Effect of vitamin E supplementation and of health status of mammary gland on immunoglobulin concentration in milk of dairy cows

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Short note

Effect of vitamin E supplementation and of health status of mammary gland on immunoglobulin concentration in milk of dairy cows

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Summary — Variation of milk IgG1, IgG2, IgM, and IgA measured by radial immunodiffusion was studied in monthly samples of 224 lactating dairy cows. Cows were assigned to one of 2 treatment groups: vitamin E supplemented and controls. Vitamin E supplementation was started when cows were dried and continued until 90 days *post partum* at the rate of 1 000 IU per cow daily and then reduced to 500 IU for the remaining lactation. There were no significant differences between the 2 treatment groups for the different Ig classes. Concentrations of IgG1 were significantly higher in milk from mastitic than non-mastitic cows.

vitamin E / mastitis / immunoglobulin / cows

Résumé — Effet de l'apport en vitamine E et de l'état sanitaire de la glande mammaire sur la concentration en immunoglobuline du lait chez les vaches laitières. *La variation de la concentration en IgG1, IgG2, IgM et IgA du lait, mesurée par immunodiffusion radiale, a été étudiée tous les mois chez les vaches laitières allaitantes. Les vaches ont été réparties en 2 groupes : un groupe a reçu un apport en vitamine E, l'autre n'a rien reçu. L'apport en vitamine E a commencé à partir du tarissement jusqu'à 90 j après le vêlage à raison de 1 000 UI/j pour chaque vache puis 500 UI/j jusqu'à la fin de la lactation. Il n'y a pas eu de différence entre les 2 groupes pour la concentration en différentes classes d'immunoglobulines. Les concentrations en IgG1 ont été significativement plus élevées dans le lait des vaches présentant des mammites cliniques que dans le lait des vaches sans mammite clinique.*

vitamine E / mammite / immunoglobuline / vache

* Correspondence and reprints

INTRODUCTION

The immunoglobulins (Ig) of milk are derived from either the central or local compartment of the immune system and are identical to those found in serum or other secretions. There are marked differences, which differ amongst species, in their relative concentrations in milk when compared with serum. Studies on the changes in level of bovine Ig associated with calving indicate that the selective transport mechanism is most active during the 2–3 weeks prior to calving and is maintained at a lower rate during lactation (Brandon *et al*, 1971). Penhale and Christie (1969) reported that mean bovine plasma and colostrum concentrations of IgG and IgM were 12.9 and 2.8 mg/ml (in plasma) and 34.1 and 4.9 mg/ml (in colostrum), respectively.

Smith *et al* (1984) reported that vitamin E supplementation reduced the incidence of clinical mastitis in dairy cows. Similar results were also reported by Batra *et al* (1991). Johnston and Chew (1984) reported no significant difference between mastitic and non-mastitic cows for vitamin A and β-carotene in milk. Considerable data are available on the relative concentrations of Ig in milk of healthy cows (Klaus *et al*, 1969; Brandon and Lascelles, 1975), but this information in mastitic cows is lacking. The objective of the present study was to determine the effect of vitamin E supplementation and presence or not of clinical mastitis on the Ig in milk during lactation of dairy cows housed indoors.

MATERIALS AND METHODS

Animals and experimental design

Cows were from a Holstein and a line crossbred from Holstein and Ayrshire lines maintained at the Animal Research Centre (McAllister *et al*, 1978). All cows that were dried off from November, 1988 to October, 1990 were used. Pregnant cows of first or later parity were randomly assigned to 1 of the 2 treatment groups taking into account breed (Holstein, Crossbreds) and parity at the time of drying off. Cows in the supplemented group received a basal ration formulated to meet the requirements of non-lactating dairy cows plus 0.5 kg ground corn containing 1 000 IU vitamin E per cow daily. Cows in the control group received the basal ration plus 0.5 kg ground corn without vitamin E supplement. Cows in the supplemented group received the same level of vitamin E (1 000 IU/cow/day) from drying to the end of the first 3 months of lactation, at which time the supplement was reduced to 500 IU per cow daily for the remaining lactation period. Composite milk samples from cows in both groups were collected at calving and once a month during the lactation period.

Rations

Dry cow ration was fed at the rate of 8.5 kg dry matter (DM) per cow daily and was composed of corn silage (15%), hay (35%), and haylage (50%). Lactating cows received forage (corn silage 70%, haylage 20%, hay 10%) mixed with concentrate in the ratio of 88% forage and 12% concentrate. Cows producing more than 7 kg of milk per day received additional concentrate at the rate of about 1 kg for every 3 kg of milk produced according to NRC recommendations (National Research Council, 1978).

Monoclonal antibody production

Murine monoclonal antibodies to bovine IgG1, IgG2, IgM, and IgA were prepared as described by Nielsen *et al* (1985). The monoclonal antibodies selected for use were all mouse isotype IgG1 with κ light chains. Murine ascites fluid was prepared in balb/c mice. Ascites fluids were used unpurified except for removal of lipids by centrifugation at 110 000 x *g* for 15 min and filter sterilization. These preparations were stored at –20 °C until use.

Statistical analysis

Data on Ig were analyzed by a least-squares procedure (SAS 1982). To test the effect of vitamin E supplementation on the milk Ig levels, (measured by radial immunodiffusion as described in Nielsen *et al*, 1985), the model (model 1) included effects of treatment, stage of lactation, and treatment by stage of lactation interaction. The measure of mastitis in each cow was evaluated at the time of sampling as "mastitic" (showing signs of clinical mastitis) or "non-mastitic" (no signs of clinical mastitis). At each sampling time ≈ 16% of the cows were mastitic. In order to test the differences in Ig levels between mastitic and non-mastitic cows, the statistical model (model 2) included the effects of mastitis status (health), stage of lactation and health by stage of lactation interaction. Differences between the control and supplemented group, as well as between mastitic and non-mastitic cows for Ig isotypes were tested by *t*-test.

RESULTS

Analysis of variance for Ig in model 1 revealed significant ($P < 0.05$) effects of stage of lactation on IgG1, IgG2, and IgA, and treatment by stage of lactation interaction on IgG1 and IgA. Effect of treatment was not significant ($P > 0.05$) for all 4 Ig isotypes (table I).

Distribution of overall Ig concentration in milk during the lactation of control and supplemented group of cows is shown in figure 1. When comparison was made between vitamin E supplemented and control group for each month of lactation there was a significant ($P < 0.05$) increase in IgG1 for month 6, IgG2 for month 3, and IgA for months 1 and 2.

Analysis of variance for Ig using model 2 showed that effects of health and stage of lactation were significant ($P < 0.05$) for IgG1 and IgG2, while the interaction of health by stage of lactation was only significant ($P < 0.05$) for IgG1 (table II). Effects of health, stage of lactation and health x stage of lactation interaction were not significant for both IgM and IgA. Mastitic cows had significantly ($P < 0.05$) higher concentration of IgG1 for month 6, and IgG2 for month 7. There were no significant differences between mastitic and non-mastitic animals for IgG2, IgM and IgA. Concentration of IgG1 was significantly ($P < 0.01$) higher in milk from mastitic than non-mastitic cows (fig 2).

Table I. Analysis of variance for immunoglobulins using model 1.

Source	*df*	*Mean squares* IgG1	IgG2	IgM	IgA
Stage of lactation	10	5 260 291**	299 245**	79 527	3 530*
Treatment	1	77 471	4 633	108 204	332
Stage x treatment	10	517 571*	49 733	50 684	3 240*
Residual [1]		271 299	85 595	95 816	1 681
R^2 (%)		24	8	4	11

[1] Residual df were 657, 476, 367, and 320 for IgG1, IgG2, IgM, and IgA, respectively. * $P < 0.05$; ** $P < 0.01$.

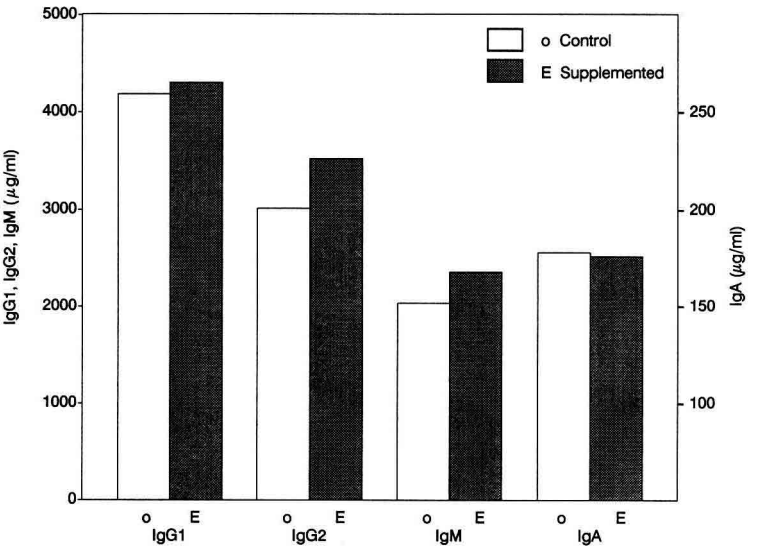

Fig 1. Distribution of mean Ig concentration in milk of control and vitamin E supplemented cows during lactation.

Table II. Analysis of variance for immunoglobulins in milk using model 2.

Source	*df*	*Mean squares* IgG1	IgG2	IgM	IgA
Stage of lactation	10	3 321 635**	346 605**	215 728	890
Health	1	1 915 893*	500 376*	3 469	3 297
Stage x health	10	1 112 606**	155 486	97 700	1 335
Residual [1]		330 495	96 596	119 006	1 740
R^2 (%)		22	13	8	10

[1] Residual df were 628, 465, 381 and 311 for IgG1, IgG2, IgM and IgA, respectively. * $P < 0.05$; ** $P < 0.01$.

DISCUSSION

Significant effect of stage of lactation on Ig isotypes was due to changes in the concentrations of Ig isotypes during the lactation. IgG is the principal Ig in the colostrum of species that transmit no or very small amounts of this to their offspring *in utero* (Butler, 1974). Guidry *et al* (1980) reported that concentrations of all milk Ig were high-

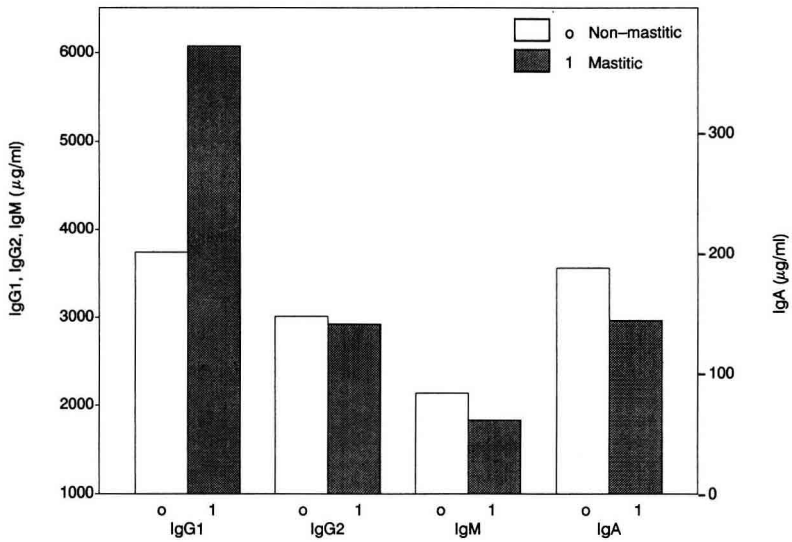

Fig 2. Distribution of mean Ig concentration in milk of mastitic and non-mastitic cows during lactation.

est in the colostrum and there was a rapid decrease in Ig levels within first few days after parturition. This parallels the transition from colostrum to normal milk. The concentration of all Ig increased during late lactation (Senft *et al,* 1976). Similar results were also observed in the present study. Mammary gland selectively transports Ig from blood to colostrum and synthesizes only a minor quantity of Ig. Larson *et al* (1980) reported that Ig in mammary secretions are both humoral, arising from the blood stream and local, arising from production by plasmacytes in the mammary gland. The bovines transfer large amounts of IgG, IgG1 in particular, from blood stream across mammary barrier in to colostrum by specific transport mechanism. Both IgM and IgA are synthetized locally in the mammary gland by plasmacytes (Brandon *et al,* 1971; Butler, 1974).

Higher concentration of IgG1 in milk from mastitic than non-mastitic cows suggested that there was some involvement of humoral immune system in elimination of the infection.

Supplementation of diets of cows with vitamin E during the dry period and lactation had no significant effect on the concentrations of Ig in milk. Concentration of IgG1 was significantly higher in milk from mastitic than non-mastitic cows.

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