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Research Article

Evaluation of the Compatibility between Different Teat Sealants and Intramammary Antimicrobials in Selective Dry Cow Treatment Protocols

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Abstract

The dry period is a crucial time in the lactation cycle, it is the period of highest susceptibility to new infection and the optimum time to cure existing intramammary infection. In selective dry-cow antimicrobial therapy, we have two different ways of intervening in the management treatments of dry period: in the first case there are dairy cows diagnosed healthy, that will be treated only with the teat sealant, in the second, dairy cows with pathogenic mastitis or high cell counts that will receive the intramammary treatment together with the sealant. One of the main prerogative of an intramammary sealant is to ensure that it remains in the teat throughout the dry period. To guarantee this, it is necessary that the sealant is characterized by good rheological properties, especially when it is administered with an antimastitis ointment. The aim of the present study was to compare the rheological behavior of several intramammary sealant suspensions present on the Italian market. To evaluate the rheological behavior of the intramammary suspensions used during the dry period, rheological comparison methods have been developed. The parameters assessed during the rheological study are Viscosity, 3 Interval Thixotropy Test (3ITT) and the viscoelastic nature of materials: Amplitude Sweep. In addition, to evaluate the sealant performance of intramammary sealants, was performed a compatibility *in-vitro* test, comparing the behavior of the products alone and in contact with two intramammary antibiotic suspensions (Fatroximin and Mastout, both manufactured by Fatro S.p.A. and used during the dry period). The results obtained highlighted that, when a sealant has good viscosity and good thixotropic behavior, is more probable that it remains in the teat, carrying out its sealing action and preventing the entry of pathogens.

Introduction

In the last few decades the misuse and overuse of antinfectives, and the increase in the development of drug resistant pathogens has required urgent actions in order to achieve a reduction in use of antimicrobials in human and in veterinary sector [1]. The new European Regulation 2019/6 on veterinary medicinal products pays particular attention to fighting antimicrobial resistance in livestock and bans the preventive use of antibiotics in groups of animals; in this law prophylaxis is defined as "administration of a medicinal product to an animal or group of animals before clinical signs of a disease, in order to prevent the occurrence of disease or infection" [2]. Udder infections are the most common reason that antinfectives are used on dairy farms. For many years, blanket therapy was the only way to treat intramammary infections during the dry period and prevent the clinical mastitis in early lactaction but now it is considered prophylactic use of antibiotics and according to the rational use of antimicrobials in dairy cows market it is discouraged [3]. The systematic dry-cow therapy means that cows without infection, which will also have a low risk of new infections in the early dry period, are given antibiotics. Unfortunately, most of the cases of mastitis are dectected through clinical signs of inflammation and without an etiology diagnosis it is difficult to know when antinfective treatments is indicated. The objective of mastitis control during the dry period is to have as less infected quarters as possible at calving. This depends on enhancing elimination of infections present at drying off and on reducing the new infection rate during the dry period. To help vets to make clinical mastitis treatment decision and to choose the best management protocol at dry-off, it is possible to implement in farm a milk quality herd plan based on on-farm culture tests, somatic cells count and by sending milk samples to a laboratory [4]. To avoid unnecessary treatments with antiinfectives, becomes necessary, the rapid identification (on-farm culture tests) of cases with no dectable pathogen because in these types of mastitis are not justifiable to use any therapeutic antibiotic treatments; in mild and moderate clinical mastitis due to gram-negative bacteria, the intramammary antimicrobial administration does not improve bacteriological cure rates. The use of intramammary antinfectives instead it is necessary in udder inflammations caused by gram-positive bacteria as it is proven that their use significantly increases the cure rate [5,6]. In selective dry-cow antimicrobial therapy we have two different ways of intervening in the management treatments of dry period: there will be dairy cows diagnosed healthy, that will be treated with only the teat sealant and heads with pathogenic mastitis or high cell counts that will receive the intramammary treatment together with the sealant. In uninfected cows, the teat sealent is used alone and to reduce the risk of new infections during the whole dry period it will have to remain intact all this time. In infected cows it can be combined with antibiotics hoping that there is no interaction between the two phases, the antibiotic and the sealant.

Artificial internal teat sealants have been developed which can overcome the low production of the natural keratin plug during the dry period because not all cows will form a plug that is effective throughout the dry period. One approach to the prevention of intramammary infections during the dry period is to use a barrier inside a healthy teat [7]. This barrier takes the form a sealant, or "stopper", consisting of a suspension of basic bismuth subnitrate in a gel matrix, administered inside the cow's teat cistern, which prevents bacteria from entering the breast. This product must remain in situ such that it does not drip from the teat, otherwise the preventive and barrier action against infections would be compromised. For this

reason, the viscosity, thixotropic and viscoelastic properties of the suspension must be such that they meet these requirements. In practice, the suspension is inserted into the teat cistern after the milking using a syringe. The administration procedure must be simple for the operator, the intramammary suspension must be fluid when it is applied, but must become more viscous once inside the teat cistern. In this way no bacteria can enter the breast (Figures 1 & 2).

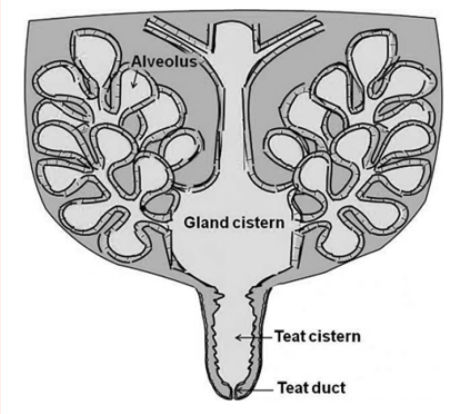

Figure 1: Cross section of a cow breast.

The study of the viscous, thixotropic and viscoelastic behaviours of materials is known as rheology. This discipline involves studying the flow and deformation of materials under applied forces using an instrument called a rheometer. The measurement of rheological properties is applicable to all materials from fluids to semi-solids such as pastes, suspensions and creams [8]. Many commonly-used materials and formulations exhibit complex rheological properties, whose viscosity and viscoelasticity may vary, depending upon the external conditions applied, such as stress, strain, timescale and temperature. Internal sample variations, such as formulation type for pharmaceuticals are the key factors that determine rheological properties. Thixotropy is a time-dependent phenomenon, the substance must recover its initial viscosity a given period of time after the force has been removed. The thixotropic behaviour is an important requisite of a sealant intramammary suspension [9-12]. An efficacious sealant intramammary suspension must have:

a. High viscosity inside the syringe: to ensure homogeneity and stability of the suspension;

b. Low viscosity during the extrusion: to ensure ease of administration;

c. High viscosity inside the teat: to ensure permanence of the product inside teat, without dripping;

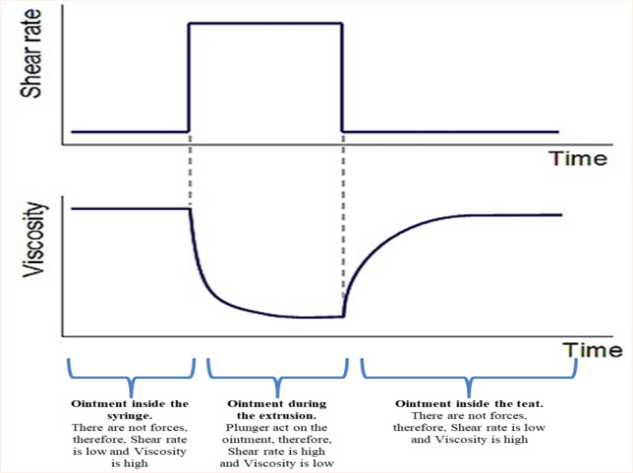

Figure 2: Schematic view of Three Interval Thixotropic Test (3ITT) of an intramammary suspension.

This phenomenon is the result the formulation losing the integrity of its internal structure temporarily, before recovering it again [13]. Another fundamental factor that determines the quality of a sealant intramammary suspension is the length of time the product takes to recover its initial viscosity after the forced applied to it has been removed. Some materials take a certain length of time to recover the original viscosity, while others recover their initial structure and viscosity immediately (Figure 3). Figure 3 illustrates the thixotropic behaviour of three different intramammary sealants. The green curve corresponds to the product having the best recovery time; in this case the initial viscosity value is recovered immediately after the applied force is removed, thus ensuring good sealant properties. The black curve indicates good recovery of the viscosity value, the fact that it takes a certain length of time means that a small amount of the intramammary suspension would leak via the teat. In the case of the red curve, the corresponding product fails to recover its initial viscosity since the applied force destroys its internal structure definitively. This represents the worst case scenario because the efficacy of the intramammary suspension is not guaranteed. To evaluate the rheological behaviour of the intramammary suspensions used during the dry period, rheological methods of comparison have been developed. These will evaluate differences between Fatroseal® (a new intramammary sealant manufactured by Fatro S.p.A.) and others four intramammary sealants present on the Italian market. The parameters assessed during the rheological study are Viscosity, 3 Interval Thixotropy Test (3ITT) and the viscoelastic nature of materials: Amplitude Sweep. In addition, to evaluate the sealant performance of intramammary sealants, was performed a compatibility *in-vitro* test, comparing the behaviour of the products alone and in contact with two intramammary antibiotic suspensions (Fatroximin and Mastout, both manufactured by Fatro S.p.A. and used during the dry period).

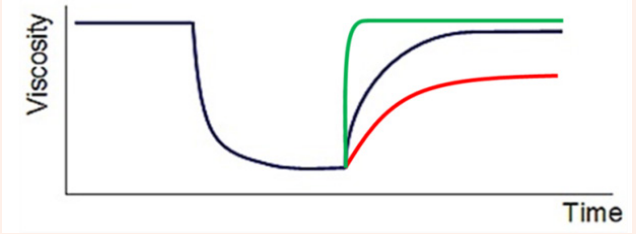

Figure 3: Recovery phase of three different intramammary sealants in 3ITT Test.

Materials and Methods

Materials

In the study were used several intramammary sealant suspensions: Fatroseal® (Fatro S.p.A.) and four intramammary sealants present on the Italian market, which are not mentioned here to avoid negative publicity, they are named samples A, B C and D. All formulation have a similar composition, in particular, they are paraffinic gels enriched with Bismuth Subnitrate (65%). In addition, were used two intramammary antibiotic suspension normally used during the dry period, Fatroximin and Mastout containing rifaximin and cefalonium respectively, both manufactured by Fatro S.p.A.. Whole unpasteurized milk, procured from local farmers, was also used to simulate the little amount of milk present in the cow breast during the dry period.

Methods

Rheological analyses: The rheological analyses (Viscosity, 3 Interval Thixotropy Test (3ITT) and Amplitude Sweep test) were performed using a Modular Compact Rheometer (MCR 92, Anton Paar GmbH, Anton-Paar-Str. 20, A-8054 Graz, Austria - Europe, serial number 82827984, software RheoCompass Light-104632) and Toolmaster™ PP50 parallel-plate geometry (Figure 4). The instrument can be operated at several temperatures, so it is possible to assess the above properties as a function of temperature. This means that it may be used to evaluate whether the reticulated three-dimensional network of the gel is susceptible to temperature variations. The devices used for carrying out the measurements are a series of plates whose geometry varies on the basis of the fluid being tested and the test to be carried out. The geometries are cone-plate and parallel-plate systems. Cone-plate geometries are suitable for all types of fluids. However, their applicability in the case of dispersions is limited to a certain maximum particle size (<10 µm). The Parallel-plate system was chosen for this study, since it is recommended for testing pastes, gels (with three-dimensional superstructure), soft solids, or highly viscous samples.

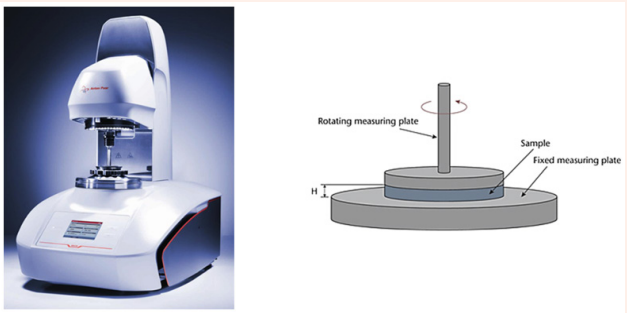

Figure 4: Photo of Modular Compact Rheometer (MCR92), Anton Paar GmbH (left) and schematic detail of parallel-plate geometry (right).

***In-vitro* compatibility test:** To evaluate the compatibility between sealants and intramammary antibiotic suspensions, a system consisting of a 10 ml syringe, connected with a perforated 15 ml vial equipped with a cap was designed. To mimic the two main drying methods, the systems were filled in two different ways (Figure 5). In addition, since even in the dry period there may be milk in the udder, 10 ml of unpasteurized milk have also been added to the systems. The test was conducted at 37 °C in a thermostat and lasted 60 days, photographing the systems at regular intervals (To, 1, 2, 7, 14, 21, 28, 35 and 60 days).

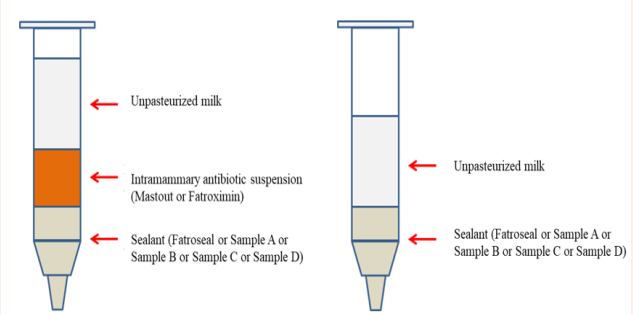

Figure 5: System filled to mimic antibiotic+sealant dry cow therapy (left) and selective dry cow therapy (right).

Results and Discussion

Rheological analyses

Rheological properties of an intramammary sealant suspension are very important, in fact, sealant efficiency is strictly connected with rheology.

Viscosity: In Figure 6 are reported the viscosity profile at several shear stress of the five sealants tested. All products can be considered non-Newtonian fluids, increasing share stress, their viscosity decreases. But, how can be seen in the Figure 6, there are significant differences among the viscosity profile of the tested products. Fatroseal and Sample A, compared to the others sealants, have the highest viscosity. Consequentially, they guarantee an effective sealing action. On the other hand, Sample B have a very low viscosity, this could reduce its sealing performance. Samples C and D have an intermediate viscosity.

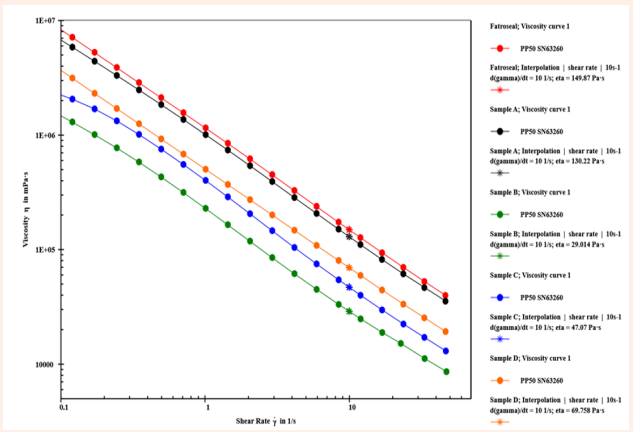

Figure 6: Fatroseal, Sample A, B, C, and D viscosity profile at several shear stress, (n=6).

Interval Thixotropy Test (3ITT): The quality of a sealant is not guaranteed only by high viscosity, the product need also to be administered. In order to guarantee a manageable administration the sealant should be more viscous in the syringe and in the teat cistern but less viscous during extrusion. As mentioned before, the thixotropic behaviour of an intramammary sealant suspension is one of the most important property. In Figure 7 is shown the thixotropic profile of the five products. All products possess a thixotropic behaviour, but there are significant differences among them. Particularly, Fatroseal and Sample A, as seen in the previous chapter, have the greatest viscosity, the difference between them stays in the recovery time. Fatroseal, as soon as share rate is reduced, return immediately to high viscosity while Sample A return to its initial viscosity slowly. The others products (Samples B, C and D), although exhibiting a thixotropic behaviour, have a lower viscosity at low share rate, therefore, the change in fluidity at high share rate is less accentuated.

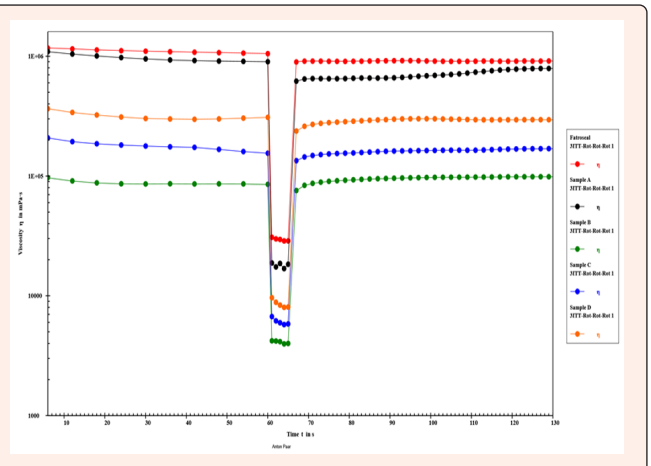

Figure 7: Fatroseal, Sample A, B, C, and D thixotropic profile, (n=6).

Amplitude sweep test: Fatroseal and the others products tested in this study are paraffinic gels in which bismuth subnitrate is disperse. These materials have a viscoelastic nature, therefore, they have an elastic component (solid) and a viscous component (liquid). In rheology, the elastic component is known as the storage modulus (G'), while the viscous portion is the loss modulus (G"). These moduli are closely associated with the deformation behaviors of materials. An intramammary sealant to have good characteristics, must have G'>G", it means that the solid component has to be prevalent on the liquid component. This guarantees that there are strong interaction forces between the components of the gel and therefore assure its stability. In particular, in the case of sealant intramammary suspension, if the liquid component (G") prevails over the solid component (G'), this would leak out of the teat, compromising product efficacy. For this reason, it is essential to determine these two components. In Figure 8 are reported the results of the Amplitude Sweep Test, it can be seen that all the products have G'>G", hence they are all gels. Nonetheless, there are significant differences between the products, in particular, with this test is possible to highlight the behavior of viscoelastic samples in the non-destructive deformation range and is useful for determining the upper limit of this range, which it is known as the Linear Viscoelastic region (abbreviated to LVE region). The LVE region indicates the range within which the test may be performed without changing the structure of the sample. Outside of this region the elastic portion (G') decreases to the point that it is less than the viscous portion (G"); thus the sample changes its nature from that of a solid to that of a liquid. It is possible to identify two points: the first is known as the Yield point (τy), beyond which the internal structure becomes softer and starts to flow; the second is the Flow point (τf), which represents the point at which the internal structure is destroyed. In Table 1 it can be noted that Yield point is similar among the products, while Flow point is significantly different. Fatroseal presents the higher value of flow point, consequentially, compared to others samples, it is needed more energy to break the gel, this probably means that it is more stable and it can guarantee a better sealant efficacy.

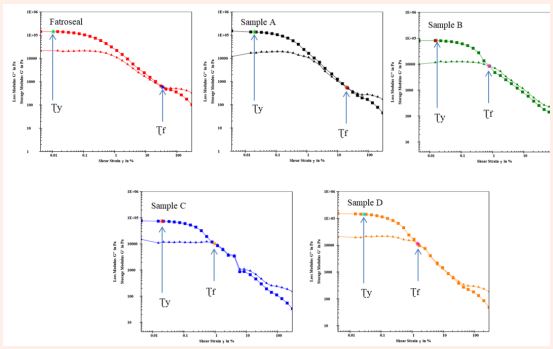

Figure 8: Fatroseal, Sample A, B, C, and D Amplitude Sweep Test, ▪=G', ▲=G", (n=6).

Table 1: Fatroseal, Sample A, B, C, and D Yield point τy and Flow point τf.

Samples	Yield point τ_y (Pa)	Flow point τ_f (Pa)
Fatroseal	15.4	293.5
Sample A	27.2	160.8
Sample B	12.9	95.38
Sample C	15.1	138.9
Sample D	23.2	146.3

***In-vitro* compatibility test**

As reported in A J Brandley, et. Al (The use of a cephalonium containing dry cow therapy and an internal teat sealant, both alone and in combination), simultaneous administration of antibiotic intramammary suspension with a sealant, could make it loses the sealant efficiency. The reason could be due to the diffusion of lipophilic excipients, like paraffin, from antibiotic suspension to sealant. This phenomenon is more pronounced if the three-dimensional network of gel is not consolidated and consequently stable. The products tested in this study behaved differently from each other and in general their behaviour followed what was seen on the rheological study. Particularly, In Table 2 and in (Figures 9-13), it can be seen that Fatroseal retains its sealant action for 60 days with all combination (Fatroximin Mastout, and milk). Sample A loses efficacy after 7 days with Fatroximin. Sample B confirms the worst performance, as seen in rheological study, indeed, loses its sealant action after one day with Fatroximin and with Mastout. Sample C and Sample D lose efficacy with Mastout after 28 days and Fatroximin after 7 days respectively. With milk only, all products have passed the test; indeed, after 60 days in all cases are present both sealant and milk.

Table 2: *In-vitro* compatibility test, ✓ retained sealing action, × lost sealing action.

Samples tested	T zero	1 day	2 days	7 days	14 days	21 days	28 days	35 days	60 days
Fatroseal®+Fatroximin	✓	✓	✓	✓	✓	✓	✓	✓	✓
Fatroseal®+Mastout	✓	✓	✓	✓	✓	✓	✓	✓	✓
Fatroseal®+Milk	✓	✓	✓	✓	✓	✓	✓	✓	✓
Sample A+Fatroximin	✓	✓	✓	×	×	×	×	×	×
Sample A+Mastout	✓	✓	✓	✓	✓	✓	✓	✓	✓
Sample A+Milk	✓	✓	✓	✓	✓	✓	✓	✓	✓
Sample B+Fatroximin	✓	×	×	×	×	×	×	×	×
Sample B+Mastout	✓	×	×	×	×	×	×	×	×
Sample B+Milk	✓	✓	✓	✓	✓	✓	✓	✓	✓
Sample C+Fatroximin	✓	✓	✓	✓	✓	✓	✓	✓	✓
Sample C+Mastout	✓	✓	✓	✓	✓	✓	×	×	×
Sample C+Milk	✓	✓	✓	✓	✓	✓	✓	✓	✓
Sample D+Fatroximin	✓	✓	✓	×	×	×	×	×	×
Sample D+Mastout	✓	✓	✓	✓	✓	✓	✓	✓	✓
Sample D+Milk	✓	✓	✓	✓	✓	✓	✓	✓	✓

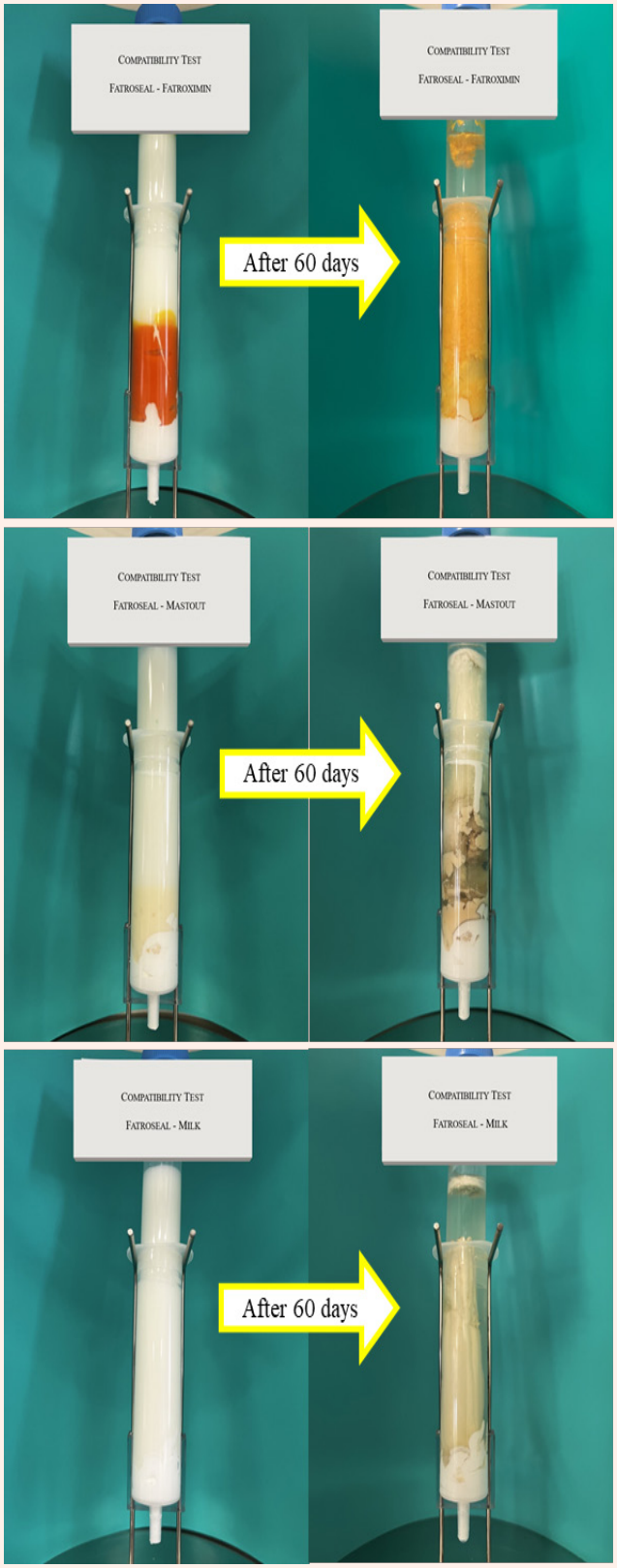

Figure 9: Fatroseal, compatibility test.

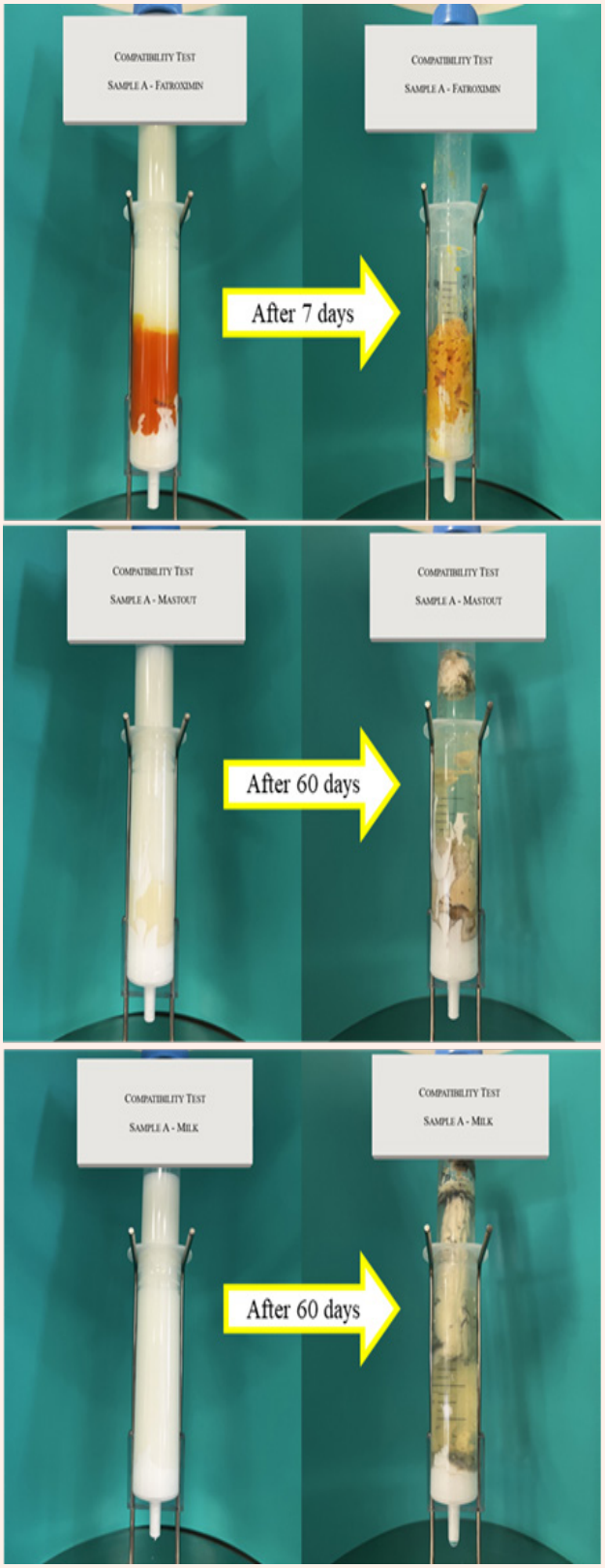

Figure 10: Sample A, compatibility test.

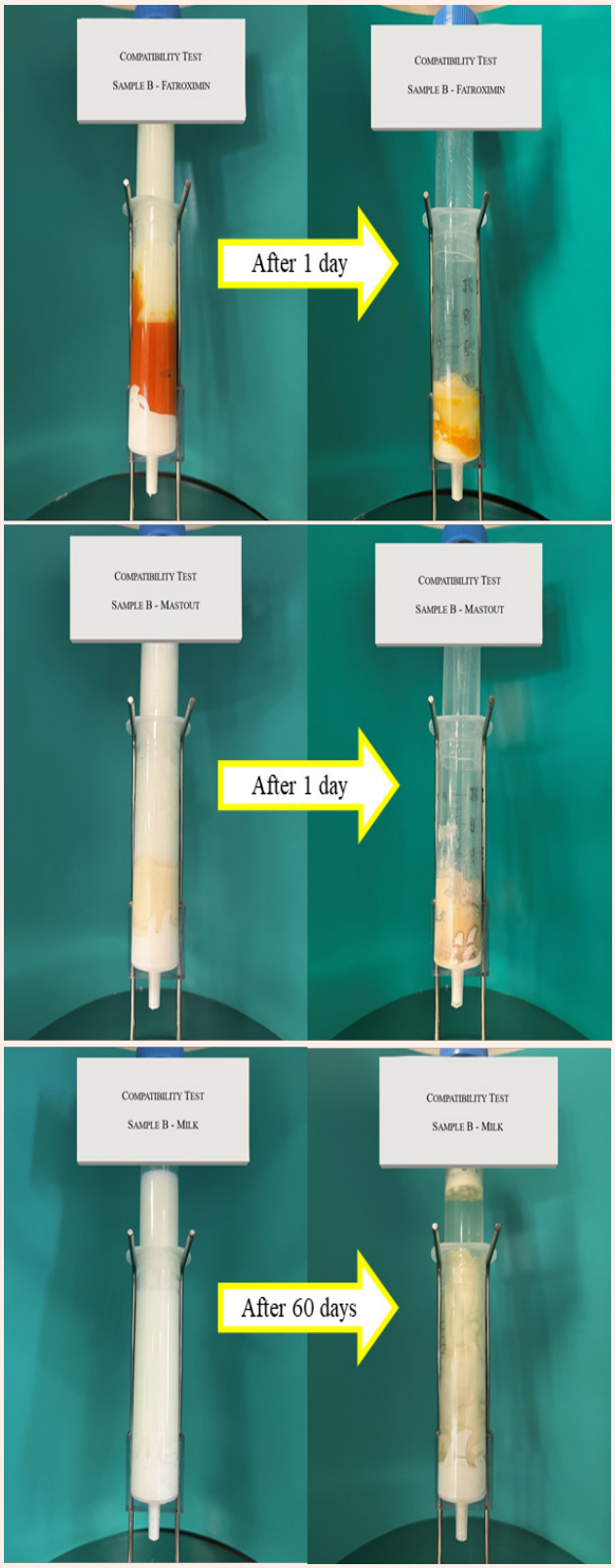

Figure 11: Sample B, compatibility test.

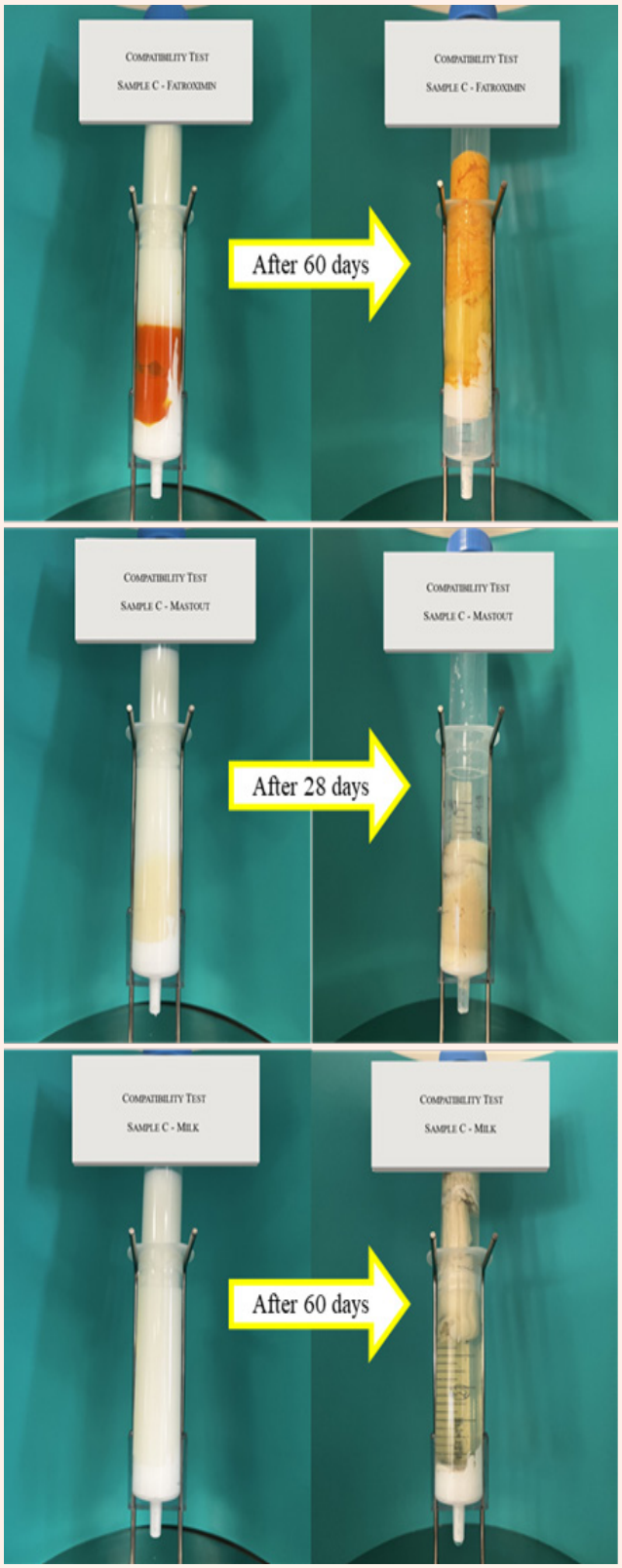

Figure 12: Sample C, compatibility test.

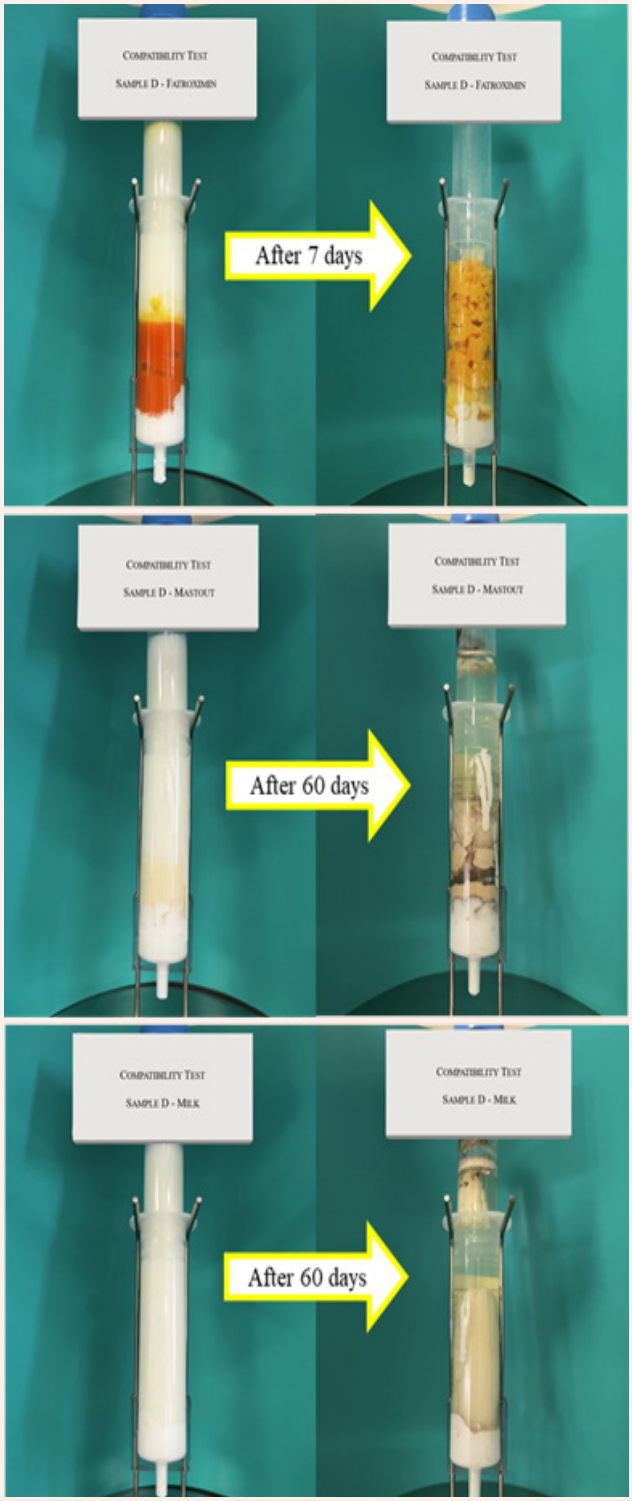

Figure 13: Sample D, compatibility test.

Conclusion

In light of the results obtained, it can be concluded that the observations reported in the literature on the partial incompatibility between sealants and antimastitis ointments are true [14-17]. In the study carried out, the loss of the sealing efficacy of Sample A, C, D and above all Sample B was demonstrated. On the other hand Fatroseal, retains its sealant efficiency for 60 day with all the ointments and milk tested. In addition Fatroseal shows also the best rheological profile. The positive results of this study prompted us to schedule a field trial to evaluate directly on the animal the behaviour of the different teat sealants during drying period.

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