

Effect of β -carotene on Serum Progesterone Level in Dairy Heifers

S.N. BARUAH

Department of Animal Gynaecology, Obstetrics and Artificial Insemination,
College of Veterinary Science, Assam Agricultural University,
Khanapara, Guwahati - 781 022

and

B.C. GOSWAMI

Department of Chemistry, Guwahati University, Guwahati 781 014, Assam.

ABSTRACT

Twenty crossbred heifers in the age group of 2-3.5 years and body varying from 130 to 190 Kg were used in the study. All the heifers were kept on a B-carotene free basic ration consisting of concentrate, @ 3 Kg per heifer per day and paddy straw, *ad libitum*. Each heifer in the control group received a daily supplement of 60,000 I.U. vitamin A in their feed while in the experimental group each heifer was given 20,000 I.U. vitamin A plus 100 mg B-Carotene daily. The heifers of the experimental group had a significantly higher level of B-carotene than the control group throughout the period of the experiment. It was observed that, although not statistically significant, the blood progesterone level during oestrous cycle was apparently higher in the heifers of the experimental group than that of the control group. But it was significantly higher during pregnancy in the heifers of the experimental group. Higher percentage (75%) of the experimental heifers had large sized corpus luteum.

—x—x—x—

The mainstay of cattle during the dry seasons of the year becomes dry fodder like paddy, wheat and oat straw which do not contain carotenoids because of depletion of natural green fodder. The green fodder contains various carotenoids of which the major portion is of B-carotene. B-carotene might play a role in the production of steroid hormone as indicated by the presence of high concentration of

B-carotene in the corpus luteum of cattle (Schultz *et al.*, (1973). Recent studies on the significance of β -carotene in bovine fertility showed that B-carotene should have a local function in the ovary which is either a specifically independent function or a local provitamin A function (Schweigert, 1990). The present study was aimed at to know the effect of B-carotene on serum progesterone level in dairy crossbred heifers.

MATERIALS AND METHODS

Twenty crossbred (Jersey x Assam local) heifers in the age group of 2-3.5 years and body weight ranging between 130 and 190 Kg were used in this study. The animals were grouped into control and experimental groups, comprising 10 heifers in each group. All the heifers were kept on a B-carotene free basic ration containing maize, wheat bran, ground nut cake, deoiled rice polish, mineral mixture, @ 3 Kg per heifer per day and the paddy straw, *ad libitum*. Each heifer in the control group was given a daily supplement of 60,000 I.U. Vitamin A (Glaxo laboratories) while in the experimental group each heifer was given 20,000 I.U. Vitamin A along with 100 mg B-carotene (10% water soluble beadlets of B-carotene, (F. Hoffmann La Roche & Co. Ltd., Basel, Switzerland). As the conversion rate of B-carotene to Vitamin

A is 1 : 400 (Hemken and Bremel, 1982), the total equivalent of vitamin A supplement in the heifers of both the groups was the same. The study was carried out till the animals attained 90 days of pregnancy.

For the assay of progesterone, blood samples were collected at 0, 3, 12, 16 and 20 day of oestrous cycle and also on day 45, 60 and 90 of pregnancy. The serum was separated from the blood samples and was stored at -20°C till assayed. The serum progesterone was estimated by double antibody radioimmuno assay using progesterone kits (Leece Diagnostics Inc. 21705, Michigan).

Extraction of β -carotene from the blood serum was carried out as described by Bieri *et al.*, (1985) : 3 ml of serum was taken in a centrifuge tube to which 3 ml of ethanol and 3 ml of diethyl ether were added. The mixture was vortexed, centrifuged and the supernatant was carefully pipetted out. The extraction was repeated two times and the pooled diethyl ether supernatant was dried with anhydrous Na_2SO_4 , evaporated in *vacuo*. The residue was dissolved in a known volume of light petroleum (B.P. $40-60^{\circ}\text{C}$) and the optical density was determined at 449 nm in a Hitachi double beam spectrophotometer (Model U-3210). The amount of β -carotene in the serum was calculated following the procedure of Britton (1985) considering E1% as 2592.

To record the size of the corpus luteum, rectal palpation was made once daily from day 9 to 13 of the oestrous cycle. Based on the size, the corpus luteum were classified as large (more than 1.5 cm diameter) and small (less than 1.0 cm diameter).

RESULTS AND DISCUSSION

The blood profile of B-carotene in the heifers of the control and experimental group is presented in Table 1. At the beginning

of the experiment, an almost equal level of B-carotene was present in the control and experimental groups of heifers (490 ± 13.60 and $481.0 \pm 9.16 \mu\text{g} / 100 \text{ ml}$ of blood serum respectively). After one month of the experiment, the level of β -carotene came down to $39.0 \mu\text{g} / 100 \text{ ml}$ in the control group, but it was $335.60 \mu\text{g} / 100 \text{ ml}$ in the experimental group. Thereafter, the blood profile of β -carotene did not show considerable rise and fall but remained between 28.10 and 40.10 335.60 and $380.83 \mu\text{g} / 100 \text{ ml}$ in the control and experimental groups respectively, the level being significantly ($P < 0.01$) higher in the experimental group.

The blood progesterone level during oestrous cycle (Table 2) did not vary significantly between the heifers of control and experimental groups which is in agreement with the observations of earlier workers (Bonsembiante *et al.*, 1983; Bindas *et al.*, 1984, Ascarelli *et al.*, 1985). However, it is evident from Table 3 that the blood progesterone values in the experimental group of heifers was apparently higher than those in the control group. A similar observation was also reported by Lotthammer (1979). The blood progesterone level during pregnancy was recorded to be higher in heifers of experimental group than in those of control group, the difference being significant on day 12, 16, 18, 60 and 90 of pregnancy (Table 3). The present finding was in conformity with that of Lotthammer (1979) and Greenberg *et al.*, (1986). It was further observed that higher proportion of the experimental heifers (75%) had large sized corpus luteum as compared to the control group. The higher blood progesterone level recorded in the experimental heifers could

be due to, large sized corpus luteum observed in this group of heifers. Jackson *et al.*, (1981) also observed a depressed production of steroid hormone in cows fed

with a low level of β -carotene diet. It might be possible that β -carotene is involved in the synthesis of progesterone in the corpus luteum of the heifers.

Table 1. Blood profile of β -carotene (g/100ml) in the heifers of control and experimental groups at different periods of the experiment.

Period (month)	Control (10) Mean \pm SE	Experimental (10) Mean \pm SE	Value of 't'
0	494.00 ^a \pm 13.60	481.00 ^a \pm 9.16	0.793
1	39.00 ^b \pm 1.96	335.60 ^d \pm 8.59	33.68**
2	40.10 ^b \pm 1.73	340.20 ^{cd} \pm 6.59	44.06**
3	39.30 ^b \pm 1.81	360.90 ^{bc} \pm 6.69	46.41**
4	37.40 ^b \pm 1.04	371.00 ^b \pm 5.40	60.70**
5	39.70 ^b \pm 1.38	365.33 ^b \pm 5.59	59.45**
6	37.20 ^b \pm 1.35	366.22 ^b \pm 4.38	75.11**
7	28.10 ^b \pm 1.18	377.50 ^b \pm 8.03	55.80**
8	29.10 ^b \pm 0.82	380.83 ^b \pm 7.91	57.86**

** (P < 0.01)

Figures in parentheses indicate number of animals

Numbers bearing similar superscripts in the column do not differ significantly.

Table 2. Levels of blood progesterone (ng/ml) in the heifers of control and experimental groups at different stages of oestrous cycle

Stages of the Oestrous cycle (days)	Control (10) Mean \pm SE	Experimental (10) Mean \pm SE	Value of 't'
0	0.43 \pm 0.06	0.38 \pm 0.06	0.22
3	0.63 \pm 0.03	0.63 \pm 0.07	0.00
12	3.40 \pm 0.23	4.11 \pm 0.33	1.61
16	2.69 \pm 0.19	3.20 \pm 0.22	1.65
18	1.84 \pm 0.23	2.27 \pm 0.17	1.59
20	0.45 \pm 0.02	0.50 \pm 0.04	0.83

Figures in the parentheses indicate number of animals.

Table 3. Levels of blood progesterone (ng/ml) in the heifers of control and experimental groups at different stages of pregnancy

Stages of Pregnancy (days)	Control (10) Mean±SE	Experimental (10) Mean±SE	Value of 't'
0	0.38±0.06	0.45±0.09	0.54
3	0.65±0.03	0.62±0.10	0.21
12	3.33±0.05	4.83±0.29	3.75**
16	3.80±0.13	5.07±0.34	2.70*
18	3.90±0.24	5.14±0.38	2.70*
20	4.20±0.11	5.10±0.34	1.92
45	4.80±0.32	5.84±0.38	1.82
60	4.95±0.12	6.40±0.37	2.84*
90	5.25±0.22	6.16±0.27	2.28*

Figures in the parentheses indicate number of animals

* (P < 0.05)

** (P < 0.01)

REFERENCES

- Ascarelli, I., Edelman, Z., Rosenberg, M. and Folman, Y. (1985) Effect of dietary carotene on fertility of high-yielding dairy cows. *Anim. Prod.* **40**: 195-207.
- Bieri, J.G., Brown, E.D. and Smith, J.C.Jr. (I) (1985) Determination of individual carotenoids in human plasma by HPLC. *J. Liq. Chrom.* **8**(3): 473-484.
- Bindas, E.M., Gwazdauskas, F.C., Aiello, R.J. Horbein, J.H., McGilliard, M.L. and Polan, C.E. (1984) Reproductive and metabolic characteristics of dairy cattle supplemented with β -carotene. *J. Dairy Sci.* **67**(6): 1249-1255.
- Bonsembiante, M., Andrighetto, I. and Bittanto, G. (1983) β -carotene and fertility in cattle: Future experimental results. *Zootecnica e Nutrizione Animale* **9**(3): 199-212 (*Anim. Breed. Abstr.*, **53**(1): No 142).
- Britton, G. (1985) General Carotenoid Methods. *Methods Enzymol.* **111** (B): 113-149.
- Greenberg, L.G., Bristol, F., Murphy, B.D. and Laarveld, B. (1986) β -carotene does not influence fertility in beef heifers. *Theriogenology* **26**(4): 491-508.
- Hemken, R.W. and Bremel, D.H. (1982) Possible role of β -carotene in improving fertility in dairy cattle. *J. Dairy Sci.* **65**(7): 1069-1073.
- Jackson, P.S., Furr, B.J.A. and Johnson, C. T. (1981) Endocrine and ovarian changes in dairy cattle fed a low β -carotene diet during an oestrous synchronization regime. *Res. on Veter. Sci.* **31**(3): 377-383.
- Lotthammer, K.H. (1979) Importance of β -carotene for the fertility of dairy cattle. *Feedstuffs* **51**(43): 16-50.
- Schultz, G., Ahlswede, G. and Grunert, E. (1973) β -carotenehalt in corpora lutea von rindern bei unterschiedlichen haltungsbedingungen. *Zuchthygiene*, **8**: 89 (cf. *Feed Stuffs* **51** (43): 16-50).
- Schweigert, F.J. (1990) β -carotene and fertility, Paper presented at 9th International Symposium on Carotenoids, Kyoto, May 25-25, 1990 P6-10.