An investigation of the β-Carotene status of Holstein cows in South Africa

By

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DECLARATION

I hereby declare that this dissertation submitted for the M.Sc. (Agric.) Animal Science: Animal Nutrition degree at the University of Pretoria is my own work and effort, conducted under supervision of Prof L.J. Erasmus, and that it has not previously been submitted by me for a degree at any other University.

Galit Machpesh
Pretoria
2013

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I dedicate this paper to my mother, Katherine Mary Liell-cock.

SUMMARY

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by

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Supervisor: Prof LJ Erasmus

Department: Animal and Wildlife Sciences

Faculty: Natural and Agricultural Sciences

Degree: MSc (Agric) Animal Science: Animal Nutrition

Experiment 1 A survey on the β-Carotene status of Holstein cows in different feeding systems

In order to make meaningful recommendations with regard to β-Carotene supplementation is it necessary to know whether cows are deficient in β-Carotene. The objective of this study was to generate data on the β-Carotene status of Holstein cows under three different feeding systems in South Africa, namely; pasture-based, silage-based and hay-based feeding systems. A survey was conducted amongst 30 farms with 10 farms utilizing each of the three systems. Twenty multiparous cows were randomly selected from each farm. Blood samples were taken from the tail vein and analysed for plasma β-Carotene using the iCheck™, a hand held spectrophotometer (BioAnalyt, GmbH, Germany). Cows were then classified as deficient (< 1.5 mg/L), marginal (1.5 to 3.5 mg/L) or optimal (>3.5 mg/L). The average plasma β-Carotene levels differed between feeding systems and concentrations were 5.53, 2.98 and 1.71 mg/L for the pasture based, hay-based and silage-based feeding systems respectively. There was a wide variation in average plasma β-Carotene concentrations in cows on farms within the different feeding systems. Average values per farm ranged between 3.84 and 10.81 mg/L for the pasture based farms, 0.91 and 5.00 mg/L for the hay-based farms and between 0.78 and 3.38 mg/L for the silage-based farms. Results suggest cows on a pasture based feeding system have optimal β-Carotene status and do not need supplementation. Cows on hay-based systems are marginal and on farm testing is recommended. Cows on silage-based systems are generally deficient and β-Carotene supplementation is recommended.

Experiment 2 Effect of prepartum β-Carotene supplementation on the postpartum β-Carotene status of Holstein cows

It has been recommended that cows be supplemented β-Carotene when blood plasma levels are deficient (< 1.5 mg/L) or marginal (< 3.5 mg/L) especially during the transition period which is characterised by low intakes and significant losses of β-Carotene through colostrum. The objective of this trial was to determine to what extent prepartum β-Carotene supplementation could maintain postpartum plasma β-Carotene concentrations above 3.5 mg/L in cows fed a lucerne hay-based TMR. Twenty multiparous Holstein cows were blocked into two groups of ten cows each and were fed either 8kg/d of a control TMR (DM) or the control diet supplemented with 1200mg of ROVIMIX® β-Carotene 10%. The experimental period was from 60d pre-partum until 56d postpartum; however the period of the β-Carotene supplementation for the one group was only from 60d prepartum until calving. Blood samples were collected from the tail vein once per week and analysed for plasma β-Carotene using the iCheck™, a handheld spectrophotometer (BioAnalyt, GmbH, Germany). Average plasma β-Carotene concentrations prepartum were higher (6.15 mg/L) ($P < 0.05$) for supplemented cows compared to the control cows (3.10 mg/L). For the first 5 weeks postpartum, plasma β-Carotene was higher ($P < 0.05$) for supplemented cows compared to control cows (3.00 mg/L vs. 1.39 mg/L), from weeks 6 to 9 there were no differences ($P > 0.05$). Overall the average postpartum plasma β-Carotene values were 1.50 mg/L for the control cows and 2.43 mg/L for the supplemented cows and did not differ. Supplemented cows maintained sufficient β-Carotene concentrations only for the first 2 weeks postpartum and were either marginal or deficient for the rest of the experimental period. Results suggest a minor carryover effect of β-Carotene after prepartum supplementation.

LIST OF ABBREVIATIONS

ADF	Acid detergent fibre
ADL	Acid detergent lignin
ARC	Agricultural Research Council
BC	β-Carotene
BF	Butter fat
Ca	Calcium
CL	Corpus luteum
CP	Crude protein
CR	Conception rate
DIM	Days in milk
DM	Dry matter
DMI	Dry matter intake
EE	Ether extract
GE	Gross energy
HPLC	High performance liquid chromatography
ICP	Inter-calving period
IGF-1	Insulin-like growth factor
IVOMD	In vitro organic matter digestibility
KZN	KwaZulu-Natal
LH	Luteinising hormone
ME	Metabolisable energy
MNL	Mononuclear leukocyte population
MUN	Milk urea nitrogen
NDF	Neutral detergent fibre
NEB	Negative energy balance
NEl	Net energy for lactation
P	Phosphorus
PMNs	Polymorphonuclear neutrophils
PR	Protein
RBP	Retinol binding protein
Rye	Rye grass
SA	South Africa
SCC	Somatic cell count

TMR	Total mixed ration
RFV	Relative feed value
UDP	Undegradable dietary protein
UHT	Ultra high treatment processed milk

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CHAPTER 1: INTRODUCTION

1.1 Overview of the South African dairy industry

The South African (SA) dairy industry is a relatively small role player in terms of worldwide milk production and only supplies around 0.5% of the total world milk production. The biggest role players globally are the USA, India, and China, producing 14.5%, 8.3% and 6% respectively of world milk production. The average milk production in SA is around 17.3 kg/cow/day compared to countries such as USA and New Zealand where the average production per cows is 30 and 14.6 kg/cow/day respectively (IFCN Dairy Report, 2011). Taking into account only cows that participate in milk recording, the SA producers are much more competitive. During the 2010 milk recording year, Holstein cows produced an average of 9 830 kg milk per lactation and Jersey cows 5 866 kg per lactation (ARC, 2011).

Data in Figure 1 shows the litres per km^2 throughout SA, with the highest volumes being in the coastal and pasture regions, the trend towards bigger herds is shown in Figure 2 with 10% of herds having more than 300 cattle in 2007, versus 26.6% in 2009 and 30.8% in 2012. The number of cows per district is shown in Figure 3, confirming the trend towards bigger herds and the movement to coastal and pasture-based areas. In a literature review by Lucy (2001) the trend in the USA is towards amalgamating the industry into larger farms. This suggests a global trend.

Figure 4 illustrates that 40% of SA herds produce between 5 and 15 litres per cow daily, it can be deduced then that these are pasture-based farms.

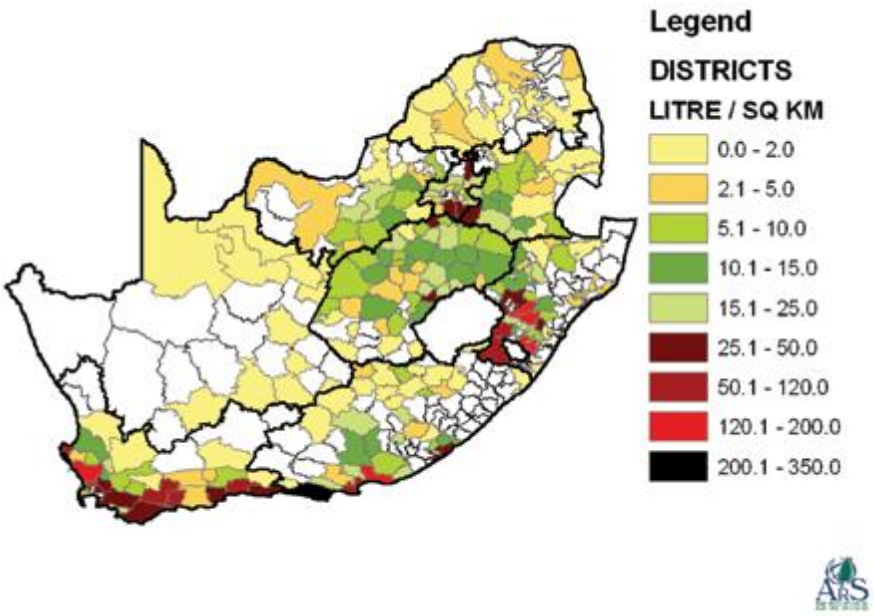

Figure 1 Milk production densities (litres/km^2) per district 2012 (Lacto Data, 2013)

The number of milk producers in SA has declined from 4 184 in 2006 to 2 123 in 2013 (Table 2), a reduction of 49% over a period of 7 years. The largest number of producer farm losses occurred in the Free State, North West and Mpumalanga, thereby increasing the total contribution of producer farms in the Cape regions and KwaZulu-Natal (KZN).

The provincial contribution to the total milk production has also changed dramatically, with the Western Cape, Eastern Cape and KZN dominating accordingly. The trend towards pasture-based farming, especially in the coastal areas is clearly visible in Table 3; this can be attributed to the more reliable rainfall in these areas as well as a more favourable climate. Due to the lower cost of feed input in pasture systems versus total mixed ration (TMR) systems, many believe that, profit margins on pasture systems are larger than those on TMR systems (Gertenbach, 2006; Lacto Data, 2013). This however, is a debatable statement and producers on well managed TMR system farms can be as productive as those on pasture-based system farms.

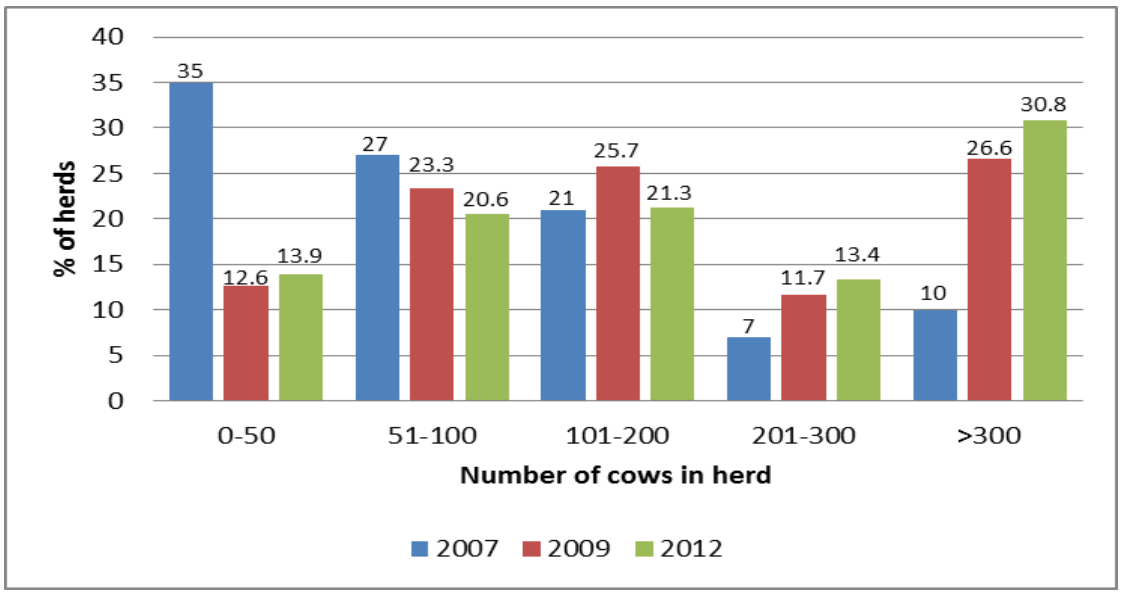

Figure 2 Size distribution of dairy herds 2007, 2009 and 2012 (Lacto Data, 2009, 2011, and 2013)

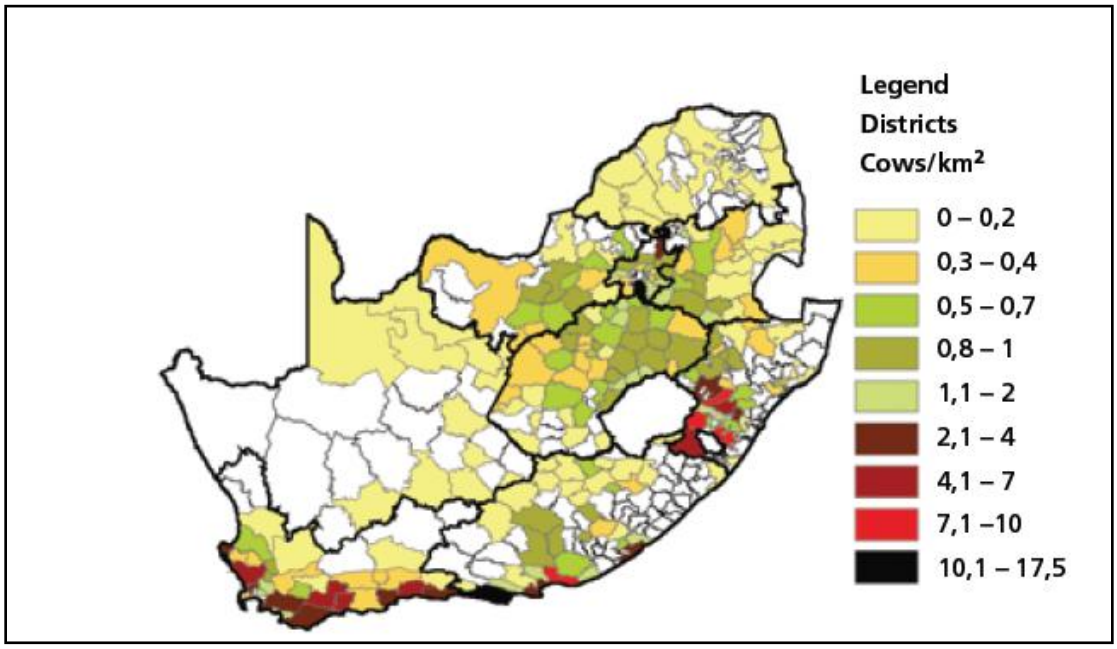

Figure 3 Cow density per district (cows/km²), 2009 (Lacto Data, 2011)

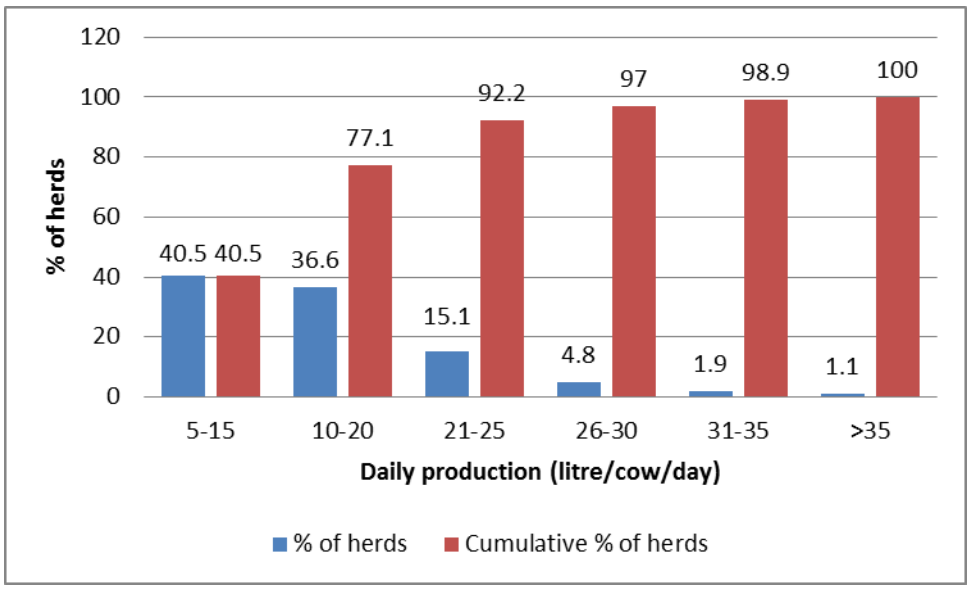

Figure 4 Distribution of herds based on daily production per cow in herd 2012 (Lacto Data, 2013)

The milk price that dairy farmers receive is always a controversial issue. Although it is fact that SA dairy farmers struggle to survive economically, the average milk price that the SA dairy producer receives compares favourably with international milk prices, as illustrated in Table 1.

Table 1 International calculated standardised raw milk producer prices, 2010 – 2013 (Lacto Data, 2013)

Country	Jan'10	Jan'11	Jan'12	Jan'13
Belgium	2,89	3,06	3,23	3,94
Germany	2,80	2,96	3,35	3,84
Denmark	2,81	2,90	3,35	3,73
Finland	3,79	3,54	4,14	4,67
France	3,37	3,01	3,58	3,90
Great Britain	2,82	2,69	3,47	4,07
Ireland	2,47	3,16	3,40	3,75
Netherlands	2,91	3,05	3,55	3,92
New Zealand	2,44	3,03	3,22	3,15
USA	2,59	2,27	3,25	3,78
*** South Africa**	3,14	2,97	3,10	3,60

Although Table 1 creates the impression that SA dairy farmers are in a good financial position, many of them leave the industry. This is mainly because of subsidies being paid by some countries resulting in an unfair advantage, high labour costs in SA, not enough protection for the SA dairy farmer with regard to milk imports, land claims, political interference beyond the farmers' control and overall unfavourable

security situation on farms. Table 2 outlines the drastic reduction in the number of milk producers per province from 2006 to 2013.

Table 2 The reduction in number of milk producers in South Africa from Jan 2006 to Jan 2013 (Lacto Data, 2013)

Province	Number of milk producers per province						
	Jan'06	Jan'07	Jan'08	Jan'09	Jan'11	Jan'12	Jan'13
Western Cape	878	827	815	795	683	647	563
Eastern Cape	422	420	407	387	314	283	270
Northern Cape	39	37	34	37	28	21	28
KwaZulu-Natal	402	385	373	373	323	322	295
Free State	1067	987	919	884	601	535	433
North West	649	596	549	540	386	352	268
Gauteng	275	245	228	217	127	126	120
Mpumalanga	407	357	302	286	201	164	130
Limpopo	45	45	38	32	23	24	16
TOTAL	4184	3899	3665	3551	2686	2474	2123

Table 3 Percentage distribution of milk production by province in South Africa in 1997 and 2012. (Lacto Data, 2013)

Province	Dec-97	Feb-12
Western Cape	22,9	27,4
Eastern Cape	13,8	24,3
Northern Cape	1,2	1
KwaZulu-Natal	15,7	23,5
Free State	18	10,5
North West	12,6	3,5
Gauteng	4,4	5,5
Mpumalanga	11	3,6
Limpopo	0,4	0,7
TOTAL	100	100

The producer price during February 2013 was around R3.50/L while the production cost for 1L of milk varied between R3.00 and R4.00. Producer prices are fixed by the milk buyers and the farmers should focus more on factors that they can control to some extent such as improving efficiency of production if they want to stay in business (Personal communication, 2013, Dr. K. Coetzee, Milk Producers Organisation, koos.coetzee@mpo.co.za).

Despite the fact that many farmers have left the industry, it remains an important agricultural industry. The dairy industry is fourth largest among the agricultural industries, adding 5.6% to the gross

Agricultural contribution, of which milk (pasteurised and UHT) makes up 80% of the liquid market, and cheeses make up 62% of the concentrated products market.

1.2 Feeding Systems in South Africa

The major feeding systems in SA are either pasture- or TMR-based feeding systems. The TMR-based systems are mainly in the Free State, Gauteng, North West, Mpumalanga and some areas of the Western Cape provinces while the pasture-based systems are predominantly in KZN and the coastal areas of the Eastern and Western Cape. The TMR systems primarily focus on either hay (lucerne, *Eragrostis curvula*, cereal straw) or silage (maize, sorghum, oats, wheat) as the roughage source. Pasture systems generally rotate between kikuyu as summer grazing and ryegrass and clover as winter grazing. Cows are then supplemented with an energy (mainly maize) mineral mix during milking, generally 2 to 3 kg concentrate twice a day. During periods of drought or scarcity of pasture, some pasture farmers might phase in a partial TMR system or feed the high producers a TMR and keep the medium and low producers on pasture.

1.3 The importance of reproduction and fertility

Apart from nutrition, one aspect that dairy producers can improve to increase the efficiency of milk production, is to revisit their reproduction management programmes to improve fertility.
Butler and Smith (1989), reported that conception rates declined from a level of 66% in 1951 to about 50% in 1973 and that during this same time period, and in the same group of dairy animals, the annual average milk production increased by 33%. In a review by Lucy (2001), the conception rate of cattle to first insemination was reported to be 65% in 1951, 40% in 1996, 45% in 1998, and 35% with the use of timed insemination in 1998, with similar trends being reported globally. Authors in the US reported an increase of 1.29 services per conception from 1972 to 1996. Figure 5, adapted from Butler (2003), shows the antagonistic effect of increasing milk production on fertility over a 50 year period with a drop of approximately 40% in the conception rate (CR) %. Between 1991 and 2000, the USA dairy industry experienced a drop of 42% in the number of dairy farms, a 6% fall in the number of cattle, and an increase in milk production of 14%. We can conclude from this data that herd sizes have increased in the USA and that cattle are producing more today than in 1991. At the same time as milk production started increasing, data showed a drop in fertility for various parameters, such as; calving interval, calving-to-conception interval, days to first service, services per conception and first service conception rate (Rajala-Schultz and Frazer, 2003).

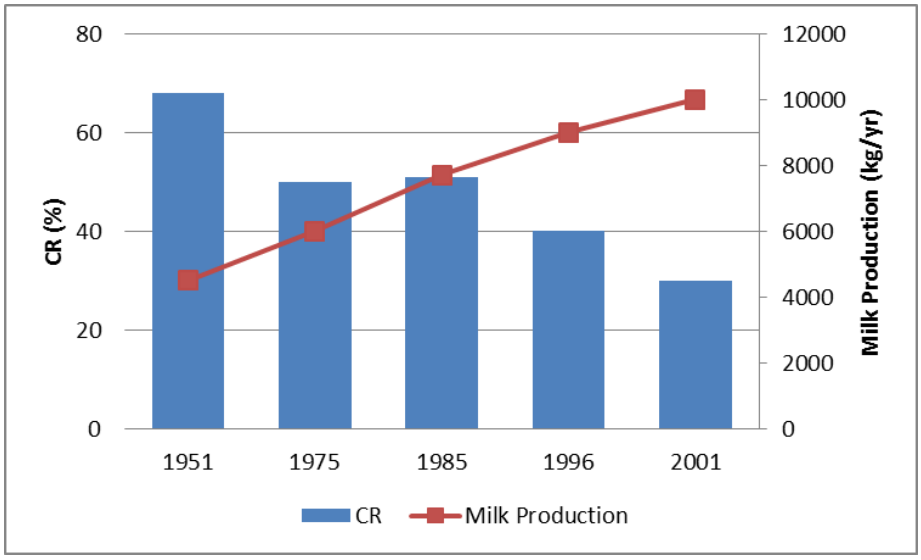

Figure 5 Milk production and fertility in dairy cows (Butler, 2003)

The average inter-calving period (ICP) for SA Holstein dairy cattle is 395 days (Mostert et al., 2010). Declining reproduction with increasing production is a worldwide trend, as previously discussed. Although proper management procedures such as; transition management, cows calving in correct body condition, correct nutrient densities and prevention of metabolic diseases, are followed, good managers still struggle with reproduction.

Because of stress on high producing cows, the role of feed additives has become increasingly important in dairy cattle nutrition and reproduction. Feed additives such as anionic salts in transition diets and buffers, ionophores and yeast products are becoming standard inclusions in lactation diets.
On the reproduction side few additives have proven themselves to consistently benefit reproduction. One additive that is currently attracting attention is β-Carotene. Benefits of supplemental β-Carotene may be related to the conversion of circulating β-Carotene to Vitamin A, specifically in the uterus and ovaries. A cow could therefore be in sufficient supply of Vitamin A but still deficient in β-Carotene because of the conversion process in the reproductive tract (Schweigert, 2003). In a recent study by de Ondarza et al (2009) it was suggested that further studies on the effect of β-Carotene on possible reproductive benefits are needed.

The aim of this study was to gain more information in the β-Carotene status of dairy cows in SA, specifically because of the important role that β-Carotene can play in improving efficiency of production through improved fertility. Two studies were conducted. In our first study we investigated the β-Carotene

status of Holstein cows under the three predominant feeding systems, namely a lucerne hay-based TMR, a silage-based TMR and a rye grass/Kikuyu based pasture system. In the second study we investigated the potential carryover effect of prepartum β-Carotene supplementation on the postpartum β-Carotene status of cows. In the following chapter a literature review on the role of β-Carotene in animal nutrition is presented.

CHAPTER 2: LITERATURE REVIEW: β-CAROTENE IN ANIMAL NUTRITION

2.1 Introduction

Retinoids are not synthesised by the body (in humans or cattle) and are therefore called essential micronutrients as they are required in small amounts from external sources such as the diet. Retinoids are available in food, firstly; via provitamin A or carotenoids in vegetables and leaves, and secondly as preformed vitamin A obtained from animal sources such as meat and milk product, i.e. the vitamin has already been previously obtained from vegetables and leaves consumed by the animal.
Provitamin A is the name given to over 600 carotenoids of which β-Carotene is one; Figure 6 shows the pathway in which β-Carotene is transformed to vitamin A. The pathway in Figure 6 illustrates the central cleavage of β-Carotene to form two molecules of retinal or one molecule of β-apo-Carotenal plus one molecule of β-ionone, both being intermediate substrates. Retinal is an intermediate in synthesis of retinoic acid and undergoes reductase to form retinol. Retinol has no biological function. It is dehydrolised to form retinyl esters creating a less toxic form of the vitamin molecule for storage. Retinyl Esters are the storage unit of vitamin A and can be hydrolysed to form retinol (Packer et al., 2004).

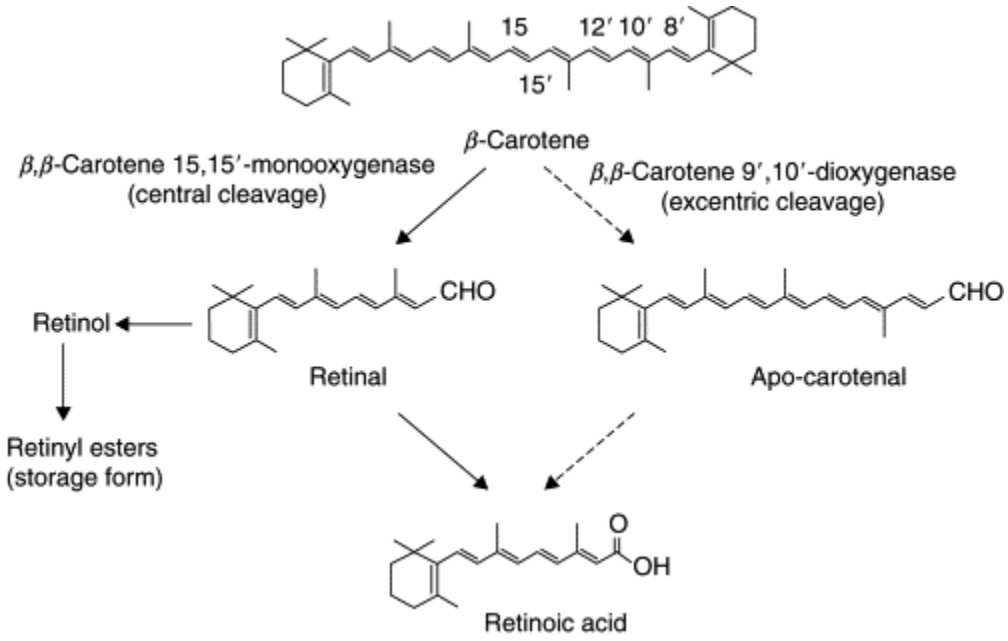

Figure 6 Possible transformations of β-Carotene in mammals (Biesalski et al., 2007)

Retinol is by definition true vitamin A, which in the body is converted to retinyl ester for storage, i.e. it is the storage form of the retinol molecule. Retinol itself has no biological purpose but is oxidised to retinal, a molecule important in vision (Packer et al., 2004). Vitamin A is virtually colourless, is soluble in fat and

is a long-chain, unsaturated alcohol possessing five double bonds. The most common form of vitamin A found in animal tissues is all-trans-vitamin A, but changes within the molecule catalysed by moisture, heat or light result in the formation of cis-forms which greatly reduce the effectiveness of vitamin A.

Vitamin A is required for a number of physiological processes, including; good vision, resistance to infectious diseases, correct functioning of epithelial cells, healthy and correct bone growth and reproduction. As the diet of most production animals consists of plants, this is the means by which they meet most of their vitamin A requirements. Different forms of vitamin A including; retinol, retinal and retinoic acid and molecular structures are shown in Figure 7.

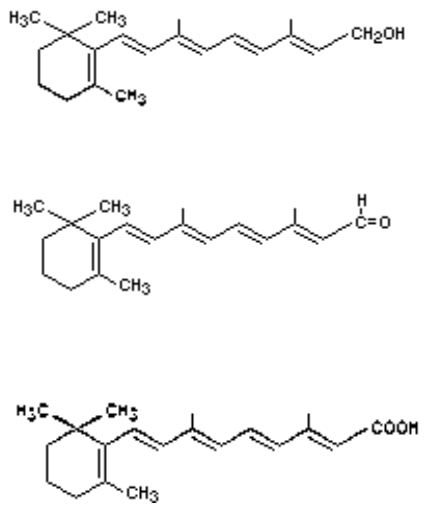

Figure 7 Molecular structure of vitamin A1 (retinol) $C_{20}H_{30}O$, retinal and retinoic Acid.

2.2 Chemical Structure of β-Carotene

Carotenoids are evident as the pigments in plants as the orange-yellow and green colour of leaves. There are over 600 types of carotenoids, but so far as we know only 60 have a function in plants and animals. Within the plant, carotenoids aid in photosynthesis and are usually found in high concentrations within the grana of chloroplasts, they are divided into two groups namely the Carotenes mostly seen as orange pigments that contain no oxygen and the xanthophylls or yellow pigments which contain oxygen.
Beta-Carotene ($C_{40}H_{56}$) also known as; Carotene, β-Carotene, provitamin A, provitamin A1 and carotene Type I, is the most occurring form of carotene and of all the carotenes has the highest activity related to vitamin A. Its molecular structure is shown in Figure 8.

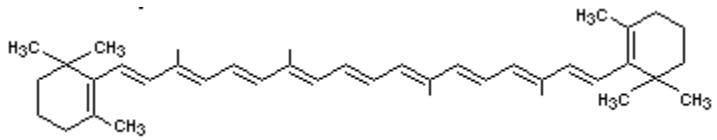

Figure 8 Molecular structure of β-Carotene

2.3 Sources of β-Carotene

Beta-Carotene is found in the green growing parts of plants and is the primary source of vitamin A for livestock, it has been established that the degree of green colour in plants is a good indicator of its Carotene content and animals on pasture are sure to get ample β-Carotene. Yellow maize has been found to have the β-Carotene of about an eighth of a good forage source. Table 4 shows the Carotene content of a number of classic forage sources.

Table 4 Typical β-Carotene concentration of feeds (mg/kg).

Carotene Source	Carotene (mg/kg)
Fresh green legumes and grasses, immature (wet basis)	33-88
Dehydrated lucerne meal, fresh, dehydrated without field curing, very bright green	242-297
Dehydrated lucerne meal after considerable time in storage, bright green	110-154
Lucerne leaf meal, bright green	120-176
Legume hays, including alfalfa, very quickly cured with minimum sun exposure, bright green, leafy	77-88
Legume hays, including alfalfa, good green colour, leafy	40-59
Legume hays, including alfalfa, partly bleached, moderate amount of green colour	20-31
Legume hays, including alfalfa, badly bleached or discoloured, traces of green colour	9-18
Non-legume hays, including timothy, cereal and prairie hays, well cured, good green colour.	20-31
Non-legume hays, average quality, bleached, some green colour	9-18
Legume silage (wet basis)	11-44
Corn and sorghum silages, medium to good green colour (wet basis)	4-22
Grains, mill feeds, protein concentrates and by-product concentrates, except yellow corn and its by-products	0.02-0.44

Source: Adapted from Maynard et al. (1979) and Scott et al. (1982), cited in McDowell, 2000.

2.3.1 Diet and plant species

Friesecke (1978) investigated plasma β-Carotene concentrations in association with the diet supplied. Dairy herds given grass and rape silage had optimal concentrations, dairy herds fed beet silage were variable and those fed maize silage or fodder beet in addition to hay or straw had deficient concentrations of plasma β-Carotene. Results showed grass silage to have a considerable amount of β-Carotene whereas pre-wilted silage, haylage and rape silage provide lower amounts, sugar beet silage generally has highly variable β-Carotene content and raw materials containing the lowest β-Carotene include maize silage, marrow, stem kale, fodder beet, turnips and hay. Lotthammer (1978) reported low β-Carotene blood values under natural conditions when animals where fed hay and concentrates containing low β-Carotene and also when maize silage was the predominant ingredient in the diet. Sugar beet was also found to supply inadequate β-Carotene, but pasture, grass silage and grass dried artificially provided sufficient β-Carotene.

Fresh roughages are a major source of β-Carotene and vitamin E. However, methods used to analyse the plant content of these vitamins do not take into consideration the amount of vitamins present in the plant actually available to the animal, as these estimates are lacking in literature due to many factors inherent to

both plants and animals that affect the amount of bio-available vitamins. As far as β-Carotene content of plants is concerned, legumes are higher in β-Carotene content than grasses at flowering but at early growth the concentrations are similar (Williams et al., 1998).

2.3.2 Stem to leaf ratio and maturity of plant species

Forages have a higher content of β-Carotene in leaves than in stem (Mc Dowell, 2000). As plants increase in stem to leaf ratio with maturity, there is an increase in DM and therefore an inverse relationship between DM and β-Carotene content. Any plant species at any stage of maturity with a higher leaf to stem ratio and a lower DM has greater β-Carotene content. Because the stem is proportionately greater at maturity and legumes have twice the amount of leaves at flowering than grasses do, the level of plant maturity affects the β-Carotene content of grasses more than legumes, therefore making legumes a superior β-Carotene source. Beta-Carotene is reduced by 90% in grasses and 60% in legumes in the time from immature to mature stages of growth (Maynard et al., 1979; Williams et al., 1998).

2.3.3 Stage of plant growth and sunlight

Forages that are cut early, before or during bloom, and are exposed to minimal sun and rain are high in β-Carotene as opposed to those cut at seeding and exposed to a lot of rain and sunlight. Generally oxidation is the method by which both β-Carotene and vitamin A are broken down; this is usually the case during hay-making when roughages are left outside to cure in the field. Breakdown occurs by enzymes that require oxygen, is catalysed by high temperatures and only terminates at total desiccation. Levels of loss are reported as high as 80% during the first 24 hours under these conditions. In lucerne it was established that pigment loss is only about 8% due to sunlight but that β-Carotene breakdown is about 28%. Fresh grasses are therefore much higher in β-Carotene content than hays (Maynard et al., 1979). When compared to results from analyses of fresh lucerne, stored hay does not compete with fresh lucerne that provides up to 4 000 mg of tocopherol per day (Bruhn & Oliver, 1978).

Beta-Carotene concentrations in plants tend to be higher in mild and moist conditions due to a reduction in the amount of sunlight reaching the plant. Temperature and light result in a larger variation of β-Carotene in plants than water does, due to light and temperature having a greater effect on leaf to stem ratio than water does (Williams et al., 1998). The effect of maturity may have a large effect on nutrient loss, the loss of tocopherol content in lucerne between the 1st and 5th cut of fresh lucerne was reported to

be about 100 mg for an animal eating 9.8 kg of lucerne daily (Bruhn & Oliver, 1978). Plasma concentrations of β-Carotene are primarily affected by the plants they ingest and therefore by season (Schweigert. 2006).

Due to the processing that ingredients of concentrates undergo there natural vitamin content is marginal. With regards to harvesting and preservation, vitamin content can vary greatly as a result of what happens to it from the time of harvest to the time of feeding. Factors reducing vitamin content include: storage, ensiling, drying, dehydration, wilting, cutting or preservative dressing. Beta-Carotene is broken down by oxidation which is increased by ultra violet light and heat, however if heat is applied in the absence of oxygen, β-Carotene loss is negligible (Williams et al., 1998). As the rate of decrease of β-Carotene content of forages relies on temperature, the amount of contact with oxygen, sunlight and the duration of storage, it is not easy to calculate, but under regular conditions this has been said to be approximately 7% per month of storage. Throughout the processing of animal feeds in a feed mill system, pressure and steam (heat and moisture) are involved, resulting in the breakdown of vitamin A and Carotene. Of the manufacturing process of feed production, the process of pelleting may be the primary cause of high vitamin loss due to the size and thickness of the pelleting die, resulting in high pressure. It is also very likely that granulated and coated vitamin A supplementation products are severed and break in this process, exposing the vitamin. Up to 40% of vitamin A that would have initially been available in the feed pre-pelleting may be destroyed during pelleting (McDowell, 2000).

Due to the various factors affecting the β-Carotene status of forages, animals generally remain in a deficient state. This is confirmed by a global survey of β-Carotene status that showed animals to be deficient in β-Carotene, substantiating that diets are not adequate to fulfil β-Carotene requirements (Byers et al., 1956). There are exceptions, however, such as dairy cows grazing on high quality green pastures.

2.4 Functions of β-Carotene

The main function of β-Carotene is that of vitamin A precursor, being converted within the gut or surrounding tissues (Ensminger and Olentine, 1978).

Reports have also shown that carotenoids have antioxidant properties; they are able to deactivate the effects of chemicals such as oxygen and free radicals, preventing the effects of potentially harmful processes such as lipid peroxidation (Olson, 1996, McDowell, 2000). Interest in β-Carotene as an antioxidant peaked when it was reported that vitamins had antioxidant effects which were believed to reduce the effect of free radicals in tissues.

Trials have shown that supplementing dairy cows with β-Carotene at 300 mg/day during the dry period reduced the occurrence of mastitis. Interestingly in the same trial mastitis was not reduced for animals only receiving vitamin A supplementation (Chew and Johnston, 1985). The function of β-Carotene as an antioxidant is further confirmed by an in vitro study by Schweigert (2003) showing the prevention of cross linking (damaging effects of free radicals) in the presence of β-Carotene, with another study showing a reduction of vitamin E but not β-Carotene during elevated levels of free radicals, suggesting that free radicals denatured vitamin E but not β-Carotene (Schweigert, 2003).

With regard to immune function, there is much evidence pointing to the ability of β-Carotene to aid in uplifting the functions of the immune system. Beta-Carotene has been revealed to aid in the response of lymphocytes, natural killer cells and macrophages. With respect to health and specifically udder health, β-Carotene supplementation has been shown to have a stabilising effect on polymorphonuclear neutrophils (PMNs) which make up the primary defence against bacteria in the udder. This is evident in dose-response trials where the incidence of intra-mammary infections and mastitis was reduced by the supplementation of β-Carotene but not of vitamin A (McDowell, 2000). It has also been proven that animals experiencing a vitamin A deficiency have a reduced mobility of natural killer cells, a reduction in the production of antibodies, a reduction in lymphocyte response and a higher inclination towards infection. This shows that vitamin A aids in regulating the immune response whereas β-Carotene has an antioxidant role in blood and milk and aids in improving the efficiency of polymorphonuclear neutrophils against *Staphylococcus aureus* (McDowell, 2000).

A number of papers have reported the positive effect of β-Carotene on reproduction. Beta-Carotene does this by effecting structures of reproduction such as follicles and the corpus luteum (Schweigert, 2006). Beta-Carotene has also been associated with increasing reproductive efficiency by increasing conception and reducing cysts (Bonsembiante el al., 1980).In the corpus luteum (CL), β-Carotene shows a higher concentration than in any other part of the cow. It has been suggested that it has a highly specific role other than that of vitamin A precursor (Buiter, 1998). When vitamin A was greatly reduced during winter feeding, β-Carotene was shown to have a positive effect on luteal progesterone (McDowell, 2000). Beta-Carotene has also been reported to have a specific function in the CL (Kirsche et al., 1987; Buiter, 1998). Arikan and Rodway (2000) reported that progesterone synthesis is at its highest when β-Carotene plasma concentrations are at their lowest. This was observed in the 3rd experiment on heifers reported by Lotthammer (1978). The heifers in that trial had received no previous β-Carotene versus heifers in trials 1 and 2. Comparisons of the CL with those of the previous trials showed clearly that CL growth in the

control group was regressed and did not reach the same mature size when compared to that of the supplemented group (Lotthammer, 1978). On the contrary, in some other studies, it was found that β-Carotene had no effect on fertility (Akordor et al., 1986), or incidence of mastitis (Oldham et al., 1991).These responses, post β-Carotene supplementation, have been suggested to be due to amount of β-Carotene fed, time of supplementation, β-Carotene status at initiation of trial, environmental effects and effects of other ingredients in the diet (Herdt and Seymour, 2006).

Results, therefore, are inconclusive, with authors reporting β-Carotene supplementation to affect reproduction positively, negatively and with some finding no response (McDowell, 2000). Nevertheless, in studies where a positive effect was reported on reproductive efficiency over a number of species, it seems very possible that β-Carotene acts as a localised precursor of vitamin A in the ovary (Schweigert, 2006).

2.5 Mode of Action

2.5.1 Absorption of β-Carotene

The process of digestion by pepsin in the stomach and proteolytic enzymes in the small intestine, break down animal feeds and liberate vitamin A and carotenoids from proteins. Bile salts within the duodenum are then able to break up large fatty masses of carotenoids and retinyl esters into smaller parts so that enzymes can digest them easily (McDowell, 2000). For vitamin A and carotenoids to be absorbed in the intestine they must become soluble in a mixed-micelle solution. In order for retinyl esters to be absorbed into the mucosal cell they must first be hydrolysed to retinol within the intestine wall. Retinol is then re-esterified in the mucosal cell by long chain fatty acids and incorporated as carotenoids into chylomicra which are transported to the general circulation by way of the lymphatic system. Chylomicrons are lipoprotein particles composed of: triglycerides, phospholipids, cholesterol and proteins. Their function is to assist in transportation of hydrophobic substances such as lipids, obtained from the diet, to other parts of the body through the water-based system of blood. The liver takes up the retinyl esters and stores them within parenchymal cells, the retinal binds to a transport protein called retinol-binding protein (RBP) thereby making retinol mobile. Retinol-binding protein in turn binds to a larger molecule called transthyretin (TTR) in the blood, making it more resistant to glomerular filtration and renal catabolism. In essence as retinol movement is regulated by the procedures that control the synthesis and secretion of RBP by the liver, RBP is solely accountable for the mobilisation of retinol from the liver to the vitamin target sites. The outcome of carotenoids is largely not understood as, unlike retinol, carotenoids are not

stored in the liver. In the general circulation, carotenoids are connected to low density lipoproteins, but the chain of events from their removal from chylomicra, until they join up with these lipoproteins, are unknown.

In review of a number of studies, regarding vitamins and health, it is clear that the role of vitamin A is well understood in its effect on disease control as opposed to β-Carotene's role in the same area. It is clear that both vitamin A and β-Carotene aid in combating infections by aiding the mechanisms of host defence, even though many papers discuss this topic, there are only a few that discuss the effects of vitamins on defence mechanisms in domestic animals (Chew, 1987).

The process whereby vitamin A is synthesized from β-Carotene is thought to mostly occur within the intestinal mucosa; however this process can also take place in the liver or other organs (McGinnis,1988) and in cattle may occur within the corpora lutea (Sklan, 1983). In order for this conversion to take place the Carotene must possess one β-ionone ring in the presence of β-Carotene -15,15-dioxygenase and retinaldehyde reductase. The function of -15,15-dioxygenase is to initiate the process of β-Carotene cleavage at the double bond in the centre of the molecule. This produces two molecules of retinol. Retinaldehyde reductase changes retinal to retinol by the process of reduction (Wolf, 1995).
Vitamin A and β-Carotene are absorbed in the intestine, the absorption of vitamin A is about 80 to 90% and β-Carotene is about 50 to 60%. In the lymph system, vitamin A is conveyed via a carrier in the form of a low density lipoprotein which takes it to the liver. The main factor effecting the secretion of RBP from the liver is the vitamin A status, as well as oestrogen, protein and zinc. Therefore in the case of vitamin A deficiency, secretion of RBP from the liver is blocked, increasing the RBP content of the liver and reducing that in blood plasma (McDowell, 2000).

There is evidence that β-Carotene is transmitted from plasma to follicular fluid by passive transfer. Only lipoproteins of high density can pass through the blood-follicle barrier due to the structure of the molecular sieve and their low molecular weight. There are two possible routes of entry for vitamin A found in follicular fluid. Firstly by crossing the blood-follicular barrier by way of transportation via carrier proteins, transfer rate by this method is influenced only by molecule charge and size, therefore implying a constant transfer rate, secondly β-Carotene is absorbed by granulose cells, converted to vitamin A and transferred to the follicular fluid via carrier proteins (Schweigert, 2006).

2.5.2 Factors affecting absorption of β-Carotene

2.5.2.1 Animal effect

With regards to animal effects, vitamins require transportation through the digestive tract, thus availability is directly proportional to transportation through the rumen and absorption in the intestine. Vitamins are released in the rumen by the action of digestion, a prerequisite to absorption, therefore the efficiency of this release is primarily important to vitamin bioavailability but this may vary with plant type.

The losses of vitamin A in the rumen that have been reported are large, approximately 40-60% but no exact values are available. Vitamin A is denatured in the rumen by chemical reactions, and rumen bacteria cause; oxidation, degradation and engulfment. It would seem that the absorption of vitamin A through the intestinal wall has not been demonstrated to date, possibly as the size of the vitamin A molecule is so large. Beta-Carotene losses in the rumen range from 3 to 32% with an average of 20%, probably due to hydrogenation; the losses of vitamin A are hypothesised to undergo reductive degradation (Potkanski et al., 1974). It could therefore be concluded that due to the broad range of feeds given and the influence of rumen fermentation the nutrient profile found in the rumen is variable and the chemical structure of vitamins undergo substantial modification (Williams et al., 1998).

2.5.2.2 Breed effect

Different breeds were found to have varying abilities to convert Carotene to vitamin A. In order of efficiency: Holstein, Ayrshire, Jersey and Guernsey. No significant ($P < 0.05$) difference was found between Holstein and Ayrshire. The different breeds had similar responses to β-Carotene and vitamin A intake but the range of plasma β-Carotene and vitamin A fluctuation varied between breeds, with vitamin A to a much lower degree than β-Carotene. Vitamin A fluctuations did not follow that of the β-Carotene levels, but seemed to have a lag effect (Sutton et al, 1945).

2.6 Reproduction

2.6.1 β-Carotene and fertility

As the milk production levels achieved by dairy cows have increased substantially over the past years, there has been an increase in the ICP, possibly as a result of a greater negative energy balance (NEB) causing late recovery of ovarian function. After calving animals experience an increase in milk production, peaking at approximately 2 months postpartum. Feed intake begins to decline prior to calving and reaches its lowest levels directly postpartum after which it increases again reaching its peak between 8 and 10 weeks postpartum. Therefore animals experience a negative energy balance for approximately 10 weeks in milk as feed intake lags behind milk production. The energy partitioning is of secondary priority for reproduction, resulting in a prolonged time period to normal ovary functioning and therefore reduced fertility (Butler, 2003).

It is well established that the steam-up period is associated with a remarkable change in hormone levels to accommodate the anticipated initiation of parturition and lactogenesis. Progesterone concentrations which were high during gestation in order to maintain pregnancy drop rapidly around 2 days prepartum, oestrogens in plasma increase during late pregnancy but drop off directly at calving, insulin concentrations decrease in plasma and growth hormone increases during the transition period stimulating the secretion of insulin-like growth factor (IGF-1) from the liver, which aids in modulating nutrition and reproduction. Animals that are managed well during the dry period express higher IGF-1 concentrations, which are higher in cows ovulating 3 weeks postpartum as compared to anovulatory cows. High IGF-1 and insulin levels are imperative for the first ovulation postpartum, the levels of which are confounded by a negative energy balance (Kawashima et al., 2009).

During the transition phase, animals experience a rapid alteration in metabolic status as a result of reduced DMI (Dry Matter Intake), foetus development and initiation of colostrum synthesis. Similarly, β-Carotene plasma concentrations decrease owing to reduced DMI and secretion into colostrum, reaching their lowest point between 7 and 14 days postpartum (Lotthammer, 1978; Bindas et al., 1984; Rakes et al., 1985; Iwanska and Strusinska, 1997; Kawashima et al., 2009), this later increases again as colostrum production is replaced by milk production. However the reduction in plasma concentrations of β-Carotene and vitamin A may be due to other factors such as low lipid levels in plasma (Iwanska and Strusinska, 1997).

Beta-Carotene is associated with host defence mechanisms, aiding in disease prevention including those related to reproduction as well as udder health via lymphocyte and phagocyte functioning (Michal et al., 1994). It has also been reported that animals experiencing early ovulation have higher β-Carotene plasma concentrations during the dry period than those experiencing anovulation. Therefore nutrition during the dry period is imperative to resumption of ovulatory functioning postpartum and β-Carotene may be a useful indicator of health, nutrition status and fertility status during the following lactation (Kawashima et al., 2009).

Kawashima et al. (2009) suggested that increasing concentrations of β-Carotene in plasma during the dry period may aid in increased reproductive performance postpartum. Plasma β-Carotene concentrations are related to the first ovulation postpartum; thus analyses of plasma β-Carotene during the dry period may be an early indication of postpartum reproductive capacity. This is confirmed in a trial by Lotthammer (1978), when the supplemented group rapidly returned to prepartum concentrations of plasma β-Carotene and continued to increase whereas the control group continued to drop and remained at concentrations of about 0.3 mg/L.

Hans Schultz in 1956 and Brüggemann and Nieser (1957) stated, for the first time, that β-Carotene had a separate role to vitamin A in cattle and that this role was positively related to fertility. Before these papers were published it was generally thought that vitamin A when supplemented at sufficient levels would remove negative effects of fertility associated with this vitamin. However after these reports it was clear that cattle have a β-Carotene requirement that cannot be met by supplementing with vitamin A (Lotthammer, 1978).

A number of reproduction problems have been reported to be due to low vitamin A or β-Carotene intake, these include: high percentage of still born or weak calves, a high incidence of retained placenta, an increase in abortions, night blindness, opthalmia, diarrhoea, reduced conception and an increase in number of services per conception. Vitamin A related deficiencies include: delayed onset of puberty and reduced libido and spermatogenesis in bulls. The following reasons were put forward by Hemken and Bremel (1982) to validate focusing on β-Carotene in relation to reproductive performance.
Low β-Carotene content of feed is related to low plasma β-Carotene concentrations, resulting in lower reproductive performance. Reproductive parameters affected by low plasma β-Carotene concentrations include; longer time between heats, reduced uterine involution, reduced conception, increase in the number of inseminations per conception, weak oestrus and an increase in the number of luteal cysts (Hemken and Bremel, 1982).

2.6.2 Effects of β-Carotene supplementation on measures of fertility

Buiter (1998) reported that a deficiency symptom related to β-Carotene is the weak or silent display of oestrus, however in some reports β-Carotene had no effect on heat display (Folman et al., 1979; Akordor et al., 1986). Akordor et al. (1986) stated that β-Carotene status of animals had no effect on the expression of oestrous, but fewer animals that received β-Carotene were treated for anoestrous as compared to the control group. Differences between studies in relation to heat detection and length of oestrus may be due to differences in the method by which this is recorded, as some authors used palpation to confirm this and others visual display of oestrus behaviour.

Reduced rates of conception have been reported to be due to the deficiency of β-Carotene (Ascarelli et al., 1985; Buiter, 1998). Conversely other authors have stated that β-Carotene has no effect on conception (Folman et al., 1979; Akordor et al., 1986). Ascarelli et al. (1985) reported that although overall β-Carotene was found to have no effect on fertility, the conception rates of the younger animals in the treatment group were two-fold higher, but that the reason for this was unclear. Beta-Carotene supplementation is said to have no effect on pregnancy rate (Byers et al., 1956; Akordor et al., 1986; Folman et al., 1987), however a report by Ducker (1984) showed evidence that animals with plasma β-Carotene concentrations above 5.75 mg/L at the time of first insemination had a significantly higher rate of pregnancy than animals with β-Carotene concentrations below this value. Ducker (1984), Rakes et al. (1985), Akordor et al. (1986) and Folman et al. (1987) stated that β-Carotene had no effect on the number of inseminations per conception, however this was contested by Buiter (1998) who found that the number of inseminations per conception increased with a deficiency of β-Carotene.

Supplemented β-Carotene has been shown to affect reproductive health by aiding in the reduction of retained placenta which could not be reduced by supplementing with vitamin A (Byers et al., 1956; Buiter, 1998). Conversely in a trial by Lotthammer (1978), no differences were observed for the incidence of retained placenta between animals supplemented with β-Carotene and those not.

It was also reported that animals receiving adequate vitamin A but not β-Carotene had a higher incidence of delayed uterine recovery after calving (Buiter, 1998). Lotthammer (1978) reported delayed involution of up to 4 days for animals with low plasma β-Carotene and Akordor et al. (1986) showed no effect of β-Carotene supplementation on uterine involution at 28 days postpartum. Animals receiving adequate vitamin A but not β-Carotene had a longer time from parturition to first heat (Buiter, 1998); others found that overall β-Carotene had no effect on the interval between calving and first heat (Ascarelli et al., 1985;

Akordor et al., 1986). Rakes et al. (1985) reported that the interval from calving to first observed oestrous was shorter for β-Carotene supplemented cows than for control cows and shorter for maize silage-fed than lucerne silage-fed cows. The reason for this was unclear but no effect on the time from parturition to first insemination was established.

Lotthammer (1978) found that heifers in a control group experienced a delay in ovulation by 24 hours on average when compared to ovulation of heifers in a supplemented group. This was confirmed in lactating cows by Byers et al. (1956) and Lotthammer (1978), who observed ovulation to be delayed by 6 days in the control group versus the supplemented group. Akordor et al. (1986) found β-Carotene supplementation to have no effect on time to first ovulation or the number of days open. Beta-Carotene supplementation had no effect on the number of days to first ovulations or the numbers of days open (Akordor et al., 1986; Folman, 1987). This was expected as the occurrence of first ovulation followed soon after initiation of supplementation. Rakes et al. (1985) reported that β-Carotene supplemented cows showed a shorter time between calving and conception but this was not significant. Ducker (1984) reported no effect on the number of days from initial insemination to conception.

In a trial by Lotthammer (1978) on lactating cows, the control group showed 31.3% embryo mortality in the first 7 weeks of pregnancy and a further 12.5% had early abortions from week 18 to 20 compared to the β-Carotene supplemented group that experienced no interrupted pregnancies or abnormalities. These findings correlated with different progesterone concentration levels, with the supplemented group having the highest progesterone concentrations, the control group animals and those that experienced mortalities and abortion having the lowest. Again it was reported that animals receiving adequate vitamin A but not β-Carotene had an increased incidence of embryo fatalities (Buiter, 1998). Beta-Carotene deficiency may then result in embryo mortalities in cows (Byers et al., 1956) and heifers (Lotthammer, 1978).

Animals receiving adequate vitamin A but not β-Carotene had a higher incidence of ovarian cysts (Eaton et al., 1972; Folman et al., 1979; Akordor et al., 1986; Buiter, 1998; Kida, 2009). Lotthammer (1978) found that heifers not receiving β-Carotene supplementation experienced a higher incidence of ovarian follicular and luteal cysts at 42.1% versus 3.1% in the supplemented group, as was the case in lactating cows. However some authors stated that β-Carotene supplementation had no consequence on the occurrence of follicular and luteal cysts (Folman et al., 1979), as well as the frequency of endometritis and pyometra (Akordor et al., 1986).
Special mention must be made of Lotthammer (1978), who conducted three experiments using German black pied heifers and one using lactating cows of the same breed, to investigate the effect of β-Carotene

on fertility. In the first experiment, the control group received no β-Carotene and 220 I.U of vitamin A, the treatment group were supplemented with 0.30 mg of β-Carotene and 100 I.U of vitamin A. This vitamin A supplementation was based on a ratio of 1:400 of β-Carotene to retinol and the test period lasted on average 50 to 52 weeks. In this experiment, the control group was significantly lower in plasma β-Carotene and higher in vitamin A than the treatment group.

The second experiment by Lotthammer (1978) consisted of 2 periods, period A lasted 29 weeks and in this time period the control group received no β-Carotene and 220 I.U of vitamin A and the treatment group received 0.3 mg of β-Carotene and 100 I.U of vitamin A. The second period or period B, lasted for 10 weeks in which the control group received 0.60 mg of β-Carotene and 100 I.U of vitamin A and the treatment group received 0.30 mg β-Carotene and 100 I.U of vitamin A.

This trial was set up in this manner in order to examine any reversibility of disorders associated with the ovaries. For the duration of one heat period, blood was taken and analysed for luteinising hormone (LH) to investigate the pre-ovulation LH peak. Results for experiment 2 during period A were the same as those for experiment 1, however in period B the β-Carotene serum concentrations increased for the control group as did vitamin A, even though vitamin A was reduced by 120 I.U per kg body weight/d.

Experiment 3, lasted 52 weeks, the control group received no β-Carotene supplementation and 220 I.U of vitamin A, the treatment group received 0.30 mg β-Carotene and 100 I.U of vitamin A. At the end of the 44th week, heifers were inseminated. Unlike heifers in experiments 1 and 2, heifers in this trial had not previously received β-Carotene. Post-insemination, both groups were tested for conception rates and the number of inseminations resulting in a confirmed pregnancy. It was found that the control group expressed longer intervals from insemination to subsequent heat as opposed to the supplemented group that did not experience a change in the interval. For all three experiments samples of the liver, thyroid, adrenal, uterus, ovaries and pituitary gland were analysed. Overall it was shown that blood serum was not a good reflection of dietary vitamin A, as the control group had lower serum levels than the supplemented group, despite higher vitamin A dosage.

Both vitamin A and β-Carotene plasma concentrations increased in cows that received β-Carotene supplementation, implying that it is possible to increase serum vitamin A concentrations even when dietary vitamin A is high. Beta-Carotene and vitamin A concentrations in sampled organs were similar to those in blood serum. However in corpora lutea the supplemented group had a value of 16.3 µg/g β-Carotene and the control group had a value of 12.1 µg/g fresh tissues but no vitamin A.

Results showed that an adequate β-Carotene supply can maintain the vitamin A status of an animal at the same level of a diet high in pre-formed vitamin A. With regards to fertility, control groups experienced an increase in the number of silent heats as well as a reduction in distinct uterine contractions and reduced

but extended secretion of vaginal mucous over a number of days, accordingly making it complicated to establish the best time for insemination and therefore conception (Lotthammer,1978).

Under field conditions, it is possible for β-Carotene plasma concentrations to be and have a negative effect on fertility. It is also possible to increase blood β-Carotene concentrations to optimal levels by supplementing the diet with β-Carotene and an increase in the concentration of blood β-Carotene is usually accompanied by an increase in reproduction performance when no other factors affect fertility (Friesecke, 1978). A survey of research results is shown in Table 5.

Table 5 is an extensive summary of responses to varying levels of β-Carotene supplementation by a number of authors at different stages of parturition and for different lengths of time.

Table 5 Summary of studies on responses to β-Carotene supplementation

Study	Animal number	Animal type	Treatment period	Supplementation (mg/cow/day)	result
Marcek et al. (1985)	62	Multiparous lactating	10 DIM for 120 days	300	No effect on occurrence of ovarian cysts
Ducker et al. (1984)	40	Heifers	90 days	300	No effect on reproductive efficiency
Ascarelli et al. (1985)	155	Multiparous dry and lactating	At dry off for 150 days	500-700	No effect on reproductive efficiency
Akordor et al. (1986)	56	Multiparous lactating	10 DIM till Confirmed in calf/culled	400	No effect on reproductive efficiency
de Ondarza & Engstrom (2009)	515	Multiparous lactating	120 days	425	Increased pregnancy rate by 1% overall
Rakes et al. (1985)	70	Multiparous lactating	At calving for 100 days	300	No significant differences
Folman et al. (1979)	20	Heifers	7-13.5 months	300	No effect on reproductive efficiency
Folman et al. (1987)	174	Multiparous dry and lactating	8 Weeks prepartum till confirmed in calf/culled	500-700	Supplementation at high and low levels may negatively affect fertility
Lotthammer (1978)	32	Multiparous lactating	7 weeks prepartum to 9 weeks postpartum	400-600	Positive effect β-Carotene on fertility
Arechiga et al. (1998)	701	Multiparous lactating	15 to 30 days postpartum	400	Positive for increased fertility in times of stress, further investigation required
Iwanska and Strusinska (1997)	50	Multiparous dry and Lactating	2 weeks prepartum up until 100 DIM	150-300	Positive effect on reproduction

2.7 β-Carotene and animal performance

2.7.1 Milk Production

Arechiga et al. (1998) conducted three experiments on two farms in the South and North of Florida to test the effects of β-Carotene supplementation on dairy cattle during heat stress. Animals were split into two groups, namely; the control group receiving no supplemental β-Carotene and the supplemental group receiving 400 mg of β-Carotene daily. Animals were supplemented from 15 days postpartum for 2 months and blood plasma was analysed for β-Carotene, retinyl palmitate, retinol, α-tocopherol and progesterone. Overall milk yield for supplemented cows showed an increased in all 3 experiments as a result of supplemental β-Carotene with increases of 11%, 6% and 7% in order. Experiment 1 ($P < 0.05$) and experiment 3 ($P < 0.01$) showed significant differences for predicted milk yield at 305 days of lactation.
Folman et al. (1987) reported that only cows in lactation 4 and higher had an increased milk yield whereas Akordor et al. (1986) reported no effect of β-Carotene supplementation on milk production. Kawashima et al. (2009) found no differences for 305 day milk yield. Rakes et al. (1985) tested the effect of β-Carotene supplementation on different diets in lactating cows with regards to milk yield but no differences were found between treatment groups for milk yield.

In a study to evaluate the effect of β-Carotene on milk yield (de Ondarza et al., 2009), 515 cows were used and treatment animals received 425 mg of β-Carotene daily. However β-Carotene supplementation had no effect on milk yield. De Ondarza and Engstrom (2009) investigated the effect of supplementing β-Carotene (425 mg/cow/d), on lactating Holstein cows with low concentrations of β-Carotene in serum (< 3 µg/mL) and adequate supplementation of vitamin A (8400 I.U/kg) to examine any effects on milk yield. Animals were supplemented for 120 days and milk production was measured. Supplementation had no effect on 3.5% FCM but there was a tendency ($P < 0.01$) for cows in early lactation and lactation 3 and higher to produce more milk. Folman et al. (1987) reported that younger animals in a supplemented group receiving β-Carotene (500 mg/d) during both the dry and lactation periods were shown to have a higher FCM yield than similar animals in the control group only receiving 69 mg of retinyl acetate per cow/day.

From the literature cited it is clear that responses to β-Carotene supplementation on milk yield is variable and is affected by various factors as discussed in the previous sections of this chapter .

2.7.2 Milk constituents

De Ondarza and Engstrom (2009) reported that β-Carotene had no effect on overall milk fat (kg/d), milk true protein % or milk true protein production; however there was a tendency ($P < 0.01$) for early lactation cows in lactation 3 or higher to produce more milk fat (kg/d). Supplementation of β-Carotene resulted in an average increase in Milk Urea Nitrogen (MUN) (mg/dL) by 2%, affecting cows in 2nd lactation and those less than 100 DIM. This is however, biologically insignificant.

In another experiment by de Ondarza et al. (2009) the percentage of milk fat was 3.25% and 3.18% for the supplemented and control group respectively. However milk fat production (kg/day) was unaffected by β-Carotene supplementation but data did show trends ($P < 0.01$) of milk fat increase in favour of the β-Carotene supplemented group. Beta-Carotene supplementation had no effect on milk true protein percentage and production. Kawashima et al. (2009) found no differences for the effect of supplemental β-Carotene on milk composition as did Rakes et al. (1985) who tested the effect of β-Carotene supplementation on milk fat percentage and found no differences between treatment groups.

As is the case with milk production, β-Carotene supplementation effects on milk composition are variable, but are mostly not affected.

2.7.3 Somatic Cell Count

In an experiment by Bindas et al. (1984), 78 cattle were assigned to either a β-Carotene supplemented group receiving 600 mg of β-Carotene daily, or a control group receiving no supplemental β-Carotene from 30 to 60 DIM. The supplemented group experienced maximum concentrations of 2.45 µg/ml β-Carotene at about week 10 and the control group of 1.50 µg/ml by about week 7. The supplemented group was found to have a lower comparative Somatic cell count (SCC) but this was not significant. Rakes et al. (1985) tested the effect of β-Carotene supplementation on different diets in lactating cows with regards to SCC and found that SCC was lower for lucerne fed cows than maize fed cows, this was also true for β-Carotene supplemented cows as compared to control or un-supplemented cows but this was not a significant difference. De Ondarza and Engstrom (2009) found β-Carotene supplementation had no effect on the SCC as did de Ondarza et al. (2009). Oldham et al. (1991) reported that neither β-Carotene nor vitamin A supplementation had an effect on reducing the SCC.

In general, β-Carotene supplementation does not seem to affect the SCC of milk, based on the results of the studies mentioned above.

2.7.4 β-Carotene levels in milk

Breed type was found to have an effect of secretion of β-Carotene levels and amount in milk, but was found to be of less importance when compared to the effect of diet on the amount of β-Carotene in milk (Nozière et al., 2006a). It has been reported that ruminants are poor absorbers of β-Carotene; this was confirmed in an experiment by Ascarelli et al. (1985) who found only a few milligrams of β-Carotene daily in milk of an un-supplemented herd. A general increase of vitamin A in milk was seen, as cows progressed in lactation, directly related to the high intake of these vitamins in the diet.
This can be associated with the low rate of uptake of β-Carotene from the blood, as these molecules are generally associated with high density proteins for transportation in ruminants, whereas molecules associated with low density lipoproteins are easily absorbed. However in a trial by Nozière et al. (2006a) plasma concentrations of β-Carotene accounted for only 20% of variation in milk levels.
Beta-Carotene levels in colostrum are high as close-up animals absorb β-Carotene to supply to calves at birth, but levels drop as milk returns to normal (Bindas et al., 1984).

2.7.5 Growth

It was suggested that β-Carotene may have a general metabolic effect on ruminant performance. Folman et al. (1987) found β-Carotene supplementation to have a positive effect on growth, this was again confirmed when Folman et al. (1979) established that a β-Carotene supplemented group experienced a higher growth rate and average daily gain than that of the control group, but this increase in body weight was only observed during the last 3 months of the trial when animals were put in the same yard, suggesting that the supplemented group had a higher DMI. A contradicting article stated that β-Carotene supplementation had no effect on the average live weight or change of live weight during the trial duration (Ducker et al., 1984).

2.8 Health

2.8.1 Fluctuating β-Carotene levels in Cattle

Katsoulos et al. (2005) reported that β-Carotene and vitamin E were lowest at calving compared to any other time, in a trial initiated at 30 days prepartum and continuing up to 10 months postpartum. Beta-Carotene concentrations during the first month in lactation were lower than plasma concentrations during the dry period.
Average plasma concentrations of β-Carotene were significantly higher in group A, which consisted of animals 4 years of age or younger, compared to group B, consisting of animals older than 4 years. This trial confirmed changes in β-Carotene from the dry period to the end of lactation, with age playing a major role in levels of fat soluble vitamins in plasma. The rapid decrease of β-Carotene plasma concentration at calving was due to lactation inception, as β-Carotene is excreted in

colostrum and lost through oxidation and a decrease in DMI at this time. Vitamin A, however, was found to experience no change.

2.8.2 Udder Health and Mastitis

Compromised udder health, which is directly related to mastitis, is one of the most economically important conditions in dairy herds. It is the single highest cause of premature culling. Farmers constantly underestimate the true cost of mastitis. The associated financial losses include; cost of medication, discarded milk not fit for human consumption, veterinarian consultation, labour, reduced milk yield, premature culling, expense of replacement animals, cost of feed for animals not producing and reduced price per litre for lower quality milk related to increased SCC. Losses in SA due to mastitis are estimated by Dr JH du Preez of the Milk Producers Organisation (MPO) to be more than R400 million per year (Personal communication, MPO, Cotton SA Building, 86 Watermeyer Street, Pretoria). Mastitis management however, remains the best means of control (Halasal et al., 2007).

Chew et al. (1982) found that a deficiency of vitamin A and β-Carotene may be linked to udder infections in cows. Proposed explanations for this include: a weakening of the udder lining due to reduced keratin secretion, resulting in a successful attack by organisms causing mastitis; reduced transportation of immunoglobulins and leukocytes to the infected area and the rate of transfer of β-Carotene and vitamin A from plasma to milk or differences in the conversion of β-Carotene to vitamin A which takes place in the intestine. Chew et al. (1982) further stated that cattle with reduced concentrations of plasma β-Carotene and vitamin A scored higher on the California Mastitis Test. Dahlquist and Chew (1985) reported that supplemental β-Carotene reduced the incidence of new udder infections acquired during the dry period. Chew and Johnston (1985) reported that supplemental β-Carotene reduced the SCC count in cattle during the lactation period. Wang et al. (1988b) found that β-Carotene supplementation had a positive effect on reducing mastitis whereas Kawashima et al. (2009) and Oldham et al. (1991) did not.

Similar to many other production measures, the effect of β-Carotene supplementation on the incidence of mastitis is variable and inconclusive.

2.8.3 Immune Function

Kawashima et al. (2009) showed that reduced plasma concentrations of β-Carotene in anovulatory cattle may correlate to inadequate support of the immune function.

In review of a number of studies regarding vitamins and health, it is clear that the role of vitamin A is well understood in its effect on disease control as opposed to β-Carotene's role in the same area. It is clear that both vitamin A and β-

Carotene aid in combating infections by aiding the mechanisms of host defence. Although many papers discuss this topic, there are only a few papers that discuss the effects of vitamins on defence mechanisms in domestic animals. Chew (1987) reported that vitamins may help in aiding mammary health. Chew (1993) stated that β-Carotene may also function as an antioxidant. This antioxidant function is further confirmed by Dembinski and Bronicki (1994), Rapoport et al. (1998) and Weiss (1998). Van den Berg et al. (2000) reported the antioxidant nature of carotenoids in ruminant diets and stated that this function aids in cell communication and immune function by shielding cells from free radical attacks.

Michal et al. (1994) reported that β-Carotene obtained from the diet was able to boost host defence mechanisms by enhancing lymphocyte and phagocyte function, reducing the occurrence of several reproductive disorders.

There are many reports of positive effects on human health by β-Carotene supplementation, including prevention of certain cancers, improved immune function, tumour restraint, aid in reduction of coronary heart disease, cataract suppression and the reduction of deterioration related to aging (Umeno et al., 2005).

2.8.4 Calf health

Kaewlamun et al. (2011a) investigated the responses to dietary β-Carotene supplementation of dairy cows during the dry period. The supplemented group received 1 g of β-Carotene daily. Results displayed an increase in plasma β-Carotene status and an increase in β-Carotene content of colostrum in the supplemented group (3.10 ± 0.23 mg/L) versus the control group (1.44 ± 0.24 mg/L).

Results reported from a number of studies show that concentrations in colostrum can range widely for β-Carotene (17.8 to 342.9 µg/dl) and vitamin A (32.9 to 450.0 µg/dl). These variations have been linked to the following factors; individual effect, breed effect, effect of lactation number, effect of diet given during the dry period and incidence of mastitis as well as the decline of both of these vitamins supplied in milk over time after calving (Foley and Otterby, 1978; Kume and Tanabe, 1993). Parrish et al. (1953) reported that absorption by calves during the first week of life of vitamin A and β-Carotene was 81-95% and only 38-65%, respectively.

In a study conducted by Nonnecke et al. (1999) 3 groups of jersey bull calves received colostrum for the first week of life and milk replacer for 7 weeks thereafter. The first group, the control group, received no supplementation of vitamin A. The second group was supplemented with 32 000 I.U vitamin A and the 3rd group was supplemented with β-Carotene equivalent to 20 000 I.U. vitamin A/day. It was found that supplemented vitamin A had an influence on the composition of mononuclear leukocyte population (MNL) or white blood cell population in calves, increasing the growth of leukocytes associated with the recognition and response to antigens. Supplemental β-Carotene had no effect on the MNL population. Vitamin A supplied in the diet to calves may help in improving the rate of development of the calf's immune system,

furthermore the vitamin A supplied to calves in milk replacer can change the bioavailability of vitamin E and the composition of the peripheral blood MNL, improving calf health.

Lotthammer (1978) reported low incidence of diarrhoea in calves when their dams were supplemented with β-Carotene. Calves with β-Carotene un-supplemented dams were shown to have much lower concentration of gamma-globulins and vitamin A versus those with supplemented dams, prior to both groups receiving colostrum. Kume and Tanabe (1993) agreed that the vitamin status of calves relies on both the amount of vitamin in colostrum, as well as that absorbed via the placenta during late pregnancy and that this is more important for vitamin A than β-Carotene, as β-Carotene is available in milk at higher levels than vitamin A. Optimal β-Carotene concentrations have also been shown to improve calf health status by reducing the magnitude and incidence of diarrhoea and pneumonia (Byers et al., 1956). Quigley et al. (1995) and Quigley and Drewry (1998) confirmed that colostrum passively transfers immunoglobulins, other proteins and nutrients to the calf which supports the immune system and results in the reduction and extent of scouring.

It is important that feed is of high quality prepartum, as this affects the vitamin status of calves via placental transfer and colostrum, consequently calves fed low vitamin colostrum require supplementation to maintain optimal health. Kume and Toharma (2001) stated that vitamin A and β-Carotene deserved further investigation in improving calf health due to their findings.

2.9 Requirements of β-Carotene

Ascarelli et al. (1985) reported a need for β-Carotene in addition to that of vitamin A, and stated that concentrations below 2 mg/L in plasma were associated with a deficiency in cattle, whereas concentrations above 3 mg/L indicate an optimal concentration of β-Carotene. Arbeiter et al. (1983) advised that animals should possess a plasma β-Carotene concentration of no less than 3 mg/L. Gül et al. (1988) stated that concentrations in plasma less than 1 mg/L signify an explicit deficit thereof. Heifers of approximately 350 kg require about 100 mg β-Carotene supplementation daily, pregnant cows require approximately 200 mg and lactating animals about 10-20 mg per kg milk production over and above the basal requirement of 100 mg daily in order to prevent a deficient plasma β-Carotene status (Buiter, 1998). Schliffka (2008) reported optimal β-Carotene plasma concentrations to be above 3.5 mg/L. Exact levels for β-Carotene supplementation have still not been established due to many contradicting trials, but there seems to be a general indication that plasma concentrations above 3 mg/L of β-Carotene will meet reproductive requirements (Friesecke, 1978; Lotthammer, 1979; Iwanska and Strusinska, 1997).

O'Fallon and Chew (1984) previously stated that a cow requires the correct amount of β-Carotene and the right ratio of β-Carotene to vitamin A, thereby allowing vitamin A to be synthesized from β-Carotene to meet its requirements. This may explain why animals supplemented only with vitamins A, D_3 and E or insufficient β-Carotene do not experience any

increase in β-Carotene or vitamin A concentrations in plasma or enhancement of reproductive measures (Iwanska and Strusinska, 1997).

Byers et al. (1956) stated that there is a strong relationship between plasma β-Carotene concentrations and β-Carotene available in the diet. It is therefore safe to assume that animals on pasture receive adequate β-Carotene to maintain plasma levels, as proposed levels of β-Carotene content include between 40-80 mg/kg for fresh pasture, 10-40 mg/kg for well-made grass silage and 2-4 mg/kg for sugar beet and maize silages which are extremely deficient in this pro-vitamin (Lotthammer, 1979; Buiter, 1998).

Vitamin requirements are hard to classify and differ greatly due to the criteria of reference (Hemken and Bremel, 1982; Williams et al., 1998). This is especially true depending on whether the method used is to prevent a symptom of a deficiency- i.e. the minimum requirement- or the level related to maximum animal performance- i.e. at what maximum level will supplementation no longer effect performance. Most recommended vitamin levels include a safety margin and are higher than the actual requirement of the animal (Williams et al., 1998).

With regards to fertility in dairy cattle, a β-Carotene concentration considered to be optimal or rather not to limit fertility was decided on at 3 mg/L of blood plasma. Friesecke (1978) reported that cows that did not conceive to one or more inseminations had an average blood concentration of 2.1 mg/L, whereas those that did conceive had an average blood β-Carotene concentration of 3 mg/L. Some authors reported values of 106 µg of β-Carotene per kg body weight to avoid elevated cerebrospinal fluid pressure, while others recommended values of between 66 and 73 µg/kg to uphold a healthy level thereof, while levels recommended to prevent night blindness and maintain normal growth were about half that required to prevent high cerebrospinal fluid pressure. Other levels suggested include prevention of papillary oedema; 24-79 µg/kg and to increase liver content of vitamin A levels between 125 and 132 µg/kg was recommended. It is widely accepted that 1 mg of β-Carotene is equal to 400 I.U of vitamin A (1:400) (Hemken and Bremel, 1982). Buiter (1998) stated that even though there is much still to investigate in terms of β-Carotene mechanisms, symptoms of a β-Carotene deficiency are enough evidence to warrant β-Carotene supplementation.

From this overview it is clear that the β-Carotene status of dairy cows is determined largely by the plant species consumed by the cow and the season. A proper β-Carotene supplementation strategy therefore would depend on knowledge of the β-Carotene status of cows under different feeding systems and stages of lactation. Responses in terms of milk production, milk composition, health and reproduction are inconsistent. Observations show that the depression in progesterone secretion by cows could be overcome by β-Carotene supplementation but not by vitamin A. Because of all the uncertainties regarding the dietary supply of vitamins, in practice vitamins are normally supplied through synthetic vitamins included in vitamin mineral premixes.

Due to the many factors that affect the β-Carotene status of dairy cows, coupled with the large variability and inconclusive results, it was decided to conduct two studies on the β-Carotene status of Holstein cows.

In the first study (Chapter 3) a survey on the effect of different feeding systems and forage types on the β-Carotene status of Holstein cows were conducted. The following hypotheses were tested:

H_o = Different feeding systems and forage types impact on the β-Carotene status of Holstein Cows
H_a= Different feeding systems and forage types have no impact on the β-Carotene status of Holstein Cows.

In the second study (Chapter 4) the effect of prepartum β-Carotene supplementation on the postpartum β-Carotene status of Holstein cows was investigated. The hypotheses tested were:

Ho = Prepartum β-Carotene supplementation will positively affect the postpartum β-Carotene status of Holstein cows, making postpartum supplementation unnecessary.
Ha= Prepartum β-Carotene supplementation will have a minor effect on postpartum β-Carotene status, therefore postpartum supplementation is needed to maintain an acceptable β-Carotene status in lactating Holstein cows.

CHAPTER 3: A SURVEY ON THE β-CAROTENE STATUS OF HOLSTEIN COWS IN DIFFERENT FEEDING SYSTEMS

3.1 Introduction

There is evidence that β-Carotene may have a nutritional role in dairy cattle nutrition apart from being a precursor for vitamin A. Beta-Carotene functions separately as an antioxidant and may enhance immunity, furthermore it may also have reproductive and udder health benefits (Chew, 1993).

Performance results however have been inconsistent. Arechiga et al. (1998) found an increase in milk production of between 6 and 11% after supplementation of 400 mg of β-Carotene while others (Rates et al., 1985) reported no effect on production. De Ondarza et al. (2009) found an increase in butterfat percentage while Rates et al. (1985) reported no effect on milk composition after β-Carotene supplementation. Wang et al. (1988b) found that cows supplemented with 300 mg β-Carotene required less mastitis treatments compared to control cows, while in a study by Oldham et al. (1991) the incidences of mastitis was not reduced.

The β-Carotene status of cows may be linked to fertility since cows with higher statuses had higher concentrations of β-Carotene in the ovary, especially in the CL (Chew et al., 1984). This potential reproductive benefit of β-Carotene may be related to the conversion of circulating β-Carotene to vitamin A, specifically in the uterus and ovaries (Schweigert, 2003). As is the case with other performance parameters, reproductive responses have been variable. Kawashima et al. (2009) found that cows that ovulated during the first follicular wave postpartum had a higher average β-Carotene concentration in plasma, compared to anovulatory cows three weeks prepartum. Lotthammer (1978 & 1979) found that β-Carotene supplementation improved conception rates, ovulation and reduced the incidence of cystic ovaries. However Bindas et al. (1984) and Marcek et al. (1985) did not see any reproductive responses to β-Carotene supplementation.

The variation in responses to supplemental β-Carotene may be related to the variation in the β-Carotene status of animals prior to supplementation. Based on an evaluation of the research conducted so far, it seems sensible to concentrate on the potential of β-Carotene to improve reproduction and fertility. In order to better evaluate the potential benefits and to make meaningful recommendations on supplementing β-Carotene, more data is needed on the β-Carotene status of SA dairy cows. Thus far no research has been conducted on the β-Carotene status of dairy cows in SA. The objective of this study was to do a survey on the β-Carotene status of Holstein cows under the three most prominent dairy feeding systems applied in SA.

3.2 Materials and Methods

3.2.1 Farms, animals and feeding systems

The three major dairy feeding regimes in SA are pasture-based systems (kikuyu/ryegrass) with maize mineral-based concentrate supplementation (hereafter referred to as System 1), predominantly lucerne hay-based TMRs (System 2) and predominantly maize silage-based TMRs (System 3). A total number of 30 farms were identified, 10 farms for each feeding regime. The pasture-based systems included 5 farms from KZN and 5 farms from the Eastern Cape (EC); the two TMR based feeding system farms were found predominantly in Gauteng (GP), Mpumalanga (MP) and the Free State (FS).

A description of the farms identified within the three feeding systems is shown in Tables 6 to 8**.** In Table 6 the pasture-based systems are described, with ryegrass being the predominant forage for most of the year. In Table 7 farms using TMR systems are described, with lucerne hay the predominant forage on most of the farms. Farms where maize silage is the predominant roughage in the TMR feeding systems are described in Table 8.

From each of the 10 herds within a feeding system, 20 healthy cows were randomly selected for blood sampling. The constant was that cows were multiparous. Ideally cows should be more than 60 DIM, this is required because β-Carotene analyses on blood plasma of cows less than 60 DIM shows more variability (Schweigert, 2010, personal communication, Institute of Physiology, Ludwig-Maximilians-University Munich, Germany), however practically on some farms this was not possible.

Table 6 Descriptions of the farms selected to represent feeding system 1, pasture-based system in the KwaZulu-Natal (KZN) Midlands area and the Eastern Cape (EC) George areas.

Farm #	Province	Roughages	Ave. litres/ cow/day	Ave. DIM [a]	BF% [b]	PR %[c]	ICP [d]
1	EC	Lucerne/Rye[e]	22.3	53.0	3.6	3.3	402
2	EC	Clover/ Rye[e]	21.1	47.6	3.6	3.3	412
3	EC	Rye[e]	21.3	39.7	3.8	3.5	412
4	EC	Kikuyu/ Rye[e] /Clover	22.3	44.5	3.5	3.3	450
5	EC	Rye[e]	28.4	26.8	3.8	3.3	406
6	KZN	Rye[e]	21	55.3	3.5	3.1	408
7	KZN	Rye[e] /Maize silage	29	45.6	3.6	3.2	409
8	KZN	Rye[e] /Maize silage	23	42.4	3.9	3.2	388
9	KZN	Rye[e]	23	41.3	3.8	3.4	380
10	KZN	Rye[e] /Kikuyu/clover	25.1	47.2	3.2	3.2	410

[a] Average days in milk, [b] Butter fat percentage, [c] Protein percentage, [d] inter-calving period, [e] Rye grass.

Table 7 Descriptions of the farms selected to represent feeding system 2, hay-based system in the Gauteng (GP) and Free State (FS) areas.

Farm #	Province and Area	Main Roughages	Ave. litres/ cow/day	Ave. DIM [a]	BF% [b]	PR %[c]	ICP [d]
11	GP (Irene)	Lucerne/Hay[e]	32.3	92.3	4.0	3.3	410
12	GP (Pretoria)	Lucerne/Hay[e]	34.6	94.7	4.0	3.1	451
13	GP (Rayton)	Maize silage/ Brewers Grain	22.5	111.1	3.9	3.3	443
14	GP (Rayton)	Maize silage/ Lucerne	27.0	186.5	3.6	3.1	423
15	GP (Pretoria)	Lucerne/Hay[e]	30.3	161.1	3.7	3.1	405
16	FS (Frankfort)	Oat hay/Tef Hay	18.0	134.0	3.8	3.1	440
17	FS (Bethlehem)	TMR	33.0	320.5	3.7	3.4	430
18	FS (Senekal)	Lucerne	19.1	96.8	3.9	3.2	325
19	FS (Marquard)	Lucerne/Hay[e]	21.0	153.6	3.7	3.2	400
20	FS (Bultfontein)	Lucerne/Peanut hay	25.2	233.3	3.6	3.3	390

[a] Average days in milk, [b] Butter fat percentage, [c] Protein percentage, [d] inter-calving period, [e] *Eragrostis curvula* hay.

Table 8 Descriptions of the farms selected to represent feeding system 3, maize silage-based system in the Gauteng (GP), North West (NW) and Mpumalanga (MP) areas.

Farm #	Province and Area	Main Roughages	Ave. litres/ cow/day	Ave. DIM [a]	BF% [b]	PR %[c]	ICP [d]
21	GP (Bapsfontein)	Maize Silage/Hay[e]	33	98.7	3.85	3.09	425
22	NW (Bethal)	Maize Silage/ Lucerne	34	268.7	3.36	3.26	463
23	GP (Rayton)	Maize Silage/Lucerne	33.3	92.3	3.6	3.1	373
24	GP (Delmas)	Lucerne/Maize	34.9	90.3	3.6	3.2	447
25	GP (Devon)	Maize Silage/Hay[e]	20.0	99.7	3.8	3.2	500
26	GP (Devon)	Maize silage/Brewers Grain/Hay[e]	31.6	183.2	3.3	3.2	418
27	MP (Greylingstad)	Maize silage/ Hay[e] / bread by-prod/Lucerne	31.0	264.7	3.5	3.2	413
28	MP (Standerton)	Maize silage/Hay[e]	35.0	194.1	3.0	3.2	402
29	MP (Morgenzon)	Maize silage/brewers grain/Hay[e]	34.0	148.3	3.5	3.2	441
30	NW (Delareyville)	Maize Silage/ Lucerne	29.8	148.2	3.6	3.3	461

[a] Average days in milk, [b] Butter fat percentage, [c] Protein percentage, [d] inter-calving period, [e] *Eragrostis curvula* hay.

3.2.2 Sample collection and Analysis

Whole blood samples were taken from the coccygeal vein (tail vein) and analysed for β-Carotene content using a hand-held portable spectrophotometer developed by Schweigert et al. (2007), through the iCheck™ procedure (BioAnalyt, GmbH, Germany). This procedure was performed on farm for real-time β-Carotene assessment. Blood analyses were done after the first morning milking. Sampling for pasture farms in KZN and Eastern Cape where done during October 2008, which is the summer or high rainfall season for both these areas. As commercial dairy farms are on irrigated Rye Grass pasture year round it is safe to assume that any fluctuations in β-Carotene plasma concentrations would not be significant. However some farmers supplement with silage during the dry months and this could greatly reduce the β-Carotene content of the feed. Both the hay-based and maize silage-based systems were sampled partly in the winter and partly in the

summer of 2009. Samples analysed in the winter were during the months of June and July and those in the summer months were during September, October and November.

An interpretation of plasma β-Carotene levels in terms of status and supplementation recommendation is shown in Table 9. Plasma concentrations >3.5 mg/L are optimal and no supplementation is needed while plasma concentrations <1.5 mg/L are deficient and a daily supplementation of 500 mg/d is recommended.

Table 9 Beta Carotene levels, status and recommended supplementation (Schweigert and Immig, 2007).

B-Carotene Level (mg/L)	Status	Recommended B-Carotene supplementation (mg)
Less than 1.5	Deficient	500
Between 1.5 and 3.5	Marginal	300
Higher than 3.5	Optimal	None

The following figures illustrate the equipment and method used to analyse plasma for β-Carotene concentrations. The ROVIMIX® β-Carotene iCheck™ portable hand held spectrophotometer kit was used to analyse plasma for β-Carotene concentrations (Figure 9) on farm. The kit consists of a carry case, hand held spectrophotometer and iCheck™ ampoules.

Figure 9 ROVIMIX® β-Carotene iCheck™ portable hand held spectrophotometer kit

Vacutainers were used to collect blood from cattle, and 1ml syringes, to accurately measure and transfer blood from vacutainers to ampules (Figure 10), for analyses by the hand held spectrophotometer.

Figure 10 Set up of equipment required for blood sampling

Approximately 10 ml of blood was gently drawn from each cow via the coccygeal or tail vein (Figure 11) using the vacutainer system, with each tube containing 9 ml NH (sodium Heparin).

Figure 11 Blood sampling from the tail veins of Holstein cattle

Blood-containing vacutainers were numbered according to the cattle placement in the crush and corresponded to a list of the cow's actual ear tag number. Vacutainers were kept out of direct sunlight (Figure 12) and blood was immediately transferred to ampules for analysis.

Figure 12 Sampled blood in numbered vacutainer blood collection tubes waiting for analyses

From each vacutainer, 0.4 ml of blood was extracted using a 1ml syringe and injected into an iEx extraction ampule (Figure 13). Each ampule was then shaken intensively for 10 seconds and then left to stand for 5minutes. Separation of the β-Carotene pigment, from the blood, into the organic fluid within the ampules is easily seen in Figure 13.

Figure 13 ICheck™ ampoules with sampled blood, absorbing β-Carotene into solution for analyses by spectrophotometry

After waiting the required 5 minutes for the separation step to take place, the extraction vial was then place in the iCheck™ hand-held photometer and tested for ß-Carotene (Figure 14).The photometer is kept out of direct sunlight and on a flat, level surface.

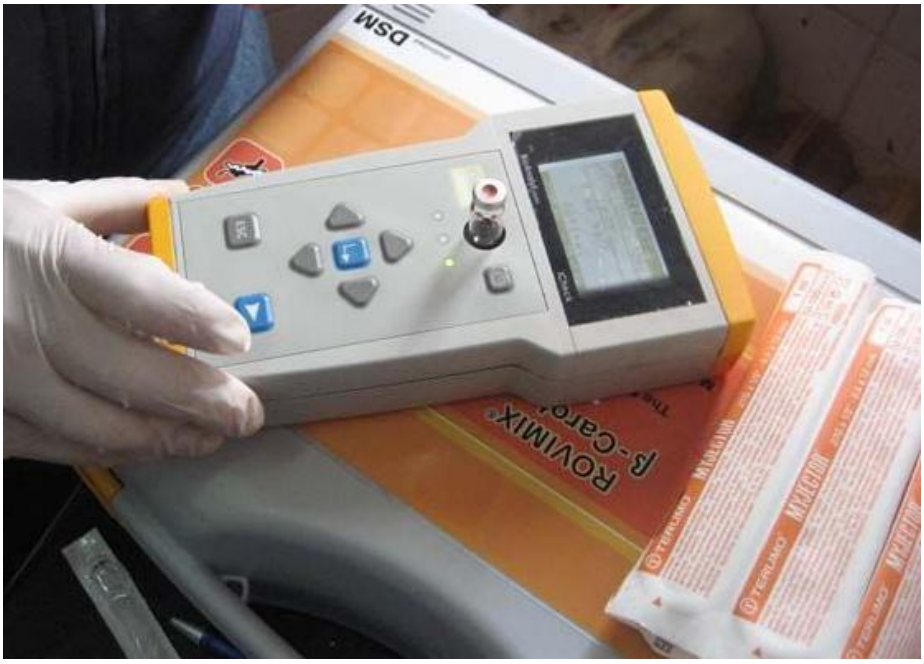

Figure 14 Ampoules with blood inserted into the iCheck™ for analysis

The analyses value is shown on the screen of the iCheck™ (Figure 15), for real-time on farm β-Carotene assessment.

Figure 15 ICheck™ screen showing analysis of sample

On the day of blood sampling, representative samples of the roughage (pasture/silage/hay) were taken, samples were placed on ice and then frozen until analysis. Milk samples were retrieved from the bulk tank at the time of bleeding and frozen until they could be analysed for β-Carotene. Diet composition for each farm was recorded.
Feed samples were analysed for Dry Matter (DM), Ash, Crude Protein (CP), acid detergent fibre (ADF), neutral detergent fibre (NDF), acid detergent lignin (ADL), gross energy (GE), ether extract (EE), calcium (Ca), phosphorus (P), Starch and In Vitro Organic Matter Digestibility (IVOMD) at the analytical laboratory of the Department of Animal and Wildlife Science at the University of Pretoria in South Africa. Beta-Carotene was analysed at the ARC (Agricultural Research Council) in Irene, using the method of Schierle et al. (1995).

All feed samples were ground through a 1mm sieve on a Retsch ZM 200 grinder, (Retsch-Allee1-5 42781, Haan, Germany). Dry matter was determined as the loss on drying at 95-100°c (method 934.01, AOAC, 2002). Ash was reported as a percentile by making use of a temperature controlled furnace at 600°C for 2 hours (Method 942.05, AOAC, 2000). Crude protein was determined by Leco analysis (Method 968.06, AOAC, 2000). The ADF and NDF analyses were performed using the Ankom 2000 Automated Fibre Analyser, (Ankom Technology, NY, USA) as per the methods supplied by the manufacturer. Acid detergent lignin was determined by first applying the ADF method to the sample and then saturation in 72% Sulphuric acid (H_2SO_4) for 3 hours stirring every 30 minutes, removing all acid by suction and washing 3 times with hot water as per method of Goering and Van Soest (1970).

Determination of GE was performed by bomb calorimetery (MC-1000 Modular calorimeter, Energy Instrumentation, 135 Knoppieslaagte, Centurion, South Africa). The ME was thus determined using the equation ME (MJ/kg DM) = 0.82 x (GE x IVOMD %) (Robinson et al., 2004). The fat content of the diet was determined by the EE method using the Soxtec System HT 1043 extraction Unit (Foss Analytical, Hillerød, Denmark, Method 920.39, AOAC, 2000). Calcium content of feed was determined by the method of Giron (1973) and sample preparation by method 935.13, AOAC, 2000. The P content was determined by ashing the sample, adding HCL and HNO_3 and boiling, using molybdovanadate reagent and a spectrophotometer at 400 nm (Method 965.17, AOAC, 2000) and sample preparation by method 968.08, AOAC, 2000. Starch content of the feed was determined by enzymatic determination of glucose by the glucose oxidase method (Method 8.202, AOAC, 1984). In vitro organic matter digestibility was determined in the feed using incubation in a water bath at 39°c, artificial saliva and a urea solution (Tilley & Terry, 1963 as modified by Engels & van der Merwe, 1967). Beta-Carotene in feed was determined by direct saponification in ethanolic solution and pigment extraction into hexane by means of EXTRELUT columns and then analysed by High Performance Liquid Chromatography (HPLC) (Schierle et al., 1995).

Milk Samples were analysed for β-Carotene at the laboratory of the ARC in Irene Gauteng by saponification to extract fat-soluble vitamins, organic sediment was evaporated in nitrogen, and the residue was read by HPLC by the method of Salo-väänänen et al. (2000).

3.2.3 Statistical analysis

Data were analysed as a randomised complete block design with general linear model (GLM) (Statistical Analysis System, 2011) for an analysis of variance to determine differences between treatments and periods. Means and standard error of the means (SEM) were calculated. The significance of differences between means was determined by the Fischers protective test (Samuels, 1989), significance was determined at $P<0.05$ and tendencies at $P<0.10$.

The linear model used is described by the following equation:

$$Y_{ij} = \mu + T_i + B_j + e_{ij}$$

Where Y_{ij} = variable studied during the period

μ = overall mean of the population

T_i = effect of the i^{the} treatment

B_j = effect of the j^{the} block

E_{ij} = error associated with each Y

3.3 Results and Discussion

3.3.1 Nutrient composition of the roughages

The nutrient composition of the roughages sampled from the three different feeding systems is shown in Tables 10, 11 and 12. The roughages collected from the pasture-based systems are ryegrass and ryegrass oversown into kikuyu and clover. The nutrient composition of these roughages is presented in Table 10. The pasture made up from 62 to 83% of the total diet and on all the farms an energy mineral supplement was fed to rectify some of the nutrient imbalances in pasture, i.e. too high in protein and low digestibility of structural carbohydrates (Marais, 2001). The CP and NDF varied between 20.4 and 27.8% for CP and between 42.3 and 53.6% for NDF, which agrees with results reported by Meeske et al. (2006) and Dugmore (1995). Meeske et al (2006), reported the following averages for both perennial and annual species over all seasons: Ash (%)10.46 CP(%)22.84, ME (MJ/kg) 10.28, NDF (%)47.4, ADF (%) 27.97, Dugmore (1995) reported a CP(%) of 20 and an ME (MJ/kg) of 10.

The roughage sampled from the TMR systems based predominantly on hay (system 2) is shown in Table 11. The quality of the *Eragrostis curvula* hay was extremely poor with an average CP of only 4.7%. The analyses for *Eragrostis curvula* hay are similar to those of Meissner et al. (1991) who reported the following average values for *Eragrostis curvula*; %IVOMD: 43.6, %NDF: 74.4 and %CP: 7. The CP content of the lucerne hay varied between 12.6 and 18.2 indicating that the quality of the lucerne hay varied between low and good quality (Peace, 2012). According to the USDA quality guidelines for alfalfa hay (2013), good quality lucerne contains 18-20% CP and a relative feed value (RFV) value of 150-170. These results are in agreement with Hassen et al. (2006) and Muller et al. (2008). Hassen et al. (2006) reported the

following average values for lucerne hay; %CP 20.4, %NDF 43.8, %Ash 8.8 and 67.7 %IVOMD and Muller et al. (2008) reported the following average values for field dried lucerne hay; %CP 17.3, %ADF 41.2, %NDF 48, %Ash 9.4.

The nutrient content of the roughages used in system 3 are shown in Table 12. Maize silage was the predominant roughage in this group of TMRs, with an average inclusion level of 38%. The average starch and NDF content agrees with results published by Meeske et al. (2000) who analysed 21 different hybrids of maize silage and reported the following average results: %DM: 33.9 ± 2.2, %CP: 6.9 ± 0.4, %IVOMD 61.1 ± 2.3, ME (MJ/kg DM): 9.41 ± 0.43, %NDF: 46.7 ± 1.9, %ADF: 25.2 ± 1.1.

Table 10 Results of feed analyses for system 1, pasture system (DM basis).

Farm no.	Roughage	% ITR[d]	% Ash	% CP	% ADF	% NDF	% ADL	ME (MJ/kg DM)	% Fat	% Ca	% P	% Starch	% IVOMD
1	Luc[a]/Rye[b]	75	10.2	20.5	27.7	45.3	4.5	9.6	2.1	0.8	0.3	0.6	72.0
2	Rye[b]	66	13.1	25.0	26.9	42.3	2.9	9.8	3.0	0.6	0.4	0.7	75.3
3	Rye[b]	64	10.6	20.4	25.6	49.8	3.1	10.1	2.6	0.5	0.3	0.4	78.3
4	Rye[b] /Kik[c]/ Clover	82.5	13.6	21.0	27.1	48.1	2.2	10.0	3.2	0.3	0.5	0.7	77.6
5	Rye[b]	71	11.5	24.5	27.8	53.6	5.5	9.9	2.5	0.4	0.3	0.6	75.3
6	Rye[b]	63	11.1	23.9	26.5	46.5	2.9	10.7	3.4	0.5	0.4	0.7	81.4
7	Rye[b]	63	12.5	20.5	32.1	55.4	7.6	10.4	3.7	0.3	0.3	0.5	78.4
8	Rye[b]	79	10.6	22.5	27.8	49.2	4.3	9.6	2.7	0.4	0.2	0.6	72.9
9	Rye[b]	71	12.0	27.8	25.8	47.6	3.0	10.2	3.8	0.4	0.4	0.2	75.3
10	Rye[b] /Kik[c]/ Clover	71	12.1	26.4	28.5	50.7	3.7	10.5	1.0	0.4	0.4	0.3	76.9

[a] Lucerne hay, [b] Rye grass, [c] Kikuyu grass, [d] Inclusion of Total Ration, this indicates the percentage that this roughage was included in the total diet.

Table 11 Results for feed analyses for system 2, TMR predominantly hay system (DM basis).

Farm no.	Roughage	% ITR[c]	% Ash	% CP	% ADF	% NDF	% ADL	ME (MJ/ kg DM)	% Fat	% Ca	% P	% Starch	% IVOMD
11	Hay[a]	12.0	4.0	5.7	43.3	78.5	6.0	5.3	1.1	0.2	0.0	0.5	39.6
11	Lucerne	17.0	7.9	17.6	28.7	34.6	7.0	9.6	1.7	1.1	0.2	0.7	73.5
12	Hay[a]	12.6	8.5	3.0	49.0	71.9	5.5	7.1	1.6	0.4	0.1	0.4	56.8
12	Lucerne	30.7	6.8	13.6	38.0	47.2	8.8	8.2	1.6	0.8	0.3	0.7	60.6
13	Maize[b]	18.0	5.1	7.1	24.7	38.5	3.6	10.4	2.9	0.1	0.1	24.1	78.7
13	Hay[a]	9.0	2.8	5.1	43.0	94.2	5.8	5.8	1.5	0.2	0.1	0.8	41.8
14	Maize[b]	28.0	4.1	7.4	22.5	37.7	2.7	10.7	2.7	0.2	0.2	26.6	79.7
14	Lucerne	13.6	7.0	12.6	42.6	53.1	13.1	6.7	1.2	0.7	0.1	0.8	52.1
15	Hay[a]	10.4	3.7	6.2	42.6	75.2	9.0	4.9	1.5	0.2	0.1	0.7	36.3
15	Lucerne	29.7	9.8	18.2	34.6	40.8	6.8	8.7	1.5	0.9	0.2	0.5	69.5
16	Oat Hay	16.7	9.3	9.2	38.1	60.6	5.2	8.0	2.3	0.2	0.1	3.2	64.1
16	Tef	16.7	7.0	3.6	32.2	59.4	4.6	8.7	1.4	0.2	0.2	12.8	69.6
18	Lucerne	26.7	6.8	18.1	44.8	57.4	10.0	7.4	2.0	0.7	0.1	0.8	56.7
19	Hay[a]	13.7	4.5	3.2	45.4	73.0	7.2	5.0	1.5	0.2	0.1	0.7	36.0
19	Lucerne	18.2	8.5	17.8	32.7	41.8	7.9	9.2	1.0	0.9	0.2	0.7	69.8
20	Lucerne	35.9	9.6	16.8	31.0	38.6	6.6	8.9	1.8	1.0	0.2	0.7	69.7

[a] *Eragrostis curvula* hay, [b] Maize silage, [c] Inclusion of Total Ration, this indicates the percentage that this roughage was included in the total diet.

Table 12 Results for feed analyses for system 3, TMR predominantly maize silage system (DM basis).

Farm no.	Roughage	% ITR[c]	% Ash	% CP	% ADF	% NDF	% ADL	ME (MJ/ kg DM)	% Fat	% Ca	% P	% Starch	% IVOMD
21	Maize[b]	30.2	3.6	6.3	24.1	40.0	2.9	10.3	2.4	0.1	0.1	33.5	76.3
21	Hay[a]	6.3	5.3	4.6	41.4	69.9	5.0	7.0	1.4	0.2	0.2	0.4	53.4
22	Maize[b]	50.8	4.1	7.0	23.2	39.4	3.7	10.7	2.2	0.2	0.2	24.6	77.5
22	Lucerne	12.1	6.6	12.0	45.5	55.6	10.5	6.9	1.2	0.6	0.2	0.4	52.0
23	Maize[b]	41.6	3.0	7.0	14.8	28.2	3.7	10.9	2.2	0.1	0.2	38.2	80.5
23	Lucerne	11.2	6.5	12.3	42.9	50.6	10.1	7.3	3.4	0.7	0.2	0.7	55.1
24	Lucerne	7.2	9.3	15.9	31.3	39.9	8.0	8.8	1.5	1.0	0.2	0.5	69.8
24	Maize[b]	49.3	3.4	7.3	25.4	37.3	3.0	10.2	2.9	0.1	0.2	32.34	75.1
24	Hay[a]	4.9	3.6	4.1	39.0	74.9	5.6	5.1	1.1	0.3	0.1	0.8	36.8
25	Hay[a]	5.0	6.9	2.0	52.6	74.8	7.7	5.6	1.2	0.2	0.1	0.7	43.3
25	Maize[b]	39.8	6.1	8.2	20.1	36.7	3.7	10.5	2.5	0.2	0.2	31.1	79.5
26	Hay[a]	7.5	3.9	6.7	42.4	76.7	7.8	6.0	1.3	0.2	0.1	0.7	44.7
26	Maize[b]	37.0	3.6	7.9	21.8	38.4	3.6	10.9	2.5	0.2	0.2	36.6	80.3
27	Hay[a]	7.6	4.1	15.8	36.9	69.4	7.0	9.1	2.3	0.3	0.1	1.0	66.1
27	Maize[b]	27.4	4.7	5.5	21.4	35.1	2.7	10.5	2.4	0.1	0.1	37.1	80.0
27	Lucerne	3.7	9.9	16.3	34.4	43.1	7.3	8.9	1.4	0.9	0.2	1.1	69.1
28	Maize[b]	39.5	4.5	6.7	29.6	50.5	3.9	10.3	2.1	0.1	0.1	20.7	76.1
28	Hay[a]	11.6	4.1	5.6	41.9	76.6	5.5	4.9	1.4	0.2	0.1	0.9	36.3
29	Lucerne	1.8	8.0	14.0	41.0	47.3	11.3	7.6	1.4	1.0	0.2	0.8	57.5
29	Hay[a]	4.6	3.3	6.3	41.4	79.5	6.6	5.7	1.6	0.2	0.1	0.8	40.4
29	Maize[b]	42.3	4.9	6.3	20.9	38.8	5.2	10.0	2.4	0.1	0.1	39.3	76.0
30	Lucerne	9.2	7.4	12.9	43.4	48.1	8.6	7.8	1.2	0.8	0.1	1.3	58.0
30	Maize[b]	24.6	5.3	7.3	24.4	37.1	3.7	10.7	2.7	0.2	0.2	37.2	81.0

[a] *Eragrostis curvula* hay, [b] Maize silage, [c] Inclusion of Total Ration, this indicates the percentage that this roughage was included in the total diet.

3.3.2 Beta-Carotene content of roughages

The β-Carotene content of the roughages sampled is shown in Table 13. Due to financial constraints it was not possible to analyse every roughage source for each farm for β-Carotene. The standard deviation in system 2 is high for β-Carotene analysis primarily due to the wide variety of roughages. Taking into account the lucerne analysis only, gives an average of 29.9 mg/kg and a standard deviation of 11.36. Overall the β-Carotene analyses for roughages show higher values from system 1 and 2 than they do from system 3. Values for these roughages as compared to those outlined in Table 4 (Typical β-Carotene concentration of feeds) indicates that; rye grass is lower than the prediction for "fresh green legumes and grasses, immature" which is between 33 and 88 mg/kg, lucerne hay meets values for "legume hays, including alfalfa, partly bleached, varying amounts of green colour from moderate to good" which is from 20 to 59 mg/kg. This large variation is also illustrated in Table 14. The lucerne hay β-Carotene levels in our study were on average higher than the pasture species, most probably because samples were taken close to the flowering stage when legumes have higher values than grasses (Williams et al., 1998). During early growth, however the values are similar. *Eragrostis curvula* hay meets specifications as outlined by Table 4 for "Non-legume hays, average quality, bleached, some green colour" at levels of between 9 and 18 mg/kg whereas β-Carotene levels for maize silage are lower than those for "corn and sorghum silages, medium to good green colour" with levels of between 14 and 22 mg/kg. The β-Carotene levels of the maize silage were the lowest of all the roughages. It is important to keep in mind that sampling technique both on farm and at the lab may have an effect on final analysis.

There is a large variation in the β-Carotene content of roughages within each of the three systems. This can be explained by the number of factors affecting β-Carotene content of roughages. The ratio of leaf to stem is one of the main factors related to levels of β-Carotene in roughages, as leaves contain the most β-Carotene anything that aids in increasing stem to leaf ratio will result in a reduction in β-Carotene in that roughage. As plants increase in maturity their stem to leaf ratio increases resulting in an increase in DM, as there an inverse relationship between DM and β-Carotene content, it is clear that β-Carotene levels in plants will reduce with an increase in maturity (Williams et al., 1998). The length of exposure to sunlight and air, as well as the temperature, also has an effect on reducing the amount of β-Carotene in plants. An increase in

temperature can increase the rate of β-Carotene loss substantially. In general, animal feed ingredients show a large variation in β-Carotene content (Bauernfeind, 1972).

Table 13 Concentrations of β-Carotene content of the main forages used in the 3 systems

Farm no.	System[a]	Sample Type	Feed β-Carotene (mg/kg)
1	1	Rye/Lucerne pasture	22.8
2	1	Rye pasture	17
6	1	Rye pasture	31.5
8	1	Rye pasture	14.7
9	1	Rye pasture	14.3
10	1	Rye/Kikuyu/Clover pasture	25
11	2	Lucerne hay	37.9
13	2	Eragrostis hay	12.8
14	2	Lucerne hay	14.2
15	2	Lucerne hay	28.9
16	2	Oat hay	1.72
19	2	Lucerne hay	38.6
21	3	Maize silage	1.26
23	3	Maize silage	0.33
25	3	Maize silage	5.21
26	3	Maize silage	2.88
27	3	Maize silage	6.25
28	3	Maize silage	6.31

[a] 1=Pasture-based, 2=Hay-based, 3= Maize silage-based.

3.3.3 Plasma β-Carotene of cows on different feeding systems

The plasma β-Carotene concentrations of cows on the pasture-based feeding system are shown in Table 14 and Figure 16. The mean plasma β-Carotene concentration for cows on the pasture-based systems was 5.53 mg/L. Nearly half (92 cows) of the tested cows fell within the ranges of 3-6 mg/L and only 31 of the 200 cows tested below 3.0 mg/L. There is a significant difference in the DMI (and therefore β-Carotene intake) of high and low producing animals and this could have contributed to the variation in plasma β-Carotene concentrations. This is especially true for pasture-based systems where there is more variation in diet quality and DMI compared to TMR systems (Kolver and Muller, 1998).

Table 14 The mean plasma β-Carotene concentration of cows fed on a pasture-based system (system 1).

Farm no.	Mean BC (mg/L)[a]	SD[b]	Max BC[c]	Min BC[d]	β-Carotene Status[e]	Predominant Roughage
1	4.47	2.075	8.32	1.53	O	Rye/Lucerne
2	5.578	1.809	8.76	1.08	O	Rye grass
3	4.031	1.658	8.47	1.55	O	Rye grass
4	3.841	2.249	8.02	1.13	O	Rye/Kik/Clover
5	4.189	2.283	11.27	1.31	O	Rye grass
6	5.62	2.052	11.07	3.19	O	Rye grass
7	4.21	0.941	5.73	2.12	O	Rye grass
8	10.181	3.67	20.4	2.94	O	Rye grass
9	6.717	5.042	16.68	1.51	O	Rye grass
10	6.52	2.069	12.86	2.85	O	Rye, Kik, Clover
Mean:	5.535	2.385	11.158	1.921	O	

[a] Mean plasma β-Carotene concentration for herd, [b] standard deviation of plasma β-Carotene within herd, [c] maximum β-Carotene value within herd, [d] minimum β-Carotene value within herd, [e] status of herd; D= deficient: < 1.5, M= marginal: >1.5, <3.5, O= optimal: >3.5.

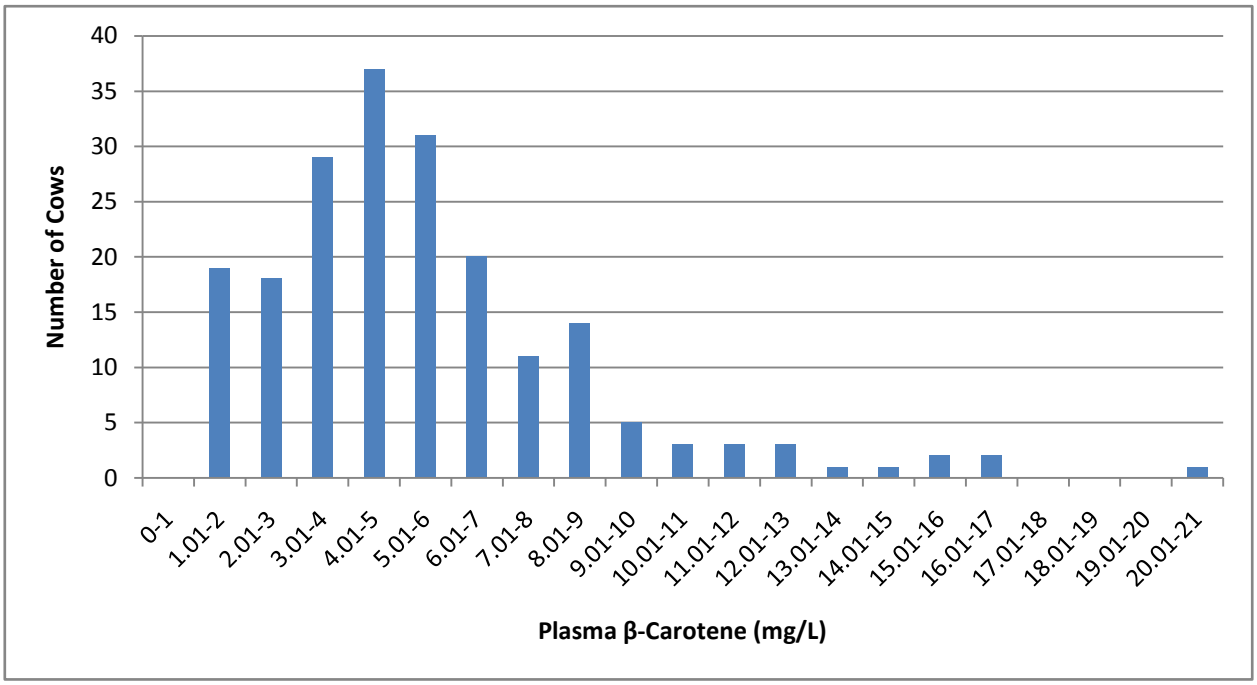

Figure 16 Number of cows per plasma β-Carotene range for cows on feeding system 1.

The overall status for the pasture systems was optimal as expected, clearly due to the diet components these animals receive such as rye grass pasture, kikuyu pasture, lucerne pasture and clover pasture, therefore β-Carotene availability is high. Farms 6 to 10 were in the KZN Midlands

area and overall show a higher β-Carotene value than farms 1 to 5 that were in the Eastern Cape area near George. The range from the average minimum value (1.921) to the average maximum value (11.158) is quite broad and illustrates the variety in β-Carotene content of the roughage fed. These values are in line with those reported by Jukola et al. (1996a), who found plasma β-Carotene values of 13.7 mg/L for cows on grass silage and 15.4 mg/L for cows on pasture and in another paper by the same authors levels of 12.9 mg/L for grass silage (Jukola et al., 1996b). Cetinayak and Ö zcan (1991) reported plasma β-Carotene concentrations on pasture for 2 groups over 2 months, namely May and September, to be; 5.75 ± 0.5 mg/L and 6.0 ± 0.46 mg/L, 5.85 ± 0.43 mg/L and 5.76 ± 0.43 mg/L respectively. In an article by Moore (1939) plasma β-Carotene of Holstein cattle was 4.83 mg/L, 7.98 mg/L and 14.46 mg/L at 2, 9 and 16 days on pasture respectively.

As the level overall for cattle on pasture systems is said to be optimal, the recommendation with regards to β-Carotene supplementation, is that cows on the pasture-based systems evaluated in this study, do not require additional β-Carotene supplementation.

The mean plasma β-Carotene concentrations for cows fed the hay-based TMR (system 2) are shown in Table 15 and Figure 17. In Table 15 the mean plasma β-Carotene concentrations were higher for cows receiving lucerne hay (except on farm 12), than those of cows receiving *Eragrostis curvula*, oat hay or *Eragrostis tef*. The lucerne hay samples were much greener with a lot of leaves compared to the grass hay and furthermore the grass hay was stored for a long period of time. According to a review by McDowell (2000) the loss of β-Carotene is approximately 7% per month, the lower plasma β-Carotene of cows receiving grass hay compared to lucerne hay, therefore was expected. By far, most of the cows (80) had β-Carotene concentrations within the range of 1.00-2 mg/L, categorising them as marginal with regard to β-Carotene status (Schweigert and Immig, 2007).

Table 15 The mean plasma β-Carotene concentration of cows fed on a hay-based TMR system (system 2).

Farm no.	Mean BC (mg/L)[a]	SD[b]	Max BC[c]	Min BC[d]	β–Carotene Status[e]	Main Roughage
11	2.086	1.035	5.25	0.49	M	Lucerne
12	0.908	0.975	3.72	-0.14	D	Lucerne
13	1.321	0.492	2.8	0.71	D	Eragrostis
14	2.153	0.63	3.66	1.23	M	Lucerne
15	3.699	0.919	5.54	1.65	O	Lucerne
16	1.77	1.037	4.65	0.75	D	oat hay/tef
17	4.41	1.217	7.06	2.4	O	TMR
18	4.682	1.528	8.02	2.66	O	Lucerne
19	3.757	1.576	7.23	1.78	O	Lucerne
20	5.006	1.201	7.3	2.96	O	Lucerne
Ave.:	2.979	1.061	5.523	1.449	M	

[a] Mean plasma β-Carotene concentration for herd, [b] standard deviation of plasma β-Carotene within herd, [c] maximum β-Carotene value within herd, [d] minimum β-Carotene value within herd, [e] status of herd; D= deficient: < 1.5, M= marginal: >1.5, <3.5, O= optimal: >3.5.ficient: < 1.5, marginal: >1.5, <3.5, optimal: >3.5.

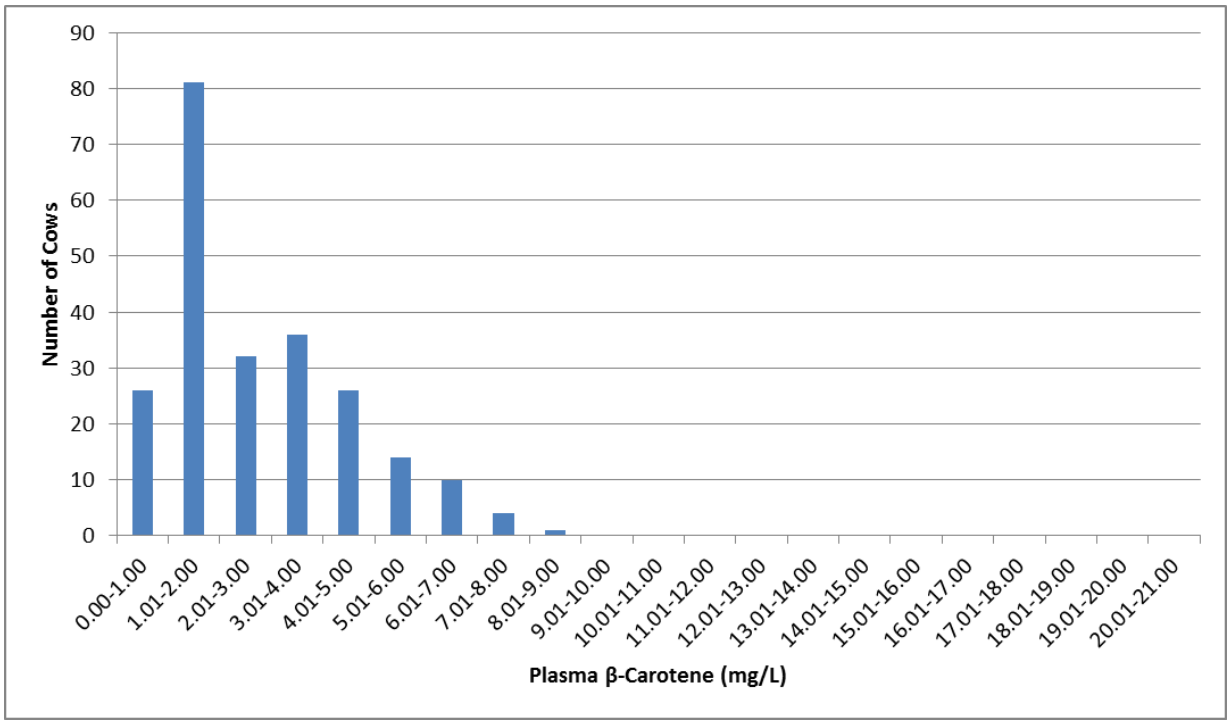

Figure 17 Number of cows per plasma β-Carotene range for cows on feeding system 2.

From the data represented in Table 16 and Figure 17, the overall status of the hay system is marginal. This suggests that these animals would benefit from supplementation of β-Carotene, which can be predicted by looking at the available diet animals in this system receive, such as lucerne, oat hay and *Eragrostis curvula*. Roughages in this system are fed as a baled source, which requires them to be cut fresh in the field, left to cure and then baled and stored. Throughout this process the amount of β-Carotene available to the animal would be reduced as opposed to roughages that were available fresh or 'green' in the field, as β-Carotene is broken down by drying, storage, heat and the baling process. The range from the average minimum β-Carotene value (1.449) to the average maximum β-Carotene value (5.523) is not as broad a range as that of system one with the average (2.979) being much lower than that of system 1. Michal et al. (1994) reported plasma β-Carotene concentrations of 4.3 μmol/L in a mixture of 80:20% ratio, hay to grass silage. Calderón et al. (2007a) reported concentrations of plasma β-Carotene for cows on a hay-based diet to be 2.58 mg/L, and on a mixed ration of 67:33% ratio hay to grass silage and lucerne protein concentrate a level of 4.28 mg/L. Nozière et al. (2006a) reported plasma β-Carotene concentrations of 2.06 mg/L for cows fed on a hay-based diet. Jukola et al. (1996a) found average plasma values for cattle on a hay-based ration to be 2.5 ± 1.07 mg/L, this is in agreement with our results.

The mean plasma β-Carotene levels for cows fed the maize silage-based TMR (system 3) are shown in Table 16 and Figure 18. The overall status of this system is shown to be either deficient or marginal, when referring to the mean concentration of 1.710, it falls within marginal status. However the blood β-Carotene values in Table 3 show 3 farms that are possible outliers (farms 22, 24, 30). These 3 farms increase the overall status of this system and had the highest percentage lucerne hay inclusion in their rations, with percentage inclusion levels of 10.2, 7.2 and 9.4%, respectively, compared to inclusion values such as 3.7% for farm 27 and 1.8% for farm 29.
The expected overall status of this system is deficient as animals in this system receive a diet low in green fresh forages, ultimately their diets are made of mostly maize silage and cut forages, resulting in low β-Carotene availability from the diet.

Table 16 The mean plasma β-Carotene concentration of cows fed on a maize silage-based TMR based (system 3).

Farm no.	Mean BC (mg/L)[a]	SD[b]	Max BC[c]	Min BC[d]	β–Carotene Status[e]	Main Roughage
21	0.782	0.371	1.46	0.11	D	Silage
22	2.833	1.138	5.6	0.66	M	Silage
23	1.2	0.459	2.12	0.45	D	Silage
24	3.384	1.217	6.86	1.67	M	Silage
25	1.133	0.665	2.72	0.14	D	Silage
26	1.256	0.493	1.94	0.3	M	Silage
27	1.305	0.497	2.06	0.07	D	Silage
28	1.029	0.354	1.59	0.32	D	Silage
29	1.717	0.479	2.57	0.82	M	Silage
30	2.46	0.883	4.14	1.05	M	Silage
Ave.:	1.71	0.655	3.106	0.559	D/M	

[a] Mean plasma β-Carotene concentration for herd, [b] standard deviation of plasma β-Carotene within herd, [c] maximum β-Carotene value within herd, [d] minimum β-Carotene value within herd, [e] status of herd; D= deficient: < 1.5, M= marginal: >1.5, <3.5, O= optimal: >3.5.

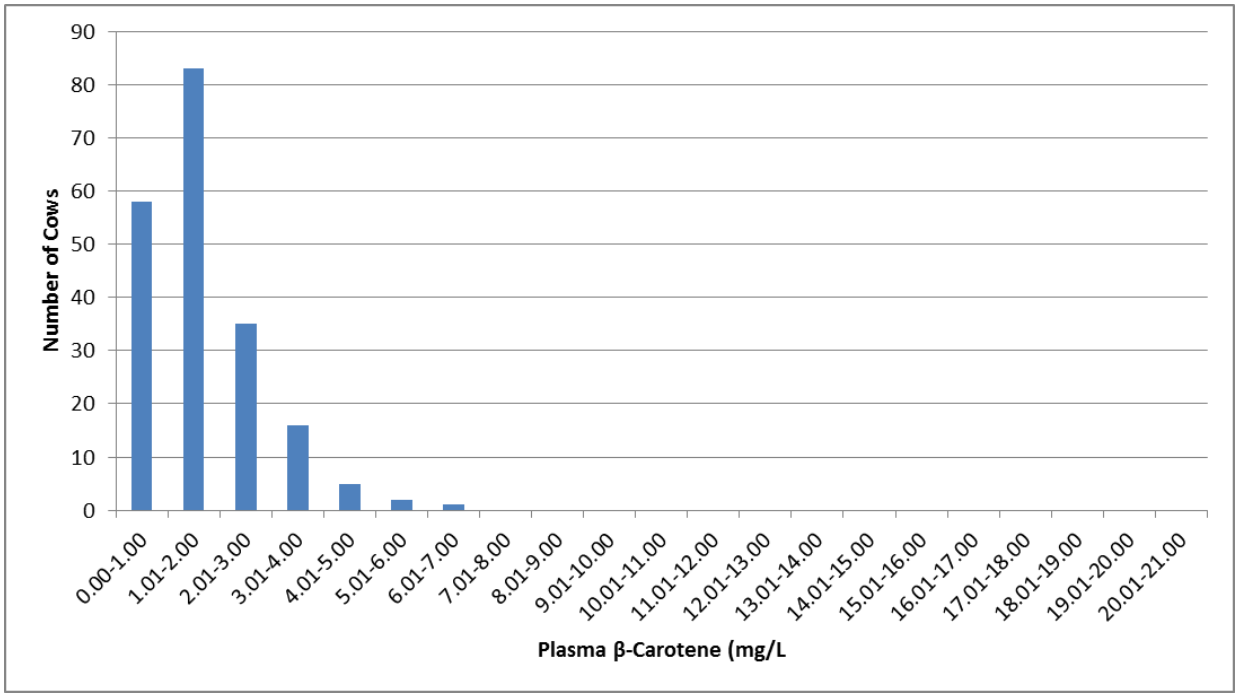

Figure 18 Number of cows per plasma β-Carotene range for cows on feeding system 3.

Kaewlamun et al. (2011a) and Ducker et al. (1984), reported plasma β-Carotene concentrations for cattle on a predominantly maize silage-based diet to be approximately 3 mg/L and 3.31mg/L respectively, both of which are higher than our findings.

A comparison of the mean plasma β-Carotene concentrations of cows on the three different feeding systems is shown in Table 17. The mean plasma β-Carotene of cows on the pasture-based system was 5.53 mg/L and differed ($P < 0.05$) from cows fed the hay and silage-based TMR's. Mean plasma β-Carotene concentration of cows receiving the lucerne hay-based TMR (2.97 mg/L) tended ($P < 0.10$) to differ from cows receiving the maize silage-based TMR (1.710 mg/L).

Table 17 A summary of the average plasma β-Carotene concentrations for cows fed a pasture-based (system 1), hay-based TMR (system 2) or a maize silage-based TMR (system 3)

System	LS Mean BC (mg/L)	SEM	# of Samples
1	5.535[a]	0.474	200
2	2.979[b]	0.474	200
3	1.710[b]	0.474	200

[ab] Means with different superscripts differ ($P < 0.05$)
[b] Means with the same superscript tend to differ ($P < 0.10$).

Jukola et al. (1996a) found a significant difference between hay-based rations and pasture-based rations on the content of plasma β-Carotene concentrations, with values of 2.5 ± 1.07 mg/L and 15.4 ± 6.15 mg/L respectively. Nozière et al. (2006b) reported significant differences in plasma blood concentrations of β-Carotene between cows fed grass silage (3.71 mg/L) and hay-based diets (1.71 mg/L). These reported differences between various rations are similar to what we found in this study, even though not all reported values are similar. However Rakes et al. (1985) found no significant differences in plasma β-Carotene concentrations between cows on maize silage- and lucerne-based diets.

3.3.4 Milk analyses for some farms

The purpose of this study was to use plasma β-Carotene as an indication of the β-Carotene status of cows. Some farmers requested, purely from an interest point of view, that we analyse their bulk tank milk samples for β-Carotene, 6 samples were taken from each system. Unfortunately 3 of the samples from farming system 2 were spoiled. Although the data is limited, the statistical analysis followed the same pattern as the results from the plasma β-Carotene concentrations. Data in Table 18 shows that milk β-Carotene from cows on pasture, was higher than ($P < 0.05$) from cows fed either a hay-based or maize silage-based TMR. Milk β-Carotene concentration from cows fed the two TMR based systems tended to differ ($P < 0.10$) from each other, with mean milk β-Carotene content from cows fed the maize silage-based diets being the lowest.

Table 18 Milk β-Carotene content from bulk tank samples taken from farms using a pasture-based (system 1), hay-based (system 2) or silage-based TMR (system 3) system.

	system 1	**system 2**	**system 3**
	Milk βC (µg/100g)	Milk βC (µg/100g)	Milk βC (µg/100g)
	27.220	1.71	1.71
	16.290	3.34	1.71
	20.770	7.21	2.39
	14.030	-	2.14
	4.490	-	2.8
	9.220	-	2.73
Average	**15.44**[a]	**4.08**[b]	**2.25**[b]

[ab] Means with different superscripts differ ($P < 0.05$)
[b] Means with the same superscript tend to differ ($P < 0.10$).

Our results are in agreement with those of Mogensen et al. (2012) who reported an average value of 17 µg/100g for 5 organic farms feeding mixtures of grass-clover silage, maize whole-crop silage and cereal whole-crop silage, and Nozière et al. (2006b) who reported milk β-Carotene values of 6.8 µg/100g for cattle on a hay-based ration and 14.2 µg/100g on a grass silage-based ration. Calderon et al. (2007a) reported a milk β-Carotene value of 9.4 µg/100g for cattle on a hay-based ration.

Mogensen et al. (2012) found that the amount of β-Carotene in milk is positively correlated to the supply in roughage fed; and stated that in general an input of 100 mg of β-Carotene supply from roughage caused an increase in milk β-Carotene of about 0.6 mg.

Variation in milk β-Carotene content is high and is related to; seasonal variation in β-Carotene content of the diet, breed and possibly the stage of lactation as well as a dilution effect as a result of milk yield. An increase in milk β-Carotene concentration is related to a reduced milk yield due to a concentration effect, while the total amount remains the same (Nozière et al., 2006b). Therefore management techniques focusing on increase milk yields will negatively affect the concentration of milk β-Carotene. However Nozière et al. (2006b) found an increase in milk β-Carotene concentration associated with no change in the plasma β-Carotene concentration, suggesting that the increase may be due to a mobilization of β-Carotene from adipose stores.

Fresh roughages are highest in vitamin concentrations, but the loss of vitamins from feed sources occurs at a number of stages within the processing of these roughages, thus only a minor proportion of the vitamins available in fresh roughages are transferred into milk (Mogensen et al., 2012).

Beta-Carotene content of milk has been in the spotlight due to its ability to reduce problems related to the oxidation of milk fat (Nicholson and St-Laurent, 1991). Beta-Carotene affects the colour and nutritional properties of dairy products (Nozière, 2006b).

3.4 Conclusion

Cows that were fed under system 1 (pasture-based) had the highest ($P < 0.05$) mean plasma β-Carotene concentration of the 3 systems, as was analysed on farm. According to Schweigert and Immig's (2007); Status and recommended supplementation Table (Table 9), the cattle on this system in SA have an optimal plasma β-Carotene status and therefore require no supplementation. With regard to System 2 (hay-based TMR system), mean plasma β-Carotene status was analysed to be marginal, thus suggesting that cows on this system require β-Carotene supplementation of approximately 300 mg/day. On some farms, where a large percentage of high quality, leafy green lucerne is fed, no supplementation would be necessary. Cows fed on hay-based TMRs therefore need to be monitored on a regular basis in order to make meaningful recommendations. Animals tested for plasma β-Carotene concentrations in system 3 (maize silage-based TMR system) had

the lowest concentrations and are therefore classified as being deficient in β-Carotene status, thus requiring supplementation of 500 mg/day. It can be concluded that the quality and type of roughage play a significant role in the overall β-Carotene status of Holstein cows fed three different feeding systems and monitoring is recommended when substituting different roughage sources.

CHAPTER 4: EFFECT OF PREPARTUM β-CAROTENE SUPPLEMENTATION ON THE POSTPARTUM β-CAROTENE STATUS OF HOLSTEIN COWS

4.1 Introduction

There is sufficient scientific evidence that circulatory β-Carotene plays a significant role in improving the fertility and health of dairy cattle and this may be specifically related to the local synthesis of vitamin A in target organs such as the ovaries, uterus and mammary gland (Schweigert, 2003). Current β-Carotene supplementation strategies are based on the research conducted by groups such as Lotthammer (1979) and Lotthammer et al. (1977). Current recommendations are as follows; supplementation at 300 mg/head/day from 3 weeks prepartum until confirmed pregnancy (Bian et al., 2007) or 300-500 mg/head/day from the beginning of the dry period until confirmation of pregnancy (Immig, 2009). Due to the cost of supplementation more research is necessary on strategic supplementation, i.e. is it more feasible to supplement for 60d during the dry period and then capitalise on a carryover effect during early lactation or perhaps supplement at a lower dose. There is very little information available on the potential of cows to store β-Carotene during the dry period and then carry it over into the early lactation period. The objective of this study was to investigate the carryover effect of β-Carotene from the dry period to the lactation period by comparing plasma β-Carotene concentrations pre- and postpartum. The experimental period was from 60 days prepartum until 56 days postpartum.

4.2 Materials and Methods

4.2.1 Animals and treatments

The trial was conducted at the University of Pretoria, Experimental Farm in Hatfield. Twenty Holstein cows were randomly selected and blocked into two groups of 10 cows according to weight and milk production during previous lactations, blocking is shown in Table 23, Appendix A. Cows were kept in single pens in order to monitor daily feed intake.

The control group was fed 8 kg (DM) of a lucerne/maize based TMR and the treatment group was fed the same diet but supplemented with 1200 mg/head/day of 10% ROVIMIX® β-Carotene from 60 days prepartum until calving. The β-Carotene supplement was mixed with a carrier and then hand mixed into the 8kg of TMR. The control group only received the carrier, a corn-starch

coated matrix of porcine gelatine and carbohydrates. The TMR was palatable because of the high quality lucerne hay and cows in general left no refusals. In addition to the TMR, cows had *ad lib* access to *Eragrostis curvula* hay and fresh water.

After calving both the experimental and control groups of animals received the same TMR *ad lib*, without any β-Carotene supplementation. The ingredient and chemical composition of the TMR is shown in Table 19.

Table 19 Ingredient and chemical composition of the TMR fed to the experimental and control groups (DM basis)

Ingredient (%DM)	% DM
"UP custom concentrate"	55.5
Yellow maize	38.3
Hominy chop	9.05
Defatted maize germ	20
Full fat soya	11.95
Soya oil cake	4
Fishmeal	4.55
Molasses meal	4
Soya oil	1.1
Limestone	1.46
Salt	0.41
Magnesium sulphate	0.21
Magnesium oxide	0.73
Urea	0.3
Vit/Min premix	0.3
Rumen buffer	0.7
Organic mineral pack	0.05
Mycotoxin binder	0.16
Rumen protected fat	2.6
Live yeast	0.01
Molasses meal	3.54
Lucerne	26.6
Eragrostis hay	14.4
Chemical composition (% DM basis)	
CP[1]	16.6
UDP[2] *(% CP)*	42.6
ME[3] *(MJ/kg DM)*	11.5
NDF[4]	32.1
Total fat	6.28
Calcium	0.94
Phosphorus	0.43
Magnesium	0.37
Potassium	1.45
Sulphur	0.2

[1] Crude protein, [2] Undegradable dietary protein, [3] Metabolisable energy, [4] Neutral detergent fibre.

Weekly samples of the TMR and the hay were analysed for β-Carotene DM, Ash, CP, ADF, NDF, ADL, GE, fat, Ca and P. Samples of the TMR and the *Eragrostis curvula* hay were taken once every 2 weeks and pooled on a 2 month basis.

4.2.2 Beta-Carotene supplement description

The β-Carotene supplement used in this trial, namely ROVIMIX® β-Carotene 10%, is brownish-red and is sold in the form of beadlets that contain β-Carotene lightly distributed in a carbohydrate containing porcine gelatine and coated in corn starch. In addition, both ethoxyquin and ascorbyl palmitate are added as sources of antioxidants. It has a molecular mass of 536.85 g/mol, its empirical formula is C40H56. Its chemical names include; β,β-Carotene; 1,18-(3,7,12,16-tetramethyl-1,3,5,7,9,11,13,15,17-octadecanonaen-1,18-diyl)-bis-(2,6,6-trimethylcyclohexene). The product is oxygen, heat, light and humidity sensitive and can be stored for up to 36 months from manufacture date in its primary sealed packaging below 15°C. It may only be used in animal feeds and is considered safe for this use. Ingestion of particles or dust of this product should be avoided. It is manufactured by DSM Nutritional Products Ltd, Basel, Switzerland.

4.2.3 Sampling and analyses

4.2.3.1 Blood sampling and analyses

Blood samples were taken weekly from the coccygeal vein (tail vein) commencing at the beginning of the dry period until 9 weeks postpartum. Samples were analysed for β-Carotene using the iCheck™ procedure (iCheck ™, BioAnalyt, GmbH, Germany). This is a hand-held portable spectrophotometer developed by Schweigert et al. (2007) for real-time on farm β-Carotene assessment. Samples were taken once a week on the same day (i.e. Friday) at the same time (0700h), just after morning milking. This is a single step separation method, done using direct whole blood and without the use of centrifugation where β-Carotene is extracted into an organic fluid within a vial. Schweigert et al. (2007), stated that this method is not affected by the regularly occurring problem of haemolysis. Blood values lower than 1.5 mg/L show a deficient status of the animal, suggesting that supplementation of β-Carotene is required, recommended at a daily dose of 500 mg β-Carotene /cow. Concentrations within the blood of between 1.5 and 3.5

mg/L are recommended to receive a minimum β-Carotene supplementation of 300 mg β-Carotene/cow/day and concentrations above 3.5 mg /L suggest that the animal has an optimal β-Carotene status (Schweigert et al., 2007).

4.2.3.2 Feed sampling and analyses

Samples of both the TMR and *Eragrostis curvula* hay were taken weekly and pooled on an eight weekly basis. Samples were analysed for DM, Ash, CP, ADF, NDF, ADL, GE, fat, Ca and P, at the analytical laboratory of the Department of Animal and Wildlife Science at the University of Pretoria in South Africa. All samples were ground through a 1mm sieve on a Retsch ZM 200 (Germany) grinder. DM was determined as the loss on drying at 95-100°C (method 934.01, AOAC, 2000). Ash was reported as a percentile by making use of a temperature controlled furnace at 600°C for 2 hours (method 942.05, AOAC, 2000). Crude protein was determined by method 968.06, AOAC (2000). The ADF and NDF were analysed using the Ankom 2000 Automated Fibre Analyser, (Ankom Technology, NY, USA) as per the methods supplied by the manufacturer. The ADL was determined by applying the ADF method to the sample and then saturating in 72% sulphuric acid (H_2SO_4) for 3 hours stirring every 30 minutes. Acid was then removed by suction and the sample was washed 3 times with hot water (Goering, H and Van Soest, P, 1970). Determination of GE was done by bomb calorimeter (MC-1000 Modular calorimeter, Energy Instrumentation, 135 Knoppieslaagte, Centurion, South Africa). The ME was determined using the equation ME (MJ/kg DM) = 0.82 x (GE x IVOMD) (Robinson et al., 2004). The fat content of the diet was determined by the EE method using the Soxtec System HT 1043 extraction Unit (Foss Analytical, Hillerød, Denmark, Method 920.39, AOAC, 2000). Calcium content of feed was determined by the method of Giron (1973). The P content was determined by ashing sample, adding HCL and HNO_3 and boiling, using molybdovanadate reagent and the use of a spectrophotometer at 400nm (Method 965.17, AOAC, 2000) and sample preparation by method 968.08,AOAC, 2000. Beta-Carotene in feed was determined by direct saponification in ethanolic solution and pigment extraction into hexane by means of EXTRELUT columns and then analysed by HPLC (Schierle et al., 1995).

4.2.4 Statistical Analysis

Data were analysed as a randomised complete block design with general linear model (GLM) (Statistical Analysis System, 2011) for an analysis of variance to determine differences between

treatments and periods. Means and standard error of the means (SEM) were calculated. The significance of differences between means was determined by the Fischers protective test (Samuels, 1989), significance was determined at $P<0.05$ and tendencies at $P<0.10$.

The linear model used is described by the following equation:

$$Y_{ij} = \mu + T_i + B_j + e_{ij}$$

Where Y_{ij} = variable studied during the period

μ = overall mean of the population

T_i = effect of the i^{the} treatment

B_j = effect of the j^{the} block

E_{ij} = error associated with each Y

4.3 Results and discussion

4.3.1 Feed Analyses

The mean analysed results for the TMR ration, on a DM basis, are shown in Table 20. The CP (14.2%), ME (10.6 MJ/kg), NDF (43.9%), Fat (4.4%), Ca (0.9%) and P (0.3%) of the diet therefore meets all the requirements of dry and transition cows (NRC, 2001). Variations between pooled data analyses can be attributed to roughage quality variation. It can be argued that β-Carotene concentrations can be attributed to the quality of the lucerne in the TMR; however these results do not correspond with the other TMR nutrient analyses. Variation in β-Carotene results can be due to sampling, storage time, storage environment, conditions during transportation as well as possible sample contamination.

Using the standard expected β-Carotene content of lucerne and *Eragrostis* hay shown in Table 4, gives a a range of 20 to 59 mg/kg of β-Carotene for lucerne hay and a range of 9 to 18 mg/kg for *Eragrostis* hay. A simple calculation using these values gives us a minimum of 6.61 mg/kg and a maximum of 18.26 mg/kg expected β-Carotene for this ration. The analysed results agree with this calculation.

The analyses for *Eragrostis curvula* hay in Table 21 are similar to those of Meissner et al. (1991) who reported the following average values for *Eragrostis curvula*; %IVOMD: 43.6, %NDF: 74.4 and %CP: 7. The β-Carotene results, with the exception of 2, agree with the results from Maynard

et al. (1979) and Scott et al. (1982), cited in McDowell (2000), who found β-Carotene values for "non-legume hays, average quality, bleached, some green colour" to vary between 9 to 18 mg/kg.

The β-Carotene results for the *Eragrostis curvula* hay on its own, are higher than those of the TMR as roughages are diluted in the TMR ration.

Table 20 Overall analyses of pooled TMR fed at 8 kg per day to dry cows (As fed basis).

Sample	Source	%DM [a]	%Ash [b]	%CP [c]	%ADF [d]	%NDF [e]	ME (MJ /kg DM) [f]	%Fat [g]	%Ca [h]	%P [i]	%IVOMD [j]	BC (mg/kg) [k]
1	TMR	86.1	6.10	14.7	22.6	35.8	9.21	4.28	0.85	0.29	74.0	7.45
2	TMR	88.8	3.16	15.3	23.5	38.0	10.8	4.65	0.87	0.30	80.8	7.84
3	TMR	89.5	5.76	14.5	22.9	37.0	9.65	4.66	0.85	0.30	75.9	10.3
4	TMR	90.6	6.54	14.1	25.3	41.1	8.91	4.05	0.73	0.29	71.7	11.2
5	TMR	90.4	6.53	13.3	27.6	42.7	9.30	3.00	0.79	0.27	72.5	8.88
6	TMR	90.1	2.94	13.6	26.8	40.6	8.76	2.82	0.82	0.26	76.7	12.4
SEM		0.68	0.68	0.30	0.86	1.09	0.30	0.33	0.02	0.01	1.35	0.80

[a] dry matter, [b] ash, [c] crude protein, [d] acid detergent fibre, [e] neutral detergent fibre, [f] metabolisable energy, [g] fat, [h] calcium, [i] phosphorus, [j] in vitro organic matter digestibility, [k] β-Carotene.

Table 21 Overall analyses of pooled *Eragrostis curvula* hay fed *ad lib* to dry cows (As fed basis).

Sample	Source	%DM [a]	%Ash [b]	%CP [c]	%ADF [d]	%NDF [e]	ME (MJ /kg DM)	%Fat [g]	%Ca [h]	%P [i]	%IVOMD [j]	BC (mg/kg) [k]
7	Hay	92.7	1.99	3.97	40.9	77.4	6.32	1.50	0.17	0.11	46.2	14.6
8	Hay	93.0	2.55	6.18	40.5	75.6	5.93	1.47	0.15	0.08	47.0	27.0
9	Hay	93.4	1.24	6.21	39.5	76.3	5.92	1.58	0.14	0.08	47.7	20.0
10	Hay	89.9	0.66	5.99	43.1	77.2	6.53	1.23	0.18	0.11	48.3	11.8
11	Hay	94.1	3.69	5.65	41.0	77.4	6.44	1.28	0.15	0.09	46.5	10.0
12	Hay	95.4	4.79	6.39	39.6	76.2	6.32	1.35	0.14	0.09	45.6	13.7
SEM		0.75	0.63	0.37	0.53	0.31	0.11	0.06	0.01	0.01	0.41	2.57

[a] dry matter, [b] ash, [c] crude protein, [d] acid detergent fibre, [e] neutral detergent fibre, [f] metabolisable energy, [g] fat, [h] calcium, [i] phosphorus, [j] in vitro organic matter digestibility, [k] β-Carotene.

4.3.2 Production data

The objective of this study was to evaluate the potential carryover effect of prepartum β-Carotene supplementation into the postpartum production phase by comparing plasma β-Carotene concentrations. The emphasis therefore was not on the effect of β-Carotene on milk production and composition. Furthermore, a literature study revealed that results of β-Carotene supplementation on milk production and composition is inconsistent and large numbers of animals would be needed to prove significance (de Ondarza et al., 2009). Therefore, only the official milk recording data per lactation of cows is reported (Table 22, ARC Irene). There were no differences in milk production or composition between the two groups. This was expected due to the inconsistent results published.

Table 22 Averages and standard deviations of lactation records for the control and treatment groups.

	Control	**Treatment**
Milk[1]	10770.2 ± 3564.1	10855.0 ±
Butter fat[2]	3.5 ± 0.2	3.8 ± 0.4
Protein[3]	3.1 ± 0.1	3.0 ± 0.1
Lactose[4]	4.8 ± 0.1	4.8 ± 0.2

[1] 305 day Milk Yield, [2]Average Butterfat % for lactation, [3]Average Protein % for lactation, [4]Average Lactose % for lactation

De Ondarza and Engstrom (2009) found that β-Carotene supplementation had no effect on milk production, which corresponds with results of Rakes et al. (1985) and Wang et al. (1998b) as well as with our results. However Chawla and Harjit (2004) reported an increase in milk production as a result of β-Carotene supplementation. Arechiga et al. (1998) found an increase in milk yield for cows supplemented with β-Carotene.

De Ondarza and Engstrom (2009) established that milk fat was higher ($P < 0.05$) for β-Carotene supplemented cows; this corresponds with results of Oldham et al. (1991), but is not in agreement with our results or that of Rakes et al. (1985). Lotthammer (1979) reported that a lower milk fat % was related to β-Carotene deficiency.
De Ondarza and Engstrom (2009) found that milk protein % was not affected by β-Carotene supplementation, which corresponds with our findings.

4.3.3 Blood analysis

The plasma β-Carotene values for both groups are shown in Figure 19. Trial commencement was at week -8, cows calved at week 0, and the trial conclusion is at week 8. Original data was for 18 weeks; however data for the first and last weeks were removed from statistical analysis due to some missing values. Asterisks show significant differences ($P < 0.05$) between groups for specific weeks.

Plasma concentrations of β-Carotene were still optimal until 2 weeks postpartum on average for the supplemented group, however individually some cows still showed optimal concentrations of up to 4 weeks postpartum. Concentrations in the control group where on average marginal prepartum and decreased to deficient concentrations at calving.

In a similar experiment conducted by Kaewlamun et al. (2011b), cattle were supplemented during the 8-week dry period. Both groups started at the same plasma β-Carotene concentration at dry off of about 3 mg/L. Cows in the supplemented group experienced a rapid increase in plasma β-Carotene and reached a peak of between 7 and 8 mg/L plasma β-Carotene at 1 month into the dry period, tapering off to reach a value of between 4 and 5 mg/L at calving. The control group gradually declined to a value close to 2 mg/L at calving.

Kawashima et al. (2010) supplemented cows with β-Carotene to test the onset of ovulation postpartum. At 21 days prepartum, both the treatment and control groups had similar β-Carotene serum concentrations of between 5.6 and 6.4 mg/L. Cows were supplemented from 21 days prepartum until calving. The supplemented group experienced a higher serum β-Carotene concentration ($P <0.01$) than the control group throughout.

Calderón et al. (2007b), divided cows into 2 groups and fed them diets different in β-Carotene content, one was fed grass silage (high β-Carotene) and the other maize silage (low β-Carotene), from 7 weeks prepartum until calving. Plasma β-Carotene concentrations were higher in the group fed grass silage ($P < 0.01$) than for those fed maize silage. Both groups started at a high plasma β-Carotene concentration of about 5 mg/L and gradually declined until calving, but the grass silage group had a concentration of about 2.15 mg/L at calving and the maize silage group had a plasma concentration of about 0.98 mg/L at calving.

All 3 trials showed similar patterns in blood β-Carotene levels to that of ours.

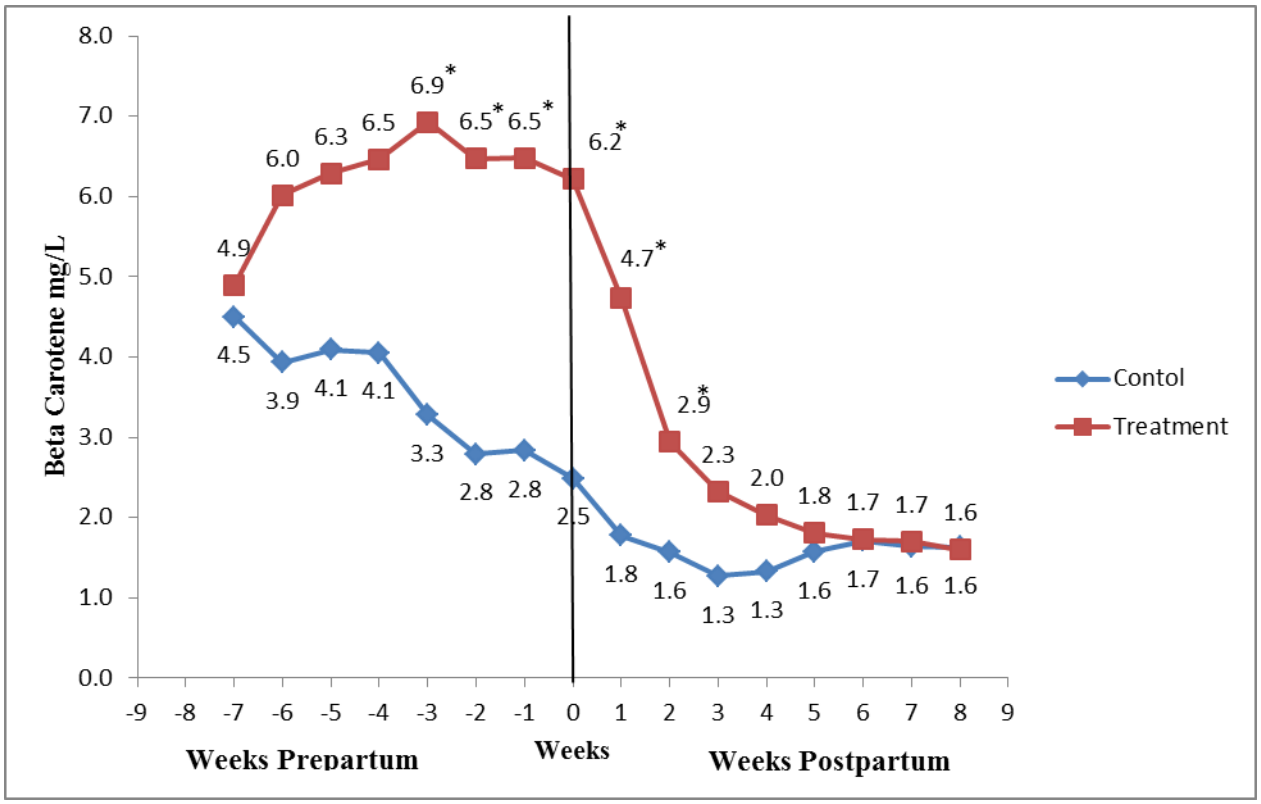

Figure 19 Overall weekly β-Carotene blood analyses for animals in both treatment groups

Authors have previously reported carryover effects of β-Carotene. Kaewlamun et al. (2011b) supplemented cows with β-Carotene during the dry period until calving and found a positive carryover effect up to 3 weeks postpartum. Yang et al. (1992) stated that β-Carotene mobilised from body reserves built up over the close-up dry period, may aid in supplying vitamin A in the granulosa cells of dominant follicles. Knight et al. (1994) found that cattle plasma concentrations went from optimal to minimal status after 28 days on a low Carotene ration.

This carryover affect can be attributed to a possible storage of β-Carotene in the cow's body. A number of sites have been suggested as storage organs of β-Carotene, these include; the CL (Hurley and Doane, 1989), liver (Yang et al., 1992) and adipose tissues (Bauernfeind. 1972, Yang et al., 1992, Strachan, 1993, Mora et al., 2000). However, Underwood (1984) stated that the liver was not a significant storage site for β-Carotene as is the case for retinol. Nozière et al. (2006) reported that adipose tissue is a major storage site of β-Carotene in cows, during medium term depletion. Knight et al (2001) showed that subcutaneous fat was a storage site for β-Carotene as seen in the yellow colour of the fat in pasture fed animals and Röhrle et al. (2011) stated that the colour of adipose tissue related to the β-Carotene content can be used to differentiate between animals fed on concentrate

based or pasture-based systems. Simonne et al. (1996) found higher levels of β-Carotene in subcutaneous adipose tissue in pasture fed animals than concentrate-fed animals. Semb (1934) proposed that the blood stream may act as a major storage reserve for β-Carotene, as did Yang et al. (1992). Hurley and Doane (1989) suggested that the β-Carotene stored in the corpus luteum may be used for CL function during times of insufficient availability from the blood. However Friesecke (1978) questioned the ability of body fat as a source of stored Carotene due to the short lifespan of fat cells, said to be approximately 4 weeks and Knight et al (2001) identified that there was no evidence to prove that this β-Carotene is mobilised or expelled from body fat reserves.
More research on this topic is urgently needed.

4.4 Conclusion

Supplemented cows maintained sufficient plasma β-Carotene concentrations until 14d postpartum and were either marginal or deficient for the rest of the experimental period.

Results suggest a minor carryover effect of β-Carotene from the dry period into early lactation when receiving supplementation during the prepartum period.

Although limited recent research demonstrated that β-Carotene plasma concentrations before calving determined when cows will produce the first dominant follicle after calving, irrespective of post calving concentrations, continuous supplementation is recommended until animals are confirmed pregnant if medium quality lucerne hay-based diets or diets of lower quality are fed, in order to maintain optimal β-Carotene plasma concentrations.

Cows having a plasma β-Carotene concentration of 1.5 mg/L require β-Carotene supplementation of 500 mg/day or 300 mg/day when concentrations are between 1.5 and 3.5 mg/L

CHAPTER 5: CRITICAL EVALUATION

5.1 Survey of β-Carotene status in different feeding systems

The results given in this survey give us a realistic indication of the current situation in SA. However the plasma β-Carotene results in the pasture-based system showed variation between concentrations of 3.8 and 10.2 over all farms. Farms in the EC showed lower average plasma β-Carotene values than those in KZN but levels of β-Carotene in grasses were similar. More information is required to understand these differences.
In order to get a clearer picture of the effect of β-Carotene content of roughages on fertility, more information is required per farm with regards to fertility measures and management techniques. This however was not the aim of the current survey.

5.2 Supplementation trial

Pooled sampling and analysis of β-Carotene in this trial gave an unclear picture of the contribution of β-Carotene by the lucerne portion to the whole ration. The *Eragrostis curvula* hay was fed *ad lib* and pooled samples showed broad variation in β-Carotene values. Daily intake could have been monitored and the number of samples analysed for β-Carotene content could have been increased to give a clearer picture of quality.
The reason this was not done was due to financial constraints on the number of samples that could be analysed for this trial.

The farm used in this trial had a small herd of about 50 milking cows and in order to get 20 dry cows for the trial the timing had to allow for cows to dry off. The trial therefore stretched over a period of 357 days from the beginning of September 2009 until the end of August 2010. This covered an entire year which may have caused a variation in the raw materials used in the ration.

It was clear that the supplemented animals were overall healthier and suffered from fewer problems than the control group. This supplemented group experienced no problems at calving or postpartum versus a number of cows in the control group that did. The reason for this was unclear and not measured, but is worthy of further investigation.

Overall there are a number of aspects that could have been added to these trials and a number of errors and sources of variation throughout that could have been anticipated with prior knowledge and could be improved if

the trial was to be repeated. Further studies may look at correlating plasma β-Carotene values across systems with fertility in herds, and management practices, as well as measuring the carryover effect of supplementing for different lengths of time and at different doses to optimise plasma β-Carotene concentrations at first ovulation postpartum.

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APPENDIX A

Table 23 Blocking of cattle into treatment groups

Cow number	Cow	Block	Treatment	Lactation No.	Total Milk Production	Weight
1	228	1	+	4	3665	645
2	6005	1	-	2	3434	635
3	5010	2	-	2	15449	590
5	5017	2	+	2	15338	620
4	7004	3	-	1	6661	485
6	4008	3	+	3	11951	660
7	5034	4	+	2	11531	670
10	6028	4	-	1	14745	470
8	303	5	+	5	11620	715
12	4032	5	-	3	15200	620
9	6031	6	+	1	10672	550
13	6040	6	-	1	12431	500
11	7005	7	+	1	10660	510
14	4039	7	-	3	11579	630
15	6023	8	-	2	9727	585
16	7029	8	+	1	6160	380
17	322	9	-	4	11026	670
19	4047	9	+	3	14758	645
18	7016	10	-	1	7720	470
20	7007	10	+	1	13698	450
				Treat. Ave	**10588**	**578**
				Control Ave.	**10797**	**566**