Effect of Vitamin E Supplementation on Plasma Antioxidant Vitamins and Immunity Status of Crossbred Cows

P. N. Chatterjee, Harjit Kaur* and N. Panda

Dairy Cattle Nutrition Division, National Dairy Research Institute, Karnal-132001, India

ABSTRACT : Twenty crossbred (HF×Tharparkar) dry pregnant cows were divided into four equal groups. They were supplemented with 1,000 I.U. α-tocopheryl acetate from 0 (group I), 15 (group II), 30 (group III) and 60 (group IV) days before parturition to 1month of lactation. All the cows were kept under similar feeding and management conditions. Blood plasma samples collected on specific days were analyzed for α-tocopherol, retinol, total antioxidant activity (FRAP), immunoglobulin and calcium. Plasma α-tocopherol concentration at 30 days prepartum averaged 3.5, 4.1, 4.4 and 3.9 μg/ml and decreased by 50.0, 41.4, 34.1 and 33.3 percent on the day of parturition in the four respective groups. After calving, plasma vitamin E started to recover earlier in groups II, III and IV as compared to group I. Mean plasma α-tocopherol concentration at 21 days postpartum was significantly higher in groups II, III and IV (2.9, 3.5 and 3.1 μg/ml) compared to group I (1.9 μg/ml) cows. Plasma retinol concentration also showed a substantial decrease in all the groups on the day of calving but recovered to its normal value at 3 weeks postpartum. Plasma total antioxidant activity averaged 901, 895, 859 and 875 μmol/l in the four respective groups on 30 days prepartum and decreased on the day of calving in all the groups, but the decrease was less in groups III and IV. Plasma immunoglobulin concentration was higher in group IV, followed by groups III, II and I, respectively, showing better immune status of vitamin E supplemented cows due to less oxidative stress. Supplementation of vitamin E resulted in higher plasma calcium concentration. The data showed that vitamin E supplementation should be started at least 30 days prepartum to reduce oxidative stress in periparturient cows. ***(Asian-Aust. J. Anim. Sci. 2003. Vol 16, No. 11 : 1614-1618)***

Key Words : Vitamin E, Retinol, Total Antioxidant Activity, Immunoglobulin, Cows

INTRODUCTION

Vitamin E is an important fat soluble antioxidant vitamin, essential for proper health, immunity and reproductive function of animals. Reactive oxygen species are continuously produced *in vivo* during normal metabolism and due to exposure to several pollutants. Vitamin E, being a free radical scavenger, can combat these harmful metabolites and prevents oxidative damage to tissues. Supplementation of vitamin E maintains proper antioxidant status of animals and improves the ability to resist infections in periparturient cows resulting in optimal udder health. Since colostrum is rich in vitamin E, circulatory level of this vitamin decreases at the time of parturition and is reported to be associated with severe health problems (Michal et al., 1994; Weiss et al., 1997; Hayangmi et al., 1999). Low plasma concentration of α-tocopherol at parturition is considered as a significant risk factor for intra-mammary infection and mastitis during first week of lactation (Goff and Stabel, 1990). Based on health and immune function of cows during peripartum period, NRC (2001) has raised the vitamin E requirement from 15 IU to 80 IU/kg feed intake. Cows with plasma concentration <2.5 μg/ml were reported to be 2.8 times more likely to have intramammary infection (IMI) than the cows having >2.5 μg/ml plasma vitamin E (Weiss et al., 1997). Jukola et al. (1996) and Weiss et al. (1997) suggested that 3.0 μg/ml of vitamin E is required at parturition for immuno-competence in dairy animals. Vitamin E supplementation in dry cows has been reported to increase their plasma vitamin E level (Campbell and Miller, 1998, Rajiv, 2001) and reduce the incidence of mastitis and retained placenta. But, information on the minimum number of days required for vitamin E supplementation during prepartum period to maintain the desired vitamin level around parturition is not precisely available. Therefore, effect of duration of prepartum vitamin E supplementation on plasma antioxidant vitamins status was studied in periparturient crossbred cows.

MATERIALS AND METHODS

Twenty crossbred Karan Fries (Tharparkar×Holstein Friesian) pregnant dry cows were selected and randomly divided into four groups namely, I, II, III and IV. They were fed as per the standard feeding practices followed at NDRI Farm and were supplemented α-tocopheryl acetate at 1,000 IU/cow from 0 (group I), 15 (group II), 30 (group III) and 60 (group IV) days before parturition to one month of lactation. The animals were kept in the general herd and were allowed to remain under open housing system throughout the period of experiment. Special care was taken during advance pregnancy of animals and they were sent to

* Corresponding Author: Harjit Kaur. Tel: +91-184-2259060, E-mail : harjit@ndri.hry.nic.in

Received November 14, 2002; Accepted July 24, 2003

Table 1. Program for HPLC analysis of retinol and α-tocopherol on a single run

Vitamin	Wavelength (nm)	Change time (min)	Retention time (min)
Retinol	325	0.00	1.98
α-tocopherol	290	4.00	6.58

maternity lot 14 days before the expected date of calving. Green fodder was offered *ad libitum* and concentrate mixture was given at milking byre. One kg concentrate mixture was given for every 2.5 to 3.0 kg of milk produced by the cows. Blood samples were collected at -30, -15, -7, 0 and +21 days of parturition. They were centrifuged and plasma obtained was utilized for the analysis of immunoglobulin (Ig), Ferric Reducing Antioxidant Power (FRAP), retinol, α-tocopherol and calcium.

Estimation of vitamins in plasma

Simultaneous estimation of retinol and α-tocopherol was carried out on HPLC using extraction procedure of Chawla and Kaur (2001). 0.5 ml of plasma was deproteinized with an equal volume of 95 percent ethanol containing 5 percent ascorbic acid. Three extractions were carried out using 2 ml petroleum ether each time to maximally extract the vitamins. The ether extract was dried under nitrogen in a water bath maintained at 37°C and was reconstituted in the mobile phase.Individual stock solutions of retinol (1 mg/ml) and α-tocopherol (2 mg/ml) were prepared in 100 percent ethanol. An aliquot was dried under nitrogen and was reconstituted in mobile phase. Working standard containing 10.0 µg/ml retinol and 20.0 µg/ml α-tocopherol was prepared. The samples were analysed on HPLC system consisting of a model 510 pump, rheodyne injector with 20 µl loop, model 486 tunable absorbance detector using multiwavelength detector and Discovery™ C-18 (USA) column (15 cm×4.6 mm) packed with 5 µm silica particles. Solvent system used was methanol and HPLC water in the ratio of 95:5. A specific programme was adopted for the separation of retinol and α-tocopherol at different wavelengths simultaneously within 8 minutes (Table 1).

Estimation of plasma total antioxidant activity

Plasma total antioxidant activity was measured by ferric reducing antioxidant power (FRAP) assay (Benzie and Strain, 1999). 100 µl of plasma sample was mixed with 3 ml of working FRAP reagent and absorbance was measured at 0 minute at 593 nm after vortexing. Thereafter, samples were placed at 37°C in a water bath and absorbance was measured after 4 minutes. Ascorbic acid standards were processed in the same way. Change in absorbance (ΔA_{593} nm) is translated into FRAP value (µM) by comparing the test samples to that of standard solution of known FRAP value. FRAP value of ascorbic acid is 2.

Plasma immunoglobulins were estimated by zinc turbidity method (McEwan and Fisher, 1970). Estimation of Calcium in Plasma was carried out on Philips Scientific Model PU9100×Atomic Absorption Spectrophotometer (AAS) using acetylene as fuel and air as oxidant. Statistical analysis of the data was carried out using two way analysis of variance technique (Snedecor and Cochran, 1980).

RESULTS AND DISCUSSION

Plasma α-tocopherol

Plasma α-tocopherol concentration at 30 days before parturition averaged 3.5, 4.1, 4.4 and 3.9 µg/ml in groups I, II, III and IV, respectively (Table 2). The plasma α-tocopherol concentration decreased during prepartum period and was lowest on the day of calving in all the four groups. A decrease in plasma vitamin E concentration at parturition in cows was first documented by Goff and Stabel (1990) and was attributed to its diversion towards colostrum production and secretion. Plasma vitamin E concentration started increasing after parturition due to reduced colostrogenesis and less secretion of vitamin E into milk.

The decrease in plamsa α-tocopherol concentration from 30 days prepartum to calving averaged 50.0, 41.4, 34.1 and 33.3 percent in four respective groups indicating comparatively less decrease in vitamin E supplemented groups. On the day of calving, the plasma α-tocopherol concentration was significantly higher ($p<0.05$) in groups II, III and IV as compared to group I (Table 2). After calving,

Table 2. Influence of Vitamin E supplementation on plasma α-tocopherol (µg/ml) in periparturient cows

Days parturition	Groups			
	I*	II*	III*	IV*
-30	3.5^{aC}±0.2	4.1^{bcD}±0.1	4.4^{cD}±0.3	3.9^{abC}±0.3
-15	2.4^{aB}±2.1	2.7^{abBC}±0.2	3.1^{bBC}±0.5	3.1^{bB}±0.1
-7	1.8^{aA}±0.1	2.3^{abAB}±0.3	2.8^{cB}±0.4	2.5^{bcAB}±0.1
0	1.5^{aA}±0.1	1.9^{abA}±0.2	2.3^{bA}±0.1	2.2^{bA}±0.1
7	1.7^{aA}±0.01	2.4^{bA}±0.2	2.9^{cB}±0.2	2.6^{bcAB}±0.1
21	1.9^{aAB}±0.2	2.9^{bC}±0.4	3.5^{cC}±0.2	3.1^{bcB}±0.1

* Group I was supplemented with α-tocopheryl acetate at 0 IU, whereas groups II, III and IV were supplemented with 1,000 IU α-tocopheryl acetate from 15, 30 and 60 days prepartum to 30 days postpartum, respectively. Day 0 means day of parturition. Values bearing, $^{a, b, c}$ superscripts in a row differ significantly ($p<0.05$). Values bearing, $^{A, B, C, D}$ superscripts in a column differ significantly ($p<0.05$). $^{C, D}$ of groups (0.45) and periods (0.56).

Table 3. Influence of Vitamin E supplementation on plasma retinol (μg/ml) in periparturient cows

Days parturition	Groups				Means±SE
	I*	II*	III*	IV*	
-30	3.3^{D}±0.2	3.0^{D}±0.2	3.1^{C}±0.2	3.4^{D}±0.0	3.2±0.1
-15	2.8^{C}±0.1	2.2^{BC}±0.3	2.3^{B}±0.1	2.3^{BC}±0.0	2.4±0.1
-7	1.9^{AB}±0.2	1.8^{AB}±0.2	2.2^{AB}±0.1	2.0^{AB}±0.3	2.0±0.1
0	1.5^{A}±0.2	1.5^{A}±0.2	1.7^{A}±0.2	1.7^{A}±0.2	1.6±0.1
7	1.9^{AB}±0.1	1.9^{AB}±0.2	2.0^{AB}±0.1	1.9^{A}±0.1	2.0±0.1
21	2.2^{B}±0.2	2.7^{CD}±0.1	2.7^{BC}±0.1	2.5^{C}±0.0	2.5±0.1
Means±SE	2.2±0.12	2.1±0.1	2.3±0.1	2.1±0.2	

* Group I was supplemented with α-tocopheryl acetate at 0 IU, whereas groups II, III and IV were supplemented with 1,000 IU α-tocopheryl acetate from 15, 30 and 60 days prepartum to 30 days postpartum, respectively. Day 0 means day of parturition. Values bearing, $^{A, B, C, D}$ superscripts in a column differ significantly ($p<0.05$). $^{C, D}$ of periods (0.48).

Table 4. Influence of Vitamin E supplementation on plasma total antioxidant activity (FRAP) in periparturient cows (μmol/l)

Days parturition	Groups				Means±SE
	I*	II*	III*	IV*	
-30	901.0^{C}±67.0	895.0^{C}±17.0	859.0^{B}±0.0	875.2^{B}±23.6	882.7±15.8
-15	797.0^{B}±59.8	793.4^{AB}±34.5	847.5^{AB}±44.2	843.7^{AB}±13.5	816.1±21.9
-7	744.0^{A}±45.5	764.8^{A}±21.6	801.5^{AB}±49.2	796.6^{AB}±35.1	775.4±18.3
0	680.4^{A}±43.2	724.2^{A}±23.9	755.0^{A}±67.3	766.4^{A}±10.6	731.5±20.8
7	778.0^{A}±30.0	765.0^{A}±50.5	811.6^{AB}±76.3	815.4^{AB}±14.9	792.5±22.9
21	844.0^{BC}±50.2	883.6^{BC}±21.9	895.6^{B}±59.6	826.5^{AB}±37.3	864.3±21.8
Means±SE	778.5±21.9	794.3±16.8	823.6±26.5	818.7±11.6	

* Group I was supplemented with α-tocopheryl acetate at 0 IU, whereas groups II, III and IV were supplemented with 1,000 IU α-tocopheryl acetate from 15, 30 and 60 days prepartum to 30 days postpartum, respectively. Day 0 means day of parturition. Values bearing, $^{A, B, C}$ superscripts in a column differ significantly ($p<0.05$). $^{C, D}$ of periods (101.7).

plasma vitamin E level started to recover earlier in groups II, III and IV as compared to group I. Mean plasma α-tocopherol concentration at 21 days postpartum was 1.9, 2.9, 3.5 and 3.1 μg/ml in the four respective groups showing higher ($p<0.05$) levels in VE supplemented cows.

Hidiroglou et al. (1997) and Weiss et al. (1997) also reported increased plasma vitamin E level with daily supplementation of 1,000 IU vitamin E in periparturient cows. Wide variation in circulatory vitamin E level in cows has been reported (Weiss et al., 1997; Cambell and Miller, 1998) which might be due to variation in previtamin status of cows or level of vitamin E supplementation. Campbell and Miller (1998) reported as low as 0.73 and 1.15 μg/ml plasma α-tocopherol in 0 and 1,000 IU vitamin E supplemented cows during dry period. Rajiv (2001) supplemented 1,000 IU vitamin E to cows at 45±3 days prepartum and recorded increased vitamin E level in supplemented cows (2.25 vs. 1.57 μg/ml) on the day of calving. The present findings support the beneficial effect of supplementing vitamin E to dry cows resulting in comparatively higher plasma α-tocopherol concentration in and around parturition and rapid regain of normal plasma vitamin E status of 3.0 μg/ml for better immune function (Weiss et al., 1997). Unsupplemented cows in group I could not reach this target even at 21 days after calving. Vitamin E supplementation at 30 and 60 days prepartum resulted in similar plasma vitamin E status as compared to 15 days prepartum supplementation (Group II) or no supplementation (Group I).

Plasma retinol

Plasma retinol concentration at 30 days prior to parturition was above 3 μg/ml in all the groups (Table 3). It started decreasing as the pregnancy advanced and was lowest on the day of parturition. The level started to increase after freshening. Substantial decline in plasma retinol level had been observed on the day of calving, which recovered after 2-3 weeks postpartum in cows (Daniel et al., 1991; Rajiv, 2001). Weiss (1998) reported plasma retinol concentration of 40 to 50 μg/100 ml in cows fed as per NRC requirements. Hidiriglou (1989) and Hogan et al. (1994) suggested that since there is less transfer of fat soluble vitamins in milk, therefore, these vitamins stay in plasma for a longer time during dry period, which results in their higher concentration. Variation in plasma retinol in the range of 0.57 to 1.21 μg/ml was observed in cows fed on non-leguminous/silage based diet (Rajiv, 2001). Higher plasma retinol concentration observed in the present study might be due to the feeding of good quality berseem fodder, which is a rich source of β-carotene.

Plasma total antioxidant activity

Plasma total antioxidant activity (FRAP value) at 30 days before calving was 901, 895, 859 and 875 μmol/l in

Table 5. Influence of Vitamin E supplementation on total plasma immunoglobulin (mg/ml) in periparturient cows

Days parturition	Groups				Means±SE
	I*	II*	III*	IV*	
-30	50.4^{aD}±3.3	47.3^{aBC}±2.5	49.7^{aB}±2.3	50.8^{aA}±1.9	49.6±1.1
-15	44.9^{aBCD}±3.4	42.7^{aB}±2.6	51.2^{bB}±4.8	47.8^{abA}±1.7	46.1±1.5
-7	40.4^{abAB}±2.3	38.8^{aAB}±1.4	39.6^{abA}±2.8	45.3^{bA}±2.3	41.0±1.2
0	34.5^{aA} ± 1.4	35.2^{aA}±1.5	38.8^{abA}±1.7	43.9^{bA}±3.0	38.1±1.3
7	42.5^{aBC}±1.5	44.1^{aBC}±3.0	43.8^{aA}±2.8	46.9^{aA}±3.6	44.4±1.4
21	48.9^{aCD}±3.1	50.0^{aC}±2.5	53.3^{aB}±1.8	50.2^{aA}±2.5	50.6±1.2
Means±SE	43.1±1.4	42.9±1.3	45.7±1.4	47.8±1.1	

* Group I was supplemented with α-tocopheryl acetate at 0 IU, whereas groups II, III and IV were supplemented with 1,000 IU α-tocopheryl acetate from 15, 30 and 60 days prepartum to 30 days postpartum, respectively. Day 0 means day of parturition. Values bearing, $^{a, b}$ superscripts in a row differ significantly ($p<0.05$). Values bearing, $^{A, B, C, D}$ superscripts in a column differ significantly ($p<0.05$). $^{C, D}$ of groups (6.1) and periods (7.5).

Table 6. Influence of Vitamin E supplementation on plasma calcium (mg/dl) in periparturient cows

Days parturition	Groups				Means±SE
	I*	II*	III*	IV*	
-30	12.2^{aD}±0.3	12.1^{aC}±0.3	12.3^{aC}±0.7	12.4^{aC}±0.5	12.3±0.2
-15	10.8^{aBC}±0.2	10.1^{aAB}±0.2	11.3^{aBC}±0.3	10.6^{aAB}±0.3	10.7±0.1
-7	9.1^{aA}±0.5	9.3^{aA}±0.5	10.5^{bAB}±0.5	9.9^{abA}±0.3	9.7±0.2
0	8.7^{aA}±0.4	9.3^{abA}±0.4	10.0^{bA}±0.3	10.0^{bAB}±0.3	9.5±0.2
7	9.7^{aAB}±0.4	11.2^{bBC}±0.2	11.1^{bD}±0.3	11.2^{bC}±0.4	10.8±0.2
21	11.0^{aCD}±0.4	11.6^{abC}±0.3	12.1^{bC}±0.2	12.4^{bC}±0.2	11.8±0.2
Means±SE	10.3±0.2	10.6±0.2	11.2±0.2	11.1±0.2	

* Group I was supplemented with α-tocopheryl acetate at 0 IU, whereas groups II, III and IV were supplemented with 1,000 IU α-tocopheryl acetate from 15, 30 and 60 days prepartum to 30 days postpartum, respectively. Day 0 means day of parturition. Values bearing, $^{a, b}$ superscripts in a row differ significantly ($p<0.05$). Values bearing, $^{A, B, C, D}$ superscripts in a column differ significantly ($p<0.05$). $^{C, D}$ of groups (1.0) and of periods (1.2).

four respective groups (Table 4). The values continued to decline till calving and became lowest on day 0 as 680, 724, 755 and 766 μmol/L in groups I to IV, respectively. Groups III and IV showed less decrease in FRAP values compared to groups I and II, and group II showed less decrease than group I. However, there was no significant effect of dietary treatments on FRAP value. Brzezinska et al. (1994) reported decrease in total antioxidant capacity with approaching parturition in cows. They observed that supplementation of vitamin E at 1,000 IU/cow/day during late gestation resulted in 33, 43 and 63 percent increase in plasma total antioxidant capacity after 2, 4 and 6 weeks of supplementation. Rajiv (2001) found a positive correlation between plasma α-tocopherol and FRAP values (r=0.63) in periparturient cows.

Plasma immunoglobulins

Total plasma immunoglobulin level was 50.4, 47.3, 49.7 and 50.8 mg/ml in four respective groups (Table 5). It started decreasing as parturition approached and became lowest on the day of parturition. The immunoglobulin concentration was significantly high ($p<0.05$) in group IV than groups I and II on the day of parturition. The levels increased after calving in all the groups. Reduction in plasma immunoglobulins level seems to be due to increased oxidative stress around parturition as evidenced by decreased plasma α-tocopherol, retinol and total antioxidant activity (FRAP) at that time (Tables 2 to 4). Supplementation of vitamin E seemed to have positive effect on Ig levels in cows.

Plasma calcium concentration

Plasma calcium level was found to decrease from 12.2, 12.1, 12.3 and 12.4 mg/dl on 30 days before parturition to 8.7, 9.3, 10.0 and 10.0 mg/dl on the day of calving in four respective groups (Table 6). Yamagishi and Natio (1997) and NRC (2001) also reported decreased Ca level in plasma at parturition in cows. The drop in Ca concentration might be due to its increased diversion for foetal growth during the last stage of pregnancy. This is an important factor for increased IMI around parturition as the streak canal and its surrounding smooth muscles loose their tonicity, thereby allowing the entry of environmental pathogens. Plasma Ca level was found to return to the original level by 21 days postpartum. Supplementation of vitamin E resulted in higher ($p<0.05$) plasma calcium concentration in the present experiment. It is inferred that vitamin E supplementation at 1,000 IU/cow/day during prepartum period improved plasma antioxidant vitamin and Ca status.

Supplementation of vitamin E should at least be started at 30 days prepartum to reduce oxidative stress in cows.

REFERENCES

Benzie, E. F. I. and J. J. Strain. 1999. Ferric reducing/antioxidant power assays: Direct measurement of total antioxidant activity

of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. Methods in Enzymology, 299:15-27.

Brzezinska, S. E., J. K. Miller, III. J. D. Quigley, J. R. Moore and F. C. Madsen. 1994. Antioxidant status of dairy cow supplemented prepartum with vitamin E and selenium. J. Dairy Sci. 77:3087-3095.

Campbell, M. H. and J. K. Miller. 1998. Effect of supplemental dietary vitamin E and Zinc on reproductive performance of dairy cows and heifers fed excess iron. J. Dairy Sci. 81:2693-2699.

Chawla, R. and H. Kaur. 2001. Isocratic HPLC method for simultaneous determination of β-carotene, retinol and α-tocopherol in feeds and blood plasma. Indian J. Dairy Sci. 54:84-90.

Daniel, L. R., B. P. Chew, T. S. Tanaka and L. W. Tjoelker. 1991. β-carotene and vitamin A effects on bovine phagocyte function *in vitro* during peripartum period. J. Dairy Sci. 74:124.

Goff, J. P. and J. R. Stabel. 1990. Decreased plasma retinol, α-tocopherol and zinc concentration during the peri parturient period : Effect of milk fever. J. Dairy Sci. 73:3195-3199.

Hayangmi, N., M. JinSan, J. YiSeok, O. H. Tacho, P. Yongho and R. H. Hong. 1999. Study of plasma β-carotene concentration in dairy cows. Korean J. Vet. Res. 39:1021-1027.

Hidiroglou, M. 1989. Mammary transfer of vitamin E in dairy cows. J. Dairy Sci. 72:1067-1071.

Hidiroglou, M., T. R. Batra and X. Zhao. 1997. Bioavailability of vitamin E compounds and the effect of supplementation on release of super oxide and hydrogen peroxide by bovine neutrophils. J. Dairy Sci. 80:187-193.

Hogan, J. S., W. P. Weiss and K. L. Smith. 1994. Efficacy of parenteral vitamin E for treatment of bovine mastitis. Agric. Practice, 15:39-42.

Jukola, E., J. Hakkarainen, H. Saloniemi and S. Sani. 1996. Blood selenium, vitamin E, vitamin A and β-carotene concentration and udder health, fertility treatments and fertility. J. Dairy Sci. 79:838-845.

McEwan, A. D. and E. W. Fisher. 1970. A turbidity test for estimation of immunoglobulins levels in neonatal calf serum. Clin. Chem. Acta. 17:155.

Michal, J. J., L. R. Heirman, T. S. Wong, B. P. Chew, M. Frigg and L. Volker. 1994. Modulatory effects of dietary β-carotene on blood and mammary leukocyte function in peri parturient dairy cows. J. Dairy Sci. 77:1408-1421.

NRC. 2001. Nutrient Requirements of Dairy Cattle. 7th Revised Edn. National Academy Press, Washington, DC.

Rajiv. 2001. Influence of β-carotene and vitamin E supplementation on udder health and immunocompetence in dairy cattle. Ph.D. Thesis, National Dairy Research Institute (Deemed University), Karnal, India.

Snedecor, G. W. and W. G. Cochran. 1980. Statistical Methods. 6th edn. Oxford and IBH Publishing Co., Calcutta.

Weiss, W. P. 1998. Requirement of fat soluble vitamins for dairy cows : A review. J. Dairy Sci. 81:2493-2501.

Weiss, W. P., J. S. Hogan, D. A. Todhunter and K. L. Smith. 1997. Effect of vitamin E supplementation in diets with a low concentration of selenium on mammary gland health of dairy cows. J. Dairy Sci. 80:1728-1737.

Yamagishi, N. and Y. Natio. 1997. Calcium metabolism in hypocalcaemia cows with myocardial lesion. J. Vet. Med. Sci. 59:71-73.