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Effect of vitamin E supplementation on neutrophil function, milk composition and plasmin activity in dairy cows in a commercial herd

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Fifty-six Holstein dairy cows from a commercial dairy herd in the Northern part of Greece were used to determine the effect of vitamin E supplementation on immune parameters, milk composition and milk quality. Cows were assigned to one of two experimental groups: control (no vitamin E supplementation) and vitamin E supplementation. Supplementation of vitamin E started 4 weeks prior to and continued up to 12 weeks after parturition. Supplementation included daily oral administration of vitamin E at 3000 i.u./cow *prepartum* and was reduced to 1000 i.u./cow *post partum*. Blood samples were collected weekly for 8 weeks starting 4 weeks before parturition, neutrophils were isolated and the following parameters were determined in neutrophils activated by phorbol myristate acetate: total cell-associated and membrane-bound urokinase plasminogen activator (u-PA) activity and superoxide production. Milk samples were collected weekly and fat, protein, lactose, somatic cell count (SCC), plasmin and plasminogen-derived activity were determined. Activated neutrophils isolated from cows that received supplemental vitamin E had higher ($P<0{\cdot}01$) total and membrane-bound u-PA activities during the first 3 weeks after parturition and higher ($P<0{\cdot}01$) superoxide production during week 1 *prepartum* and week 1 *post partum* compared with the corresponding values of activated neutrophils isolated from control cows. Vitamin E supplementation had no effect ($P=0{\cdot}28$) on plasminogen-derived activity in milk. Milk obtained from cows that received supplemental vitamin E had SCC lower by 25% ($P<0{\cdot}05$) and plasmin lower by 30% ($P<0{\cdot}01$) than corresponding values in milk obtained from control cows. The reduction in plasmin as a result of vitamin E supplementation is very beneficial to the dairy industry because plasmin reduces the cheese-yielding capacity of milk, affects the coagulating properties of milk and its overall ability to withstand processing during cheesemaking. In conclusion, vitamin E supplementation had positive effects on the function of bovine neutrophils and milk quality in a commercial dairy herd.

Keywords: Vitamin E, neutrophil function, plasmin activity, dairy cows.

The capacity of nutrients to modulate the actions of the immune system is increasingly recognized. The decline in immune function during the periparturient period is an excellent example of the interaction between availability of certain nutrients and immune function in dairy cows. How this immunosuppression occurs is not completely understood. It is certain, however, that endocrine changes and availability of critical nutrients including vitamin E are implicated (Goff & Horst, 1997; Smith et al. 1997).

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Vitamin E plays a critical role in the periparturient decline in immune function. Politis et al. (1996) and Smith et al. (1997) reported that dietary supplementation of dairy cows with vitamin E prevents the decline in immune system activity and function. This decline is thought to contribute to increased rates of mastitis in dairy herds during the periparturient period. A more rapid influx of neutrophils into the mammary gland along with increased phagocytosis and superoxide production by neutrophils occurs in cows receiving supplemental vitamin E (Politis et al. 1996; Smith et al. 1997). More recently, Politis et al. (2001) reported that neutrophils isolated from cows receiving supplemental

vitamin E had higher membrane-bound urokinase-plasminogen activator (u-PA) and u-PA mRNA than neutrophils isolated from control cows at week 1 *post partum*. This is thought to facilitate the ability of neutrophils to extravasate and reach the mammary gland. Most of the results suggesting that vitamin E prevents the decline in immune function during the periparturient period come from North America where cows are likely to encounter significantly different levels of oxidative stress than cows in the EU (Allison & Laven, 2000). More studies are needed to examine the effect of vitamin E supplementation on neutrophil function during the periparturient period in commercial dairy herds in European countries.

The periparturient period is associated with many new intramammary infections that contribute significantly to mastitis and reduced milk quality in dairy herds as shown by an increase in the somatic cell count (SCC) of milk (Smith et al. 1997). Plasmin is a serine proteinase that occurs in bovine milk together with its inactive zymogen, plasminogen (Politis et al. 1989). Plasmin hydrolyses two caseins (α_s- and β-casein). The extent of proteolysis caused by plasmin reduces the cheese-yielding capacity of milk, affects the coagulating properties of milk and its overall ability to withstand processing during cheesemaking. Higher plasmin activity is associated with increased SCC in milk (Politis et al. 1989). Because vitamin E supplementation affects milk SCC (Politis et al. 1995; Smith et al. 1997; Baldi et al. 2000), it follows that vitamin E supplementation may affect the plasmin–plasminogen system in milk. However, there is no published information on the effects of vitamin E supplementation of dairy cows on the plasmin–plasminogen system in bovine milk.

The objective of the present study was to determine the effect of vitamin E supplementation on the u-PA system and superoxide production by neutrophils in cows in a commercial dairy herd in Greece. Furthermore, the effect of vitamin E supplementation on SCC and the plasmin–plasminogen system of bovine milk was examined.

Materials and Methods

Animals and experimental design

Fifty-six Holstein dairy cows from a total of about 400 lactating cows housed in a commercial dairy herd in the northern part of Greece were assigned to one of two experimental groups: control and vitamin E-supplemented. Prior to assignment, cows were chosen on the basis of similar parity, milk production in the previous lactation and their health history. All cows received each day a basal amount of 300 i.u. of vitamin E (all-rac α-tocopheryl acetate; Roche Vitamins Ltd., Basel, Switzerland). Cows in the supplemented group received the normal diet plus a concentrate containing 3000 i.u. of vitamin E during the non-lactating and 1000 i.u. during the lactating period added to 0·5 kg of corn. Care was taken to ensure that cows ingested the entire corn ration. Cows in the control group received the same diet including the concentrate without the vitamin E supplement. Dietary supplementation started 4 weeks before expected parturition and continued for up to 12 weeks after parturition.

Only 50 cows (25 cows per experimental group) that calved within ±4 d of anticipated calving were used throughout the experimental period. Average sampling days for the 4 weeks prior to parturition (weeks −4, −3, −2 and −1) were 29·0, 22·0, 14·6 and 7·5 d, respectively. All cows were housed in the same barn unit in individual tie stalls. Approximately 3 d before expected calving, cows were moved to individual box stalls and maintained on the same diet that was fed during the non-lactating period. Approximately 3 d after calving, cows were moved to tie stalls.

Diets (dry matter basis) fed during the non lactating period were 42% corn silage, 40% straw hay, 9·6% soybean meal and 8·4% molasses. After parturition, the diet was switched to one consisting of (dry matter basis) 48·5% corn silage, 16·5% soybean meal, 14% alfalfa hay, 12% corn, 3·7% molasses, 1·7% rumen-protected fat and 3·6% vitamin and mineral premix.

Blood collection; vitamin E analyses; neutrophil isolation

Blood samples were collected weekly for 8 weeks starting 4 weeks prior to parturition for vitamin E analyses and neutrophil isolation. Samples were obtained from 10 control and 10 treatment cows. Plasma vitamin E concentration was determined by HPLC (Baldi et al. 2000).

Neutrophils were isolated as described by Politis et al. (2003). Briefly, 30 ml of heparinized (10 i.u. of heparin per ml) venous blood was layered onto 20 ml of a sodium metrizoate (95 g/l)-polysaccharide gradient (56 g/l) with a specific gravity of 1·077 g/cm^3 (Sigma Chemical Co., St. Louis, MO, USA), then centrifuged at 500 ***g*** for 45 min. Cells from the bottom layer were washed twice in RPMI-1640 medium (Sigma) and the red blood cells were lysed by addition of 20 ml of sterile distilled water, followed by addition of sterile saline solution (NaCl, 27 g/l) to restore isotonicity. Cells were pelleted by centrifugation at 200 ***g*** for 10 min and resuspended in RPMI-1640 medium containing fetal bovine serum (FBS; 100 ml/l; Sigma) at a concentration of 1×10^7 cells/ml. Cell viability was assessed by trypan blue dye exclusion (Sigma) and was always high (>95%).

Determination of total cell-associated u-PA activity

Neutrophils (2×10^6/ml) were resuspended in 500 µl of Hanks balanced salt solution (HBSS; Sigma) containing 20 mM-HEPES and 81 mM-phorbol myristate acetate (PMA; Sigma). After incubation for 30 min at 37 °C, neutrophils were washed three times with HBSS. Neutrophils were then lysed by addition of 500 µl of 1 mM-sodium bicarbonate, centrifuged at 12 000 ***g*** for 3 min, aliquotted and stored at −80 °C. Activity of u-PA in aliquots of lysed neutrophils was determined as described by Politis et al. (2003).

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The assay system utilized the enzymically active u-PA present within the lysed neutrophils to convert exogenously supplied plasminogen to active plasmin. Plasmin, so produced, was subsequently allowed to attack the chromogenic substrate Val-Leu-Lys-*p*-nitroaniline adjacent to lysine and liberate the free chromophore *p*-nitroaniline. In this system, changes in colour were directly related to plasmin concentrations and, therefore, indirectly to u-PA activity.

Determination of membrane-bound u-PA activity

Membrane-bound u-PA is catalytically active (Politis et al. 2002, 2003) and, thus, its activity can be measured in 'live' PMA-stimulated and neutrophils. Neutrophils (2×10^6/ml) were resuspended in 500 µl of HBSS containing 20 mM-HEPES and 81 mM-PMA. After incubation for 30 min at 37 °C, neutrophils were washed three times with HBSS and, finally, resuspended at various cell concentrations in 250 µl of 100 mM-Tris buffer (pH 8·0) containing 100 mM-NaCl, plasminogen (50 µg/ml; Sigma), and 0·6 mM-Val-Leu-Lys-*p*-nitroanilide (Sigma). The reaction mixture was incubated for up to 90 min and absorbance at 405 nm was measured at 15-min intervals using a microtitre plate reader. The rate of formation of *p*-nitroaniline was calculated from the linear part of the curve for absorbance *v*. time. A sample without plasminogen served as control.

To eliminate the possibility that some u-PA was secreted in the medium, in control wells neutrophils were removed by centrifugation, at the end of the incubation period. No detectable activity was found in these samples indicating that neutrophils remained intact and no u-PA was released during the incubation period. Therefore, any changes in colour were due to conversion of plasminogen to plasmin by catalytically active u-PA present on the cell membrane.

Determination of free u-PA binding sites on the cell membrane of neutrophils

The u-PA binding sites present on the cell membrane of bovine neutrophils may or may not be fully saturated (Politis et al. 2003). Free, unoccupied u-PA binding sites can be fully saturated following incubation of neutrophils with purified u-PA. Thus, the difference of membrane-bound u-PA before and after incubation with u-PA reflects the presence of free u-PA binding sites on the cell membrane of neutrophils.

Neutrophils (2×10^6/ml) were resuspended in 500 µl of HBSS containing 20 mM-HEPES with or without 81 mM-PMA. After incubation for 30 min at 37 °C, neutrophils were washed three times with HBSS and resuspended in 500 µl of HBSS plus 20 mM-HEPES containing purified u-PA (Sigma; 10 units/ml) to fully saturate all u-PA binding sites on the cell membrane of neutrophils (Politis et al. 2003). After incubation for another 30 min at 37 °C, neutrophils were washed three times with HBSS, and then resuspended at various cell concentrations in 250 µl of 100-mM Tris buffer (pH 8·0) for the determination of the membrane-bound u-PA as described above.

Determination of superoxide anion production by neutrophils

Superoxide anion production as a direct indicator of respiratory burst activation was measured by superoxide dismutase-inhibitable reduction of ferricytochrome C (Politis et al. 1995). Cells (2×10^6/ml) suspended in 1·4 ml of HBSS were preincubated in the presence or absence of 10 µl of superoxide dismutase (3 mg/ml; Sigma) for 2 min. Then, 0·1 ml of type-IV cytochrome C (35 mg/ml; Sigma) was added, followed by addition of 1·5 ml of PMA (2 µg/ml). After 10 min, the reaction was terminated by placing tubes on ice, followed by centrifugation at 1500 ***g*** for 20 min. The supernatant was collected and the absorbance was measured at 550 nm. The amount of superoxide produced was calculated as the difference between the amounts reduced in the presence and absence of superoxide dismutase.

Milk composition

Individual milk samples were collected weekly for 12 weeks after parturition from all cows of both groups. Samples were analysed for fat, protein and lactose by infrared method using a MILKOSCAN 133 (Foss Electric, Hillerød, Denmark) calibrated against the Mojonnier method for fat, Kjeldahl for protein and the polarimetric method for lactose according to official methods (AOAC, 1980). SCC was determined with a Fossomatic cell counter (Foss Electric). Plasmin and plasminogen-derived activities in milk were determined by a colourimetric method (Politis et al. 1989).

Statistical analysis

Results were analysed by use of a mixed linear model with autoregressive (order 1) covariance structure because of the repeated measures per subject (cow). The model included the effect of treatment, the week of treatment, the treatment by week interaction as fixed effects and the effect of cow nested within treatment as a random effect. Logarithmic transformations (ln) of the SCC and plasmin activity in milk were used in statistical analysis. All analyses were carried out using the MIXED procedure of SAS (2001). Results are presented as least square means with SE. A Scheffe adjustment for group comparison(s) was employed in the analysis. Level of significance was set at $P<0·05$ unless otherwise specified.

Results

Vitamin E analyses

Plasma vitamin E concentrations in control cows decreased progressively *prepartum*, were lowest 1 week before up to

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Table 1. Mean plasma concentrations of vitamin E (μg/ml) in 10 control cows and 10 cows that received supplemental vitamin E beginning 4 weeks prior to parturition (−4, −3, −2, −1) and continuing for 4 weeks after parturition

	Vitamin E (μg/ml)	
Week	Control	Supplemented
−4	2·71[a,e]	2·71[a]
−3	2·29[a,d]	2·85[a]
−2	1·71[b,d]	2·82[a†]
−1	1·23[c]	1·97[b†]
1	1·09[c]	1·89[b†]
2	1·87[d]	3·38[a,c]†
3	2·58[a]	4·38[c,d]†
4	3·52[e]	5·86[c,d]†
Pooled SE	0·12	

† Values were significantly different from those of control cows: $P<0·01$
[a,b,c,d,e] Values within a column without a common superscript letter are significantly different: $P<0·05$

1 week after parturition, and increased steadily thereafter (Table 1). Cows in the treatment group had higher ($P<0·01$) plasma concentrations of vitamin E than control cows, starting 2 weeks before and continuing up to 4 weeks after parturition. Similarly to their control counterparts, treatment cows showed their lowest concentrations of plasma vitamin E during the period starting 1 week before up to 1 week after parturition (Table 1).

Immunological parameters

Total cell-associated and membrane-bound u-PA activities in neutrophils of both groups were lowest *prepartum* and the first week after parturition and increased steadily thereafter (Table 2). Neutrophils isolated from treatment cows had significantly higher total cell-associated u-PA activity starting 1 week before up to 3 weeks after parturition and higher membrane-bound u-PA activity during the first 3 weeks after parturition compared with corresponding values of neutrophils isolated from control cows (Table 2).

There were no statistically significant differences in the ratio of membrane-bound to total cell-associated u-PA activity in neutrophils isolated from control and treatment cows. Most PA (>90%) in neutrophils of both groups appeared to be present on the cell membrane.

Superoxide production of neutrophils isolated from control cows remained constant during the first 3 weeks *prepartum* (weeks –4, −3 and –2), were lowest 1 week before up to 1 week after parturition, and increased steadily thereafter (Table 3). Vitamin E administration prevented the decline in superoxide production by neutrophils during the period starting 1 week before up to 1 week after parturition. There were no significant effects of vitamin E supplementation on superoxide production by neutrophils during the first three weeks *prepartum* (−4, −3 and −2) and during weeks 2, 3 and 4 *post partum*.

Table 2. Total cell-associated and membrane-bound urokinase plasminogen activator (u-PA) activity (absorbance/h) in neutrophils obtained from 10 control cows and 10 cows that received supplemental vitamin E beginning 4 weeks prior to parturition and continuing for 4 weeks after parturition

	u-PA activity (ΔA/h)			
	Total		Membrane-bound	
Week	Control	Supplemented	Control	Supplemented
−4	0·09[a]	0·09[a]	0·09[a]	0·08[a]
−3	0·14[b,c]	0·12[a,b]	0·10[a]	0·13[b]
−2	0·12[a,b,c]	0·12[a,b]	0·11[a,b]	0·11[a,b]
−1	0·12[a,b,c]	0·21[c]†	0·09[a]	0·20[a]†
1	0·12[a,b,c]	0·27[c,d]†	0·10[a]	0·27[c,d]†
2	0·17[c]	0·37[d]†	0·15[b]	0·36[d]†
3	0·36[d]	0·54[e]†	0·35[c]	0·51[e]†
4	0·45[d]	0·54[e]	0·43[c]	0·52[e]
Pooled SE	0·12			

† Values were significantly different from those of control cows: $P<0·01$
[a,b,c,d,e] Values within a column without a common superscript letter are significantly different: $P<0·05$

Table 3. Superoxide production (nmol/10^6 cells) in neutrophils obtained from 10 control cows and 10 cows that received supplemental vitamin E beginning 4 weeks prior to parturition and continuing for 4 weeks after parturition

	Superoxide (nmol/10^6 cells)	
Week	Control	Supplemented
−4	2·82[a,c]	2·58[a]
−3	2·60[a,c]	2·60[a,b]
−2	2·36[a]	2·63[a,b]
−1	1·53[b]	2·60[a,b]†
1	1·24[b]	2·60[a]†
2	2·68[a,c]	3·38[a,b,c]
3	3·31[c,d]	3·45[b,c]
4	3·89[d]	4·43[c]
Pooled SE	0·11	

† Values were significantly different from those of control cows: $P<0·01$
[a,b,c,d] Values within a column without a common superscript letter are significantly different: $P<0·05$

Milk composition and SCC

There were no significant effects of vitamin E supplementation on milk concentrations of fat, protein or lactose throughout the experiment (Table 4). Vitamin E supplementation resulted in a 29% reduction ($P<0·05$) in milk SCC.

Plasmin–plasminogen system in milk

There were no significant effects on plasminogen-derived activity between the two experimental groups. Plasmin activity was reduced by 30% ($P<0·01$) in treatment cows (Table 5). The ratio of plasmin to plasminogen was no different between the two experimental groups but there was a tendency ($P=0·07$) for higher conversion rate of plasminogen to plasmin in control cows.

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Table 4. Effect of vitamin E supplementation on milk composition and milk SCC

Values are means with SEM for 275 determinations

	Control	Supplemented	*P* value
Fat (%)	4·14 ± 0·16	4·17 ± 0·06	0·73
Protein (%)	3·47 ± 0·17	3·42 ± 0·17	0·59
Lactose (%)	4·75 ± 0·08	4·78 ± 0·08	0·76
ln SCC/ml	4·70 ± 0·12	4·36 ± 0·12	0·04
SCC/ml × 10^{-3}	109·9	78·3	

Table 5. Effect of vitamin E supplementation on plasmin and plasminogen-derived activities (absorbance/h) and the ratio of plasmin/plasminogen in bovine milk

Values are means with SEM for 275 determinations

	Control	Supplemented	*P* value
Plasmin	0·13 ± 0·01	0·09 ± 0·01	0·01
Plasminogen	0·88 ± 0·02	0·85 ± 0·02	0·27
Ratio	0·44 ± 0·02	0·39 ± 0·02	0·07

Discussion

The first finding from the present study is that vitamin E supplementation affected the immune system of dairy cows housed in a commercial dairy herd during the periparturient period. This conclusion is supported by three key observations: the increased total cell-associated and membrane-bound u-PA activities and superoxide production by neutrophils isolated from treatment cows.

A previous study showed that vitamin E supplementation to be associated with higher chemotactic activity and faster migration of neutrophils towards the mammary gland (Politis et al. 1996). Because membrane-bound u-PA is involved in extravasation and migration of neutrophils to inflamed tissues (Blasi, 1993), it follows that neutrophils isolated from treatment cows, which had higher membrane-bound u-PA (Table 2), had an enhanced ability to migrate. Membrane-bound u-PA in neutrophils isolated from both groups was low *prepartum*. Membrane-bound u-PA increased steadily after parturition in both groups but the rate of the increase was faster in treatment cows. It appears that there was a delay of 4 weeks for vitamin E to affect the u-PA system. Furthermore, the effect was transient because there were no significant effects of vitamin E on the u-PA system of bovine neutrophils during week 4 *post partum*. The reason for the diminished effect of vitamin E on the u-PA system by the fourth week *post partum* maybe that circulating vitamin E of the control cows had reached the critical level of 3 μg/ml. These results are similar to those previously reported on the effect of vitamin E supplementation on the u-PA system (Politis et al. 2001); the only difference was that the previous study showed that vitamin E supplementation to be effective for a very short period (week 1 *post partum*). Differences between the two studies may be due to the fact that the present study was done in a commercial dairy herd in Greece and the previous study was under more controlled conditions in a North American University herd; apart from the obvious differences in climate, the two herds differed in their feeding and management and they probably differed in terms of oxidative stress.

It is interesting that the ratio of membrane-bound to total cell-associated u-PA activity was not affected by vitamin E supplementation and the majority (>90%) of u-PA in neutrophils of both groups was present on the cell membrane. Thus, the higher membrane-bound u-PA in neutrophils isolated from vitamin E supplemented cows was due to an increase in total cell-associated u-PA and not to a translocation of pre-existing u-PA within the neutrophil cell interior to the cell membrane.

Superoxide production by neutrophils isolated from control cows was lowest during a period of 2 weeks, starting week 1 *prepartum*, and it coincided with the lowest circulating vitamin E concentrations (Tables 1 and 3). The drop in superoxide production during this period was blocked in treatment cows. These results are similar to those reported by Politis et al. (1995). Other work shows that decreased phagocytosis and intracellular kill occur in parallel with decreased circulating vitamin E during the periparturient period (Hogan et al. 1992; Smith et al. 1997). In contrast, Hidiroglou et al. (1997) report that vitamin E supplementation with 1000 i.u./d during the periparturient period has no effect on the superoxide anion production by bovine neutrophils. Once again, differences between studies may be due to differences in oxidative stress, the level of vitamin E supplementation and management practices.

The second finding from the present study was that vitamin E supplementation of dairy cows in a commercial herd had positive effects on milk quality. This conclusion is supported by two observations: milk SCC was reduced by 29% and plasmin activity was reduced by 30% in treated cows. The reduction of milk SCC in treated cows is similar to that reported by Baldi et al. (2000) and Politis et al. (1995). The present and the two previous studies are similar in that supplementation was at high levels (2000–3000 i.u./cow per day) and this supplementation lasted for several weeks and was sufficient to block the drop in levels of circulating vitamin E during the periparturient period. Weiss et al. (1990) and Smith et al. (1997) investigated the association between vitamin E supplementation and clinical mastitis and reached the same conclusion: vitamin E supplementation is associated with reduced cases of clinical mastitis and a reduction in the duration of symptoms. More specifically, Weiss et al. (1990) reported that cases of clinical mastitis were only 2·6% for cows supplemented daily with 4000 i.u./cow during the last 2 weeks of the dry period and 2000 i.u./cow during lactation and 25% in cows supplemented daily with 100 i.u. of vitamin E/cow during the dry period and lactation. It appears that high levels of vitamin E supplementation, particularly during the periparturient period, are essential to affect the immune system and prevent mastitis.

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Two field studies show no association between circulating vitamin E levels and milk SCC (Erskine et al. 1987; Jukola et al. 1996). Differences in the conclusions from previous studies may be explained by differences in management, differences in the manner of vitamin E supplementation (oral *v.* i.m. injection), and the level of oxidative stress. It is clear, however, that the results of the present study and the majority of previous studies strongly suggest that high levels of vitamin E supplementation (>2000 i.u./cow per day) during the periparturient period reduce milk SCC.

A new finding from the present study is the association between vitamin E supplementation and the plasmin–plasminogen system in milk. This association is clearly relevant to cheesemaking because high levels of plasmin are associated with poor coagulating properties of milk, lower cheese-yielding capacity and production of cheese of inferior quality and composition. Our results showed that vitamin E supplementation reduced plasmin activity in milk by about 30% but it had no effect on plasminogen-derived activity. Therefore, the ratio of plasmin/plasminogen (an index of the conversion rate of plasminogen to plasmin) was lower in treated cows. Thus, vitamin E supplementation appears to interfere with the rate of conversion of the inactive plasminogen to active plasmin. To the best of our knowledge, this is the first report of an association between vitamin E supplementation and plasmin activity in milk.

The most likely explanation for reduced plasmin in milk from treated cows is that changes in plasmin follow closely those of milk SCC. It is known that increased levels of plasmin in milk are observed in milk with elevated SCC (Politis et al. 1989). In fact, somatic cells isolated from milk are known to contain plasminogen activators (Heegard et al. 1994). Thus, the decrease in milk SCC in treated cows would be expected to result in a decreased rate of conversion of plasminogen to plasmin in bovine milk. Against this notion, our results showed that vitamin E supplementation reduced milk SCC from 110 000/ml to 78 000/ml. Although statistically significant, this reduction in SCC does not fully account for the response because a reduction in plasmin activity of the magnitude of 30% would require a much greater effect of vitamin E on SCC. Thus, the reduction of plasmin can be only partly explained by the reduction in milk SCC. The mechanism whereby vitamin E supplementation affects the plasmin–plasminogen system in milk will be addressed in our future investigations.

In conclusion, vitamin E supplementation of dairy cows in a commercial herd resulted in increased membrane-bound u-PA and superoxide production by bovine neutrophils. Furthermore, milk obtained from cows that received supplemental vitamin E supplementation had lower SCC and plasmin activity.

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