THE UNIVERSITY OF MANITOBA

EFFECT OF BETA-CAROTENE ON THE REPRODUCTIVE PERFORMANCE OF HOLSTEIN HEIFERS AND COWS

by

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ABSTRACT

Three long-term experiments were conducted to investigate the role of beta-carotene (β-carotene) in the reproductive function of Holstein heifers and cows. Experiment 1 was a year-long study involving two groups of 8 cows each, that calved between January and April (group 1) and July and October (group 2) respectively. The cows received rations adequate in nutrients according to NRC (1978) standards and had access to pasture in the summer months. The concentration of β-carotene in the forages fed ranged from 0 to 6.9 mg/kg DM for corn silage to 101.5 to 228.1 mg/kg DM for pasture. Mean serum β-carotene concentration during the year ranged from about 240 µg/100 ml in March to over 600 µg/100 ml in June and July. Mean serum vitamin A concentration ranged from about 31 µg/100 ml in April to about 53 µg/100 ml in June. During the calving period, mean serum β-carotene concentration was higher ($P<.05$) in group 2 cows compared to group 1 cows but mean serum vitamin A levels did not differ between the two groups. Correlation between serum β-carotene and vitamin A was significant ($P<.05$) and positive. Mean interval to ovulation (IO) and services/conception (SC) did not differ ($P>.05$) between the two groups but interval to uterine involution (IUI) and interval to first service were longer ($P<.05$) in group 1 compared to group 2. Mean days open (DO) was somewhat longer ($P = .06$) in group 1 than in group 2 cows. Partial correlation coefficients for serum β-carotene and vitamin A concentrations during the calving period and each of the reproductive parameters were not significant ($P>.05$).

In experiment 2, 37 cows were assigned to a 2x2x3 factorial experi-

ment from 4 weeks prepartum to 12 weeks postpartum. The 3 factors were: 2 levels of a β-carotene treatment - a no carotene supplement (B1; supplying only vitamin A) and a β-carotene supplement (B2; supplying only β-carotene); 2 levels of a deworming treatment - no treatment (T1) and one injection of 0.044 ml/kg BW of tramisol (Tram) at parturition (T2); 3 ration (Rat) treatments - R1 (control with 9.7% corn), R2 (with 7.9% extruded canola seed in place of corn) and R3 (with 7.9% canola whole seed in place of corn). Beta-carotene supplementation raised ($P<.01$) serum β-carotene concentration in the B2 group but had no effect on serum vitamin A. Tram and Rat treatments had no effect on serum β-carotene and vitamin A concentrations. Beta-carotene supplementation had no effect ($P>.05$) on IO, IUI, SC and DO. None of these parameters were affected by Tram and Rat treatments. Reproductive disorders, clinical mastitis and milk somatic cell count were not affected ($P>.05$) by any of the three factors. Apart from a higher milk yield ($P<.05$) for cows on T2, the three factors had no independent effects ($P>.05$) on milk production parameters.

In experiment 3, 20 5-month-old heifers were assigned to either a control (C; supplying only vitamin A) or a β-carotene supplemented (BC; supplying only β-carotene) grain ration in a completely randomized design. Heifers received, ad libitum, a low-carotene basal forage of corn silage, old weathered hay and/or straw plus 2 to 2.5 kg/hd of the appropriate grain ration. Serum β-carotene and vitamin A concentrations were higher ($P<.05$) in BC heifers. Average daily gain was improved with β-carotene supplementation ($P<.05$). Beta-carotene supplementation had no effect ($P>.05$) on mean age and body weight at puberty; estrous cycle length and SC. Serum concentration of progesterone (P_4), estradiol-

17β, LH and FSH from prepuberty to day 17 of the first estrous cycle was not affected (P>.05) by β-carotene supplementation. Total P_4 output during the estrous cycle did not differ (P>.05) between C and BC heifers. However, the intensity of estrus was increased (P<.01) by β-carotene supplementation, and the interval (hrs) from LH peak to ovulation was longer (P<.01) in the C heifers.

DEDICATION

To the memory of Frederick C.T. Tekpetey,

for a lifetime of commitment and

sacrifice to fulfill an

unshakeable belief in

his childrens' education

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INTRODUCTION

Efficient reproductive management is now recognized as an essential and integral part of a profitable dairy operation. The literature indicates that 3 to 6% of the milking herd is culled annually for reproductive reasons (Fosgate 1965; Batra et al. 1971; Allaire et al. 1977). Nutrition is one of the environmental factors that influences reproductive efficiency in cattle. Energy and protein deficiency are two nutritional factors whose adverse effects on reproductive performance have been extensively researched. However, adverse effects of the deficiency of some specific minerals and vitamins on reproduction have been reported as well (Zintzen 1974).

The necessity of vitamin A for optimum reproduction in cattle and other species has been clearly established (Thompson 1975). Beta-carotene is one of many plant carotenoids that is converted in the intestinal mucosa of animals into vitamin A (Goodman and Olson 1969). As a nutrient, β-carotene has been traditionally regarded solely as the principal precursor of vitamin A in animals. Within the last decade, research findings in Europe have suggested a new specific role for β-carotene in the reproductive function of dairy cattle, which is independent of its vitamin A activity. The European studies have indicated a positive correlation between dietary intake of β-carotene and reproductive performance in dairy cattle (Friesecke 1978). Furthermore, irrespective of adequate vitamin A supply, β-carotene deficiency in dairy heifers and cows was found to be associated with silent heat; delay of ovulation; higher incidence of cystic ovaries; more services per conception; re-

duced conception rate; lower progesterone output and increased incidence of early embryonic mortality and abortion (Lotthammer 1979b).

The results of field trials conducted in Canada and the United States between 1978 and 1980 have generally supported the earlier European findings (Olentine 1982). At the time this project was initiated in October of 1980, there were no published reports of controlled studies in North America on the reproductive effects of β-carotene. However one Israeli study involving Friesian heifers indicated that β-carotene deficiency had no adverse effects on the reproductive performance of the heifers (Folman et al. 1979). If the suggested role of β-carotene in bovine reproduction is universal, then it seems reasonable to hypothesize that β-carotene supplementation to cattle on low-carotene diets would improve reproductive performance. Therefore, three long-term controlled experiments with Holstein heifers and cows were conducted to investigate the suggested new role of β-carotene in bovine reproductive function under North American conditions.

LITERATURE REVIEW

Most of the literature on β-carotene prior to the early 1970's is related to its vitamin A activity. Although the conversion of β-carotene to vitamin A and the importance of vitamin A for optimum reproduction have been established, the form of vitamin A necessary for reproduction and the physiological basis for its influence on reproduction are not well known.

Recent suggestions of a specific role for β-carotene in bovine reproductive function were based primarily on observed improved reproductive performance in vitamin A-sufficient cattle that were supplemented with β-carotene. However, other reports have provided inconsistent and/or contradictory evidence on the role of β-carotene in bovine reproduction.

The initial portion of this review will deal with the connection between β-carotene and vitamin A, factors affecting carotene utilization and the presence and metabolism of intact β-carotene in the body. Subsequently, an overview of the available data on the vitamin A-independent effect of β-carotene on reproduction and other body functions will be presented. Finally, the suggested β-carotene requirements for cattle will be discussed.

Beta-carotene as a Precursor of Vitamin A

There are over 80 naturally-occurring carotenes, but only 15 are known to have vitamin A activity (Schweiter and Isler 1967). Beta-carotene has the highest vitamin A activity of all the carotenes (Deuel et al. 1945).

Earlier studies demonstrated an association between carotene intake

by animals and vitamin A status. As early as 1919, Steenbock and Goss recognized an association between the yellowness of feeds (an indication of carotene content) and vitamin A activity. But another report by Palmer and Kempster (1919) indicated that yellow-pigmented feeds were not the only sources of vitamin A. They corrected poor growth and reproductive development in vitamin A-deficient birds by feeding them non-pigmented pork liver, even though the birds's shanks, comb and egg yolks remained pale. A decade later, Moore (1929) demonstrated conclusively that vitamin A accumulated in the liver of vitamin A depleted rats after they were fed highly purified carotene that could not have contained any preformed vitamin A. This finding established carotene as a precursor of vitamin A.

Hemken and Bremel (1982) have stated that most of the literature prior to 1950 in which the essentiality of vitamin A for optimum reproduction was established involved studies that used carotene as the primary source of vitamin A. Some symptoms of reproductive problems associated with a low intake of vitamin A or its precursor were described by Hart and Guilbert (1933). These included birth of dead or weak calves, a high incidence of retained placentae, and some abortions. Conception rates were lower for cattle fed 44 compared with 84 μg carotene per kg body weight (Kuhlman and Gallup 1942). Another report discussed by Asdell (1949) in his review contended that cattle fed diets containing 27 to 47 mg/animal/day of carotene required 2.0 services per conception as compared with 1.4 services per conception for cattle fed 48 to 72 mg of carotene/animal/day.

The conversion of β-carotene to vitamin A occurs primarily in the intestinal mucosa during the absorption of dietary β-carotene and other

carotenoid precursors of vitamin A. Moore (1930) was the first to report the conversion of carotene to vitamin A in the intestinal mucosa of rats. Subsequently, it was confirmed that when β-carotene was fed to rats, vitamin A appeared in the intestinal wall before an appreciable amount was found in the liver (Mattson et al. 1947; Wiese et al. 1947). Other studies identifying the intestinal wall as the site for the conversion of β-carotene to vitamin A were conducted with rabbits and goats (Goodwin and Gregory 1948); with pigs and sheep (Thompson et al. 1950); with chicks (Cheng and Deuel 1950; Sibbald and Olsen 1958) and with cattle (Elliot 1949; Stallcup and Herman 1950).

The biochemical processes involved in the conversion of carotene to vitamin A have been established (Goodman and Olson 1969). In the intestinal mucosa cells, β-carotene is first cleaved at its central double bond by a dioxygenase to yield (ideally) two molecules of retinaldehyde. The retinaldehyde is converted by a reductase to retinol, which then becomes esterified for transport (initially through the lymphatic vessels) to the liver. In the liver hydrolysis, reesterification and storage, primarily as retinyl palmitate, occurs. The efficiency of β-carotene conversion to vitamin A is greater in the intestinal mucosa than in other tissues probably because of the excellent system for emulsifying lipids in the intestine thus making carotene more accessible to the enzyme systems (McGillivray 1960).

Factors Affecting Carotene Utilization

Apart from the wide variation in carotene content of feeds, there are numerous factors that affect the utilization of carotene. A discussion

of all these is beyond the scope of this review. However, some of the more relevant ones are presented in this section.

Level of Carotene Fed

Of the various nutritional factors that can affect the utilization of carotene, the most decisive one appears to be the amount of carotene consumed. The efficiency of carotene utilization is highest when it is consumed at relatively low levels (Rubin and DeRitter 1954; Branion and Emslie 1966). Absorption is impaired at high dosage rates since apparent digestibility and absorption coefficients decreased at higher dosage rates (Bauernfeind et al. 1981). Furthermore, as the amounts of carotene ingested increased, the efficiency of its conversion to vitamin A decreased markedly (Erwin et al. 1957; Myers et al. 1959; Grifo et al. 1960).

This phenomenon has been demonstrated in several species of animals using both natural and synthetic forms of β-carotene. For example in the rat, a carotene to vitamin A conversion ratio of 2:1 is obtained at low intake levels but this ratio could increase to 6:1 to 10:1 at higher levels of intake (Bauernfeind et al. 1981). Similarly, in the chicken, a 2:1 ratio has been suggested for low intake levels but a 4:1 to 10:1 ratio is more likely at higher levels of intake (Marusich and Bauernfeind 1963; Flegal et al. 1971). Myers et al. (1959) reported a ratio of 5 to 6:1 for pigs at low intake levels but an 8 to 10:1 ratio at higher intake levels. In sheep the ratio varies from 5 to 6:1 at low intake levels to 13:1 at high intake levels (Rouseau et al. 1956; Myers et al. 1959). In dairy cattle, data presented in a review by Bauernfeind et al. (1981)

suggest that the ratio could vary from 5:1 at low intake levels to as high as 20:1 when intake is very high. Aside from the level of carotene consumed, numerous trials have shown that after periods of deficiency, carotene is poorly utilized (Byers et al. 1956; Erwin et al. 1957; Myers et al. 1959). The longer a deficiency of carotene was maintained, the greater the deterioration of the animal's ability to subsequently convert carotene to vitamin A (Grifo et al. 1960).

Pre-intestinal Losses

A number of papers indicate that there is considerable pre-intestinal degradation of carotene, particularly in the ruminant. King et al. (1962) demonstrated by means of *in vitro* and *in vivo* studies that about 40% of ingested carotene and vitamin A were destroyed in the reticulo-rumen of sheep. Potkanski et al. (1974) have reported pre-intestinal carotene losses of 25-33% of dietary intakes when alfalfa meal or carotene in stabilized beadlets were the sole source of carotene. Losses were greater with the alfalfa meal than with the beadlets. Others have also reported appreciable losses of carotene due to pre-intestinal destruction (Davidson and Seo 1963; Pugh and Garner 1963). However Keating et al. (1964) found no effect of *in vitro* fermentation on loss of carotene, although there was some loss of vitamin A acetate.

There is very little information on how carotene is destroyed in the reticulo-rumen. The preintestinal degradation of carotene may be by oxidation since clear stomach extracts, in the presence of methyl linoleate, were found to oxidise carotene while extracts from the small intestine were less active (Hove 1943).

Dietary Fat

Since β-carotene is a precursor of a fat soluble vitamin, it is used more effectively in the presence of fat or oil. In the absence of fat, carotene may not be absorbed from the intestine. Ahmad (1937) demonstrated that rats on a low fat diet were unable to absorb much carotene but when 10% fat was incorporated in the diet, utilization of the carotene was almost complete. Beta-carotene and vitamin A utilization were both better in rats on diets containing 5% lard compared to those whose diet had no added fat (Burns et al. 1951). Shaw and Deuel (1944) observed a correlation between the quantities of fat in the diet and carotene utilization by rats. An improvement in carotene absorption by the addition of fat in the diet has also been observed in chickens (Russell et al. 1942). Similar findings in human subjects and in other animals have also been reported (Van Eckelen and Pannevis 1938; Thompson et al. 1949). Recently, Ashes et al. (1982) reported that feeding cows with diets containing protected sunflower oil seed or protected tallow raised the concentration of plasma β-carotene over that observed for control cows. Conversely, according to Hibbs (1980), excessive amounts of fat in the diet may be detrimental since the addition of large amounts of fat by feeding large amounts of raw soybeans can reduce carotene absorption.

The type of fat also influences carotene utilization. Beta-carotene was 5 to 6 times more effective for rats when dissolved in peanut oil than when dissolved in cottenseed oil (Dyer et al. 1934). As a solvent for carotene, soybean oil was better than cottonseed oil, linseed oil and corn oil; while butterfat and coconut oil were the worst (Sherman 1941).

This would suggest that unsaturated oils are more effective in promoting carotene absorption than saturated fat. However, in contrast to the previous finding, Adlersberg et al. (1949) found butter to be a better promoter of carotene absorption than cottonseed oil.

The findings of Brown and Bloor (1945) indicated that chain length of dietary fatty acids influenced carotene absorption. They observed that carotene absorption by rats from raw carrots was highest when low molecular weight fatty acids were given. A depression in absorption occurred as fatty acids with longer chain lengths were fed. For dietary fat to be effective in improving carotene utilization, the fat must be stable in order to enhance carotene preservation by tocopherols and other antioxidants. The development of rancidity in dietary fat causes the destruction of carotene and vitamin A giving rise to deficiency symptoms (Matsushima and Dowe 1954).

Dietary Proteins and Vitamins

Dietary protein and β-carotene and vitamin A metabolism are also closely related. Both quantitative and qualitative protein deficiency appear to impair carotene utilization. Dietary carotene was much more effectively utilized by rats when high-quality protein (casein) rather than low-quality protein (zein or gluten) was used (Fraps 1946; James and El Gindi 1953). A number of workers have concluded that either the absorption of carotene or its conversion to vitamin A is directly dependent on the amount of protein intake (Arnich and Pederson 1956; Matthews and Beaton 1963). In his review of the literature, Campbell (1964) suggested that protein deficiency in an animal may result in

reduced absorption of carotene and retarded mobilization of hepatic reserves of retinol. Olson (1969) has indicated that the synthesis of a number of key proteins involved in the utilization of provitamin A carotenoids and in the absorption and transport of vitamin A is depressed by protein deficiency.

The three key vitamins that appear to influence carotene utilization are vitamins A, D and E. Administration of massive doses of vitamin A have been reported to lower serum, liver, skin and egg yolk carotenoid values in poultry (Donovan et al. 1961; Dua et al. 1966) and serum and milk fat carotene content in cattle (Blaxter et al. 1946; Jacobson et al. 1950). Vitamin A is believed to interfere with the absorption of carotene from the intestinal tract possibly due to competition between the two since they are structurally related (Bauernfeind et al. 1981). There is no solid evidence to support other indirect mechanisms by which vitamin A affects carotene utilization although the effects of thiouracil and high vitamin A levels on carotenoid utilization appear to be similar (Dua et al. 1968).

Obviously other fat-soluble vitamins influence carotene utilization through other mechanisms. Hendricks et al. (1967) reported that the utilization of β-carotene is depressed by high levels of vitamin D. On the other hand, high carotene intake also has anti-vitamin D effects (Hibbs 1980).

It is generally recognized that both carotene and vitamin A are more effectively utilized when fed with optimal vitamin E intake. More (1940) made the original observation that when vitamin E is administered with vitamin A or carotene, a greater storage of vitamin A in the liver

occurs as a result of improved stability of vitamin A and carotene in the gut. It has since been repeatedly confirmed by other workers that vitamin E exerts a protective effect on vitamin A and to a greater extent on β-carotene at various intake levels (Hickman et al. 1944; Lemley et al. 1947; Goldharber et al. 1950; Ames 1969). In enhancing the efficiency of carotene and vitamin A utilization, vitamin E acts as an antioxidant in the gastrointestinal tract to protect β-carotene and vitamin A from destruction (Quackenbush et al. 1942). However, the amount of vitamin E fed is critical since large doses of vitamin E may adversely affect carotene and vitamin A utilization (Johnson and Bauman 1948). More (1957) has postulated that at high dose levels, vitamin E inhibits the oxidation phase of retinol formation, but at lower levels it prevents oxidative destruction of carotene.

Nitrates

Research has linked the high nitrate content of forages heavily fertilized with nitrogen with impaired utilization of carotene (Muhrer et al. 1955; Cullison and Ward 1965). However, several researchers have demonstrated that nitrite and not nitrate is really responsible for the impairment of carotene and vitamin A utilization (Davidson and Seo 1963; Olson et al. 1963; Pugh and Garner 1963; Roberts and Sell 1963). In the ruminant, nitrate can be converted to nitrite by rumen microbial reduction. In his work with the rat, Phillips (1966) concluded that nitrite inhibited carotene and vitamin A utilization by direct oxidation of the two compounds. There was no interference with absorption, enzymatic processes associated with conversion of carotene or liver function. Other

workers have suggested that the nitrate effect may involve a combination of direct oxidation and interference with absorption (Sell and Roberts 1963; Adams et al. 1966). The impairment of carotene utilization by nitrite may also involve an indirect inhibition of the conversion of carotene to vitamin A by oxidising haemoglobin which is thought to participate in the conversion (Pfander 1962). The destruction of carotene by nitrite is pH dependent; the rate of carotene destruction increasing as pH decreases (Olson et al. 1963).

Presence of Intact Beta-carotene in Body Fluids and Tissues

The biochemistry of carotene conversion to vitamin A, as presently understood, is most applicable to the rat, the experimental model for most of the experiments on this subject. The main features of the biosynthetic pathway from carotene to vitamin A are thought to be similar in all animals. However, there are some species, breed and individual animal differences in the intestinal metabolism of carotene. Some species in addition to converting carotene to vitamin A, absorb intact carotenoids while others absorb little or no carotenoids.

The rat and pig absorb and accumulate virtually no carotenoids beyond the intestinal mucosa (Thompson et al. 1949; Olson 1964). Human beings absorb and accumulate both carotene and xanthophylls in their body tissues (Krinsky et al. 1958). Among the domestic mammals, cattle and horses absorb and accumulate fairly substantial amounts of carotene (Phillips 1966). The presence of carotenes in the plasma and body fat of the horse was reported in the early literature (Garton et al. 1964). The carotene content of horse plasma was found to fluctuate seasonally

probably due to the alternating influence of fresh pasture and dry forage (Vander Noot et al. 1965). The absorption and accumulation of carotenes in the body by cattle has been widely reported (Parish et al. 1947; Ganguly et al. 1953; Chew et al. 1983). The level of carotenoids in the plasma of cattle also shows a marked seasonal variation reflecting fluctuations in dietary intake (Marsh and Swingle 1960).

Variations in plasma carotene levels among different breeds of cattle have been reported (Hibbs et al. 1949; Krukowsky et al. 1950). For example, under similar feeding conditions Guernseys accumulate greater quantities of carotene in their tissues than do Holsteins (Goodwin 1954). Bauman et al. (1934) reported that Guernsey milk is richer in carotenoids than Friesian or Ayrshire milk. The breed differences in the accumulation of carotene in body tissues of cattle may reflect, in part, differences in intestinal carotene metabolism. More et al. (1943) reported that to prevent increased cerebrospinal fluid pressure, Guernseys required 1.13 times as much, and Jerseys 1.07 times as much carotene as did Holsteins. To maintain marginal plasma vitamin A concentrations with slight liver storage, Elliot (1949) noted a carotene requirement for Guernseys that was 1.7 times that for Holsteins. Depending on the criteria used, Holsteins converted carotene to vitamin A 1.4 to 1.8 times as efficiently as Guernseys (Eaton et al. 1959). Kapoor and Ranjhan (1974) have reported that the absorption of carotene in Holsteins and crossbreds was better than in the Hariana breed.

Sheep and goats are among the mammalian species that have little or no carotenoids in their body tissues. Early reports (Goodwin 1954) indicated the presence of some carotenes in plasma, colostrum and liver of

sheep, particularly when they were on fresh pasture. Low levels of carotene have been found in the plasma (5-15 µg/100 ml; Pierce 1946), colostrum and milk (8-10 µg/100 ml; Pierce 1947) of sheep. Chanda (1953) observed that goat colostrum contained traces of β-carotene, whereas milk did not.

Aside from blood, colostrum, liver and body fat, which are the principal sites for carotene accumulation, other body tissues in species that absorb carotene have been found to contain the pigment. Carotenoids are deposited in body organs and tissues such as the adrenals, bile, corpus luteum (CL), corpus rubrum, iris, kidney, muscle, ovaries, epithelium, pituitary, placenta, testes and thymus (Bauernfeind et al. 1981). The high concentration of β-carotene in a specialized reproductive organ and tissue such as the ovary and CL is of particular interest since that raised the initial speculation that β-carotene may play a specific role in the ovarian function of cattle (Lotthammer 1979b).

Earlier studies by Schultz (1956), Bruggerman and Niesar (1957) and more recent ones by Schultz et al. (1973) and Ahlswede and Lotthammer (1978) revealed that the CL of cattle contains a high concentration of β-carotene but no vitamin A. Stowe and Marteniuk (1982) observed significant positive correlations between carotene in bovine corpora lutea and serum carotene. However, contrary to the earlier reports, they found a small but inconsistent quantity of vitamin A, as retinyl palmitate in the corpora lutea. On the other hand, Chew et al. (1983) found β-carotene in both the follicular fluid and corpora lutea of the cow, while vitamin A was found in the follicular fluid but not in the CL.

It is not clear how vitamin A activity is derived from absorbed intact carotenes in most species. Some cleavage of carotenes to retinol can occur in the liver of the rat through the action of a dioxygenase with properties similar to that found in the intestine (Olson and Hayaishi 1965). A number of workers have demonstrated that the rat can convert carotene injected into the blood to vitamin A (Beiri and Pollard 1954; McGillivray et al. 1956; Worker 1956). Extra intestinal tissues such as the liver, kidney and lung were suggested to be involved in this conversion. In the calf, Kon et al. (1955) and McGillivray et al. (1956) found no evidence of the conversion of intravenously administered carotene to vitamin A. Ganguly and Murphy (1967) have concluded that extra intestinal conversion is less efficient in those species that regularly circulate carotene in the blood. Yet a recent in vitro study by Sklan (1983) has revealed carotene cleavage activity in the bovine CL with properties similar to those reported for the intestinal mucosa and liver in other species. Sklan (1983) found that the carotene cleavage activity increased from a rate in regressing CL which was comparable to that of intestinal mucosa, to a rate in mid-cycle CL which was more than two-fold that of intestinal mucosa. There was very little retinol esterification for storage in the CL, prompting Sklan to hypothesize that carotene is stored in and converted by the CL to retinol for immediate use by that organ.

Effect of Beta-carotene on Bovine Reproduction

Field Studies

As previously indicated, the initial speculation about a vitamin

A-independent role for β-carotene in bovine reproduction was prompted by the reported high concentrations of β-carotene in the ovary and CL. Field studies conducted by Seitaritis (1963) and Konnerman (1967) provided initial indirect evidence in support of this hypothesis. Seitaritis (1963) examined bovine serum vitamin A and carotene values at specific stages of the ovarian cycle and found an association between low carotene levels and high incidences of ovarian cysts. Konnerman (1967) observed that the interval between first insemination and conception in cows was shorter when β-carotene supplementation was increased. Consequently, cows fed the high levels of carotene had shorter calving intervals. Subsequent reports suggested a correlation between progesterone output by the CL and the carotene status of cattle. Schultz et al. (1974) observed that the progesterone concentration in the CL of slaughter cows was higher in summer than in winter; paralleling the seasonal variation in β-carotene concentration in the CL. Anwandter (1974), on the other hand, did not find much seasonal difference in the progesterone output of the CL but noted that the rise of the progesterone profile during the estrous cycle was delayed in winter.

Several other field studies have produced data which generally support the hypothesis that β-carotene influences reproductive performance. However, like many field studies, these trials were often not adequately controlled. Mingazov (1977) observed a first service conception rate of 57% for carotene-supplemented Red Steppe cows compared to 39% for unsupplemented controls. Despite this, the final plasma carotene concentration in the supplemented group was within the range (100-200 µg/100 ml) classified as critical by Friesecke (1978). The results of three

trials conducted in Denmark on the β-carotene effect on fertility of cattle were reviewed by Friesecke (1978). In the first trial, β-carotene administration by injection reduced the interval from calving to conception and services per conception and doubled the conception rate after first insemination. Similar results were obtained in the second trial involving oral carotene supplementation, even though the control cows had high plasma carotene levels (>300 μg/100 ml). Again there was improvement in conception rate with carotene supplementation in the third trial.

In a field trial conducted in the United Kingdom (Cooke and Comben 1978) half of a commercial dairy herd of 182 cows was fed grass silage (high in carotene) and the other half was fed corn silage (low in carotene). No supplemental β-carotene was given. The results showed that cows fed the high carotene diet had a higher conception rate after first insemination and fewer overall services per conception than those on the low carotene diet.

A review of data from β-carotene supplementation field trials in four United States and five Canadian herds was presented by Olentine (1982). The results showed that supplemental β-carotene appeared to be useful in maintaining normal reproduction in dairy cows. Carotene-supplemented cows on the average had fewer days open and fewer services per conception than cows not receiving supplemental β-carotene. In three of these trials, reproductive performance data of the carotene-supplemented cows were compared with that of cows in the herd one year before the carotene supplementation. Such a comparison is not valid since interim culling of the herd on the basis of fertility problems could have

influenced the reproductive performance of the remaining herd that subsequently received the carotene supplementation.

In his review, Stowe (1984) presented the results of an unpublished field study by Studer at Carnation Farms that showed no beneficial effect of carotene supplementation. No significant differences in the rate of first-service conception, services per conception and days open were observed for control and β-carotene (300 mg/head/day) supplemented cows.

Controlled Studies

The validity of the growing evidence regarding a possible specific role for β-carotene in bovine reproduction was first tested in controlled studies conducted in Germany. A number of recent reviews concerning these initial studies and subsequent ones have been published (Friesecke 1978; Lotthammer 1978; Lotthammer 1979b; Stowe 1984).

In all the German studies, the Black Pied breed of cattle was used. Heifers were used in the first three studies in which all animals received a basal low-carotene diet. Half of the heifers received 100 I.U. vitamin A plus 0.3 mg β-carotene per kg body weight while their control counterparts received only vitamin A equal to the total vitamin A equivalent received by the carotene-supplemented groups. In all three studies, the low-carotene heifers showed disturbances of reproductive function which were linked directly or indirectly to ovarian function. Compared to the carotene-supplemented groups, the low-carotene heifers showed prolonged and poorly defined estrus (silent heat); a longer interval from both onset of estrus and pre-ovulatory LH peak to ovulation by up to 24 hours; and a higher incidence of ovarian cysts (Meyer et al. 1975;

Lotthammer et al. 1976; Lotthammer and Ahlswede 1977; Schams et al. 1977). While the preovulatory LH peak values were not significantly different between the two groups of heifers (Schams et al. 1977), the CL of the low-carotene heifers developed more slowly and remained smaller (Lotthammer et al. 1976). As a result of the ovarian disorders, conception rate to one or two inseminations was markedly lower and overall services per conception was higher in the low-carotene heifers compared to the carotene supplemented ones. In one experiment, the time lag between onset of estrus and ovulation was reduced considerably in low-carotene heifers when (after 29 weeks of carotene deficiency) they were given supplemental β-carotene for 7 to 10 weeks (Meyer et al. 1975).

A study was also conducted with cows in which the levels of vitamin A and β-carotene given to control and carotene-supplemented cows respectively, were based on milk yield. Beta-carotene deficiency in the control group also produced detrimental effects on postpartum reproduction. Uterine involution and the resumption of ovarian function after parturition were delayed in these cows compared to the carotene-supplemented ones (Lotthammer et al. 1978). Estrus in the control cows, as in the low-carotene heifers, was weak and ovulation was delayed by an average of 13 hours in comparison to the carotene supplemented cows. There was a slower rise and lower levels of progesterone during the estrous cycle in the control cows. Furthermore, the β-carotene-deficient cows had a high rate of embryonic mortality between day 37 to 45 of gestation and a high rate of early abortion in the 18th to 20th weeks of gestation.

Folman et al. (1979) attempted to confirm the positive German findings using similar carotene treatment procedures on Israeli Friesian

heifers. In contrast to the previous findings, they observed no differences in duration of standing heat, length of the estrous cycle, plasma progesterone and LH concentrations, interval from LH peak to ovulation and conception rate to 1 or 2 inseminations between control and carotene-supplemented heifers. However, average growth rate was higher for the carotene supplemented group compared to the controls. Folman et al. (1979) suggested that the use of a teaser bull for estrus detection may have markedly improved the conception rate particularly in the control heifers which had extremely low serum β-carotene levels. It is worth noting that the peak serum β-carotene concentration of the carotene-supplemented heifers in the study by Folman et al. (1979) was far lower than that of carotene-supplemented heifers in the Meyer et al. (1975) and Lotthammer et al. (1976) experiments.

In a study with Brown Swiss and Simmental heifers, Bonsembiante et al. (1980) observed 20% irregular estrous cycles which occurred primarily in the low-carotene cows. They found no positive effect of carotene supplementation on number of cows in heat, although the carotene-supplemented cows appeared to have longer and more evident periods of estrus than the low-carotene cows. The results of a more recent study with Charolais by the same workers (Bonsembiante et al. 1983) showed a higher incidence of irregular estrous cycles (46 vs 31%) and weak estrus (31 vs 19%); a lower fertility rate at first insemination (27.9 vs 50%); and more services per conception (3.9 vs 2.7) in low-carotene heifers compared to those supplemented with β-carotene. Carotene supplementation did not affect serum progesterone concentration at day 21 post-insemination in this study.

Jackson (1981) studied the effect of carotene deficiency on reproductive performance by feeding a low-carotene diet to group 1 of 12 Friesian cows for varying periods of time (2, 4 and 5 weeks). The cows were estrus synchronized with 2 injections of cloprostenol given 11 days apart, followed by inseminations at 72 and 96 hours after the second injection. Jackson (1981) found that mean plasma carotene was inversely correlated with the number of days on the carotene-depleting diet and the number of services per conception. An evaluation of the endocrine and ovarian changes in these cows revealed that cows with lower serum β-carotene values showed cyclic irregularities and an apparent depression of progesterone and estradiol production (Jackson et al. 1981). LH and FSH did not appear to be affected by the β-carotene status of the cows. Despite the positive indications in support of a role for β-carotene in bovine reproduction, Jackson and his associates acknowledged that the small number of animals used in their study precluded reaching definite conclusions. Ducker et al. (1984), using a larger number of Friesian heifers (160), fed a ration based on corn silage to study the effect of β-carotene on fertility. Beta-carotene supplementation (300 mg/head/day) did not improve live weight gain and neither did it improve reproductive performance when compared to control heifers receiving only vitamin A. However, within the carotene-supplemented heifers, there was a significant negative correlation between serum β-carotene concentration at first AI and the number of days from that time to established pregnancy.

Results of other controlled studies conducted mostly with Holsteins, at North American research centers have shown either inconsistent or no

positive effects of carotene supplementation on bovine reproduction. A study with 32 Jersey and Holstein cows showed that 7 of the 9 cases of cystic ovaries diagnosed occurred in the carotene-supplemented cows (Bremel et al. 1982). Services per conception were not different between the control and supplemented groups. Studies involving Holstein heifers divided into a low-carotene control and a carotene-supplemented group (300 mg/head/day) for test periods of 6 to 8 weeks have been reported by Wang et al. (1982) and Wang and Larson (1983). Estrus was synchronized with prostaglandin ($PGF_{2\alpha}$) injection at the end of the test periods. In three trials, onset of estrus after $PGF_{2\alpha}$ injection occurred earlier in the control heifers then in the carotene-supplemented group (Wang et al. 1982). The intervals from $PGF_{2\alpha}$ injection to preovulatory LH peak and to ovulation were also shorter in the control heifers. No significant differences were found among treatments in the number of heifers that expressed heat, the serum progesterone concentration or the conception rates (in 2 trials, conception rate tended to be higher with carotene supplementation) in any of the trials. Estrual activity (measured as mounts observed per heifer) was not influenced by carotene supplementation (Wang and Larson 1983). In another study with cows, β-carotene supplementation had no effect on interval to uterine involution, intervals to first ovulation and observed estrus postpartum, incidence of cystic follicles, peak serum progesterone level, conception rate to first service and days open (Larson et al. 1983). Similar findings of no significant differences between carotene-supplemented and control Holstein cows for interval to first heat, interval to first service, days open and services per conception were reported by Bindas et al. (1983).

The lack of significant improvement in the overall fertility of Holstein cows with carotene supplementation has also been reported by Akordor et al. (1984). They observed a significant ($P<.05$) effect of β-carotene supplementation on services per conception but no effect on days open, intervals to uterine involution and first breeding and conception rate. Carotene supplementation also did not improve the interval from calving to first estrus and services per conception in beef heifers (Greenberg et al. 1984).

Effect of Beta-carotene on Production, Health and Some Metabolic Parameters in Dairy Cattle

There is little information on the effect of β-carotene on milk yield. Lotthammer (1979a) reported that, in comparison to carotene-supplemented cows, milk fat production in carotene-deficient cows was significantly depressed, especially at peak lactation. In other studies, feeding supplemental β-carotene had no effect on milk yield (Akordor et al. 1984), but serum β-carotene concentration was negatively correlated ($r = -.44$; $P<.02$) with solids-corrected milk yield (Larson et al. 1983).

Calves born to carotene-deficient cows showed a higher incidence of diarrhea and a higher mortality rate in the first week of life than the calves of carotene-supplemented cows (Lotthammer et al. 1978). This was probably due to differences in colostrum composition of the dams since the colostrum (and subsequently the milk) of carotene-deficient cows showed lower levels of β-carotene and vitamin A than that of the carotene-supplemented cows. Furthermore, the calves of the carotene-supplemented group had, after their first meal, higher immunoglobulin levels than

calves of the deficient group.

Beta-carotene deficiency also had effects on certain parameters of metabolism and organic functions in lactating cows. Serum glutamic oxaloacetic transaminase (GOT) activity in carotene-deficient cows increased after calving and stayed at levels significantly higher than those of the supplemented group (Lotthammer 1978). The GOT values of carotene-deficient cows with pregnancy abnormalities were even greater. This was interpreted as being due to greater stress on the liver as a result of deficiency of carotene. There was also a higher serum thyroxine level in the carotene-deficient cows. However, serum thyroxine levels in carotene-deficient cows with abnormal pregnancy were lower than those with normal pregnancy.

Chew et al. (1982) have suggested a possible link between the severity of mastitis in Holstein cows and low levels of serum β-carotene. They observed that cows with lower plasma β-carotene, vitamin A and total vitamin A equivalent had higher California Mastitis Test scores than cows with higher plasma β-carotene and vitamin A.

Suggested Beta-carotene Requirement for Bovine Reproduction

The exact β-carotene requirements for optimum reproduction in cattle have not been established. However, based solely on the German experiments with the Black Pied breed, a blood β-carotene classification system has been adopted to define a baseline above which β-carotene status may be considered satisfactory. According to this classification (Friesecke 1978), blood β-carotene values between 0 to 100 µg/100 ml are considered deficient; 100 to 200 is critical; 200 to 300 is suspect; and values above

300 µg/100 ml are considered sufficient.

In the experience of the German researchers, young cattle require a daily intake of about 100 mg β-carotene to maintain sufficient serum levels of β-carotene (Lotthammer 1979b). Dairy cows require a daily intake of about 200 mg during the dry period and for maintenance. During lactation, cows require a further 20 mg of β-carotene per litre of milk produced. According to Lotthammer (1979b), a slight deficit in β-carotene intake can apparently be partly compensated for by more efficient absorption. Nevertheless, the suggested minimum daily intake for lactating cows is 300 mg per head.

GENERAL MATERIALS AND METHODS

Serum Beta-carotene and Vitamin A Analysis

Extraction and Chromatography Procedures

Beta-carotene and vitamin A were extracted by vortexing 1.0 ml of serum and 2.0 ml cyclohexane in the presence of 2.0 ml ethanol and 1.0 ml distilled water. After a 1 minute extraction, the extraction tubes were centrifuged for 10 minutes at 500xg to achieve a clear separation of the liquid phases. Then 1.5 ml of the upper cyclohexane phase containing the carotenoids and vitamin A was chromatographed on a deactivated Al_2O_3 column to separate the two compounds. The carotenoids were first eluted from the column with 2 to 2.5 ml of petroleum ether. The column was eluted a second time with 6 ml of a solvent mixture (85 parts petroleum ether:15 parts ether; v/v) to recover vitamin A.

Beta-carotene Determination

Beta-carotene was determined using the procedures described by Brubacher and Vuilleumier (1980). The eluate containing the carotenoids was dried in a 40°C water-bath under a stream of nitrogen gas. The residue was dissolved in 1.5 ml cyclohexane and transferred into a 1 cm semi-micro cuvette (1.5 ml capacity). Absorbance was read at 450 nM on a Gilford Spectrophotometer 2400 (Gilford Instrument Laboratories Inc., Ohio), against a cyclohexane blank. Beta-carotene concentration was calculated using a value of 2500 as the absorbance at 450 nM of a 1% solution of β-carotene in cyclohexane in a 1 cm cuvette.

Vitamin A Determination

Serum vitamin A concentration was determined using a modification of the trifluoroacetic acid (TFA) reagent method described by Neeld and Pearson (1963). The eluate containing vitamin A was dried in a 40°C water-bath under a stream of nitrogen gas. Overdrying of the samples was carefully avoided. The residue was dissolved in 0.5 ml of chloroform and 0.3 ml was transferred into a 1 cm cuvette (3.5 ml capacity). After adding 2 drops of acetic anhydride, the cuvette was placed on a Gilford Spectrophotometer 2400 set at 620 nM against a TFA reagent blank (1 vol TFA:2 vols chloroform). Then 3.0 ml of TFA reagent was rapidly added to the mixture and the absorbance was read 30 secs later. Vitamin A concentration was extrapolated from a standard curve prepared using 0.1, 0.3, 0.6, 0.9 and 1.5 µg/ml solutions of vitamin A acetate.

Recovery of Beta-carotene and Vitamin A After Extraction and Chromatography

To determine the efficiency of extraction and recovery after chromatography, known amounts of β-carotene and vitamin A were analysed in duplicate as described above. Recoveries after extraction and chromatography for 6.0, 8.5 and 9.0 µg of added β-carotene averaged 102±3.7%. Recoveries after extraction alone and after extraction and chromatography for 0.3, 0.6, 1.5, 3.0 and 6.0 µg of added vitamin A acetate averaged 94.3±3.2% and 90.1±2.4%, respectively.

Single determinations of β-carotene and vitamin A were made for the experimental samples due to the large number (862) of samples analysed. To evaluate intra- and inter-assay variation for the β-carotene and

vitamin A determinations, 6 replicate determinations per assay run on a pool serum sample were done on 3 occasions (beginning, middle and end) during the analysis period. The intra- and inter-assay coefficients of variation for a mean β-carotene concentration of 354.6±2.8 µg/100 ml were 4.5% and 3.9%, respectively. For a mean vitamin A concentration of 31.8±.9 µg/100 ml, the intra- and inter-assay coefficients of variation were 7.5% and 14.5%, respectively.

Beta-carotene Analysis in Forages

The analysis was done according to the standard carotene assay method for feedstuffs (A.O.A.C. 1980).

Dry Forages (Hay and Alfalfa Pellets)

The carotenoids were extracted exhaustively overnight from about 2 g of finely ground samples in a brown flask using 30 ml of an acetone-hexane mixture (3:7; v/v). The extract was decanted through glass wool in the stem of a funnel into a flask and diluted to 100 ml with hexane.

The pigment in the total extract was separated by chromatography on a column of a 1:1 mixture (w/w) of activated magnesia (Sea Sorb 43, Fisher) and diatomaceous earth (Hyflo Super-cell). The column was eluted with a mixture of acetone and hexane (1:9, v/v) to recover all the yellow carotenoids in the eluate into a flask in a 40^{o}C water-bath. After allowing the eluate to cool to room temperature, it was diluted to 100 ml with a mixture of acetone and hexane (1:9, v/v). The absorbance of the carotene solution was determined on a Bausch and Lomb Spectronic 20 calorimeter (Bausch and Lomb Inc., Rochester, NY) at a wavelength of

435 nM. The concentration of carotene (expressed as β-carotene) was extrapolated from a calibration curve prepared using 100, 200, 300 and 400 μg pure β-carotene per 100 ml acetone-hexane (1:9, v/v) solution.

Fresh Pasture and Silages

The carotenoids were extracted by blending 2 to 5 g finely cut and/or ground samples with 40 ml acetone, 60 ml hexane and 0.1 g $MgCO_3$ for 5 minutes. The extract was decanted through glass wool in a large funnel into a 500 ml separatory funnel containing 100 ml of water. After washing the residue twice with acetone into the separatory funnel, the total mixture was shaken for 1 minute, allowed to settle and the lower aqueous layer was run off. The organic layer was washed 5 more times with 100 ml portions of water. The organic layer was then transferred into a volumetric flask containing 9 ml acetone and diluted to 100 ml with hexane. Chromatography of the extract and the determination of absorbance and β-carotene concentration was as described above for the dry forages.

Milk Progesterone Assay

A non-extraction radioimmunoassay (RIA) procedure essentially similar to that described by Heap et al. (1973) was used for the determination of progesterone concentration in milk. The milk samples were first mixed well and diluted 10-fold with 0.05 M sodium phosphate buffer solution (PBS, pH 7.0). The assay procedure involved pipetting 0.5 ml of standard progesterone solutions (0, 25, 50, 100, 200, 400 and 800 pg/tube) or 0.5 ml PBS plus 0.1 ml of the diluted milk samples into duplicate tubes. Aliquots (0.1 ml) of a diluted milk (10-fold) taken from

a non-cycling, non-pregnant cow that had no detectable progesterone was added to all the standard tubes. Then 0.1 ml of an appropriately diluted progesterone antiserum (#11-9/4/80 obtained from N.C. Rawlings, Dept. of Vet. Physiology, Univ. of Saskatchewan) and 0.1 ml of [1,2-^{3}H] progesterone (≈10,000 cpm) was added to all tubes and incubated overnight at 4^{o}C. The following day, the bound progesterone was separated by precipitating the free progesterone with 0.5 ml charcoal suspension in PBS, followed by a 10 minute incubation at 4^{o}C and centrifugation at 800xg for 10 minutes. The supernatant containing the antibody-bound progesterone was decanted into plastic vials containing 4 ml scintillation fluid and counted. The concentration of progesterone in the milk samples extrapolated from the standard curve.

EXPERIMENT 1

Seasonal Variation in Serum Beta-carotene and vitamin A and their Association with Postpartum Reproductive Performance of Holstein Cows

Cattle, particularly range cattle, exhibit a marked seasonal variation in blood and milk concentration of β-carotene and vitamin A which reflects fluctuations in dietary intake of carotene (Marsh and Swingle 1960; Kirchgessner et al. 1967). Beta-carotene levels usually peak during the summer months when animals have access to pasture. During the winter months when the animals are fed stored forage and other low-carotene diets, the milk and blood levels of β-carotene tend to drop drastically.

In Canada, some dairy herds are confined year-round and are thus totally dependent on stored forage. Under such conditions, animals not receiving enough good quality hay and haylage are likely to have deficient (<100 μg/100 ml) levels of blood carotene. In a survey of eight herds in Ontario and Quebec, Harvey and Smith (1979) reported that serum β-carotene levels were low (<300 μg/100 ml) in five of the herds. The animals in these herds were fed primarily corn silage and grain rations with no more than 4.5 kg of hay per cow per day.

In temperate regions, seasonal variation in reproductive performance of cattle has been reported (Ortavant et al. 1964; Boyd 1977; DeKruif 1978). For example, spring-calving beef and dairy cows have been reported to have a longer interval from calving to first ovulation than autumn calvers (Bulman and Lamming 1978; King and Hurnik 1980; Peters

and Riley 1982b). Holtman (1979) reported that the 60 to 90-day non-return rates for a number of Quebec dairy herds bred by AI during a 10-year period showed marked seasonal fluctuations. The non-return rates were low in the winter months but improved gradually during spring and summer reaching a peak in September and October.

Thibbault et al. (1966) have suggested that photoperiod might be involved in the seasonality of reproductive activity of the cow and Peters and Riley (1982b) have demonstrated a negative correlation between photoperiod during late pregnancy and the onset of postpartum ovarian cycles. However, most workers suggest that nutritional management is largely responsible for the seasonal variation in cow fertility. While energy and probably protein supply may be the major nutritional factors that are involved, β-carotene intake might also be important since the seasonal variation in blood carotene appears to coincide with the fluctuations in bovine fertility.

Konnerman (1967) originally noted a correlation between conception rate in cows and β-carotene intake based on a statistical survey. Subsequent controlled studies led to the suggestion that β-carotene has a specific role independent of vitamin A in bovine reproduction (Lotthammer 1979b). Numerous trials conducted in Europe (Friesecke 1979; Cooke and Comben 1978; Jackson 1981; Bosenbiante et al. 1983) and North America (Olentine 1982) have generally provided evidence in support of this hypothesis. In contrast, other workers have observed inconsistent, marginal and/or no beneficial effects of β-carotene supplementation on bovine reproduction (Folman et al. 1979; Bremel et al. 1982; Wang et al. 1982; Larson et al. 1983; Ducker et al. 1984).

This experiment was initiated as a preliminary survey with the following objectives in mind:

1) To determine the levels of serum β-carotene and vitamin A in winter-housed cows that received adequate amounts of good quality forage in winter and had access to pasture in summer.

2) To determine if the postpartum reproductive performance of these cows was influenced by mean serum carotene concentrations during the prepartum to early postpartum period.

Materials and Methods

Animals

Sixteen Holstein-Friesian cows from the University of Manitoba dairy herd were studied from October 1980 to September 1981. The cows were selected such that half of them calved between January and April, Group 1 and the other half between July and October, Group 2. The mean ages of the cows were 3.9 (2-6) and 4.0 (2-7) years for groups 1 and 2, respectively. Rations adequate in all nutrients according to NRC (1978) standards were fed. Grain was fed (based on milk yield) prior to both morning and afternoon milkings while silage and hay were fed (free choice) between milkings and at night. Water was available at all times.

The cows were housed in a stanchion barn and kept indoors throughout the winter. In summer, the warm days of spring and fall the milking cows were allowed outside to graze pasture between 08:00 and 13:30 hours. The dry cows were usually left on pasture during this period and their diets were supplemented with silage if necessary.

Serum Beta-carotene and Vitamin A Concentration

Blood samples for serum β-carotene and vitamin A analysis were taken by a puncture of the coccygeal vein/artery from all cows at two week intervals throughout the experimental period. The samples were kept overnight in the dark at 4°C, centrifuged and the serum stored at -20°C until analysed. Monthly serum pools were prepared for each cow and analysed for β-carotene and vitamin A according to procedures described previously in the general materials and methods section.

Forage Beta-carotene Concentration

Conserved forages (hay, silages, haylage, alfalfa pellets) fed during the experimental period were sampled at weekly intervals. The silage and haylage samples were stored frozen while the hay and alfalfa pellets were stored at 4°C in the dark. Monthly composites were prepared from these samples for β-carotene analysis. During the grazing season, pasture was sampled once a month from the field the cows were grazing at the time and analysed on the same day for β-carotene concentration. The analysis for β-carotene in the various forage samples was carried out according to the procedures described previously.

Milk Progesterone and Interval to First Ovulation (IO)

Composite milk samples (from all four teats of the udder) for progesterone analysis were taken every other day from each cow from day 7 to 40 postpartum. The samples were preserved with potassium dichromate (1 tablet/sample) and stored at 4°C until analysed, which was usually within one week of sampling. The assay procedure for milk progesterone

was described in the general materials and methods section. The intra- and inter-assay coefficients of variation for a pooled milk sample were 11.6 and 12.9% respectively (n = 22 assays).

The postpartum progesterone profiles obtained from the milk analysis for each cow was used to estimate the interval to first postpartum ovulation. Ovulation was presumed to have occurred when milk progesterone concentration increased progressively from levels lower than 1 ng/ml to levels greater than 5 ng/ml within a period of 10 consecutive days. Ovulation was usually confirmed by rectal palpation for a CL on the ovary.

Ovarian and Uterine Condition

Each cow was palpated twice a week to evaluate uterine involution and to check the ovaries for the confirmation of ovulation. The interval to full uterine involution was estimated based on the palpations. Artificial inseminations usually began at the first estrus after day 60 postpartum.

Statistics

The intra- and inter-assay coefficients of variation were calculated according to the method of Rodbard (1971).

The experimental data were analysed using computer programs outlined in the Statistical Analysis Systems (SAS) User's Guide (1982 Ed.). A General Linear Model (GLM) procedure was used for the analysis of the reproduction data. A split-plot analysis with season of calving as main plot and month as subplot was used for the serum β-carotene and vitamin A data. Mean differences for all parameters were tested by the Student

Newman Keul (SNK) test (Snedecor and Cochran 1967).

To determine the correlation between serum and reproductive parameters, a period extending from 1 month prepartum to 2 months postpartum (calving period) was defined for each cow and the mean serum β-carotene and vitamin A concentrations during this period were used in the computation. The correlation coefficients for all parameters were adjusted for month and/or season of calving effects.

Results

Forage Beta-carotene Concentration

The concentration of β-carotene in the various forages fed throughout the experimental period is presented in Table 1. Different types of good quality hay from different sources were fed throughout the experiment. As a result, the concentrations of β-carotene in hay were generally high but there was considerable monthly variation ranging from 23.3 to 62.7 mg/kg DM.

The corn silage fed was from a batch freshly ensiled one week before the trial began. A severe frost two weeks prior to harvesting the corn had killed the plants resulting in very low initial β-carotene concentration (6.9 mg/kg DM) in the silage which deteriorated with time in storage resulting in non-detectable β-carotene by the third month of the trial. Corn silage was not available from May to September.

Fababean silage was fed from November to March. Unlike corn silage, fababean silage had a high concentration of β-carotene (35.4 to 42.5 mg/kg DM) which decreased only slightly with storage. Rye silage, fed in May

Table 1. Mean* concentration of β-carotene (mg/kg DM) in the forages fed to animals throughout the experimental period

	Type of forage						
Month	Hay	Corn silage	Fababean silage	Rye silage	Pasture	Grass haylage	Alfalfa pellets
Oct. 1980	49.5	6.9	-	-	-	-	-
Nov. 1980	38.1	1.6	42.5	-	-	-	-
Dec. 1980	50.7	ND	39.5	-	-	-	-
Jan. 1981	33.3	ND	35.4	-	-	-	-
Feb. 1981	62.7	ND	37.4	-	-	-	-
Mar. 1981	31.9	ND	36.9	-	-	-	-
Apr. 1981	48.0	ND	-	-	-	-	-
May 1981	35.1	-	-	48.5	-	-	-
June 1981	45.0	-	-	44.4	102.5	-	-
July 1981	44.1	-	-	-	228.1	-	-
Aug. 1981	26.9	-	-	-	101.5	31.1	72.2
Sept.1981	23.3	-	-	-	113.3	33.4	50.6

ND - Not detectable.

* - Mean of duplicate determinations.

and June, also had high levels of β-carotene (48.5 and 44.4 mg/kg DM, respectively).

Pasture which was grazed from June to September contained the highest concentrations of β-carotene (101.5 to 228.1 mg/kg DM) compared to all other forages. The greatest carotene concentrations in pasture were seen in July.

Grass haylage and alfalfa pellets were fed in August and September. Although the β-carotene concentrations in grass haylage were relatively high (31.1 and 33.4 mg/kg DM), they were much lower than the β-carotene levels in pasture from which the haylage was prepared. Alfalfa pellets fed during August and September had β-carotene concentrations of 72.2 and 50.6 mg/kg DM, respectively.

Serum Beta-carotene and Vitamin A

As shown in Table 2, there was a wide variation in the serum concentration of β-carotene and vitamin A throughout the experimental period and also during the calving period. This wide range of β-carotene and vitamin A concentrations represent both individual animal differences and monthly variations which appeared to reflect the differences in the levels of carotene in the forages fed at different times of the experimental period.

Mean monthly serum β-carotene concentrations fluctuated between 239.5±13.8 and 339±19.3 μg/100 ml from October to May when the main sources of carotene were hay and the silages (Figure 1). However, none of the monthly differences during this period were significant. Serum β-carotene concentration rose significantly ($P<.01$) to over 600 μg/100 ml in June

Table 2. Overall summary of serum β-carotene and vitamin A data

Item	Entire experimental period	Calving period
Number of cows	16	16
Number of observations	192	16
β-carotene conc.* (μg/100 ml)	108.8-1194.4	156.3-883.2
Vitamin A conc.* (μg/100 ml)	20.0-80.0	29.6-57.5

*Range of values.

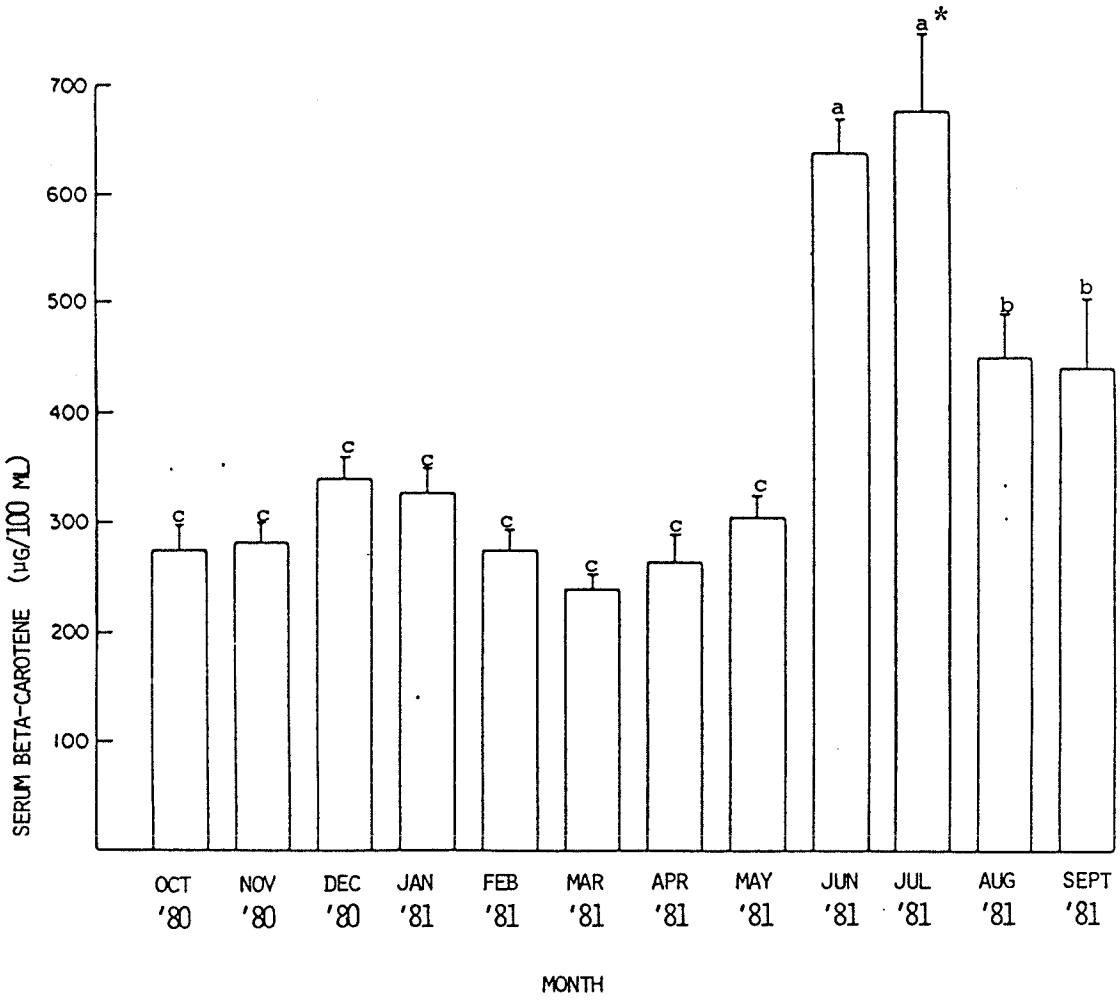

Figure 1. Mean (±SE) monthly serum β-carotene concentration for 16 Holstein cows.

*Means with different superscripts are significantly different ($P<.05$).

and July when pasture became available to the cows. Although pasture concentration of carotene in July was more than double that in June, the serum β-carotene concentrations during those two months were not significantly ($P<.05$) different. Mean serum β-carotene concentrations in August and September (450.2±40.4 and 441.6±62.6 µg/100 ml, respectively) were not significantly ($P>.05$) different from each other. However, both levels were significantly ($P<.05$) lower than the levels in June and July and higher than those from October to May.

Mean serum vitamin A level was significantly ($P<.05$) higher in June (52.7±2.8 µg/100 ml) than in July (46.4±2.5 µg/100 ml) (Figure 2) although serum β-carotene concentration was slightly lower ($P>.05$) in June than in July (Figure 1). The serum vitamin A concentrations in both months were significantly ($P<.05$) higher than the vitamin A concentrations in all the other months. The mean serum vitamin A concentrations in those other months fluctuated between 31.4±2.4 and 40.3±2.2 µg/100 ml with the differences among months being generally non-significant. The only exceptions were differences ($P<.05$) between November (40.3±2.2 µg/100 ml) and March (32.2±1.7 µg/100 ml) and April (31.4±2.4 µg/100 ml).

Mean serum β-carotene concentration for the entire experimental period was not significantly different between group 1 (381.7±25.6 µg/100 ml) and group 2 (370.8±25.6 µg/100 ml) cows (Table 3). However, during the calving period, mean serum β-carotene concentration was significantly ($P<.01$) higher for group 2 cows (477.5±46.5 µg/100 ml) compared to group 1 cows (229.3±46.5 µg/100 ml). There were no significant ($P>.05$) differences in mean serum vitamin A levels between the two groups of cows for both the entire experimental period and the calving period. There were significant

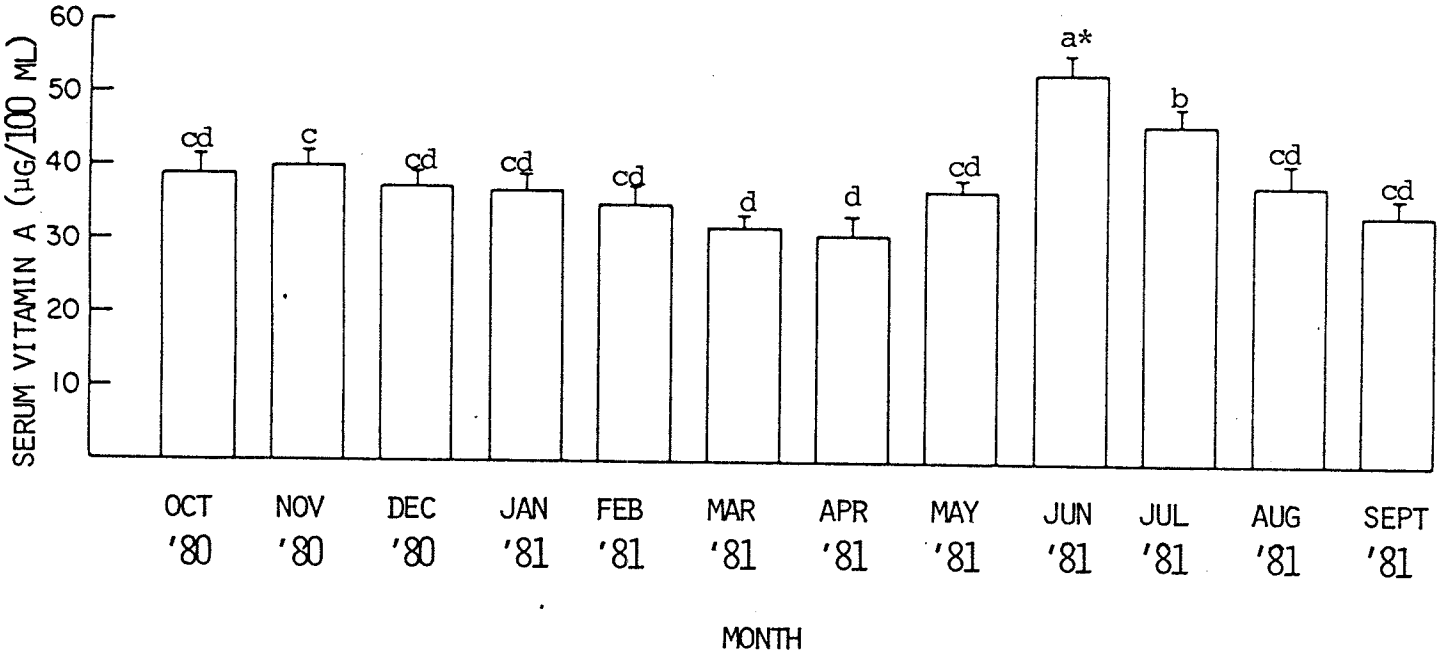

Figure 2. Mean (±SE) monthly serum vitamin A concentration for 16 Holstein cows.

*Means with different superscripts are significantly different (P<.05).

Table 3. Mean (±SE) serum β-carotene and vitamin A concentrations for the entire experimental period and during the calving period for the two groups of cows

	Cow groups	
	Group 1	Group 2
Serum parameter	January-April calving	July-October calving
	µg/100 ml	
β-carotene:		
a) Entire expt. period	381.7 (25.6)[a*]	370.8 (25.6)[a]
b) Calving period	229.3 (46.5)[b]	477.8 (46.5)[c]
Vitamin A:		
a) Entire expt. period	38.4 (2.4)[d]	38.2 (2.4)[d]
b) Calving period	32.9 (2.5)[e]	36.7 (2.5)[e]

*Means within a row having different superscripts are significantly different, $P<.01$.

and positive correlations between serum β-carotene and vitamin A concentrations for the two periods (Table 4).

Reproductive Performance

A wide range of values (Table 5) were obtained for the reproductive parameters, especially the interval to first service (IFS) and days open (DO). The missing data for interval to first ovulation (IO) represent two cows from group 1 and one cow from group 2 that did not ovulate within the first 40 days postpartum. In all three cows, milk progesterone level remained lower than 1 ng/ml throughout the 40-day early postpartum period. Another cow in group 1 was not rebred because of a management decision. Conception rate for all the cows was generally poor; conception rates after 1, 2 and 3 inseminations being 33.3%, 60% and 66.7%, respectively.

The mean IO and services/conception (SC) did not differ significantly between the two groups of cows (Table 5). However the interval to uterine involution (IUI) and IFS were significantly ($P<.05$) longer in the group 1 cows compared to group 2 cows. Mean DO was also longer in group 1 cows (201.0±32.2) compared to group 2 cows (109.3±30.1) with the difference between the two groups approaching significance ($P = .058$). One cow in group 1, with a previous record of being difficult to get pregnant, had an exceptionally long DO of 385 days.

Serum β-carotene concentration had a negative but low correlation with IO, IUI, SC and DO; and a positive but low correlation with IFS (Table 6). None of the correlation coefficient values were significant ($P>.05$).

Table 4. Partial correlation between the serum β-carotene and vitamin A parameters

Parameters	*Partial correlation coefficient	Level of significance
Entire period:		
β-carotene and vitamin A	0.34	.0001
Calving period:		
β-carotene and vitamin A	0.67	.02

*Partial or residual correlation with the effects of month, season of calving and cow removed.

Table 5. Means ±SE and range of the postpartum reproductive parameters for the two groups of cows

Parameter	Group 1 Jan.-April calving	Group 2 July-Oct. calving	Overall range	
Interval to ovulation (days)	22.7±2.6[a*]	25.6±2.4[a]	15-33	(13)**
Interval to uterine involution (weeks)	6.3±.4[b]	4.1±.4[c]	3-9	(16)
Services/conception	2.9±.8[d]	2.9±.7[d]	1-7	(15)
Interval to first service (days)	129.1±15.5[e]	76.4±14.5[f]	47-243	(15)
Days open	201.0±32.2[g]	109.3±30.1[g]	47-385	(15)

*Row means with different superscripts are significantly different ($P<.05$).

**Numbers in parenthesis indicate the number of observation for 16 animals.

Table 6. Partial correlation coefficients for serum β-carotene concentration during the calving period and the reproductive parameters

Reproductive parameter	β-carotene concentration	Statistical significance
Interval to ovulation	-0.15*	NS
Interval to uterine involution	-0.01	NS
Services/conception	-0.24	NS
Interval to first service	0.20	NS
Days open	-0.12	NS

*Correlation coefficient based on 12 observations and adjusted for the effect of season of calving.

NS = non-significant (P>.05).

Presented in Table 7 are the partial correlation coefficients between serum vitamin A concentration during the calving period and the reproductive parameters. The correlations were negative and non-significant ($P>.05$) for IUI and SC, and positive and non-significant for IO, IFS and DO.

Discussion

The values obtained for the concentration of β-carotene in the forages fed in this trial fell within the range of values for such forages published by the National Academy of Sciences (1971). The literature relating to the levels of carotene in forages and the losses that occur during conservation of fresh forages into hay, haylage and silage and also during storage of conserved forages, has been extensively reviewed (Friesecke 1964; Aitken and Hankin 1970; Thompson 1975). The considerable variation in β-carotene levels found in hays fed in this trial is supported by the wide variation in β-carotene concentration in hay reported in the literature. The variation in the level of carotene in hay may be due primarily to large losses (50-90%) that occur during the process of hay-making depending upon the methods used, the weather and the time required to cure (Friesecke 1964). Therefore, although hay is usually a good source of β-carotene it may also be an unreliable source. The variation in the β-carotene content of the hays used in the present trial probably reflect both the initial carotene content of the plant materials and the conditions under which they were made since they were obtained from different sources.

The high levels of β-carotene in the fababean and rye silages and the small losses that occurred with storage indicate that these silages can be a good source of β-carotene. Silage tends to reflect the initial

Table 7. Partial correlation coefficients for serum vitamin A concentration during the calving period and the reproduction parameters

Reproductive parameter	Vitamin A concentration	Statistical significance
Interval to ovulation	0.19*	NS
Interval to uterine involution	-0.09	NS
Services/conception	-0.21	NS
Interval to first service	0.36	NS
Days open	0.09	NS

*Correlation coefficient based on 12 observations and adjusted for the effect of season of calving.

NS = non-significant (P>.05).

carotene content of the plant material much better than hay does since losses in well-preserved silage appear to be much smaller (Friesecke 1978). Lotthammer (1979b)has indicated that many green fodder silages may have sufficient carotene concentration and thus require no supplementation. In the present trial, corn silage had a low initial carotene content which deteriorated rapidly to undetectable levels of the third month of storage. This observation is supported by indications in the literature that corn silage is generally a very poor source of carotene initially, and that rapid deterioration during storage makes it even poorer (Friesecke 1978). One possible reason for the very low carotene content of the corn silage fed in the present trial might be the exposure of the corn plants to frost prior to ensiling. Phipps (1978) has indicated that carotene content of corn forage decreased markedly when the crop was exposed to frost.

The fact that the highest concentrations of carotene were found in pasture is supported by the general opinion in the literature that good pasture is the best and most reliable source of β-carotene for cattle. The extremely large β-carotene value for pasture in July compared to the other months probably reflects differences in the condition of the pasture being grazed at the time of sampling. Unlike the other months, the July pasture sample was taken on a day the cows had been transferred onto a new fresh paddock. The substantially lower levels of β-carotene in grass haylage compared to the pasture from which it was derived may be due largely to losses during the process of making the haylage.

The cows obtained their supply of β-carotene almost exclusively from the forages fed since concentrate rations contain practically no carotene and no supplemental β-carotene was fed. This explains why the monthly

variation in mean serum concentration of β-carotene and to a lesser extent vitamin A appeared to parallel differences in β-carotene concentration of the forages fed at different times during the trial. The combination of hay and silages fed from October to May succeeded in maintaining mean serum β-carotene concentration at levels (300 µg/100 ml or higher) considered to be sufficient by Friesecke (1978). During this period a few of the cows had serum β-carotene levels within the suspect to critical range but these were not sustained for a long time.

The high supply of β-carotene from pasture in June and July was responsible for the significant increase in serum β-carotene during these two months. However, the fact that differences in mean serum β-carotene concentration between the two months was not significant despite an apparent doubling of carotene supply from pasture in July, might be evidence in support of the earlier suggestion that the pasture carotene value for July reflected the condition of the pasture at the time of sampling. On the other hand, mean serum β-carotene levels were significantly lower in August and September compared to June although the pasture carotene levels in the three months were comparable and high-carotene grass haylage and alfalfa pellets were also fed in August and September. This appears to suggest a possible impairment of carotene absorption at high β-carotene intakes, as indicated previously by Bauernfeind et al. (1981). Furthermore, the significantly lower serum vitamin A levels in July compared to June despite slightly higher β-carotene levels in July appear to confirm previous reports that the efficiency of β-carotene conversion to vitamin A is markedly decreased at high carotene intakes (Myers et al. 1959; Grifo et al. 1960).

The group 2 cows that had a significantly shorter IUI and IFS and a shorter DO that approached significance, calved during a period when mean serum β-carotene (but not vitamin A) level was significantly higher. However the low partial correlations between serum β-carotene and vitamin A concentration during the calving period and all the reproductive parameters suggest a lack of any significant independent effects of β-carotene and vitamin A on the reproductive performance of the cows. Reproductive performance may be influenced by several factors, with confounding effects, which make it difficult to determine the relative importance of each factor. Since serum β-carotene and vitamin A levels throughout the trial were generally adequate, these two factors were probably not limiting to the extent as to adversely affect reproductive performance. Seasonal effects unrelated to nutrition may largely account for the differences between the two groups of cows for IUI, IFS and DO. As suggested by Peters and Riley (1982b) photoperiod is one such seasonal factor which may play a role in the seasonality of reproductive activity of cows in temperate regions. In the present trial however, lights in the barn were on a time clock such that cows received 16 hours of light throughout the year.

Summary and Conclusions

Feeding a combination of hay and silages (some high in carotene) ensured that adequate levels of serum β-carotene were maintained in cows during fall, winter and spring. There was a sharp rise in serum β-carotene levels during the summer months when the cows were on pasture. However, there appeared to be an impairment in the absorption of β-carotene and

the efficiency of carotene conversion to vitamin A in the cows at high carotene intakes.

The improvement in IUI, IFS and DO for cows that calved from July to October when serum β-carotene levels were higher may be due largely to a seasonal effect independent of serum β-carotene levels. In the present trial, β-carotene and vitamin A did not appear to be the limiting factors in the reproductive performance of the cows.

Hay was a good but variable source of β-carotene. The fababean and rye silages used in this trial were relatively high in β-carotene with little or none in the corn silage.

EXPERIMENT II

Reproductive Performance of Holstein Cows on Low or High Beta-carotene Diets During Early Lactation.

Calving interval is one of many factors that influence the productivity of the dairy cow. The optimum calving interval required for maximum milk yield has been suggested to be 12 months (Louca and Legates 1968). To achieve such a calving interval, the cow must be pregnant again no later than 80 to 85 days postpartum. Therefore efficient reproductive management during the early postpartum period is essential.

In the dairy cow, the critical early postpartum period coincides with a period of increasing milk production. Some workers have found an association between high milk yield and reduced fertility (Carman 1955; Marion and Gier 1968; Spalding et al. 1975), while others suggest that there is no such relationship (Smith and Legates 1962; Bulman and Lamming 1978). The adverse effects of high milk yield on postpartum reproduction may be due, in part, to inadequate nutritional status during a period of high nutritional demand. High yielding cows in early lactation are often in a state of negative energy balance (Coppock et al. 1974). Low energy intake in pre- and postpartum cows is associated with an extended period of anestrus (Wiltbank et al. 1964; Dunn et al. 1969; Oxenreider and Wagner 1971). Furthermore, positive relationships between nutritional status, body weight and body condition score and fertility in both dairy and beef cows have been reported (Youdan and King 1977; Kilkenny 1979; Somerville et al. 1979). Although energy deficiency is probably the most important

nutritive factor that affects fertility the deficiency of other specific nutrients, particularly the vitamins and minerals, have been shown to adversely affect fertility in the cow (Zintzen 1974).

Lotthammer et al. (1978) were the first to report that β-carotene deficiency during the early postpartum period in the dairy cow had adverse effects on reproductive performance. There had been earlier reports of adverse effects of β-carotene deficiency on the fertility of heifers (Lotthammer and Ahlswede 1977; Lotthammer et al. 1976; Schams et al. 1977). In the study by Lotthammer et al. (1978), the reproductive performance of Black Pied cows fed low-carotene diets with or without β-carotene supplementation (according to milk yield) were compared. The trial extended from 7 weeks prepartum to week 6 and 9 of the next gestation for the cows with and without β-carotene supplementation, respectively. The observed adverse effects of β-carotene deficiency on postpartum reproduction included delayed resumption of ovarian function and uterine involution, weak signs of estrus, delayed ovulation and high incidences of early embryonic death and abortion. Another aspect of this study indicated that during lactation, the β-carotene deficient cows had lower levels of milk fat, especially at peak lactation (Lotthammer 1979a).

Jackson (1981), in a rather limited study, also observed that β-carotene deficiency had adverse effects on the fertility of Holstein cows. In addition, Chew et al. (1982) recently reported that low levels of vitamin A and β-carotene in plasma were associated with the severity of mastitis in cows. Contrarily, in other studies with a variety of breeds, β-carotene supplementation had marginal or no improvement on post-

partum reproductive performance (Bonsembiante et al. 1980; Bremel et al. 1982; Wang et al. 1982; Bindas et al. 1983; Akordor et al. 1984). Unlike the study by Lotthammer et al. (1978), β-carotene supplementation during early lactation in virtually all of the other studies were at a fixed and lower level. Also, the trial periods did not cover the early postpartum period in some of these studies.

In many herds, the high energy and protein requirements of the cow during early lactation are met by feeding high levels of concentrate and corn silage which are low in β-carotene. Therefore the effects of β-carotene deficiency on reproductive performance during this critical postpartum period needs to be clearly established. The present study investigated the effects of feeding low-carotene diets, in comparison to high β-carotene supplementation (based on milk yield), from prepartum through the early postpartum period on various reproductive, productive and health parameters of Holstein cows.

Materials and Methods

Animals

Thirty-seven Holstein-Friesian cows from the University of Manitoba dairy herd were studied from 4 weeks prepartum to 12 weeks postpartum. They were housed in a stanchion barn and kept indoors throughout the experimental period.

Experimental Design and Treatments

A 2x2x3 factorial arrangement in a completely randomized design was used, resulting in 12 treatment combinations with 3 cows per treatment

combination except one which had 4 cows. The cows were assigned such that the treatment combinations were reasonably balanced for cow age and milk yield potential (based on their preceding lactation). The 3 factors were:

1) Beta-carotene treatment at 2 levels - the cows were fed either a control supplement (B1) that supplied only vitamin A or a β-carotene supplement (B2) that contained only β-carotene. The amounts of vitamin A or β-carotene supplied to each cow varied with milk yield according to the protocol shown in Table 8; and provided equal vitamin A based on 400 IU vitamin A/mg β-carotene.

2) A deworming (Anthelmintic) treatment at 2 levels - the cows received either no treatment (T1) or one injection (0.044 ml/kg body weight) of the drug Tramisol (Levamisole) at parturition (T2).

3) Three experimental rations were employed: a control ration (R1) containing 9.7% corn, a ration containing 7.9% extruded canola seed in place of corn (R2) or a ration containing 7.9% whole seed canola in place of corn (R3). The inclusion of canola seed in rations R2 and R3 was designed to increase the fat content of those rations, with no change in protein level.

During the 4 weeks before parturition, each cow received, ad libitum, a basal ration of weathered low-carotene hay (<10 mg β-carotene/kg DM) plus about 1 kg/day of concentrate containing either the B1 or B2 supplement. The three experimental rations were fed as complete feeds containing 62.8% concentrate, 17.6% weathered low-carotene hay and 19.6% fababean silage (37.2 mg β-carotene/kg DM, initially) during the lactation period. The B1 or B2 supplements were given as a top-dressing during this period. The ingredient and nutrient composition of the experimental rations are shown in Table 9. The Net Energy for Lactation (NE_L) level was

Table 8. Daily supply of β-carotene and vitamin A per cow

Milk production (L/day)	B1 Vitamin A (I.U.)	B2 β-carotene* (mg)
Dry cows	80,000	200
Lactating cows:		
<20	160,000	400
20 to 29	240,000	600
30 to 39	320,000	800
>40	400,000	1,000

Vitamins D and E - Similar for both treatments

Vitamin D - 6,000 I.U.

Vitamin E - 2 I.U. for every 1,000 I.U. of vitamin A equivalent

*Source = Rovimix β-carotene 10% (Roche).

Table 9. Ingredient and nutrient composition of the experimental rations

Ingredients	Ingredient or nutrient composition		
	R1	R2	R3
	(g/kg)		
Barley	360.5	419.5	419.5
Corn	97.0	-	-
Canola meal	125.6	85.3	85.3
Canola seed (extruded)	-	79.0	-
Canola seed (whole)	-	-	79.0
Molasses	15.7	15.0	15.0
Urea (feed grade)	5.0	5.0	5.0
Trace mineral premix*	4.5	4.5	4.5
Salt (cobalt-iodized)	3.0	3.0	3.0
Limestone	9.2	9.2	9.2
Phosphorus supplement (biophos)	7.5	7.5	7.5
Hay	176	176	176
Fababean silage	196	196	196
Nutrients (proximate analysis)			
Crude protein (%)	17.57	17.56	17.56
NE_L (Mcal/kg)	1.61	1.76	1.76
Ca (%)	0.87	0.83	0.83
P (%)	0.68	0.67	0.67

*Trace mineral premix supplied per kg diet: 0.43 g $CuSO_4$, 0.28 g ZnO, 0.28 g MnO_2-H_2O (Prince) and 10 g MgO (Maggox).

slightly higher for R2 and R3 but all other nutrients were virtually similar for the three rations.

The β-carotene treatment formed the basis of this study. However, the other treatments had to be superimposed on this treatment to accommodate other research with the limited number of lactating cows that were available.

Serum Beta-carotene and Vitamin A

Blood samples for β-carotene and vitamin A analysis were taken, using a vacutainer, from the coccygeal vein/artery from all cows at weekly intervals. After overnight storage in the dark at $4^{o}C$, the blood samples were centrifuged and the sera harvested were stored at $-20^{o}C$ until analysed. During the lactation period, the β-carotene and vitamin A analyses were performed on bi-weekly serum pools. The analytical procedures for β-carotene and vitamin A determinations were described in the general materials and methods section.

Milk Progesterone, Fat and Somatic Cell Counts (SCC)

Composite milk samples for progesterone analysis were taken daily from all cows from parturition to day 60 postpartum and on day 21 post-service. All samples were preserved with potassium dichromate and stored at $4^{o}C$ until analysed, which was usually within one week of sampling. The assay procedure for the determination of milk progesterone was described earlier in the general materials and methods section. The intra- and inter-assay coefficients of variation for a pooled milk sample were 5.1% and 13.4% respectively (n = 22 assays). The postpartum milk pro-

gesterone profile generated for each cow was used to estimate the interval to first ovulation (IO). Ovulation was presumed to have occurred when milk progesterone concentration increased progressively from levels lower than 1 ng/ml to levels greater than 5 ng/ml within a period of 10 days. The milk progesterone data for the day 21 post-service samples were used for pregnancy diagnosis.

For milk fat (MF) analysis, weekly composite samples from the morning and evening milkings were taken for each cow. The milk fat analyses were done by infrared analysis on Milko-Scan 203 (A.S.N. Foss Electric, Denmark) at the Manitoba Agriculture Dairy Laboratory.

Composite aseptic milk samples from all 4 quarters of the udder were taken at parturition and every 4 weeks thereafter during the morning and evening milkings to evaluate milk somatic cell counts as an index of sub-clinical mastitis. The morning and evening milk samples for each cow were composited and SCC analysis was done with an electronic cell counter (Somaton; Coulter Electronics).

Ovarian and Uterine Examination, Breeding and Pregnancy Diagnosis

Weekly palpations were performed on each cow to evaluate uterine involution and to check the general condition of the reproductive tract and ovaries. First postpartum ovulation was usually confirmed by these palpations.

Artificial insemination of the cows began around day 60 postpartum. Two inseminators were used for AI service, but the number of first services by each insemination was reasonably balanced between control and carotene-supplemented cows: inseminator 1 performed 8 and 5 inseminations; inseminator 2 did 9 and 10 inseminations, respectively.

Other Data

Data were compiled for weekly milk yield and fat-corrected milk. Data were also collected for such health parameters as cystic ovaries, metritis, retained placenta, uterine infection, loss of fetus due to abortion or early embryonic death and clinical mastitis. Criteria for the diagnosis of health parameters were defined as follows: Cystic ovaries - presence of enlarged follicular or luteal structures on the ovary that persisted beyond the length of a normal estrous cycle; metritis - severe inflammation of the uterus as detected by palpation and accompanied by a pusy discharge; retained placenta - placenta membranes that remained attached to the uterine wall beyond 48 hours post calving; clinical mastitis - severe inflammation of at least one quarter of the udder due to infection that required antibiotic treatment.

Statistics

The intra- and inter-assay coefficients of variation for milk progesterone were calculated according to the method of Rodbard (1971).

The experimental data were analysed using the GLM and Analysis of Variance (ANOVA) procedures outlined in SAS-82. Split-plot analyses were employed for the data on serum β-carotene and vitamin A; SCC; and the milk production parameters. Reproductive parameters such as intervals to ovulation and uterine involution, services/conception and days open were analysed by factorial analysis. Mean differences for all dependent variables were tested by the SNK test (Snedecor and Cochran 1967). Differences in serum β-carotene concentration over the trial period between the low-carotene and β-carotene supplemented cows were tested by T-test (Snedecor and Cochran 1967). Chi-square analysis (Snedecor and Cochran 1967) was used for the data on conception rate and the incidence of disorders.

Results

Serum Beta-carotene and Vitamin A

Serum concentration of β-carotene was significantly ($P<.01$) elevated by β-carotene supplementation. As shown in Figure 3, the mean serum concentration of β-carotene was high initially for both the low-carotene (B1; 549.7±50.9 µg/100 ml) and the carotene-supplemented (B2; 502.7±47.2 µg/100 ml) cows. Serum β-carotene concentration declined sharply during the first 4 weeks of the trial reaching levels of 190.2±22.6 µg/100 ml for B1 and 280.8±22.4 µg/100 ml for B2 cows by one week prior to calving (week -1). Serum β-carotene concentration in the B1 (control) cows remained low, between 100 and 200 µg/100 ml, for the rest of the experimental period. In contrast, serum β-carotene concentration in the B2 (supplemented) cows increased steadily from week -1 to a mean peak level of 750.2±60.2 µg/100 ml at the end of the trial. The differences in the concentration of serum β-carotene between the two groups of cows became significant ($P<.01$) by week -1 and remained significant throughout the rest of the trial period.

The ration and tramisol treatments did not have significant ($P>.05$) independent effects on serum β-carotene concentration. However, there was a significant ($P<.05$) β-carotene x ration interaction which is illustrated in Figure 4. In the B1 cows, feeding the whole seed canola ration (R3) in comparison to the control ration (R1) appeared to increase serum carotene concentration, while the extruded canola ration (R2) had no effect. On the other hand, in the B2 cows R2 was more effective than R3 in raising serum β-carotene concentration over the levels obtained on R1.

Beta-carotene supplementation did not affect ($P>.05$) serum vitamin A levels and neither did the ration nor tramisol treatments. For the B1 and B2 cows, mean serum vitamin A did not change appreciably from the

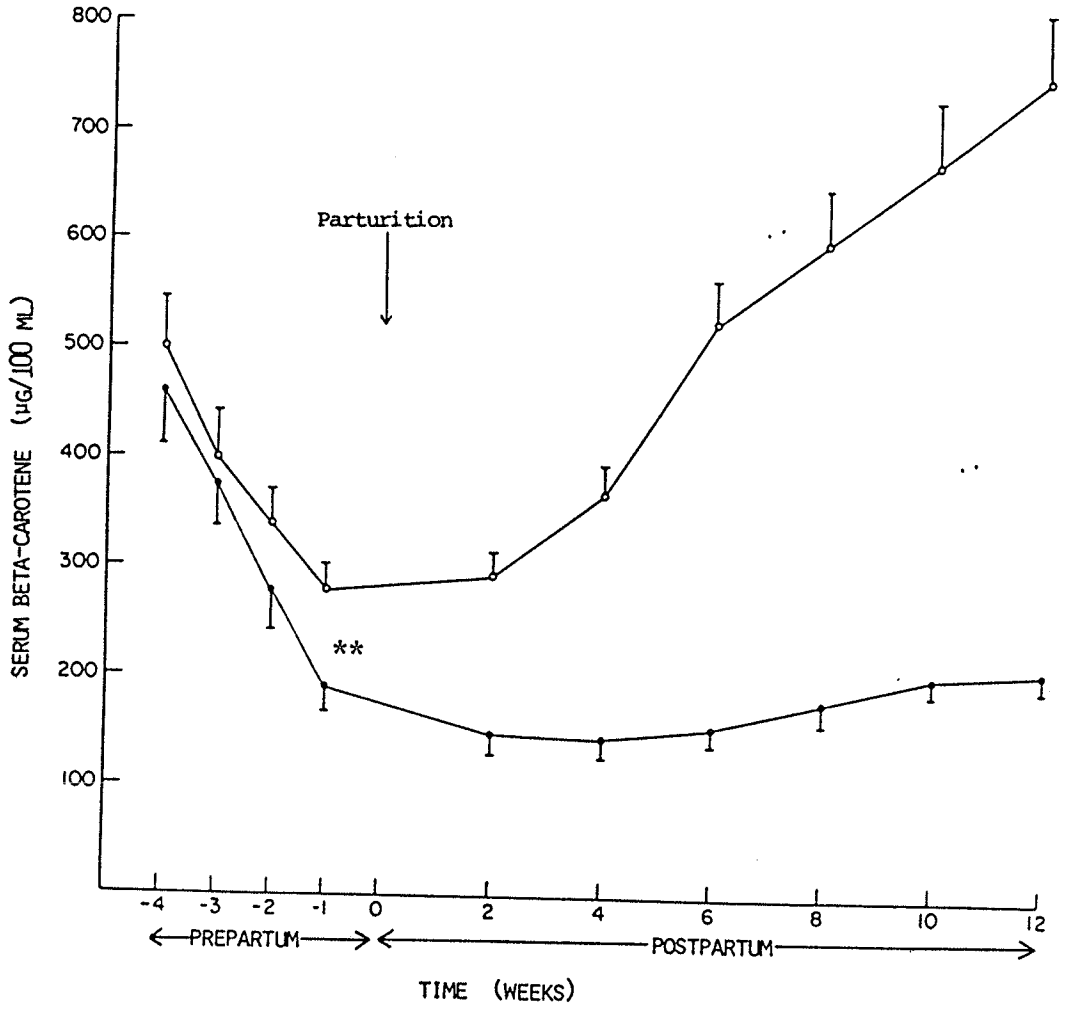

Figure 3. Mean (±SE) serum β-carotene concentration for control (B1; ●——●——●) and carotene supplemented (B2; o——o——o) cows.

**Differences between B1 and B2 were highly significant ($P<.01$) from week -1 onwards.

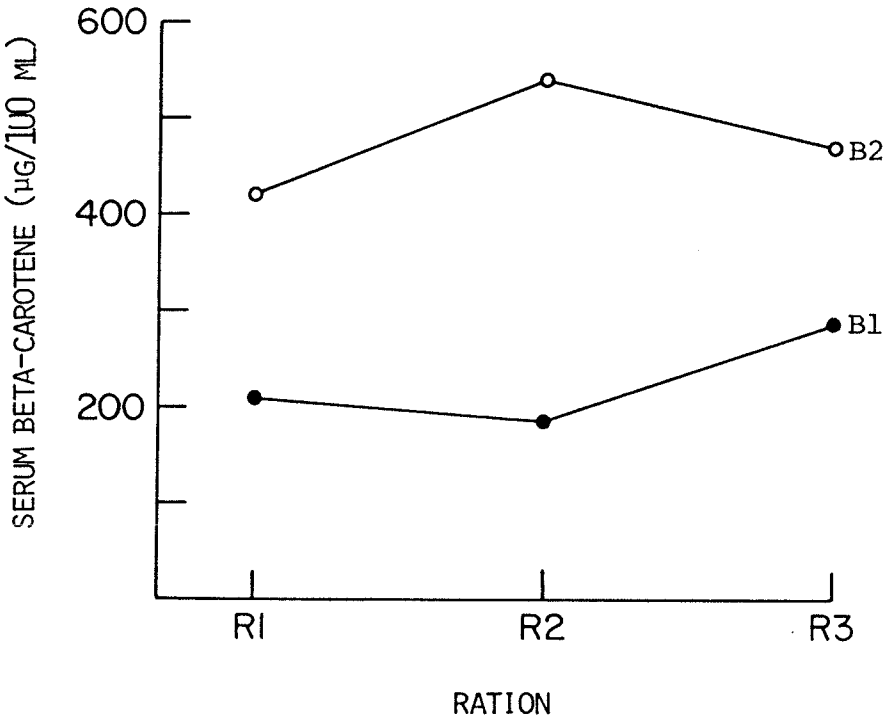

Figure 4. Two-way interaction between β-carotene (B1 = control; B2 = carotene supplemented cows) and ration (R1 = control; R2 = extruded canola; R3 = whole seed canola) treatments for serum β-carotene concentration ($P<.05$).

beginning of the trial to week 2 postpartum (Figure 5). After week 2 postpartum, serum vitamin A levels in both groups of cows increased slowly but steadily throughout the rest of the trial period.

Reproductive Performance, Reproductive Disorders and Mastitis

Interval to first ovulation postpartum (IO) ranged from 7 to 40 days (Table 10). The three missing observations for IO were for three cows (1 on control; 2 on β-carotene supplementation) that failed to ovulate during the 60-day postpartum period when milk samples were taken for progesterone analysis. The control cow had milk progesterone values that fluctuated between 1.9 and 6.5 ng/ml with no cyclical pattern from day 15 to 60 postpartum and was diagnosed by palpation to have had a follicular cyst. The other two cows had low milk progesterone values (<1 ng/ml) throughout the 60-day postpartum period. Interval to uterine involution (IUI), services/conception (SC) and days open (DO) ranged from 2 to 9 weeks, 1 to 5 and 60 to 217 days respectively. Overall conception rate to first service (CR) was rather low (31.3%) but was improved considerably after 2 (68.8%) and 3 (87.5%) services. The missing observations for SC, CR and DO represent five cows (2 on control; 3 on β-carotene supplementation) that were culled for failing to conceive after five or more inseminations.

The mean IO was about 4 days longer in the low-carotene cows compared to those supplemented with β-carotene but this difference was not significant (P>.05) (Table 10). Mean IUI was appreciably longer (P = .08) in the low-carotene cows (4.9±.3 weeks) compared to the β-carotene supplemented cows (4.1±.4 weeks). The low-carotene cows also had a

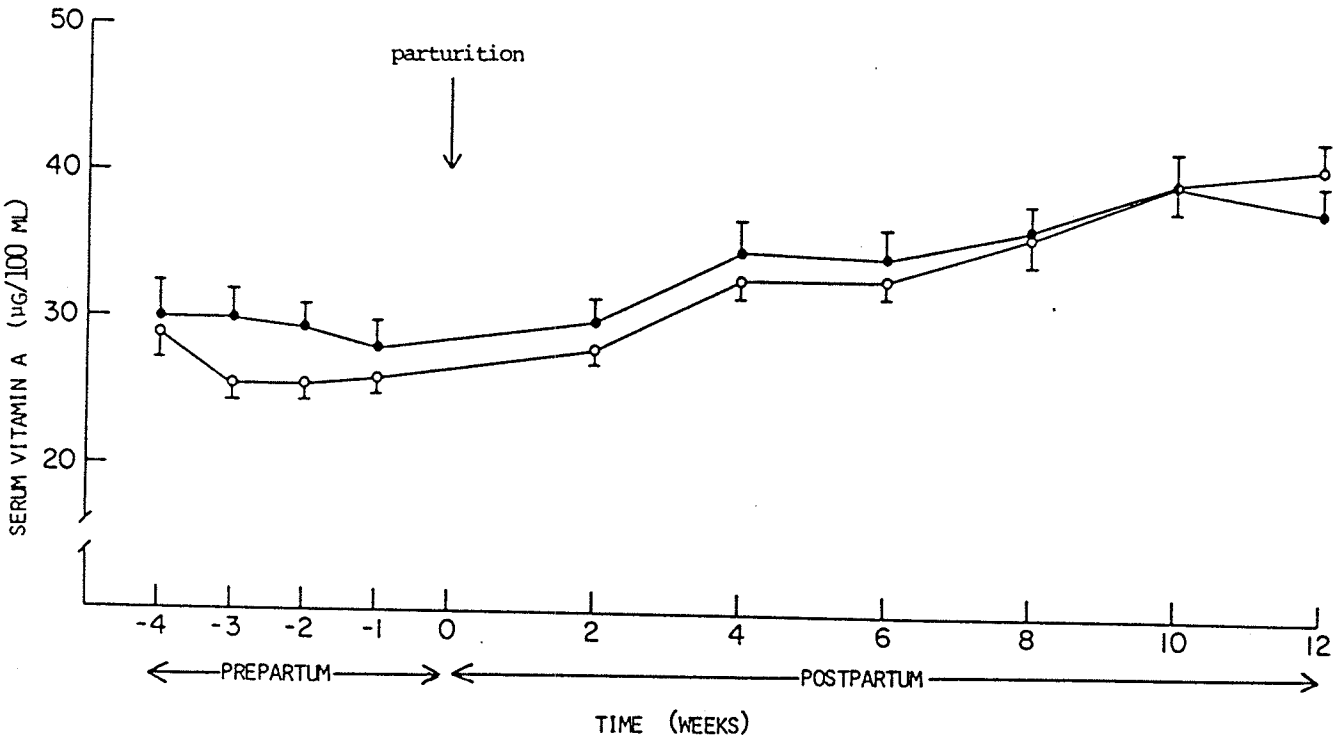

Figure 5. Mean (±SE) serum vitamin A concentration for control (●——●——●) on carotene supplemented (o——o——o) cows.

Table 10. Mean of reproductive parameters for control and β-carotene supplemented cows

Reproductive parameter	Treatment		Overall mean or range
	Control	β-carotene	
	Mean ±SE		
Interval to ovulation (days)	23.3±2.0 (18)*	19.2±2.2 (16)	7-40 (34)
Interval to uterine involution (weeks)	4.95±0.3 (19)	4.1±0.4 (18)	2-9 (37)
Services/conception	2.4±.3 (17)	1.9±.3 (15)	1-5 (32)
Conception rate to first service (%)	17.6 (3/17)	46.7 (7/15)	31.3 (32)
Days open	119.9±9.1 (17)	108.5±9.6 (15)	60-217 (32)

*Numbers in parenthesis indicate the number of animals.

substantially lower conception rate to first insemination (17.6%) than the β-carotene supplemented cows (46.7%) (X^2 = 3.1; df = 1; P = .08). Beta-carotene supplementation did not significantly improve SC and DO, although the 1.9 SC for the carotene-supplemented cows represented a 20.8% improvement over the 2.4 SC for the low-carotene cows.

None of the ration treatments influenced any of the reproductive parameters (Table 11). With respect to the effect of tramisol treatment on reproductive performance, IUI was somewhat longer (P = .08) in cows not treated with tramisol (5.0±.4 weeks) compared to those treated with tramisol (4.1±.3 weeks) (Table 12). Tramisol treatment had no significant effect on the other 4 reproductive parameters.

The incidence of cystic ovaries and clinical mastitis tended to be higher in the low-carotene cows (31.6% in each case) than in the β-carotene supplemented cows (11.1% in each case) (Table 13). The incidences of metritis (3 cases) and loss of fetus (2 by abortion and 1 early embryonic death) occurred only in cows not supplemented with β-carotene. The cow presumed to have had the case of early embryonic death returned to cyclicity after she had been confirmed pregnant by both milk progesterone diagnosis and palpation. The incidence of retained placenta and uterine infection, as evidenced by a dirty pus discharge, were comparable in the two groups of cows. Chi-square analysis showed no significant differences between the low-carotene and β-carotene supplemented cows for all the above disorders.

With the exception of fetal loss, the incidence of the other disorders for cows on the three ration treatments (Table 14) did not differ significantly (P>.05). All three cases of fetal loss occurred in cows on the

Table 11. Mean of reproductive parameters for cows on control, extruded canola and whole seed canola rations

Reproductive parameter	Ration treatments		
	Control	Extruded canola	Whole seed canola
	Mean ±SE		
Interval to ovulation (days)	19.9±2.5 (12)*	24.2±2.6 (11)	20.1±2.6 (11)
Interval to uterine involution (weeks)	4.7±0.4 (13)	4.4±0.4 (12)	4.5±0.4 (12)
Services/conception	2.1±0.3 (11)	2.5±0.4 (10)	1.9±0.3 (11)
Conception rate to first service (%)	18.2 (2/11)	30.0 (3/10)	36.4 (4/11)
Days open	110.2±11.3(11)	130.1±11.8(10)	104.8±11.3(11)

*Numbers in parenthesis indicate number of animals.

Table 12. Mean of reproductive parameters for control and tramisol treated cows

Reproductive parameter	Tramisol treatments	
	Control	.044 ml Tramisol/kg BW
	Mean ±SE	
Interval to ovulation (days)	21.6±2.0 (18)*	21.1±2.2 (16)
Interval to uterine involution (weeks)	5.0±0.4 (18)	4.1±0.3 (19)
Services/conception	1.9±0.3 (14)	2.3±0.3 (18)
Conception rate to first service (%)	42.9 (6/14)	22.2 (4/18)
Days open	103.1±10.0(14)	123.5±8.8 (18)

*Numbers in parenthesis indicate number of animals.

Table 13. Effect of β-carotene treatment on the incidence of reproductive disorders and clinical mastitis

	β-carotene treatments	
Disorder	Control	β-carotene supplementation
Cystic ovary (%)	31.6 (6/19)*	11.1 (2/18)
Metritis (%)	15.8 (3/19)	0 (0/18)
Loss of fetus (%)	15.8 (3/19)	0 (0/18)
Uterine infection (pus discharge) (%)	21.1 (4/19)	16.7 (3/18)
Retained placenta (%)	5.3 (1/19)	11.1 (2/18)
Clinical mastitis (%)	31.6 (6/19)	11.1 (2/18)

*Numbers in parenthesis indicate number of animals.

Table 14. Effect of ration treatment on the incidence of reproductive disorders and clinical mastitis

	Ration treatments		
Disorder	Control	Extruded canola	Whole seed canola
Cystic ovary (%)	30.8 (4/13)*	33.3 (4/12)	0 (0/12)
Metritis (%)	7.7 (1/13)	16.7 (2/12)	0 (0/12)
Loss of fetus (%)	23.1 (3/13)[a]	0 (0/12)[b]	0 (0/12)[b]
Uterine infection (%)	30.8 (4/13)	16.7 (2/12)	8.3 (1/12)
Retained placenta (%)	15.3 (2/13)	8.3 (1/12)	0 (0/12)
Clinical mastitis (%)	23.1 (3/13)	8.3 (1/12)	33.3 (4/12)

*Numbers in parenthesis indicate number of animals.

[a,b]Row values with different superscripts are significantly different using chi-square ($P<.05$).

control ration which was significantly ($P<.05$) higher than in the other two ration treatments.

For the tramisol treated (T2) and non-treated (T1) cows, there were no significant differences for the incidence of any of the disorders, although mastitis tended to be higher for T1 than for T2 cows (Table 15).

Milk SCC as an Index of Subclinical Mastitis

The milk SCC varied widely among animals and also among sampling periods within animals. The SCC values obtained ranged from $3x10^4$ to $14x10^6$ cells/ml. In general, SCC was significantly ($P<.05$) higher in the samples taken at parturition compared to the samples at other periods. There was a tendency for milk SCC to be lower in the β-carotene supplemented and tramisol injected cows compared to their respective controls (Table 16). However, the observed differences among treatment groups for all three factors were not significant ($P>.05$).

Milk Production

The milk production data forms a major part of another thesis, therefore it is not presented in extensive detail here. Milk yield (MY), milk fat (MF) and fat-corrected milk (FCM) tended to be higher for the carotene-supplemented cows compared to the controls although the differences were not significant ($P>.05$) (Table 17).

As shown in Table 18, cows on the extruded canola ration (R2) had a significantly ($P<.05$) higher MY than those on the control ration (R1). Milk yield of cows on the whole seed canola ration (R3) did not differ significantly from those on either R1 or R2. MF and FCM for the cows on R2 and R3 tended to be higher but not significantly different from those on R1.

Table 15. Effect of Tramisol treatment on the incidence of reproductive disorders and clinical mastitis

	Tramisol treatments	
Disorder	Control	0.044 ml Tramisol/kg BW
Cystic ovary (%)	22.2 (4/18)*	21.1 (4/19)
Metritis (%)	11.1 (2/18)	5.3 (1/19)
Loss of fetus (%)	11.1 (2/18)	5.3 (1/19)
Uterine infection	22.2 (4/18)	15.8 (3/19)
Retained placenta	0 (0/18)	15.8 (3/19)
Clinical mastitis	33.3 (6/18)	10.5 (2/19)

*Numbers in parenthesis indicate number of animals.

Table 16. Effect of the three factors on milk somatic cell counts (cells/ml)

Factor	*Mean ±SE somatic cell count (x1000)
Beta-carotene:	
Control group:	641.8±147.8
β-carotene supplemented group:	366.1±151.8
Ration:	
Control:	392.0±178.6
Extruded canola:	386.2±185.9
Whole seed canola:	754.4±185.9
Tramisol:	
Control:	662.1±151.8
Tramisol injection:	361.4±147.8

*Mean of milk SCC for 4 sampling periods.

Table 17. Effect of β-carotene treatment on milk production

Production parameter	β-carotene treatments	
	Control	β-carotene supplementation
	(Mean ±SE)	
Milk yield (kg/day)	27.96±1.7	29.53±1.8
Milk fat (kg/day)	0.84±.05	0.89±.05
FCM (kg/day)	23.74±1.3	25.19±1.4

FCM = 0.4 M + 15 F

where M = amount of milk (kg)
F = amount of butterfat (kg).

Table 18. Effect of ration treatment on milk production

Production parameter	Ration treatments		
	Control	Extruded canola	Whole seed canola
	(Mean ±SE)		
Milk yield (kg/day)	25.22±2.1[a*]	33.03±2.2[b]	28.2±2.2[ab]
Milk fat (kg/day)	0.79±.06	0.89±.06	0.92±.06
FCM (kg/day)	21.96±1.6	26.53±1.7	25.06±1.7

*Row means with different superscripts are significantly different ($P<.05$).

FCM = 0.4 M + 15 F

where M = amoung of milk (kg)
F = amount of butterfat (kg).

With respect to the tramisol treatment, all three milk parameters tended to be higher, though not significantly ($P>.05$), for the tramisol treated cows compared to their control counterparts (Table 19).

Although β-carotene supplementation had no significant independent effects on the milk production parameters, there was a significant ($P<.05$) β-carotene x tramisol x week interaction for MY and highly significant ($P<.01$) β-carotene x ration x week interactions for MF and FCM. The β-carotene x tramisol x week interaction for MY, illustrated in Figure 6, shows that for the cows not treated with tramisol, MY was equal or marginally higher in B1 than in B2 cows for all weeks except for weeks 1, 3 and 4. On the other hand, MY in all weeks were appreciably higher in B2 than B1 cows treated with tramisol. Therefore, the data suggests synergistic effect between β-carotene supplementation and tramisol treatment for higher milk yield.

Figure 7 illustrates the rather complex β-carotene x ration x week interaction for MF. In general, there were wide weekly fluctuations in all treatment groups for MF. For cows on ration R1, MF was higher in B2 than in B1 cows for all weeks except week 11. The differences in MF between B2 and B1 cows on ration R1 tended to be larger at the beginning of lactation, becoming smaller as lactation progressed. For the cows on ration R2, MF production decreased from 1.07±.1 kg/day in week 1 to 0.74±.07 kg/day in week 5 of lactation after which the mean MF levels fluctuated between 0.84±.1 and 0.73±.07 kg/day. In contrast, MF in B2 cows on ration R2 increased from 0.89±.04 kg/day in week 1 to a peak of 1.01±.1 kg/day in week 4, after which MF levels were higher in B2 than in B1 cows for the remaining weeks of the trial. For cows on R3, there

Table 19. Effect of tramisol treatment on milk production

Production parameter	Treatment	
	Control	.044 ml tramisol/kg BW
	(Mean ±SE)	
Milk yield (kg/day)	26.50±1.7	31.07±1.8
Milk fat (kg/day)	0.84±.05	0.89±.05
FCM (kg/day)	23.21±1.3	25.76±1.4

FCM = 0.4 M + 15 F

where M = amount of milk (kg)
F = amount of butterfat (kg).

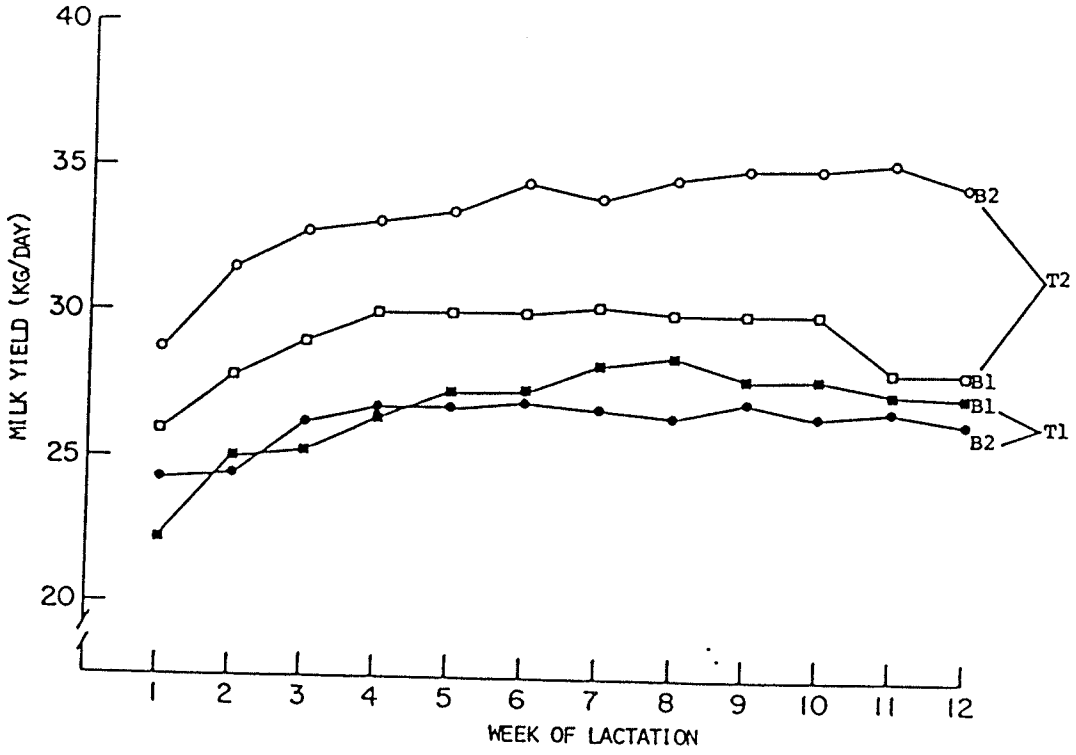

Figure 6. Three-way interaction between β-carotene treatment (B1 = control; B2 = carotene-supplemented cows); tramisol treatment (T1 = control; T2 = .044 ml/kg BW tramisol) and week for milk yield ($P<.05$).

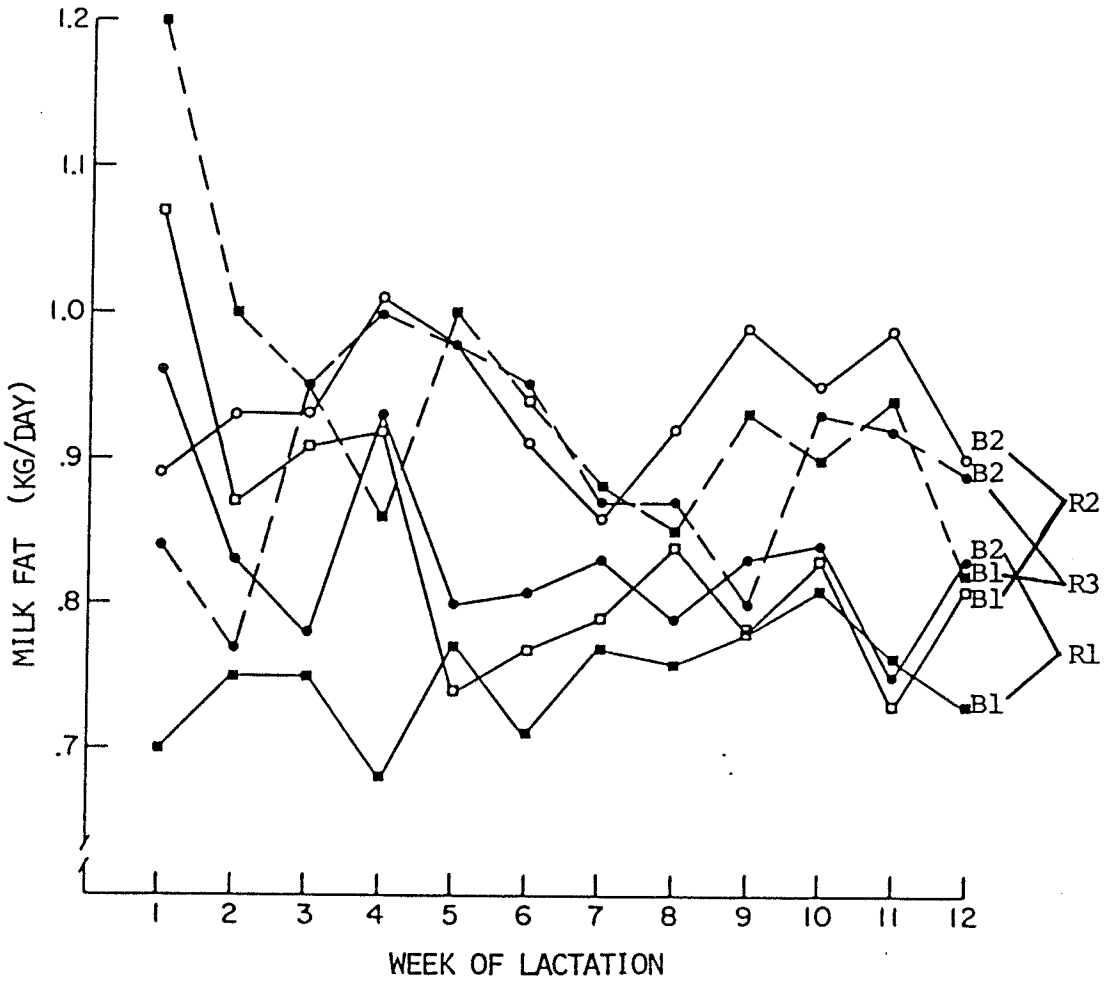

Figure 7. Three-way interaction between β-carotene treatment (B1 = control; B2 = carotene supplemented cows); ration treatment (R1 = control; R2 = extruded canola; R3 = whole seed canola); and week for milk fat ($P<.01$).

was a decrease in MF for B1 from 1.2±.1 kg/day in week 1 to 0.86±.1 kg/day in week 4, as opposed to an increase in MF for B2 from 0.84±.1 kg/day to 1.01±.16 kg/day during the same period. From week 5 to week 12, MF levels in B1 and B2 cows on ration R3 were generally comparable except in week 9 when MF was lower in B2 and in week 12 when MF was lower in B1.

The β-carotene x ration x week interaction for FCM is illustrated in Figure 8. For cows on ration R1, FCM was consistently higher in B2 than in B1 cows during the 12 week lactation period. The higher FCM in B2 cows compared to B1 cows appeared to be even greater on ration R2, especially from week 5 onwards. In contrast to rations R1 and R2, FCM was generally higher for B1 cows than for B2 cows on ration R3, particularly in the first two weeks of lactation.

Discussion

As expected, β-carotene supplementation resulted in significantly higher serum β-carotene levels in the supplemented cows. The shape of the serum β-carotene profiles for the low-carotene and β-carotene supplemented cows was similar to those obtained by Lotthammer et al. (1978). However, in the present trial, serum β-carotene levels in the low-carotene cows were higher than the suggested deficient level of less than 100 µg/100 ml probably due to the high-carotene fababean silage (37.2 mg β-carotene/kg DM initially) fed in the postpartum rations. The significant β-carotene x ration interaction for serum β-carotene concentration may be indicative of the effect of the higher fat levels in rations R2 and R3 (with the fat being more available in R2) due to the canola seeds in

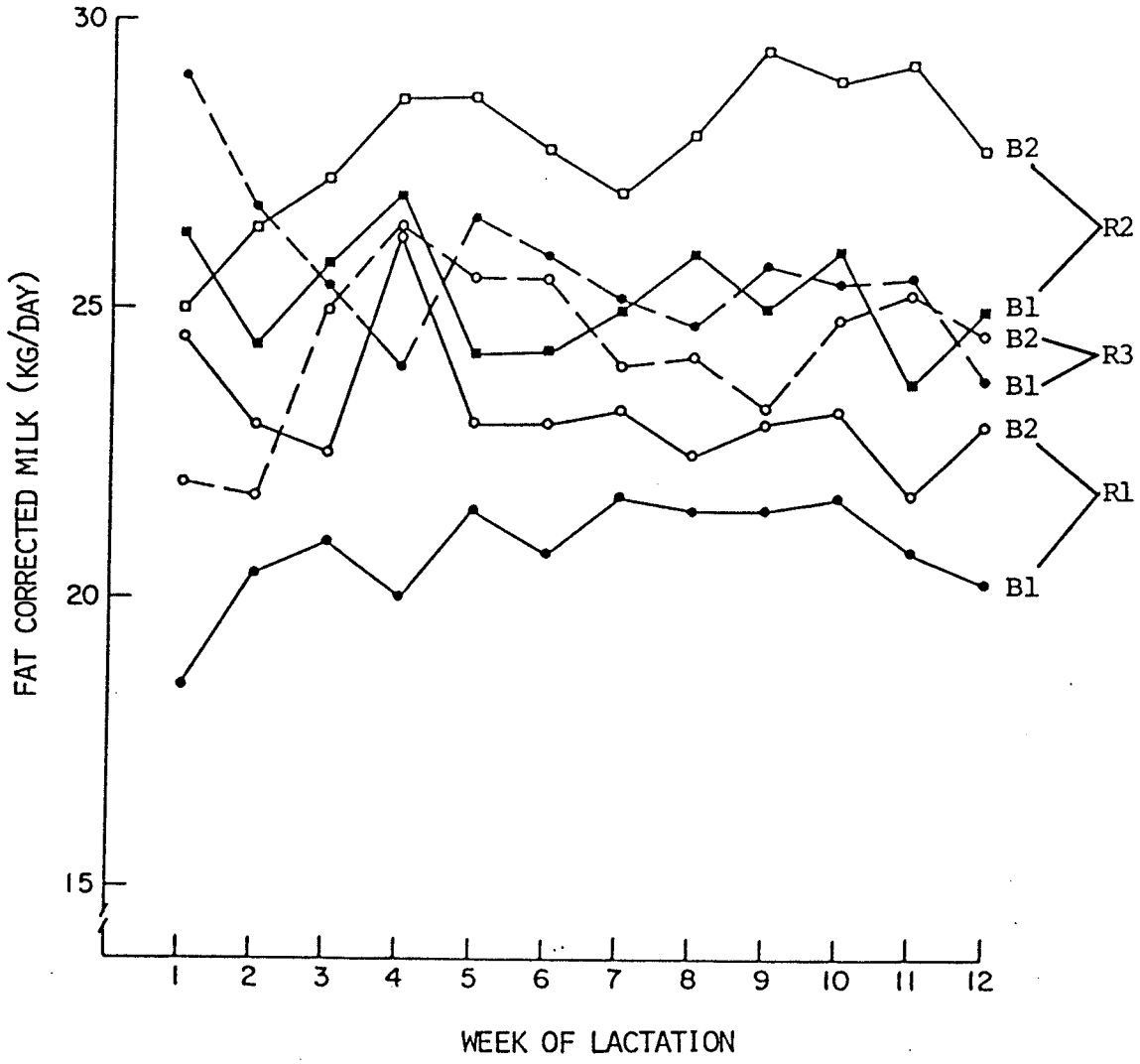

Figure 8. Three-way interaction between β-carotene treatment (B1 = control; B2 = carotene supplemented); ration treatment (R1 = control; R2 = extruded canola; R3 = whole seed canola) and week for fat corrected milk ($P<.01$).

those rations. Increasing the fat level in dairy rations by the addition of sunflower whole seeds protected by encapsulation has been shown to increase plasma β-carotene concentration in cows (Ashes et al. 1982).

Mean serum vitamin A levels did not differ between control and carotene-supplemented cows, although the supplemented cows received no vitamin A, because on a ratio of 1 mg β-carotene to 400 I.U. vitamin A, the B1 and B2 supplements were equal in vitamin A equivalent. The rise in serum vitamin A as lactation progressed in both cow groups may be due to the increased supplementation of both vitamin A and β-carotene at higher milk production levels as lactation progressed. The vitamin A allowances during lactation in the present trial were 1.3 to 6.5 times the highest NRC (1978) requirement. Vitamin A supplementation was at a calculated level to counterbalance the amount fed as β-carotene.

Although β-carotene supplementation had some positive effects on IUI and CR to first insemination in the present trial, the effects were not as pronounced as those reported by Lotthammer et al. (1978). The serum β-carotene status of the low-carotene group might be partly responsible for the extremely low CR to first service in this group (17.6%). There also appeared to be an inseminator effect on CR since one of the two inseminators used for AI services obtained a higher CR to first service (36.8%; 7 of 19) than the other (23.1%; 3 of 13). However, since the number of first services by each inseminator were reasonably balanced for the control and carotene-supplemented groups, the inseminator effect with respect to the difference between the two groups for first service CR would be minor. Despite the very high serum β-carotene levels in the carotene-supplemented group, CR to first service in this group (46.7%) was considerably lower

than the average CR to first service by AI of 66% obtained for Holsteins in a survey involving 2254 herds (Foote 1978). Evidently other factors, including perhaps stress due to the palpations during the present trial, contributed to the low overall CR to first service which was improved substantially after 2 and 3 services. In other studies, CR to first service in carotene-supplemented cows have ranged from 38.1% (Larson et al. 1983) to 58.8% (Bonsembiante et al. 1980).

The lack of a significant effect of β-carotene supplementation on IO, SC and DO is supported by similar findings by Larson et al. (1983) and Bindas et al. (1983). In addition, β-carotene supplementation had no effect on IUI and CR according to Larson et al. (1983). Bosembiante et al. (1980) reported that β-carotene supplementation improved CR to first service but had no effect on cows observed in heat and SC; while Akordor et al. (1984) observed an improvement in SC but not in days to first breeding, DO, IUI and CR to first service following β-carotene supplementation to cows.

Contrary to the findings of Lotthammer et al. (1978) β-carotene supplementation did not significantly affect the incidence of cystic ovaries, metritis, fetal loss, uterine infection and retained placenta in this trial. The present finding concerning cystic ovaries is supported by other studies which showed no effect of β-carotene supplementation on the incidence (Bremel et al. 1982; Larson et al. 1983; Marcek et al. 1985).

In spite of the very high level of β-carotene supplementation in the present trial, overall response in postpartum reproductive performance of the carotene-supplemented cows over the controls was marginal. A possible explanation for the inability of carotene supplementation to sub-

stantially improve reproductive performance might be that the β-carotene status of the control cows, which was above deficient levels according to current guidelines, did not adversely affect reproductive function. In the study by Lotthammer et al. (1978), poor postpartum fertility in the low-carotene cows was associated with serum β-carotene levels of less than 50 μg/100 ml. However in a study with cows, serum β-carotene concentrations of 150-200 μg/100 ml, similar to the levels in the low-carotene cows of the present trial, were associated with reduced fertility (Jackson 1981); whereas serum β-carotene levels as low as 30 μg/100 ml in another study with heifers did not influence reproductive performance (Folman et al. 1979). These findings, including the present one, suggest that the level of β-carotene deficiency that would adversely affect fertility in the cow probably depends on the extent of the animal's predisposition to numerous other detrimental factors which together cause fertility disturbances. Lotthammer (1979b) has suggested that β-carotene deficiency may only enhance the effects of other common detrimental factors so that β-carotene supplementation alone is unlikely to abolish the fertility problem. Alternatively, if conditions are otherwise optimal, β-carotene deficiency alone, especially for short periods of time, may be unlikely to cause serious fertility problems. This was probably the situation in the present trial since overall reproductive performance was generally good except for the low CR to first service which was markedly improved after the second insemination.

Since the ration treatments had no independent effects on serum β-carotene status, it was not surprising that they did not have independent effects on the reproductive parameters as well. Similarly, the

tramisol treatment which had no independent effect on serum β-carotene status was not expected to and did not influence most of the reproductive parameters. Therefore the somewhat shorter IU for the cows treated with tramisol cannot be explained. Studies concerning anthelmintic (deworming) treatment in dairy cattle have not indicated any effects on reproductive performance.

The fact that both ration and tramisol parameters had no significant effects on the incidence of reproductive disorders provide additional evidence that those two factors did not influence the fertility of the cows. Although, the three cases of fetal loss on ration R1 was significant ($P<.05$) there is no apparent connection between the ration treatments and fetal loss.

Based on a study which revealed an association between the severity of mastitis and low levels of plasma vitamin A and β-carotene in cows, Chew et al. (1982) suggested that plasma β-carotene levels of less than 200 μg/100 ml may be indicative of deficiency in relation to udder health in Holstein cows. The authors hypothesized that a deficiency of vitamin A and β-carotene may impair the functional integrity of the epithelial lining of the mammary secretory system leading to a successful invasion and establishment of mastitis causative organisms. The serum β-carotene levels for the low-carotene cows in the present trial were within the deficiency range for udder health suggested by Chew et al. (1982), but the trend for a higher incidence of clinical mastitis in these cows was not significant. However, milk SCC, as an index of subclinical mastitis, tended to be higher in the low-carotene cows.

The basis for the use of milk SCC as an index of subclinical mastitis is that while about 60% of the cells in milk from a normal healthy

mammary gland are epithelial, irritation of the mammary gland due to infection may increase dramatically the number of neutrophils and in particular the polymorphonuclear leucocytes to 90-95% of the total cells in milk (Schalm et al. 1971). The SCC is therefore a sensitive indicator of inflammation in the mammary gland. A SCC threshold value of 500,000 cells/ml of fore milk sampled asceptically from an individual quarter at normal milking from cows in normal lactation is accepted by the International Dairy Federation (IDF Bulletin, 1979) as an index of non-specific mastitis. However, since the predictability of SCC for diagnosis of intra-mammary infection depends on the sensitivity and specificity of the SCC and on prevalence of infection (Martin 1977), McDermott et al. (1982) have suggested that SCC threshold values should vary between herds and be altered within a herd as level of mammary infection varies.

If the 500,000 cells/ml SCC threshold value were applied to the SCC data in Table 16, the low-carotene cows, the cows on ration R3 and the cows not treated with tramisol could be considered to have had subclinical mastitis. There may be a physiological explanation, as proposed by Chew et al. (1982), for the high milk SCC value in the low-carotene cows; but that explanation cannot be applied to the high SCC values for the cows on ration R3 or those not treated with tramisol.

Although β-carotene supplementation and anthelmintic treatment had no independent effects on any of the milk production parameters, interaction between the two treatments produced a synergistic effect on milk yield. In other studies, β-carotene supplementation was reported to increase milk fat percent particularly at peak lactation (Lotthammer

1979a) but did not influence milk yield (Akordor et al. 1984). Anthelmintic administration to clinically normal dairy cows that have access to pasture elicited a positive response in milk yield (Todd et al. 1972; Bliss and Todd 1973, 1974 and 1976) which may be indicative of subclinical parasitism due to infection with internal nematodes on pasture. Under drylot housing conditions, anthelmintic treatment of cows failed to elicit a positive response in milk yield probably because infection with parasites was too low to affect milk yield (Glenn et al. 1982). Since the cows in the present trial had no access to pasture during the trial period, infection with parasites was probably low and, therefore, anthelmintic treatment alone did not improve milk yield.

The higher MY on ration R2 could be attributed to the higher energy density of that ration due to the additional fat provided by the extruded canola seed. Ration R3, which was equally high in fat, was unable to improve milk yield probably because the protective coat of the whole seed canola made the fat in the seeds less available. Unpublished growth data from a study with dairy calves conducted at the University of Manitoba (Sharma et al. unpublished data) indicated a low availability of fat from whole canola seeds. Beta-carotene supplementation and the high fat rations had no independent effects on MF and FCM, but the β-carotene x ration x week interactions for MF and FCM indicated that in general a combination of β-carotene supplementation and ration R2 tended to be more effective than a combination of R3 and β-carotene supplementation in improving MF and FCM as lactation progressed. This may be a reflection of the β-carotene x ration interaction for serum β-carotene concentration.

Summary and Conclusions

High levels of β-carotene supplementation to lactating Holstein cows significantly increased serum β-carotene concentration. Serum vitamin A levels in the control and carotene-supplemented cows were similar although the latter group received no vitamin A. As a fat supplement in the dairy ration, extruded canola seed was more effective than whole canola seed in increasing serum β-carotene concentration in the carotene-supplemented cows but not in the low-carotene cows. Anthelmintic treatment had no influence on serum β-carotene and vitamin A levels.

Beta-carotene supplementation had some positive effects on IUI and CR to first service but did not influence IO, SC and DO. The incidence of cystic ovaries, metritis, fetal loss, uterine infection and retained placenta were also not affected by β-carotene supplementation. The overall positive response in reproductive performance to the high β-carotene supplementation was marginal probably because the effects of other detrimental factors on reproduction were minimal. The low carotene status of the unsupplemented cows did not seem to have a substantial adverse affect on reproductive function.

There was a tendency for β-carotene supplementation and anthelmintic treatment to be associated with a lower incidence of clinical mastitis. This conclusion was supported by somewhat lower milk SCC, an index of subclinical mastitis, in the β-carotene supplemented and anthelmintic treated cows.

Interaction between β-carotene supplementation and anthelmintic treatment produced a synergistic effect for higher milk yield. However

neither treatment, had any independent effects on MY, MF and FCM. As a fat supplement in the ration, extruded canola seed improved milk yield but whole canola seed did not, probably because the fat was less available from the whole canola seed. A combination of β-carotene supplementation and extruded canola seed as a fat supplement tended to be more effective than a combination of β-carotene supplementation and whole canola seed in improving MF and FCM as lactation progressed.

EXPERIMENT III

Reproductive Performance of Prepubertal Holstein Heifers on Low or High Beta-carotene Diets

Variations in the age at which an animal attains puberty have been attributed to both genetic and environmental factors (Grass et al. 1982). Nutrition is a major environmental factor that affects sexual maturity (Zintzen 1974). Heifers on a low plane of nutrition (primarily energy and protein) took longer to reach puberty than heifers on a high plane of nutrition (Menge et al. 1960; Short and Bellows 1971; Grass et al. 1982).

The necessity of vitamin A for optimum reproduction has been established for cattle as well as other species (Thompson 1975). Hart and Guilbert (1933) described a number of reproductive problems associated with a low intake of vitamin A or its precursor. Bulls fed vitamin A deficient diets had delayed puberty and reduced libido and spermatogenesis (Hodgson et al. 1946; Erb et al. 1947).

Recent studies in Germany have indicated that in spite of adequate vitamin A status, β-carotene deficiency adversely affected reproductive performance in heifers (Lotthammer 1979b). However, other workers have found no relationship between serum β-carotene levels and reproductive performance in heifers (Folman et al. 1979; Wang et al. 1982; Ducker et al. 1984). Folman et al. (1979) reported that although mean daily gain was significantly ($P<.02$) higher for β-carotene supplemented Friesian heifers compared to unsupplemented controls, age at first ovulation (puberty) was identical for both groups. No other studies involving

heifers have specifically examined β-carotene effects on the onset of puberty. Furthermore, there is little information on reproductive hormone response, particularly during prepuberty, to low levels of serum β-carotene.

The objectives of this study were to determine if β-carotene deficiency adversely affected growth rate, onset of puberty and subsequent reproductive performance of young Holstein heifers. Pre- and post-pubertal serum concentrations of gonadotropic and steroid hormones associated with reproduction were also determined to evaluate their response to β-carotene deficiency.

Materials and Methods

Animals

Twenty 5-month old Holstein-Friesian heifers from the University of Manitoba dairy herd were employed in this study. After a three week pre-experimental period during which the heifers received a low-carotene diet to reduce their serum carotene levels, the heifers were assigned alternatively to either a low-carotene control diet (B1) or a β-carotene supplemented diet (B2). The heifers were on trial for approximately 10 months.

Heifers were weighed at the beginning of the trial, at first observed estrus (puberty) and at the end of every month for weight gain assessment. Heifers on the same dietary treatment were housed as a group in pens located in the same barn that housed the lactating herd. Heifers remained indoors throughout the experimental period.

Experimental Diets and Feeding

All heifers were fed ad libitum, a low-carotene (<10 mg/kg DM) basal roughage of corn silage, old weathered hay and/or straw. In addition each heifer received 2 to 2.5 kg of a concentrate daily that supplied 21,200 I.U. vitamin A or 105 mg β-carotene (Rovimix 10%, Roche) to the B1 and B2 heifers respectively. The ingredient composition of the concentrates is presented in Table 20.

Blood Sampling and Handling

Blood for the analysis of serum concentration of β-carotene, vitamin A and reproductive hormones in each heifer was initially obtained twice a week via venipuncture of the jugular vein. Beginning on the day of first observed estrus, the frequency of sampling was increased to every second day through two estrous cycles. At the end of the second cycle, sampling frequency was returned to twice a week for the rest of the experimental period.

For the determination of interval from LH peak to ovulation, one estrus in each heifer was selected for a 24-hour sampling regime at 2 hour intervals beginning shortly after the initial signs of heat were observed. At the end of the 24-hour period, additional samples were taken at 12 to 24 hour intervals until ovulation was confirmed by palpation.

All samples were stored overnight in the dark at $4^{o}C$, centrifuged and the harvested serum stored at $-20^{o}C$ until analysed.

Table 20. Ingredient composition of the concentrate rations

Ingredient	Treatment	
	B1	B2
	(g/kg)	
Barley	637	637
Oats	100	100
Fababean meal	200	200
Molasses	20	20
Limestone	10	10
Trace-mineralized salt*	10	10
Phosphorous supplement (biophos)	10	10
Urea (feed grade)	8	8
Vitamin premix I**	5	-
Vitamin premix II***	-	5

*Trace-mineralized salt supplied per kg concentrate: 9.7 g NaCl, .04 g Zn, .028 g I, .016 g Fe, .012 g Mn, .0033 g Cu and .0004 g Co.

**Vitamin premix I supplied per kg concentrate: 8480 I.U. vitamin A, 660 I.U. vitamin D and 17 I.U. vitamin E.

***Vitamin premix II supplied per kg concentrate: 42 mg β-carotene, 660 I.U. vitamin D and 17 I.U. vitamin E.

Serum Beta-carotene and Vitamin A Analysis

Beta-carotene and vitamin A analyses were done on weekly pooled subsamples for the first six weeks and then on 4 weekly pools for the rest of the experimental period for each heifer. The analytical procedures for β-carotene and vitamin A determinations were described previously in the general materials and methods section.

Hormone Radioimmunoassays (RIA)

The concentrations of serum reproductive hormones were determined using RIA procedures previously described in detail (Connor 1976) and routinely used in our laboratory. The assays were set up to ensure that serum samples from an equal number of heifers from each treatment were included in each assay.

Estradiol-17 (E)

The RIA procedure used for serum E was essentially that of Yu et al. (1974) but without column chromatography. A 2.0 ml aliquot of serum sample was extracted with ether and assayed using an estradiol-17β antiserum (#76-22/7/77), obtained from N.C. Rawlings (Department of Veterinary Physiology, WCVM, University of Saskatchewan). This antiserum was reported to have cross-reactivities of 5.6% with estrone; 0.35% with estradiol-17α and <0.01% with progesterone, testosterone and cortisol (N.C. Rawlings, personal communication). The sensitivity of the assay defined as 95% B/B_o (where B is the total amount of E trace bound to antibody and B_o is the amount of E trace bound to antibody in the absence of unlabelled hormone) was 1.65 pg/ml. The mean extraction efficiency

was 80.3±1.9% (n = 3 assays) and this value was used to correct all determinations for procedural losses.

Due to insufficient serum samples for some heifers, only single E determinations were made for 5 heifers on each treatment. A pooled sample of heifer serum (assayed in duplicate) was included in each assay for intra- and inter-assay coefficient of variation determinations which were 19.2% and 14.1%, respectively (n = 3 assays).

Progesterone

Serum progesterone concentration was determined by the RIA procedure of Abraham et al. (1971) as modified by Yuthasastrakosol (1975). A 0.5 ml aliquot of serum was extracted with ether and assayed using antiserum (#11-9/4/80), obtained from N.C. Rawlings (Dept. of Vet. Physiology, WCVM, University of Saskatchewan). This antiserum was reported to cross-react with cholesterol (0.4%), testosterone (0.04%) estradiol-17β (<0.01%) and cortisol (<0.01%) (N.C. Rawlings, personal communication). The mean sensitivity of the assay was 47.9 pg/ml. Mean extraction efficiency was 74.8±1.2% (n = 8 assays) and this value was used to correct all determinations for procedural losses.

Duplicate determinations were made on all samples collected for all heifers. A pooled sample of heifer serum (assayed in duplicate) was included in each assay for intra- and inter-assay coefficients of variation determinations which were 4.4% and 16.8%, respectively (n = 8 assays).

Luteinizing Hormone (LH)

Serum LH concentration was measured by the double antibody RIA procedure previously described by Howland (1972) using anti-ovine LH serum (#7-17/7/81), obtained from N.C. Rawlings (Dept. of Vet. Physiology, WCVM, University of Saskatchewan). The trace used was a purified ovine LH (NIH-LH-S21) labelled with ^{125}I using a modification of the method of Greenwood et al. (1963). LH values were expressed as ng/ml of NIH-LH-S14 standard. The mean sensitivity of the assay was 0.26 ng/ml. The use of an ovine LH standard for bovine LH determinations was justified by the demonstration of parallelism between the ovine standard curve and an inhibition curve for serial dilutions of bovine serum (Figure 9).

Duplicate determinations were made on all samples collected from all heifers from the beginning of the trial to the end of the first estrous cycle; and on the 24-hour samples. Intra- and inter-assay coefficients of variation of 13.1% and 19.1%, respectively (n = 4 assays) were obtained using a pooled serum sample (assayed in duplicate) that was included in each assay.

Follicle Stimulating Hormone (FSH)

Serum FSH was measured by the double-antibody RIA procedure of Cheng (1978). A rabbit anti-bovine FSH serum was used at an initial dilution of 1:60,000 in phosphate-EDTA buffer containing rabbit serum. A trace of purified bFSH labelled with ^{125}I was used in the assay system. Anti-rabbit gamma globulin serum was used to separate bound from free hormone. FSH values were expressed as ng/ml of USDA-FSH-BP3 standard (28 x NIH-

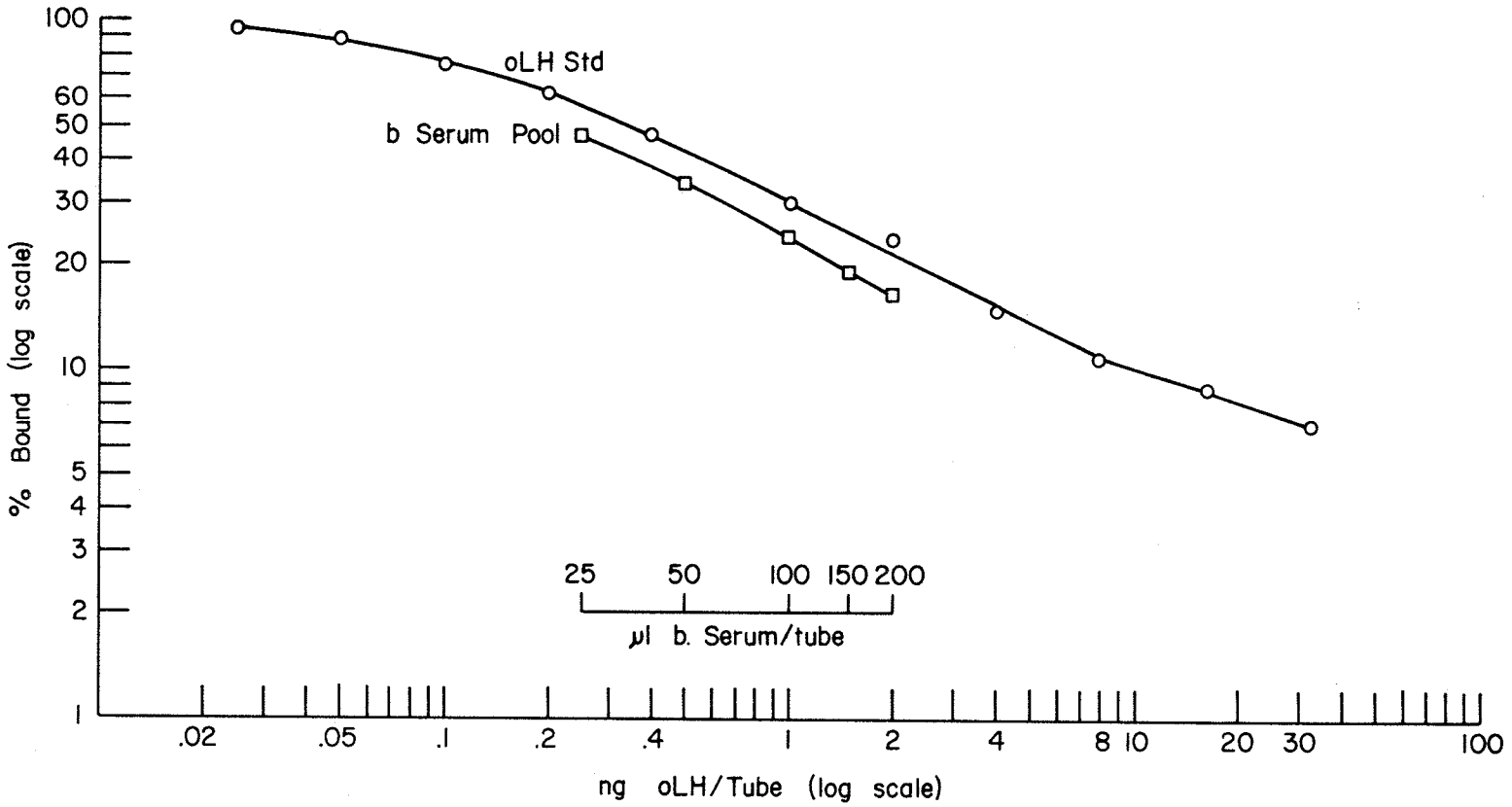

Figure 9. Inhibition curves obtained for serial dilutions of oLH standard and bovine serum (pool).

FSH-S1). The mean sensitivity of the assay was 0.06 ng/ml.

Duplicate determinations were made on all samples collected from all heifers from the beginning of the trial to the end of the first estrous cycle. Intra- and inter-assay coefficients of variation of 9.9% and 4.4%, respectively (n = 3 assays) were obtained using a pooled serum sample (assayed in duplicate) that was included in each assay.

Evaluation and Interpretation of Hormone Data

The serum progesterone profile generated for each heifer was used to determine ovulation dates and to estimate the length of the estrous cycles. Ovulation was presumed to have occurred when serum progesterone values exceeded 1 ng/ml for at least 3 consecutive samples (at a sampling frequency of 2x a week, this corresponds to a luteal phase of 8 to 14 days). Estimates of the amount of progesterone produced during the estrous cycle, for all cycles in which blood was sampled every second day, was determined by calculating the area under the progesterone curve assuming a baseline of zero. An on-line computer program that calculates area from a series of coordinates was used for the area determinations.

The 24-hour LH data was used to roughly estimate the interval from LH peak to ovulation defined as the time interval from the highest pre-ovulatory LH value to 8:00 AM on the day of ovulation as determined by palpation. The LH peak occurred before the 24-hour sampling began in 8 heifers, but the descending limb of the LH profile was obtained. From the LH profiles of 10 heifers in which LH peaks were obtained, the mean interval from LH peak to post-peak basal LH level was estimated to be 6 hours. Using this value, the interval to ovulation was extrapolated

for the 8 heifers whose LH peaks were missed. The LH profile in 2 other heifers indicated that they were sampled at the wrong time.

All hormone data for each heifer from the beginning of the trial to day 17 of the first estrous cycle were categorized based on changes in progesterone concentration into six periods as follows: beginning to day -22; day -21 to day -8; day -7 to day -1; day 0 (day of first ovulation); day 1 to day 8 and day 9 to day 17. The mean hormone concentrations for each period were computed and subjected to statistical analysis.

Estrus Observation and Intensity Rating

Three observations of 10 to 15 minute duration for estrual activity were made each day in the morning, at mid-day and in the evening. A fourth observation was made at night between 20:00 and 22:00 hours. The nightly observations were terminated after the first estrus in all heifers had been detected.

Each observed estrus was subjectively rated for intensity or strength on a scale of 1 to 4, based on criteria similar to those described by Lotthammer et al. (1976): 1 = silent heat (no visible signs of estrus); 2 = weak heat (moist and turgid vulva; restless but no mounting activity); 3 = strong heat (clear mucus discharge from vulva; restlessness; mounting of herd mates; 4 = very strong heat (clear mucus discharge from vulva; restlessness; standing to be mounted by herd mates without resistance).

Palpation

In conjunction with the 24-hour sampling regime, each heifer was palpated once a day until ovulation was confirmed.

The heifers were bred by A.I. at the end of the experimental period. They weighed in excess of 350 kg each at that time and were 15 to 16 months of age. The heifers were palpated between day 45 and 55 post-service for pregnancy diagnosis.

Statistics

The intra- and inter-assay coefficients of variation were calculated according to the method of Rodbard (1971).

The experimental data were analysed using the GLM and ANOVA procedures of SAS-82. The data on serum β-carotene, vitamin A and reproductive hormone concentrations were analysed by split-plot analysis. The reproduction data and the body weight and average daily gain data were analysed by ANOVA. Mean differences for all dependent variables were tested by SNK (Snedecor and Cochran 1967).

Results

Serum Beta-carotene and Vitamin A

Beta-carotene supplementation significantly ($P<.01$) increased serum β-carotene concentration (Figure 10). Mean serum β-carotene levels in the control heifers were generally less than 50 µg/100 ml throughout the experimental period. In contrast, mean serum β-carotene concentration in the carotene-supplemented heifers increased sharply from about 40 µg/100 ml in week 1 to over 140 µg/100 ml by week 5. Beyond week 5, mean serum β-carotene levels in this group fluctuated between approximately 150 and 190 µg/100 ml except for a drop to about 130 µg/100 ml in week 38. In general, there was a large variation in serum β-carotene concentration, particularly in the β-carotene supplemented heifers.

To simplify the analysis, and in view of the fact that changes in serum parameters following β-carotene supplementation usually became apparent within 4 weeks, statistical analysis of the serum β-carotene

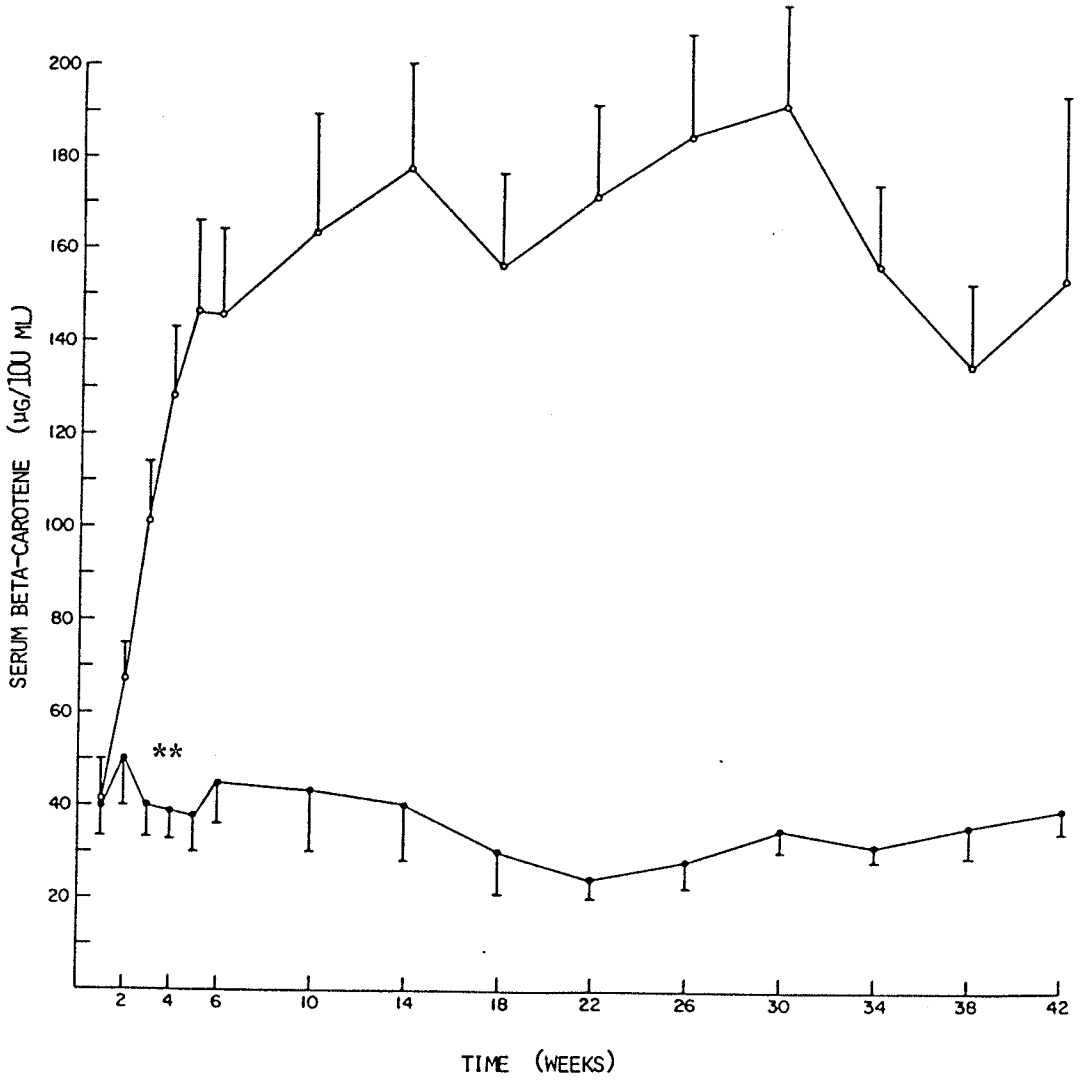

Figure 10. Mean (±SE) serum β-carotene concentration for control (B1; ●——●——●) and carotene supplemented (B2; o——o——o) heifers during the trial.

**Differences between B1 and B2 were highly significant ($P<.01$) from week 3 onwards.

and vitamin A parameters were performed on data for the first six weeks only. For serum β-carotene concentration, mean differences between control and carotene-supplemented heifers became highly significant ($P<.01$) by week 3 and undoubtedly remained significant for the rest of the trial period.

Mean serum vitamin A concentration was significantly higher ($P<.05$) in the carotene-supplemented heifers than in the control heifers during the first six weeks of the trial (Figure 11). Mean serum vitamin A levels in the two groups of heifers were identical in weeks 10, 14 and 42. From weeks 18 to 34, serum vitamin A levels tended to be higher in β-carotene supplemented heifers compared to control heifers.

Growth Rate, Age and Body Weight at Puberty

Mean body weights at the beginning of trial were similar for control (152.6±5.7) and β-carotene supplemented (157.7±3.3 kg) heifers (Figure 12). Mean monthly body weights during the trial period were usually higher (except for months 3-6) for the β-carotene supplemented heifers. At the end of the trial, overall average daily gain was significantly ($P<.05$) higher for carotene-supplemented heifers (753.8±17.1 g/day) compared to control heifers (682.9±23.7 g/day).

Beta-carotene supplemented heifers reached puberty (age at first observed estrus) approximately 30 days earlier and at a mean body weight approximately 14 kg lighter than their control counterparts (Table 21). However, the differences between control and carotene-supplemented heifers for both age and body weight at puberty were not significant ($P>.05$).

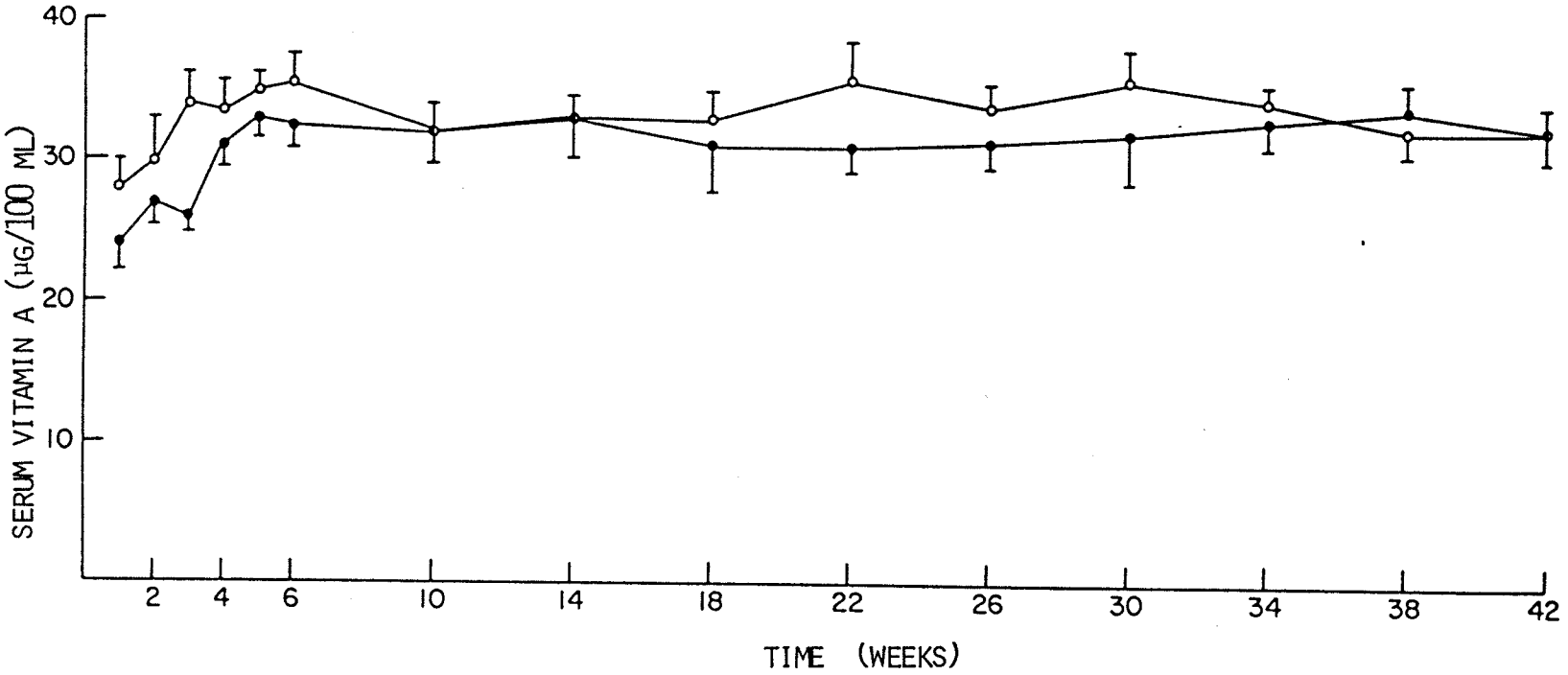

Figure 11. Mean (±SE) serum vitamin A concentration for control (B1; ●——●——●) and carotene supplemented (B2; o——o——o) heifers.

*Mean vitamin A concentration was significantly ($P<.05$) higher in B2 than in B1 during the first 6 weeks.

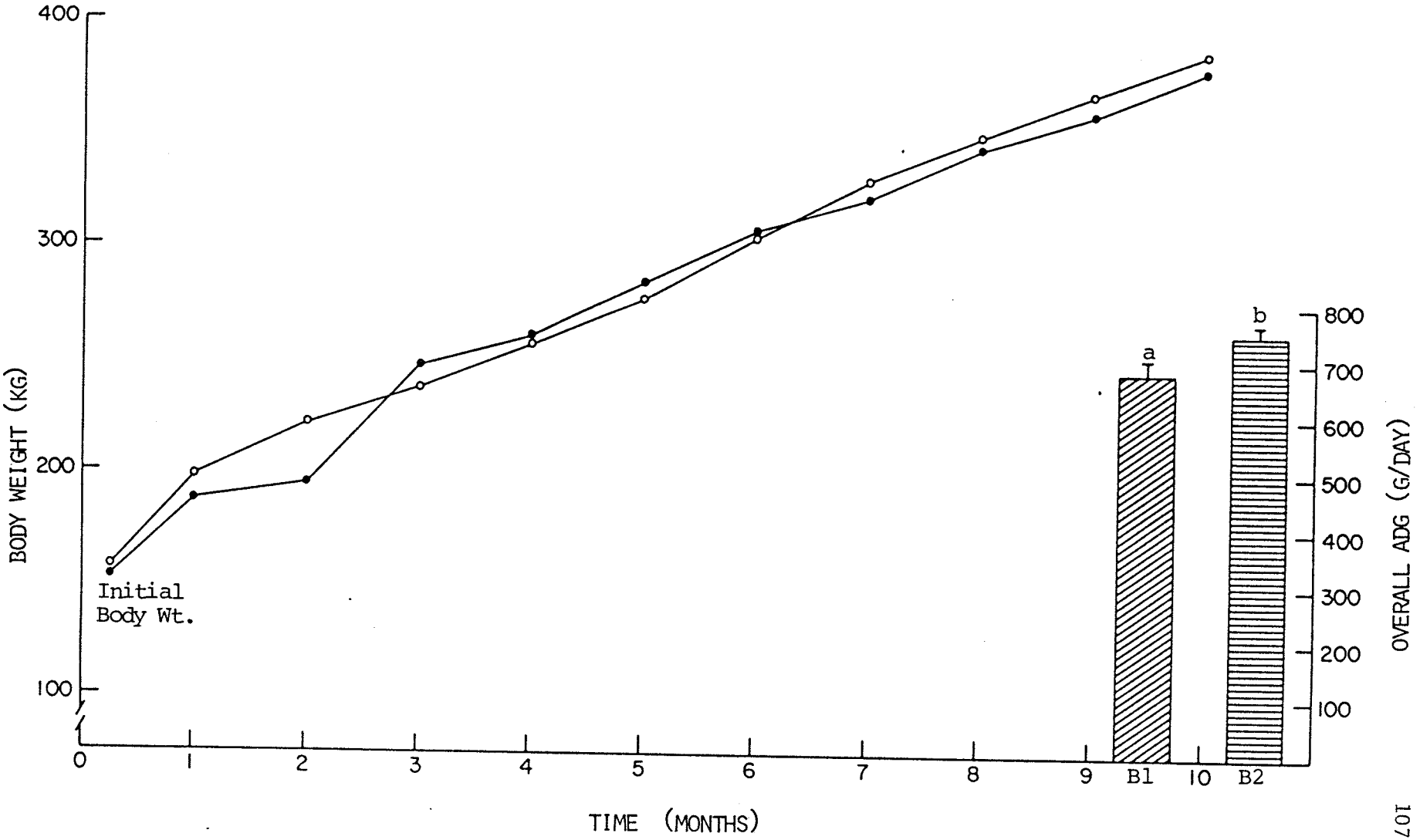

Figure 12. Mean monthly body weights during the trial and overall average daily gain (ADG; ±SE) for control (B1; ●——●——●) and carotene supplemented (B2; o——o——o) heifers.
a,b - Means significantly different (P<.05).

Table 21. Mean (±SE) age and body weight at puberty for control and β-carotene supplemented heifers

Parameter	Treatment	
	Control	β-carotene
Age (days)	329.5 (17.9)	299.7 (17.9)
Body weight (kg)	282.4 (13.0)	268.6 (13.0)

Reproductive Performance

The number of ovulations prior to the first observed estrus (puberty; OPP) based on serum progesterone profiles averaged 1.1±.3 and 1.2±.3 for control and carotene-supplemented heifers respectively and did not differ significantly between the two (Table 22). The data further indicated that estrus associated with first ovulation was detected in 30% (3 of 10) of control heifers and 20% (2 of 10) of carotene-supplemented heifers. However, 40% of control heifers had 2 ovulations prior to puberty whereas only one (10%) carotene-supplemented heifer had more than one ovulation prior to puberty. This particular heifer was very aggressive most of the time making it difficult to confirm her first estrus until after 5 ovulations.

Mean intensity or strength of the behavioural signs of estrus was significantly ($P<.01$) higher in the carotene-supplemented heifers (3.85± .1 rating) than in the controls (2.88±.1 rating).

The interval from preovulatory LH peak to ovulation based on palpation was significantly ($P<.01$) longer in control heifers (44±3 hours) compared to carotene-supplemented heifers (30±3 hours) by approximately 14 hours. However, services per conception were acceptable and not significantly ($P>.05$) different for control (1.4±.2) and carotene-supplemented (1.3±.2) heifers. First service conception rates were 70% and 80% for control and carotene-supplemented heifers, respectively.

Beta-carotene supplementation did not significantly ($P>.05$) affect the mean lengths of the first and second estrous cycles and the mean length of all subsequent cycles (Table 23). The cycle lengths ranged

Table 22. Least square means (±SE) of reproductive performance parameters for control and β-carotene supplemented heifers

	Treatment	
Parameter	Control	β-carotene supplementation
No. of ovulations prior to puberty	1.1 (.3)	1.2 (.3)
Intensity of estrus	2.88 (.1)[a*]	3.85 (.1)[b]
Interval from LH peak to ovulation (hrs)	44 (3)[c]	30 (3)[d]
Services/conception	1.4 (.2)	1.3 (.2)

*Row means with different superscripts are significantly different ($P<0.01$).

from 13 to 25 days and irrespective of treatment, the mean length of the first cycle (18.2±.4 days) was significantly ($P<.01$) shorter than the mean lengths of the second cycle (20.1±.4 days) and all subsequent cycles (20.5±.4 days).

Reproductive Hormone Response

Serum progesterone profile prior to and following first ovulation was similar in both control and carotene-supplemented heifers. As shown in Figure 13, mean serum progesterone levels during prepuberty in both heifer groups were less than 1 ng/ml up to about 6 days before first ovulation. There was a transient rise in serum progesterone concentration between day -6 and day -1 which peaked at a mean value of 1.76±.4 ng/ml for both heifer groups on day -4. Serum progesterone concentration decreased to the typical concentration of less than 1 ng/ml on the day of first ovulation then rose again to follow the typical cyclic profile.

The levels of serum progesterone during the first estrous cycle appeared to be higher in the control heifers compared to the carotene-supplemented heifers. However mean estimates of the amounts of progesterone produced during the estrous cycle, calculated as area under the cyclic progesterone curve (Table 24), did not differ significantly ($P>.05$) between control (68.3±4.1) and carotene-supplemented heifers (61.2±4.3).

Mean serum progesterone concentration from prepuberty to day 17 of the first estrous cycle, divided into six periods as shown in Table 24, did not differ significantly between treatment groups for all periods except period 6 (day 9 to 17 of the first cycle). Mean serum progesterone

Table 23. Least square means (±SE) of estrous cycle lengths for control and β-carotene supplemented heifers

Item	Treatment		
	Control	β-carotene	Combined data
First cycle (days)	18.3 (.6)	18.1 (.6)	18.2 (.4)[a*]
Second cycle (days)	19.9 (.6)	20.3 (.6)	20.1 (.4)[b]
Mean of all subsequent cycles (days)	19.9 (.6)	21.0 (.6)	20.5 (.4)[b]

*Means in the same column with different superscripts are significantly different (P<.01).

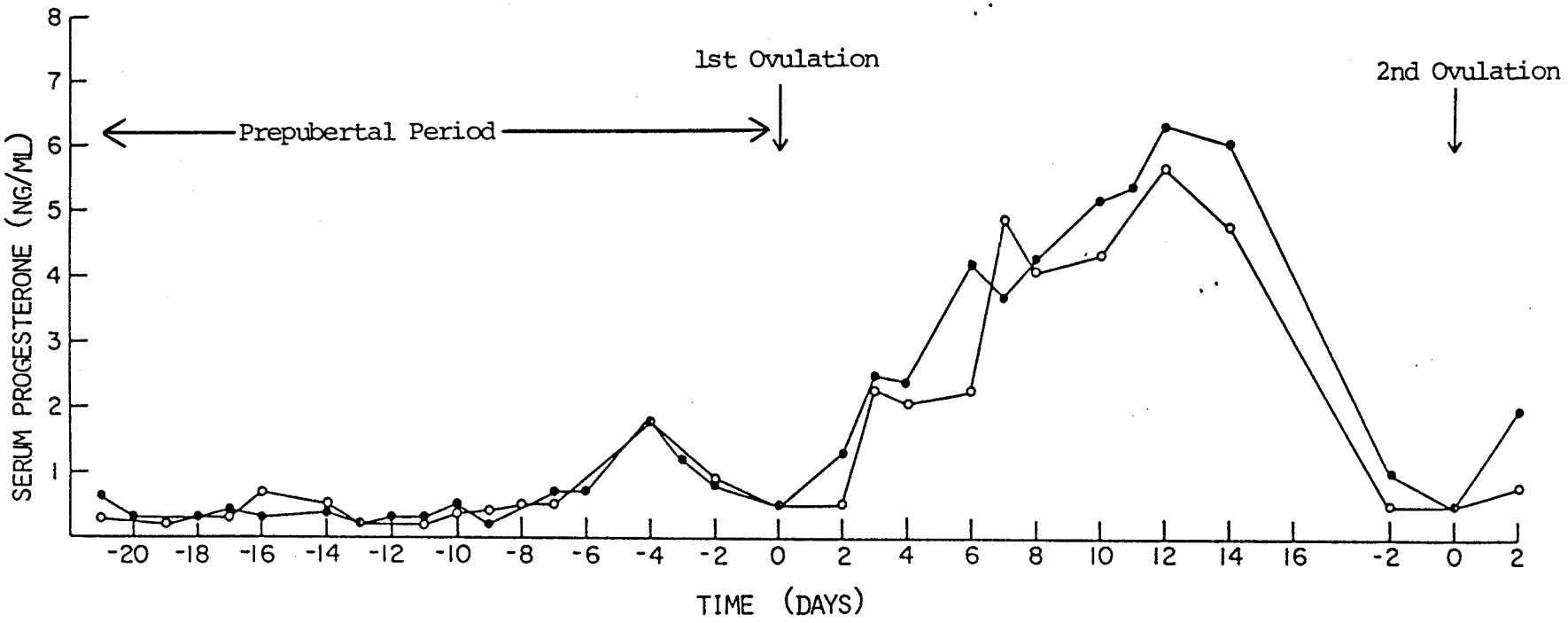

Figure 13. Mean serum progesterone profile from 21 days prior to first ovulation through the first estrous cycle for control (●——●——●) and carotene-supplemented (o——o——o) heifers.

Table 24. Least square means (±SE) of serum progesterone (ng/ml) from prepuberty to day 17 of the first cycle and area under the progesterone profiles for control and β-carotene supplemented heifers

Sampling periods	Days before or after 1st ovulation	Treatment: Control	Treatment: β-carotene	Combined data
1	Beginning of sampling to -22	.31 (.18)	.39 (.18)	.35 (.13)[a]
2	-21 to -8	.39 (.18)	.37 (.18)	.38 (.13)[a]
3	-7 to -1	1.06 (.18)	1.16 (.18)	1.11 (.13)[b]
4	0 (1st ovulation)	.48 (.18)	.48 (.18)	.48 (.13)[a]
5	1 to 8	3.04 (.18)	3.17 (.18)	3.11 (.13)[c]
6	9 to 17	5.6 (.18)[e]	4.71 (.19)[f]	5.16 (.13)[d]
	Area under cyclic progesterone profile (arbitrary units)	68.3 (4.1)	61.2 (4.3)	
	# cycles	14	14	

[a,b,c,d] Column means with different superscripts are significantly different ($P<.05$).

[e,f] Row means with different superscripts are significantly different ($P<.01$).

concentration during this period was significantly ($P<.01$) higher in control heifers (5.6±.18 ng/ml) than in carotene-supplemented heifers (4.71±.19 ng/ml). Irrespective of treatment, there were significant period differences. Serum progesterone concentration during the first two periods of prepuberty average less than 1 ng/ml for each period and did not differ from each other or from the mean serum progesterone level on the day of first ovulation (combined data, Table 24). During the 1-week period prior to first ovulation (days -7 to -1) mean serum progesterone level was significantly ($P<.05$) higher than the mean levels for the two previous periods or the mean level on the day of first ovulation. During the first 8 days following ovulation (period 5), which corresponds to the early luteal phase of the cycle, mean serum progesterone concentration was significantly ($P<.05$) higher than all previous levels. Mean serum progesterone concentration further increased significantly ($P<.05$) during days 9 to 17 of the first estrous cycle, which corresponds to the mid luteal phase of the cycle.

Mean serum estradiol-17β concentrations for control and carotene-supplemented heifers from prepuberty to day 17 of the first estrous cycle, divided into six periods, are shown in Table 25. There were no significant treatment differences in mean serum estradiol-17β concentration for all periods except periods 3 (days -7 to -1) and 6 (days 9 to 17). In period 3, mean serum estradiol-17β level was significantly ($P<.05$) higher in control heifers than in carotene-supplemented heifers, while the reverse was true in period 6. For the combined data, there was no significant period effect on mean serum estradiol-17β concentration.

Table 25. Least square means (±SE) of serum estradiol-17β (pg/ml) from prepuberty to day 17 of the first estrous cycle for control and β-carotene supplemented heifers

Sampling periods	Days before or after 1st ovulation	Treatment		Combined data
		Control	β-carotene	
1	Beginning of sampling -22	5.58 (.76)	5.14 (.76)	5.36 (.54)
2	-21 to -8	5.95 (.76)	5.72 (.76)	5.84 (.54)
3	-7 to -1	6.75 (.76)[a]	4.19 (.76)[b]	5.47 (.54)
4	0 (1st ovulation)	5.6 (.76)	4.36 (.76)	4.98 (.54)
5	1 to 8	6.26 (.76)	5.28 (.76)	5.77 (.54)
6	9 to 17	5.05 (.76)[c]	7.51 (.76)[d]	6.28 (.54)

[a,b,c,d]Row means with different superscripts are significantly different ($P<.05$).

Serum LH levels from prepuberty to day 17 of the first cycle were not significantly ($P>.05$) influenced by β-carotene supplementation (Table 26). For the combined data, there was a significant period effect. Mean serum LH levels for the first 4 periods from prepuberty to day of first ovulation and also during period 6 (days 9 to 17) were not significantly ($P>.05$) different from each other although mean LH levels in periods 3 (days -7 to -1) and 2 (days -21 to -8), in particular, tended to be higher. The lowest mean serum LH level of 0.7±.11 ng/ml seen in period 5 (days 1 to 8), corresponding to the early luteal phase of the cycle, differed significantly ($P<.05$) from mean LH level in period 2 (1.23±.11 ng/ml) but did not differ from mean LH levels in the other periods.

There were no significant ($P>.05$) differences between control and carotene-supplemented heifers for mean serum FSH concentrations for any of the periods from prepuberty to day 17 of the first estrous cycle (Table 27). On the other hand, there were significant period differences for the combined data. Mean serum FSH concentrations for periods 1, 2 and 4 were significantly ($P<.05$) higher than the mean levels during the 2 post-ovulation periods. Mean serum FSH concentrations were similar for the first two periods of prepuberty (2.63±.14 vs 2.84±.14 ng/ml). Mean FSH level in period 3 (2.22±.14 ng/ml) was significantly ($P<.05$) lower than the mean level in period 2 (2.84±.14 ng/ml) but similar to the mean level in period 1 (2.63±.14 ng/ml). Mean FSH concentration on the day of first ovulation did not differ ($P>.05$) from the mean FSH levels for all 3 previous periods. As well, serum FSH levels during the last 2 periods were similar.

Table 26. Least square means (±SE) of serum LH (ng NIH-LH-S14/ml) from prepuberty to Day 17 of the first estrous cycle for control and β-carotene supplemented heifers

Sampling periods	Days before or after 1st ovulation	Treatment		Combined data
		Control	β-carotene	
1	Beginning of sampling to -22	0.89 (.16)	0.91 (.16)	0.90 (.11)[ab]
2	-21 to -8	1.14 (.16)	1.31 (.16)	1.23 (.11)[a]
3	-7 to -1	1.03 (.16)	0.99 (.16)	1.01 (.11)[ab]
4	0 (1st ovulation)	0.93 (.16)	0.84 (.16)	0.88 (.11)[ab]
5	1 to 8	0.65 (.16)	0.76 (.16)	0.70 (.11)[b]
6	9 to 17	0.75 (.17)	1.15 (.17)	0.95 (.11)[ab]

[a,b]Column means with different superscripts are significantly different ($P<.05$).

Table 27. Least square means (±SE) of serum FSH (ng USDA-FSH-BP3/ml) from prepuberty to Day 17 of the first estrous cycle for control and β-carotene supplemented heifers

Sampling periods	Days before or after 1st ovulation	Treatment		Combined data
		Control	β-carotene	
1	Beginning of sampling to -22	2.44 (.2)	2.82 (.2)	2.63 (.14)^{ab}
2	-21 to -8	2.6 (.2)	3.09 (.2)	2.84 (.14)^{a}
3	-7 to -1	2.08 (.2)	2.36 (.2)	2.22 (.14)^{bc}
4	0 (1st ovulation)	2.38 (.2)	2.72 (.2)	2.55 (.14)^{ab}
5	1 to 8	1.79 (.2)	2.19 (.2)	1.99 (.14)^{cd}
6	9 to 17	1.74 (.2)	1.63 (.2)	1.69 (.15)^{d}

^{a,b,c,d}Column means with different superscripts are significantly different ($P<.05$).

Discussion

Serum β-carotene levels in the noncarotene-supplemented control heifers of the present experiment were substantially lower than the suggested deficiency level (Friesecke 1978) and comparable to serum β-carotene levels in carotene-deprived heifers reported by Lotthammer and Ahlswede (1977) and Folman et al. (1979). Although β-carotene supplementation significantly increased serum β-carotene levels, the mean β-carotene peak levels in the supplemented heifers were still below the suggested optimum level of 300 µg/100 ml. This observation agrees with results reported by Lotthammer and Ahlswede (1977) and Folman et al. (1979).

In the present experiment, serum vitamin A levels were higher in the carotene-supplemented heifers although they received no vitamin A. This may be due to the fact that the vitamin A equivalent of the daily β-carotene supplement of 105 mg (42,000 I.U. vitamin A) was twice the level of vitamin A supplied to the control heifers (21,200 I.U.).

In agreement with a similar observation by Folman et al. (1979), β-carotene supplementation in the present trial significantly improved growth rate but had no influence on age at puberty. On the contrary, Ducker et al. (1984) reported that β-carotene supplementation (300 mg/head/day) did not affect growth rate in heifers receiving a ration based on corn silage. This is probably because the non-supplemented heifers of Ducker et al. (1984) had relatively high serum β-carotene levels (231-331 µg/100 ml) which were higher than the peak serum β-carotene levels in the supplemented heifers of the present experiment (190 µg/100 ml)

and that of Folman et al. (1979) (219 µg/100 ml). The requirement of β-carotene, per se, for growth (if it is required for growth) may probably be much lower than that for reproduction, as is the case for vitamin A (Hemken and Bremel 1982). Thus, the serum β-carotene levels in the unsupplemented heifers of Ducker et al. (1984) may have satisfied the requirements for growth.

On average, there was one ovulation before first observed estrus in both treatment groups which indicates that first estrus was generally silent and undetectable despite the carotene supplementation. However, the fact that 4 control heifers had 2 ovulations before puberty compared to 1 carotene-supplemented heifer which had more than 1 ovulation before puberty appear to support the observation that estrus in the low-carotene control heifers was more difficult to detect due to their lower intensity of estrus.

The lower intensity of estrus and longer interval from preovulatory LH peak to ovulation in the control heifers of the present experiment, are supported by similar observations of Schams et al. (1977); but inconsistent with observations by others that β-carotene supplementation did not influence estrual activity and/or interval from LH peak to ovulation in heifers (Folman et al. 1979; Wang et al. 1982; Wang and Larson 1983). The frequency of the 24-hour blood sampling regime and the accompanying palpation employed in the present experiment did not permit a precise estimation of the interval from LH peak to ovulation. The mean interval of 30±3 hours for the carotene-supplemented heifers in comparison to a reported normal mean interval from onset of estrus to ovulation of 24.4±6.4 hours for Holstein cows (Refsal and Seguin 1980)

suggests an overestimation of the length of the interval in the present experiment.

In spite of the extremely low serum β-carotene levels in control heifers and the fact that serum β-carotene levels in the supplemented heifers were less than optimum, services per conception and conception rate were very good and did not differ between the two groups. This is contrary to observations by Lotthammer et al. (1976) but consistent with reports by Folman et al. (1979) and Wang et al. (1982). In the present trial, the satisfactory conception rate in both heifer groups despite the lower intensity of estrus in the controls may be attributed to the close observation for estrus and the fact that records were kept of each estrus enabling a fairly accurate prediction of the next one. The lack of a significant effect of carotene supplementation on the length of estrous cycle also agrees with a similar observation by Folman et al. (1979).

Contrary to the report by Schultz et al. (1974) that progesterone concentration in the CL was lower in cows with low serum β-carotene, β-carotene supplementation in this experiment did not significantly affect total serum progesterone output during the estrous cycle. Other workers have similarly found no differences in blood progesterone concentrations between carotene supplemented and unsupplemented heifers (Folman et al. 1979; Wang et al. 1982). The pre- and post-pubertal profile and levels of serum progesterone in this experiment are consistent with previous reports (Donaldson et al. 1970; Gonzalez-Padilla et al. 1975; Berardinelli et al. 1979). The transient rise in serum progesterone prior to first ovulation observed in this experiment and previously re-

ported by others (Gonzalez-Padilla et al. 1975; Berardinelli et al. 1979; Schams et al. 1981) is thought to originate from luteinized immature follicles (Gonzalez-Padilla et al. 1975; Berardinelli et al. 1979). The apparent high levels of prepubertal serum LH and FSH particularly during the 2-week period before the transient rise in serum progesterone in the present trial, appear to support the "luteinized follicle" theory.

Estrogen is the hormone responsible for the behavioural expression of estrus in the female animal (Bearden and Fuquay 1980). Since intensity of estrus in the present trial and as previously reported by Schams et al. (1977) was higher in the carotene-supplemented heifers, one would have expected serum estradiol-17β levels to be higher in those heifers. However, serum estradiol-17β concentration was not affected by β-carotene supplementation in the present experiment. This was even more surprising in view of the reported high concentrations of β-carotene in the follicular fluid of cows (Chew et al. 1983).

Gonzalez-Padilla et al. (1975) reported that serum estradiol-17β levels in prepubertal beef heifers were high up until about day 40 before the first preovulatory LH peak, then decreased to levels which remained constant until the end of sampling on day 16 of the first cycle. In the present experiment, mean serum estradiol-17β levels for all heifers did not change significantly over time from approximately 5 months of age to day 17 of the first estrous cycle.

The fact that serum LH and FSH levels in the present trial were not significantly affected by β-carotene supplementation may be an indication that the effect of β-carotene on reproduction may not be mediated at the hypothalamic or pituitary level. For the combined data of all

heifers in the present trial, mean prepubertal serum LH levels were generally similar to the levels after first ovulation except for the higher mean LH value for period 2 (day -21 to -8) compared to period 5 (days 1 to 8) (Table 26). Mean serum FSH levels on the other hand, were generally higher during prepuberty compared to the post-ovulation period. In contrast, Gonzalez-Padilla et al. (1975) observed no marked changes in serum FSH levels in beef heifers as puberty approached or during the first estrous cycle but mean prepubertal LH concentrations were higher than that observed after first ovulation. On the other hand, Schams et al. (1981) have described a biphasic profile for prepubertal basal gonadotropin levels in which mean serum LH and FSH values in heifers increased from birth to 3 months of age, and then decreased reaching the initial levels at 5 and 6 months of age before increasing again to a second peak at 9 months of age.

The serum FSH values obtained in this experiment were approximately 20 times lower than the values reported by Gonzalez-Padilla et al. (1975), a fact probably related to the high potency of the USDA-FSH-BP3 standard (28xNIH-FSH-S1) used for the FSH assays in this experiment.

Summary and Conclusions

Beta-carotene supplementation of 105 mg/head/day to prepubertal heifers on a low-carotene diet significantly increased serum β-carotene levels; although mean peak serum carotene concentrations following the carotene supplementation were less than the suggested optimum of 300 µg/100 ml. Serum vitamin A levels were higher in carotene-supplemented heifers than in unsupplemented controls that received a lower equivalent of vitamin A.

Beta-carotene supplementation significantly improved average daily gain of the heifers but had no influence on mean age and body weight at puberty.

The estrous activity that accompanied first ovulation tended to be silent and undetectable in both carotene-supplemented and unsupplemented heifers. However, in general estrus was more difficult to detect in the low-carotene control heifers due to their lower intensity of estrus. The interval from preovulatory LH peak to ovulation was significantly longer in the low-carotene control heifers. The effect of the low carotene status in the control heifers on services per conception and conception rate to first insemination were probably ameliorated by the close observations for estrus. Mean length of the estrous cycles were not influenced by β-carotene supplementation, but in general the first estrous cycle was shorter than the subsequent cycles by about 2 days.

Serum progesterone concentration from prepuberty to day 17 of the first estrous cycle as well as total serum progesterone output during the estrous cycle were not affected by β-carotene supplementation. However, there were significant period differences in serum progesterone concentration in both heifer groups. The lower intensity of estrus in the control heifers did not appear to be due to lower estradiol-17β output since serum estradiol-17β concentration from puberty to day 17 of the first estrous cycle was not affected by β-carotene supplementation. As well, there were no significant period differences in mean serum estradiol-17β concentration from prepuberty to day 17 of the first estrous cycle.

Beta-carotene supplementation had no influence on mean serum LH and

FSH concentrations from prepuberty to day 17 of the first estrous cycle. However, there was a significant period effect on both serum LH and FSH concentrations. The apparent high mean serum concentrations of FSH and to a lesser extent LH during the 2-week period prior to the prepubertal transient rise in serum progesterone provide some evidence in support of the theory that the prepubertal transient rise in serum progesterone originated from luteinized immature follicles.

GENERAL DISCUSSION

Despite efforts to demonstrate a vitamin A-independent role for β-carotene in bovine reproduction, the results of the experiments reported in this thesis and those of other studies reviewed do not conclusively resolve the issue of whether β-carotene, per se, is required for optimal bovine reproductive performance. The original German studies showed consistently that β-carotene deficiency had significant adverse effects on bovine reproduction which were alleviated by β-carotene supplementation (Lotthammer 1979b). In the experiments reported here, some of the reproductive parameters evaluated, such as IUI and CR (Experiment 2) and intensity of estrus and interval from LH peak to ovulation (Experiment 3), were substantially improved by β-carotene supplementation. However, most of the reproductive parameters observed had marginal or no improvement following β-carotene supplementation. Other workers have similarly reported that β-carotene supplementation improved some reproductive parameters but not others (Bonsembiante et al. 1980; Akordor et al. 1984). On the other hand, there are studies in which β-carotene supplementation did not improve any of the reproductive parameters (Folman et al. 1979; Wang and Larson 1983; Larson et al. 1983; Ducker et al. 1984).

In view of the lack of response to β-carotene supplementation for the many reproductive parameters in the experiments reported here and in other studies, one might conclude that β-carotene has no specific role in bovine reproductive function. However, there could be a number of reasons for the discrepancy in reproductive response to β-carotene

supplementation between the various studies. Differences in the breed of cattle used in the different studies may account for some of the discrepancy in reproductive response to β-carotene supplementation. It is known that there are breed differences in the absorption and accumulation of carotene in the tissues of cattle (Goodwin 1954) which may reflect differences in the efficiency of β-carotene conversion to vitamin A (Eaton et al. 1959). Therefore it seems logical to hypothesize that bovine breeds that are genetically less efficient in the conversion of β-carotene to vitamin A and hence accumulate greater amounts of β-carotene in their tissues might be more responsive to β-carotene.

It is not known how long a β-carotene deficiency must be maintained before its adverse effects on reproductive performance are manifested. In the case of vitamin A deficiency in bulls, other gross symptoms of deficiency occurred before reproductive failure (Erb et al. 1947; Hodgson et al. 1946). If animals prior to coming on trial had received adequate levels of β-carotene for extended periods of time, β-carotene deficiency as indicated by blood carotene levels during a short trial period may not affect reproductive function. There are variations between the various studies with respect to the β-carotene status of experimental animals before coming on trial and the length of the trial periods.

Another factor in the discrepancy of results between studies is the reliability of blood β-carotene level as an indicator of true deficiency status. The current guidelines for blood β-carotene levels were based entirely on European studies. Variations in carotene-assay methods between experiments and breed differences in β-carotene absorption and accumulation invalidates the applicability of those guidelines to all trials.

Perhaps the most critical reason for differences in reproductive response to β-carotene supplementation might be interaction between β-carotene deficiency and other detrimental factors that affect reproductive efficiency such as prior nutritional status of the heifers and cows and other pre-existing fertility problems. Lotthammer (1979b) has suggested that β-carotene deficiency may aggravate the effects of other common detrimental factors, such as poorly balanced nutrient and mineral ratios and hepatic disorders, so that β-carotene supplementation alone is unlikely to abolish the fertility problem. In some β-carotene studies, particularly the field trials, some management factors such as estrus detection is likely to be improved during the trial period thus resulting in a favourable response to the β-carotene supplementation.

If indeed β-carotene has a specific role in bovine reproductive function, its mechanism of action is not yet known. The results of experiment 3 reported here failed to show any association between β-carotene status and endocrine parameters although others (Schultz et al. 1974; Jackson et al. 1981) have suggested the existence of such an association. Jackson et al. (1981) hypothesized mechanisms by which β-carotene may be involved in ovarian steroidogenesis. They suggested that since blood cholesterol makes a significant contribution to progesterone produced by the bovine CL (Bartosik et al. 1967), β-carotene in association with lipoproteins may be involved in the efficient transfer of cholesterol from blood to luteal tissue. Jackson et al. (1981) indicated that support for their suggestion comes from the correlation between blood levels of β-carotene and cholesterol demonstrated by

Lotthammer and Ahlswede (1977) and the reduced output of progesterone by rat luteal tissue deficient in cholesterol (Everett 1947). In contrast, Greenberg et al. (1985) recently observed no significant (P>.05) effect of β-carotene status in beef heifers on plasma and CL concentrations of cholesterol and progesterone and plasma concentration of lipoproteins. An alternative suggestion by Jackson et al. (1981) was that β-carotene may be involved directly with the early stages of steroidogenesis. A recent demonstration of carotene cleavage activity in bovine CL by Sklan (1983) led to the hypothesis that β-carotene may serve a storage capacity in the CL to be converted to vitamin A when needed by that organ. This hypothesis might explain a possible involvement of β-carotene in steroidogenesis in the CL since vitamin A is believed to be necessary for the synthesis of the adrenocortical hormones glucocorticosteroids and progesterone (Zintzen 1974).

The ultimate question remains under what conditions would β-carotene supplementation be advisable. The results of experiment 1 reported here suggest that animals fed a combination of good hay and silages such as faba-bean and rye silages that are moderately high in β-carotene, even without access to pasture, would maintain good serum β-carotene levels. On the other hand, availability of pasture to animals during the summer months would be more desirable. In practice, β-carotene deficiency is likely to be a problem only if high levels of low-carotene forages, such as corn silage and badly weathered or old hay, are fed for extended periods of time. Therefore β-carotene supplementation might be advisable in herds with inexplicable poor fertility problems when fed diets containing

large quantities of low-carotene forages. Selective monitoring of serum β-carotene concentration in such herds should provide a basis for a decision on the appropriateness of β-carotene supplementation. The results of experiments 2 and 3 suggest that there should be no need for vitamin A in the concentrate ration if β-carotene is being supplemented. At the current cost for β-carotene supplementation, the response would have to be substantial to economically justify carotene supplementation.

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Appendix I

Analysis of variance tables and raw data for Experiment 1

Table 1. Analysis of variance table for serum beta-carotene and vitamin A concentration for the entire experimental period

Source of variation	df	MS	F
Variable: Serum β-carotene			
Season of calving (per cal)	1	5681.1	0.34 NS
Cow (per cal)	14	62806.7	3.8 **
Month	11	343132.7	20.8 **
Month (per cal)	11	24334.0	1.47 NS
Error	154	16509.8	
Total	191		
Variable: Serum vitamin A			
Season of calving (per cal)	1	3.6	0.07 NS
Cow (per cal)	14	534.3	10.28 **
Month	11	582.1	11.20 **
Month (per cal)	11	59.6	1.15 NS
Error	154	51.97	
Total	191		

** $P \leq .01$

NS = Not significant.

Table 2. Analysis of variance table for serum beta-carotene and vitamin A during the calving period

Source of variation	df	MS	F
Variable: Serum β-carotene			
Season of calving	1	246909.6	14.24 **
Error	14	17334.4	
Total	15		
Variable: Serum vitamin A			
Season of calving	1	57.4	1.11 NS
Error	14	51.8	
Total	15		

** $P \leq .01$

NS = Not significant.

Table 3. Analysis of variance table for the reproduction data

Source of variation	df	MS	F
Variable: Interval to ovulation			
Season of calving	1	27.3	0.65 NS
Error	11	41.7	
Total	12		
Variable: Interval to uterine involution			
Season of calving	1	18.1	13.8 **
Error	14	1.31	
Total	15		
Variable: Services per conception			
Season of calving	1	0.0	0.0 NS
Error	13	4.4	
Total	14		
Variable: Interval to first service			
Season of calving	1	10395.3	6.2 *
Error	13	1671.6	
Total	14		
Variable: Days open			
Season of calving	1	31427.4	4.3 NS
Error	13	7238.1	
Total	14		

* $P \leq .05$

** $P \leq .01$

NS = Not significant.

Table 4. Serum β-carotene concentration (µg/100 mL) throughout experimental period

Heifer/cow	Month											
	Oct. 1980	Nov. 1980	Dec. 1980	Jan. 1981	Feb. 1981	Mar. 1981	April 1981	May 1981	June 1981	July 1981	Aug. 1981	Sept. 1981
Group 1: Calving Jan.-April												
1) F. Holly	204	215.2	241.2	120.4	152.4	194	182	273.6	725.6	1016	479.2	731.2
2) C. Ann	364.4	172.4	249.2	280	134.4	210.8	177.6	278.4	537.3	536	318.4	507.6
3) R. Lana	317.5	378.4	442.4	448.8	375.2	196	207.2	252	779.2	469.6	354.4	484.8
4) Valerie	324.8	313.6	446.4	420	382.4	205.6	214.4	314.4	696.8	1132	831.2	1033.6
5) S. Vision	292	341.6	296.8	393.6	274.4	243.2	171.2	209.6	466.4	763.2	567.2	220
6) M. Joanna	391.2	368.8	420	334.4	292	267.2	202.4	270.4	581.6	581.6	426.4	201.6
7) B. Leona	233.6	255.2	277.6	274.4	219.2	155.2	108.8	204.8	528	700	530.4	143.2
8) S. Queen	414.4	469.6	450.4	392.8	320.8	324	238.4	349.6	608	576	538.4	431.2
$\bar{x}$	305.2	314.4	353	330.1	268.9	224.5	187.8	269.1	615.7	721.8	505.7	469.2
Group 2: Calving July-Oct.												
1) P. Beatrice	296	284.8	316.8	404.8	189.6	227.2	271.2	296	512.8	525.6	360.8	429.6
2) Flossy	136	261.6	314.4	296.8	294.4	191.2	309.6	326.4	734.4	963.2	404	415.2
3) Primette	208	232	267.2	246.4	295.2	200.8	175.2	188.8	519.2	248.8	224	392
4) Peggy	244.8	277.6	406.4	349.6	280.8	313.6	463.2	472	955.2	799.2	328	238.4
5) Rosa	372.8	296.8	456.8	420	407.2	338.4	465.6	449.6	928	326.4	426.4	359.2
6) Queenie	300.8	230.4	280	296	264	219.2	333.6	336	519.2	472.8	310.4	209.6
7) Emmy	136.8	203.2	288.8	256	256	235.2	333.6	349.6	432.8	496	360	384.8
8) Royalle	173.6	232.8	272	300	272.8	309.6	397.6	304	673.6	1194.4	744	883.2
$\bar{x}$	233.6	252.4	325.3	321.2	282.5	254.4	343.7	340.3	659.4	628.3	394.7	413.9

Table 5. Serum vitamin A concentration (µg/100 ml) throughout experimental period

Cow/heifer	Month											
	Oct. 1980	Nov. 1980	Dec. 1980	Jan. 1981	Feb. 1981	Mar. 1981	April 1981	May 1981	June 1981	July 1981	Aug. 1981	Sept. 1981
Group 1: Calving Jan.-April												
1) Holly	28.8	30	27.5	27.5	37.5	45	27.5	35	55	50	30	30
2) C. Ann	40	35	27.5	27.5	31	40	37.5	36.3	36.3	37.5	27.5	28.8
3) Lana	57.5	40	55	52.5	50	32.5	42.5	37.5	50	55	57.5	55
4) Valerie	40	42.5	35	42.5	37.5	30	27.5	30	50	50	30	42.5
5) Vision	37.5	40	42.5	35	27.5	35	30	35	42.5	52.5	47.5	35
6) Joanna	40	40	32.5	42.5	35	35	27.5	42.5	70	55	37.5	32.5
7) Leona	40	35	32.5	32.5	27.5	30	27.5	35	65	57.5	47.5	40
8) S. Queen	25	40	35	35	25	32.5	25	32.5	42.5	40	37.5	27.5
$\bar{x}$	38.6	37.8	35.9	36.9	33.9	35	30.6	35.5	51.4	49.7	39.4	36.4
Group 2: Calving July-Oct.												
1) Beatrice	37.5	35	47.5	37.5	27.5	35	20	37.5	45	37.5	27.5	35
2) Flossy	20	35	27.5	27.5	27.5	25	32.5	35	45	42.5	50	35
3) Primette	32.5	37.5	35	35	27.5	25	22.5	35	50	30	27.5	40
4) Peggy	35	35	42.5	35	32.5	27.5	27.5	35	55	55	30	22.5
5) Rosa	62.5	62.5	50	55	60	30	45	37.5	80	35	35	32.5
6) Queenie	32.5	32.5	27.5	30	30	20	20	27.5	45	40	27.5	20
7) Emmy	40	50	37.5	30	32.5	27.5	35	45	55	40	27.5	22.5
8) Royalle	50	55	45	42.5	50	45	55	52.5	57.5	65	67.5	42.5
$\bar{x}$	38.8	42.8	39.1	36.6	35.9	26.3	32.2	38.1	54.1	43.1	36.6	31.3

Table 6. Serum beta-carotene concentration (µg/100 mL) during the calving period

Heifer/cow	Month of calving	Month prepartum 1	Month postpartum 1	Month postpartum 2	Mean
Group 1:					
F. Holly	Jan.'81	241.2	120.4	152.4	171.3
C. Ann	" "	249.2	280	134.4	221.2
R. Lana	Mar.'81	375.2	196	207.2	259.5
Valerie	" "	382.4	205.6	214.4	267.5
S. Vision	April '81	243.2	171.2	209.6	208
M. Joanna	" "	267.2	202.4	270.4	246.7
B. Leona	" "	155.2	108.8	204.8	156.3
S. Queen	" "	324	238.4	349.6	304
Group 2:					
P. Beatrice	Aug. '81	525.6	360.8	429.6	438.7
Flossy	Sept.'81	404	415.2	-	409.6
Primette	July '81	519.2	248.8	224	330.6
Peggy	Aug. '81	799.2	328	238.4	455.2
Rosa	July '81	928	326.4	426.4	560.3
Queenie	Aug. '81	472.8	310.4	209.6	330.9
Emmy	Aug. '81	496	360	384.8	413.6
Royalle	Oct. '81	883.2	-	-	883.2

Table 7. Serum vitamin A concentration (µg/100 ml) during the calving period

Heifer/cow	Month of calving	Month prepartum	Month postpartum		
		1	1	2	Mean
Group 1:					
F. Holly	Jan.'81	27.5	27.5	37.5	32.5
C. Ann	" "	27.5	27.5	31	29.3
R. Lana	Mar.'81	50	32.5	42.5	41.7
Valerie	" "	37.5	30	27.5	31.7
S. Vision	April '81	35	30	35	32.5
M. Joanna	" "	35	27.5	42.5	35
B. Leona	" "	30	27.5	35	30.8
S. Queen	" "	32.5	25	32.5	30
Group 2:					
P. Beatrice	Aug. '81	37.5	27.5	35	33.3
Flossy	Sept.'81	27.5	35	-	31.3
Primette	July '81	45	30	27.5	34.2
Peggy	Aug. '81	55	30	22.5	35.8
Rosa	July '81	80	35	35	57.5
Queenie	Aug. '81	40	27.5	20	29.2
Emmy	" "	40	27.5	22.5	30
Royalle	Oct. '81	42.5	-	-	42.5

Table 8. Reproduction data

Cow	*Interval to 1st ovulation postpartum (days)	**Interval to uterine involution (weeks)	Services per conception	Interval to 1st service (days)	Days open
Group 1: Calving period - Jan.-April 1981					
1) F. Holly	21	4	7	108	286
2) C. Ann	18	7	–	–	–
3) R. Lana	28	6	2	117	218
4) O. Valerie	33	6	1	99	99
5) S. Vision	21	5	1	100	100
6) M. Joanna	–	9	2	160	221
7) B. Leona	–	7	5	243	385
8) S. Queen	15	6	2	77	98
$\bar{x}$	22.7	6.3	2.9	129.2	201
Group 2: Calving period - July-Oct. 1981					
1) P. Beatrice	30	4	2	72	106
2) L. Flossy	16	4	5	99	127
3) D. Primette	31	5	1	52	52
4) N. Peggy	19	4	6	94	222
5) M. Rosa	28	4	1	83	83
6) S. Queenie	32	5	3	69	113
7) S. Emmy	23	4	1	47	47
8) L. Royalle	–	3	4	95	124
$\bar{x}$	25.3	4.1	2.9	76.4	109.3

*Based on milk progesterone profiles.

**Uterine involution assessed via rectal palpation.

Appendix II

Analysis of variance tables and raw data for Experiment 2

Table 1. Analysis of variance table for serum beta-carotene and vitamin A concentration

		Serum β-carotene		Serum vitamin A	
Source of variation	df	MS	F	MS	F
Rat	2	96485	1.2 NS	209.7	0.8 NS
βcar	1	4613461	59.3 **	269.4	1.1 NS
Ratxβcar	2	302915	3.9 *	609.9	2.4 NS
Tram	1	102538	1.3 NS	34.1	0.1 NS
RatxTram	2	135024	1.7 NS	140.2	0.6 NS
βcarxTram	1	235869	3.0 NS	74.3	0.3 NS
RatxβcarxTram	2	189777	2.4 NS	199.1	0.8 NS
Cow (RatxβcarxTram)					
Error a	25	77806		249.4	
Week	9	346504	31.4 **	820.3	27.8 **
RatxWeek	18	14565	1.3 NS	45.3	1.5 NS
βcarxWeek	9	335850	30.4 **	53.5	1.8 NS
RatxβcarxWeek	18	14160	1.3 NS	40.8	1.4 NS
TramxWeek	9	3348	0.3 NS	14.8	0.5 NS
RatxTramxWeek	18	16254	1.5 NS	37.2	1.3 NS
βcarxTramxWeek	9	4920	0.4 NS	26.2	0.9 NS
RatxβcarxTramxWeek	18	6253	0.6 NS	16.1	0.5 NS
Error b	210	11034		29.5	

* $P \leq .05$

** $P \leq .01$

NS = Not significant.

Table 2. Analysis of variance table for interval to ovulation and interval to uterine involution

Source of variation	Interval to ovulation			Interval to uterine involution		
	df	MS	F	df	MS	F
Rat	2	70.4	0.9 NS	2	0.38	0.2 NS
βcar	1	106.7	1.4 NS	1	7.3	3.2 *
Ratxβcar	2	55.6	0.7 NS	2	2.0	0.9 NS
Tram	1	0.5	0.01NS	1	7.3	3.2 *
RatxTram	2	22.9	0.3 NS	2	0.7	0.3 NS
βcarxTram	1	264.2	3.5 NS	1	4.0	1.7 NS
RatxβcarxTram	2	16.2	0.2 NS	2	0.6	0.3 NS
Error	22	74.8		25	2.3	
Total	33			36		

* $P \leq .01$

NS = Not significant.

Table 3. Analysis of variance table for services per conception and days open

Source of variation	df	Services per conception		Days open	
		MS	F	MS	F
Rat	2	0.7	0.5 NS	1068	0.8 NS
βcar	1	1.1	0.8 NS	901	0.6 NS
Ratxβcar	2	0.4	0.3 NS	3798	2.7 NS
Tram	1	1.9	1.5 NS	4052	2.9 NS
RatxTram	2	2.3	1.8 NS	2828	2.0 NS
βcarxTram	1	0.8	0.7 NS	388	0.3 NS
RatxβcarxTram	2	0.5	0.4 NS	286	0.2 NS
Error	20	1.3		1395	
Total	31				

NS = Not significant.

Table 4. Analysis of variance table for somatic cell counts

Source of variation	df	MS	F
Rat	2	2080639	1.4 NS
βcar	1	3262336	2.2 NS
Ratxβcar	2	508588	0.3 NS
Tram	1	3170583	2.1 NS
RatxTram	2	1077561	0.7 NS
βcarxTram	1	3846907	2.6 NS
RatxβcarxTram	2	1851718	1.2 NS
Cow (RatxβcarxTram)			
Error a	25	1501795	
Week	3	13219647	8.0 **
RatxWeek	6	649160	0.4 NS
βcarxWeek	3	2156122	1.3 NS
Ratx βcarx Week	6	280112	0.2 NS
TramxWeek	3	1075405	0.6 NS
RatxTramxWeek	6	648439	0.4 NS
βcarxTramxWeek	3	3512523	2.1 NS
RatxβcarxTramxWeek	6	1061283	0.6 NS
Error b	75	1659333	

** $P \leq .01$

NS = Not significant.

Table 5. Analysis of variance table for MY, MF and FCM

Source of variation	df	MY		MF		FCM	
		MS	F	MS	F	MS	F
Rat	2	2024	2.9 NS	0.6	1.2 NS	705	1.7 NS
βcar	1	324	0.5 NS	0.2	0.4 NS	208	0.5 NS
Ratxβcar	2	281	0.4 NS	0.2	0.4 NS	184	0.4 NS
Tram	1	2442	3.6 NS	0.3	0.6 NS	802	1.9 NS
RatxTram	2	725	1.1 NS	0.5	1.0 NS	370	0.9 NS
βcarxTram	1	444	0.6 NS	0.2	0.4 NS	215	0.5 NS
RatxβcarxTram	2	80	0.1 NS	0.08	0.2 NS	62.7	0.2 NS
Cow (RatxβcarxTram) Error a	25	680		0.5		410	
Week	11	67	17.7 **	.04	1.8 *	8.2	1.1 NS
RatxWeek	22	9.9	2.6 **	.015	0.7 NS	4.0	0.6 NS
βcarxWeek	11	3.2	0.8 NS	.04	1.8 *	10.6	1.5 NS
Ratxβcarxweek	22	2.9	0.7 NS	0.043	1.9 **	13.3	1.9 **
TramxWeek	11	1.1	0.3 NS	0.01	0.4 NS	2.0	0.3 NS
RatxTramxWeek	22	2.3	0.6 NS	0.013	0.6 NS	2.9	0.4 NS
βcarxTramxWeek	11	8.0	2.1 *	0.018	0.8 NS	7.3	1.0 NS
RatxβcarxTramxWeek	22	3.3	0.9 NS	0.03	1.3 NS	7.1	1.0 NS
Error b	275	3.8		.022		7.0	

* $P \leq .05$

** $P \leq .01$

NS = Not significant.

Table 6. Serum ß-carotene concentration (µg/100 mL) throughout experimental period

	Weeks relative to parturition									
	Prepartum				Postpartum					
Heifer/cow	-4	-3	-2	-1	2	4	6	8	10	12
Low carotene group										
Control diet/no Tramisol										
B. Royal	689.6	448.8	324.8	224	76	88	64	54.4	79.2	126.4
Monarch	-	322.4	212.8	156	80.8	107.2	169.6	172	233	292.8
Norma	348	251.2	149.6	89.6	136.8	140	172	184.8	216	208.8
Control diet/Tramisol										
Patsy	-	-	775.2	476	180.8	105.6	89.6	105.6	146.4	146.4
S. Visigoth	280	216	151.2	108	96.8	72	60.8	85.6	129.6	109.6
Wanda	388.8	300.8	183.2	147.2	140.8	149.6	172	150.4	194.4	165.6
J. Visigoth	-	-	-	-	490.4	395.2	213.6	313.6	271.2	280
Extruded canola/no Tramisol										
Laura	334.4	293.6	197.6	201.6	110.4	102.4	81.6	142.4	179.4	190.4
Anyra	412.8	270.4	179.2	106.4	87.2	78.4	103.2	130.4	200.8	184.8
Holly	745.6	424.8	332.8	230.4	126.4	101.6	112	97.6	110.4	138.4
Extruded canola/Tramisol										
S. Kathy	347.2	258.4	211.2	167.2	72.8	105.6	282.4	309.6	302.4	223.2
Bebe	227.2	99.2	75.2	77.6	118.4	112	116	104	147.2	164
J. Queen	348	253.6	172.8	111.2	108	102.4	116.8	180.8	181.6	136.6
Whole seed canola/no Tramisol										
Vivan	-	638.4	384	228.8	160	104	163.2	210.4	328.8	322.4
J. Leona	827.2	694.4	469.6	309.6	237.6	256	264.8	344	293.6	366.4
Rosa	412.8	336	314.4	217.6	187.2	275.2	307.2	312	286.4	304.8
Whole seed canola/Tramisol										
Lorna	-	665.6	315.2	184.8	191.2	184.8	181.6	167.2	250	311
S. Rose	395.2	308	196	128	92	131.2	140.8	147.2	142.4	175.2
T. Bonny	680	561.6	400.8	259.2	162.4	178.4	180.8	236.8	184	191.2
Mean	459.8	379.0	280.3	190.2	150.3	146.8	157.5	181.5	204.0	212.5
Carotene supplemented group										
Control diet/no Tramisol										
Empress	357.6	283.2	283.2	227.2	196.8	272.0	281.6	332	313.6	336
Fern	364.8	239.2	190.4	172	193.6	220	328	413.6	564.8	572
Joyce	-	-	251.2	216.8	378.4	408.8	826.4	906.4	1036	1226
Control diet/Tramisol										
Lenorette	529.6	364.8	329.6	269.6	194.4	278.4	479.2	422.4	401.6	388
Lorell	-	-	-	-	260.8	262.4	262.4	276	379.2	438.4
Joan	654.4	590.4	483.2	289.6	273.6	512	688	743.2	725.6	733.6
Extruded canola/no Tramisol										
Lottie	630.4	392	280	198.4	322.4	313.6	544.8	463.2	573.6	591.2
Wendy	310.4	355.2	296.8	273.6	219.2	191.2	248.8	563.2	678.4	806.4
Emmeline	336.8	300.8	274.4	224	236	313.6	504	579.2	692.8	714.4
Extruded canola/Tramisol										
Lea	1057.6	959.2	724	571.2	284	356.8	668	794.4	924	950.4
Arlene	498.4	431.2	384	317.6	428.8	576.8	785.6	1052	1215	1224
Nettie	444	354.4	375.2	332	320	366.4	583.2	843.2	968	1005
Whole seed canola/no Tramisol										
Lanie	392	420	397.6	327.2	280	461.6	582.4	502.4	626.4	802.4
Della	628	444	331.2	236	180.8	445.6	595.2	615.2	715.2	869.6
Patti	328	304	251.2	240.8	320	338.4	472.8	491.2	444.8	530.4
Whole seed canola/Tramisol										
Roxy	566.4	430.4	392.8	384	483.2	508	655.2	665.6	676	870.4
S. Leona	556	380.4	288	242.4	353.6	530.4	542.4	674.4	640	788
S. Regal	388	168.8	228	250.4	369.6	340	443.2	465.6	558.4	657.6
Mean	502.7	401.1	338.9	280.8	294.2	372	527.2	600.2	674.1	750.2

Table 7. Serum vitamin A concentration (μg/100 mL) throughout experimental period

	Weeks relative to parturition									
	Prepartum				Postpartum					
Heifer/cow	4	3	2	1	2	4	6	8	10	12
Low carotene group										
Control diet/no Tramisol										
B. Royal	32.5	32.5	27.5	42.5	32.5	47.5	55	50	55	50
Monarch	-	27.5	27.5	25	25	27.5	35	37.5	35	40
Norma	35	40	32.5	20	20	35	42.5	32.5	42.5	30
Control diet/Tramisol										
Patsy	-	-	35	35	35	50	42.5	42.5	55	55
S. Visigoth	27.5	27.5	35	35	35	30	30	35	37.5	32.5
Wanda	25.0	22.5	20	20	25.0	27.5	27.5	30	30	35
J. Visigoth	-	-	-	-	47.5	50	50	50	42.5	50
Extruded canola/no Tramisol										
Laura	22.5	25	22.5	37.5	35	27.5	25	27.5	27.5	25
Amyra	27.5	25	37.5	32.5	35	42.5	35	40	35	37.5
Holly	20	20	20	20	22.5	45	30	40	40	37.5
Extruded canola/Tramisol										
S. Kathy	20	22.5	25	20	22.5	27.5	25	30	27.5	27.5
Bebe	35	32.5	32.5	35.0	30	42.5	42.5	40	42.5	42.5
J. Queen	27.5	30	30	27.5	32.5	30	30	37.5	37.5	37.5
Whole seed canola/no Tramisol										
Vivian	-	30	30	22.5	20	22.5	30	27.5	32.5	27.5
J. Leona	52.5	50	35	30	27.5	27.5	27.5	27.5	50	47.5
Rosa	42.5	40	37.5	27.5	35	37.5	35	42.5	47.5	42.5
Whole seed canola/Tramisol										
Lorna	-	25	22.5	20	27.5	30	27.5	42.5	52.5	50
S. Rose	32.5	42.5	40	35	35	35	42.5	35	40	32.5
T. Bonny	22.5	20	22.5	22.5	25	27.5	25	27.5	27.5	27.5
Mean	30.2	30.1	29.4	26.4	29.9	34.9	34.6	36.6	39.9	38.3
Carotene supplemented group										
Control diet/no Tramisol										
Empress	27.5	22.5	27.5	25	27.5	27.5	27.5	32.5	35	30
Fern	27.5	22.5	27.5	25	25	47.5	35	40	37.5	52.5
Joyce	-	-	27.5	27.5	27.5	32.5	42.5	42.5	50	52.5
Control diet/Tramisol										
Lenorette	25	22.5	22.5	22.5	27.5	35	35	50	55	45
Lorell	-	-	-	-	25	25	27.5	27.5	40	35
Joan	35	27.5	27.5	22.5	25	30	35	32.5	32.5	35
Extruded canola/no Tramisol										
Lottie	20	22.5	20	22.5	35	30	27.5	35	35	35
Wendy	20	20	22.5	20	25	37.5	37.5	42.5	42.5	42.5
Emmeline	35	35	35	35	27.5	30	32.5	37.5	40	52.5
Extruded canola/Tramisol										
Lea	50	32.5	27.5	27.5	27.5	35	42.5	42.5	55	55
Arlene	30	27.5	27.5	27.5	32.5	42.5	35	55	52.5	52.5
Nettie	30	35	25	30	32.5	37.5	35	37.5	35	42.5
Whole seed canola/no Tramisol										
Lanie	22.5	22.5	27.5	25	22.5	25	25	22.5	32.5	35
Della	30	27.5	22.5	35	30	27.5	30	27.5	35	35
Patti	27.5	25	22.5	27.5	27.5	32.5	30	30	27.5	30
Whole seed canola/Tramisol										
Roxy	27.5	22.5	22.5	25	27.5	32.5	27.5	27.5	37.5	35
S. Leona	30	22.5	22.5	20	25	27.5	35	35	40	35
S. Regal	25	22.5	30	22.5	40	37.5	37.5	37.5	37.5	35
Mean	28.9	25.6	25.7	25.9	28.3	32.9	33.2	36.4	40	40.8

Table 8. Reproduction data

Heifer/cow	Interval to ovulation (days)	Interval to uterine involution (weeks)	Days open	Services/conception
Low carotent group				
Control diet/no Tramisol				
B. Royal	17	9	-	-
Monarch	14	5	95	2
Norma	40	4	60	1
Control diet/Tramisol				
Patsy	16	4	122	3
S. Visigoth	13	3	93	2
Wanda	21	6	103	3
J. Visigoth	-	3	115	2
Extruded canola/no Tramisol				
Laura	20	6	177	4
Amyra	36	7	87	1
Holly	25	5	114	3
Extruded canola/Tramisol				
S. Kathy	17	7	217	5
Bebe	15	4	131	2
J. Queen	38	3	172	2
Whole seed canola/no Tramisol				
Vivian	35	7	125	2
J. Leona	25	5	132	3
Rosa	24	4	-	-
Whole seed canola/Tramisol				
Lorna	13	5	89	2
S. Rose	31	4	141	2
T. Bonny	19	3	65	1
$\bar{x}$	23.3	4.95	119.9	2.3
Carotene supplemented group				
Control diet/no Tramisol				
Empress	13	7	-	-
Fern	20	4	130	2
Joyce	26	3	90	1
Control diet/Tramisol				
Lenorette	13	4	207	4
Lorell	13	6	94	2
Joan	33	3	103	1
Extruded canola/no Tramisol				
Lottie	26	5	66	1
Wendy	13	4	75	1
Emmeline	22	3	-	-
Extruded canola/Tramisol				
Lea	-	3	147	4
Arlene	24	2	-	-
Nettie	30	4	115	2
Whole seed canola/no Tramisol				
Lanie	13	4	110	2
Della	7	4	68	1
Patti	13	4	114	3
Whole seed canola/Tramisol				
Roxy	17	6	84	1
S. Leona	-	4	161	3
S. Regal	24	4	64	1
$\bar{x}$	19.2	4.1	108.5	1.9

Table 9. Data on incidence of reproductive and other disorders

Heifer/cow	Ovarian cyst	Metritis	Lost fetus	Puss discharge	Retained placenta	Mastitis
Low carotene group						
Control diet/no Tramisol						
B. Royal	Y	Y	Y	N	N	Y
Monarch	Y	N	N	N	N	N
Norma	N	N	Y	Y	N	Y
Control diet/Tramisol						
Patsy	N	N	Y	N	Y	N
S. Visigoth	N	N	N	N	N	N
Wanda	N	N	N	Y	N	Y
J. Visigoth	Y	N	N	N	N	N
Extruded canola/no Tramisol						
Laura	N	Y	N	N	N	N
Amyra	Y	N	N	N	N	N
Holly	Y	N	N	Y	N	N
Extruded canola/Tramisol						
S. Kathy	N	Y	N	N	N	N
Bebe	N	N	N	N	N	N
J. Queen	Y	N	N	N	N	N
Whole seed canola/no Tramisol						
Vivian	N	N	N	N	N	Y
J. Leona	N	N	N	N	N	Y
Rosa	N	N	N	N	N	N
Whole seed canola/Tramisol						
Lorna	N	N	N	N	N	N
S. Rose	N	N	N	N	N	N
T. Bonny	N	N	N	Y	N	Y
Overall incidence (%)	31.6	15.8	15.8	21.1	5.3	31.6
Carotene supplemented group						
Control diet/no Tramisol						
Empress	N	N	N	N	N	N
Fern	N	N	N	Y	N	N
Joyce	N	N	N	N	N	N
Control diet/Tramisol						
Lenorette	Y	N	N	N	N	N
Lorell	N	N	N	Y	Y	N
Joan	N	N	N	N	N	N
Extruded canola/no Tramisol						
Lottie	N	N	N	N	N	N
Wendy	N	N	N	N	N	N
Emmeline	N	N	N	Y	N	Y
Extruded canola/Tramisol						
Lea	N	N	N	N	Y	N
Arlene	Y	N	N	N	N	N
Nettie	N	N	N	N	N	N
Whole seed canola/no Tramisol						
Lanie	N	N	N	N	N	N
Della	N	N	N	N	N	Y
Patti	N	N	N	N	N	N
Whole seed canola/Tramisol						
Roxy	N	N	N	N	N	N
S. Leona	N	N	N	N	N	N
S. Regal	N	N	N	N	N	N
Overall incidence (%)	11.1	0	0	16.7	11.1	11.1

Y = Yes

N = No

Table 10. Somatic cell counts per ml of milk (x 1000)

Heifer/cow	Weeks postpartum				$\bar{x}$
	Week 1	Week 4	Week 8	Week 12	
Low carotene group					
Control diet/no Tramisol					
B. Royal	3200	490	81	80	962.8
Monarch	160	70	50	90	92.5
Norma	3500	490	150	100	1060
Control diet/Tramisol					
Patsy	820	170	120	80	297.5
S. Visigoth	260	130	110	120	155
Wanda	760	80	80	140	265
J. Visigoth	270	150	73	300	198.3
Extruded canola/no Tramisol					
Laura	1100	110	150	290	412.5
Amyra	160	80	160	93	123.3
Holly	3700	280	90	86	1039
Extruded canola/Tramisol					
S. Kathy	3000	190	80	54	831
Bebe	1600	210	110	46	491.5
J. Queen	370	120	100	110	175
Whole seed canola/no Tramisol					
Vivian	14000	650	310	410	3842.5
J. Leona	210	3800	110	180	1075
Rosa	180	150	90	180	150
Whole seed canola/Tramisol					
Lorna	600	90	60	120	217.5
S. Rose	530	380	120	160	297.5
T. Bonny	480	170	280	1100	507.5
Carotene supplemented group					
Control diet/no Tramisol					
Empress	490	140	130	320	270
Fern	110	50	50	100	77.5
Joyce	1200	70	200	130	400
Control diet/Tramisol					
Lenorette	2500	130	70	60	690
Lorell	160	340	110	60	167.5
Joan	1500	170	100	70	460
Extruded canola/no Tramisol					
Lottie	330	90	100	110	157.5
Wendy	340	110	90	270	202.5
Emmeline	500	900	640	690	682.5
Extruded canola/Tramisol					
Lea	150	120	80	90	110
Arlene	250	60	90	30	107.5
Nettie	600	110	210	290	302.5
Whole seed canola/no Tramisol					
Lanie	2100	290	240	230	715
Della	670	140	400	490	425
Patti	450	140	120	210	230
Whole seed canola/Tramisol					
Roxy	1100	140	210	130	395
S. Leona	910	100	70	70	287.5
S. Regal	3000	290	160	190	910

Table 11. Data for MY, MF and FCM

Index

Ration 1 (Rat = 1) - Control ration
Ration 2 (Rat = 2) - Extruded canola
Ration 3 (Rat = 3) - Whole seed canola
B1 - Low carotene group
B2 - Carotene supplemented group
T1 - No Tramisol
T2 - Tramisol treatment

Cow/heifer

1 - B. Royal; 2 - Monarch; 3 - Norma
4 - Patsy; 5 - S. Visigoth; 6 - J. Visigoth; 37 - Wanda
13 - Laura; 14 - Amyra; 15 - Holly
16 - Kathy; 17 - Bebe; 18 - J. Queen
26 - Vivian; 27 - J. Leona; 25 - Rosa
28 - Lorna; 29 - S. Rose; 30 - T. Bonny

7 - Empress; 8 - Fern; 9 - Joyce
10 - Lenorett; 11 - Lorell; 12 - Joan
20 - Lottie; 21 - Wendy; 19 - Emmeline
24 - Lea; 22 - Arlene; 23 - Nettie
31 - Lanie; 32 - Della; 33 - Patti
34 - Roxy; 35 - S. Leona; 36 - Regal

Treatment combination

B1 - R1 - T1
B1 - R1 - T2
B1 - R2 - T1
B1 - R2 - T2
B1 - R3 - T1
B1 - R3 - T2

B2 - R1 - T1
B2 - R1 - T2
B2 - R2 - T1
B2 - R2 - T2
B2 - R3 - T1
B2 - R3 - T2

RAT=1

COW	WEEK	MY	MF	FCM
1	1	16.72	0.771	18.25
1	2	20.19	0.755	19.41
1	3	24.58	0.595	18.75
1	4	26.10	0.736	21.48
1	5	27.82	0.924	24.99
1	6	27.37	0.772	22.53
1	7	28.70	0.921	25.30
1	8	28.73	0.928	25.42
1	9	29.45	0.984	26.53
1	10	28.80	1.063	27.46
1	11	29.12	1.060	27.55
1	12	28.83	1.072	27.62
2	1	16.40	0.479	13.74
2	2	18.31	0.626	16.72
2	3	18.93	0.697	18.02
2	4	20.55	0.674	18.33
2	5	18.86	0.587	16.35
2	6	18.54	0.393	13.31
2	7	17.99	0.622	16.53
2	8	18.05	0.484	14.48
2	9	17.34	0.940	21.03
2	10	16.92	0.613	15.95
2	11	16.98	0.530	14.74
2	12	16.85	0.637	16.29
3	1	15.26	0.751	17.37
3	2	21.23	0.707	19.10
3	3	21.59	0.915	22.37
3	4	24.35	0.833	22.23
3	5	24.61	1.196	27.79
3	6	23.54	0.899	22.90
3	7	23.57	0.990	24.28
3	8	25.26	0.909	23.74
3	9	21.92	0.846	21.46
3	10	21.20	1.018	23.75
3	11	21.69	0.863	21.62
3	12	21.95	0.817	21.03
4	1	19.87	0.775	19.57
4	2	22.66	0.700	19.57
4	3	22.50	0.428	15.41
4	4	21.85	0.426	15.13
4	5	22.08	0.671	18.90
4	6	23.08	0.667	19.24
4	7	23.25	0.665	19.27
4	8	22.40	0.688	19.28
4	9	20.00	0.480	15.20
4	10	20.55	0.649	17.96
4	11	20.71	0.576	16.92
4	12	19.74	0.529	15.83
5	1	18.57	0.724	18.29
5	2	20.58	0.677	18.39
5	3	21.33	0.663	18.48
5	4	22.01	0.742	19.93
5	5	23.25	0.678	20.04
5	6	24.54	0.785	21.60

RAT=1

COW	WEEK	MY	MF	FCM
5	7	26.01	0.749	21.64
5	8	25.46	0.789	22.02
5	9	25.52	0.852	22.99
5	10	24.84	0.753	21.22
5	11	22.60	0.755	20.36
5	12	24.94	0.698	20.45
6	1	37.80	0.805	27.21
6	2	39.19	1.262	34.60
6	3	40.55	1.322	36.05
6	4	39.35	0.582	24.48
6	5	40.58	0.722	27.07
6	6	40.81	0.604	25.38
6	7	39.09	0.735	26.66
6	8	38.99	0.795	27.53
6	9	38.21	0.657	25.14
6	10	38.02	0.989	30.04
6	11	33.47	0.844	26.04
6	12	30.81	0.786	24.11
7	1	25.36	0.913	23.84
7	2	24.38	0.873	22.85
7	3	25.32	0.861	23.05
7	4	24.90	0.687	20.27
7	5	24.35	0.881	22.96
7	6	24.03	0.904	23.16
7	7	24.42	0.784	21.52
7	8	25.39	0.642	19.79
7	9	26.20	0.679	20.66
7	10	24.84	0.805	22.01
7	11	27.05	0.741	21.93
7	12	27.73	0.821	23.40
8	1	18.70	0.626	16.88
8	2	19.06	0.431	14.08
8	3	19.22	0.336	12.73
8	4	20.23	0.817	20.35
8	5	19.80	0.665	18.80
8	6	20.58	0.650	17.99
8	7	19.84	0.678	18.11
8	8	19.64	0.621	17.17
8	9	19.68	0.679	18.05
8	10	20.46	0.661	18.09
8	11	20.68	0.695	18.70
8	12	19.90	0.681	18.17
9	1	34.71	1.194	31.79
9	2	35.78	1.145	31.49
9	3	36.40	1.034	30.06
9	4	36.98	1.287	43.10
9	5	36.10	1.101	30.96
9	6	35.75	1.194	32.21
9	7	34.58	1.245	32.50
9	8	31.98	0.982	27.52
9	9	31.46	1.151	29.86
9	10	30.23	0.937	26.15
9	11	29.03	0.810	23.76
9	12	28.38	1.084	27.61

RAT=1

COW	WEEK	MY	MF	FCM
10	1	19.42	0.980	22.47
10	2	21.69	0.772	20.26
10	3	22.44	0.713	19.68
10	4	23.08	0.801	21.25
10	5	23.93	0.636	19.12
10	6	23.86	0.692	19.93
10	7	23.54	0.640	19.02
10	8	23.31	0.937	23.38
10	9	23.86	0.835	22.07
10	10	24.06	0.818	21.89
10	11	23.57	0.646	19.12
10	12	24.19	0.750	20.92
11	1	15.81	0.756	17.66
11	2	17.30	0.703	17.46
11	3	17.86	0.661	17.05
11	4	17.04	0.590	15.66
11	5	18.47	0.567	15.90
11	6	17.73	0.606	16.18
11	7	17.92	0.690	17.52
11	8	17.53	0.726	17.90
11	9	17.34	0.641	16.56
11	10	17.89	0.801	19.18
11	11	17.89	0.805	19.23
11	12	17.63	0.730	18.00
12	1	36.62	1.300	34.15
12	2	38.41	1.041	30.98
12	3	40.26	1.091	32.47
12	4	41.36	1.382	37.27
12	5	41.27	0.974	31.11
12	6	42.24	0.803	28.93
12	7	41.95	0.931	30.75
12	8	42.08	0.812	29.01
12	9	41.75	0.969	31.23
12	10	41.66	1.029	32.10
12	11	40.88	0.801	28.37
12	12	39.38	0.945	29.93
37	1	15.49	0.623	15.53
37	2	17.99	0.540	15.29
37	3	19.16	0.632	17.14
37	4	18.67	0.780	19.17
37	5	17.79	0.578	15.79
37	6	19.29	0.816	19.95
37	7	19.42	0.699	18.25
37	8	18.34	0.737	18.40
37	9	19.03	0.719	18.40
37	10	18.31	0.586	16.11
37	11	19.51	0.730	18.75
37	12	19.06	0.607	16.71

RAT=2

COW	WEEK	MY	MF	FCM
13	1	24.71	0.830	22.34
13	2	26.98	0.850	23.54
13	3	26.53	0.753	21.91
13	4	27.04	0.982	25.54
13	5	26.92	0.619	20.05
13	6	25.29	0.703	20.66
13	7	26.27	0.783	22.25
13	8	27.70	0.676	21.21
13	9	27.99	0.677	21.39
13	10	25.26	0.722	20.94
13	11	27.60	0.690	21.39
13	12	28.93	0.723	22.42
14	1	15.84	0.602	15.37
14	2	18.86	0.619	16.83
14	3	20.03	0.587	16.82
14	4	20.68	0.625	17.64
14	5	21.75	0.626	18.10
14	6	20.55	0.668	18.24
14	7	22.83	0.628	18.55
14	8	23.47	0.559	17.77
14	9	22.53	0.622	18.34
14	10	23.96	0.616	18.82
14	11	21.79	0.538	16.79
14	12	22.66	0.721	19.87
15	1	25.20	1.144	27.24
15	2	28.44	0.865	24.35
15	3	32.53	0.852	25.80
15	4	35.81	0.945	28.51
15	5	36.20	0.862	27.40
15	6	35.88	0.796	26.30
15	7	36.14	0.820	26.76
15	8	35.52	0.881	27.42
15	9	37.24	0.935	28.92
15	10	38.57	0.806	27.52
15	11	37.01	0.744	25.96
15	12	35.10	0.927	27.94
16	1	29.29	1.315	31.44
16	2	33.67	0.862	26.40
16	3	37.40	1.264	33.92
16	4	44.58	1.337	37.89
16	5	43.31	0.931	31.29
16	6	40.68	0.602	25.30
16	7	44.29	1.036	33.26
16	8	44.87	1.382	38.68
16	9	43.99	1.095	34.03
16	10	44.22	1.229	36.13
16	11	43.28	1.021	32.63
16	12	44.32	1.232	36.21
17	1	27.70	1.440	32.68
17	2	29.06	0.912	25.31
17	3	31.07	0.945	26.60
17	4	33.64	0.794	25.36
17	5	34.58	0.512	21.51
17	6	33.34	0.380	19.04

RAT=2

COW	WEEK	MY	MF	FCM
17	7	33.54	0.610	22.57
17	8	33.54	0.594	22.32
17	9	33.21	0.717	24.05
17	10	32.44	0.629	22.41
17	11	32.66	0.608	22.18
17	12	30.42	0.773	23.76
18	1	32.53	1.061	28.92
18	2	32.70	1.107	29.71
18	3	33.96	1.032	29.07
18	4	35.10	0.821	26.36
18	5	34.48	0.907	27.39
18	6	35.75	1.451	36.07
18	7	35.62	0.876	27.39
18	8	34.38	0.949	27.99
18	9	36.23	0.616	23.73
18	10	35.26	1.026	29.49
18	11	28.80	0.783	23.27
18	12	31.46	0.488	19.90
19	1	30.03	0.997	26.97
19	2	35.55	0.811	26.38
19	3	40.23	0.969	30.63
19	4	38.18	1.191	33.14
19	5	39.32	0.896	29.17
19	6	40.03	1.017	31.27
19	7	41.20	0.849	29.21
19	8	41.14	1.045	32.12
19	9	42.56	0.818	29.28
19	10	42.79	0.920	30.92
19	11	42.18	0.877	30.03
19	12	38.99	0.873	28.70
20	1	18.38	0.731	18.32
20	2	17.96	0.707	17.79
20	3	19.61	0.686	18.14
20	4	22.37	0.846	21.63
20	5	23.15	0.810	21.41
20	6	23.67	0.497	16.92
20	7	22.73	0.532	17.07
20	8	23.02	0.826	21.60
20	9	23.83	0.827	21.94
20	10	23.08	0.845	21.91
20	11	23.67	0.750	20.72
20	12	23.80	0.505	17.09
21	1	23.25	0.814	21.50
21	2	22.08	0.857	21.68
21	3	23.96	0.819	21.88
21	4	24.94	0.913	23.66
21	5	25.68	0.814	22.48
21	6	26.36	0.962	24.98
21	7	26.98	0.842	23.42
21	8	26.14	0.944	24.61
21	9	28.15	0.954	25.57
21	10	28.41	0.920	25.17
21	11	26.62	0.980	25.35
21	12	27.76	0.955	25.43

RAT=2

COW	WEEK	MY	MF	FCM
22	1	34.54	0.912	27.50
22	2	39.32	0.794	27.64
22	3	39.54	1.068	31.83
22	4	38.38	0.764	26.81
22	5	42.21	1.017	32.14
22	6	43.34	0.828	29.76
22	7	41.56	0.781	28.34
22	8	41.46	0.597	25.54
22	9	43.47	1.000	32.39
22	10	43.70	0.861	30.39
22	11	42.53	0.808	29.13
22	12	41.88	0.854	29.57
23	1	40.13	1.011	31.22
23	2	42.30	1.519	39.70
23	3	43.12	1.457	39.11
23	4	43.60	1.578	41.12
23	5	42.44	1.464	38.93
23	6	41.95	1.263	35.72
23	7	43.93	1.476	39.71
23	8	45.13	1.196	35.99
23	9	44.03	1.506	40.20
23	10	43.83	1.271	36.60
23	11	43.83	1.429	38.97
23	12	42.66	1.425	38.44
24	1	27.01	0.875	23.93
24	2	30.78	0.874	25.42
24	3	34.03	0.575	22.24
24	4	33.51	0.797	25.36
24	5	35.91	0.901	27.88
24	6	36.56	0.896	28.06
24	7	35.94	0.665	24.35
24	8	37.92	0.929	29.11
24	9	37.37	0.837	27.50
24	10	36.40	0.881	27.77
24	11	39.25	1.084	31.42
24	12	39.16	0.807	27.76

RAT=3

COW	WEEK	MY	MF	FCM
25	1	32.04	1.442	34.45
25	2	34.38	1.162	31.19
25	3	33.93	1.187	31.38
25	4	34.03	0.755	24.94
25	5	31.53	0.744	23.77
25	6	35.46	0.759	25.56
25	7	36.49	0.974	29.21
25	8	36.79	0.942	28.84
25	9	34.19	0.913	27.37
25	10	35.10	0.941	28.15
25	11	33.54	1.033	28.91
25	12	31.72	0.996	27.63

RAT=3

COW	WEEK	MY	MF	FCM
26	1	25.39	1.178	27.83
26	2	24.06	0.931	23.59
26	3	23.64	0.671	19.52
26	4	28.77	1.056	27.34
26	5	30.71	1.053	28.27
26	6	30.55	0.972	26.79
26	7	31.36	0.963	26.99
26	8	30.65	0.824	24.78
26	9	28.28	0.894	24.72
26	10	29.54	0.869	24.85
26	11	28.93	0.943	25.72
26	12	27.96	0.895	24.60
27	1	29.68	1.249	30.61
27	2	32.66	1.176	30.70
27	3	26.14	1.275	29.59
27	4	21.20	0.568	17.00
27	5	27.56	0.954	25.33
27	6	29.22	0.964	26.15
27	7	30.91	0.921	26.18
27	8	30.29	0.957	26.48
27	9	30.71	0.872	25.37
27	10	30.88	1.000	27.36
27	11	30.16	0.899	25.55
27	12	31.36	0.809	24.68
28	1	21.59	0.721	19.45
28	2	23.25	0.674	19.41
28	3	24.68	0.698	20.34
28	4	24.35	0.638	19.31
28	5	24.84	1.416	31.17
28	6	22.79	0.709	19.75
28	7	23.12	0.687	19.55
28	8	24.68	0.679	20.05
28	9	25.39	0.762	21.58
28	10	25.10	0.718	20.81
28	11	24.35	0.808	21.87
28	12	25.29	0.670	20.17
29	1	27.92	1.245	29.85
29	2	27.60	0.836	23.58
29	3	28.28	0.956	25.65
29	4	28.90	0.925	25.43
29	5	27.96	0.917	24.94
29	6	29.90	0.945	26.13
29	7	29.64	0.895	25.29
29	8	28.90	0.838	24.13
29	9	30.94	1.033	27.88
29	10	31.14	0.866	25.40
29	11	29.16	0.939	25.74
29	12	29.16	0.787	23.47
30	1	29.16	1.397	32.61
30	2	31.40	1.243	31.21
30	3	31.46	0.884	25.85
30	4	30.10	1.201	30.05
30	5	29.97	0.947	26.19
30	6	28.86	1.293	30.94

RAT=3

COW	WEEK	MY	MF	FCM
30	7	28.34	0.856	24.18
30	8	28.38	0.854	24.16
30	9	28.67	1.078	27.64
30	10	28.38	0.976	25.99
30	11	24.61	1.009	24.98
30	12	25.03	0.809	22.14
31	1	30.35	0.795	24.07
31	2	29.25	0.854	24.51
31	3	27.56	0.910	24.67
31	4	29.32	1.126	28.61
31	5	25.32	0.859	23.01
31	6	27.14	0.928	24.78
31	7	26.62	0.916	24.39
31	8	25.88	0.841	22.97
31	9	26.20	0.660	20.38
31	10	23.15	0.662	19.19
31	11	25.68	0.950	24.53
31	12	26.53	0.934	24.62
32	1	34.74	0.973	28.49
32	2	29.45	0.660	21.67
32	3	36.36	1.404	35.60
32	4	36.59	1.412	35.82
32	5	37.47	1.311	34.66
32	6	37.27	1.319	34.70
32	7	35.39	0.860	27.06
32	8	36.20	1.238	33.05
32	9	35.97	1.140	31.50
32	10	35.97	1.273	32.52
32	11	37.04	1.271	33.88
32	12	35.20	1.000	29.07
33	1	11.07	0.407	10.54
33	2	11.98	0.443	11.44
33	3	13.38	0.475	12.47
33	4	14.19	0.443	12.32
33	5	14.64	0.483	13.11
33	6	13.54	0.408	11.53
33	7	14.48	0.484	13.05
33	8	14.16	0.478	12.84
33	9	13.41	0.410	11.52
33	10	14.22	0.454	12.49
33	11	14.12	0.511	13.32
33	12	13.38	0.575	13.98
34	1	14.71	0.524	13.74
34	2	20.36	0.519	15.93
34	3	20.13	0.656	17.90
34	4	22.56	0.659	18.91
34	5	23.12	0.728	20.17
34	6	24.54	0.680	20.02
34	7	24.35	0.601	18.76
34	8	24.48	0.597	18.75
34	9	25.46	0.611	19.35
34	10	26.17	0.701	20.99
34	11	27.18	0.799	22.85
34	12	25.91	0.725	21.25

RAT=3

COW	WEEK	MY	MF	FCM
35	1	39.94	1.350	36.22
35	2	41.42	1.135	33.57
35	3	43.25	1.297	36.76
35	4	43.99	1.434	39.11
35	5	41.17	1.457	38.33
35	6	42.21	1.321	36.70
35	7	40.32	1.524	38.98
35	8	41.72	1.026	32.08
35	9	43.44	1.025	32.75
35	10	42.73	1.380	37.79
35	11	41.66	0.966	31.16
35	12	41.92	1.090	33.11
36	1	19.06	1.018	22.89
36	2	21.40	1.003	23.61
36	3	22.63	0.941	23.17
36	4	22.04	1.003	23.86
36	5	22.18	1.016	24.10
36	6	24.71	1.060	25.78
36	7	23.96	0.829	22.02
36	8	25.20	1.038	25.65
36	9	26.17	0.937	24.52
36	10	24.61	1.098	26.31
36	11	25.49	1.050	25.95
36	12	26.01	0.996	25.34

Appendix III

Analysis of variance tables and raw data for Experiment 3

Table 1. Analysis of variance table for serum beta-carotene and vitamin A concentration

Source of variation	Serum β-carotene			Serum vitamin A		
	df	MS	F	df	MS	F
Trt	1	117562.8	24.2 **	1	429.4	5.12 *
Cow (Trt)-Error a	18	4859.0		18	83.8	
Week	5	8909.7	12.11 **	5	221.3	8.78 **
Trt x week	5	10065.5	13.68 **	5	21.5	0.85 NS
Error b	90	735.8		90	25.2	

* $P \leq .05$

** $P \leq .01$

NS = Not significant.

Table 2. Analysis of variance table for initial body weight and average daily gain

Source of variation	Initial body weight			Average daily gain		
	df	MS	F	df	MS	F
Trt	1	105.8	0.46NS	1	25169.5	5.85*
Error	18	230.17		18	4303.5	
Total	19			19		

*P<.05

NS = not significant (P>.05).

Table 3. Analysis of variance table for age at puberty, weight at puberty and intensity of estrus

Source of variation	Age at puberty			Weight at puberty			Intensity of estrus		
	df	MS	F	df	MS	F	df	MS	F
Trt	1	4440.2	1.38NS	1	952.2	0.56NS	1	4.71	38.76**
Error	18	3215.4		18	1689.3		18	0.12	
Total	19			19			19		

**P<.01

NS = not significant (P>.05).

Table 4. Analysis of variance table for cycle length, ILHPO, area under P_4 profile and services/conception

	Cycle length			ILHPO			Area under P_4			Services/conception		
Source of variation	df	MS	F	df	MS	F	df	MS	F	df	MS	F
Trt	1	2.65	0.49NS	1	854.2	7.97*	1	212.9	1.41NS	1	0.05	0.11NS
Error				16	107.1		15	150.7		18	0.47	
Cow (Trt) - Error a	18	5.39										
Cyn	2	29.49	7.77**									
TrtxCyn	2	1.99	0.52NS									
Error b	36	3.79										
Total	59			17			16			19		

*P<.05

**P<.01

NS = not significant (P>.05).

Cyn = cycle number.

ILHPO = interval from LH peak to ovulation.

Table 5. Analysis of variance table for serum progesterone, estradiol-17β, LH and FSH concentration from prepuberty to day 17 of first cycle

Source of variation	Progesterone			Estradiol-17β			LH			FSH		
	df	MS	F	df	MS	F	df	MS	F	df	MS	F
Trt	1	0.32	0.41 NS	1	3.7	0.2 NS	1	0.26	0.3 NS	1	2.58	1.2 NS
Cow (Trt) - Error a	18	0.79		8	17.6		18	0.77		18	2.2	
Period (Per)	5	74.2	231.9 **	5	2.0	0.7 NS	5	0.59	2.4 *	5	3.66	9.1 **
TrtxPer	5	0.73	2.3 *	5	6.9	2.4 *	5	0.15	0.6 NS	5	0.2	0.5 NS
Error b	89	0.31		40	2.9		89	0.25		89	0.4	

* $P \leq .05$

** $P \leq .01$

NS = Not significant.

Table 6. Serum β-carotene concentration (μg/100 ml) throughout the experimental period

Heifer	Week on trial														
	1	2	3	4	5	6	10	14	18	22	26	30	34	38	42
Control group															
1. 24-80	48	48.8	51.2	44	65.6	72.8	76.8	73.6	86.4	26.4	72	59.2	32	40.8	56
2. 28-80	39.2	113.6	38.4	62.4	77.6	84.8	124	133.6	81.6	28	37.6	53.6	27.2	45.6	51.2
3. 32-80	36.8	32.8	48	49.6	61.6	76	97.6	57.6	20	23.2	25.6	26.4	28.8	35.2	44
4. 42-80	40.8	40	31.2	40.8	47.2	54.4	17.6	36	17.6	12.8	11.2	20.8	27.2	26.4	18.4
5. 44-80	36	47.2	51.2	68	48	31.2	11.2	30.4	12.8	16	27.2	36	55.2	26.4	20.8
6. 49-80	8.8	8.8	2.4	8.8	11.2	58.4	13.6	4	2.4	14.4	25.6	20	22.4	21.6	-
7. 57-80	24	34.4	41.6	46.4	23.2	23.2	28.8	23.2	27.2	16.8	9.6	7.2	26.4	84.8	48.8
8. 6-81	36.8	42.4	20	20	16.8	14.4	31.2	19.2	6.4	14.4	9.6	49.6	40	34.4	-
9. 16-81	91.2	100.8	89.6	44	25.6	20	24	13.6	18.4	52.8	33.6	35.2	32	-	-
10. 19-81	36	35.2	29.6	9.6	3.2	11.2	6.4	10.4	24.8	38.4	27.2	41.6	23.2	11.2	37.6
$\bar{x}$	39.7	50.4	40.3	39.4	38	44.6	43.1	40.2	29.8	24.3	27.9	35	31.4	36.3	39.5
Carotene supplemented group															
1. 26-80	52	81.6	42.4	160	234.4	234.4	276	249.6	255.2	308.8	240	208.8	160	173.6	126.4
2. 30-80	42.4	88	164.8	233.6	263.2	244.8	324.8	351.2	266.4	200	258.4	268	192	215	287.2
3. 36-80	9.6	24.8	65.6	77.6	84.8	87.2	140	159.2	68	81.6	72	100	89.6	78.4	73.6
4. 43-80	41.6	82.4	148	113.6	104.8	148.8	116	134.4	166.4	209.6	185.6	212	172.8	140	-
5. 45-80	29.6	61.6	149.6	171.2	184	161.6	162.4	158.4	152	196.8	137.6	177.6	127.2	88	83.2
6. 50-80	6.4	47.2	66.4	78.4	97.6	116	109.6	117.6	100.8	121.6	100	43.2	70.4	156.8	266.4
7. 4-81	63.2	77.6	76	118.4	141.6	114.4	155.2	127.2	116.8	148.8	259.2	165.6	214.4	218.4	-
8. 13-81	15.2	62.4	86.4	106.4	127.2	145.6	64	153.6	135.2	125.6	156	248.8	237.6	82.4	-
9. 17-81	102.4	112	118.4	123.2	155.2	144	88.8	194.4	171.4	174.4	171.2	234.4	192	-	-
10. 21-81	48	33.6	88.8	97.6	70.4	52.8	190.4	127.2	123.2	143.2	261.6	246.4	102.4	61.6	85.6
$\bar{x}$	41	67.1	100.6	128	146.3	145	162.7	177	155.5	171	184.2	190.5	155.8	134.9	153.7

Table 7. Serum vitamin A concentration (μg/100 ml) throughout the experimental period

Heifer	Week on trial														
	1	2	3	4	5	6	10	14	18	22	26	30	34	38	42
Control group															
1. 24-80	27.5	35	30	35	35	37.5	30	35	27.5	35	30	27.5	35	37.5	32.5
2. 28-80	27.5	22.5	25	30	37.5	40	40	45	45	42.5	42.5	50	35	30	27.5
3. 32-80	27.5	30	30	27.5	35	32.5	35	27.5	27.5	30	35	25	27.5	27.5	27.5
4. 42-80	32.5	30	30	35	35	30	22.5	22.5	27.5	27.5	27.5	27.5	30	35	35
5. 44-80	30	22.5	25	27.5	25	22.5	20	30	27.5	30	32.5	30	30	42.5	42.5
6. 49-80	20	27.5	25	35	40	30	35	27.5	27.5	25	25	35	47.5	32.5	-
7. 57-80	27.5	32.5	27.5	35	27.5	27.5	27.5	25	25	27.5	25	27.5	27.5	27.5	25
8. 6-81	15	20	20	20	35	32.5	35	47.5	55	30	27.5	27.5	27.5	35	-
9. 16-81	15	27.5	27.5	30	30	32.5	42.5	42.5	30	35	45	37.5	35	-	-
10. 19-81	15	20	20	32.5	32.5	40	30	30	20	25	25	35	32.5	37.5	37.5
$\bar{x}$	23.8	26.8	26	30.8	33.3	32.5	31.8	33.3	31.3	30.8	31.5	32.3	32.8	33.9	32.5
Carotene supplemented group															
1. 26-80	25	20	35	25	30	45	27.5	35	40	50	37.5	42.5	35	35	35
2. 30-80	27.5	30	30	35	35	35	40	42.5	42.5	40	40	47.5	37.5	22.5	32.5
3. 36-80	25	27.5	35	35	35	27.5	27.5	35	27.5	27.5	30	27.5	27.5	25	35
4. 43-80	40	45	42.5	35	32.5	40	35	35	35	32.5	35	37.5	35	40	-
5. 45-80	35	27.5	35	42.5	42.5	32.5	27.5	30	25	35	27.5	30	35	35	27.5
6. 50-80	27.5	45	45	32.5	35	35	32.5	27.5	27.5	27.5	27.5	32.5	35	37.5	37.5
7. 4-81	15	20	20	27.5	32.5	25	27.5	35	35	30	37.5	27.5	37.5	35	-
8. 13-81	25	27.5	32.5	27.5	40	42.5	35	27.5	27.5	32.5	30	35	35	35	-
9. 17-81	32.5	30	27.5	40	37.5	37.5	35	37.5	35	47.5	35	37.5	32.5	-	-
10. 21-81	27.5	25	35	35	32.5	35	32.5	27.5	37.5	35	42.5	42.5	35	27.5	27.5
$\bar{x}$	28	29.8	33.8	33.5	35.3	35.5	32	33.3	33.3	35.8	34.3	36	34.5	32.5	32.5

Table 8A. Summary of growth performance data

Heifer	Initial body wt.(kg)	Average daily gain (gm)	Age at puberty (days)	Wt. at puberty (kg)
Control group				
24-80	133	619.8	306	270
28-80	132	645.7	320	204
32-80	143	567.6	345	326
42-80	172	602.5	230	258
44-80	152	699.6	280	244
49-80	163	721.6	396	324
57-80	164	670.9	418	318
6-81	154	795.3	344	306
16-81	183	782.9	305	294
19-81	130	723	351	280
Carotene supplemented group				
26-80	143	690.2	435	373
30-80	150	678.6	263	272
36-80	143	812.9	318	288
43-80	173	768.5	255	250
45-80	163	807.4	308	276
50-80	165	690.5	318	270
4-81	166	734.7	286	256
13-81	166	756.1	333	249
17-81	155	825.2	255	243
21-81	153	774.3	226	209

Table 8B. Monthly body weights (kg) throughout the experimental period

Heifer	Month on trial									
	1	2	3	4	5	6	7	8	9	10
Control group										
24-80	-	-	-	-	-	304	310	330	364	362
28-80	-	-	-	-	-	312	321	341	368	385
32-80	-	-	-	-	295	311	332	359	368	379
42-80	-	-	288	300	329	354	360	359	360	400
44-80	-	-	237	259	291	311	310	340	329	355
49-80	-	209	224	242	270	285	305	335	343	402
57-80	188	210	232	254	264	269	297	306	340	373
6-81	163	196	221	252	277	308	311	332	357	392
16-81	229	269	292	294	312	343	370	401	410	-
19-81	168	193	214	224	250	279	308	343	356	372
Mean	187	195.4	247.9	260.7	286	307.6	322.4	344.6	359.5	380
Carotene supplemented group										
26-80	-	-	-	-	-	323	343	366	373	394
30-80	-	-	-	-	270	307	342	352	355	377
36-80	-	-	-	245	272	294	315	331	378	362
43-80	-	-	253	295	234	333	356	361	390	414
45-80	-	-	212	242	312	296	310	330	338	378
50-80	190	213	234	254	270	266	288	316	359	386
4-81	188	207	234	248	271	298	302	333	366	388
13-81	208	240	249	259	295	325	358	376	379	-
17-81	198	236	255	267	298	325	362	379	397	-
21-81	186	213	227	244	270	297	336	359	376	403
Mean	194	221.8	237.7	256.8	276.9	306.4	331.2	350.3	371.1	387.8

Table 8C. Mean daily weight gain (grams/day) throughout the experimental period

Heifer	Month on trial									
	1	2	3	4	5	6	7	8	9	10
Control group										
24-80	-	-	-	-	880*	214	690	970	-71	1036
28-80	-	-	-	-	950*	321	690	770	607	536
32-80	-	-	-	900	571*	724	770	321	393	294
42-80	-	-*	990	429	1000	714	214	-36	29	1480
44-80	-	810	786	1103*	571	-36	1070	-324	963	1353
49-80	740	536	620	800	536	714	882*	296	1735	357
57-80	629	786	786	294	185	824*	321	1214	1100	-
6-81	1178	893	912	926	912*	107	750	833	1060	867
16-81	1088	1480	882	71*	643	1033	818	1033	281	-
19-81	824	926	618	357	929	900	879	1167	406	280
Carotene supplemented group										
26-80	-	-	-	-	870*	1070	793	229	750	429
30-80	-	-	-*	750	1320	1207	286	107	786	294
36-80	-	-	700	964	759*	600	571	1679	-470	1700
43-80	-	720*	1500	586	600	821	179	853	889	-
45-80	-	730	1070	1000*	714	500	714	235	1480	824
50-80	450	820	724	571	571*	-143	647	1037	1265	964
4-81	679	964	412*	852	794	143	1107	1100	667	-
13-81	618	1185	265	357	1286*	1000	1000	800	94	-
17-81	1000	1407*	559	429	1107	900	1120	567	563	-
21-81	1000*	412	607	929	900	1182	767	531	474	-

*Month of puberty.

Table 9. Summary of reproduction data

Heifers	Interval: LH peak to ovulation (hrs)	Area under progesterone curve (arbitrary units)	Mean intensity of heat	Services/ conception	No. of ovulations prior to 1st estrus
Control group					
24-80	53	79 (2)*	3.4 (5)*	1	0
28-80	68	-	2.75 (4)	2	1
32-80	44	89 (2)	3.3 (3)	1	2
42-80	29	67.5 (2)	2.75 (4)	2	1
44-80	41	49 (1)	2.43 (7)	3	0
49-80	-	69 (1)	2.25 (4)	1	1
57-80	51	62 (1)	3.5 (4)	1	2
6-81	42	55.5 (2)	3.0 (4)	1	2
16-81	34	58 (1)	3.2 (5)	1	0
19-81	38	85.5 (2)	2.2 (5)	1	2
Carotene supplemented group					
26-80	41	55 (1)	3.67 (3)	1	5
30-80	-	69.5 (2)	3.88 (8)	3	0
36-80	35	50.5 (2)	3.75 (4)	1	1
43-80	22	60 (1)	3.67 (3)	1	1
45-80	26	55 (2)	4 (4)	1	1
50-80	37	-	4 (7)	1	0
4-81	26	80.5 (2)	4 (8)	1	1
13-81	18	-	3.8 (5)	1	1
17-81	44	52.5 (2)	3.86 (7)	2	1
21-81	27	66.5 (2)	3.86 (7)	1	1

*No. of observations.

Table 10. Preovulating LH and progesterone (P_4) profiles for all heifers - 24 hour collection at 2-hr intervals plus additional samples

A. Control group

Heifer 24-80				Heifer 28-80				Heifer 32-80			
Collection		LH	P_4	Collection		LH	P_4	Collection		LH	P_4
Time	Hour	(ng/ml)	(ng/ml)	Time	Hour	(ng/ml)	(ng/ml)	Time	Hour	(ng/ml)	(ng/ml)
9:00pm D1	0	0.84	0.84	12:00noon D1	0	3.7	.73	4:00pm D1	0	2.8	.9
	2	2.25	0.91		2	1.7	.65		2	.85	.91
1:00am D2	4	11.0	0.80		4	1.08	.63		4	1.4	.86
	2	11.5	0.90		6	0.95	.65		6	1.13	.86
	8	8.0	0.98		8	0.7	.69		8	1.08	1.09
	10	2.9	0.81		10	0.6	.73	2:00am D2	10	1.05	0.94
	12	1.5	0.77		12	0.78	.72		12	1.35	.89
	14	0.75	0.75	2:00am D2	14	0.63	.75		14	.95	.89
	16	0.73	0.90		16	0.6	.79		16	1.08	.99
	18	0.7	0.75		18	0.33	.76		18	1.53	.77
	20	0.49	0.71		20	0.55	.78		20	21.0	.97
	22	0.7	0.77		22	0.83	.84		22	18.0	.90
1:00pm D3	41	0.2	0.73		24	0.77	.86		24	9.0	.84
10:00am D4	61*	0.43	0.87		32	0.73	.9	1:00pm D3	45	.9	.79
	85	0.46	1.88	8:00am D3	44	1.05	.78	9:00am D4	65*	.93	.56
	D12		7.11	10:00am D4	68*	0.65	.97		D7		2.6
	D15		7.11		D9		5.01		D11		6.71
	D19		8.66		D12		6.26		D14		7.87
	D22		.8		D16		0.59		D18		7.19
	D26		.38						D21		1.23

Continued .....

Table 10 - Continued

Heifer 42-80				Heifer 44-80				Heifer 49-80			
Collection		LH	P_4	Collection		LH	P_4	Collection		LH	P_4
Time	Hour	(ng/ml)	(ng/ml)	Time	Hour	(ng/ml)	(ng/ml)	Time	Hour	(ng/ml)	(ng/ml)
7:00pm D1	0	2.6	.55	9:00pm D1	0	0.9	0.74	9:00pm D1	0	1.4	6.24
	2	2.1	.52		2	1.08	0.75		2	.36	5.37
	4	4.55	.62	1:00am D2	4	2.5	0.63	1:00am D2	4	.48	5.76
1:00am D2	6	5.0	.49		6	0.95	0.67		6	1.1	6.48
	8	8.5	.49		8	1.15	0.54		8	.7	5.31
	10	5.25	.61		10	3.25	0.59		10	.8	6.22
	12	3.4	.60		12	0.75	0.67		12	3.35	4.97
	14	1.68	.49		14	1.15	0.58		14	.43	5.78
	16	1.45	.55		16	1.08	0.61		16	.65	5.28
	18	1.63	.41		18	0.88	0.60		18	.95	5.31
	20	1.21	.65		20	0.88	0.66		20	.93	6.82
	22	0.93	.58		22	1.05	0.67		22	.53	4.59
	24	1.15	.53	1:00pm D3	41*	1.03	0.59	10:00am D3	38	.65	4.81
11:00am D3	40*	1.13	.56		62	0.88	0.48				
	D6		1.78		86	0.78	0.60				
	D9		4.67		D12		3.25				
	D13		6.44		D15		3.92				
	D16		5.92		D19		5.0				
	D20		1.02		D22		2.33				
					D26		0.74				
					D29		1.52				

Continued .....

Table 10 - Continued

Heifer 57-80				Heifer 6-81				Heifer 16-81			
Collection		LH	P_4	Collection		LH	P_4	Collection		LH	P_4
Time	Hour	(ng/ml)	(ng/ml)	Time	Hour	(ng/ml)	(ng/ml)	Time	Hour	(ng/ml)	(ng/ml)
9:00am D1	0	1.25	.43	2:00pm D1	0	36.0	.79	10:00am D1	0	1.35	.95
	2	.95	.44		2	35.0	.83		2	2.3	.74
	4	.83	.51		4	13.5	.77		4	3.05	1.0
	6	.63	.42		6	3.3	.85		6	1.5	.68
	8	.63	.39		8	1.8	.92		8	1.38	.69
	10	.63	.9		10	1.15	.69		10	10.25	.59
	12	.6	.45	2:00am D2	12	0.93	.64		12	39.0*	.80
	14	.5	.45		14	0.83	.55		14	32.0	1.09
1:00am D2	16	.32	.54		16	0.8	.60	2:00am D2	16	8.5	.79
	18	.45	.46		18	0.98	.69		18	2.6	.75
	20	.5	.50		20	1.05	.64		20	1.23	.67
	22	.5	.54		22	1.05	.48		22	.88	.58
	24	.6	.41		24	1.1	.59		24	.85	.64
	35	.58	.59	10:00am D3	44*	1.15	.60		31	.83	.53
10:00am D3	49*	.83	.62		D6		1.27	10:00am D3	48*	.7	.58
	D5		1.66		D10		3.85		D7		2.98
	D7		3.44		D13		3.78		D10		5.55
	D9		3.84		D17		4.63		D14		6.79
	D11		6.04		D20		3.59		D17		5.79
	D13		6.34		D24		0.19		D21		4.33
	D15		5.74		D27		1.50		D24		0.67
	D17		7.25						D28		1.40
	D19		2.32								
	D21		0.37								
	D23		0.19								

Continued ....

Table 10 - Continued

Heifer 19-81

Collection Time	Hour	LH (ng/ml)	P_4 (ng/ml)
10:00am D1	0	1.0	.75
	2	2.0	1.03
	4	1.3	.92
	6	2.6	1.93
	8	52.5	1.91
	10	25.0	1.7
	12	5.23	1.08
	14	1.9	2.09
	16	1.15	1.02
	18	.9	2.23
	20	.98	1.70
	22	.63	.76
	24	.58	.93
9:00am D3	47*	.53	1.17
	D5		1.27
	D8		4.39
	D12		7.35
	D15		7.22
	D19		7.45
	D22		1.05
	D26		0.38
	D29		2.0

Continued .....

Table 10 - Continued

B. Carotene supplemented group

Heifer 26-80				Heifer 30-80				Heifer 36-80			
Collection		LH	P_4	Collection		LH	P_4	Collection		LH	P_4
Time	Hour	(ng/ml)	(ng/ml)	Time	Hour	(ng/ml)	(ng/ml)	Time	Hour	(ng/ml)	(ng/ml)
9:00pm D1	0	1.05	.73	9:00pm D1	0	.21	7.42	9:00pm D1	0	17.5	0.65
	2	1.03	.69		2	.73	7.46		2	10.3	0.64
1:00am D2	4	1.23	.74	1:00am D2	4	.7	7.33	1:00am D2	4	3.9	0.69
	6	.95	.71		6	.7	7.84		6	2.0	0.69
	8	1.1	.64		8	.78	7.33		8	1.1	0.58
	10	1.0	.69		10	.68	7.63		10	1.0	0.58
	12	1.05	.65		12	.6	7.04		12	0.38	0.48
	14	1.03	.67		14	.8	6.89		14	0.63	0.40
	16	.9	.50		16	.58	7.18		16	0.73	0.60
	18	1.0	.60		18	.7	6.38		18	0.63	0.50
	20	1.15	.70		20	.78	7.46		20	0.95	0.48
	22	2.1	.81		22	.6	6.59		22	0.8	0.50
1:00pm D3	41*	1.65	.54	1:00pm D3	41	1.75	3.71	1:00pm D3	41*	0.5	0.55
10:00am	62	2.4	.62	10:00am D4	62	.95	1.22		62	0.73	0.54
10:00pm	86	1.03	.55		86	.9	1.03		86	0.47	0.75
	D12		2.97						D12		6.30
	D15		5.2						D15		4.86
	D19		6.49						D19		8.56
	D22		5.49						D22		.30
	D26		1.08						D26		.26
									D29		2.12

Continued .....

Table 10 - Continued

Heifer 43-80				Heifer 45-80				Heifer 50-80			
Collection		LH	P_4	Collection		LH	P_4	Collection		LH	P_4
Time	Hour	(ng/ml)	(ng/ml)	Time	Hour	(ng/ml)	(ng/ml)	Time	Hour	(ng/ml)	(ng/ml)
10:00am D1	0	8.0	.66	10:00am D1	0	1.55	.49	7:00pm D1	0	52.0	.63
	2	4.9	.49		2	1.28	.49		2	50.25	.63
	4	2.95	.58		4	1.6	.48		4	11.5	.55
	6	1.5	.54		6	1.03	.48	1:00am D2	6	5.0	.66
	8	1.48	.56		8	.83	.37		8	1.8	.59
	10	.73	.54		10	.73	.52		10	1.05	.51
	12	.83	.56		12	.88	.46		12	1.08	.57
	14	.75	.67		14	.63	.34		14	.37	.46
2:00am D2	16	.78	.64	2:00am D2	16	.8	.39		16	.7	.54
	18	1.08	.53		18	.93	.22		18	.83	.45
	20	1.3	.58		20	.7	.27		20	.73	.43
	22*	.78	.54		22*	.8	.34		22	.73	.51
	34	1.08	.41		24	1.0	.41	10:00am D3	39*	.9	.39
8:00am D3	46	.75	.66	9:00am D3	36	1.5	.81		D6		1.12
	D5		1.55		D6		2.23		D10		3.86
	D8		3.81		D9		4.6		D13		3.98
	D12		5.46		D13		7.1		D17		3.68
	D15		7.89		D16		5.54		D20		.43
	D19		2.33		D20		0.85		D24		.69
					D23		1.49		D27		3.26

Continued .....

Table 10 - Continued

Heifer 4-81				Heifer 13-81				Heifer 17-81			
Collection		LH	P_4	Collection		LH	P_4	Collection		LH	P_4
Time	Hour	(ng/ml)	(ng/ml)	Time	Hour	(ng/ml)	(ng/ml)	Time	Hour	(ng/ml)	(ng/ml)
10:00am D1	0	2.65	.76	9:00am D1	0	2.05	.38	2:00pm D1	0	3.3	.69
	2	1.35	.64		2	2.35	.38		2	2.25	.58
	4	.95	.58		4	5.5	.46		4	1.45	.62
	6	.78	.46		6	23.0	.54		6	.95	.55
	8	.8	.52		8	17.5	.62		8	.83	.52
	10	1.05	.56		10	5.75	.51		10	.7	.70
	12	.95	.43		12	2.95	.50	2:00am D2	12	.6	.50
	14	.9	.46		14	1.28	.45		14	.6	.60
2:00am D2	16	.7	.43	1:00am D2	16	1.13	.49		16	.58	.70
	18	.63	.50		18	.88	.75		18	.55	.60
	20	.8	.46		20	.75	.37		20	.49	.54
	22*	.63	.57		22	.63	.54		22	.6	.64
	24	.63	.40		24*	.68	.44		24	.58	.44
9:00am D3	48	1.03	.54		31	.83	.70		30	.68	.63
	D5		1.96	10:00am D3	49	.65	.93	12:00noon D3	46*	.83	.72
	D8		5.87		D6		1.89		D4		0.52
	D12		6.02		D10		4.11		D7		2.6
	D15		5.87		D13		4.17		D11		6.71
	D19		0.64		D17		4.57		D14		5.57
	D22		.38		D20		3.97		D18		7.07
	D26		4.43		D24		0.39		D21		0.96
					D27		0.78		D25		0.36
					D31		4.24		D28		0.68
									D32		2.03

Continued .....

Table 10 - Continued

Heifer 21-81			
Collection Time	Hour	LH (ng/ml)	P_4 (ng/ml)
11:00am D1	0	.93	.63
	2	.95	.62
	4	.98	.62
	6	1.08	.52
	8	.93	.53
	10	.95	.75
	12	.95	.70
1:00am D2	14	.88	.57
	16	.83	.56
	18	.88	.79
	20	.9	.61
	22*	1.08	.71
	35	.95	.87
10:00am D3	48	1.18	1.04
	D6		2.40
	D10		6.33
	D13		5.08
	D17		9.05
	D20		7.23
	D24		0.69
	D27		1.68

*Day of ovulation based on palpation.

Table 11. Progesterone profiles for all cycles in which blood samples were taken every other day

Heifer	Cycle #	Day of cycle 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	-2	-1	0
Control group																						
24-80	1	.32		1.55		3.46		5.44		6.19		6.49		8.66		7.73			3.71	.96		.53
"	2	.53		2.54		5.2		6.19		7.42		8.04		8.66		8.04			-	2.78		.6
32-80	3	.72		.92		3.05		6.03		6.30		6.57		7.87		7.20		8.56	-	.89		.31
"	4	.31		2.5		5.34		6.57		6.44		8.9		7.87		7.87		-	-	.86		.56
42-80	2	.34		1.9		3.42		5.06		6.05		6.18		8.88		7.56		-	-	.66		.54
"	3	.54		.89		2.01		4.54		4.21		5.79		-		-		-	2.14	.58		.3
44-80	1	.91		1.72		1.76		3.92		3.18		3.99		5.48		5.75		-	-	2.84		.59
49-80	1	.25		.85		1.99		3.65		3.32		6.34		4.65		5.07		7.25	-	-		.32
57-80	3	.37		1.11		2.48		3.38		4.35		5.97		5.55		6.05		-	-	1.57		.53
6-81	3	.22		.88		2.54		3.59		4.37		5.09		5.74		5.61		-	1.04	.38		.16
"	4	.16		1.43		2.41		3.26		2.93		4.89		5.54		4.83		-	-	1.27		.29
16-81	1	.31		1.16		2.97		3.59		4.49		4.52		6.77		5.02		-	-	.32		.22
19-81	3	.28		1.08		4.04		5.3		-		5.58		6.97		7.78		5.9	1.7	.45		.3
"	4	.3		.37		2.11		4.23		5.77		6.43		6.61		9.00		5.93	-	.79		.27
Carotene supplemented group																						
26-80	6	.96		1.02		2.10		3.77		5.2		5.51		5.88		5.88		5.88	4.83	1.27		1.24
30-80	1	.44		.66		1.22		1.14		4.01		-		-		-		-	-	.66		.61
"	2	.61		1.72		3.82		4.38		7.51		6.88		7.51		8.14		-	-	1.91		.2
36-80	2	.82		1.06		-		1.88		4.93		4.04		5.07		6.03		6.03	-	1.37		.64
"	3	.64		.86		1.23		3.29		5.14		5.20		5.41		4.93		5.20	-	.57		.31
43-80	1	.34		.37		1.51		3.22		4.21		2.1		6.58		6.12		4.7	-	.69		.29
45-80	2	.74		.81		.91		2.67		3.58		5.14		6.35		4.33		4.46	4.12	.50		.36
"	3	.36		1.18		3.04		4.6		5.41		4.39		6.22		5.14		3.58	-	-		.78
4-81	2	.39		.59		1.17		4.69		6.85		7.17		6.2		5.54		5.0	-	.55		.24
"	3	.24		1.63		4.89		5.87		7.50		-		8.8		6.85		-	-	.75		.29
17-81	2	.35		.37		.78		2.02		2.85		3.91		3.45		4.07		4.85	4.3	-		.47
"	3	.47		.69		1.51		3.47		4.25		4.86		5.96		6.37		6.13	-	.65		.28
21-81	2	.38		1.99		4.78		4.86		7.29		4.3		5.3		-		-	-	.57		.26
"	3	.26		1.38		2.28		2.98		7.42		6.04		3.95		7.55		5.71	-	.53		.37

Table 12. Serum progesterone profile from 21 days prior to first ovulation through the first estrous cycle

Day from first ovulation

Heifer	-21	-20	-19	-18	-17	-16	-15	-14	-13	-12	-11	-10	-9	-8	-7	-6	-5	-4	-3	-2	-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	-2	-1	0	1	2
Control group																																												
24-80	.38			.32				.58			.46				.56			2.97				.32		1.55		3.46		5.44		6.19		6.49		8.66		7.73			3.71	.96		.53		2.54
28-80	1.72				.84			.41				.69			.58				2.28			.63				1.8			2.75				4.63			5.26						.72		1.81
32-80	.29				.63			.46				.35			.37				1.23			.37				4.93			6.16				7.53			7.19						.4		
42-80	.11			.17				.27			.33				1.28			.47				.89			2.89				3.22			4.13								.36		.34		1.9
44-80		.48		.50		.59				.28		.62			.50	.95		1.83		1.08		.91		1.72		1.76		3.92		3.18		3.99		5.48		5.75				2.84		.59		1.28
49-80		.13				.10			.19				.15			.21		1.12		.75		.25		.85		1.99		3.65		3.32		6.34		4.65		5.07		7.25		.32		.66		1.39
57-80	.22			.22				.22			.21				.8			2.4				.32			2.05				4.47			5.19				6.64						.42		
6-81	.17				.14			.12				.13			1.21				.27			.19				1.63			2.38				4.04									.53		
16-81		.28			.29				.26			.26				.83			.74	.65		.31		1.16		2.97		3.59		4.49		4.52		6.77		5.02				.32		.22		3.32
19-81	.95				.30			.21				.85			.42				1.23			.65				.74			3.21							6.04						.26		
Mean	.55	.30		.3	.44	.33		.36	.23	.28	.33	.48	.15		.72	.66		1.76	1.15	.83		.48		1.32	2.47	2.41		4.15	3.7	4.3		5.21	5.4	6.39		6.09		7.25	3.71	.96		.47		2.04
Carotene supplemented group																																												
26-80	.52				.39			1.39				.41		.36				3.96				.65				3.71			5.57					5.94								.80		
30-80		.66				.94			.3				.66		.47		.6	.72		.72		.44		.66		1.22		1.14		4.01										.66		.61		1.72
36-80	.58			.49				.56			.25				1.08			1.34				.89			3.42				4.52			3.77										.82		1.06
43-80	.52			.33		.36		.49		.16		.45		.53		1.78		3.29		1.05		.34		.37		1.51		3.22		4.21		2.1		6.58		6.12		4.6		.69		.29		.39
45-80	.74			.29				.56			.28				.35			1.05				.45			1.15				3.58			4.06				4.46						.74		.81
50-80	.15			.14				.13			.28				.21			.85				.32		.59		1.94		2.48				3.08		4.59		3.38				.42		.21		.48
4-81	.13			.23				.34			.29				.23			1.96				.14			2.61				6.52			5.87				4.24						.39		.59
13-81	.09		.19		.15		.07		.16		.12		.16		.45			1.96				.37			2.18				5.41			4.83										.14		
17-81	.23			.23				.23			.25				.76			.89				.44			1.77				3.3			5.99							3.16	.37		.35		.37
21-81	.23			.17				.3			.15				.71			1.59				.71			2.57				5.52			5.38				5.98						.36		
Mean	.33	.66	.19	.27	.27	.65	.07	.5	.23	.16	.23	.43	.41	.45	.53	1.78	.6	1.76		.89		.48		.54	2.28	2.1		2.28	4.92	4.11		4.39		5.7		4.84		4.6	3.16	.54		.47		.77

Table 13. Summary of cycle length data

Heifer	Length of 1st cycle (days)	Length of 2nd cycle (days)	Mean length of subsequent cycles (days)
Control group			
24-80	21	18	21.5 (8)*
28-80	19	17	19.5 (4)
32-80	19	21	20 (4)
42-80	15	18	18.9 (9)
44-80	18	18	20 (4)
49-80	21	20	16 (1)
57-80	18	20	20 (2)
6-81	15	21	20.4 (5)
16-81	19	21	21 (3)
19-81	18	25	22 (6)
Mean	18.3	19.9	19.9
Carotene supplemented group			
26-80	15	20	20.3 (4)*
30-80	13	18	20.5 (8)
36-80	15	20	20.8 (6)
43-80	21	22	19.6 (5)
45-80	17	24	21.3 (6)
50-80	20	18	21.3 (3)
4-81	18	20	19.1 (7)
13-81	21	21	23.7 (3)
17-81	20	20	21.5 (6)
21-81	21	20	21.8 (10)
Mean	18.1	20.3	21

*Number of observations.

Table 14. Mean serum progesterone concentration (ng/ml) from beginning of trial to Day (D)17 of first cycle

Heifer	Beginning of trial to -D22	-D21 to -D8	-D7 to -D1	(Puberty) D0	D1 to D8	D9 to D17
Control group						
24-80	.63 (31)*	.44 (4)	1.77 (2)	.32	4.16 (4)	6.65 (4)
28-80	.38 (45)	.92 (4)	1.43 (2)	.63	2.28 (2)	4.95 (2)
32-80	.27 (38)	.43 (4)	.8 (2)	.34	5.55 (2)	7.36 (2)
42-80	.22 (11)	.22 (4)	.88 (2)	.89	3.06 (2)	4.73 (1)
44-80	.49 (32)	.51 (6)	1.09 (4)	.91	2.65 (4)	5.07 (3)
49-80	.16 (58)	.14 (4)	.69 (3)	.25	2.45 (4)	5.83 (4)
57-80	.26 (53)	.22 (4)	1.6 (2)	.32	3.26 (2)	5.92 (2)
6-81	.13 (34)	.14 (4)	.74 (2)	.19	2.0 (2)	4.04 (1)
16-81	.26 (31)	.27 (4)	.74 (3)	.31	3.05 (4)	5.44 (3)
19-81	.34 (32)	.58 (4)	.83 (2)	.65	1.98 (2)	6.04 (1)
$\bar{x}$	.31 (365)	.39 (42)	1.13 (24)	.48 (10)	3.04 (28)	5.6 (23)
Carotene supplemented group						
26-80	.53 (46)	.68 (4)	2.16 (2)	.65	4.64 (2)	5.95 (1)
30-80	.65 (26)	.64 (4)	.63 (4)	.44	1.76 (4)	-
36-80	.46 (37)	.47 (4)	1.21 (2)	.89	3.97 (2)	3.77 (1)
43-80	.48 (18)	.41 (7)	2.04 (3)	.34	2.33 (4)	4.85 (4)
45-80	.57 (33)	.47 (4)	0.7 (2)	.45	2.37 (2)	4.26 (2)
50-80	.23 (41)	.18 (4)	.53 (2)	.32	1.69 (3)	3.68 (3)
4-81	.29 (21)	.25 (4)	1.10 (2)	.14	4.57 (2)	5.06 (2)
13-81	.13 (40)	.13 (7)	1.21 (2)	.37	3.80 (2)	4.83 (1)
17-81	.27 (11)	.24 (4)	.83 (2)	.44	2.54 (2)	4.58 (2)
21-81	.24 (3)	.21 (4)	1.15 (2)	.71	4.05 (2)	5.68 (2)
$\bar{x}$	.39 (276)	.37 (46)	1.16 (23)	.48 (10)	3.17 (25)	4.27 (18)

*Number of samples

Table 15. Mean serum estradiol-17β concentration (pg/ml) from beginning of trial to Day (D)17 of first cycle

Heifer	Beginning of trial to -D22	-D21 to -D8	-D7 to -D1	(Puberty) D0	D1 to D8	D9 to D17
Control group						
49-80	4.0 (58)*	2.7 (4)	4.5 (3)	4.1	4.93 (4)	2.98 (4)
57-80	6.3 (48)	7.65 (4)	7.1 (2)	5.2	4.65 (2)	6.6 (1)
6-81	8.7 (34)	9.4 (4)	9.4 (2)	6.8	7.9 (2)	5.6 (2)
16-81	4.8 (30)	6.35 (4)	8.63 (3)	5.0	8.55 (4)	5.28 (4)
19-81	4.1 (30)	3.63 (4)	4.1 (2)	6.9	5.25 (2)	4.8 (1)
$\bar{x}$	5.58 (200)	5.95 (20)	6.75 (12)	5.6 (5)	6.26 (14)	5.05 (12)
Carotene supplemented group						
50-80	5.6 (41)	4.50 (4)	4.65 (2)	8.2	9.7 (4)	11.0 (3)
4-81	7.8 (21)	9.85 (4)	5.35 (2)	3.2	5.05 (2)	13.25 (2)
13-81	4.5 (40)	6.24 (7)	3.35 (2)	3.6	4.75 (2)	4.5 (2)
17-81	4.9 (11)	3.83 (4)	3.95 (2)	3.5	3.95 (2)	3.68 (4)
21-81	2.9 (3)	4.2 (4)	3.65 (2)	3.3	2.95 (2)	5.1 (2)
$\bar{x}$	5.14 (116)	5.72 (23)	4.19 (10)	4.36 (5)	5.28 (12)	7.5 (13)

*Number of samples.

Table 16. Mean serum LH concentration (ng/ml) from beginning of trial to Day (D)17 of first cycle

Heifer	Beginning of trial to -D22	-D21 to -D8	-D7 to -D1	(Puberty) D0	D1 to D8	D9 to D17
Control group						
24-80	.73 (31)*	.26 (4)	.19 (2)	.14	.31 (4)	.33 (4)
28-80	1.02 (45)	1.77 (4)	.51 (2)	2.75	.64 (2)	.3 (2)
32-80	.96 (38)	1.52 (4)	.45 (2)	.53	.22 (2)	.28 (2)
42-80	1.13 (11)	1.36 (4)	.82 (2)	1.45	1.33 (2)	1.88 (1)
44-80	.66 (32)	1.38 (6)	1.07 (4)	.63	.78 (4)	.65 (3)
49-80	.88 (58)	.85 (4)	.39 (3)	.35	.41 (4)	.45 (4)
57-80	.61 (53)	.79 (4)	.71 (2)	.45	.43 (2)	.64 (2)
6-81	1.03 (34)	1.02 (4)	1.24 (2)	.8	.93 (2)	1.5 (2)
16-81	.89 (31)	.88 (4)	3.56 (3)	.58	.63 (4)	.89 (4)
19-81	1.0 (32)	1.57 (4)	1.37 (2)	1.6	.8 (2)	.58 (1)
$\bar{x}$	.89 (365)	1.14 (42)	1.03 (24)	.93 (10)	.65 (28)	.75 (23)
Carotene supplemented group						
26-80	.44 (46)	.35 (5)	.18 (1)	.38	.23 (2)	.23 (1)
30-80	.85 (26)	1.39 (4)	1.74 (4)	.58	1.25 (3)	2.2 (2)
36-80	.85 (37)	1.97 (4)	.69 (2)	.65	.55 (3)	-
43-80	1.77 (18)	3.48 (7)	.74 (3)	1.83	.71 (4)	1.8 (4)
45-80	1.07 (33)	1.21 (4)	1.6 (2)	1.2	.82 (2)	.74 (2)
50-80	.98 (41)	.75 (4)	.9 (2)	.55	.91 (4)	1.35 (3)
4-81	.84 (21)	1.18 (4)	.97 (2)	.7	.6 (2)	.6 (2)
13-81	.87 (40)	.84 (7)	.93 (2)	.75	1.12 (2)	1.65 (2)
17-81	.75 (11)	.89 (4)	1.49 (2)	1.1	.57 (2)	1.18 (3)
21-81	.71 (3)	1.07 (4)	.63 (2)	.65	.79 (2)	.57 (2)
$\bar{x}$	.91 (276)	1.31 (47)	.99 (22)	.84 (10)	.76 (26)	1.15 (20)

*Number of samples.

Table 17. Mean serum FSH concentration (ng/ml) from beginning of trial to Day (D)17 of first cycle

Heifer	Beginning of trial to -D22	-D21 to -D8	-D7 to -D1	(Puberty) D0	D1 to D8	D9 to D17
Control group						
24-80	1.58 (31)*	1.65 (4)	1.1 (2)	1.7	1.0 (4)	1.2 (4)
28-80	2.52 (45)	3.68 (4)	1.9 (2)	3.2	1.9 (2)	1.2 (2)
32-80	2.57 (38)	1.88 (4)	1.9 (2)	2.0	1.2 (2)	1.4 (2)
42-80	1.98 (11)	2.48 (4)	2.5 (2)	2.5	2.3 (2)	3.1 (1)
44-80	1.87 (32)	2.73 (6)	2.18 (4)	2.3	3.15 (4)	1.17 (3)
49-80	3.04 (58)	1.78 (4)	1.5 (3)	2.5	1.48 (4)	1.3 (4)
57-80	3.07 (53)	3.2 (4)	2.5 (2)	2.3	3.05 (2)	2.35 (2)
6-81	2.35 (34)	1.78 (4)	1.35 (2)	1.4	.9 (2)	2.15 (2)
16-81	2.94 (31)	3.33 (4)	3.15 (2)	2.8	1.1 (4)	1.95 (4)
19-81	2.47 (32)	3.5 (4)	2.7 (2)	3.1	1.8 (2)	1.6 (1)
$\bar{x}$	2.44 (365)	2.6 (42)	2.08 (23)	2.38	1.79 (28)	1.74 (25)
Carotent supplemented group						
26-80	2.13 (46)	2.26 (5)	1.3 (1)	1.9	2.0 (2)	1.2 (1)
30-80	2.77 (26)	2.3 (4)	2.78 (4)	2.0	3.2 (4)	-
36-80	2.35 (37)	2.58 (4)	1.0 (2)	1.5	1.35 (2)	.8 (1)
43-80	4.06 (18)	5.09 (7)	1.77 (3)	5.2	2.75 (4)	2.57 (3)
45-80	2.24 (33)	1.98 (4)	2.5 (2)	1.1	1.65 (2)	.85 (2)
50-80	3.22 (41)	2.95 (4)	2.05 (2)	1.8	2.33 (3)	2.5 (3)
4-81	3.84 (21)	3.95 (4)	3.35 (2)	2.6	2.7 (2)	1.95 (2)
13-81	2.1 (40)	1.7 (7)	2.05 (2)	2.9	1.8 (2)	2.1 (1)
17-81	3.11 (11)	4.78 (4)	4.6 (2)	4.5	2.55 (2)	2.0 (2)
21-81	2.33 (3)	3.23 (4)	2.15 (2)	3.7	1.55 (2)	.75 (2)
$\bar{x}$	2.82 (276)	3.08 (47)	2.36 (22)	2.72	2.19 (25)	1.63 (17)

*Number of samples.