing period, bright orange in colour, characteristic of a lactic acid smell. It did not become cloudy, and did not fade after 6, 12, and 24 months after production (Table 2). Changes in the teat dip solution with lactic acid sensual properties were not detected.

Measuring the pH value showed that it remained stable ($P > 0.05$). Evaluation in a real-time programme showed that the pH value did not change statistically significantly. The pH value was in the range of 2.6–2.8, so it was found that the teat dip solution met the initially set requirements. After 24 months, the average value of pH decreased slightly – 0.01 ($P > 0.05$) in the samples from the three tested series in the real-time stability program. After correlation analysis evaluation, it was found that there were statistically significant linear relationships between pH in all teat dip solutions lots. The pH value stability test showed that the teat dip solution containing lactic acid properties did not change significantly ($P > 0.05$) during the 24-months stability testing period. The obtained data are presented in Table 2.

Teat dip solutions viscosity decreased insignificantly during the real-time stability program ($P > 0.05$). A decrease of viscosity was determined after 6 months to 33 mPa*s, compared with the manufactured product: it was 3.4% lower than after production after 6 months, 7.5% after 12 months, and 12.7% after 24 months (Table 2).

The microbiological quality of the new formulated teat dip solutions containing lactic acid was assessed after preparation (emulsion) and the samples were stored and tested at intervals specified in the real stability program (Table 2).

High manufacturing standards of hygiene and components with antimicrobial activity ensure that microbial growth was mostly inhibited in the newly manufactured teat dip solution (Table 2). Total aerobic bacteria count was 1.0×10^1 CFU/g (mL). Microorganisms such *P. aeruginosa, S. aureus, C. albicans* in 0.1g of the tested final teat dip solution samples were not detected. During the accelerated stability program, colour, homogeneity, and odour of the new formulated teat dip solution remained

Table 2. The new model teat dip solution containing lactic acid stability data

Time of evaluation	Homogeneity	Colour	Smell	pH	Viscosity, mPa*s	Total count of aerobic bacteria CFU/g (mL)	*P. aeruginosa*, *S. aureus*, *C. albicans*
Batch No. 1							
After 24 h	Homogeneous	Orange	Specific lactic acid	2.7	940	$< 1.0 \times 10^1$	Unidentified
After 6 months	Homogeneous	Orange	Specific lactic acid	2.6	910	$< 1.0 \times 10^1$	Unidentified
After 12 months	Homogeneous	Orange	Specific lactic acid	2.7	870	$< 1.0 \times 10^1$	Unidentified
After 24 months	Homogeneous	Orange	Specific lactic acid	2.6	820	$< 1.0 \times 10^1$	Unidentified
Batch No. 2							
After 24 h	Homogeneous	Orange	Specific lactic acid	2.8	940	$< 1.0 \times 10^1$	Unidentified
After 6 months	Homogeneous	Orange	Specific lactic acid	2.8	900	$< 1.0 \times 10^1$	Unidentified
After 12 months	Homogeneous	Orange	Specific lactic acid	2.8	870	$< 1.0 \times 10^1$	Unidentified
After 24 months	Homogeneous	Orange	Specific lactic acid	2.6	830	$< 1.0 \times 10^1$	Unidentified
Batch No. 3							
After 24 h	Homogeneous	Orange	Specific lactic acid	2.6	930	$< 1.0 \times 10^1$	Unidentified
After 6 months	Homogeneous	Orange	Specific lactic acid	2.6	900	$< 1.0 \times 10^1$	Unidentified
After 12 months	Homogeneous	Orange	Specific lactic acid	2.7	860	$< 1.0 \times 10^1$	Unidentified
After 24 months	Homogeneous	Orange	Specific lactic acid	2.6	830	$< 1.0 \times 10^1$	Unidentified

unchanged (Table 3). After the centrifugation tests (speed 45 rpm, time 8 min), the investigated samples of the product did not change; they remained stable during the entire study. Samples are considered stable if the product phase does not separate during centrifugation at the specified mode. At this stage of testing, the total number of aerobic microorganisms in 1 g (mL) did not exceed the permitted amounts, no microbial strains of *P. aeruginosa*, *S. aureus*, and *C. albicans* were detected. The accelerated stability program did not detect possible microbiological contamination.

No pH value deviation from the initial value was observed (Table 3) when samples were stored at a lower temperature. It can also be stated that no change in pH value occurred in the samples during freezing/heating cycles and these data remain stable; no statistically reliable decrease in pH value ($P > 0.05$) was found. From the data presented in Table 3, it can be seen that the harder experimental conditions did not have a statistically significant effect on the pH value; the pH value varies in stable and appropriate characteristic limits of the product. During this study, antibacterial activity (*in vitro*) of teat dip solutions was determined by the ability to reduce the total number of microorganisms used in testing. Results were evaluated according to the standard.

The teat dip solution antimicrobial efficiency test was carried out by evaluating bactericidal tests against reference strains *S. aureus S. uberis*, and *E. coli*, according to the EN 1656 standard. The teat dip solution is considered as bactericidal effective if the logarithm value obtained during the test exceeds 5. The test was performed with 80%, 50% and 10% teat dip solution concentrations. Bactericidal testing showed that 80% and 50% teat dip solution concentrations were bactericidal active against *S. uberis* ATCC 19436 and *E. coli* ATCC 10536 strains (log R > 5, Table 4). After diluting the teat dip solution to 10%, it was not effective in the performed study.

Discussion

The new formulated teat dip solution contains components such a thickener, an antimicrobial active ingredient, moisturizing, teat skin-friendly ingredients, and water. When developing new products and preparing chemical mixtures, it is important that the product is not harmful to the animal and friendly to the environment. For teat dip solutions and other skin surface disinfectants, classification of the chemical mixture is very important. Therefore, during the creation of a new formula teat dip solution, the

Table 3. The new model teat dip solution containing lactic acid data during accelerated stability program

Time of evaluation	Homogeneity	Colour	Smell	pH	Total aerobic microorganisms count CFU/g (mL)	Centrifugation, speed 45 rpm, time 8 min
Batch No. 1						
After manufacturing	Homogenic	Orange	Specific lactic acid	2.7	$< 1.0 \times 10^1$	Stabile
Heating/cooling cycles (10 d. at +4°C; 10 d. +45°C; 10 d. +4°C; 10 d. +45°C; 10 d. 15°C)	Homogenic	Orange	Specific lactic acid	2.7	$< 1.0 \times 10^1$	Stabile
After 2 months. keep at +45°C	Homogenic	Orange	Specific lactic acid	2.6	$< 1.0 \times 10^1$	Stabile
Batch No. 2						
After manufacturing	Homogenic	Orange	Specific lactic acid	2.8	$< 1.0 \times 10^1$	Stabile
Heating/cooling cycles (10 d. +4°C; 10 d. +45°C; 10 d. +4°C; 10 d. +45°C; 10 d. 15°C)	Homogenic	Orange	Specific lactic acid	2.7	$< 1.0 \times 10^1$	Stabile
After 2 months at +45° C	Homogenic	Orange	Specific lactic acid	2.8	$< 1.0 \times 10^1$	Stabile
Batch No. 3						
After manufacturing	Homogenic	Orange	Specific lactic acid	2.6	$< 1.0 \times 10^1$	Stabile
Heating/cooling cycles (10 d. +4°C; 10 d. +45°C; 10 d. +4°C; 10 d. +45°C; 10 d. 15°C)	Homogenic	Orange	Specific lactic acid	2.7	$< 1.0 \times 10^1$	Stabile
After 2 months at +45°C	Homogenic	Orange	Specific lactic acid	2.6	$< 1.0 \times 10^1$	Stabile

classification of the used thickener and antimicrobial substance was taken into account according to the European Parliament and Council Regulation (EC) No. 1272/2008. Xanthan gum is a natural polymer and does not pose a risk to animal health or the environment under this regulation. Also, lactic acid is recognized as safe for use in animal husbandry for surface disinfection.

Disinfectant effect and sufficient thickness are important in biocidal teat dip solutions. Viscosity should be sufficient to form a non-drop emulsion when the teat dip solution is applied to the skin of the teat. Therefore, viscosity is an important physicochemical parameter of the teat dip solution, reflecting the quality of the product (Elella, 2020; Petri, 2015). In our study, we paid high attention to obtain the required viscosity. In the study, we chose the natural material that forms the viscosity in the new formula product. Xanthan gum is sufficient when inserted into the product at 0.67%. The product viscosity was statistically reliably stable throughout the entire study period, i.e., 24 months after manufacture.

A disinfectant is a necessary component in teat dip. In veterinary and animal husbandry, one of the most widely used biocides is lactic acid. Lactic acid is recognized by the US and the European Union Commission as a safe food additive for health and its quantities are unlimited. This compound is environmentally friendly and exhibits antimicrobial activity (Storton, 2010). Also, the 2017 EU regulation

Table 4. Antimicrobial activity (log R) of the new model teat dip solutions containing lactic acid (80%, 50%, 10%) according to standard UNI EN 1656

Test microorganisms	Test suspension	Results		
		80%	50%	10%
S. aureus ATCC 6538	10^{-6}: >330–>330* 10^{-7}: 46–35* N: 4.05 x 10^{8} No: 4.05 x 10^{7} log No: 7.61	Vc: >330–>330 Na > 3300 log Na > 3.52 log R < 4.09 Not active	Vc: >330–>330 Na > 3300 log Na > 3.52 log R < 4.09 Not active	Vc: >330–>330 Na > 3300 log Na > 3.52 log R < 4.09 Not active
S. uberis ATCC 19436	10^{-6}: >330–>330* 10^{-7}: 42–30* N: 3.60 x 10^{8} No: 3.60 x 10^{7} log No: 7.56	Vc: <14–<14 Na < 140 log Na < 2.15 log R > 5.41 Active	Vc: <14–<14 Na < 140 log Na < 2.15 log R > 5.41 Active	Vc: >330–>330 Na > 3300 log Na > 3.52 log R < 4.04 Not active
E. coli ATCC 10536	10^{-6}: >330–>330* 10^{-7}: 44–49* N: 4.65 x 10^{8} No: 4.65 x 10^{7} log No: 7.67	Vc: <14 – <14 Na < 140 log Na < 2.15 log R < 5.52 Active	Vc: <14–<14 Na < 140 log Na = 2.15 log R = 5.52 Active	Vc: >330–>330 Na > 3300 log Na > 3.52 log R < 4.15 Not active

* number of colonies (CFU) in test suspension dilutions; N = number of CFU/mL of the test suspension; Vc = viable count; Na = number of CFU/mL of the test mixture; R = reduction in viability. No – number of CFU/mL in the test mixture (diluted).

(https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX:32017R2002) confirmed that lactic acid can be used in different types of veterinary products intended for animal skin or surface disinfection. Lactic acid products have been shown to have antibacterial activities against Gram-positive and Gram-negative bacteria (Boomsma et al., 2015). These ingredients are used in food and cosmetics as preservatives and also have low toxicity; lactic acid is less sensitive to skin irritation (Alsaheb et al., 2015). Many commercial teat antiseptic products have various active ingredients including iodine, hydrogen peroxide, chlorine, and chlorhexidine. Some may cause skin irritation and bacterial resistance (Sadakane et al., 2015). Therefore, pre-parations of natural products are desirable. The study of Chivero and Gohtani (2015) states that teat dip solutions with lactic acid protect the teat canal from possible infections or inflammations, and the moisturizer film remains effective until the first washing of the skin.

Our newly formulated teat dip solution showed a bactericidal effect. It was confirmed by *in vitro* study against reference isolates. Our study according to the EN 1656 standard showed bactericidal activity at 80% and 50% concentration to reference cultures, as log R > 5 was obtained. World scientists have analyzed the antibacterial effectiveness of teat dip containing lactic acid. Extensive research has been conducted (Fitzpatrick et al., 2021). These researchers tested products containing lactic acid as the main active ingredient. Lactic acid concentrations in these products ranged from 1.76% w/w to 8% w/w. For streptococcal isolates, the product with 5% w/w lactic acid resulted in a numerically largest bacterial reduction of 70.1%. The product with 2% lactic acid combined with 0.6% w/w chlorhexidine obtained the largest bacterial reduction of 100% against staphylococcal isolates. The product with 1.6% w/w lactic acid combined with hydrogen peroxide was found to result in bacterial reductions of 89.9% and 59.4% for streptococcal and staphylococcal isolates, respectively. This study suggests that some teat disinfectant products achieve a higher reduction in bacterial levels against different specific bacterial groups on teat skin than other teat disinfectant products. However, Chotigarpa (2019) found that no relationship was observed between a higher concentration of active ingredient and increased effectiveness. The scientist notes that the use of a lactic acid-based teat disinfectant reduced the bacterial load on the teat skin and decreased the prevalence of mastitis due to coliforms such as *E. coli*. Important studies on reduced susceptibility to lactic acid teat disinfectants have not been published yet; only resistance mechanisms of lactic acid bacteria coping with acid stress have been observed (Wang, 2018; Schwenker, 2022).

In assessing the quality of pharmaceutical products, stability of physio-chemical parameters is important. When conducting stability studies for biocidal products, it is important to determine whether the quality of disinfectant products is sufficient during the entire shelf life, whether the product maintains its original physical and chemical properties. In case of a decrease of viscosity, film would not form on the skin, which ensures the lowest disinfectant properties by the inability to keep the active substances where they are needed. The importance of stability studies is also defined in Regulation (EU) of the European Parliament and Council no. 528/2012, Title 2 of Annex III, which states that in order to register a

biocidal product in the European Union, it is necessary to submit the results of stability studies at low and ambient temperatures, according to which the shelf life of the biocidal product is determined. For this purpose, two types of stability programs were implemented: real-time and accelerated stability. In real-time stability studies, the product was stored under recommended conditions and monitored for changes in product properties. When testing the product in an accelerated way, the product is placed under stressful conditions: +45°C temperature, heating-freezing cycle. Both real-time and accelerated stability program tests are designed to reliably assess the following aspects: stability and physical integrity of products, chemical and microbiological stability. After carrying out stability tests of the samples and evaluating the obtained results, it was observed that the teat dip solution maintained sufficient stability when stored at low and room temperature; the pH and viscosity results of the samples also showed the stability of the composition. During the stability study, it was found that the storage conditions did not significantly affect the physical product appearance and chemical characteristics of the new formulated teat dip solution.

Microbiological quality control of pharmaceutical products is necessary to ensure their safety for the environment, the animal and health of the husbandry service personnel. Microbiological contamination of the new formulated teat dip solution was not detected by real and accelerated product stability programs. At accelerated stability program conditions, microbiological contamination did not initiate.

Conclusions

After determining the appropriate thickener xanthan gum and lactic acid concentrations, a new teat dip solution was formulated that also contains glycerol, sorbitol, oat and calendula extracts.

At real and accelerated stability program, the teat dip solution kept unchanged physical parameters such as colour, appearance, homogeneity, smell and viscosity. The pH of the teat dip solution during the study varied slightly.

The teat dip solution showed bactericidal activity against reference *E. coli* and *S. uberis* bacteria (log R > 5). The antimicrobial effect to *S. aureus* was insufficient and the considered product was not effective against this bacteria.

Conflict of Interests

The authors declare that there is no conflict of interests.

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