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Effectiveness of dry cow therapy comprising antibiotic treatment, internal teat sealant, and α-tocopherol against new intramammary infections in cows

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Abstract

The aim of this study was to evaluate the preventive effectiveness of dry cow therapy based on antibiotic, internal teat sealant, and α-tocopherol administered separately or in various combinations at drying-off. The study was performed on 322 uninfected quarters of 95 cows originating from three dairy herds. The new intramammary infection rates after calving were measured to evaluate the effectiveness. The quarters were divided into six groups differing in treatment, namely: control group (group C, n = 40) and five treatment groups. Treatment groups were arranged as follows: group A (antibiotic alone, n = 81), group AS (antibiotic + sealant, n = 40), group AST (antibiotic + sealant + α-tocopherol, n = 40), group T (α-tocopherol alone, n = 40), group S (sealant alone, n = 81). New infection rate amounted to 47.5% in group C. The treatment in group AST significantly prevented from the occurrence of new intramammary infections (12.5%, $P < 0.05$), especially those caused by major pathogens. Antibiotic treatment alone (group A) did not prevent from new infections (34.6%, $P > 0.05$), although the use of the sealant alone (group S) decreased the risk of new infection (24.7%, $P < 0.05$). A decrease in new infection rate (25%, $P < 0.05$) was also observed in AS group treated with the combination of the sealant and antibiotic. α -tocopherol supplementation alone (group T) had no overall effect on new infections (35%, $P > 0.05$). Increased α-tocopherol level ($P < 0.05$) was detected after calving in the quarters from cows that received α-tocopherol injections. In conclusion, the combination of antibiotic, internal teat sealant, and α-tocopherol used in dry cow therapy showed a significantly better preventive effect against new intramammary infections, than the therapeutics administered separately.

Keywords: cow, mastitis, dry cow therapy, antibiotic, α-tocopherol, sealant.

Introduction

Mastitis is an endemic and costly disease that causes an economic damage through milk production losses, use of drugs, discarded milk, veterinary and labour costs, decreased milk quality, and culling. Therefore, mastitis is a concern for the dairy industry and needs effective prevention programme from the quarter and cow level to the herd level.

Physiological, histological, ultrastructural, immunocytological, and biochemical changes in the mammary gland have been evaluated in three distinct stages during the dry period. These are: active involution stage, which begins with the cessation of milking and includes the first 30 d of the dry period; steady state involution stage in which mammary glands are completely involuted, and colostrogenesis stage, which is characterised by colostrum formation and initiation of lactation, and begins two weeks before parturition. The mammary gland becomes more exposed to mastitis pathogens, particularly at the beginning and end of the dry period (25). During

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active involution and colostrogenesis, some predisposing factors for new intrammamary infection (IMI) are obtained. These factors increase intramammary pressure due to milk accumulation, cessation of flushing effects of milking, decreased phagocyte accumulation in the dry period, keratin plug formation failures and loss, and incomplete closing of the teat canal. Additionally, cessations of teat dipping and decreased antibiotic concentration at the end of dry period predispose the udder to bacterial penetration. Invading pathogens during the dry period may be also insensitive to the active ingredients of antibiotics used in the dry cow therapy (DCT). Existing or newly formed IMI during the dry period can cause mastitis during the early lactation (7). Therefore, the control programme applied at drying off aims to eliminate existing pathogens and prevent from new infections (4). In particular, the approaches such as vaccination, antibiotic treatments, external and internal teat sealing, vitamin and mineral administrations are suggested for the dry period in cows. Especially, injection or supplementation of α-tocopherol and selenium were reported to be supportive for mammary gland immune system (6, 26).

Tocopherols are known to be chain-breaking antioxidants in plasma and milk (19). α-tocopherol acts as a scavenger of free radicals, which mediate activation of phagocytes, resulting in damage to the related tissues (10). During the peripartum period, especially around the calving, plasma α-tocopherol concentration decreases (22) and adversely affects the immune response of the mammary gland (15).

The aim of this study was to evaluate the preventive effects of dry cow therapy based on the use of antibiotic, internal teat sealant, and α-tocopherol treatments, which were applied separately or in combination to uninfected quarters of cow's udder at drying-off.

Material and Methods

Herd selection and cows. The study was performed on three herds, which had similar feeding: 80% forage (80% corn silage, 10% alfa alfa hay, and 10% wheat straw) and 20% compound feeds (18%-19% crude protein and 2700 kcal/kg). Mastitis control programmes including dry cow treatment with antibiotics, milking hygiene, vaccination against mastitis, teat dipping, and milking by automatic systems twice a day, with an average milk yield of 22-25 kg/day, and had 72-93 lactating cows per year. The cows were housed in rubber mat-bedded free stall barns. According to farm records, monthly bulk tank somatic cell count (BTSCC) of the herds ranged from 150 000 to 350 000 cells/mL. Regular bacteriological data of previous clinical cases could not be found in the farm records. Dry period length varied between 56 to 65 d. The herds were all located in the same vicinity of Ankara, Turkey.

According to the farm records, 95 Holstein cows, which were at the end of the 2^{nd}-3^{rd} lactation and had no clinical signs of mastitis, such as fever or swelling, and had not received antibiotic and anti-inflammatory treatment within the last 30 d, were chosen for the study. The milk samples were collected from individual udder quarters, of which 322 quarters out of 380 were included in the study as uninfected according to bacteriological examination.

Study design and drug administration. The cows were divided into six groups: one control and five treatment groups, treated with various drugs separately or in combination. The following drugs were used: antibiotic for DCT - including 600 mg of cloxacillin (Orbenin Extra DC®, Pfizer Animal Health, Italy), internal teat sealant consisting of bismuth sub-nitrate in a paraffin basis (65% w/w, 2.6 g in 4 g) (Orbeseal®, Pfizer Animal Health, Ireland), and α-tocopherol solution consisting of 150 mg (d, l)-α-tocopherol-acetate and 1.67 mg/mL of pentahidra-disodium selenite (Selen E Sol®, Richter Pharma AG, Austria). Groups were arranged as follows: group C (control, n = 40), group A (antibiotic alone, n = 81), group AS (antibiotic + sealant, n = 40), group AST (antibiotic + sealant + α-tocopherol, n = 40), group T (α-tocopherol alone, n = 40), and group S (sealant alone, n = 81). Treatments were applied to each udder quarter, considering lactation number and milk level at drying off to be homogenous during the treatments.

Internal teat sealant and DCT-antibiotic were administered intramammary after the last milking of lactation by an aseptic drug administration procedure. A dose of 1500 mg of (d, l)-α-tocopherol-acetate (recommended dose by manufacturer) was injected intramuscularly (in the injection triangle of the neck) at the beginning of the dry period and the same dose was repeated 15 d before the expected calving date. The injection procedure was established according to the previous report (26), that suggested repeated injection of α-tocopherol-acetate in dry period, and according to the study by Hogan *et al.* (15) who reported using decreased α-tocopherol concentration in plasma 7 to 10 d prior to calving.

Sampling procedure. Two consecutive milk samples were collected from all cows one week before drying off and on the day of drying off to detect uninfected quarters. Milk samples were collected aseptically into 15 mL sterile plastic tubes (LP Italiana SPA, Italy) (14, 20) and transported to the laboratory in iceboxes within 3 h after collection and processed within 24 h. The samples were stored at 4ºC until processing. Sampling was repeated from the same uninfected quarters before drying-off, and on days 5 and 10 after calving, consecutively (14). Two samples were collected on day 5; one for α-tocopherol and one for bacteriological analysis. A single sample

was collected on day 10 for bacteriological evaluation. The samples collected for bacteriology were transported to the laboratory in iceboxes. The samples for α-tocopherol analysis were stored at -80°C until the day of examination.

Bacteriological procedure. Bacteriological examination was performed according to Quinn *et al.* (28). Ten microlitres of the sample was spread separately on MacConkey and blood agars containing 5% of defibrinated sheep blood. The plates were incubated at 37°C for 24 and 48 h, respectively. Colonies were classified according to Gram staining, morphology, and degree of haemolysis in order to determine IMI, contamination, and mixed infections.

Evaluation of culture results. The culture results of the milk samples were evaluated according to the procedure described in a previous report (20). Briefly, the quarters, which had one-three different pure colonies with >500 cfu/mL of bacterial species, were considered to have an IMI. A milk sample was defined as contaminated when more than three different colonies were detected. The quarters, which had two to three pure colonies, were considered as having a mixed infection. Major pathogens, such as *Staphylococcus aureus* (*S. aureus*), *Streptococcus uberis* (*S. uberis*), *Escherichia coli* (*E. coli*), *Streptococcus agalactiae* (*S. agalactiae*) were considered as the causative agents for the mixed infection. Sampling was repeated if the culture results were negative or contaminated, to avoid false negative and false positive results, both before drying off and after calving. The quarters were considered as uninfected when the culture results were negative in both samplings. The quarters were considered as infected when the results of the second sampling were positive. Coagulase negative *Staphylococcus* sp. (CNS) was considered as the minor pathogen. When the quarters, which were uninfected at drying off, became infected after calving, it was evaluated as new infection.

Milk α-tocopherol analysis. Milk α-tocopherol was analysed according to the procedure described by Rammel *et al.* (29). Two millilitres of ethanol, 1 mL of methanol, 1 mL of KOH (10 M), and 1 mL of 15% ascorbic acid were added into the glass tube (Wheaton, Cat. No. 358646, USA). One millilitre of milk sample was added to the mixture and vortexed for 10 s. The mixture was kept in a water bath for 10 min at 60°C, and then vortexed for further 10 s. This procedure was repeated three times. After the water bath procedure, the mixture was kept on ice for 5 min, and subsequently 5 mL of hexane was added to the mixture and vortexed for 10 s before shaking for 2 min. The homogenised mixture was centrifuged at 500 × g for 3 min at 4°C, and then the clear hexane phase was collected into a 10 mL glass vial and placed into a vacuum incubator (Heraeus, VTR 5036, Germany) at 60°C. The vial was kept in the incubator until the hexane phase was completely vacuumed. One millilitre of methanol was added to the vial containing α-tocopherol residue and vortexed for 10 s. The methanol was collected by a plastic injector and passed through 0.2 mm Millipore filters. The sample (0.1 mL) was analysed using high-performance liquid chromatography (HPLC, Dionex Softron, Germany), and the fluorescent detector determined the result (μg/mL).

Statistical analysis. The available risk factors that could affect the occurrence of new IMI were subjected to the logistic regression. The effect of treatment on new infections occurring at the beginning of lactation was evaluated by the Chi-square test. After log transformation, α-tocopherol levels were analysed by ANCOVA, in which the length of the dry period was considered covariate and quarter within group was the random term (SAS, 2002). The statistical significance was evaluated at $P < 0.05$.

Results

Statistical analysis revealed that only the length of the dry period constituted a significant risk factor (Table 1). According to the bacteriological results, overall new infection rates were 47.5%, 34.6%, 25%, 12.5%, 35%, and 24.7% for group C, A, AS, AST, T, and S, respectively. *S. aureus* was the predominant pathogen in groups C (n = 10), A (n = 10), AS (n = 2), and S (n = 5), whereas *S. uberis* (n = 3) and *E. coli* (n = 3) were predominant in group T. However, none of the major pathogens were isolated from the quarters in group AST. CNS was the minor pathogen in groups C (n = 4), A (n = 12), AS (n = 8), AST (n = 4), and S (n = 4), whereas *Bacillus* sp. was the minor in group T (n = 6) (Table 2).

Group C had the highest new infection rate at the beginning of lactation. Surprisingly, the new infection rate in group A was higher than in group C and group T. Additionally, the prevalence of *Staphylococcus* sp. was significantly higher in group A than in group T. Although the combined use of antibiotics and sealants (group AS) had similar new infection rates in comparison to the sealant treatments alone ($P > 0.05$), the major pathogens were more active within group S than in group AS. According to bacteriological results, minimum new infection rates were detected in group AST ($P < 0.05$) (Table 2).

Because logistic regression revealed that the length of the dry period was a risk factor for the occurrence of new IMI (Table 1), α-tocopherol data was subjected to correction based on the dry period length. According to the HPLC results, mean α-tocopherol levels were 0.22 ± 0.04, 0.27 ± 0.03, 0.28 ± 0.04, 0.99 ± 0.04, 0.75 ± 0.04, and 0.41 ± 0.03 μg/mL for group C, A, AS, AST, T, and S respectively. As expected, a significant difference in the concentration of α-tocopherol was found in the

α-tocopherol injected groups (groups AST and T, P < 0.0001) (Table 3). In terms of bacterial growth and bacterial species, the mean level of α-tocopherol was 0.43 ± 0.02 and 0.32 ± 0.03 μg/mL in uninfected and infected quarters, respectively (P < 0.04). The average level of α-tocopherol was 0.29 ± 0.06, 0.28 ± 0.06, 0.57 ± 0.20, 0.44 ± 0.13, 0.53 ± 0.11, 0.33 ± 0.11, and 0.26 ± 0.15 for CNS (n = 33), *S. aureus* (n = 28), *S. dysgalactiae* (n = 3), *S. agalactiae* (n = 7), *Bacillus* sp. (n = 10), *S. uberis* (n = 10), and *E. coli* (n = 6) respectively (P < 0.03, Table 3). α-tocopherol concentration was significantly lower in *E. coli* infected quarters (Table 3).

Table 1. Analysis of maximum likelihood estimates for possible risk factors for new intramammary infections during the dry period (P < 0.05)

Parameter	DF	Estimate	SE	Wald Chi-Square	P<
Intercept	1	-6.5	3.88	2.83	0.09
Group	1	0.11	0.14	0.70	0.40
Herd	1	-0.07	0.15	0.16	0.70
Cow	1	0.003	0.009	0.13	0.72
Quarter	1	0.060	0.11	0.28	0.59
Dry period length	1	0.11	0.06	3.03	0.08
Odds ratio estimates					
Effect		Point estimate		95% CI	
Group		1.12		0.86 - 1.46	
Herd		0.94		0.70 - 1.27	
Cow		1.00		0.99 - 1.02	
Quarter		1.06		0.85 - 1.33	
Dry period length		1.12		0.99 - 1.27	
Association of predicted probabilities and observed responses					
Concordant %	59.9	Sommer's D		0.20	
Discordant %	39.4	Gamma		0.21	
Tied %	0.7	Tau-a		0.09	
Pairs	21090	c		0.60	

Table 2. Bacteriology and new intramammary infection rates after calving in response to various preventative approaches

	Groups[1]					
Bacteriology	C (n)	A (n)	AS (n)	AST (n)	T (n)	S (n)
Culture negative	21	53	30	35	26	61
Culture positive	19^{a}	28^{ab}	10^{bc}	5^{c}	14^{ab}	20^{bc}
Major pathogens						
S. aureus	10	10	2	0	1	5
S. agalactiae	3	2	0	0	0	2
S. uberis	1	3	0	0	3	3
S. dysgalactiae	0	0	0	0	0	2
E. coli	0	1	0	0	3	2
Minor pathogens						
CNS	4	12	8	4	1	4
Bacillus sp.	1	0	0	1	6	2
New inf. rate (%)	47.5	34.6	25.0	12.5	35.0	24.7

[1]C - untreated quarters (control), A - quarters treated with antibiotic, AS - quarters treated with antibiotic plus internal teat-sealant, AST - quarters treated with antibiotic, internal teat sealant, and α-tocopherol, T - quarters from α-tocopherol injected cows, S - quarters treated with internal teat-sealant. Different superscripts represent statistical difference at P < 0.05 level among groups

Table 3. Changes in tocopherol concentration caused by treatment and pathogens*

Groups	α-tocopherol (μg/mL) ± SE
Group C	0.22 ± 0.04^{d}
Group A	0.27 ± 0.03^{d}
Group AS	0.28 ± 0.04^{d}
Group AST	0.99 ± 0.04^{a}
Group T	0.75 ± 0.04^{b}
Group S	0.41 ± 0.03^{c}
Bacterial culture	
Uninfected	0.43 ± 0.02^{a}
Infected	0.32 ± 0.03^{b}
Pathogens	
CNS	0.29 ± 0.06^{ba}
S. aureus	0.28 ± 0.06^{ba}
S. dysgalactiae	0.57 ± 0.20^{a}
S. agalactiae	0.44 ± 0.13^{ab}
Bacillus sp.	0.53 ± 0.11^{a}
S. uberis	0.33 ± 0.11^{ba}
E. coli	0.26 ± 0.15^{b}

*The dry period length was used as covariate. The probability of significance was $P < 0.0001$ for treatment, $P < 0.04$ for bacterial culture, and $P < 0.03$ for bacterial agent

Discussion

The mammary gland is markedly susceptible to new IMI during the dry period due to keratin plug formation failures and keratin plug losses (7). According to Bradley and Green (7), and Green *et al.* (13), IMI caused by environmental microorganisms usually occurs in early involution and prior to parturition. Additionally, the infections, which started in the dry period, are thought to be responsible for 60% of the new environmental mastitis cases that occur within the next lactation (7). In the study, almost half of the uninfected quarters in control group became infected during dry period. Particularly *Staphylococcus* sp., which could colonise teat apex, canal, and skin, was active in this period. This result showed the importance of aforementioned predisposition factors and the necessity of the mastitis control programmes.

Dry cow therapy based on antibiotic treatment, which is a part of the mastitis control programme, is used to eliminate existing IMI and prevent new infections occurring in dry period. The previous reports stated that the DCT with antibiotics prevented infections, which occur in the first trimester of lactation (5). Conversely, in this study-antibiotic treatment alone was not found to be preventive against new infections occurring in the dry period. This result was associated with a possible decrease in antibiotic concentration in therapeutic levels, and keratin plug failures at the end of the dry period, as stated by other authors (12). Prolonged dry period might be another factor predisposing for new IMI, probably leading to low antibiotic concentration and/or yielding below minimal inhibitory concentration (MIC) (3). In the present study, the dry period length was tending to increase the occurrence of new IMI ($P < 0.08$, Table 1), which should be elucidated in further experiments involving large number of animals.

Another preventative approach against mastitis is an internal teat sealant, which is a physical barrier in the teat canal during the dry period. In previous studies, internal teat sealant was reported to be as effective as antibiotic treatment at drying off, and it was suggested as an alternative for antibiotic treatment (4). In the study, antibiotic and sealant were used either alone (groups A, S) or in combination (group AS). The new infection rate in group S was lower than that of group A ($P < 0.05$). In contrast with this finding, Huxley *et al.* (18) reported similar postpartum new IMI rates in quarters which were treated by internal teat sealant and antibiotic at drying-off. However, the authors (18) suggested a significant difference in new IMI aetiology, as fewer quarters were infected with *E. coli*, *Enterobacteriaceae*, and major pathogens at the beginning of lactation following the teat sealant treatment alone at drying off. In the presented study, there was no significant difference in the rates of IMI due to major pathogens. On the other hand, a significant difference between group A and group S was observed in regard to higher rate of new IMI of *Staphylococcus* sp. (both for *S. aureus* and CNS) in group A. This result was associated with characteristics of the pathogen. Namely, many staphylococci are a part of normal udder and teat skin microflora and they can colonise the teat apex (27). *Staphylococcus* sp., which exists in distal streak canal in dry period, are also isolated after calving (23). In the case of lowered resistance such as leakage from the quarters due to increased intramammary pressure and lower concentration of natural protective factors (lactoferrin, immunoglobulins, and phagocytic cells) (9) and antibiotics (12) at the end of dry period, the bacterium can penetrate the teat canal and cause infection in early lactation (23). Additionally, cloxacillin might be ineffective against to *S. aureus*, which could penetrate streak canal and survive within neutrophils. Due to failure of cloxacillin to kill intracellular

staphylococci (11), the new infection rate might increase. This study demonstrated that internal teat sealant might support the antibiotic by restricting (forming a physical barrier) staphylococcal penetration. However, infection due to *E. coli*, which was reported to be resistant to the long acting antibiotics (9), was limited in quarters treated with antibiotic. Moreover, it was not isolated from control quarters. In our opinion, this difference may be due to climatic and environmental conditions in Turkey, which are different than in the UK. Although the sealant treatment was more successful and preferable than antibiotic treatment alone, the non-antibiotic treatments had some risk of iatrogenic contamination. Pathogens present around the teat sphincter can penetrate into the quarters when aseptic infusion techniques are not used.

In previous studies, long-acting antibiotics and internal teat sealant were also used in combination at drying-off (3, 12, 21). As some authors found the combination treatment more effective than antibiotic and sealant alone (3, 12, 21), the effect of combination in uninfected quarters was less clear in the study by Bradley *et al*. (7). In our study, the new infection rate was lower in group AS than that in group A. In addition to new infection rates, fewer quarters were infected with *S. aureus* in group AS than in group A (Table 2), and none of the quarters was infected with environmental microorganisms in group AS. This result also supported observations of previous studies (7) in which a decrease in staphylococcal and streptococcal infection following combined treatment was reported. CNS was the most frequently isolated bacteria in the present study. The teat canal colonisation characteristics of CNS, which was mentioned above, might be responsible for higher CNS infection rates during the dry period. Godden *et al.* (12) also used the combination of antibiotic and teat sealant in uninfected quarters at the beginning of dry period and also reported high new CNS infection rates at 1-3 and 6-8 DIM (days in milk).

Interestingly, in overall evaluation, the new infection rate in group S was similar to the rate in group AS ($P > 0.05$). However, this does not mean that there is no effect of antibiotic used in combination with other type of treatment. We found less IMI due to major pathogens in group AS than in group S. A double effect might be provided in group AS. Namely, when the sealant limited the pathogen penetration during dry period, the antibiotic might have eliminated the major pathogens, which overcame the physical barrier in AS treatment. Although, the new infection rate in this study supported the observations of Bradley *et al*. (8), who reported that sealant treatment alone was as preventive as antibiotic-sealant combination in uninfected quarters, the combination was more preventive against major pathogens, which could cause severe damages in the mammary gland. In addition to new IMI, aetiology and bacterial species should be taken into consideration in evaluation of efficiency of dry cow therapy. As a result, the benefits of antibiotics in DCT were low unless it was combined with other treatment choices. However, this combination raises the costs of treatment, so the strategies for dry period must be evaluated economically. Berry *et al.* (5) reported little economic differences between antibiotic and internal teat sealant treatments for cows, which had uninfected quarters at drying-off. The same authors reported additional costs for antibiotic therapy when compared to the teat sealant during *Corynebacterium* sp. and CNS infections. This study showed that the use of the internal teat sealant alone in uninfected quarters was more effective against new CNS infections than the antibiotic treatment alone. Hence, internal teat sealant may reduce the aforementioned economic disparity, and may help in the control of antibiotic resistance and the residue problem, which is essential to the public health. Nevertheless, the probability of an infection occurring due to contagious and environmental pathogens such as *Streptococcus* sp. should be considered in those quarters.

There were various aspects concerning the role of α-tocopherol (parentally or dietary) in mastitis. Some authors suggested it to be an important factor to reduce the incidence of clinical mastitis, by decreasing severity of clinical signs, shortening the duration of infections (30), and promoting the intracellular killing of bacteria by neutrophils (17). On the other hand, some authors (22) suggested no association between α-tocopherol and mastitis. Furthermore, Bouwstra *et al.* (6) reported that a high dose (3000 IU/d) of vitamin E caused increased reactive oxygen molecules, malondialdehyde, and relative risk for clinical mastitis.

In the present study, milk α-tocopherol concentration was considerably higher in α-tocopherol injected groups than in the non-injected groups. On the other hand, because α-tocopherol level was not detected before drying-off, the rate of increase could not be evaluated. Double injection of 1500 mg of α-tocopherol during the dry period provided a remarkable prevention against new IMI caused mainly by *Staphylococcus* sp. This result supports Ndiweni and Finch findings (24), who reported that α-tocopherol supplementation under *in vitro* conditions increased phagocytosis and intracellular eradication of *S. aureus* by neutrophils. In our study, α-tocopherol injection alone was not effective in prevention of new infections by other pathogens (*i.e. Bacillus* sp.*)* occurring during the dry period. This result was associated with the variation in endogenous α-tocopherol secretion, which was induced by pathogens, and differences in phagocytic capabilities, which were affected by α-tocopherol content (derived from fat globules or neutrophils). The α-tocopherol originating from fat globules could not be distinguished as original α-tocopherol in phagocytosis (2). Although, α-tocopherol injections supported the milk α-tocopherol level, the content of endogenous α-tocopherol might play a determinative role in

phagocytosis of these pathogens in the study. LeBlanc *et al*. (22) also declared that there was no association between serum α-tocopherol concentration and the risk of mastitis shortly after calving. Conversely, the combined use of sealant and antibiotics (group AS) was considerably successful against new infections; however, it did not provide as much prevention as treatment in group AST. According to this result, α-tocopherol should be accepted as a supportive approach to the main mastitis control programmes rather than as a primary approach.

Major sources of α-tocopherol in milk are fat globule membranes and neutrophils. Due to neutrophil increase and fat globule decrease during IMI, α-tocopherol concentration may show variations. Some authors declared an increase in α-tocopherol concentration (2, 16), but others reported a decrease during IMI (1). A previous study reported the excessive phagocyte accumulation and α-tocopherol concentration in clinical mastitis due to *E. coli* (16). However, in our study, it was lower in quarters infected with *E. coli*. Additionally, the mean α-tocopherol concentration was lower in the infected quarters than in uninfected. This result might be associated with two reasons, which had reciprocal interaction. Firstly, none of the infected quarters showed clinical symptoms. Thus, phagocyte infiltration might not be as excessive as in clinical cases, and this indicates that the decrease may be associated with decreased milk fat production. Although, α-tocopherol concentration was higher in quarters infected with *S. dysgalactiae*, this result could not be evaluated due to inadequate number of infected quarters with the pathogen. Secondly, lower α-tocopherol level may lead to delayed immune response and can cause susceptibility to infections, due to poor killing ability of phagocytes (17). Briefly, the absence of α-tocopherol may be either a result, or potentially be a reason of new IMI. Moreover, the role (result or reason) may change depending on the type of mastitis pathogen. This result should be evaluated in further studies.

This study was performed to evaluate the preventive effects of antibiotic, internal teat sealant, and vitamin E used in DCT alone and in combination against new IMI, which started in the dry period and existed at the beginning of next lactation. Up to 47.5% of the control quarters showed new infections during the dry period. Nearly half of the uninfected mammary quarters can become infected if mastitis control programmes are not performed at drying-off. Because of the physiological properties of the mammary gland in the dry period, which cause predisposition to mastitis in early lactation, mastitis control procedures should be performed at drying-off in order to avoid serious economic losses in subsequent lactation. Another important issue is making decision on suitable procedures, which have some advantages and disadvantages as mentioned above. Some specific realities in the herd such as the most prevalent mastitis pathogens and treatment costs should be taken into consideration. Antibiotics which activity may be negatively influenced by several factors in the dry period, and internal teat sealant alone can be insufficient in preventing new IMI during the dry period. Because of this, the herds can minimise the IMI risk caused by major pathogens (especially for *S. aureus* and *Streptococcus* sp.) by using intramammary antibiotics and internal teat sealant combination treatment. Although α-tocopherol has a limited effect on eliminating or preventing new IMI, it may contribute to increasing of immune response against pathogen invasion. It appears that the combination of antibiotic, internal teat sealant, and α-tocopherol injections in dry cow therapy should be used in combination to prevent new IMI in the dry period. Otherwise, precalving risk period for new infections remains to be a problem even when the cows are treated with antibiotics, sealent, or α-tocopherol alone.

Conflict of Interest Statement: The authors declare that there is no conflict of interest.

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