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Praful Chandra Bhatnagar
Senior Subject Matter Specialist, Animal Science, Krishi Vigyan Kendra, Udaipur, Rajasthan, India

JL Choudhary
Professor, Department of Animal Production, Rajasthan College of Agriculture, Maharana Pratap University of Agriculture and Technology, Udaipur, Rajasthan, India

B Bhardwaj
Joint Director, Department of Animal Husbandry, Government of Rajasthan, Udaipur, Rajasthan, India

Chandr Shakhar
Business Manager Ruminants, ANH, DSM Nutritional Products India Private Limited, Powai, Mumbai, India

Deepak Kumar Sharma
Assistant professor, Department of Veterinary Microbiology, CVAS, Navania, Vallabhnagar, Udaipur, Rajasthan, India

Corresponding Author:
Praful Chandra Bhatnagar
Senior Subject Matter Specialist, Animal Science, Krishi Vigyan Kendra, Udaipur, Rajasthan, India

Synergic effect of β-carotene in reproductive functioning of dairy cows

Praful Chandra Bhatnagar, JL Choudhary, B Bhardwaj, Chandr Shakhar and Deepak Kumar Sharma

Abstract
Effect of beta carotene on multiparous infertile dairy cows was assessed in this study. A total of 135 dairy cows were divided into three groups, each of 45 cows. Group–I supplemented balance feed without minerals and vitamins while group II supplemented balance feed with minerals and vitamins without β-Carotene and group–III supplemented balance feed with minerals and vitamins with β-Carotene. Plasma β-Carotene concentrations were much higher in the β-Carotene group III {3·02 mg/L *v. control (group 1)* 1.32 mg/L} at 90 days. Conception rate was greatly improved by β-Carotene supplementation in cows (group III): conception rates at first group were 64.44% *v.* 37.77%.

Keywords: β-carotene, reproductive performance

Introduction
In the country, cows and buffaloes are the principle milk producing animals contributing more than 96.5% of total milk production [1]. These animal species in comparison to other species have better feed conversion efficiency, greater resistance to diseases and higher milk fat percentages. Livestock productivity or production efficiency is to a large extent dependent on reproductive performance. Proper nutrition could encourage mediocre biological types to reach their genetic potential and also alleviate the negative effects of a harsh physical environment. Deficiencies of various trace minerals, inadequate vitamin intake, energy, protein imbalance and excessive protein intakes are mentioned as contributive to infertility and poor reproductive performance [2].
Antioxidant effect of β-carotene, the precursor of vitamin A, and influences on reproductive and thyroid functions is very well recorded [3, 4]. One mg of β-carotene is equivalent to 400 IU of vitamin A [5]. Many reproductive disorders have been recorded due to β-carotene deficiency in cows *i.e.* delays ovulation, increases abortions and early embryonic death rates, silent oestrus, increase tendencies of ovarian cysts, Retention of fetal membranes and metritis risk, steal birth or weak, and blind calves and reduces fertility etc. [6]. Daily requirement of an adult cow is 0.18 mg of β-carotene/kg body weight in order to maintain its normal reproductive functions [3]. The corpus luteum contains a high level of β-carotene [6, 7]. However, it does not contain vitamin A and low fertility is related to a low level of β-carotene [6, 7]. High β-carotene level influenced the release of ovarian steroid [6]. Effect of β-carotene on secretion of LH from the hypothalamus has also been observed by other researchers [8] and thus increased progesterone production. Normal level of β-carotene in serum of cattle l is 300-1200 µg/100 ml, subnormal level - 100-200 µg/100 ml and level of 9-100 µg/100 ml is regarded as deficiency [9, 4].
Present study gives an insight about the effects of oral β-Carotene supplementation on reproductive performance of dairy cows in field conditions at subtropical areas of southern Rajasthan.

Materials and Methods
Selection of animals
A total of 135 infertile dairy cows were selected for study from commercial dairy farms, village animals and animals from *gaushalas* of Udaipur district. These animals were further subdivided into three feed groups as follows-

Feed groups

Total 135 infertile animals were divided into three groups Group I (45 cows) was supplemented with two kg balance feed (composition table-1)/day/animal and Group II (45 cows) were supplemented with two kg balance feed+ added Vitamins enriched chelated mineral mixture(70 gm/day/animal) and Group III (45 cows) were supplemented with two kg balance feed + Vitamin enriched chelated mineral mixture(70 gm/day/animal) with β-Carotene(500 mg /day/animal). These animals were fed as above concentrate with same quantity of roughage and water ad-libitum. Supplementation was commenced for 60 days. It was started in selected infertile cows (having infertility problem *i.e.* anestrous/ repeat breeding).

Sampling and estrus monitoring

Blood samples (10 ml) were collected from the jugular vein of each animal 4 times during Experiments. The first sample was taken immediately prior to β-carotene supplementation, which commenced on day zero. Samples were taken at 30 days interval and lastly at 90 days of start of supplementation. The blood samples were collected in vacutainer, after clotting serum was separated by clot breaking and centrifugation, and it was stored at -20ºC for β-carotene estimation. Signs of estrus were monitored daily in morning and evening and recorded. The animal was bred a standing estrus.

β-carotene estimation

β-carotene value was determined using β-Carotene estimation meter (iCheck™ carotene meter, BioAnalyt Germany) directly from blood serum and results has been recorded in mg/L.

Breeding and Pregnancy diagnosis

The animals were daily observed for sign of estrus in morning and evening and when they showed sign of standing estrous were artificially inseminated with good quality frozen semen. All the inseminated cows which did not return to estrus were examined either through rectum palpation at 35-45 days

Results

In our study we assess the effect of β-Carotene in reproductive performance in 135 dairy cows (three different groups- Group I, Group II and Group III in each of 45 cows). Beta carotene level start to increase at second record *i.e.* at 30 days and significant increase more than double was observed in group–III animals, whereas in control group animals increase was non-significant. The signs of estrus were noted in all group animals but the observable signs i.e. ropy clear discharge, and standing while mounting by other animals etc. were more pronounced in group-III animals. The pregnancy diagnosis revealed highest conception rate in group-III animals (64.44%) followed by Group-II (46.66%) and lowest in group-I (37.77%).

Table 1: Balanced Feed Composition

Ingredients	Qty (kg.)
Maize	20.0
Barley	13.0
Moong Churi	7.5
Molasses	4.0
Rice Polish	5.0
GNC	20.0
Guar Korma	10.0
By Pass Fat	1.5
DORB	10.0
DCP	3.0
Salt	1.6
Calcite	3.7
Na bi Carb	0.375
MgO	0.125
Toxin Binder	0.2
Total	100.0

Table 2: Mean value of β-Carotene in different groups (mg/L) at every 30 days of interval

S. No.	Days	Group I	Group II	Group III	SE
1	0	0.71	0.92	0.88	0.04
2	30	0.95	1.24	1.86	0.06
3	60	1.20	1.52	2.57	0.07
4	90	1.32	1.63	3.02	0.10

Table 3: Effect of β-Carotene on conception of dairy cows

Group	No. of animals selected	AI performed	No. of animals conceived	Conception rate
I	45	45	17	37.77%
II	45	45	21	46.66%
III	45	45	29	64.44%

Table 4: Effect of beta carotene on pregnancy of animals as per feed groups

			Pregnancy		Total
			Non-Pregnant	Pregnant	
Feed	1	Count	28	17	45
		% within Pregnancy	41.20%	25.40%	33.30%
	2	Count	24	21	45
		% within Pregnancy	35.30%	31.30%	33.30%
	3	Count	16	29	45
		% within Pregnancy	23.50%	43.30%	33.30%
Total		Count	68	67	135
		% within Pregnancy	100.00%	100.00%	100.00%

If we compare the animals within the group which are formed with and without β-carotene, the non-pregnant percentage is in decreasing trend (41.2% to 23.5%) when we proceeds from

group I to group III and reverse trend has been found in pregnant percentage (25.4% to 43.3%).

Discussion

Negative impacts of β-carotene deficiency on bovine fertility have been well documented [10]. In addition to infertility it's effect has been also documented in abortion, night blindness, increase in the birth of weak and sick calves, weakening the oestrus symptoms, and delay in ovulation are other negative outcomes related to deficiency of vitamin A and β-carotene [3, 4, 6, 11].

The initial analysis of β-carotene levels at just before start of β-carotene supplementation in cows with chronic fertility impairment have shown a value of β-carotene serum level is 0.71-0.92 mg/L in all three groups. This level was lower than the physiological levels (>2 mg/L) in lactating dairy cows as reported by other authors [7, 12, 13].

The oral supplementation of 500 mg β-carotene resulted in an increase of the serum concentration to a mean level of 1.86 mg/L at day 30 in group-III animals, whereas no significant increase was observed in rest two control group I and II. Its level has further increased at 60 and 90 days of supplementation up to 2.57 and 3.02 respectively.

Analyzing the results of our study it is necessary to emphasize that we have selected animal deficit with basic nutrition as well as with vitamin and mineral supplementation and having chronic infertility problems. It is common practice in rural conditions in India to fed quality concentrate to lactating animals and maintenance ration to infertile animals so selected animals were deficient in all term of balance ration. And we used beta carotene as a solution of infertility problems along with balance concentrate feed. Though improvement of the reproductive functions was observed in control group-I and II also because of balance concentrate mixture (group-I) and vitamin+ mineral mixture (group-II) supplementation. But conception rate was highest among three groups in group-III, as concluded by other authors [14, 15, 16].

β-Carotene alone or as pro vitamin A has a significant role in infertile animals. However, it is necessary to clarify the overall mechanisms of the vitamin A action in fertility [3, 17] before the discussion on the effects of β-carotene on fertility of dairy cows. Role of β-Carotene supplementation (90 days) had also significant effect on fertility documented [18] they reported positive influence of β-carotene and vitamin A supplementation on the conception rate. But they worked on animals with normal reproductive status.

To conclude our study gives first glimpse on effects of balance diet, mineral and vitamin supplementation and effect of beta carotene on infertile conditions in subtropical areas of Rajasthan. Our results showed that balance feed along with mineral mixture can improve the infertility condition up to some extent. Whereas beta carotene can solve the infertility problem in a better way if animal has deficiency of it.

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