

CONCENTRATIONS OF SERUM VITAMINS A, E AND C AND β -CAROTENE DURING PREGNANCY IN COWS

HAMİT YILDIZ¹, ERDAL KAYGUSUZUOĞLU¹ AND ÖMER KIZIL²

¹Department of Obstetrics and Gynaecology, ²Department of Internal Disease, Faculty of Veterinary Medicine, University of Firat, 23119 Elazig, Turkey
e-mail: yildizhamit@yahoo.com

Received for publication January 14, 2005.

Abstract

The concentrations of serum vitamins A, E and C, and β -carotene in 12 cows, between 3-8 years of age, were determined during pregnancy and after delivery. The compounds were assigned spectrophotometrically. It was observed that the levels of vitamins E and C differed during the pregnancy. The level of vitamin A in the 3rd month of pregnancy was lower than that in the 7th month and the level of β -carotene in the 4th month was lower than that in the 6th and 7th months. It was also noted that the level of vitamin A after delivery was lower than that in months 1, 2, 5, 6, 7, 8 and 9 of pregnancy, but in the 3rd and 4th months, no statistical difference was observed. Vitamin E level was also lower after parturition than the value noted in the 9th month. The level of β -carotene was lower than the values noted in the 1st, 6th, 7th, 8th, and 9th months as well. The vitamin C level was found to be significantly lower after delivery. It was noted that there was positive correlation between contents of vitamin A and β -carotene during the whole period of pregnancy. It was also noted a positive correlation ($P < 0.01$) between vitamin E and β -carotene in the 6th month of pregnancy. It was concluded that the addition of β -carotene and vitamins A, E and C in sufficient amounts to the rations of the animals before delivery would be beneficial.

Key words: cows, pregnancy, vitamin A, vitamin E, vitamin C, β -carotene.

Vitamins and minerals are necessary for animal biological development and reproduction. One of the compounds is vitamin A. The source of the vitamin are carotenoids, in particular existing in plants β -carotene which, after obtaining with feed, is converted within the body into vitamin A (14).

The deficiency of vitamin A in cows may cause infertility, abortion, retained placenta, blind foetus, irregularity in sexual cycle, suboestrus, anoestrus, delayed ovulation and increase in the rates of endometritis (13, 14). In respect of the studies carried out (13, 14), it has been suggested that oestrus indications in the cows that were fed the feeds rich in β -carotene became more indicative, the rate of pregnancy increased and cystic ovary incidence was reduced.

Furthermore, it was also pointed out that the breeding was affected negatively in cows that were fed the feeds containing β -carotene at high levels (8).

It was also noted that the diseases such as the mastitis, retained placenta and metritis were observed in cows suffering from vitamin E deficiency. It was observed that the incidence of metritis and retained placenta in cows treated with vitamin E 14 d before delivery was lower compared to the control cows (5, 7). It was also suggested that vitamin C injected to the sheep during the breeding season increased the levels of plasma oestrogen and progesterone in addition to the fertility; so, there was a relation between vitamin C and these steroid hormones (11).

Towards the final term of pregnancy, it is observed that the cows have larger mammae and an indicative increase in colostrum and milk synthesis. Vitamin A and E and β -carotene levels decrease during the last months of pregnancy and at the delivery these substances are utilized for colostrum and milk synthesis and foetus growth (5, 6, 9). Such reduction occurring in these periods causes mastitis and puerperal diseases. It has been noted that the incidence of mastitis, metritis, and retained placenta becomes lower in cows fed additional β -carotene and vitamins A and E during the last months of pregnancy than control cows (5, 20). In various researches, it was pointed out that there was an indicative correlation between plasma β -carotene and vitamin E (2, 21) and β -carotene and vitamin A (15, 21) contents in pregnant cows and heifers.

This study was carried out to examine the serum concentration of β -carotene and vitamins A, E and C during the pregnancy and after delivery as well as the respective relation among them.

Material and Methods

Twelve cows (5 Holstein, 5 Swiss-Brown and 2 Simmental), between 3 and 8 years of age, were used in the study. The material was chosen among animals reared at Research and Implementation Farm of the Firat University.

All the animals were under the same care and feeding conditions. The chemical composition of feed concentrates given to the animals was as follows: dry matter - 93.75%; ash - 5.09%; crude fiber - 9.75%; crude protein - 15.18%; ether extract - 5.8%; organic matter - 88.66%; barley - 70.5%; sunflowerseed meal - 17.5%; soybean - 7.5%; limestone - 3.0%; DCP (dicalcium phosphate) - 0.5%; salt - 0.5%; vitamins - 0.25% and trace elements - 0.25%. Composition of forage feed was as follows: dry matter - 95.2%; ash - 9.47%; crude fiber - 35.0%; crude protein - 3.3%; ether extract - 3.2% and organic matter - 85.73%. The ration was given in the morning and evening.

The oestrus of the animals was synchronized with PGF_{2α}. The cows at the oestrus were mated with a bull. Following mating on the 30th d, a blood sample of 10 ml was taken from each animal from *v. jugularis* into sterile tubes, oestrus and insemination data were recorded. The pregnancy of the animals was determined by B mode ultrasonography between the 30th and 35th d after insemination. Then the blood samples were taken each month during the pregnancy and within 24 h following the delivery. The blood samples were kept at room temperature for 2 h in order to obtain the serum. The serum taken into the centrifuge tubes was centrifuged at 3000 rpm for 15 min. The serum which was taken into serum preservation tubes was stored at -20°C until the analysis was performed.

Vitamin A and β-carotene values were determined by using Suzuki and Katoh's method (24) and vitamin E levels were determined

spectrophotometrically according to Martinek's method (19). The level of vitamin C was measured by phosphotungstic acid method of Kyaw (17) with Shimadzu UV-1208, UV-VIS spectrophotometer. The results were expressed as mean ± SEM. In respect of statistical calculations, the variations among the pregnancy months were found by implementing the ANOVA and the significance of such variations was also noted according to Duncan Test. The correlation coefficients among the parameters were determined by Pearson test. (12). A value of P<0.05 was considered statistically significant.

Results

Mean serum levels of vitamins A, E and C, and β-carotene and monthly variations in 12 cows were shown in Table 1. It was demonstrated that the post-pregnancy vitamin A, C and E and β-carotene levels were lower than those in the pregnancy period.

Throughout the pregnancy period the correlation between serum vitamin A and β-carotene and vitamin E and β-carotene was summarized in Table 2. Significant positive correlation was found between vitamin A and β-carotene levels during the whole pregnancy period. Positive correlation was demonstrated also between vitamin E and β-carotene concentration (P<0.01) in the 6th month of pregnancy.

Table 1
Mean serum levels of vitamins A, E and C and β-carotene during pregnancy and after parturition

Months of pregnancy	Compounds			
	Vit. A (µg/dl)	Vit. E (mg/dl)	Vit. C (mg/dl)	β-carotene (µg/dl)
1	52.97±3.11	0.224±0.02	0.90±0.08	216.82±12.84
2	49.76±3.11	0.183±0.01	0.77±0.04	199.43±8.24
3	45.60±2.15 ^c	0.202±0.01	0.65±0.03	202.77±10.62
4	46.43±2.52	0.172±0.01	0.70±0.03	188.21±9.80 ^f
5	47.87±1.74	0.196±0.06	0.78±0.06	198.06±6.73
6	50.70±2.10	0.209±0.01	0.69±0.04	224.26±8.01
7	53.85±2.94	0.189±0.01	0.73±0.04	223.30±11.30
8	50.90±2.63	0.269±0.08	0.68±0.03	216.37±15.19
9	47.97±1.22	0.282±0.08	0.75±0.03	218.34±5.70
Parturition	40.22±1.53 ^a	0.152±0.04 ^b	0.59±0.02 ^c	180.44±3.52 ^d

^a P<0.001- compared to months 1, 2, 5, 6, 7, 8 and 9; ^b P<0.05- compared to month 9; ^c P<0.001- compared to months 1, 2 and 5; ^d P<0.01- compared to months 1, 6, 7, 8 and 9; ^e P<0.001- compared to month 7; ^f P<0.01 compared to months 6 and 7.

Table 2
Relationships between vitamin A and E, and β-carotene during pregnancy

Compounds	Pregnancy period (months)					Parturition
	1	2	4	6	7	
Vit A - β-carotene	0.72**	0.61*	0.69*	0.79**	0.65*	0.79**
Vit E - β-carotene	0.26	-0.36	0.51	0.71**	0.25	0.61

* P<0.05; ** P<0.01.

Discussion

Plasma vitamin A levels were determined sequentially as 0.35, 0.25, 0.21 and 0.18 $\mu\text{g}/\text{ml}$ and there was a decrease accordingly to progress of the pregnancy between 0-2.5, 2.5-5, 5-7.5, and 7.5-9 months in Holstein cows (16). Vlcek *et al.* (25), stated serum vitamin A level as $43.62 \pm 13.49 \mu\text{g}/\text{dl}$ in the last month of pregnancy, and Bouda *et al.* (4) found that vitamin A level ($43.4 \pm 5.7 \mu\text{g}/\text{dl}$) in the pre-delivery period was lower ($48.2 \pm 8.0 \mu\text{g}/\text{dl}$) than that in post-delivery period ($P < 0.01$). It was determined that the serum vitamin A levels during pregnancy period were similar to the findings of other researchers (4, 16, 25). Post-partum serum vitamin A level was shown to be lower ($P < 0.001$) than that in months 1, 2, 5, 6, 7, 8, and 9 of pregnancy, but in the 3rd and 4th months, no statistical difference was observed.

According to Aksakal *et al.* (1) plasma β -carotene and vitamin E levels between the 2nd and 3rd months of pregnancy were 1.14 ± 0.62 and $1.64 \pm 0.37 \mu\text{g}/\text{ml}$. In a similar study of Aksakal *et al.* (2), β -carotene levels were sequentially 177, 190, 168, 263, 125 and 101 $\mu\text{g}/\text{dl}$ in the 5th, 6th, 7th, 8th, and 9th months of pregnancy, and the E vitamin levels were 328, 324, 297, 264, 230 and 198 $\mu\text{g}/\text{dl}$. The authors demonstrated that plasma vitamin E and β -carotene levels decreased continually in the late pregnancy period, and the minimum levels were observed in the delivery period. Lynch (18) stated blood serum vitamin E level as 137 $\mu\text{g}/\text{dl}$ in the 6th and 7th months of pregnancy, and Kara *et al.* (16) found plasma vitamin E levels as 2.81 and 2.56 $\mu\text{g}/\text{ml}$ in the 5-7.5 and 7.5-9 months of pregnancy in Holstein cows. The level decreased accordingly to the progress of the pregnancy and the decrease occurred due to the growing of the foetus.

It was demonstrated in this study that the serum vitamin E and β -carotene levels were similar to the results obtained by other researchers (1, 2, 16, 18). We found no difference in the serum vitamin E level among the months of pregnancy, however, only the values in the 9th month were higher ($P < 0.05$) compared to the post-delivery period. The β -carotene levels were less than in the 1st, 6th, 7th, 8th, and 9th months of the pregnancy ($P < 0.01$).

It was found that the plasma vitamin C levels of the pregnant and non-pregnant cows were 2.39 ± 0.39 and $2.85 \pm 0.22 \text{ mg}/\text{ml}$, respectively (21). In other study (10), vitamin C levels were $5.09 \pm 0.28 \mu\text{g}/\text{ml}$ in the 5th-6th months of pregnancy. According to Bouda *et al.* (3), levels of vitamin C were sequentially 610.0 ± 170.0 and $760.0 \pm 120.0 \mu\text{g}/\text{dl}$ in the blood taken 2-5 weeks before and 2-4 weeks after delivery, and that there was a difference between the given values.

In this research, the levels of vitamin C in the 5th, 6th, and 9th months of pregnancy were the same as the findings of other authors. The level of vitamin C reached the maximum level in the 1st month of the pregnancy, and the minimum level at the delivery. It was also noted that the level at the delivery period was lower than those in the 1st, 2nd and 5th months of

pregnancy ($P < 0.001$). Such a decrease at the delivery period might result from the transfer of vitamin C from the blood into the colostrum, and from the stress caused by giving birth, as the stress, in general, causes a decrease in vitamin C content (3, 22).

In the 2nd and 3rd months of pregnancy, Aksakal *et al.* (1) observed in cows a significant correlation between the plasma carotene and vitamin E in June ($r = 0.71$) and in December ($r = 0.52$). In other study (2), correlation between the plasma β -carotene and vitamin E was $r = 0.85$ in the 6th month of pregnancy. In this research, positive correlation 0.71 ($P < 0.01$) was demonstrated between the β -carotene and vitamin E levels in the 6th month of the pregnancy. The correlation may be related to development of the *corpus luteum* which provides the continuation of pregnancy, as the vitamin E and β -carotene levels in the *corpus luteum* in the luteal phase is reported to be higher compared to the other phases of the cycle (23).

Some researchers (15, 21) reported a significant correlation between plasma vitamin A and β -carotene levels in the pregnancy and post-partum periods in cows. In this research, significant positive correlation was found between serum vitamin A and β -carotene levels during pregnancy period. The results are similar to the data obtained by other authors (15, 21).

It was stated that vitamin A and E, and β -carotene levels decreased in pregnant cows, reaching the minimum values at the birth period, and started to re-increase in the post-partum period (9, 15). The decrease resulted from the utilization of the compounds for the colostrum and milk synthesis accordingly to the growing of the foetus (6, 9, 15). The reduced concentration of antioxidants affects the immune system and phagocytic activity of cells and results in an increase in the incidence of mastitis and puerperal diseases in pregnancy, delivery, and post-partum periods (5, 6, 9).

In this research, the vitamin A and E, and β -carotene levels in the 9th month and at delivery period were 47.97 ± 1.22 and $40.22 \pm 1.53 \mu\text{g}/\text{dl}$; 0.282 ± 0.08 and $0.152 \pm 0.04 \mu\text{g}/\text{dl}$; 218.34 ± 5.70 and $180.44 \pm 3.52 \mu\text{g}/\text{dl}$, respectively. It was found out that contents of these antioxidants were lower in the post-partum period compared to the pregnancy. The results obtained in this research were similar to findings reported by other authors (5, 6, 19).

In conclusion, it was found out that no fluctuations occurred in the vitamin A, E and C, and β -carotene levels during the pregnancy period. The presented research shows that the biggest changes in the concentration of these compounds occurred at the parturition. We would recommend that the antioxidants should be added to feed for pregnant animals in order to prevent such diseases as mastitis, metritis, and retained placenta.

References

1. Aksakal M., Karakilcik A.Z., Kalkan C., Çay M., Naziroglu M.: Levels of β -carotene and vitamin E at

- various stages of reproductivity in cows. Turk J Vet Anim Sci 1995, **19**, 59-64.
2. Aksakal M., Karakilcik A.Z., Kalkan C.: The effects of breed, age, season, pregnancy and lactation on blood plasma β -carotene and vitamin E values of cows and heifers. Turk J Vet Anim Sci 1990, **14**, 320-333.
 3. Bouda J., Jagos P., Dvorak R., Ondrova J.: Vitamin E and C in the blood of cows and their calves fed from buckets. Acta Vet Brno 1980, **49**, 53-58.
 4. Bouda J., Jagos P., Dvorak V.L., Hamsik V.: Vitamin A and carotene metabolism in cows and their calves fed from buckets. Acta Vet Brno 1980, **49**, 45-52.
 5. Chawla R., Kaur H.: Plasma antioxidant vitamin status of periparturient cows supplemented with α -tocopherol and β -carotene. Anim Feed Sci Technol 2004, **114**, 279-285.
 6. Daniel L.R., Chew B.P., Tanaka T.S., Tjoelker L.W.: β -carotene and vitamin A effects on bovine phagocyte function *in vitro* during peripartum period. J Dairy Sci 1991, **74**, 124-128.
 7. Erkinse R.J., Bartlet P.C., Herdt T., Gaston P.: Effects of parenteral administration of vitamin E on health of periparturient dairy cows. J Am Vet Med Assoc 1997, **211**, 466-469.
 8. Folman Y., Ascarelli I., Kraus D., Barash H.: Adverse effect of β -carotene in diet on fertility of dairy cows. J Dairy Sci 1987, **70**, 357-366.
 9. Goff J.P., Stabel J.R.: Decreased plasma retinol, α -tocopherol and zinc concentration during the periparturient period: effect of milk fever. J Dairy Sci 1990, **73**, 3195-3199.
 10. Haliloglu S., Serpek B., Baspinar N., Erdem H., Bulut Z.: The relationship between ascorbic acid, oestradiol 17 β and progesterone in plasma and ovaries in pregnant Holstein cows. Turk J Vet Anim Sci 2002, **26**, 639-644.
 11. Haliloglu S., Serpek B.: The effects of plasma vitamin C and ceruloplasmin levels and exogen vitamin C supplementation on reproduction in sheep. Turk J Vet Anim Sci 2000, **24**, 403-411.
 12. Hayran M., Ozdemir O.: Computer statistics and medicine. Physicians Publishing unity, Ankara, 1996. pp. 320-355.
 13. Hemken R.W., Bremel D.H.: Possible role of β -carotene in improving fertility in dairy cattle. J Dairy Sci 1982, **65**, 1069-1073.
 14. Hurley W.L., Doane R.M.: Recent developments in the roles of vitamins and minerals in reproduction. J Dairy Sci 1989, **72**, 784-804.
 15. Johnston L.A., Chew B.P.: Peripartum changes of plasma and milk vitamin A and beta-carotene among dairy cows with or without mastitis. J Dairy Sci 1984, **67**, 1832-1840.
 16. Kara H., Karatas F., Servi K., Akar Y., Konar V.: Investigation of levels plasma adrenalin, noradrenalin, vitamins A and E at various of pregnancy periods in Holstein cattle. F U J of Health Sci 2001, **15**, 171-174.
 17. Kyaw A.: A simple colorimetric method for ascorbic acid determination in blood plasma. Clin Chim Acta 1978, **86**, 153-157.
 18. Lynch G.P.: Changes of tocopherols in blood serum of cows fed hay or silage. J Dairy Sci 1983, **66**, 1461-1465.
 19. Martinek R.G.: Method for determination of vitamin E (total tocopherols) in serum. Clin Chem 1964, **10**, 1078-1086.
 20. Michal J.J., Heirman L.R., Wong T.S., Chew B.P., Frigg M., Voker L.: Modulatory effects of dietary β -carotene on blood and mammary leukocyte function in periparturient dairy cows. J Dairy Sci 1994, **77**, 1408-1421.
 21. Naziroglu M., Gur S.: Antioxidants and lipid peroxidation levels of blood and cervical mucus in cows in relation to pregnancy. Dtsch Tierarzti Wschr 2000, **107**, 374-376.
 22. Roth J.A., Kaeberle M.L.: *In vivo* effect of ascorbic acid on neutrophil function in healthy and dexamethasone-treated cattle. Am J Vet Res 1985, **46**, 2434-2436.
 23. Schweigert F.J.: Research note: changes in the concentration of beta-carotene, alpha-tocopherol and retinol in the bovine *corpus luteum* during the ovarian cycle. Arch Tierernahr 2003, **57**, 307-310.
 24. Suzuki C., Katoh N.: A simple and cheap method for measuring serum vitamin A in cattle using a spectrophotometer. Jpn J Vet Sci 1990, **52**, 1282-1284.
 25. Vlcek Z., Nedbalkova J., Svobodova R., Chlupac J.: Vitamin A in the blood serum, liver, *corpora lutea*, placentomas and fetal fluids of cows and in the blood serum and liver of their fetuses in the latter third of pregnancy. Acta Vet Brno 1980, **49**, 37-44.