EFFECT OF BETA-CAROTENE SUPPLEMENTATION ON FERTILITY OF DAIRY CATTLE IN KENYA

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DECLARATION

This thesis was compiled by the author, based on work done by himself, and has not been presented for a degree in any other University.

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To loving Nancy and my parents.

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Abstract

A field feeding trial was conducted to determine the effect of ß-carotene supplementation on the fertility of dairy cows, and disease resistance in cows and their calves. Using stratified randomization according to parity and milk yield, 95 ayrshire cows were allocated to treatment in a randomized complete block design in three Agricultural Development Corporation (ADC) farms viz: Olngantongo, Katuke, and Zea in Trans Nzoia District of Kenya. Concentrate feeds, were formulated, one to supply 75 mg/kg of ß-carotene and the other to supply no ß-carotene,for the test and control group, respectively. In addition, both feeds were fortified with 9.38 mg/kg of vitamin E. The feeding trial commenced two weeks prepartum and continued for three months postpartum. Blood samples were taken to monitor plasma ß-carotene status. Blood samples for ß-carotene analyses were taken 14 days prepartum, and then day one, and 14-90 days post-partum depending on time of heat, to show the ß-carotene trend. Pasture and concentrate feed samples were also taken to determine the ß-carotene intake. Animals were observed for puerperal conditions, oestrous intensity, and all dates of heat and insemination were recorded. Pregnancy diagnosis was done 70 days post-insemination. Data were collected to determine cows' fertility performance, mastitis, calf diarrhoea, milk yield, and butterfat.

ß-carotene supplementation improved ($P < 0.05$) the conception rate, insemination index, and oestrous intensity while service period, uterine involution and incidence of mastitis, metritis, retained placenta, and calf diarrhoea were not affected ($P > 0.05$). The improvement of some of the fertility indicators suggests that ß-carotene supplementation is beneficial to dairy cattle especially under conditions where the pasture supplies less ß-carotene to the cows than was the case at the time of this study.

1. INTRODUCTION

Bovine fertility is affected by nutritional and non nutritional factors. The non nutritional factors include micro-climate of the stable, lay-out of the stable, hygiene and genetic manipulations.

Severe underfeeding or overfeeding may cause irregularity of the oestrous cycle and thus affect conception and may lead to higher incidence of dystocia. The most direct relationship between nutrition and fertility exist in the first three months of lactation when the energy demand of the high-producing dairy cow becomes increasingly difficult to sustain. Energy deficiency leads to delayed or silent heat and if conception occurs, placental development is affected and may cause abortion. Protein deficiency on the other hand may disturb hormone metabolism and hence fertility, although reduction in milk production is the first indication and fertility becomes secondary.

In addition to energy and proteins, minerals have a significant effect on fertility. Deficiency of either calcium or phosphorus or a wide calcium:phosphorus ratio or deficiency of sodium leads to irregularity of oestrous, silent heat or cessation of the cycle. The trace elements implicated directly with bovine fertility are copper, cobalt, zinc, manganese, iodine, and selenium. In addition vitamins A, E and B_{12} have also been shown to influence fertility performance. These vitamins are added in feeds as preformed

vitamins except for vitamin B_{12} which is synthesised by rumen microorganisms from dietary cobalt. Each of these vitamins is involved in a number of varied metabolic and physiological functions. Thus vitamin A is important in maintenance of the integrity of the epithelial membranes, while vitamin E is an important biological antioxidant. Vitamin E is also involved in synthesis of gonadotrophins,while vitamin B_{12} is involved in energy metabolism in the ruminant animal.

The main source of vitamin A for ruminants is ß-carotene which is available in abundant quantities from green foliage. In the intestinal mucosa, ß-carotene is converted to vitamin A. The importance of ß-carotene had been viewed solely as a source of vitamin A until 1955. Since then, stabilized vitamin A has become available for use in animal feeds and research has extended into the comparison between vitamin A and ß-carotene. It was not until the 1970s that ß-carotene was suspected to have effects independent of its role as a vitamin A precursor in bovine reproduction.

Various types of forage are rich in ß-carotene, however, this decreases as the plant matures or dries up. Concentrates on the other hand contain practically no ß-carotene. In the course of the 1970s, increase in labour cost, changes in the concentrates:roughage ratio of normal diets, and the increase in average milk yield per cow, has lead to higher ß-carotene requirements. ß-carotene content in good quality hay or silage is just adequate for maintenance and

production of a maximum 10 litres of milk per day. This may explain why the best yielders show poor fertility if the diet does not meet the animals daily requirements. ß-carotene deficiency is not a problem all year round, there being ample amounts in fresh green grass during certain period of the year. It is generally accepted that fertility declines in winter (temperate) and the dry season (tropics), this could however be due to a host of other factors. However, experimental evidence from the temperate regions indicates that ß-carotene deficiency contribute to decreased bovine fertility and other ß-carotene associated functions. No documented evidence was available to the author that ß-carotene affects reprodu-ctive performance in the tropics. In Kenya a calving interval as long as 471 days has been reported for animals under stall feeding or partial stall feeding production systems fed mostly on napier grass, maize stover, fodder maize, and sugar cane tops. Whereas improved reproductive performance,as a result of supplementation in the temperate region indicates that ß-carotene is one of the most limiting factors, this may not be the case in the tropics. This study is designed to investigate the effect of ß-carotene supplementation during what was expected to be a dry season on dairy cattle reproduction in the tropics.

2.0 OBJECTIVES

2.1 General objectives

1. To determine the effect of ß-carotene supplementation on reproduction in dairy cattle.
2. To determine the effect of ß-carotene supplementation on resistance to diseases.
3. To determine the effect of ß-carotene supplementation on milk production.

2.2 Specific objectives

(i).To determine the effect of ß-carotene supplementation on service period.

(ii).To determine the effect of ß-carotene supplementation on repeat breeding,conception rate, and insemination index.

(iii).To determine the effect of ß-carotene supplementation on the incidence of ovarian cysts and state.

(iv). To determine the effect of ß-carotene supplementation on the incidence of mastitis.

(v). To determine the effect of ß-carotene supplementation on the incidence of retained foetal membranes and metritis.

(vi). To determine the effect of ß-carotene supplementation on the vitality and viability of the calves.

(vii). To determine the effect of ß-carotene supplementation on milk yield and composition.

3. LITERATURE REVIEW

3.1 General information on ß-carotene

3.1.1 Essence of ß-carotene supplementation

Modern dairy industry has improved through breeding for desired traits, feeding and management. However, this development has been associated with a constant decline in the productive life of individual animals. Fertility problems and loss of quarters due to mastitis are among the main causes of this decline (Lindner and Gadient,1981). In the modern intensive dairy production system the animals have been taken off pasture and are mostly fed conserved and stored forage. Processing coupled with storage brings about a reduction in the ß-carotene content of forages which means that animals receive less ß-carotene from such forages and hence the need for ß-carotene supplementation.

For purposes of storage, forage is harvested when mature in most cases, and as such, the micronutrients and macronutrients, including carotenes content, which are known to decrease with advancing age, are very low. It has been shown that silage reflects the initial carotene content of the plant material much better than hay does (Kalac and McDonald,1981). However clostridial silage has high proportions of less active stereoisomers. Wilting of the forage to be ensiled also tends to cause heavy losses as may the use of makeshift silos. The highest proportion of the initial carotene content of fodder plants is conserved when

the material is artificially dehydrated (Friesecke, 1978).

Chew, (1983) stated that ß-carotene deficiency is common under modern intensive dairy production systems because: (i) one cannot safely rely on forage for supply of ß-carotene since ß-carotene is readily destroyed through field curing and prolonged storage, (ii) the modern high producing cow excretes substantial amounts of vitamin A and ß-carotene through the milk, and (iii) ß-carotene is not usually provided in the concentrate, the proportions of which have continued to increase in the diet.

3.1.2. Provitamins

Vitamin A does not exist as such in plants but is present as precursors or provitamins. These provitamins in the form of certain carotenoids can readily be converted in the mucosal cells and hepatic cells into vitamin A. Excluding the cis- and trans- isomers, approximately 600 carotenoids have been characterized in nature, the most abundant being, fucoxanthin, neoxanthin and violaxanthin which are nutritionally inactive (Olson, 1989). At least 80 provitamins are known and include α, ß, and τ-carotenes, and cryptozanthin which are present in higher plants, and myxoxanthin found in blue-green algae. Of these the most widely distributed and active is ß-carotene. It is generally accepted that all carotenoids with unsubstituted ß-carotene moiety have a provitamin A activity. Jensene, (1987)

reported that ß-carotene occurs as cis-and trans-isomers. In nature the cis/trans ratio is not fixed but depends on the biological source. This ratio is in addition to source also influenced by light, heat, acid, and solvents (Burton and Ingold, 1984). Different stereoisomers exhibit different physico-chemical and sometimes different biological properties (Jensene, 1987).

There is increasing evidence that various dietary carotenoids in themselves, irrespective of whether or not they have provitamin A activity, are some of the most powerful antioxidants known. They are capable of neutralizing the cytotoxic effects of substances like singlet oxygen and other free radicals that are increasingly being implicated in various disease states such as cancer (McLaren, 1990). Xanthophyll is a carotenoid with no nutritive value, but is the main pigment responsible for the yellow colour of egg yolk and is nutritionally desirable because it enhances the aesthetic value of meat, eggs, and other food products.

Pure ß-carotene is red in colour, although in solution it appears yellowish-orange. ß-carotene, like other provitamins, is fat soluble and is usually associated with chlorophyll in plants, and as such, greenness is usually a good indication of ß-carotene content. Green feeds are excellent sources of ß-carotene while dry feeds are poor sources. ß-carotene rapidly loses its potency by oxidation, especially at high temperature, exposure to air and sunlight

such that large losses can occur during sun-drying of crops. Carotenes also occur in certain animal tissues such as the body fat of cattle and horses but not in sheep or pigs (Ribaya-Mercado, *et al.*, 1989). They are also found in bird's feathers, egg yolk and butterfat.

3.1.3 ß-carotene content of feeds

All vegetable feedstuffs contain ß-carotene. However, only a few of these feedstuffs actually affect the quantitative levels of ß-carotene in the dairy cow. The most important of these is fresh forage. Fresh grass is an excellent source of ß-carotene with an average content of 254 mg/kg dry matter (Bieber-Wlaschny,1988). Endogenous ß-carotene is highly unstable in feedstuffs. The factors considered most important in affecting the concentration of ß-carotene in forages include method of preservation, duration of storage, preservation conditions, processing methods, application of nitrogen fertilizers and pelleting (Bieber-Wlaschny,1988). Other factors such as plant species, cultivars, day lengths, and application of certain pesticides have also been reported to affect ß-carotene concentration in forage (Kalac and McDonald, 1981).

Preservation and storage can cause considerable losses. Although ß-carotene loss due to duration of storage and preservation conditions depends on light intensity, moisture and heat, in most situations the longer the duration of

storage the more the ß-carotene degradation (Kalac,1980). Processing methods of the fresh material have been found to alter ß-carotene content of feed. Ensiling has been reported to result in lower losses than drying, usually necessary for hay making. However, artificial drying methods cause less destruction than natural drying. Owango,(1978) found that oven dried lucerne had four times more ß-carotene than air dried lucerne, while Bieber-Wlaschny,(1988) reported that artificially dried lucerne which was then preserved with anti-oxidants had more ß-carotene than air dried lucerne. Putnam,(1983) further noted that processing procedures have a profound influence on ß-carotene availability to the animal. The researcher observed that some modern feedmills are poorly designed such that micronutrients including ß-carotene are also subjected to the hammermill processes, which destroys the protective coat subjecting the synthetic active ingredients to oxidative degradation. During processing, ß-carotene is destroyed and/or the stability of ß-carotene reduced in presence of trace minerals and high temperatures (Putnam, 1983). Thus as a result of pre-conditioning meals with steam during the pelleting process, the stability of the ß-carotene in such feeds is reduced.

Application of nitrogenous fertilizers has been found to increase ß-carotene concentration in the forage in addition to promoting forage growth. The ß-carotene content is affected by plant species and also varies within the same

plant. Lindner and Gadient, (1981) reported higher ß-carotene levels in silage made from grass than that made from maize. Grains have also been shown to have a lower ß-carotene content than other plant parts (Smith, 1980). This results in very low ß-carotene levels in concentrates which are mostly constituted from grains (Putnam, 1983).

In ruminants, substantial amount of ß-carotene is oxidized or cleaved in the rumen due to bacterial activity and presence of oxidizing feed ingredients. ß-carotene was reported to be better utilized when fed together with Vitamin E (Mingazov, 1977a). This apparent associative effect of vitamin E was explained later by the findings of Bieber-Wlaschny,(1988) when he established the anti-oxidant effect of vitamin E on ß-carotene and vitamin A.

3.1.4 Synthetic ß-carotene

This is usually in the form of a red-brown, fine granular powder which is finely dispersed in a matrix of gelatine and carbohydrates. However, synthetic crystalline or oil based forms of ß-carotene are also available. The synthetic form of ß-carotene is mainly trans isomers, though the cis/trans ratio which is similar to that of carrots is not stable but changes with heat, light, acid, and solvents the same way as that from natural sources (Burton and Ingold, 1984). It has been reported that the bio-availability of crystalline or oil based ß-carotene is better than that of

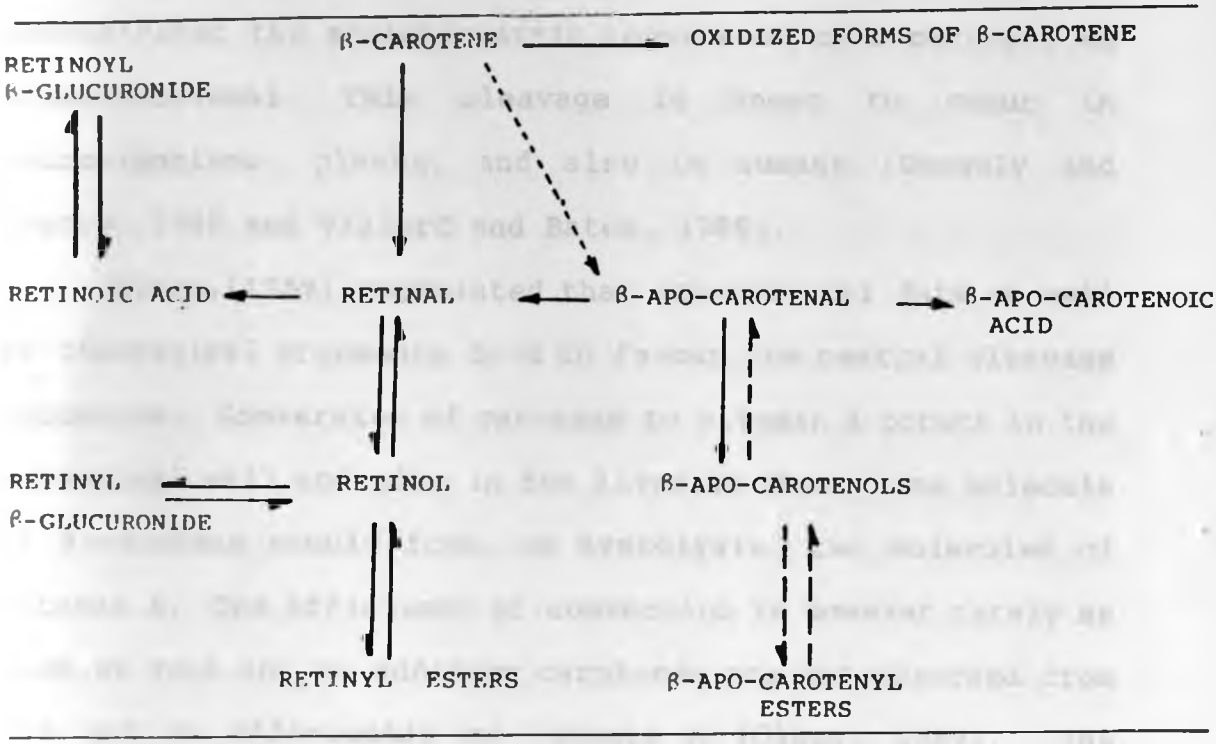
natural origin. Synthetic ß-carotene has also been found to be more stable in concentrate and mineral mixtures for reasonable periods of storage (Roche,1989). Further work has shown that moisture is an important factor in the stability of this compound. The manufactures claim that after manufacture, synthetic ß-carotene can stay for up to one year without loss of potency.

3.2. Metabolism and function of ß-carotene

3.2.1 ß-carotene metabolism

Glover,(1960) and Sharma *et al.*,(1977) discussed the various pathways in which carotenoids might be converted to vitamin A as shown in Figure 1. These researchers indicated that two primary oxidative reactions might occur, one at the central 15,15' double bond (central cleavage) and the other at one or more of the other double bonds (excentric cleavage). The central cleavage yields two molecules of vitamin A while excentric cleavage yields one short and one long ß-apo-carotenal, such as cyclocitral and ß-apo-8'-carotenal. Ganguly and Sastry, (1985), and Villard and Bates,(1986) confirmed earlier reports by Bendich,(1987) and Olson, (1969) that ß-carotenoid 15,15'-dioxygenase is the enzyme responsible for central cleavage of ß-carotene. This enzyme has been partly purified and characterized from intestinal mucosa of various animal species.

Figure 1: Suggested β-carotene catabolism (Sharma et al., 1977)



KEY

——— Known pathway

— — — Probable but undemonstrated pathway

------ Unknown but theoritically possible pathway

The enzyme catalyzing excentric cleavage has however not been characterized, though Juttner and Hoflacher, (1985) demonstrated the stoichiometric conversion of ß-carotene to ß-apo-carotenal. This cleavage is known to occur in microorganisms, plants, and also in humans (Ganguly and Sastry, 1985 and Villard and Bates, 1986).

Olson,(1989) postulated that experimental data as well as theoretical arguments tend to favour the central cleavage mechanism. Conversion of carotene to vitamin A occurs in the intestinal wall and also in the liver.In theory one molecule of ß-carotene should form, on hydrolysis two molecules of vitamin A. The efficiency of conversion is however rarely as high as this and in addition carotenes are not absorbed from the gut as efficiently as Vitamin A (Olson, 1989). The Agricultural Research Council, (1978,1980) states that 6µg, 1-4µg, and 5-8µg of ß-carotene in the diet are equivalent to 1µg of vitamin A alcohol in pig, poultry, and ruminants, respectively while NRC, (1978) and Lotthammer, (1979) considered 1 mg of ß-carotene to be equivalent to 400 IU of vitamin A in cattle.

3.2.2 Factors affecting conversion of ß-carotene to vitamin A

Olson, (1989) managed to partly purify the enzyme ß-carotenoid 15,15' dioxygenase from the intestines and other organs of several unnamed species. This enzyme was

demonstrated to require molecular oxygen. This author further reported that the enzyme was inhibited by sulphydryl-binding reagents and ferrous ion chelating agents and had a pH optimum of between 7.5-8.5 . Activity of ß-carotenoid 15,15' dioxygenase of the intestine is also affected by both the protein content of the diet and vitamin A status of the animal.

Various researchers have reported that high supplementation with vitamin A has a depressing effect on the conversion of ß-carotene to vitamin A (Kalyanakrishnan, *et al.*, 1951 and Wise, *et al.*, 1947), and as such more ß-carotene is saved for other functions. Thompson, (1975) stated that once a ß-carotene molecule has been split or terminally catabolized, it could not be resynthesized again. For this reason it might be desirable to keep the amount of ß-carotene which is converted into vitamin A to a minimum by ensuring that the daily ration supplied optimum amounts of vitamin A. Villard and Bates, (1986) and Grownowska-Senger and Wolf, (1970) found that ß-carotenoid 15,15' dioxygenase activity in vitamin A-sufficient rats is 50% that in vitamin A depleted rats which suggests a homeostatic control mechanism for carotenoid cleavage. These researchers also suggested that the dietary carotenoids may induce activity of the intestinal enzyme. Grownowska- Senger and Wolf, (1970) also reported that the activity of the intestinal enzyme is depressed by approximately 50% in rats receiving low (5%)

protein diets. Kamath and Arnrich, (1973) reported that the effects of high protein intake did not give consistent results. In one study however, appear- ance of retinyl esters from ß-carotene in the intestine and liver of rats fed a 40% protein diet was twice that of rats fed a 10% protein diet. Another factor, quoted by NRC (1978), affecting conversion ratio is biological source of the carotene. Meinecke, *et al.*, (1986) found that ß-carotene given as an intramuscular injection is utilized immediately by the body and not stored as Vitamin A. They observed that ß-carotene was rapidly absorbed and metabolized with a half-life of about four hours.

Thompson,(1975) reported a considerable variation in the efficiency of converting ß-carotene to vitamin A by different species. Goodman *et al.*, (1966) and Blomstrand and Werner,(1967) reported that approximately 15% of ß-carotene is absorbed unchanged in cattle, horses, and humans, while in most other species, dietary ß-carotene is largely converted to vitamin A before absorption (Ribaya-Mercado *et al.*, 1989). They further reported absence of ß-carotene in blood plasma of guinea pigs and rabbits.

3.2.3. Physiological functions of vitamin A

Besides the well known functions of vitamin A in the visual process, vitamin A is also known to have many systematic effects on various physiological processes

including growth, embryonic development, fertility, haemopoiesis, immune response, epithelial cell differentiation, synthesis of corticosterone, prophylaxis of neoplasms and bone development. On epithelial cell differentiation, deficiency affects the major barrier to infection in the bronchial, gastrointestinal, urinary and other epithelia. Weakening of these barriers by keratinizing metaplasia is probably the mechanism as a result of which the incidence, severity and duration of various infections are increased (World Health organization, 1982).

3.2.4. Physiological function of ß-carotene

ß-carotene can be said to have all the functions of vitamin A. However this is indirect as it has to be converted by a dioxygenase to vitamin A. In addition, work done in Germany (Meyer *et al.*, (1975), Lotthammer *et al.*, (1976), Lotthammer and Ahlswede,(1977), Schams *et al.*, (1977), and Ahlswede and Lotthammer,(1978) indicated that ß-carotene has specific functions of its own independent of vitamin A. These include wound healing, prevention of mastitis, anti-carcinogenic, growth promoter, metabolism (activation of the thyroid), and influence on butterfat content in milk. Of the functions shared by both vitamin A and ß-carotene, the major ones being fertility and immune response,the latter has been found to be more potent.

3.2.5 Speculated mode of action of ß-carotene in influencing reproduction

Very little is known yet about the non-vitamin A related mode of action of ß-carotene. However in recent years various hypotheses have been put forward which may partly explain the physiological mechanisms. It has been thought for some time that the ability of the corpus luteum to synthesise progesterone depends on its ß-carotene supplies (schultz *et al.*, 1973). Jackson, (1981) and Meinecke *et al.*, (1984) showed that the synthesis of steroid hormones by the ovaries is reduced in cows with low plasma ß-carotene levels. Lotthammer *et al.*,(1978) found a positive correlation between cholesterol levels and ß-carotene concentration. Many of the symptoms occurring with low plasma ß-carotene levels can be explained in the light of the intricate interrelationship between reproductive hormones and reduced synthesis of these hormones in ß-carotene deficiency. Moreover, Schweigert *et al.*,(1986) found that the intrafollicular vitamin A concentration depends on ß-carotene in plasma and is correlated with follicle quality, follicle size, and intrafollicular oestradiol-17ß. These findings further confirm the independent role of ß-carotene in ovulation,and more generally, to the fertility of dairy cows. Kovanen *et al.*,(1979), suggested that since the low-density-lipoprotein associated cholesterol fraction in blood is the main substrate unit for ovarian production of steroid

hormones, ß-carotene could thus affect hormone synthesis through the metabolism of cholesterol. Jackson, (1982) postulated the theoretical sites for such action to be:

(a) In the liver by facilitating production of cholesterol from cholesterol ester.

(b) In the blood by acting as cholesterol "transport" agent.

(c) In the ovary by assisting the uptake of cholesterol and/or by facilitating the production of progesterone.

(d) At the gonads during steroidogenesis as an anti-oxidant.

The hypothesis and speculations in this area must nevertheless be left open in the absence of definitive proof but it is a fact that bovine fertility is better (Rensburg and Des vos, 1962), plasma cholesterol levels higher (Sinha *et al.*, 1981), and return to cyclicity post-partum quicker (Peters and Riley, 1982), during seasons when plenty of green forage is available. However,these researchers suggest that it is important to differentiate between a possible ß-carotene nutritional effect from those of day length, temperature and other nutritional factors, which require detailed and lengthy field studies supported by in vitro laboratory work.

3.2.6 Some roles of ß-carotene

3.2.6.1. Anti-carcinogenic nature of ß-carotenes

Of late there has been increasing interest in the potential of ß-carotene to provide protection again certain types of human cancer. Epidemiological studies indicate an inverse relationship between dietary intake of foods rich in ß-carotene and the occurrence of lung cancer,(Bendich, 1987, and Colditz *et al.*, 1985). Riegger, (1989) reported that ß-carotene is a promising micronutrient in the prevention of lung, cervix, oesophagus and stomach cancer. It has also been shown that ß-carotene has the potential to prevent certain types of animal tumors as well as their regression (Alam and Alam, 1985). Theories on causation of cancer suggest that free radicals play some part in this condition (Richter 1988 and Linnane *et al.*, 1989). ß-carotene is thought to prevent cancer because it is a potent quencher of singlet oxygen and other free radicals (Ganguly and Sastry, 1985, Burton and Ingold, 1984). Epidemiological data indicate that risk reduction for some types of cancer is more related to the intake of food rich in ß-carotene than to the intake of vitamin A (Colditz *et al.*, 1985). The same researchers suggest a similar function for canthaxanthin, a carotenoid without vitamin A activity.

3.2.6.2 **Growth promoter**

Not much work has been carried out on the growth promoter function of ß-carotene. However, Parigi-Bini, *et al.*,(1983) and Kormann and Schlachter,(1984) in experiments with ß-carotene and vitamin A supplementation of rabbits reported an 8% improvement in growth at 40 ppm ß-carotene intake. With graded levels of ß-carotene,it was found that 50 ppm ß-carotene gave maximum growth rate and feed conversion efficiency.

3.2.6.3. **Wound healing**

Gerber and Erdman, (1982) investigated the effect of retinol and ß-carotene supplementation on the rate of wound healing in rats and concluded that both vitamin A and ß-carotene increased wound repair during the initial 5 days inflammatory phase of wound healing. In this study, ß-carotene was found to have twice the potency of retinol, implying that it may have a specific function independent of vitamin A activity in wound healing.

3.2.6.4. **Immune Response**

Like other antioxidants, ß-carotene has been shown to have immuno-enhancement properties. Lotthammer, (1978) demonstrated that calves from ß-carotene supplemented dams had higher antibody titre than those from dams which were not supplemented. This finding is not unusual bearing in mind

that other vitamins/vitamin like compounds have been shown to affect the immune response. Seifter *et al.*,(1982) examined the effect of ß-carotene in mice at six dietary levels (0-270 m/g) and found that ß-carotene increased the thymic weight, stimulated allograft rejection, and inhibited virally induced tumour growth. Further work by Bendich, (1987) showed that both ß-carotene and canthaxanthin added to rat diets at 2000 mg/kg for a minimum period of 10 weeks, modestly but consistently enhanced T and B lymphocytes function. These researchers hypothesized that the immuno-enhancement from these experiments were due to the antioxidant function of ß-carotene per se than due to ß-carotene vitamin A independent effects.

3.3 ß-carotene requirements

The evidence available indicate that ß-carotene improves reproduction in dairy cattle. It has also been shown that ß-carotene supply from pastures varies with season. It is therefore important to establish ß-carotene requirement in dairy cattle. Jackson, (1982) pointed out that critical plasma concentration for normal fertility was a matter of debate but he quoted some researchers as suggesting this to be between 100-300 µg%. In 1978, Lotthammer gave the ß-carotene requirement for young cattle to be about 100 gm/day which also applies to dairy cows during the dry period. Later, Lotthammer, (1985) calculated the daily

vitamin A - independent requirement of ß-carotene per animal to be 300-400 mg. Indeed, this researcher categorized plasma concentration below 400 µg% as questionable for adequate supplies in dairy cows, while concentrations below 250 µg% and 150 µg% are critical and deficient for optimum fertility, respectively.

Requirement of the dairy cow for ß-carotene will however depend on the amount of milk produced and the amount of vitamin A in the diet. During lactation cows require an additional 20 mg of ß-carotene per litre of milk produced, such that a cow giving 25 litres should receive 600 mg of ß-carotene (Roche, 1978). Experiments carried out by Lotthammer, (1985) established levels for ß-carotene plasma concentration as an aid in deciding on ß-carotene supplementation needs and where this is necessary, by how much. Friesecke,(1978) indicated that ß-carotene would only improve fertility if it is the most limiting factor but it should not be considered a remedy for all bovine fertility problems. Hoffmann La Roche, (1989) recommends supplementation of ß-carotene as shown in Table 1.

3.4. Role of ß-carotene on performance of farm animals

3.4.1. Effects of ß-carotene supplementation in pigs

The reported effects of ß-carotene in performance of cattle prompted studies in other animal species. Sciaraffia,

(1981) investigated the effect of graded levels of ß-carotene on the ß-carotene content of the ovaries of gilts. While Sciaraffia,(1981) used feeding levels in the range of 0.1-1.6 mg per kg live weight per day, the highest plasma ß-carotene content was observed with a supplement of 0.2 mg ß-carotene per kg live weight per day. Chew, (1981), on the other hand, investigated the effect of supplementation on the uterine-specific-proteins (a basic glycoprotein with iron-binding capacity and an acidic protein with immuno suppression capabilities) reported to regulate embryonic development and survival. The results of the two studies is summarized in the Table 2. In a later study, Pingel, (1983) showed that ß-carotene supplementation resulted in a more intense, longer, and earlier oestrous than in the control group . In the same study, the litter size and number of piglets born alive were higher with supplementation, while return to oestrous after service was nil compared to 18% in the control group. The conclusion that can be drawn from these results is that ß-carotene may have specific functions on reproduction in pigs.

3.4.2 Effect of ß-carotene supplementation in horses

The importance of ß-carotene on equine fertility was not documented, in terms of gynaecological criteria as it had for cattle, until 1981. However a pilot study (Lindner and Gadient, 1981) reported marked beneficial effects of

Table 1. Specific ß-carotene indication for various functions in different species.

Animal species	Supplementation mg/head/day	Supplementation mg/kg feed	Application time	Duration days
		Reproduction		
Cow	300-500	____	3 wks before calving up to conception	100
Bull	300-500	____	During winter	150
Sow	200-400	____	2 weeks preweaning up to 2 weeks postweaning	60
Mare	400-600	____	3 wks before foaling	60
Rabbit	_____	30-40	Throughout lactation period	30
		General health		
Calf (immune response)	100-150	____	Milk replacer	30
Cow (mastitis prevention)	300	____	6 weeks before up to 10 weeks after calving.	110

Source:Roche Information Service, 1989.

TABLE 2. **Effect of ß-carotene on reproduction in pigs**

Parameter	Control without vit.A & ß-carotene	Vit.A only	Vit.A + ß-carotene	Source
No. of *corpora lutea*/gilt	12.5	12.6	20.2	Sciaraffia (1981)
Uterine-specific proteins (mg)				
Basic	4.3	10.6	15.0	Chew (1981)
Acidic	1.7	6.5	6.9	Chew (1981)

ß-carotene supplementation on disturbances of fertility; atrophy of the ovaries, disturbance of follicle maturation, delayed ovulation, in mares. These researchers observed that in horses, as in cattle, seasonal fluctuation, in ß-carotene supply are reflected in the blood ß-carotene levels. Schubert and Henning,(1983) carried out a trial to assess the effect of ß-carotene (230-700 mg/mare/day) and Vitamin A (40,000 IU/mare/day) supplementation on fertility and reported variable responses among different horse types. Trotter and Serum mares required 1.4 services less per mare for conception, the abortion rate was three times less, and the conception rate 24% higher in the supplemented group than in the control group. In the same study, it was noted that ß-carotene supplemented mares had more than double the titre of pregnant mare serum gonadotrophin (PMSG) compared to control animals. Holst, (1984) investigated the effect of ß-carotene supplementation on ponies and found that conception rate in supplemented ponies was better (67%) than control (25%). In addition oestrus symptoms in the ponies receiving ß-carotene supplementation were stronger and early embryonic mortality tended to be reduced. This clearly indicates that ß-carotene supplementation improves reproduction in horses.

3.4.3 Effect of ß-carotene supplementation in dairy cattle

3.4.3.1 Vitality of dairy calves

ß-carotene and vitamin A supply via the colostrum has been shown to have a definitive effect on vitality of the newborn calves and on their resistance to early calfhood diseases (Konermann and Abou el Fadle,1966). In 1978, Lotthammer investigated the effect of ß-carotene on calf diarrhoea and found that, by the third day of life, only 12.5% of the calves from animals receiving ß-carotene supplementation had diarrhoea compared to 35.3% for the control group. The difference was most pronounced at 12 days of age when the supplemented group recorded 18.8% and the control group 52.9% diarrhoeal incidence. From the same study,it was shown that calf mortality was 11.7% in the control group while in the treatment group no mortality was recorded by one week of age. The conclusion drawn from these results was that ß-carotene supplementation significantly reduced calf diarrhoea. These differences in vitality were probably due to differences in composition of the colostrum. The colostrum and the subsequent milk, from the control group had lower levels of vitamin A and of ß- carotene than from those supplemented with ß-carotene. In addition, the calves of the supplemented group had higher immunoglobulin levels than the calves of the unsupplemented group after the first feed. From this study, Lotthammer, (1978) concluded that

calves from cows with ß-carotene deficiency have weaker defenses immediately after birth and are therefore more susceptible to diarrhoea than calves from cows adequately supplied with ß-carotene during the late gestation period.

3.4.3.2. Breeding bulls

Weiss,(1975) demonstrated that ß-carotene possesses a spermatogenic function which is distinct from that of vitamin A. ß-carotene deficient bulls had lighter adrenal cortex, lower concentration of ß-carotene in testicular parenchyma, low proportions of motile spermatozoa, an increased proportion of deformed spermatozoa,and less glandular tissue in the seminal vesicles. This suggests participation of ß-carotene in the synthesis of testosterone. It has been postulated that ß-carotene does not act on the seminal epithelia but acts on the leydig's cells (Lange, 1977). However, adequate information is not available to elucidate the specific role of ß-carotene on spermatogenesis.

3.4.3.3 Mastitis in dairy cattle

Mastitis has been one of the main problems in the dairy industry. A diseased udder causes a reduction in milk production as well as condemnation of the milk due to high bacterial counts. Use of antibiotics is being discouraged because of bacterial resistance and presence of antibiotic residues in the milk and meat. Hence alternative,

non-antibiotic, methods are being tried to overcome these shortcomings. Such methods of therapy include vaccination and nutritional prophylaxis (Chew,1983). ß-carotene is one of the nutrients thought to reduce incidence of mastitis. Chew *et al.*,(1983) carried out a study to investigate the possible relationship between incidence of mastitis and ß-carotene and vitamin A content in various diets. The results of the work showed a high incidence of mastitis, associated with low levels of ß-carotene and vitamin A in the plasma, while mastitic cows tended to have lower (3-4 times) vitamin A but higher (30-40 times) ß-carotene content in the milk compared to plasma. Cows with negative or trace California mastitis test scores had an average ß-carotene plasma level of 215 µg%, while those with high mastitis scores averaged 150-185 µg%. In a subsequent study, Chew, (1983) reported that only 13% of the animals receiving ß-carotene had mastitis compared to 67% for the control group. Vitamin A at either low or high levels of supplementation had less effect on mastitis incidence than ß-carotene. From these two studies it is not clear whether ß-carotene merely acts as a readily available and concentrated pool of vitamin A, or whether ß-carotene may have a more direct role. However, the benefits of a nutritional approach to mastitis control are that the cow will more likely be able to defend herself against most mastitic organisms possibly through the maintenance of a healthy protective epithelial barrier and an enhanced immune

system, but in addition,in the process of maintaining the udder health, the entire body systems are likely to benefit. Such an approach would result in less antibiotic resistance and reduced antibiotic residues in animal products.

3.4.3.4 **Effect on milk composition**

Lotthammer,(1979) noted that ß-carotene deficiency affects milk composition, particularly during peak lactation. The possible explanation could be due to better ruminal milieu and consequently more favourable conditions for the ruminal function and bacterial activity. ß-carotene supplementation is said to increase butter fat which is desirable in milk. However information on the mode of action is not available.

3.4.3.5. **Dairy cow fertility**

Bovine corpus luteum has been found to contain higher concentrations of ß-carotene than other tissues unlike in other species (Ahlswede and Lotthammer, 1978). This high ß-carotene content in the corpus luteum is dependent on plasma ß-carotene (Anwandter, 1974). However, Ahlswede and Lotthammer, (1978) found no correlation between plasma and hepatic ß-carotene in dairy cattle. In addition, the plasma ß-carotene content for cattle at various stages of the oestrous cycle is not uniform. Seitaridis,(1963) found that *luteal* phase had the highest concentration followed by

follicular phase while cows with ovarian cysts had the lowest amounts. No such variation was observed with vitamin A. These researchers also reported that the content of ß-carotene in the corpus luteum and in other tissues depended on the type of feed the animals were on. Indeed, the concentration of ß-carotene in these tissues and blood plasma varies with season or type of forage offered (Anwandter, 1974). This was found to be higher when the pasture was green in summer and low in winter when feeding was on hay. In the tropics one would also expect low ß-carotene in foliage during the dry season. Schultz *et al.*,(1973) surveyed the ß-carotene and progesterone concentration in the corpus luteum at slaughter and not only did they confirm that ß-carotene levels were two to three times higher in summer than in winter but also found a similar pattern for progesterone levels. These findings were also supported by Meyer *et al.*,(1975) and Lotthammer and Ahlswede, (1977) who showed that plasma ß-carotene is a reflection of supplementary ß-carotene or the pasture quality.

Effect of ß-carotene on conception was studied by Konermann, (1974) who reported that first insemination to conception interval was shortened by ten days when ß-carotene supply to the herd was increased by 100 mg/day. Calving interval and the time-lag between onset of oestrus and ovulation were also shortened. The duration of oestrus was shorter with 0.5 hours while the incidence of cystic ovaries

was nil compared to 50% in the control group. The interval between peak luteinizing hormone (LH) and ovulation was delayed by almost 24 hours in the ß-carotene deficient heifers. Schams *et al.*, (1977) also reported a maximum delay of ovulation to be 72.5 hours compared to 49 hours after peak LH in deficient cows and animals given ß-carotene supplements, respectively. Meyer *et al.*,(1975) also found that the corpora lutea of ß-carotene deficient heifers were smaller than those of the control group and reached maximum size four days later. Similar results were reported by Schams *et al.*, (1977) who also noted that cystic changes in the ovaries in the form of *lutea* and/or follicular cysts developed in 45% of the heifers with the lowest ß-carotene levels both in the blood and in various tissues. However, Schams and associates also found the level of vitamin A in the blood of the ß-carotene deficient heifers to be significantly lower than that in the control group, despite a more than double supply of dietary vitamin A in the deficient group. These researchers concluded that with deficiency of ß-carotene, for which low blood plasma levels are indicative, ovulation is delayed by about one day, the duration of oestrus is extended and the whole development of the *corpus luteum* is drastically retarded and impaired. This may in turn explain the abnormalities in the production of progesterone observed by Anwandter, (1974) and Schultz *et al.*, (1973). Lotthammer *et al.*,(1976) observed reproductive

disturbances such as prolonged, poorly defined oestrous and nymphomania, observed as swelling of the vulva, moist and reddened vaginal vestibule with a low discharge of mucus during non-oestrous parts of the cycle, in heifers deprived of ß-carotene for a period 6 to 7 weeks, which was attributed directly or indirectly to the ovary. In a later study Lotthammer,(1978) observed that insemination at the wrong time risks infection of the vagina which may lead to further complication as a result of purulent inflammation.

Mingazov, (1977b) did field trials on ß-carotene supplementation of dairy cows and reported that ß-carotene supplementation improved conception after the first insemination from 39 to 57 per cent. The differences persisted in the same order of magnitude during the second insemination. ß-carotene supplementation reduced the number of inseminations per conception from 2.0 to 1.4. Lotthammer, (1978) attributed the poor conception in ß-carotene deficient cows to ovarian disorders and also observed that if conception occurred at all, embryonic death at around sixth and seventh week of gestation and risk of early abortion was imminent.

Cooke and Comben, (1978) investigated the effect of seasons on a dairy herd fed maize or grass silage. No ß-carotene was offered as it was expected that the difference in grass silage and maize silage was sufficient to significantly affect the ß-carotene status of the two groups.

ß-carotene content of grass and maize silage was 45.2 and 13.0 mg/kg DM, respectively. These researchers observed that the plasma concentration of ß-carotene in the cows fed maize silage was low in autumn and dropped further in the course of winter whereas those fed grass silage maintained the levels well above 700 µg/100 ml. Conception rate after the first insemination was about 35% higher in cows fed grass silage compared to those on maize silage. However the fertility problems observed while the animals were on maize silage were reversed when the cows were put on pasture.

Wetherill, (1965) carried out a study to investigate the effect of ß-carotene on puerperal problems in cattle. From this study, it was observed that blood carotene level of 300 µg% or more corrected or essentially decreased puerperal problems. Similar findings have been reported by Mihalka, (1981) who investigated the influence of ß-carotene status on puerperal diseases in a Hungarian dairy cattle herd. Puerperal problems such as delayed uterine involution and retained placenta were increased when cows were ß-carotene deficient (Mihalka, 1981) and a decrease in puerperal disorders improved conception rate. Lotthammer, (1978) reported no ß-carotene effect on the incidences of retained placenta, although Akordor *et al.*,(1986) and Inaba *et al.*, (1986) found a direct correlation between retention of placenta and the plasma concentration of vitamin A and ß-carotene. It has been suggested that ß-carotene either

plays a specific vitamin A independent role in reproduction of cattle, or it acts on uterus-placenta separation after conversion into vitamin A (Inaba *et al.*, 1986).

UNIVERSITY OF NAIROBI

4.0 MATERIALS AND METHODS

4.1 Experimental location

The feeding trial was carried out in the Agricultural Development Corporation (ADC) farms in Kitale area of Trans Nzoia District. Kitale is in Western Kenya on the slopes of Mount Elgon. It is an agricultural area where mixed farming is practised, with dairying being an important enterprise,a fact which was taken into account in setting up the experiment. The district lies 1° North, 35° East with an elevation of 2134 meters. It has an average annual rainfall of about 1000 mm and an average annual temperature of about 18° C. The rainfall distribution is unimodal, with rains peaking between April and August as shown in Table 3b. The dry season occurs between september and March.

The ADC farms were chosen for the experiment because a large number of dairy cows on a well defined management programmes were available. Besides, ADC has a modern feed mill which could be used for production of concentrate feeds according to the specifications of this study. Another important fact worth mentioning was that the ADC management was willing to cooperate in this study. The farms used for the study were Olngantongo, Katuke and Zea which were within a reasonable distance from each other and also from the feed mill. These farms are located in different microclimates as rainfall data in Table 3a show.

Table 3a. **Amount and distribution of rainfall in experimental location five months before and after start of the experiment.(mm)**

NAMANDALA[1]

1990					1989				
Jan	Feb	Mar	Apr	May	Aug	Sep	Oct	Nov	Dec
12.4	103.4	93.4	214.8	55.7	135.2	91.2	156.3	76.9	184.1
SABWANI[2]									
19.8	90.1	140.7	143.7	77.5	79.3	80.6	54.2	39.7	122.4
Mean rainfall of five stations in and around the experimental location (1989-1990)									
28	92	122	190	114	111.2	87.4	119.8	61.0	101.9

Table 3b. **Mean rainfall (mm) of five stations in and around the experimental location (1980-1989)**

Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
12	28	100	168	158	105	136	135	91	81	72	31

1. Represents conditions similar to those of Katuke.
2. Represents conditions similar to those of Olngantongo and Zea.

Source: Kenya, Meteorological Department, Nairobi.

This experiment was carried out between January and April which was expected to be a dry season as shown in Table 3b. Rainfall data during the period of the feeding trial, (January-April) and five months prior to commencement of trial is shown in Table 3a.

4.2 Animals.

The main breeds of dairy cattle in the ADC farms at Kitale are Friesian, Guernsey, Ayrshire, Brown Swiss and Jersey. However in the farms used for this study, only Ayrshires were raised. These farms had a total of 3,900 animals, with Olngantongo raising 1,300, while Katuke and Zea raised 1,400 and 1,200 respectively. The experimental animals were selected from these three herds based on the following criteria :-

(i) they were to calve down within the planned experimental period.

(ii) they did not have a history of repeat breeding, abortion, stillbirths and retention of foetal membranes.

(iii) they were not beyond the eighth parity. Two heifers were also included in each treatment group.

(iv) they were to have a fair to good body condition score.

Allotment of the animals to either the treatment or control group was done by stratified randomization according to milk yield in the previous lactation, body condition and

parity. For purposes of allocation to treatment group, milk yield status for the pregnant heifers was based on the mothers milk production records.The means of parity, age, and milk yield after randomization is shown in Table 4. The experimental animals in each farm were herded together and received the same management (one herdsman and one inseminator) and the same milking environment.

4.3 Experimental diets.

A control and a test diet were formulated as shown in Table 5. Rovimix ß-carotene 10% was added to the test diet to provide 75 mg of ß-carotene per kg of the diet[1]. An extra 9.38 mg of vitamin E in the form of Rovimix E-25 was added to both control and test diets, to protect ß-carotene from oxidation and to improve absorption of ß-carotene as suggested by Mingazov, (1977a) and to ensure that both diets received the same amount of Vitamin E[2]. The feed was produced in a three chamber mill (grinding, mixing, and pelleting) with a capacity of 2000 kg. The main feed components were measured according to the formula used at the feed mill and introduced into the hammermill.

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1. Rovimix ß-carotene 10%: 1 kg of Rovimix 10% contains 1000 mg ß-carotene.
2. Rovimix E-25: 1 g contains 250 mg of dl-α-tocopheryl acetate,(250 IU Vitamin E).

These products were donated by Hoffmann La Roche, Basle, Switzerland.

Table 4. **Mean parity, age, and milk yield of experimental animals.**

	Olngatongo		Katuke		Zea		Mean	
	Test	Control	Test	Control	Test	Control	Test	Control
Number of animals	15	17	14	15	12	13	41	45
Mean parity	2.3	2.7	2.5	3.0	2.9	3.2	2.5	2.9
Mean age (months)	61.5	71.1	66.4	77.2	72.5	80.0	66.4	75.8
Milk yield (kg/305 days)	3477	3473	3363	3235	3929	4043	3889	3584

Test:With ß-carotene; Control: No ß-carotene

Table 5: Composition of the experimental diets (%)

Ingredients	Control	Test
Reject maize	44.16	44.16
Reject wheat	7.95	7.95
Wheat bran	19.88	19.88
Molasses	5.00	5.00
Fish meal	3.75	3.75
Cotton seed cake	15.41	15.41
Urea	0.50	0.50
Limestone	2.04	2.04
Salt (NaCl)	1.11	1.11
Vitamin/mineral premix [1]	0.10	0.10
Extra vitamin E (Rovmix 25%)	0.0037	0.0037
ß-carotene (Rovimix 10%)	0.0	0.075
DE,Kcal/kg (calculated)	3263.480	3263.480

1: Zoodry VM 902-Premix supplied the following per kilogram feed vitamin A 10000 IU, vitamin D 1000 IU, vitamin E 10 mg, cobalt 2.5 mg, iron 80 mg, manganese 50 mg, zinc 45 mg, copper 6 mg, magnesium 100 mg, and iodine 0.8 mg.

Likewise ß-carotene, vitamin E, and vitamin/mineral premix were measured according to the formula and fed directly into the mixing chamber.

The compounded feed was then mixed thoroughly in this chamber after which the meal was preconditioned with steam. This was followed by mechanical pressing to form pellets. The two diets were fed to the experimental animals as described in Section 4.4.

4.4 Feeding and management of milking cows and calves.

All the experimental animals were on pasture which contributed the bulk of the total feed intake. They were grazed in herds of about 150 animals in paddocks of about 4 hectares for a period of one to two weeks depending on the amount of available pasture. All experimental animals in any one farm were grazed with other cows but were allocated to one herd. Rotational grazing was practised between the paddocks and where paddocking had not been done, strip grazing was done with electric fence. The animals were offered the concentrate feed at the rate of 3.5 kg to 4.5 kg per day, depending on the level of milk production, in two meals during the morning and evening milking. Besides grazing and the concentrates feed, the animals were sometimes fed on maize silage or kibbled reject maize, but this formed a small proportion of the total intake and never exceeded 2 kg and 0.5 kg/day (as is) respectively whenever it was fed.

Animals were drafted into the experiment two weeks prepartum, when the ordinary steaming up ration was replaced with the experimental diet which was offered at 2 kg per head per day. Two days before the expected calving date, or when calving was imminent, the animals were moved to the maternity paddock, to facilitate better attention during calving. The cows were then transferred from the maternity paddock to the lactating herd after expulsion of the foetal membranes.

After birth, the calf was allowed to remain with the dam for the first 3-4 days of life to ensure *ad libitum* feeding of colostrum. The calves were ear tagged within this period. The calves were then housed in individual calf-pens for the first month of age. Bull calves were disposed of at seven days of age. Since one of the aims of this study was to evaluate the effect of ß-carotene supplementation of dams on the viability and vitality of the calves, calves born to the animals in the experiment were fed on milk from the respective treatment group. The calves were bucket fed two litres of milk twice a day from the third day of life to weaning at three months of age. After one month of age, the calves were let out on pasture. The calves were prepared for an early weaning by introduction of "calf early weaner pellets" at the beginning of the third month of age. Calves showing any sign of disease were promptly attended to by a veterinarian.

4.5. Sample collection

4.5.1 Milk samples

The animals were hand milked and yields recorded per milking from which individual and herd totals of daily yields were determined, starting from 3-4 days after calving. Milk samples for composition analysis were first taken at two weeks after calving and at two week intervals thereafter until the end of the experiment. These samples were taken during the morning milking for each experimental animal. A total of 400 samples were taken, preserved in formalin (5 drops of 40% formalin in 200 ml of milk), and then deep frozen. The samples were kept for periods ranging from two weeks to three months as it was not possible to concurrently do the field and laboratory work. Procedure for determination of milk fat is given in Section 4.7.4

4.5.2. Blood samples for ß-carotene determination.

Blood samples were first taken two weeks before calving, i.e just prior to starting of the feeding trial, the second sample was obtained 24 hours post-partum and finally a sample was obtained any other time when the animal was on heat. Animals which did not show any heat during the experimental period were sampled at the end of the feeding trial. The blood samples were obtained by jugular venepuncture and put in heparinized bottles. The heparinized blood samples were then centrifuged at 1200 g for 15 minutes and the plasma

decanted into plastic tubes and deep frozen awaiting analyses.

4.5.3. Pasture samples.

Pasture samples were taken monthly during the experimental period. By simulating a grazing cow, adequate pasture sample was taken by hand grabbing across the diagonal of the grazing field. The samples were oven dried, hammer milled through a 1 mm sieve and then stored awaiting laboratory analysis.

4.5.4. Concentrate feed samples.

Feed samples were taken before and after pelleting for proximate analyses and ß-carotene concentration determination. A total of four samples were taken for each feed type in either the meal or pellet form since the feed mixing was done two times in the course of the experimental period.

4.6 Parameters measured.

4.6.1 Retained foetal membranes and uterine infection.

A few days before the expected calving dates, cows were kept in maternity paddocks for observation. Unnecessary interference during parturition was avoided. An animal was considered to have retained the foetal membranes if twelve hours expired between the calf and placental expulsion

(Julien and Conrad, 1976). Thereafter animals were observed for bloody, purulent or unusual vaginal discharge. Any of these signs was recorded positive for uterine infection. The severity of uterine infection was not categorized but was recorded on an all or none basis.

4.6.2 Uterine state and ovarian function

All the experimental animals were assessed for uterine involution and ovarian function by rectal palpation two weeks after parturition. This was recorded as the diameter of the uterine horns, and the functional structure of the ovaries. Repeat of rectal palpation was done for animals which did not show heat signs by 30 days post-partum to check for ovarian cysts or ovarian dysfunction. Uterine palpation was also done whenever an animal was suspected to have endometritis.

4.6.3. Service period,insemination index and conception rate.

Parturition and heat dates were recorded. An animal was considered due for service if she was on heat, was not suffering from metritis and was over 45 days postpartum. Repeat breeders were served any time they came on heat up to the third heat after which an obstetrical examination was carried out. Animals which developed puerperal problems, were served after the condition subsided. Service period was calculated as the period between parturition and successful

insemination. Services per conception or insemination index was calculated as the number of services leading to the same successful insemination. Pregnancy diagnosis by rectal palpation was done around seventy days post service to avoid embryo interference.

4.6.4 Heat intensity

Heat signs were observed by farm personnel with the help of a teaser bull which was penial deviated. The characteristics observed and recorded were, amount of mucous, duration of active oestrous and activity of the animal. The amount of mucous was scored as plenty, little and none or not noticed and assigned numerical scores of 3, 2 and 1, respectively. Activity of animal during oestrous was scored as, very aggressive, aggressive or vigorous, active and indicative with numerical scores of 4, 3, 2 and 1, respectively. The heat intensity index was derived as the sum of the scores in all the characteristics considered.

4.6.5 Mastitis

Californian mastitis test kit was used to test for mastitis in all experimental animals, first at 2 weeks post-partum and then fortnightly thereafter. Animals with clinical mastitis at parturition were recorded positive. Scoring for mastitis, based on viscosity and gel formation, was characterized as subclinical, mild and severe, where the

latter two represented clinical mastitis, This scoring was then given values whereby severe was 3 and subclinical and mild were given 1 and 2, respectively. An animal or quarter(s) with no mastitis during the test were given a value of zero. Regardless of the fact that clinical mastitis were being treated, records were kept of when mastitis was first observed, and when recovery occurred to give duration of the condition.

4.6.6 Calf diarrhoea and viability

All calves were kept under constant observation for diarrhoea, and unthriftiness. Any calf showing diarrhoea or unthriftness was noted and attended by a veterinarian. Mortality records were also kept. The duration of diarrhoea and unthriftness was recorded.

4.7 Laboratory analyses

4.7.1 Chemical assay of ß-carotene in plasma.

The method used was as described by Brubacher and Vuillermier, 1974 (see Appendix 1). This involved extraction of ß-carotene and other carotenoids with cyclohexane. The ß-carotene was separated from other carotenoids by chromatography using deactivated aluminium oxide column. ß-carotene was then eluted using hexane and petroleum ether fraction. This elute was evaporated to dryness and the residue picked with cyclohexane. The absorbance of the

cyclohexane solution was determined using a spectrophotometer (Beckman, model 24) in a 1 cm cuvette at 455 nm wavelength. The concentration of ß-carotene was calculated by taking 2500 to be the extinction coefficient of 1% ß-carotene in cyclohexane in 1 cm cuvette.

4.7.2 **Chemical assay of ß-carotene in forage.**

ß-carotene assay from forage was carried out as described by Seibold and Bassler, 1974 (see Appendix 2). The forage was dried to a moisture content of less than 15% and comminuted to 100% passage through a 0.8 mm mesh screen. Carotene and Xanthophyll were exclusively extracted with a hexane-acetone mixture at room temperature. The extract was saponified with methanoic potassium hydroxide to destroy the chlorophyll. An aliquot of the clear supernatant was evaporated to dryness and the residue picked up in petroleum ether. This extract was then passed through an aluminium oxide column and ß-carotene eluted with petroleum ether while xanthophyll was eluted with absolute ethanol sequentially. Absorbance of the two solutions were determined using a spectrophotometer (Beckman, model 24) in a 1 cm cuvette at 450 nm wave length. Concentration of ß-carotene and xanthophyll were calculated by taking 2600 and 2500 respectively to be extinction coefficient of 1% ß-carotene in a 1 cm cuvette at 450 nm.

4.7.3 Chemical assay of ß-carotene in admixed feed.

ß-carotene assay was carried out as described by Manz, 1977 (see Appendix 3). A known quantity of feed was suspended in distilled water to which trypsin and pepsin were added to digest the gelatine coat protecting ß-carotene from oxidation. ß-carotene was then extracted with chloroform solution. Water in the chloroform solution was then exsiccated with anhydrous sodium sulphate. The chloroform solution was then evaporated to dryness and the residue picked up with hexane. Saponification was not done as the fat content in the feeds was low (see Table 6). The extract was then passed through an aluminium oxide chromatographic column to exclude other carotenoids. ß-carotene was eluted with n-hexane and iodine was added to isomerize ß-carotene. The extract was dried again and picked up with cyclohexane. Absorbance of this cyclohexane solution was determined using a spectrophotometer (Beckman, model 24) in a 1 cm cuvette at 454 nm wavelength. The concentration of ß-carotene was calculated by taking 2230 to be the extinction coefficient of a 1% ß-carotene cyclohexane solution in a 1 cm cuvette at 454 nm.

4.7.4 Milk butterfat assay.

The milk butter fat was assayed using the standard Garber method. 10.0 ml of sulphuric acid (density 1.825) was pipetted into a butyrometer, followed by 11 ml of the milk

sample. 1 ml of amyl alcohol (density 0.815) was added and the mixture thoroughly shaken to ensure complete hydrolysis of proteins by the sulphuric acid. The mixture was then placed in a water bath at 70° C for 10 minutes, followed by centrifuging at 2000 g for 5 minutes. The butyrometer were then incubated in the water bath for a further 5 minutes before reading the percentage butter fat. The milk butterfat from each animal was analyzed in duplicate after pooling the first 4 milk samples collected from each cow during the experiment period.

4.7.5 Proximate analyses.

Proximate composition of the experimental diets and the pasture were determined according to the standard procedures (Association of Official Analytical Chemists, 1984).

4.7.6 Statistical analysis

Data collected were analyzed by Least Squares Maximum Likelihood Computer Program (Harvey, 1987), using the model shown:

$$Y_{ijkl} = \mu + F_i + T_j + P_k + b(m_{ijkl}) + e_{ijkl}$$

Where Y_{ijkl} = observation in the i^{th} farm in j^{th} treatment and in k^{th} parity.

μ = overall mean

F_i = Effect of the i^{th} farm

T_j = Effect of the j^{th} treatment

P_k = Effect of the k^{th} parity

b = the regression on milk yield(m_{ijkl}) of the individual (x_{ijkl}) in kg.

e_{ijkl} = residue component.

5. RESULTS AND DISCUSSION

5.1 General observations

The pasture composition varied between the farms and even within the farms. Improved pastures predominated and natural pastures were mostly in paddocks where rotational crop growing was not practised. The predominant grasses were, Kikuyu grass (*Pennisetum clandestinum*), Mbarara rhodes (*Chloris gayana*), Nandi setaria (*Setaria sphacelata*), Stargrass (*Cynodon dactylon*), and Signal grass (*Brachiaria brizantha*) with Chloris gayana being the most dominant grass in the three farms. The mean monthly rainfall recorded in 1980-1989 in and around the experimental location from December to April was 31, 12, 28, 100 and 168 mm, respectively, as compared to that of 1990 during the same period which was 101.9, 28, 92, 122 and 190 mm in the same order. Thus, the rainfall was heavier than is normal for the area during this part of the year and the pastures remained green throughout the experimental period. All experimental cows calved down as planned within a period of three weeks. The first calving occurred in mid-January and the last ones occurred three weeks later.

5.2 composition of feeds

Concentrate feed samples were taken in January and March when these feeds were being compounded. Analyses of these together with pasture samples collected at the same time was not done until the end of the feeding trial in May. The mean ß-carotene content of the test diet in meal form was 72.0 and 74.0 mg/kg DM for the feed produced in January and March, respectively, while the corresponding values for the pellet form were 30.0 mg/kg and 50.00 mg/kg. The control diet in either meal or pellet form had a ß-carotene content of 0.1 mg/kg. The mean pasture ß-carotene content was 16.0 and 44.0 mg/kg DM for samples taken in January and March, respectively. The pasture ß-carotene content varied between the farms which may be explained by the differences in microclimate as well as the fact that rotation of animals between paddocks was not synchronized among the farms and samples were taken at the same time in all the farms which resulted in different pasture conditions at the time of sampling. The pastures were of good quality with a mean crude protein content of 9.4%. The chemical composition of both the compounded feeds and pastures is shown in Table 6.

The difference in ß-carotene content between the meal and pellet forms of the concentrate diet could be due to the pelleting action or the extra processing in preparation of the samples for analyses. ß-carotene loss due to pelleting, has been reported by Konerman and Abou el Fadle,(1966),

Table 6. **Chemical composition of the feeds (D.M. basis)**

	Concentrates		Pastures		
	Meal	Pellets	Olngan-tongo	Katuke	Zea
Crude protein,%	18.50	18.50	11.3	8.61	8.4
Crude fibre,%	8.42	8.42	33.3	36.1	35.0
Ether extract,%	6.62	6.62	4.0	3.5	2.0
Ash,%	7.45	7.45	11.8	11.4	8.4
Nitrogen free extract,%	49.19	49.19	33.6	32.5	39.8
Phosphorus,%	0.63	0.63	0.30	0.28	0.25
Calcium,%	1.53	1.60	0.42	0.69	0.43
ß-Carotene, ppm					
Test					
January	72.0	30.0	14.4	23.0	10.9
March	74.0	50.0	42.0	65.0	25.0
Control					
January	0.1	0.1	____	____	____
March	0.1	0.1	____	____	____

Shields *et al.*, (1982) and Bieber-Wlaschny,(1988). This loss was attributed not only to the physical destruction of the gelatine coat but also to the steam preconditioning and the subsequent rise in temperature in these feeds during the pelleting process. Storage has been found to be accompanied by decrease in ß-carotene content of feeds due to oxidative degradation. This would be expected, since ß-carotene like other antioxidants is spent in the process of quenching oxidative processes occurring in the compounded feed.

Thus, the differences in ß-carotene content from the pasture and concentrate feed samples collected in January and March could be due to differences in duration of storage. This effect appears to have been more pronounced in pasture samples which is in agreement with findings of Kalac, (1980) who reported rapid degradation of ß-carotene from natural origin catalyzed by intrinsic haematins in plants. Another contributing factor to higher pasture ß-carotene content in March, was the heavier rainfall in the month of March than in December-January as shown in Table 3, as the sampling was done in mid January and end of March.

5.3 ß-carotene intake.

The experimental animals received ß-carotene from pasture and the concentrate feed. The estimated ß-carotene intake as well as the procedure used in deriving this is shown in Table 7. Since the animals were under free grazing

Table 7. Estimated total daily ß-carotene intake (mg/day)

	With ß-carotene	No ß-carotene
Average body weight, Kg	496.38	512.09
Expected average dry matter intake,kg/day	11.00	11.00
Concentrate (a)	3.60	3.60
Pasture (b)	7.40	7.40
Average ß-carotene content, mg/kg DM		
Concentrate (c)	80.30	0.11
Pasture (d)		
Olngantongo	27.70	27.70
Katuke	44.00	44.00
Zea	17.50	17.50
ß-carotene intake(mg/day) from:-		
Concentrates (a*c)	300.00	0.40
Pasture (b*d)		
Olngantongo	204.98	204.98
Katuke	325.60	325.60
Zea	129.50	129.50
Estimated ß-carotene intake(a*c)+(b*d)		
Olngantongo	504.98	205.38
Katuke	625.60	326.00
Zea	429.50	129.90
Estimated mean intake,mg/day	520.03	220.43

rather than stall fed, actual feed intake could not be determined. For purposes of estmating ß-carotene intake, established feed intake values adjusted for animal live weights and milk yield were used (ARC,1980). The average dry matter intake per day was thus estimated as 11.0 kg of which 3.6 kg was supplied as concentrate and the balance of 7.4 kg was assumed to be supplied from pastures. Daily ß-carotene intake, from the concentrate supplement ranged from 300 mg - 383 mg/cow, while pasture supplied about 220.4 mg/head/day. The estimated total daily ß-carotene intake in the test and control group was 520.0 and 220.4 mg, respectively. Thus from these figures only the test group had an intake above the 300 mg/head/day minimum threshold (Lotthammer,1978 and Jackson, 1982).

From the estimated ß-carotene intake only the test group received adequate ß-carotene to cater for production of 15.3 kg of milk per day recorded in this study. Lotthammer,(1978) recommended 100 mg of ß-carotene per day for maintenance and an allowance of 20 mg ß-carotene per litre of milk. Green pastures have been reported to have ample ß-carotene and supplementation is usually not necessary where adequate quantities of this are available (NRC,1980, Lindner and Gadient, 1981 and Bieber-Wlaschny, 1988). On average, the ß-carotene content of the forage in this study was higher than the 12-15 mg/kg; equivalent to 30 mg/day/100 kg live weight; which was suggested by Bonsembiante *et al*, (1980) as being

adequate for preventing its negative influence on fertility.

5.4 Plasma ß-carotene

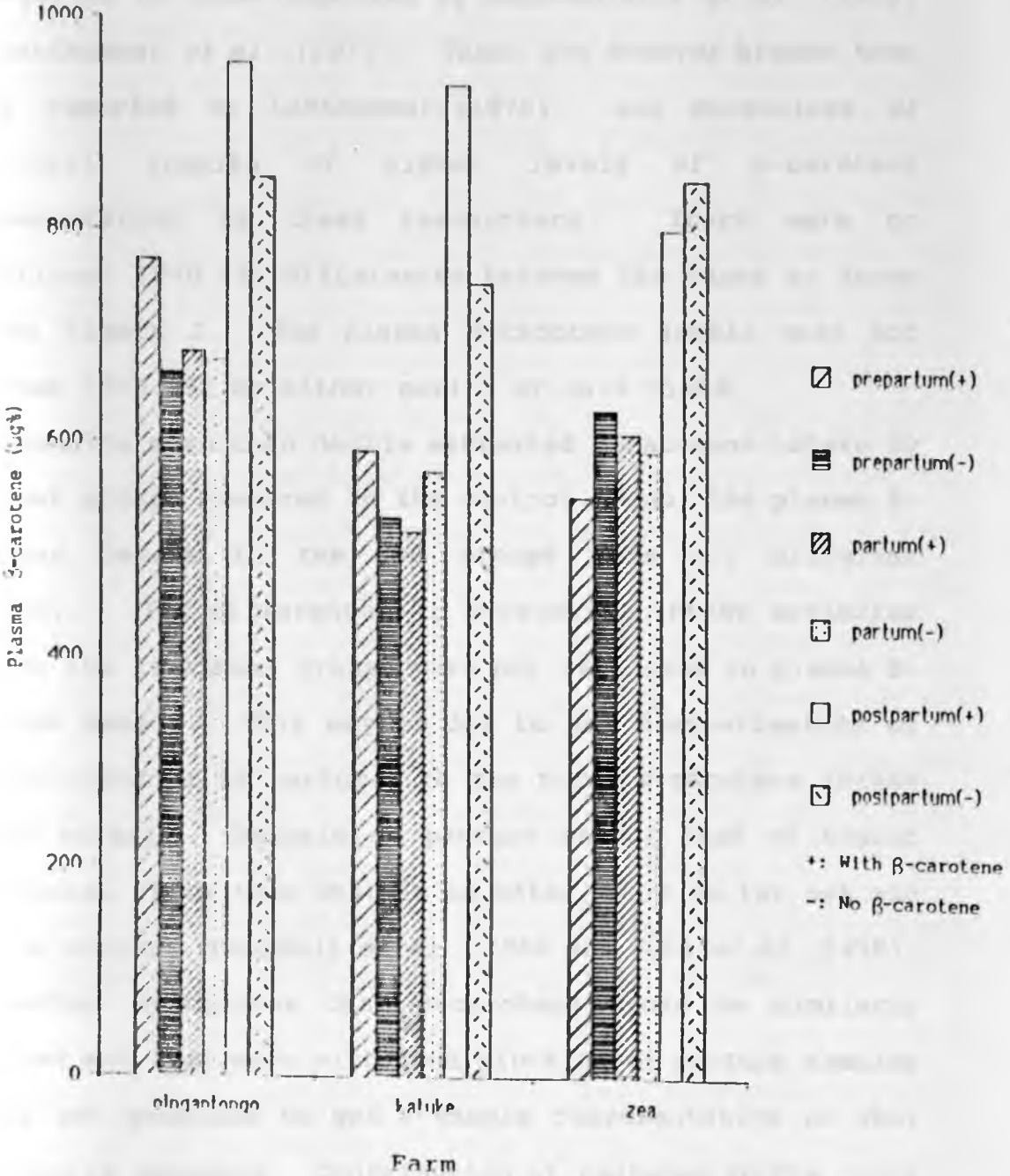
ß-carotene was determined in plasma samples collected two days before the start of the supplementation, at partum and at 2-12 weeks post partum as described in Section 4.5.2. Plasma ß-carotene levels are shown in Table 8. The mean plasma ß-carotene values for animals on the test diet were 679.7, 572.7 and 955.1 µg while those for the control group were 628.0, 589.0 and 831.1 µg% plasma prepartum, at partum and postpartum, respectively. The values were not significantly different between the treatment groups although the supplemented group had consistently higher values. The mean plasma ß-carotene levels prepartum, at partum and post-partum were 654.02, 580.85, and 858.1, µg %, respectively. Post-partum plasma ß-carotene levels were higher ($P<0.05$) than prepartum and partum levels. Prepartum and partum levels were not significantly ($P>0.05$) different. Wang *et al.*,(1988) used a similar supplementation level as in this study and reported a plasma ß-carotene level of between 200-300 µg % while Lotthammer *et al.*,(1976) using similar levels of supplementation reported plasma levels of 918 µg %. Variable plasma ß-carotene levels may be observed despite similar levels of supplementation. Such differences may be attributed to breed (Ribaya-Mercado *et al.*,1989), nitrites in feed (Ullrey,1972), vitamin E, dietary proteins, energy

Table 8. Effect of ß-carotene supplementation and physiological state on plasma ß-carotene (µg%)

	Prepartum	Partum	Postpartum	Mean
With ß-carotene	679.7±56.0[a]	572.7±47.0[a]	955.1±75.5[a]	735.8±36.0[a]
No ß-carotene	628.3±50.0[a]	589.0±46.0[a]	831.0±63.5[a]	682.8±32.8[a]
Mean	654.0±39.3[*]	580.8±37.3[*]	898.1±40.6[**]	

a: means within a column with the same superscripts are not significantly different (P>0.05).
* : Means within a row with different number of asterisks are significantly different (P<0.05).

Figure 2. Variation in Plasma β-carotene within farms and physiological states



density, and calcium:phosphorus ratio in the feed (Jackson, 1982). The plasma ß-carotene levels observed in this study are similar to those reported by Bonsembiante *et al.*,(1980) and Lotthammer *et al.*,(1976). These are however higher than those reported by Lotthammer,(1978) and Huszenicza *et al.*,(1984) inspite of higher levels of ß-carotene supplementation by these researchers. There were no significant ($P>0.05$) differences between the farms as shown in the Figure 2. The plasma ß-carotene levels were not affected ($P>0.05$) by either parity or milk yield.

Despite more than double estimated ß-carotene intake by the test group, compared to the control group, the plasma ß-carotene levels in the two groups were not different ($P<0.05$). The differences in ß-carotene intake estimates between the treatment groups were not reflected in plasma ß-carotene levels. This may be due to an underestimation of the contribution of pastures to the total ß-carotene intake by the animals. Animals on pasture select feed of higher nutritional value than what is on offer, more so for ash and protein content (Campbell *et al.*, 1968 and Cook *et al.*,1958). The author speculates that ß-carotene would be similarly affected and that even with hand plucking of pasture samples it was not possible to get a sample representative of what the animals consumed. Contribution of pastures to the total dry matter intake was not measured and the value of 7.4 kg used in calculating the ß-carotene intake may have been an

underestimate. These together with storage of samples before analysis and processing for analysis could have resulted in underestimation of the total ß-carotene intake. In addition, the efficiency with which ß-carotene is absorbed from the gastrointestinal tract varies with dietary levels of ß-carotene being higher with low dietary supply (Lotthammer, 1978). However, both the test and the control group had levels well above the 300 µg% plasma which according to Wetherill, (1965), Lotthammer, (1978), and Jackson, (1982) constitute the critical minimum for optimum fertility.

Use of plasma ß-carotene as an indicator of intake is not reliable because variation not only exist among different trials but also between animals and season (Bonsembiante et al., 1983 and Meinecke *et al.*, 1986). Differences in the absorptive efficiency of intact ß-carotene between various breeds of cattle has also been suggested by Ribaya-Mercado *et al.*,(1989). The Ayrshire breed used in this study may respond differently to the documented Friesian, Black pied cows, Black-and-tan cows, and Holstein. Wang *et al.*, (1988) observed that Holstein cows did not respond to ß-carotene supplementation in a manner similar to the Germany Black Pied cattle. Similarly, Rakes *et al.*, (1985) and Graves-Hoagland *et al.*,(1989) observed that plasma ß-carotene concentration was higher in Jersey cattle than in Holsteins.

The trend in plasma ß-carotene values from prepartum to partum observed in this study is similar to that reported by

Lotthammer, (1978) and Varlygin and Vertyanyna, (1986). Though not significant (P>0.05) a drop in plasma ß-carotene soon after parturition was noted. This is speculated to be due to increased mammary gland uptake during the synthesis of colostrum (Wang *et al.*,1988 and Lotthammer, 1978). Postpartum increase of plasma ß-carotene is thought to be due to decreased mammary gland uptake with the transition from colostrum to normal milk synthesis (Lotthammer, 1978, Stampfer, 1982, Varlygin and Vertyanyna, 1986 and Pivnyak *et al.*,1987).

5.5 Milk yield and composition

The mean daily milk yield for the test and control animals was 15.3 kg and 15.4 kg which indicates that ß-carotene supplementation had no effect on milk yield. As expected, milk production increased with increase in parity with animals in eighth parity giving the highest yields. These results agree with those of Lotthammer *et al.*,(1978) and Akordor et al,(1986) who reported that ß-carotene supplementation had no effect on milk yield. However, results of this study differ with those obtained by Starikova, (1985) and Varlygin and Vertyanyna,(1986) who reported that ß-carotene supplementation was coupled with increase in milk production. The lack of significant differences in milk yield between treatments observed in this study may be due to the low milk yield levels compared to the

over 30 kg per day reported by Starikova, (1985) and Akordor, *et al.*, (1986). These low yields in both groups suggest that there were other limiting factors and that ß-carotene was not necessarily limiting. There were no differences in milk yield between farms which indicate that the cows' potential and the feed composition was fairly similar.

Analysis of milk composition revealed that the mean butterfat content was similar in supplemented and control group at 3.5% and 3.4%, respectively, as shown in Table 9. Parity and milk yield had no significant ($P>0.05$) effect on butterfat. These results are in agreement with the findings of Varlygin and Vertyanyna,(1986) but are different from those obtained by Lotthammer *et al.*,(1978) and Popov, (1983) who found that ß-carotene supplementation was accompanied by increased butterfat content. There is scarcity of literature on the effect of ß-carotene on milk composition and none of the reports available to the author offered satisfactory scientific explanation for the reported effects. Butterfat is the most variable component of milk. It is affected by management factors, weather, genetic factors, age, stage of lactation, stage of milking and nutrition. Whether ß-carotene supplementation increases butterfat or not remains to be confirmed. Rumen fermentation is an important factor in the determination of butterfat. It is speculated that ß-carotene improves the rumen environment in favour of the butterfat precursors mostly during peak lactation (Lotthammer, 1979).

Table 9. Effect of ß-carotene supplementation on milk yield and composition

Parameter	With ß-carotene	No ß-carotene
Milk yield, kg/cow/day.	15.3±0.54[a]	15.4±0.50[a]
Butter fat,%	3.5±0.16[a]	3.4±0.14[a]

a: means within a row with the same superscripts are not significantly (P>0.05) different.

Farm effect on butterfat was not significant (P>0.05) and the minor differences observed can be attributed to variations in milk yield. The farms with the lowest milk yield had the highest butterfat content.

5.6 Fertility aspects.

5.6.1 Service period

The mean service period for all the 79 animals used in the determination of service period was 80.8 days, which was shorter than that reported by Lindner and Gadient,(1981). This period was half that reported in Kenya (Valker, 1990). The mean service period of the test group was 76.4 while that of the control group was 85.3 days. These means were however not significantly different (P>0.05). Parity and milk yield had no significant (P>0.05) effect on service period. However, service period was increasing with increase in milk yield.

Lack of significant difference in service period after ß-carotene supplementation has also been reported by Rakes *et al.*,(1984) who supplemented ß-carotene at a rate of 300 mg/head/day. However, Akordor *et al.*,(1986), Lotthammer *et al.*, (1976), Lotthammer and Alhswede, (1977), Schubert and Henning,(1983) and Huszenicza *et al.*,(1984), despite variable levels of ß-carotene supplementation, have reported significant shortening of the service period. Although the

farm effect was not significant, the difference in service period between the farms took the trend of the pasture ß-carotene content. Service period may be affected by management factors as well as the state of the uterus and the ovary. In this study there appears to be a correlation between service period and puerperal conditions.

This study confirms the findings of Lindner and Gadient, (1981) and Smith, (1980) that supplementation only improves service period if plasma ß-carotene level prior to supplementation is less than 300 µg%. This being a field study, there was no control of ß-carotene intake from the pastures and it appears that the animals were able to attain the minimum levels of ß-carotene recommended by various researchers. In a field study, Lindner and Gadient,(1981) concluded that ß-carotene deficiency would not arise when cattle were out on fresh pasture. They observed that such fresh pastures could ensure blood plasma levels of 500 µg% or more.

5.6.2 Insemination index and conception rate

The effect of ß-carotene supplementation on insemination index and conception rate are shown in Table 10. ß-carotene supplementation had a significant ($P<0.05$) effect on insemination index. The mean insemination indices were 1.4 and 2.0 for the test and control group, respectively. Parity and milk yield had no significant ($P>0.05$) effect on

Table 10. Effect of ß-carotene supplementation on service period, insemination index and conception rates

Parameter	With ß-carotene.	No ß-carotene
Service period, days	76.4±5.38[a]	85.3±4.87[a]
Insemination index,	1.4±0.19[a]	2.0±0.17[b]
Conception rates,%		
After first insemination	67.5±16.92[a]	38.0±6.40[b]
After second insemination	87.5±3.70[a]	64.3±0.61[b]

ab : means within a row with different superscripts are significantly ($P<0.05$) different.

insemination index.

The mean conception rates after the first service were 67.5% and 38.0% and these increased to 87.5 and 64.3% after the second insemination for the test and control group, respectively. The ß-carotene supplemented group attained better ($P<0.05$) conception rates after both the first and second insemination. The ß-carotene supplemented group required 15% services less for conception than the control group. These results compare well with those of Lotthammer *et al.*, (1976) who reported a 15% improvement in insemination index. Similarly Sokolovskaya and Skopets,(1986), Varlygin and Veryanyna, (1986), Tekpetey *et al.*,(1987), Seitaridis,(1963), Meyer *et al.*,(1975) and Ahlswede and Lotthammer,(1978) have reported increases in conception rate and improvement in insemination index with ß-carotene supplementation. However, Rakes *et al.*,(1984) and Akordor *et al.*,(1986) reported no improvement in insemination index on supplementation. Insemination index and conception rates may be influenced by inseminator, semen quality and the health of the uterus. As shown later, ß-carotene supplementation had no significant ($P>0.05$) effect on incidence of retained placenta, and metritis. In addition Muller and Owens,(1973) found that high incidence of metritis and retained placenta were not always followed by low conception rates or high insemination indices. Various researchers have tried to explain their results of improvement in conception rates and

insemination indices with ß-carotene supplementation. The suggestions put forward include, better oestrus signs and thus heat detection (Tekpetey *et al.*,1987), faster follicular ripening and thus earlier ovulation (Meinecke *et al.*,1986) and finally better phagocytic activity and humoral immunity to good uterine health and thus conception (Varlygin and Vertyanyna,1986). However, Sokolovskaya and Skopets,(1986) suggested that the most probable explanation was better sperm permeability, elasticity, and density of cervical mucous.

From this study, the heat intensity, heat duration, and ovarian activity, were better in the test group than in the control group and these may have contributed to better conception, due to better heat detection and insemination timing. Improvement of conception rates and insemination indices while other fertility parameters like service period were not significantly changed was not unusual in that Bonsembiante *et al.*,(1980) reported similar results with a low serum ß-carotene level of 228 µg% serum.

5.6.3 Heat Intensity

The results for heat intensity, determined as an index in 72 cows are shown in Table 11. ß-carotene supplementation had a significant ($P<0.05$) effect on heat intensity index which was 21.2 and 18.3 for the test and control groups, respectively. However, the components of heat intensity index were not similarly affected by ß-carotene supplementation.

Table 11. Effect of ß-carotene supplementation on parameters constituting heat intensity index

Parameters	With ß-carotene	No ß-carotene
Vulva signs	2.28 ± 1.407[a]	1.76 ± 0.127[b]
Heat activity	3.27 ± 0.136[a]	2.98 ± 0.118[a]
Mucous discharge	3.59 ± 0.136[a]	3.45 ± 0.122[a]
Heat duration	12.10 ± 0.560[a]	10.07 ± 0.506[b]
Heat intensity index [1]	21.23 ± 0.774[a]	18.25 ± 0.700[b]

ab: Mean within a row with different superscript are significantly ($P<0.05$) different.
1: Heat intensity index is an aggregate of the parameters listed in the table.

Thus no significant (P>0.05) effect was observed for either mucous discharge or heat activity. Heat intensity index varied greatly between the farms which could be attributed to the subjective scoring method used. Parity and milk yield had no significant (P>0.05) effect on heat intensity since the animals received ß-carotene according to the milk yield. Similar findings have been obtained by various researchers such as Lotthammer *et al.*,(1978) and Jackson, (1982) who reported better oestrus in general and greater heat activity, respectively with ß-carotene supplementation. Meinecke *et al.*,(1986) and Tekpety *et al.*, (1987) reported a short and intensive heat. However, Wang *et al.*,(1988) reported no improvement on duration of heat with ß-carotene supplementation.

The supplemented cows had a longer duration of heat than the control cows. In temperate regions some researchers have reported that the ß-carotene supplemented cows had shorter heat duration than the control group (Friesecke,1978; Lotthammer *et al.*,1976 and Meinecke *et al.*,1986). In addition the heat duration values reported by these workers were longer than those from this study. Meinecke *et al.*,(1986) reported values of 23.0 and 31.0 hours for the test and control groups, respectively compared to 12.1 and 10.1 hours for the respective groups in this study. These discrepancies might be due to heat signs passing unnoticed as the cows were not being stall fed, biases of the heat detector or too weak

signs of heat to be noted when they started or ended.

5.7 Puerperal conditions

5.7.1 Retained placenta, metritis and uterine involution.

Effect of ß-carotene supplementation on retained placenta, metritis and uterine involution is shown in Table 12. The data were collected from 94 cows. ß-carotene supplementation had no significant ($P>0.05$) effect on retained placenta, metritis, and uterine involution. The incidence rate of retained placentae was 9% and 8% for test and control groups, respectively, while that of metritis was 16% and 17% in the same order. Incomplete uterine involution, fourteen days postpartum was 33.3% for both groups. Variation in response to ß-carotene supplementation between the farms was not significant, despite different levels of estimated ß-carotene intake. Parity and milk yield had no significant effect on retained placenta, metritis and uterine involution. Not all animals with retained placenta developed metritis. However, most of the cows with incomplete uterine involution fourteen days postpartum had metritis or developed metritis thereafter.

The results obtained from this study are in agreement with those of Lotthammer, (1978) who reported that ß-carotene supplementation did not affect placenta retention. Huszenicza *et al.*,(1984) Akordor *et al.*, (1986),

Table 12. Effect of ß-carotene supplementation on puerperal conditions and ovarian state

Parameter	With ß-carotene	No ß-carotene
Retained placenta,%	9.0±3.8[a]	8.0±9.8[a]
Metritis,%	16.0±1.08[a]	17.0±13.6[a]
Incomplete uterine involution, %	33.3±2.5[a]	33.3±10.1[a]
Ovarian cyst,%	2.4±3.8[a]	11.0±4.8[a]
Ovarian activity, %	64.3±10.2[a]	43.2±6.7[a]

a: means within a row with the same superscripts are not significantly different (P > 0.05)

Inaba *et al.*,(1986) found a direct correlation between retention of placenta and the plasma ß-carotene concentration. Again in agrement with these latter researchers differences between treatments would not be expected since the two treatment groups had similar plasma ß-carotene levels. The mean incidence of retained placenta in this study is in the expected range of 8% in normal calving, 25-55% in dystocia and 11% in all calvings, in a well managed herd (Arthur *et al.*,1982). These values were however, higher than those obtained in ß-carotene supplemented herds as reported by Huszenicza *et al.*,(1984) and Inaba *et al.*,(1986). Placenta retention is not only influenced by the nutrition of the animal but also by its genetic predisposition and is more common in Ayrshire, than Friesian cows (Muller and Owens, 1973). In addition, a two week prepartum supplementation period might have been too short for a positive response.

The results of this study are similar to those of Wang *et al.*,(1988) who found no effect of supplementation on metritis. Mihalka, (1981) also reported that puerperal diseases and fertility were strongly correlated to plasma ß-carotene. However, in this study no significant difference was observed between the test and control the group. This can be explained by the similar plasma ß-carotene levels between the two groups. Failure to obtain significant differences in uterine involution can be similarly explained. Farm effect on metritis was not significant. The lack of

significant differences in these parameters suggest that, ß-carotene was not limiting.

5.7.2 Ovarian cysts and activity

Ovarian status was determined by palpation per rectum in 87 cows. The data collected did not differentiate between luteal and follicular cysts. The rate of incidence of cysts in the ß-carotene supplemented group was lower, though not significantly ($P>0.05$) different from the control group at 2.4% and 11%, respectively. This is shown in Table 12. Though not statistically significant, the incidence of ovarian cysts may be biologically significant. Presence of ovarian cysts and failure to take any action when they are detected may keep the cow for long periods without showing cyclicity. Milk yield, parity and farm had no significant effect on the incidence of ovarian cysts. These results are in agreement with those of Meyer *et al.*,(1975), Lotthammer *et al.*,(1976), Lotthammer and Alhswede, (1977) and Lotthammer, (1978), who used supplementation levels ranging from 300 mg/day to 600 mg/day. However, Akordor *et al.*,(1986) reported fewer cysts in ß-carotene supplemented cows than in control cows. Lotthammer and Ahlswede,(1977) found that ovarian cysts developed when the plasma ß-carotene levels dropped beyond 200 µg%.

The activity or quiescence of the ovaries was determined fourteen days postpartum. It was found that 64% of the

supplemented animals had a functional structure while only 43% of the control group had a functional structure. However, these differences were not statistically significant. These results are in agreement with those of Wang, *et al.*,(1988) who used a similar level of ß-carotene supplementation. Quiescence of the ovary postpartum present a bigger problem than the presence of cystic ovaries, though in both there are no oestrus cycles. Wang *et al.*, 1988) who used Holstein cows observed that ß-carotene supplementation did not have an effect on the ovarian activity. The results of ovarian activity observed in this study were in agreement with those reported by Graves-Hoagland *et al.*,(1989) who reported ovarian function to be positively related to plasma ß-carotene. Abnormal ovarian activity was reported to occur when milk yield exceeded 20 litres/day (Bulman and Lamming, 1978).

5.8 Disease Resistance

5.8.1 Mastitis.

Animals were scored for mastitis every two weeks on a 0-4 (negative-severest) point score. The mean scores were 0.87 and 0.93 for the test and control group respectively as shown in Table 13. Milk yield and farm had no significant ($P<0.05$) effect on mastitis score, while parity had a significant ($P>0.05$) effect. Heifers and cows in the second, third, and eighth parity had a lower mastitis score than cows in the forth

Table 13. Effect of ß-carotene supplementation on infection and duration of mastitis

Parameter	With ß-carotene	No ß-carotene
Mastitis score,	0.87±0.15[a]	0.93±0.13[a]
mastitis duration,days	20.1±0.19[a]	24.2±1.59[a]
Mastitis incidence,%		
No mastitis	33.3±9.4[a]	22.7±2.09[a]
Sub-clinical mastitis	43.5±4.7[a]	50.0±10.8[a]
Severe mastitis	22.2±14.8[a]	27.3±13.2[a]

a: means within a row with the same superscripts are not significantly (P>0.05) different.

to seventh parity, which was most probably due to the fact that there were too few animals in the extreme parity groups.

Regardless of whether animals were treated for mastitis or not, duration of the condition was shorter though not significantly (P>0.05) in supplemented animals than in control group with means of 20.1 and 24.2 days, respectively. Parity had a significant effect (P>0.05) on the duration of mastitis. High yielding cows had mastitisfor a longer period than those with lower yields.

The results obtained in this study agree with those of Lotthammer, (1978), Chew *et al.*,(1982) and Glazer *et al.*,(1988) who reported a decrease in both severity and duration of mastitis with ß-carotene supplementation. Due to antibiotic treatment of clinical cases, the effects of ß-carotene on severity and duration on mastitis were not expressed fully. To avoid these confounding effects of antibiotic treatment, cases of no mastitis, subclinical mastitis, and severe mastitis infection were determined. The results of this analysis showed that only 66.6% of the test animals had mastitis at any time during the experimental period compared to 77.3% in the control group. Animals which suffered severe mastitis at any time of the experiment were 22.2% for the test group and 27.5% for the control group. However, these differences were not significant (P>0.05) as would be expected since the animals exhibited high but similar plasma ß-carotene concentrations in the two treatment

groups.

5.8.2 Calf Diarrhoea

All calves from experimental animals were used to determine the resistance to enteric infection in the first week of life. The percentage occurrence for the test and control group were 4.9 and 10.9, respectively. The diarrhoea incidences in the subsequent weeks was determined in female calves only. The results of diarrhoeal incidences are shown in Table 14. Though, the supplemented group had fewer diarrhoeal cases, the differences between treatments were not significant ($P>0.05$) except at four weeks of age. Effects of farm, and milk yield on diarrhoeal incidences were not significant ($P>0.05$). Two calves in each group died during the experimental period. Similar improvements in calf diarrhoea after supplementation of dams with ß-carotene have been reported by Lotthammer, (1978, 1979), Dembinski,(1986), Dembinski *et al.*,(1986), Varlygin and Vertyanyna,(1986), Pivnyak *et al.*, (1987), Dryanovski *et al.*,(1988), Bardos *et al.*,(1989) and Skland *et al.*,(1989). In addition, the incidence of diarrhoea and mortality recorded in this study are lower than those reported by Lotthammer *et al.*,(1978) which may be a reflection of the higher plasma ß-carotene levels in this study. Lack of statistical differences between treatments may be due to the fact that the dam's plasma ß-carotene levels were not

Table 14. Effect of ß-carotene supplementation on incidence of calf diarrhoea (%)[1]

Week	1	2	3	4*	5	6	Mean
With ß-carotene	4.9(37)	10.8(14)	23.5(13)	2.9(13)	3.0(12)	6.1(12)	8.5
No ß-carotene	10.9(45)	27.0(23)	32.4(21)	23.5(21)	12.1(21)	12.1(21)	19.7
Mean	7.9(82)	18.9(37)	27.9(34)	13.2(34)	7.5(33)	9.1(33)	14.1

1: Figures in parenthesis represent the number of calves.
* : means between the treatment groups within this week were significantly ($P<0.05$) different

significantly different (P>0.05). Lotthammer *et al.*,(1978), Jagos *et al.*,(1979), Pivnyak *et al.*,(1987) and Bardos *et al.*,(1989) not only demonstrated that ß-carotene supplementation decreased susceptibility to diseases and subsequent mortality, but also that gamma-globulins in the calves increased after the first colostrum meal. Colostrum from ß-carotene supplemented cows was richer in fat, vitamin A, and ß-carotene.

6.0 CONCLUSION.

The purpose of this study was to investigate the effect of ß-carotene supplementation on the fertility of dairy cattle, incidence of mastitis, and calf diarrhoea. The results of the study were:

1. Blood ß-carotene levels were not significantly different between the test and control groups, despite differences in estimated levels of intake.
2. ß-carotene supplementation significantly improved conception rate, insemination index and heat intensity but had no significant effect on incidences of ovarian cysts, retained placenta, metritis, ovarian activity and service period.
3. Severity and duration of mastitis were improved though not significantly after supplementation. Likewise, calf vitality and viability were not significantly affected by ß-carotene supplementation of dams.
4. No differences were observed in milk yield and composition due to ß-carotene supplementation.
5. There was an indication that pelleting and storage decreased ß-carotene content in concentrate feeds.

The conclusion drawn from this study is that ß-carotene supplementation does improve or is beneficial to dairy cattle fertility and production. Among the parameters considered, results suggest that ß-carotene was not a limiting factor to performance of animals in the experiment. In addition the

plasma ß-carotene level which is an indicator of ß-carotene status in cattle was similar between treatment groups. The diet for both groups had other fertility enhancing factors in the form of vitamins A and E. The ß-carotene content of pastures and therefore intake from pastures was relatively high, thus even before the start of the trial, it is suspected that the animals were in good ß-carotene status since the vegetation remained relatively unchanged during the trial. On average, the milk production levels were relatively low and would not have caused an appreciable stress on reproduction. Therefore, the response to supplementation would not have been expected to be significant in all the parameters considered. This study contrast with most of the published work which has been carried out under controlled conditions whereby supplementation was compared with severe ß-carotene deficiency of as low as 9µg% (Marsh and Swingle, 1960). Such deficiency was developed by feeding the animals with several years old hay, maize silage, straw and concentrates. In such cases supplementation with ß-carotene was found to be very responsive.

7. SCOPE FOR FURTHER WORK

In this study , the effect of ß-carotene supplementation on ayrshire cows under field conditions was investigated. ß-carotene intake could not be ascertained and it appears that the pastures supplied adequate quantities of ß-carotene to the grazing cows. However, the period of supplementation was only 14 weeks and may have been too short for manifestation of the beneficial effects of ß-carotene supplementation. The supplementation of ß-carotene over a longer period, especially over periods of time when ß-carotene supply from pastures is likely to be limiting should be investigated. Such a study should include evaluation of all the common roughages used in cattle feeding at various times of the year. The amount and periods when supplementation is necessary should also be investigated together with the economics of supplementation in Kenya.

The above should be investigated for the common dairy breeds in Kenya as it appears that there are differences between breeds in their responce to ß-carotene supplementation. With the increasing awareness that ß-carotene is capable of curbing cancer, it may be useful to establish the health value of milk from supplemented and un-supplemented cows.

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9. APPENDICES

Appendix 1. Chemical assay of ß-carotene in plasma.

1. Reagents.

Ethanol, Cyclohexane, Aluminium oxide, Petroleum ether hexane (50:50), Distilled water

2. Glassware and equipment

Erlenmeyer flasks (150ml), Graduated pipettes , Centrifuge tubes with glass stopper, Centrifuge , Vacuum rotary evaporator, Chromatography column with a 200 mm length and an inside diameter of 6 mm, 1 cm semi-microcuvette , Spectrophotometer.

3. Chromatography columns

Deactivate aluminium oxide (Al_2O_3) adsorbent with distilled water at a ratio of 100:18(g/ml). Allow the deactivation to take place for one hour in room temperature. Suspend the deactivated aluminium oxide in petroleum ether hexane fraction and pipetted slowly into a chromatographic column. Pack the column with the Al_2O_3 to a height of 5 cm and ensure it is covered with a 3 mm thin layer of petroleum ether-hexane fraction.

4. Method

a. Thaw the assay samples and allow them to reach room temperature.

b. Pipette 0.5 ml of the plasma sample into 1.0 ml of distilled water in a centrifuge tube.

c. Add 2.0 ml of ethanol followed by 2.0 ml of cyclohexane into the previous mixture.

d. Stopper the tube and mix thoroughly the contents by shaking.

e. Centrifuge the mixture at 1300 g for 5 minutes, and pipette 1.5 ml of the clear cyclohexane supernatant into an aluminium oxide column.

f. Elute the ß-carotene with 2 ml of petroleum ether-hexane fraction (1:1) and evaporate to dryness. Pick up the residue in 1.5 ml of cyclohexane and determine the absorbance in a 1 cm cuvette against that of a blank cyclohexane at 455 nm in a spectrophotometer.

g. Calculate the ß-carotene concentration by taking 2500 to be the extinction coefficient of 1% ß-carotene cyclohexane solution in a 1 cm cuvette.

Appendix 2. Chemical assay of ß-carotene in forage

1. Reagents

Ethanol 96%(v/v), Petroleum ether with a boiling range 30-40°C, Aluminium oxide for chromatography, Anhydrous sodium sulphate analytical grade, n-Hexane or petroleum ether with boiling range 50-75°C, Acetone, Mixture of n-hexane or petroleum ether and acetone 70+30 (v/v), Methanoic potassium hydroxide solution 40% (w/v)

2. Glassware and equipment

Erlenmeyer flask, pipettes, Graduated flasks, Graduated cylinders with stopper, Water bath, Vacuum rotary evaporator, Chromatography tubes in glass with height 300 mm and inside diameter of 10-15 mm, spectrophotometer.

3. Chromatography columns

Deactivate aluminium oxide (Al_2O_3) adsorbent with double distilled water at a ratio of 90:9(g/ml). Allow the deactivation to take place for 12 hour in room temperature. Suspend the deactivated aluminium oxide in petroleum ether and then pipette it slowly into a chromatographic column. Pack the column with Al_2O_3 sediment to a 15 cm high. Cover this pack with a 2 cm thick layer of anhydrous sodium sulphate. Ensure this column was always under a layer of petroleum ether.

4. Methods

a. Dry green fodder in a oven to moisture content of less than 15%, and then comminute this sample to 100%

passage in a 0.8 mm mesh screen. Coat all the glassware with an aluminium foil to prevent photo-oxidation.

b. Transfer 2.0 g of the sample into a graduated flask and add 15 ml of hexane-acetone mixture (70-30 v/v).

c. Allow the mixture to stand overnight under nitrogen.

d. Add 1.0 ml of methanoic KOH, 40% (W/v) and shake vigorously then allow to stand.

e. To the above mixture, add 35 ml of n-hexane,shake again and allow to stand.

f. Take 5 ml aliquot of the supernatant and evaporate to dryness under partial vacuum. Pick the residue in 5 ml of petroleum ether, and pass this extract through an aluminium oxide chromatographic column.

g. Elute ß-carotene with petroleum either, and xanthophyll with ethanol sequentially in different flasks.

h. Dilute the two elute to 25 ml mark and determine the absorbance in a 1 cm cuvette against that of the respective blank at 450 nm in a spectrophotometer.

i. Calculate the ß-carotene concentration by taking 2600 to be the extinction coefficient of 1% carotene petroleum ether solution in a 1 cm cuvette at 450 nm wavelength.

Appendix 3. Chemical assay of ß-carotene in admixed feed.

1. Reagents

Distilled water, Trypsin, Pepsin, Ammonium solution 10%, Hydrochloric acid 1 N, pH Indicator paper(above pH 3), Chloroform, Unhydrous sodium sulphate, Absolute ethanol, n-hexane.

2. Glassware and equipment

Round bottomed flask (300-500 ml), centrifuge tubes (200 ml),Erlenmeyer flasks (250 ml), Filter Funnel, Shaking Machine, centrifuge, Vacuum rotary evaporator, Water bath with agitator, Glass rod, Cotton or glass wool, Chromatography tubes.

3. Chromatography columns

Deactivate aluminium oxide (Al_2O_3) adsorbent with double distilled water at a ratio of 90:9(g/ml). Allow the deactivation to take place for 12 hour in room temperature. First pack the column with a 0.5 cm thick layer of washed sea-sand, then suspend the deactivated aluminium oxide in n-hexane slowly to a height of 10 cm. Ensure that Al_2O_3 sediment is covered with 3 mm n-hexane layer.

4. Methods

a. Suspend 20.0 g of the sample into 50 ml of distilled water in a flask.

b. Add 100 mg of trypsin + pepsin (1:1) followed by 3 ml of 10% ammonium solution.

c. Place the mixture in a water bath at 50 °C with a

agitator for 30 minutes

d. Adjust the pH of the mixture to 3 using HCL.

e. Extract ß-carotene with 50 ml of chloroform.

f. Centrifuge the chloroform/water/feed emulsion at 300 g for 15 minutes. Discard the aqueous phase and transfer the chloroform phase into another flask.

g. Exsiccate the chloroform solution with anhydrous sodium sulphate.

h. Take a 50 ml aliquot of the chloroform solution and evaporate to dryness. Pick up the residue in 5 ml of n-hexane. Pass this extract through a chromatographic column.

i. Evaporate the elute from the chromatographic column to dryness and pick it up in 5 ml of cyclohexane. Add 1 mcl of a 1% Iodine solution and then keep the solution in dark for 3 hours before reading the absorbance.

j. Determine the absorbance of this solution in a 1 cm cuvette against a blank cyclohexane at 454 nm in a spectrophotometer.

k. Calculate the ß-carotene in the sample by taking 2230 to be the extinction coefficient of a 1% ß-carotene cyclohexane solution in a 1 cm cuvette at 454 nm wavelength.

Appendix 4: Analysis of variance tables

4.1 Prepartum plasma ß-carotene

Source	d.f.	Sum of squares	Mean squares	f	prob
Total	68	5.638			
Treatment	1	.041	.041	.494	.485
Farm	2	.477	.238	2.879	.065
Parity	7	.126	.018	.218	.979
Regressions					
Milk yield	1	.106	.106	1.285	.262
Error	56	4.637	.083		

4.2 Partum plasma ß-carotene

Source	d.f.	sum of squares	mean squares	f	prob
Total	74	4.093			
Treatment	1	.005	.005	.093	.762
Farm	2	.226	.113	2.180	.122
Parity	8	.522	.065	1.261	.280
Regressions					
Milk yield	1	.001	.001	.002	.969
Error	61	3.157	.052		

4.3 Post Partum ß-carotene

Source	d.f.	Sum of squares	Mean squares	f	prob
Total	58	5.626			
Treatment	1	.202	.202	2.077	.156
Farm	2	.011	.005	.055	.947
Parity	8	.733	.092	.939	.494
Regressions					
Milk yield	1	.018	.018	.185	.669
Error	45	4.386	.097		

4.4 Plasma ß-carotene at various physiological states

Source	d.f.	Sum of squares	Mean squares	f	prob
Total	205	18.375			
Farm	2	.584	.292	4.107	.018
Treatment	1	.078	.078	1.102	.295
Parity	8	.651	.081	1.144	.336
Physiol. State	2	3.096	1.548	21.776	.000
Regressions					
Milk yield	1	.005	.005	.067	.797
Error	188	13.365	.071		

4.5 Milk butterfat

Source	d.f.	Sum of squares	Mean squares	f	prob
Total	77	45.587			
Treatment	1	.066	.066	.116	.734
Farm	2	.891	.445	.790	.458
Parity	8	7.927	.991	1.757	.102
Regressions					
Milk yield	1	.013	.013	.023	.880
Error	64	36.088	.564		

4.6 Service period and insemination index

		Mean square	
source	d.f.	Service period	Insemination index
Treatment	1	1306.323	6.724*
Farm	2	117.514	.940
Parity	8	391.117	.327
Regressions			
Milk yield	1	646.317	.090
Error	59	632.738	.782

* :significant at P<0.05

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4.7 Metritis, uterine involution and ovarian cysts

Source	d.f.	mean squares		
		Metritis	Uterine involution	Ovarian cysts
Treatment	1	.024	.004	4877.324
Farm	2	.005	.309	245.687
Parity	8	.054	.050	295.593
Regressions				
Milk yield	1	.062	.059	0.067
Error	70	.139	.234	1018.089

4.8 Heat intensity index and its constitituents

Source	d.f.	mean squares				
		V. signs	H. act	M. dis	H. dur	Heat intensity
Treatment	1	4.425*	1.346	.324	67.820*	147.171*
Farm	2	.295	.498	.675	71.604*	98.807*
Parity	8	.904	.298	.159	3.488	6.393
Regressions						
Milk yield	1	.441	.446	.017	2.415	3.827
Error	59	.434	.373	.403	6.856	13.125

key: V. signs :vulva signs; H. act :heat activity;
M. dis :mucous discharge; H. dur :heat duration

* :significant at P<0.05

4.9 Mastitis infection

Source	d.f.	Mean squares		
		Week	Severity	Duration
treatment	1	.000	4.341	1242.534
Farm	2	1.221	5.055	1176.252*
Parity	7	.301	7.717*	3602.788*
Treatment x farm	2	.269	1.350	49.792
Regressions				
Milk yield	1	.597	.772	923.971
Error	347	1.944	2.378	364.001

* : significant at P<0.05

4.10 Calf diarrhoea at one week of age

Source	d.f.	Sum of squares	mean squares	f	prob
Total	80	10.888			
Farm	2	1.254	.627	5.809	.005
Treatment	1	.192	.192	1.782	.187
Parity	8	1.537	.192	1.780	.097
Sex	1	.573	.573	5.311	.024
Farm x treatment	2	.318	.159	1.473	.237
Regressions					
Milk yield	1	.148	.148	1.369	.246
Error	64	6.906	.108		

4.11 Calf diarrhoear at various weeks of age

		Mean squares				
Source	d.f.	2	3	4	5	6
Treatment	1	.092	.011	1.534*	.349	.001
Farm	2	.595	.170	.285	.214	.210
Parity	6	.126	.317	.323	.124	.334
Treatment x farm	2	.492	.271	.121	.108	.207
Error	20	.239	.249	.191	.293	.236

* : significant at $P<0.05$