Effect of β carotene supplementation on oestrous synchronisation and milk production of Saanen goats

By

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Declaration

I, Dominic Lado Marino Gore declare that the thesis/dissertation, which I hereby submit for the degree MSc (AgricS) Animal Science: Production Physiology at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

Signature……..…

Date:……

Dedication

This work is dedicated to my mother, Lina Denya, wife Nancy Marcelino, son Tombe Dominic and to my sisters and brothers.

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Abstract

Goats play a major role in the life of rural populations, especially in the Sub-Sahara Africa. The use of nutritional supplements such as β-carotene and the reproductive management techniques can lead to improved goat productivity. β-carotene is a carotenoid with an antioxidant activity, it plays beneficial role in getting rid of free oxygen radicals. Due to its antioxidant activity, the hypothesis is that β-carotene will improve reproductive and milk production parameters of Saanen goats. The present study firstly evaluated the effect of β-carotene and synchronisation protocol on ovarian activity and fertility of Saanen goats. Secondly, it evaluated the effect of β-carotene supplementation on milk yield and components. A total of 60 Saanen does aged 1-6 years were used. In the first experiment, the factors in the design were supplementation (β-carotene supplemented versus non-supplemented) and oestrous synchronisation protocol (equine chorionic gonadotropin (eCG) versus male effect). The supplemented group was dosed with β-carotene 100 mg/goat/day for 60 days starting from 28 days before oestrous synchronisation. For the oestrous synchronisation protocols, all animals were inserted with controlled internal drug release devices (CIDR) for 11 days and were intramuscularly injected with prostaglandin at CIDR withdrawal. For eCG group, does were injected with 300 IU eCG, while for male effect group, bucks wearing aprons were introduced at CIDR removal. Blood samples were collected for evaluation of progesterone (P4), oestradiol-17β concentration and glutathione peroxidase (GPx) activity. The ultrasonographic scanning was performed to measure the number and size of follicles, corpora lutea (CL) size, and pregnancy diagnosis. The onset and duration of oestrus were monitored using bucks wearing aprons. In the second experiment, the animals were divided into two groups (β-carotene supplemented versus non-supplemented). The animals were dosed with 50 mg/goat/day from the drying off period until kidding which was approximately a period of two months. The colostrum samples were collected three days postpartum and the ordinary milk samples were collected once a week for a month. The milk collected was analysed for the milk yield, fat, protein, lactose and somatic cells count. All the data were analysed using the GLM procedures and categorical modelling (CATMOD) procedures of SAS (version 9.4; 2014) while the correlation was analysed using Pearson correlation of SPSS (Version 23.0; 2015). β-carotene supplementation and synchronisation protocol had no significant effect on body weight, response to oestrus, onset and duration of oestrus, oestradiol-17β concentration, number of follicles, size of largest follicle and CL, gestation length, birth weight, and litter size.

However, β-carotene supplementation had increased plasma P4 concentration and GPx activity. There was a significantly positive correlation between the CL size and P4 concentration regardless of β-carotene supplementation. The synchronisation protocol had a significant effect on conception rate. The male effect group had higher conception rate (97%) than the eCG (72%) group. β-carotene supplementation had no significant effect on milk yield and components. Milk type had a significant effect on the milk components of Saanen goats. Therefore, it can be concluded that male effect can improve conception rate and may be used to replace eCG on oestrous synchronisation of Saanen goats primed with progesterone. β-carotene supplementation during the breeding period may play a beneficial role during embryo implantation and development as a result of increased progesterone concentration and glutathione peroxidase activity. Supplementation of Saanen goats with β-carotene during the drying off period has no beneficial effect on milk yield and components.

Keywords: Goat, β-carotene, oestrous synchronisation, artificial insemination, milk components

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List of Abbreviations

AI: Artificial insemination

CATMOD: Categorical modelling

CIDR: Controlled internal drug release

CL: Corpus luteum

DM: Dry matter

DMR: Duncan multiple range test

eCG: Equine chorionic gonadotropin

FCM: Bentley Flow Cytometer

FGA: Fluorogestone acetate

FSH: Follicle stimulating hormone

FTS: Bentley Fourier Transform Spectrometer

GLM: General linear model

GnRH: Gonadotropin releasing hormone

GPx: Glutathione peroxidase

hCG: Human chorionic gonadotropin

LH: Luteinizing hormone

MAP: Medroxyprogesterone

Me: Male effect

MGA: Melengestrol Acetate

P4: Progesterone

PGF2α: Prostaglandin F2α

SCC: Somatic cell counts

SE: Standard error

TMR: Total mixed ration

CHAPTER ONE

1. INTRODUCTION

1.1 Overview

The current growing world human population is expected to reach 9.6 billion in 2050, and this growth will be mainly in developing countries (United Nations Report, 2013). More than half of this population growth will be in Africa and in particular Sub-Sahara African countries. South Africa is one of the countries with the large human population in Africa. Most of Sub-Sahara Africa rural populations are living in poverty and high level of malnutrition, especially children. In South Africa most of its rural population experienced high level of poverty and malnutrition (Norris *et al*., 2011).

Farm animals are expected to contribute in feeding this growing human population and address the issue of poverty and malnutrition. Farm animals, such as cattle, sheep, goats and poultry are the most widely kept animals in the world, providing milk, meat and eggs. In many countries, rural poor communities prefer to keep goats over the other livestock species. This has been attributed to the fact that goat eats little, occupies a small area, produces enough milk for the average nuclear family and easy to manage (Aziz, 2010). Additionally, goat has short generation interval and high reproductive efficiency. Goat mainly provides both meat and milk to rural populations (Haenlein, 2004) which are the sources of protein to these populations. Therefore, goats play a crucial role in supporting the livelihood of rural population especially those farming in the communal land (Webb & Mamabolo, 2004).

Africa is the second largest continent in terms of goat population after Asia. Africa and Asia owned 33.8% and 55.9% of total world goat population, respectively (Aziz, 2010). Goats are mainly categorised into dairy and meat producing goats in the world. The goat breeds known for milk production include Saanen, Toggenburg, Alpine and Nubian while for meat production include Boer and Pedi (Ngambi *et al*., 2013). Nevertheless, almost all the goat breeds originated in Africa are primarily known for meat production but can also provide milk and other products.

Dairy goats produce about 15.2 million metric tonnes (MT) of milk, accounting for about 2% of the total amount of milk produced globally by livestock species (Ngambi *et al*., 2013). In Africa, Sudan and Somalia are ranked third and ninth, respectively, of top ten countries in

terms of goat milk produced. Most African or tropical local goat breeds are poor milk producers. However, the temperate goat breeds such as Saanen, Toggenburg and Alpine are among the best dairy goat breeds in the world. These milk producing breeds were imported from Europe in an attempt to improve the dairy goat industry, and are the main dairy goats in South Africa (Pieters, 2007).

Despite the fact that these European goat breeds are good milk producers, proper feeding and efficient reproductive management system are important aspects in improving dairy goat industry. Reproduction in temperate goat breeds can be affected by factors such as season and nutrition. Goats are seasonal breeders; their breeding season is only during the short day photoperiod (autumn), under natural conditions. The seasonality is especially exhibited by temperate breeds while most tropical breeds, such as those found in South Africa, are less seasonal. Saanen goats in South Africa maintained their seasonal sexual activity. These seasonal sexual activities in goat affects the dispersal of production over the year and this is a problem both in dairy and meat production systems which attempt to have a constant production year-round (Fatet *et al*., 2011).

Reproductive technologies such as oestrous synchronisation together with artificial insemination can be used in the reproductive management of goats (Leboeuf *et al*., 1998). By using these techniques goats are able to reproduce during both the breeding and non-breeding seasons (Dogan *et al*., 2005). Artificial insemination in goats provides high selection intensity and adequate genetic evaluation (Leboeuf *et al*., 1998). Oestrous synchronisation on the other hand is a valuable management tool that has been successfully employed to implement AI efficiently, especially in ruminants (Kusina *et al*., 2000). It is a method in which hormones are applied to bring animals in to oestrus more or less at the same time. The purpose of synchronising oestrus is to inseminate or breed animals at a particular time during the breeding season or induce out of season oestrus (Dogan *et al*., 2005). Oestrous synchronisation methods are based on the control of the corpus luteum lifespan using either prostaglandins to initiate oestrus or progesterone to prevent the occurrence of oestrus (Martemucci and D'Alessandro, 2010). Progesterone or its analogue is commonly used for oestrous synchronisation in goats. However, besides the use of progesterone or its analogue alone or in combination with other hormones to induce oestrus, other methods include the use of artificial light, melatonin implants and the male effect (Pietroski *et al*., 2013).

The most commonly used progesterone or its analogues intravaginal devices for oestrous synchronisation in goats are medroxyprogesterone (MAP), flurogestoneacetate (FGA) and controlled internal drug release devices (CIDR) (Motlomelo *et al*., 2002; Ramukhithi *et al*., 2012). However, CIDR has become the most preferred method as vaginal secretions are easily discharged without being retained by the device and also because it can be easily removed (Motlomelo *et al*., 2002; Romano, 2004). These intravaginal devices can be used with or without co-treatments such as equine chorionic gonadotropins (eCG), human chorionic gonadotropins (hCG) or both. For fixed time artificial insemination, CIDR in conjunction with eCG have been used effectively and regularly in different goat breeds (Moore *et al*. 1988; Ritar *et al*., 1990). Regardless of the effectiveness of eCG in ovulation, there are limitations for its use. Repeated use of eCG led to reduced fertility in goats inseminated at fixed time. This reduction in fertility was attributed to the presence of circulating anti eCG antibodies in the plasma of goats treated with eCG (Baril *et al*., 1996; Roy *et al*., 1999).

The problem associated with the use of eCG as a co-treatment in oestrous synchronisation has led seeking of alternative treatments. Some studies have used male effect following priming with progesterone pessary. Male introduction induces onset of oestrus in goats and ewes synchronised with progestagen pessary during the breeding season (Romano, 1998; Romano *et al*., 2000). Male introduction not only reduces the time between sponge removal and oestrous onset but also reduced variation in the onset of oestrus (Romano, 1998). Apart from improving oestrous parameters, male introduction is an inexpensive alternative co-treatment in progesterone based synchronisation protocol in non-cycling females (Wildeus, 2000).

On the other hand, the response to oestrous synchronisation regardless of method used still depends on animal body condition. Nutritional supplements such as β-carotene plays important role such as stress reduction, especially in highly producing dairy animals. β-carotene, is a provitamin A that also acts independently from vitamin A (Chew *et al*., 1993; Sies & Stahl, 1995). β-carotene has gained popularity because of its possible importance as an antioxidant in reproductive performance of farm animals (Schweigert *et al*., 2002). It is involved in the steroidogenic process in the ovary by getting rid of free radical formation (Rapoport *et al*., 1998). β-carotene plays a crucial role in many reproductive functions through it antioxidant activity and immune function. In goats, supplementation with β-carotene positively affects the ovarian activity by increasing the number of follicles, corpus

luteum and progesterone concentration (Arellano-Rodriguez *et al*., 2007, 2009; Meza-Herrera *et al*., 2013ab). All these physiological effects of β-carotene can have a significant impact on the oestrous cycle activity and eventually fertility in does. Although, no previous evidence about the effect of β-carotene on oestrous and fertility parameters in does, there are some reports in other animals about the effect of β-carotene on oestrous and fertility parameters. In a review study, it was mentioned that Cattle fed on diets lower in β-carotene had reduced intensity of oestrus and conception rate (Hemken & Bremel, 1982). In addition, cattle supplemented with β-carotene had increased pregnancy rate and milk yield (De Ondarza *et al*., 2009). However, other studies found no effect of β-carotene in reproduction. β-carotene supplementation did not changed somatic cell counts in milk, luteinising hormone and progesterone concentrations or improves reproductive efficiency in cattle (Bindas *et al*., 1984). Therefore, this study will evaluate the effect of β-carotene on oestrous response and reproduction performance following oestrous synchronisation with different protocols and artificial insemination (AI).

1.2 Problem statement

In goats, there are limited studies on the effect of β-carotene supplementation on oestrous activity, fertility, milk yield and components. From those studies conducted on cattle, pigs, rats and rabbits, contradictory results have been obtained (Kumar *et al*., 2010). Therefore, the controversial reports and limited information in goats concerning the effect of β-carotene on ovarian activity, fertility and production performance had led to the need for such studies to be conducted in goats. On the other hand, the repeated use of eCG in oestrous synchronisation reduces fertility in goats due to presence of circulating anti-eCG antibodies in plasma. As such, there is need to look for an alternative co-treatment with no immunological reaction to gonadotropic agent such as the male effect.

1.3. Aim of the study

The overall aim of the study was to evaluate the effect of supplemental β-carotene following two oestrous synchronisation protocols on reproductive and productive performance of Saanen goats.

1.4. Objectives of the study

- To evaluate the effect of β-carotene and synchronisation protocol on ovarian activity and fertility of Saanen goats.
- To evaluate the effect of β-carotene supplementation on milk yield and milk components of Saanen goats.

1.5. Hypotheses of the study

H_1: β-carotene supplementation and synchronisation protocol will improve ovarian activity and fertility of Saanen goats.

H_1: β-carotene supplementation will improve milk yield and components of Saanen goats.

CHAPTER TWO

2. LITERATURE REVIEW

2.1 Introduction

Reproductive performance in farm animals is influenced by various factors. These factors are mainly the genetic merit, physical environment, nutrition and management (Smith & Akinbajimo, 2000). Reproductive technologies in farm animals have enabled farmers to improve both the biology and management efficiency of their livestock (Gordon, 2004). These reproductive technologies include oestrous synchronisation, artificial insemination, embryo transfer and many others. Oestrous synchronisation is a management tool used to provide tight synchronised oestrus and give acceptable fertility after mating during or outside breeding season (Kusina *et al*., 2000; Wildeus, 2000). There are many different methods and protocols being used for oestrous synchronisation and induction in goats. However, there is a need to improve the efficiency and address limitations of these methods and protocols (Wildeus, 2000).

On the other hand, lack of proper nutrition can negatively affect reproductive efficiency. Nutrition plays both direct and indirect roles in ruminant fertility (Robinson *et al*., 2006). It directly influences ovulation, fertilisation, embryo survival and establishment of pregnancy through supply of specific nutrients. Vitamin A is an essential dietary supplement in animal nutrition required to support animal life (McDonald, 2000). Its deficiency can have a detrimental effect on reproduction in both male and female animals. Vitamin A does not exist in plants, however, its precursors (carotenoids) such as β-carotene are found in plant materials. Besides, β-carotene nutritional benefits as a provitamin A, it also functions independently as an antioxidant which can enhance immunity with possible reproductive and mammary benefits (Chew, 1993). Its deficiency can lead to prolonged oestrus, delayed ovulation, reduced oestrus signs, low conception rate and low progesterone concentrations (Hemken & Bremel, 1982; Rakes *et al*., 1985; Arikan & Rodway, 2000).

Therefore, the purpose of this review was firstly to discuss on the current knowledge of the oestrous synchronisation methods and protocols, and their effect on the reproductive parameters in goats. Secondly, the chapter reviews the influence of β-carotene on the production and reproductive performance of goats. However, due to limited information in

the literature about β-carotene supplementation in goats, studies on other related animal species such as cattle, sheep, pigs, rats were also reviewed.

2.2 Oestrous synchronisation methods in goats

Efficient oestrous synchronisation in goats normally involves manipulation of the luteal phase of the oestrus cycle. The manipulation of luteal phase depends on either extending the life span of corpora lutea (CL) or by regressing them prematurely. Usually exogenous progesterone is used to extend the luteal phase while prostaglandin F2α (PGF2α) is applied to shorten it (Wildeus, 2000). Both hormones simulate natural control during the oestrous cycle. Progesterone (P4) is produced by the CL during the luteal phase and PGF2α is produced by the uterus at the end of the luteal phase to regress the CL. P4 inhibits the release of luteinising hormone (LH) and as a result can influence ovulation (Pietroski *et al*., 2013).

Oestrous synchronisation methods using P4 include intravaginal devices (medroxyprogesterone (MAP), fluorogestone acetate (FGA), controlled internal drug release (CIDR), norgestomet implants and melengestrol acetate (MGA). Other oestrous synchronisation methods include the use of PGF2α, male effect, light and melatonin. These methods can apply co-treatments like equine chorionic gonadotropins (eCG) and human chorionic gonadotropins (hCG) for oestrous synchronisation to tighten oestrus onset and improve ovulation rate. Among all these methods, progesterone and its analogues are widely used for oestrous synchronisation in goats. It has been indicated that efficient oestrous synchronisation is normally achieved when P4 or its analogue is used (Romano, 2004) in does during or outside the breeding season (Dogan *et al*., 2005).

2.2.1 Oestrous synchronisation using progesterone or in combination with eCG

The most commonly used combination for oestrous synchronisation in goats involves intravaginal devices impregnated with progesterone in combination with eCG (Motlomelo *et al*., 2002; Kor *et al*., 2011). Currently, the commonly used intravaginal devices include FGA, MAP and CIDR (Motlomelo *et al*., 2002; Ramukhithi *et al*., 2012). These devices are equally effective in oestrous synchronisation (Romano, 1996; Motlomelo *et al*., 2002; Romano, 2004). Some studies in goats have tested the effectiveness of these intravaginal devices without the addition of co-treatments. In goats, the intravaginal devices impregnated with P4 were inserted for 13 days during the breeding season and there was no difference in the response to oestrus, duration of oestrus, and kidding rate between MAP, FGA and CIDR

(Romano 2004). However, the difference was related to the onset of oestrus with FGA leading to earlier onset of oestrus compared to CIDR and MAP (Romano, 2004). Additionally, Motlomelo *et al*. (2002) reported earlier onset of oestrus in CIDR group compared to MAP and FGA. This early onset of oestrus has been attributed to differences in rate of absorption and metabolization of each P4 (Romano, 1996). Progesterone treatments with MAP or FGA intravaginal progestagen sponges or combinations with PGF2α are equally efficient in synchronising oestrous in non-lactating does during the natural breeding season (Dogan *et al*., 2005).

Progesterone based protocol in combination with eCG may improve follicular development and trigger ovulation, thus allowing artificial insemination (AI) at fixed time (Wheaton *et al*., 1993; Wildeus, 2000; Baldassarre & Karatzas, 2004; Holtz, 2005; Abecia *et al*. 2012; Inya & Sumretprasong, 2013). Generally, the recommended doses are from 200 to 600 IU of eCG, however, the dose may vary according to breed, season, weight and age of each animal. (Baldassarre & Karatzas, 2004; Holtz, 2005; Fatet *et al*. 2011; Inya & Sumretprasong, 2013). The response to oestrus is an important factor following treatments with synchronisation agents. If an animal responded to oestrus, it shows it will likely ovulate and may conceive if mated. The response to oestrus following synchronisation with different types of P4 or its analogues in combination with eCG has been reported. It is clear that the response to oestrus is more or less the same regardless of intravaginal P4 pessaries used in combination with eCG. In the trial conducted in goats, the intravaginal devices (MAP, FGA and CIDR) were inserted for a period of 16 days and at pessaries withdrawal, 300 IU of eCG was injected intramuscularly (Motlomelo *et al*., 2002). These authors found that the response to oestrus was similar for MAP, FGA and CIDR. Similarly, it was reported that the oestrous response in ewes between CIDR and MAP treatment was similar when pessaries were inserted for 12 days and injected with 500 IU eCG during the non-breeding season (Hashemi *et al*., 2006). Moreover, intravaginal pessaries were inserted for 14 days and 350 IU of eCG was administered intramuscularly at pessaries removal, and the response to oestrus for CIDR and FGA was found to be similar (Kor *et al*., 2011).

It is apparent that addition of co-treatments such as eCG into P4 protocol improves the response to oestrus. Previous studies have reported the effect of oestrous synchronisation using P4 alone or in combination with eCG on response to oestrus. The response to oestrus was higher for CIDR when combined with eCG and lower when CIDR was used without eCG (Oliveira *et al*., 2001). In addition, lower response to oestrus was recorded in goats that

was synchronised using CIDR and FGA only than those in combination with eCG (Omontese *et al*. 2013ab).

Onset of oestrus is one of the most important aspects in the reproductive management as it can indicate when goat can be mated. Synchronising oestrus with P4 in combination with eCG can lead to earlier and better tighten onset of oestrus. In two separate studies conducted in Red Sokoto goats by Omontese *et al*. (2013ab), the first study inserted CIDR and FGA for a 15 days and at pessaries withdrawal does were injected intramuscularly with 400 IU of eCG. In the second study FGA was inserted for 14 days and 200 IU of eCG was injected intramuscularly at pessaries withdrawal. These authors found that in both studies the time to the onset of oestrus was earlier in CIDR+eCG and FGA+ eCG groups compared to CIDR and FGA. Additionally, it has been reported that the addition of eCG into FGA treated ewes has shortened the time to the onset of oestrus (Amer & Hazzaa, 2009). This earlier onset of oestrus may be attributed to the FSH and LH-like activity of eCG (Abecia *et al*., 2012) which stimulates follicular growth and maturation which result in ovulation (Leboeuf *et al*., 1998). The duration of oestrus has been noted to be prolonged by the addition of eCG into progesterone protocol. It was found that the duration of oestrus is longer in goats treated with CIDR+ eCG and FGA+ eCG compared to those treated only with CIDR and FGA (Omontese *et al*., 2012; Omontese *et al*., 2013ab).

Synchronisation of oestrus with P4 in combination with eCG promotes the fertility parameters in sheep and goats. This improvement in fertility has been attributed to the fact that eCG tightens the onset of oestrus, promote follicular development and triggers ovulation (Leboeuf *et al*., 1998) and as a result may lead to high chances of conception. In sheep, FGA was inserted for 12 days and at pessary withdrawal one group was injected with 500 IU of eCG and the other group was not (Amer & Hazza, 2009). These authors recorded higher conception rate in the FGA+ eCG group compared to FGA group. Additionally, higher conception rate was recorded in goats synchronised with MAP in combination with 500 IU of eCG compared to those synchronised using MAP alone (Greyling & Van Niekerk, 1991). Contrary, CIDR and FGA was inserted for 15 days and injected goats with 400 IU of eCG at pessaries withdrawal, these authors found no differences on the conception rate between CIDR, CIDR+eCG, FGA and FGA+ eCG groups (Omontese *et al*., 2013a).

Despite the fact that the addition of eCG into oestrous synchronisation protocol has been acknowledged to improve fertility parameters, still its challenge is that, when used repeatedly

for oestrous synchronisation in goats, it reduces the fertility (Baril *et al*., 1996; Roy *et al*., 1999). The low fertility in goats inseminated at fixed time AI after eCG treatment was attributed to the delayed occurrence of oestrus and the pre-ovulatory LH surge (Roy *et al*., 1999). The delay in the occurrence of oestrus and the pre-ovulatory LH surge is due to the presence of circulating anti-eCG antibodies in plasma developed following successive use of eCG.

2.2.2 Use of prostaglandins (PGF2α) in oestrous synchronisation

Synchronisation of oestrus in sheep and goats can alternatively be done through the induction of luteolysis to eliminate the corpus luteum and induce a subsequent follicular phase with ovulation (Abecia *et al*. 2012). Prostaglandin (PGF2α) or its analogue is commonly used to cause luteolysis in goats and it is only effective in the presence of active corpus luteum. Therefore, is only during the breeding season when goats are actively cycling that oestrus can be synchronised with PGF2α or its analogues (Gordon, 1997; Bitaraf *et al*. 2007).

The corpora lutea can be responsive to PGF2α from day 3 of the oestrous cycle until the day of natural luteolysis (Rubianes *et al*., 2003; Abecia *et al*., 2012). Normally a double injections of PGF2α 9-10 days apart is recommended, this is due to the impossibility of knowing the phase of the oestrus cycle in a group of female animals (Abecia *et al*., 2012) to achieve effective synchronisation. In the studies using PGF2α or its analogue, researchers have reported higher response to oestrus with double injections. It was noted that the response to oestrus was 100% when goats were injected intramuscularly with double injections of 125 µg PGF2α analogue 13 days apart (Ahmed *et al*., 1998). Similarly, 97% of the goats responded to oestrus when injected with 250 µg of PGF2α analogue 12 days apart (Bitaraf *et al*., 2007). Higher response to oestrus had been reported after the second injection of PGF2α compared to the first injection (Roman 1998).

With respect to the onset and duration of oestrus in sheep and goats, double injection of PGF2α has been reported with contradicting results. Using a double injection of PGF2α (Ahmed *et al*., 1998) has reported longer time to onset of oestrus in ewes while earlier onset of oestrus was observed in does (Bitaraf *et al*., 2007; Andrabi *et al*., 2015). For the duration of oestrus following double injection of PGF2α analogue, (Ahmed *et al*., 1998; Andrabi *et al*., 2015) have recorded longer duration of oestrus compared to shorter duration of oestrus recorded by Bitaraf *et al*. (2007).

The application of double injections of PGF2α for oestrous synchronisation has been reported with variable results on the conception rate. It has been noted that the conception rate was 90% following double injection of PGF2α analogue 10 days apart (Ogunbiyi *et al*., 1980). In addition, the conception rate of 77.8% was recorded following double injection of PGF2α analogue 13 days apart (Ahmed *et al*., 1998). Furthermore the pregnancy rate of 78.9% was recorded when double injection was implemented at the interval of 12 days (Andrabi *et al*., 2015). However, lower conception rate of 58.1% has been reported after double injection with PGF2α (Greyling &Van Niekerk, 1986).

2.2.3 Oestrous synchronisation using the male effect

The male effect refers to the introduction of males in a group of seasonally anoestrus females which results in LH release and eventually to synchronise ovulation (Chemineau, 1983; Martin *et al*., 1986; Gelez & Fabre-Nys, 2004). In both sheep and goats, oestrus can be induced with the strategic exposure of anoestrous does (Chemineau, 1987) and ewes (Martin *et al*., 1986; Wildeus, 2000) to intact males or androgen treated castrates. The mechanism through which the male effect induces LH release and synchronise ovulation in anoestrous female is through the release of pheromones by the male and detected by the vomeronasal organ (VNO) in the female (Booth & Webb, 2011). These pheromones cause an immediate increase in the number and amplitude of LH pulses and the preovulatory surge of LH to start ovulation (Chemineau, 1987). This induced oestrus is associated with a first ovulation in 2 to 3 days and is usually silent, of low fertility and with premature regression of the first corpus luteum (Wildeus, 2000). And the second ovulation 5 days later is accompanied by a fertile oestrus with a luteal phase of normal length (Wildeus, 2000).

The buck effect effectively induces oestrus in goats outside the breeding season. It was asserted that the fertile oestrus can be induced in Saanen goats using sexually active buck outside breeding season (Véliz *et al*., 2009). The oestrus can be induced by either using buck effect or by both buck effect and P4 priming. Priming with P4 before buck introduction reduces the time to the onset of oestrus in goats. It was found that the time to onset of oestrus was shorter in P4 primed goats that were later subjected to buck effect compared to those only subjected to buck effect without progesterone priming (Gonzalez-Bulnes *et al*., 2006; Véliz *et al*., 2009). Additionally, goats treated with P4 and introduced to male have higher response to oestrus compared to those only subjected to male (Gonzalez-Bulnes *et al*., 2006).

Though the male effect does not cause ovulation during the breeding season in sheep and goats, it has a profound effect on their reproductive axis (Delgadillo *et al*., 2009). The exposure of males to cyclic goats can stimulate LH secretion and alter oestrous synchronisation (Hawken *et al*., 2009; Delgadillo *et al*., 2009). Oestrous synchronisation using male effect primed with P4 or PGF2α during the breeding season is effective to synchronise oestrus. Exposure of does to bucks following administration of progesterone during the breeding season causes early onset of oestrus and ovulation. Romano (1998) conducted a study on the effect of buck on the onset of oestrus in goats during the breeding season. The does were synchronised for oestrus by using two doses of PGF2α analogue injected at 12 days interval or using progestagen intravaginal pessaries impregnated with FGA or MAP over a 12 day period. Aproned teaser bucks were exposed for 36 h after termination of the oestrous synchronisation treatment. The author found that the onset of oestrus was earlier for does exposed to male compared to those in the control group. The time of male introduction after the end of the synchronisation treatment has an impact on the onset of oestrus. Immediate exposure to a ram at sponge removal causes onset of oestrus in ewes synchronised during the breeding season (Romano, 2000). The onset of oestrus occurred earlier for sheep introduced to male immediately after sponge removal compared to those exposed after 48 h from sponge removal. This author contended that the ram effect not only reduced the time between sponge removal and onset of oestrus, but also reduced variation in the onset of oestrus.

2.3 β-carotene and its implication in animal nutrition

2.3.1 Structure of β-carotene

β-carotene is a primary precursor for vitamin A, which belongs to the family carotenoids. Carotenoids are natural coloured pigments which are biosynthesised by higher plants, bacteria, algae, and yeasts (Namitha & Negi, 2010). More than 600 carotenoids are characterised structurally, and depending on their structure are classified into two groups; carotenes which contain hydrocarbons only and these include α-carotenes, β-carotene and lycopene, and xanthophylls which comprise of hydrocarbons and oxygen, such as lutein and zeaxanthin (McDonald, 2000; Namitha & Negi, 2010). Carotenoids are antioxidants, having immune functions and play role in intercellular communication (Skibsted, 2012; Stephensen, 2013). However, animals are unable to synthesise carotenoids *denovo*, as such, they rely on

the diet to supply these pro-vitamin A compounds (Biesalski *et al*., 2007). β-carotene is a sub-group of carotenes with a chemical formula $C_{40}H_{56}$ (figure 2.1).

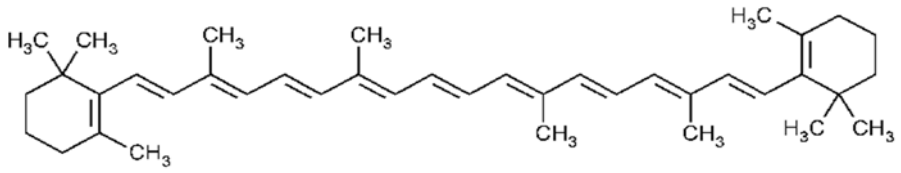

Figure 2.1 Molecular structure of β-carotene

2.3.2 Conversion of β-carotene to retinol

β-carotene like other carotenoids is mainly converted into vitamin A in the intestinal mucosa as well as in the liver and other body tissues (McDonald, 2000; Borel *et al*., 2005). The enzymes responsible for the conversion of β-carotene to vitamin A are β, β-carotene 15, 15´-monooxygenase which splits β-carotene molecule through central cleavage and β, β-carotene 9´, 10´-dioxygenase which cleavages through eccentric cleavage. β-carotene is converted into two molecules of retinal through central cleavage and into one molecule each of β-apo-carotenal and β-ionone through eccentric cleavage (Figure 2.2) (Biesalski *et al*., 2007).

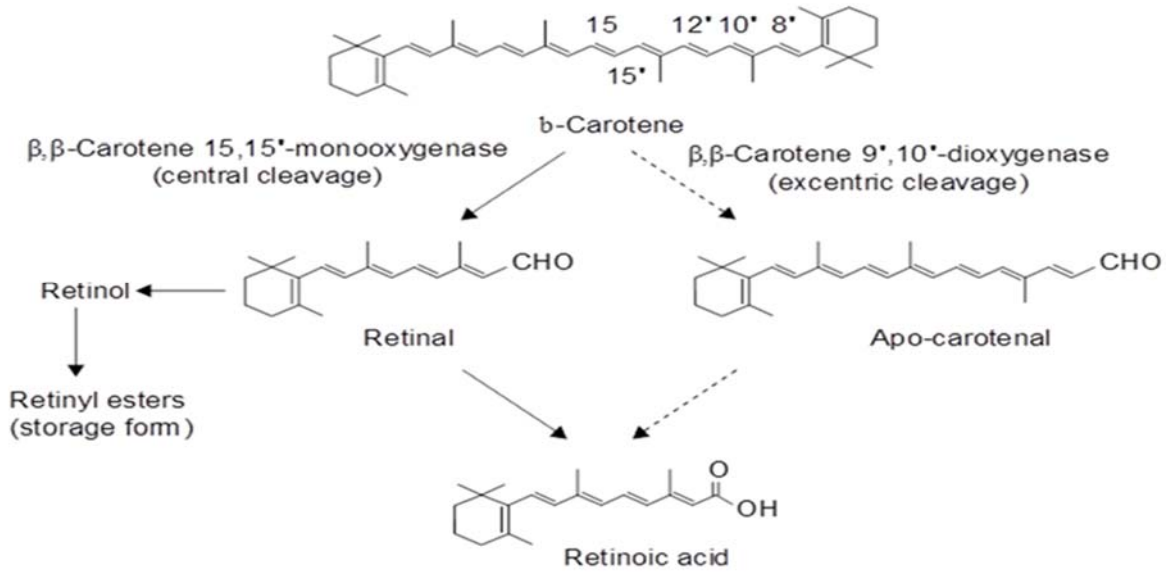

Figure 2.2 Mechanisms of β-carotene conversion through both central and eccentric cleavage (Biesalski *et al*., 2007).

2.3.3 Absorption of β-carotene

β-carotene absorption follows the absorption pathway of dietary fat because of the lipid-soluble characteristics of carotenoids (Deming & Erdman, 1999). Generally, absorption of carotenoids involves; discharge of the carotenoids through both mechanical and enzymatic breakdown of the food matrix, emulsification of the carotenoids by the lipids, transfer into the mixed micelles in the intestinal lumen, uptake into the intestinal mucosa, incorporation into the chylomicrons and final secretion to the lymphatic system (Deming & Erdman, 1999). β-carotene absorption differs in mammalian species and the absorption is between 50% to 60 % in the intestine (McDonald, 2000). Mammalian species such as cattle and horses, absorb β-carotene intact more compared to sheep, goats and rabbits which absorb a minimal quantity (McDonald, 2000). The absorption and metabolic pathway of carotenoid is indicated in (Figure 2.3).

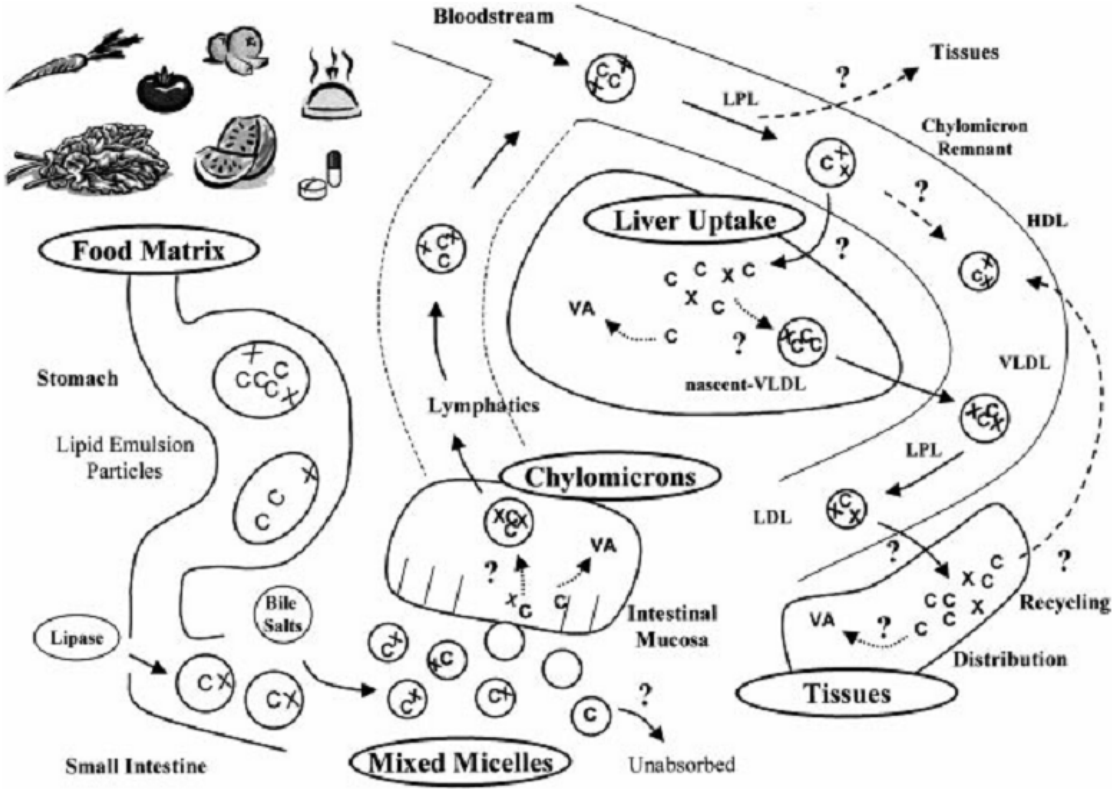

Figure 2.3 Absorption and metabolism pathway of carotenoid (Deming & Erdman, 1999).

C, carotene; X, xanthophyll; LPL, lipoprotein lipase; HDL, high density lipoprotein; VLDL, very low density lipoprotein; LDL, low density lipoprotein.

2.3.4 Factors affecting β-carotene absorption

2.3.4.1 Species difference in absorption of β-carotene

In ruminants, digestion and absorption of some nutrients vary from one species to another and also within one species. With regards to β-carotene absorption, there is a clear species difference between and within species, more especially in the intestine where most of its conversion takes place. It has been noted that sheep and goats convert β-carotene efficiently in their intestinal mucosa while cattle are poor converters of β-carotene (McDonald, 2000). This difference is attributed to variation in the activities of enzymes responsible for conversion of β-carotene to vitamin A. It has been clearly noted lower activity of β, β-carotene 15, 15'monooxygenase in cattle intestine as compared to goats which have high β, β-carotene 15, 15'monooxygenase activities (Mora *et al*., 2000). This reflects a higher conversion rate of β-carotene in the intestine of goats than in cattle. It has been indicated that the concentration of β-carotene in both serum and fat in sheep and goats were not detectable compared to cattle in which β-carotene concentration is dominant (Yang *et al*., 1992). These authors also reported that cattle have a higher concentration of β-carotene in the liver compared to sheep and goats while in milk Sheep and goats have higher vitamin A concentration compared to cow's milk; this is because sheep and goats convert most of their β-carotene to retinol (Park *et al*., 2007).

2.3.4.2 Nutritional status of the Animal

Any nutritional imbalance may interfere with many vital processes in the animal body. The nutritional status of the animal has been reported to influence the absorption of β-carotene. This nutritional influence on the absorption of β-carotene has been demonstrated through the influence of vitamin A, fat and protein status of the animal on the intestinal enzymatic activity responsible for absorption of β-carotene (Lakshman *et al*., 1996; Biesalski *et al*., 2007). The activity of the intestinal enzyme β, β-carotene 15, 15'monooxygenase indicates β-carotene conversion process, with high activity indicating increase in conversion rate and vice versa. β-carotene conversion rate increases when an animal is deficient in vitamin A and protein. When vitamin A and dietary protein is optimal, the rate of β-carotene conversion reduces. It was shown that there was higher activity of β, β-carotene 15, 15'monooxygenase in rats that were deficient in vitamin A (Parvin *et al*., 2000). This higher enzymatic activity is associated with the increase in conversion of β-carotene to vitamin A, is likely to meet the

vitamin A requirement in the body. It has been reported that the activity of β, β-carotene 15, 15'monooxygenase was decreased in protein deficient rats (Parvin *et al*., 2000). Additionally, in mid-level dietary protein there was an increased intestinal activity of 15, 15'dioxygenase in rats (Hosotani & Kitagawa, 2005).

2.3.5 Functions of β-carotene

The primary function of β-carotene is that of being the precursor of vitamin A. However, it is also noted to play other functions independently of provitamin A. Carotenoids are antioxidants involved in scavenging both singlet molecular oxygen and peroxyl radicals (Ramadan *et al*., 2001; Stahl & Sies, 2003). β-carotene has been shown to help in the body defense system and thus play a role in immune function in cows (Chew *et al*., 1987) which may lead to improve reproductive processes. β-carotene as an antioxidant was reported to prevent harmful effects of free oxygen radicals during steroidogenesis (Arellano-Rodriguez *et al*., 2009). The mechanism through which GPx activity reduces hydrogen peroxide and organic hydroperoxides is by using glutathione (GSH) as an electron donor (Kamiloglu & Beytut, 2005). It has been reported that injection of sheep with β-carotene increased erythrocyte glutathione peroxidase (GPx) activity (Kamiloglu & Beytut, 2005). These authors mentioned that the mechanism behind the increase in the activity of erythrocyte GPx activity is not known. Increase in GPx activity may play role in reducing the free radicals. β-carotene has been implicated to increase and promote the killing ability of leukocytes in the body. Supplementation of buffalo with β-carotene has enhanced the killing ability of polymorphonuclear leukocytes (PMN) (Ramadan *et al*., 2001). Polymorphonuclear leukocytes have been noted to be the major defense line against bacteria in the mammary gland (McDonald, 2000). Additionally, Chew (1996), noted that β-carotene stimulated the growth of the thymus gland and increased the number of thymic small lymphocytes. Moreover, it was mentioned that β-carotene supplementation may promote phagocytic cell killing ability in bovine blood and mammary gland during the peripartum period (Daniel *et al*. 1991). This beneficial effect of β-carotene in the killing ability of the defense cells in the mammary gland may play a positive role in improving the milk somatic cell counts.

2.3.6 Effect of β-carotene on reproductive hormones

Hormones play a major role in regulating different physiological processes which occur in the animal body. Reproductive processes in animals are almost entirely regulated by the

hormones. In female animals the most important of these hormones include gonadotrophin releasing hormone (GnRH), progesterone (P4), luteinising hormone (LH), oestradiol and follicle stimulating hormone (FSH). Some nutritional supplements can influence the concentration of these hormones and as a result may have an influence on reproduction. A number of studies have reported either positive effect or no effect of supplemental β-carotene on concentration of reproductive hormones in various animal species. However, limited studies have been conducted in goats, therefore studies from other species were discussed. Concerning P4 concentrations, contradicting results have been reported. It was found that the P4 concentration was increased through supplemental β-carotene in cattle (Greenberg, 1986) and goats (Arellano-Rodriguez *et al*., 2009). Additionally, supplemental β-carotene has increased plasma P4 concentration in canine during the oestrous cycle (Weng *et al*., 2000). It has been noted that β-carotene was not detectable in CL of non-supplemented dogs while its concentration in the CL of supplemented dogs increases in a dose dependent manner (Weng *et al*., 2000). Also β-carotene concentration in CL increases during dioestrus and especially during pregnancy, suggesting its role in regulation of luteal functions (Haliloglu *et al*., 2002). The presence of β-carotene in the CL may play a role in the progesterone synthesis (Arikan & Rodway, 2000). β-carotene, through its antioxidant activity of quenching singlex oxygen and hydroxyl radicals, which cause lipid peroxidation and cross-linking of membrane lipids (Stahl *et al*., 1997) can reduce steroidogenic cytochrome P450 and cholesterol side-chain cleavage activity in adrenal and ovarian tissue (Young *et al*., 1995). Contrary, β-carotene supplementation in cattle has been shown to have no positive effect on P4 concentrations in the blood (Bindas *et al*., 1984; Wang *et al*., 1987, 1988b; Kaewlamum, 2010; Trojačanec *et al*., 2012). Additionally, supplemental β-carotene had no effect on milk P4 concentration (Rakes *et al*., 1985). These differences in P4 concentrations following β-carotene supplementation may be attributed to variations in β-carotene concentrations in the diet, the blood concentration of β-carotene, the level, time and the duration of supplementation (Kaewlamun, 2010).

Luteinising hormone is an important hormone which is responsible in triggering ovulation in most mammalian animals, it always peaks just before ovulation. Based on the prior studies, it seems that supplemental β-carotene does not have positive effect on the LH concentration. In goats, LH concentration was reduced in β-carotene supplemented group (Meza-Herrera *et al*., 2013b). However, in other animal species, LH concentration was not influenced by β-carotene supplementation in ewes (Brozos, 2006), cows (Bindas *et al*., 1984; Wang *et al*.,

1988a) and rabbits (Tek *et al*., 2002). Lack of the beneficial effect of β-carotene on LH concentration may be attributed to the fact that β-carotene exploits LHRH-independent mechanism to positively influence the ovarian activity and as such does not have effect on LH concentration (Mezza-Herrera *et al*., 2013b).

Oestradiol-17β is an important hormone in the process of ovulation, high oestradiol-17β concentration will cause positive feedback in which large quantities of GnRH is released to stimulate a preovulatory surge of LH. With regards to oestradiol-17β concentration following β-carotene supplementation, variable results have been reported. Cows supplemented with β-carotene had higher blood oestradiol-17β concentration (Trojačanec *et al*., 2012). Contrarily, supplementation of canine with β-carotene did not have an effect on plasma oestradiol-17β concentration (Weng *et al*., 2000). The differences in oestradiol-17β concentration in these studies may be attributed to the doses of β-carotene used and species of animal. In the former study, cattle were supplemented with 200 mg of β-carotene while in the later study, dogs were supplemented with 50 mg of β-carotene.

2.3.7 Effect of β-carotene on ovarian activity and fertility

In ruminant species the reproductive performance has been reported to be improved by β-carotene supplementation (Arechiga *et al*., 1998). However, several studies have reported varying results on the effect of β-carotene supplementation on ovarian activity and fertility in ruminants. In goats, there are limited studies conducted, and as a result, studies from other species such as cattle were reviewed. Supplemental β-carotene did not positively affect the ovarian activity reflected in normal ovulation, anovulation, regular cycle and persistent corpus luteum in cattle (Kaewlamun, 2010). In addition, mean diameter of both the follicles and corpora lutea were not positively influenced by supplemental β-carotene in cattle (Trojačanec *et al*., 2012). Contrary, β-carotene supplementation has increased total follicles, total corpus luteum and total ovarian activity (Arellano-Rodriguez *et al*., 2007; Meza-Herrera *et al*., 2013b). Concerning the oestrous parameters, β-carotene supplementation had no effect on the duration and incidence of oestrus in cows (Folman *et al*., 1979; Wang *et al*., 1988b). The lack of beneficial effect of β-carotene on oestrous parameters may be attributed to the failure of β-carotene to exert its influence on the oestradiol-17β and LH concentrations. Oestrous parameters, such as the onset and the duration of oestrus can be influenced by oestradiol-17β and LH concentration. High oestradiol-17β levels causes onset of oestrus and a surge in gonadotrophin releasing hormone (GnRH) that lead to preovulatory LH peak at

oestrus and thus ovulation occurs toward the end of oestrus (Rahman *et al*., 2008). It has been reported that LH concentration was not positively influenced by β-carotene supplementation in ewes (Brozos, 2006), cows (Bindas *et al*., 1984; Wang *et al*., 1988a) and rabbits (Tek *et al*., 2002). In addition, oestradiol-17β was not affected by supplementation of β-carotene in canine (Weng *et al*., 2000). However, some studies have noted improved oestrous performance in β-carotene supplemented heifers. In addition, β-carotene deficient heifers exhibited less intense signs of oestrus (Wang *et al*., 1982).

The overall influence of β-carotene supplementation on ovarian and oestrual activity may have a direct influence on the fertility of animal. Conception rate can negatively be affected when there is a wide variation in the onset of oestrus when fixed time artificial insemination (AI) is implemented. The conception rate is an important aspect in the reproductive management, its rate always has an impact on the farm income. Therefore, nutritional supplements that aim to increase the conception rate are highly promoted. Antioxidants such as β-carotene has been implicated to improve conception rate. It has been noted that β-carotene supplementation for an extended period of time may improve pregnancy rate (Aréchiga *et al*., 1998; De Ondarza *et al*., 2009; Ay *et al*., 2012). Out of seven 21 day periods of β-carotene supplementation, pregnancy rate was only increased during the last two 21 day periods of β-carotene supplementation in cattle (De Ondarza *et al*., 2009). Similarly, higher rates of pregnancy were recorded in cows supplemented with β-carotene (Trojačanec *et al*., 2012; Aréchiga *et al*. (1998). It was mentioned that the prolonged supplementation with β-carotene is necessary to increase tissue concentrations of β-carotene molecule to positively affect pregnancy rate (Aréchiga *et al*., 1998). Therefore, the antioxidant activity of β-carotene might have provided a favourable uterine environment for implantation and embryo development (Weng *et al*., 2000; Ay *et al*., 2012) which subsequently might led to improved pregnancy rate, especially during hot season. This may indicate the role of β-carotene in stress reduction during the hot season. However, other reports regarding the influence of β-carotene on the conception rate have noted a lack of beneficial effect. Contrary, several studies in cattle have reported that β-carotene supplementation had no positive effect on conception rate (Folman *et al*., 1979; Wang *et al*., 1982; Bindas *et al*., 1984; Wang *et al*., 1988ab). Additionally, De Ondarza *et al*. (2009) had noted that β-carotene supplementation of 425 mg/cow/day for seven 21-day periods did not improve the overall pregnancy rate.

2.3.8 Effects of β-carotene supplementation on milk yield and components

β-carotene with its antioxidant activity has been reported to possibly have a beneficial impact on mammary function (Chew, 1993). However, milk yield and components following β-carotene supplementation has been reported with variable outcomes. In goats, there are limited studies with regard to the influence of β-carotene on milk yield and components. In cows, it has been reported that milk yield was not improved with supplemental β-carotene (Rakes *et al*., 1985; Wang *et al*., 1987; De Ondarza *et al*., 2009; De Ondarza & Engstrom 2009). Similarly, in sheep, there was no beneficial effect of supplemental β-carotene on milk yield (Brozos, 2006). Contrary, it has been reported that supplemental β-carotene increases milk production in cattle (Oldham *et al*., 1991; Aréchiga *et al*., 1998). The increase of milk yield in β-carotene supplemented group was attributed to high β-carotene antioxidant activity in the mammary gland, which has helped to improve alveolar epithelial cell function (Aréchiga *et al*., 1998). However, the differences in the influence of supplemental β-carotene on milk yield had been attributed to variations in β-carotene concentrations in diet, the blood concentration of β-carotene, the level, time and duration of β-carotene supplementation (Kaewlamun, 2010).

Regarding the influence of β-carotene supplementation on milk components, studies have reported conflicting results. β-carotene supplementation had no positive effect on milk fat, protein and lactose composition (Kaewlamun, 2010; Machpesh, 2013). In addition, it was noted that milk protein percentage remains unchanged following supplemental β-carotene (De Ondarza *et al*., 2009). Contrary, it was reported that supplemental β-carotene increases milk fat percentage (De Ondarza *et al.,* 2009), but Oldham *et al*. (1991) found lower fat % in cows supplemented with β-carotene. Two possible modes of action on the increase in milk fat were suggested by De Ondarza *et al*. (2009); firstly, through the positive effect of β-carotene on the rumen cellulolytic bacteria, as observed by Hino *et al*. (1993) and secondly through alteration of rumen biohydrogenation and reduced formation of trans-10 isomers in the rumen resulting in less fat depression. However, the differences in milk composition from these studies may be attributed to variations in β-carotene concentrations in the diet, the blood concentration of β-carotene, the level, time and the duration of supplementation (Kaewlamun, 2010).

Somatic cell count (SCC) is an important index in dairy industry that is used as an indicator of milk quality for grading purposes (Reneau, 2001; Yang & Li, 2015). The lower the SCC in

milk, the higher is its quality and the opposite is true. Concerning the influence of supplemental β-carotene on milk SCC, De Ondarza *et al.* (2009), had reported no positive effect of β-carotene on SCC in high producing Holstein cows. Similarly, SCC was not positively influenced by β-carotene supplementation during the dry-off and early lactation period (Bindas, 1984). Moreover, SCC was not affected by β-carotene supplementation (Wang *et al.*, 1987; Oldham *et al.*, 1991). In contrast, SCC was lower in β-carotene supplemented cows compared to those that were not supplemented (Rakes *et al.*, 1985; Wang *et al.*, 1988b). This reduction in SCC may be attributed to the protection of functional integrity of epithelium lining of the mammary secretory system by β-carotene antioxidant activity. It was mentioned that β-carotene deficiency may impair the functional integrity of epithelium lining of the mammary secretory system, leading to invasion and establishment of mastitis organisms (Rakes *et al.*, 1985). However, SCC may also be influenced by other factors such as breed, stage of lactation, age, oestrus, milk production and management conditions (Poutrel *et al.*, 1997).

2.3.9 Effect of β-carotene supplementation on body weight

Some nutritional supplements may cause metabolic changes that can influence both the body weight and condition score of an animal. These metabolic changes may modulate the endocrinological pathways that will possibly interfere with reproductive performance of an animal. In goats there are limited studies regarding the influence of β-carotene on the body weight, therefore, studies from other related farm animal species were used. It was revealed that supplemental β-carotene has no positive effect on the body weight in goats (Arellano-Rodriguez *et al.*, 2007; Meza-Herrera *et al.*, 2011; Meza-Herrera *et al.*, 2013ab). However, some studies have reported higher growth rate in heifers supplemented with β-carotene. Folman *et al.* (1979) fed a gelatin capsule containing 0.3 mg β-carotene/kg body weight and had noted that heifers given β-carotene had a higher growth rate compared to those heifers not obtain it. Although Folman *et al.* (1979) in their study observed increase in weight gain, but did not attribute it to the effect of supplemental β-carotene, and instead they attributed it to high consumption of feed by supplemented group. Similarly, Greenberg *et al.* (1986) in their trial, supplemented heifers with β-carotene 625 mg/head/day and they observed the difference in weight gain during the last trimester and postpartum. However, the reasons behind the positive effect of β-carotene on weight gain were not stated. However, it has been reported that addition of β-carotene can improve rumen bacteria function and digestion of

cellulose (Hino *et al*., 1993). Therefore, the increase in weight gain after β-carotene supplementation may be attributed to improved rumen function and cellulose digestion. However, the differences in these studies may be attributed to species, time and duration of supplementation and level of β-carotene. With regards to birth weight, it has been reported that β-carotene supplementation had no positive effect on birth weight of calves (Greenberg *et al*., 1986; Kaewlamun, 2010) and piglets (Kostoglou *et al*., 2000). There was limited information on the effect of β-carotene supplementation in goats.

2.3.10 Summary

From this literature overview, it is clear that oestrous synchronisation using progesterone in combination with a co-treatment such as eCG could be used efficiently to improve fertility in goats. However, it was noted that the use of eCG repeatedly for oestrous synchronisation reduces fertility in goats. Male effect has been reported to induce oestrus, increase LH concentration and initiates ovulation in goats. On the other hand, previous studies on the effect of β-carotene supplementation on production and reproduction parameters in goats have been limited and inconsistent. Therefore, this study sought to evaluate the effect of β-carotene supplementation on production and reproduction parameters in Saanen goats and also whether the addition of male effect into progesterone primed oestrous synchronisation could be as effective as addition of eCG.

CHAPTER THREE

3. MATERIALS AND METHODS

3.1 Ethical approval

The materials and procedures of this study were approved by the animal ethics committee of the University of Pretoria (Project no.EC108-14).

3.2 Experimental site and duration

This experiment was started in February 2015 during the breeding season and ended in late October 2015, one month after the kidding season. The experiment was conducted at the experimental farm of the University of Pretoria, South Africa. The site is located at latitude 25°44'30" S, longitude 28°15'30" E, at the elevation of 1360 meters above sea level. The climate is sub-tropical with warm and humid condition in summer. However, in winter the climate is dry, cold and sunny. The average rainfall in summer is approximately 650 mm (Van Niekerk *et al*., 2009).

3.3 Experimental animals, feeding and management system

A total of 60 female Saanen goats age 1-6 years were used in this experiment. All the experimental animals were fed with a total mixed ration (TMR) composed of a mixture of Lucerne, *Eragrostis curvula* hay, maize meal, molasses and high protein concentrate (Table 3.1). Water access was *ad libitum* throughout the experimental period. The animals were kept under intensive system.

Table 3.1 Percentages of feed ingredients for the total mixed ration

Ingredients	**Amount % DM**	**Amount as fed kg/animal**
Lucerne hay	43.81	1.600
Eragrostis hay	24.10	0.900
Maize meal	16.06	0.600
Molasses	9.05	0.350
High protein concentrate	6.98	0.250
Total	100	3.700

3.4 Experimental design and treatments

Sixty female Saanen goats were allocated into one of the following two groups and balanced per group based on their weight, age and parity during the breeding season; Group 1: β-carotene supplemented (n=30) and Group 2: non-supplemented (n=30) group. Each of the two groups were sub-divided into two sub-groups according to the oestrous synchronisation protocol; (A) Progesterone (CIDR) + Prostaglandin (PGF2α) + equine chorionic gonadotropin (eCG) and (B) Progesterone (CIDR) + Prostaglandin (PGF2α) + male effect (Me). The experimental design was 2*2 factorial as indicated in the Table 3.2.

Table 3.2 The experimental treatments and number of animals per group

β-carotene supplementation	**CIDR + PGF2α + eCG**	**CIDR + PGF2α + Me**
β-carotene non-supplemented group	Group 1A(n=15)	Group 2A(n=15)
β-carotene supplemented group	Group 1B(n=15)	Group 2B(n=15)

3.5 β-carotene supplementation

For the β-carotene groups, goats were individually supplemented with β-carotene given orally (100 mg/goat /day) (Pennville Pty Ltd, Animal nutrient solutions, South Africa) for a period of 60 days started from 28 day before oestrous synchronisation until 17 day post artificial insemination (AI). And also goats were individually supplemented orally with β-carotene (50 mg/goat/day) during the pregnancy period, starting from first day of drying off until kidding (approximately 40 days). Each 10 g paste contained 100 mg β-carotene according to the company instructions, and it was dissolved in water and administered to animals orally using a syringe. For the supplementation during the pregnancy, the concentration was adjusted to 50 mg of β-carotene and also administered orally.

3.6 Oestrous synchronisation protocols

Two synchronisation protocols were used in this experiment. The animals were group into two according to the protocols; (1) CIDR + PGF2α + eCG group and (2) CIDR + PGF2α + Me group. All animals regardless of their group were treated with progesterone using

controlled internal drug releasing device (CIDR-G) (Pfizer, New Zealand) containing 0.3 g progesterone left intravaginally for 11 days. At CIDR removal, all does were injected intramuscularly with 150µg PGF2α analogue (Intervet Schering-Plough Animal Health, South Africa). In addition, at CIDR removal, all does in group 1 were injected intramuscularly with 300 IU (2.5 ml) of eCG (Intervet Schering-Plough Animal Health, South Africa). While in group 2, two aproned bucks were introduced immediately at CIDR removal and left with the females throughout for 72 hours. The female goats were isolated from the males for a period of one month before male introduction. The males were kept away at a distance of approximately 1 kilometre.

3.7 Semen collection and artificial insemination (AI)

3.7.1 Semen collection

The semen for AI was collected from 5 bucks using an electro ejaculator (Ramsem, South Africa) as described by Sundararaman *et al*. (2007) with some few modification. Briefly, the buck was restrained in a lateral recumbency position, faeces were removed and the urethral opening was cleaned. Then the rectal probe was lubricated with a lubricating jelly (Glycerine K-Y Gel) (Johnson & Johnson Pty Ltd, South Africa), and inserted in to the rectum of a restrained buck at approximately 10 cm. By using the manual control knob of the instrument the power output of 3-5 volts was generated and held for 4-5 sec, and again brought to 0. This procedure was repeated after a rest period, equal to the duration of electrical stimulation until ejaculation took place. During collection and examination, the semen was protected from temperature shock and exposure to direct sunlight. Following semen collection, each ejaculate was immediately evaluated for semen mass motility. The mass motility of semen was assessed by placing 10 µl semen on a pre-warmed stage (37°C) glass slide 76x26x1mm and covered with a cover slip 18x18mm (LASEC Pty Ltd, South Africa) and then examined on a pre-warmed stage microscope (Olympus Cx21). The semen sample was scored using a scale ranging from 0 (no wave movement) to 5 (extreme wave movement) as described by Dogan *et al*. (2005). Only semen with mass motility equal or more than 3 was used.

3.7.2 Artificial insemination

The artificial insemination procedure was carried out by an experienced animal scientist. The procedures followed were as described previously by Steyn (2005). Briefly, a speculum with

a build in light source and pipette connected to a 1 ml syringe were used. The animals were restrained by placing the hindquarters over a rail with the head facing downwards and the front legs standing on the floor. The rail height was from 80-90 cm. The hind legs of the doe was secured by one assistant. The vulva of doe was wiped with a paper towel to prevent contamination of semen during insemination procedure. The speculum was lubricated with a lubricating jelly and inserted carefully into the vagina to locate the cervical opening. Thereafter, the inseminating pipette was loaded to the syringe plunger. Each animal was inseminated with 0.2 ml fresh undiluted semen with a concentration of 300-800×10^6 sperm. All does were inseminated twice cervically at fixed times of 48 and 60 h following CIDR withdrawal (Motlomelo *et al*., 2002).

3.8 Data collection and analysis

3.8.1 Weighing

All the animals in the trial were weighed at two weeks interval throughout the experimental period. Each kid weight was taken immediately after kidding. Body weight (kg) was determined with a LS4H electronic scale attached to the hang crate of internal dimensions 1250mm x 500 mm (TAL-TEC Ltd, South Africa).

3.8.2 Blood sampling for hormonal and enzyme assay

Five goats from each treatment group were selected using the same method of their allocation into different treatments, for blood samples collection. Blood samples (4.0 ml) through jugular veni-puncture were collected in BD vacutainer tubes (Lithium heparin 4.0 ml.13x75 mm). The blood samples were collected at CIDR insertion, 8 h and 48 h after CIDR removal as well as 12 days post AI. Then immediately after collection, the blood samples were centrifuged at 3000 × g for 20 minutes. The blood plasma recovered was then stored at -20°C until assayed for progesterone, oestradiol-17 β and glutathione peroxidase activity.

3.8.3 Analysis of hormones and enzyme activity

Progesterone and oestradiol concentration as well as glutathione peroxidase activity level were analysed using commercial kits using FC Microplate Photometer Thermo Scientific Multiskan®. The plasma progesterone and oestradiol-17β concentrations were analysed using progesterone ELISA DE1561 kit (Demeditec-Germany) and oestradiol-17β ELISA DE2693

kit (Demeditec-Germany) while for glutathione peroxidase activity glutathione peroxidase assay kit (ab102530-Abcam) was used.

3.8.4 Ultrasonographic evaluation of ovarian activity

A real-time B-mode ultrasound scanner (Aloka, 500 SSD, Japan) equipped with a transrectal 7.5-MHz linear array probe (UST-660-7.5 model) was used to evaluate all the ovarian activity and pregnancy diagnosis. The animal was restrained in a standing position. The rectal probe was lubricated with a lubricating jelly before the probe was inserted in to the rectum. The ultrasonographic evaluation of ovarian follicular development (number of follicles and the size of preovulatory follicles) was performed at 21 and 28 day of supplementation period before oestrous synchronisation and also performed at AI day. The follicle diameter of 2 mm and above were measured during the study. At day 12 after AI, ultrasound was performed for corpus luteum detection and measurement of corpus luteum size. The pregnancy diagnosis was also performed at day 35 after AI using the same rectal probe.

3.8.5 Oestrous onset and duration monitoring

The onset and duration of oestrous were monitored after CIDR withdrawal using two bucks wearing aprons for a period of 72 hours at 8 hours interval (6:00 am, 2:00 pm and 10:00 pm). The female was considered on oestrus when it accepted mounting by the buck and out of oestrus when it did not allow to be mounted.

3.8.6 Evaluation of reproductive parameters

The following parameters were evaluated: Oestrous response, as number of does in oestrus/number of total does treated*100. The Onset of oestrus, as the time from CIDR removal to first acceptance of mounting. The duration of oestrus, as the time from the first acceptance of mounting to the first refusal of mounting. And also the conception rate was evaluated as the number of does conceived/number of total does mated *100. The gestation length, as the period starting from the AI date until kidding. Litter size, as the number of kids born per does kidded and birth weight as the average weight of kids in each treatment at birth.

3.8.7 Milk collection and analysis

3.8.7.1 Milk collection

All the goats were milked every morning and evening, daily, using milking machine. The milk yield for each goat was recorded at each milking for a period of 30 days starting 5 days after kidding and at the same period milk samples were also collected once a week from five goats of each group. The milk samples collected were preserved in milk sampling vials provided by the Lactolab Pty Ltd using a Broad Spectrum Microtabs II tablet (Advanced Instruments, Inc. Norwood, Massachusetts, USA), a preservative containing Bronopol and Natamycin prevent growth of bacteria, yeast and mold. Colostrum samples were collected once a day in the morning for three consecutive days, starting from the first day of kidding.

3.8.7.2 Milk analysis

All the colostrum and milk samples were analysed for protein, fat and lactose percentage as well as somatic cell counts. After milk collection, the samples were transported for analysis to the Lactolab Pty Ltd laboratory at the Agricultural Research Council, Irene, Pretoria. The milk samples were analysed for somatic cell counts with the aid of the Bentley Flow Cytometer (Chaska, Minnesota 55318, USA). For milk protein, fat and lactose percentage using the Bentley Fourier Transform Spectrometer (Chaska, Minnesota 55318, USA). Each of the colostrum and milk samples was pooled during the analyses into β-carotene supplemented and non-supplemented group.

3.9 Statistical analysis

The data were subjected to statistical analysis using the general linear model (GLM) for onset and duration of oestrus, number and size of follicles, corpus luteum size, gestation length, litter size and kidding weight, and categorical modelling (CATMOD) procedures for oestrous response and conception rate of SAS (version 9.4; 2014, Inc. Cary Institute, North Carolina, USA). The model used for these parameters included the fixed effects of synchronisation and supplementation. In the supplementation group, the body weight, progesterone and oestrdiol-17β concentrations, glutathione peroxidase and milk yield as well as the milk components were compared using student's t-test. The milk composition and quality between the milk types (Colostrum and ordinary milk) as well as the effect of sex on kidding weight were

compared using student's t-test. The kidding weight of different litter sizes was analysed using completely randomised design (CRD) and Duncan multiple range test (DMR) was used to separate their means. The means were considered significant at $P<0.05$. Correlation between the corpora lutea size and Progesterone concentration was analysed using Pearson correlation of SPSS (Version 23.0; 2015, Armonk, NY. IBM Corp).

CHAPTER FOUR

4. RESULTS

4.1 Effect of β-carotene supplementation on ovarian activity and fertility of Saanen does following oestrous synchronisation

One animal was removed from the experiment due to reproductive tract abnormality. Supplementation of β-carotene during the breeding period had no positive effect (P>0.05) on the body weight of does (fig.4. 1), number of follicles, size of largest follicle and size of corpus luteum (Table 4.1). The response to oestrus, onset and duration of oestrus, and conception rate was also not affected (P>0.05) by β-carotene supplementation (Table 4.2). β-carotene supplementation had significantly (P<0.05) increased the progesterone concentration (8.85±0.41 ng/ml versus 7.49±0.41 ng/ml) at day 12 post artificial insemination (AI) (Table 4.3). Oestradiol-17β and P4 concentrations were not influenced by supplemental β-carotene 8 h and 48 h following CIDR withdrawal (Table 4.3). The glutathione peroxidase activity was significantly (P<0.05) increased by β-carotene supplementation before CIDR insertion and 8 h, 48 h and 12 days following CIDR withdrawal (Table 4.3). There was a significantly positive correlation (r=0.70; P<0.01) between the size of CL and P4 concentration on day 12 post AI in both the groups.

The synchronisation protocol did not affected the response to oestrus, onset and duration of induced oestrous period (P>0.05) (Table 4.2). However, the synchronisation protocol had a significant effect (P<0.05) on conception rate (Table 4.2). The male effect group had significantly (P<0.05) higher conception rate (97%) than the eCG group (72%). On the other hand, the oestrous synchronisation protocol did not have a significant effect on the number of follicles, size of largest follicles, and size of corpus luteum (P>0.05) (Table 4.1). There was no interaction effect between β-carotene supplementation and the synchronisation protocol on the parameters measured as shown in Table 4.1, 4.2 and 4.4.

β-carotene supplementation did not affected (P>0.05) the gestation length, kids birth weight, and litter size as shown in Table 4.4. However, regardless of β-carotene supplementation, sex had a significant effect (P<0.05) on the kids birth weight as shown in Table 4.5. Male kids had higher (P<0.05) birth weight compared to their females counterparts. No significant difference between singletons and twins with regard to birth weight was observed. However, singletons were significantly heavier than triplets (P<0.05). Quadruplets and quintuplets had significantly (P<0.05) lower birth weight than singletons, twins and triplets (Table 4.5).

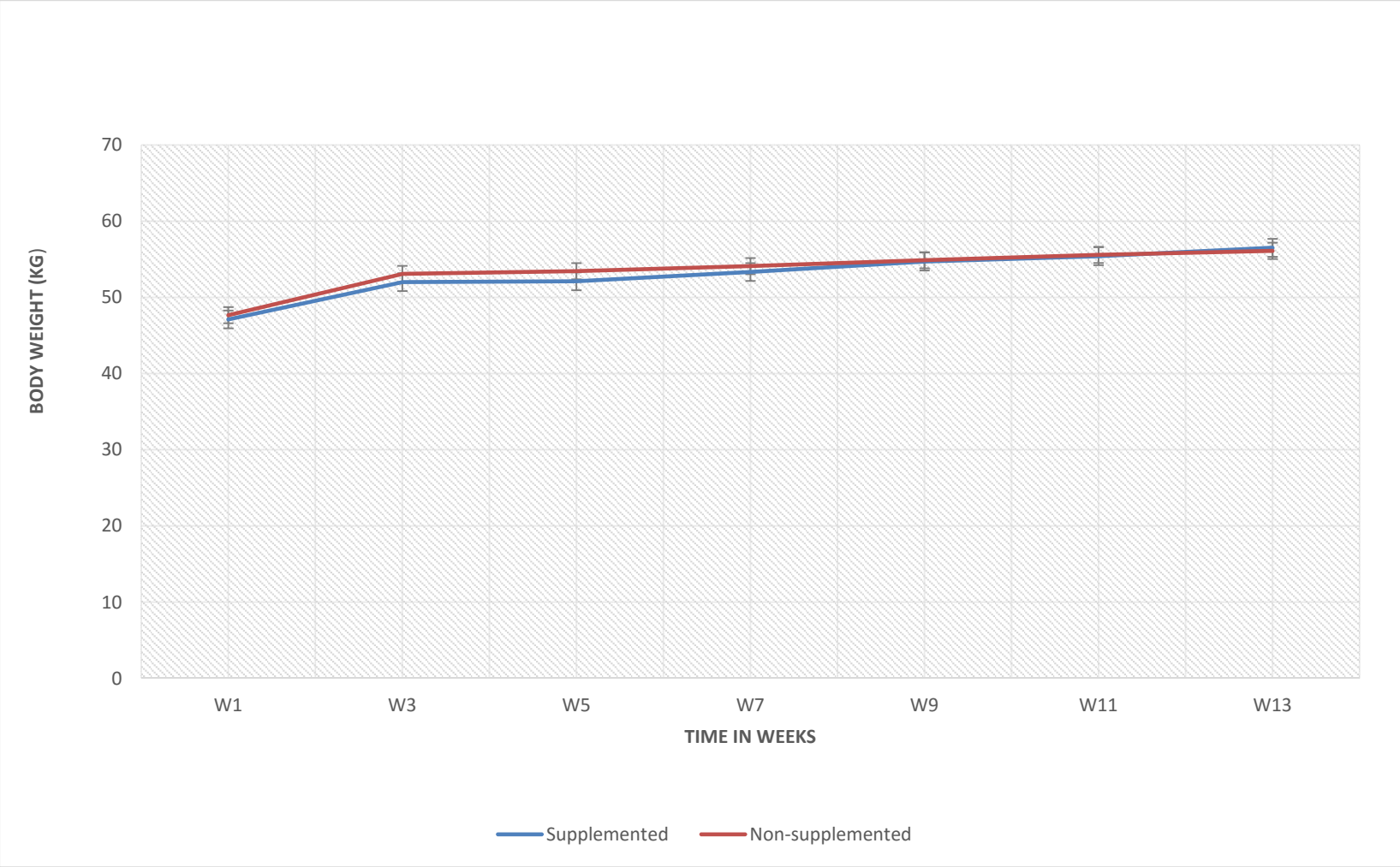

Fig. 4.1 Mean body weight of Saanen does supplemented with β-carotene

Table 4.1 Effect of β-carotene supplementation and synchronisation protocols on ovarian activity of Saanen does.

	Number of follicles (Mean±SE)			Size of largest follicle(mm) (Mean±SE)			Size of corpus luteum(mm) (Mean±SE)
	Day 21	Day 28	48 h	Day 21	Day 28	48 h	Day 12
Supplementation	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**
β-carotene supplemented	3.94±0.55	3.13±0.31	2.31±0.37	8.53±0.38	8.34±0.35	5.75±0.74	16.13±0.58
Non-supplemented	4.19±0.50	3.63±0.31	1.88±0.37	8.13±0.34	8.46±0.35	6.63±0.74	15.08±0.58
Synchronisation	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**
eCG	3.83±0.54	3.44±0.31	2.75±0.37	8.35±0.37	8.44±0.35	6.03±0.74	15.90±0.58
Me	4.29±0.52	3.31±0.31	1.86±0.53	8.31±0.36	8.36±0.35	6.35±0.74	15.31±0.58
Interaction	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**
β-carotene supplementedxeCG	3.17±0.81	2.87±0.43	2.75±0.53	8.65±0.56	8.39±0.50	4.85±1.04	15.35±0.81
β-carotene supplementedxMe	4.71±0.75	3.38±0.43	1.88±0.53	8.40±0.52	8.29±0.50	6.65±1.04	14.80±0.81
Non-supplementedxeCG	4.50±0.70	4.00±0.43	2.75±0.53	8.05±0.47	8.50±0.50	7.21±1.04	16.45±0.81
Non-supplementedxMe	3.88±0.70	3.25±0.43	2.31±0.45	8.23±0.49	8.43±0.50	6.05±1.04	15.81±0.81
Overall	4.10±0.63	3.38±0.37	2.31±0.37	8.31±0.44	8.29±0.43	6.19±0.89	15.60 ±0.70

ns: not significant, eCG: equine chorionic gonadotropin, Me: Male effect, SE: Standard error, mm: millimeter, day 21 and 28: following the start of supplementation before oestrous sychronisation, 48 h: following CIDR withdrawal, day12: following artificial insemination

Table 4.2 Effect of β-carotene supplementation and synchronisation protocol on oestrous response and conception rate of Saanen does.

	Oestrous response (%)	Onset of oestrus (h) (Mean±SE)	Duration of oestrus (h) (Mean±SE)	Conception rate (%)
Supplementation	**ns**	**ns**	**ns**	**ns**
β-carotene supplemented	100	28.41±0.91	30.06±0.81	87
Non-supplemented	100	26.00±0.91	30.04±0.81	83
Synchronisation	**ns**	**ns**	**ns**	*****
eCG	97	28.29±0.94	29.43±0.83	72
Me	100	26.13±0.90	30.67±0.80	97
Interaction	**ns**	**ns**	**ns**	**ns**
β-carotene supplementedxeCG	100	28.00±1.32	29.14±1.17	71
β-carotene supplementedxMe	100	24.00±1.32	30.93±1.13	93
Non-supplementedxeCG	93	28.57±1.32	29.71±1.17	73
Non-supplementedxMe	100	28.27±1.28	30.40±1.13	100
Overall mean	99	27.17±1.11	30.07±1.00	85

ns: not significant; superscript * show significant difference at (P<0.05), eCG: equine chorionic gonadotropin, Me: Male effect, SE: Standard error, h: hour

Table 4.3 Effect of supplemental β-carotene on hormonal concentration and glutathione peroxidase activity of Saanen goats

Hormones and Glutathione peroxidase activity (Mean±SE)	Time							
	At CIDR insertion		8 h following CIDR withdrawal		48 h following CIDR withdrawal		12 days following AI	
	Suppl	Non-suppl	Suppl	Non-suppl	Suppl	Non-suppl	Suppl	Non-suppl
Progesterone (ng/ml) (Mean±SE)	3.92±0.41[a]	4.41±0.41[a]	1.59±0.11[a]	1.67±0.11[a]	1.11±0.02[a]	1.08±0.02[a]	8.85±0.41[a]	7.49±0.41[b]
Oestradiol-17β (pg/ml) (Mean±SE)	1.39±0.24[a]	1.47±0.24[a]	3.90±0.13[a]	3.85±0.13[a]	9.12±0.42[a]	8.70±0.42[a]	0.69±0.16[a]	0.75±0.16[a]
Glutathione peroxidase activity (mU/ml) (Mean±SE)	1.10±0.08[a]	0.70±0.08[b]	1.22±0.12[a]	0.68±0.12[b]	1.17±0.11[a]	0.59±0.11[b]	1.17±0.08[a]	0.62±0.08[b]

Values in the same row with different letters are significantly different at $P<0.05$.

Table 4.4 Effect of β-carotene supplementation and oestrous synchronisation protocols on kidding weight, gestation length and litter size

	Kidding weight (kg)(Mean±SE)	Gestation length (days)(Mean±SE)	Litter size (Mean±SE)
Supplementation	**ns**	**ns**	**ns**
Supplemented	2.98±0.10	150.40±0.46	2.13±0.19
Non- supplemented	2.95±0.11	150.33±0.51	2.25±0.21
Oestrous synchronisation	**ns**	**ns**	**ns**
eCG (Mean±SE)	2.86±0.10	150.40±0.53	2.25±0.22
Me (Mean±SE)	3.07±0.12	150.33±0.43	2.13±0.18
Interaction	**ns**	**ns**	**ns**
Non-supplementedxeCG	2.67±0.18	150.00±0.79	2.00±0.33
Non-supplementedxMe	3.22±0.15	150.67±0.64	2.25±0.27
SupplementedxeCG	3.04±0.12	150.80±0.71	2.50±0.29
SupplementedxMe	2.92±0.14	150.00±0.58	2.00±0.25
Overall mean	3.18±0.11	150.36±0.48	2.18±0.20

ns: not significant, eCG: equine chorionic gonadotropin, Me: Male effect, SE: Standard error

Table 4.5 Effect of sex and litter size on the kidding weight of Saanen kids

Parameters	Kidding weight (Kg)(Mean±SE)
Sex	
Male	3.17±0.11[a]
Female	2.75±0.10[b]
Litter size	
Single	3.96±0.20[a]
Twin	3.42±0.10[ab]
Triplet	2.92±0.13[b]
Quadruplet	2.55±0.19[c]
Quintuplet	1.97±0.29[c]

Values in the same column with different letters are significantly different at P<0.05

4.2 Effect of β-carotene supplementation on the milk yield and milk components of Saanen goats

One animal was removed from the experiment due to reproductive tract abnormality. The result which shows the effect of supplemental β-carotene on milk yield, components and quality is presented in Table 4.6. β-carotene supplementation did not improve any of the milk parameters measured. The mean total milk yield and daily milk yield over 30 days period during early lactation did not differ significantly (P>0.05) between β-carotene supplemented group and non-supplemented group. The normal milk fat %, protein %, lactose % and the SCC were not significantly affected (P>0.05) by β-carotene supplementation. Moreover, the colostral fat %, protein %, lactose and the SCC were not significantly affected (P>0.05) by β-carotene supplementation. The influence of milk type on milk components and quality is presented in Table 4.7. Milk type had a significant effect on the milk components and quality. The fat %, protein % and SCC in colostrum were significantly higher (P<0.05) compared to normal milk. However, lactose was lower (P<0.05) in colostrum compared to normal milk lactose.

Table 4.6 Milk yield and components of Saanen goats supplemented with β-carotene

Parameters	**β-carotene supplemented group (Mean±SE)**	**Non- supplemented group (Mean±SE)**	**Level of significance**
Milk yield (30 days period), kg	108.78±5.48	107.30±6.13	ns
Milk yield, kg/day	3.63±0.18	3.58±0.20	ns
Milk fat %	3.76±0.23	4.06±0.23	ns
Milk protein %	3.29±0.08	3.39±0.08	ns
Milk lactose %	4.57±0.05	4.54±0.05	ns
Milk SCC x 1000 cells/ml	881.55±207.50	930.53±207.50	ns
Colostral fat%	6.89±0.47	6.56±0.45	ns
Colostral protein %	7.33±0.54	6.30±0.52	ns
Colostral lactose %	3.62±0.14	3.73±0.14	ns
Colostral SCC x 1000 cells/ml	2213.86±344.33	2030.10±332.66	ns

ns: non-significant difference, SCC: somatic cell count, SE: Standard error

Table 4.7 Effect of milk type on milk composition and quality of Saanen goats

Milk type	Fat % (Mean±SE)	Protein % (Mean±SE)	Lactose % (Mean±SE)	SCC x 1000 cells /ml (Mean±SE)
Colostrum	6.72±0.26[a]	6.81±0.25[a]	3.68±0.07[b]	2121.98±203.11[b]
Milk	3.91±0.22[b]	3.34±0.21[b]	4.55±0.06[a]	906.04±172.84[a]

Values in the same column with different letters are significantly different at P<0.05, SCC: somatic cell count, SE: Standard error

CHAPTER FIVE

5. DISCUSSION

5.1 Effect of β-carotene supplementation on ovarian activity and fertility of Saanen does following oestrous synchronisation

β-carotene as an antioxidant has been reported with variable influences on the reproductive parameters in farm animals. In goats, limited studies have been reported about the influence of β-carotene supplementation on body weight, response to oestrus, onset and duration of oestrus, number of follicles, size of largest follicle, corpus luteum size, progesterone (P4) concentration, oestrdiol-17β concentration, glutathione peroxidase activity (GPx), conception rate, gestation length, birth weight and litter size. Therefore, findings from the other related farm animal species were used to compare and contrast the results of the present study. Previous studies in goats have revealed that β-carotene supplementation had no positive effect on the body weight (Arellano-Rodriguez *et al*., 2007; Meza-Herrera *et al*., 2011; Meza-Herrera *et al*., 2013ab). The results of the previous studies concur with the present study in which β-carotene did not have influence on body weight of Saanen does. However, some studies in cattle have recorded higher growth rate in heifers supplemented with β-carotene (Folman *et al*., 1979; Greenberg *et al*., 1986). Folman *et al*. (1979) did not attribute the higher growth rate to the effect of β-carotene but rather to high consumption of more feed by supplemented group. It has been reported that β-carotene can improve rumen bacteria and digestion of cellulose (Hino *et al*., 1993). The increase in weight gain following β-carotene supplementation may be attributed to the improved rumen bacteria function and cellulose digestion. However, lack of beneficial effect of β-carotene on body weight in the present study may be attributed to the lower doses of β-carotene supplemented as the values given were calculated based on those suggested in cattle. To date, there are no approved supplemental doses of β-carotene neither in cattle nor in goats.

Previous findings (Arellano-Rodriguez *et al*., 2009; Meza-Herrera *et al*., 2013b) reported that supplemental β-carotene had improved the ovarian activity in terms of total number of follicles and corpora lutea. However, the present study did not show positive effect of β-carotene on the ovarian activity reflected in the number of follicles, size of largest follicle and size of corpora lutea. The difference between the present and the previous findings in terms of number of follicles is not clear as the doses of β-carotene supplemented in the present

study is higher (100 mg) and in the previous study is lower (50 mg). In the present study, supplemental β-carotene increased the plasma P4 concentration and GPx activity without influencing oestradiol-17β concentration. The high P4 concentration following β-carotene supplementation in this study is supported by numerous reports (Greenberg, 1986; Weng *et al*., 2000; Arellano-Rodriguez *et al*., 2009). The increase in plasma P4 concentration on day 12 following AI might be attributed to the involvement of β-carotene in the steroidogenic process. It had been observed that β-carotene concentration in the CL of dogs supplemented with β-carotene increased in a dose dependent manner (Weng *et al*., 2000) and may play a role in the progesterone synthesis (Arikan & Rodway, 2000; Weng *et al*., 2000), prevents lipid peroxidation by removing oxygen free radicals (Young *et al*., 1995). During steroidogenesis, cholesterol is converted to pregnenolone catalysed by the mitochondrial cholesterol side chain cleavage cytochrome P450 enzyme. This enzyme produces oxygen free radicals which may be detrimental to its activities, however, antioxidants play role of removing these oxygen free radicals (Young *et al*., 1995). However, the lack of positive effect of β-carotene on P4 concentration 8 h and 48 h post CIDR withdrawal may be due to the fact that CL is already undergoing luteolysis and no steroidogenic process occurring in which β-carotene is speculated unable to exert its influence. The increase in plasma GPx activity in the present study due to β-carotene supplementation is in agreement with (Kamiloglou & Beytut, 2005) who reported that, β-carotene injection prior to breeding and during pregnancy had improved the antioxidant activity of GPx in sheep. It had been noted that the increase in blood plasma GPx activity is associated with an increase in protection from oxygen free radicals during oxidative stress (Festila *et al*., 2012). The positive influence of β-carotene in scavenging free radicals is important to protect cells from the negative effects of free radicals and maintain the integrity and activity of immune cells against pathogen (Ramadan *et al*., 2001). The observation that oestradiol-17β concentration was not affected by supplemental β-carotene is in agreement with the report of Weng *et al*. (2000), who reported lack of beneficial effect of β-carotene supplementation on oestradiol-17β concentration in canines.

In agreement with the present findings, other studies in cattle have reported that supplemental β-carotene had no effect on the response to oestrus and onset of oestrus (Folman *et al*., 1979; Wang *et al*., 1982; Wang *et al*., 1987; Aréchiga *et al*., 1998). Conversely, Wang *et al*. (1982) reported that β-carotene supplementation did shortened the onset of oestrus following synchronised oestrus with prostaglandin analogue. The result on the duration of oestrus in the

present study is consistent with other studies in cattle (Folman *et al*., 1979; Wang *et al*., 1982; Wang *et al*., 1988b). The lack of beneficial effect of β-carotene on oestrous parameters in the present study may be attributed to the failure of β-carotene to exert its effect on the oestradiol-17β and LH concentrations. These two hormones, especially oestradiol-17β play important role in controlling the oestrous behaviours. Therefore, any supplemental nutrient that affects these hormones may influence the oestrus behaviour. A number of studies have reported that β-carotene supplementation had no positive influence on conception rate in cattle (Folman *et al*., 1979; Wang *et al*., 1982; Bindas *et al*., 1984; Wang *et al*., 1988ab). These findings support our present finding with respect to the conception rate. Contrary, it has been noted that supplemental β-carotene did increase pregnancy rate at 120 days postpartum when cows were supplemented with β-carotene for an extended period of more than 90 days (Aréchiga *et al*., 1998). Another contrary study noted that β-carotene supplementation after 105 day increased pregnancy rate in cattle (De Ondarza *et al*., 2009). These studies have argued that the conception rate increases with the increase in the duration of β-carotene supplementation. It has been noted that prolonged supplementation with β-carotene is required to uplift tissue concentration of β-carotene molecule to positively affect the pregnancy rate (Aréchiga *et al*., 1998). The high concentration of β-carotene has been attributed to boost its antioxidant activity and thus providing conducive uterine environment for implantation and embryo development (Weng *et al*., 2000; Ay *et al*., 2012) which might have led to improved conception rate in these studies.

The present findings that β-carotene supplementation had no effect to kid birth weight agree with other studies reported in cattle and pigs. It has been reported that β-carotene supplementation did not affect birth weight of calves (Greenberg *et al*., 1986; Kaewlamun, 2010) and piglets (Kostoglou *et al*., 2000). Contrary, Brief & Chew (1985) reported heavier litter weight at birth in pigs supplemented with β-carotene. Studies on the influence of supplemental β-carotene on gestation length and litter size could not be found to compare and contrast with the present study.

The mean diameter of the largest follicle in the present study was not affected by the synchronisation protocol, however the values recorded at 48 h following CIDR withdrawal when the anmals were on oestrus are consistent with previous study that reported the diameter of largest follicle to be 7.3 mm 6.9 mm and 6.3 mm in Beetal, Teddy and Neuquen-Criollo goats, respectively (Cueto *et al*., 2006; Riaz *et al*., 2013). The mean diameter of 9.7±0.3 mm was recorded in Saanen goats during the natural oestrous period (Ginther & Kot,

1994) and is in agreement with the result of the present study for the largest follicle diameters recorded in day 21 and 28 during the supplementation period respectively. The mean total number of follicles recorded in the present study during the oestrus is lower compared to findings of previous study in Cabra del Guadarrama Spanish goat breed that recorded 13.1 follicles following synchronised oestrus using progesterone analogue (Fernandez-Moro *et al*., 2008).

The mean diameter of the corpus luteum (CL) in the present study was larger (15.60±0.70 mm) compared to CL of 12.3±1.2 mm in diameter reported by Bukar *et al*. (2012). The difference between the present and the previous study with regards to CL diameter may be attributed to the time when the scanning was performed and the type of synchronisation protocol used. In the present study the scanning was performed 12 days after artificial insemination while in the previous study, animals were scanned 4 days after ovulation. It has been noted that after ovulation, the CL starts to form and continue to increase in size, reaches its maximum size on day 9 in normal oestrous cycles of cattle (Kayacik *et al*., 2006).

The findings of the present study with regard to the oestrous parameters have confirmed the hypothesis that the synchronisation protocol could have no effect on the oestrus parameters. The onset of oestrus was not positively affected by the synchronisation protocols in the present study. However, numerically the onset of oestrus following CIDR withdrawal was earlier in the male effect group (26.13±0.90 h) compared to the eCG group (28.29±0.94 h). We could not compare the present findings with other related studies because of limited reports. There was no beneficial effect of oestrous synchronisation protocol on the duration of oestrus in the present study. The lack of positive effect of the oestrous synchronisation protocol on the duration of oestrus may be due to the similar effect of both eCG and male effect to induce LH surge. Comparing the present results with other studies, the duration of oestrus in the eCG group was shorter (Oliveira *et al*., 2001; Motlomelo *et al*., 2002). Oliveira *et al*. (2001) reported the onset of oestrus of 36.0±2.64 h following synchronised oestrus using CIDR combined with eCG. Additionally, the duration of oestrus was 35.2±0.7 h following synchronised oestrus using CIDR combined with eCG in indigenous and Boer goats (Motlomelo *et al*., 2002). Response to oestrus was not positively affected by the synchronisation protocol in the present study. This may be attributed to the similar effect of both eCG and male effect to induce LH surge from the hypothalamus. The results of oestrous response in the eCG group for this study is in agreement with other studies (Oliveira *et al*.,

2001; Motlomelo *et al*., 2002; Hashemi *et al*., 2006; Kor *et al*., 2011; Omontese *et al*., 2013a) following synchronised oestrus using CIDR combined with eCG.

The conception rate in the male effect group was higher compared to that of eCG group. The cause of improvement in the conception rate recorded in the male effect group is not clear, but may be ascribed to the physical presence attributes of male. However, previous studies could not be found to support or reject this finding. This positive influence of buck effect on conception rate may possibly encourage the use of male effect when synchronising oestrus in P4 primed goats and avoid the wide application of eCG which has been implicated to reduce fertility in goats when used repeatedly.

The gestation length, birth weight and the litter size were not affected by the synchronisation protocol. The present study recorded extended overall gestation length of 150.36 days, this is in agreement with other previous studies (Lehloenya *et al*., 2005; Bitaraf *et al*., 2007; Kor *et al*., 2011) but disagrees with (Khanum *et al*., 2008) who recorded shorter gestation length of 144.8 days. It was found that small size breeds like Pakistani Dwarf goat, Black Bengal goat and West African Dwarf goat have shorter gestation length compared to large breeds of goat such as Toggenburg, Saanen and Nubian (Khanum *et al*., 2008). The present study recorded litter size of 2.18 that is in line with the overall litter size of 2.2 and 2.0 recorded in Boer and Nguni goats, respectively (Lehloenya *et al*., 2005). However, other studies recorded lower overall litter size of 1.38 (Kor *et al*., 2011) and 1.15 (Kausar *et al*., 2009). Breed may also contribute into the differences observed.

The positive correlation between the CL size and P4 concentration in the present study is in agreement with studies in sheep (Gonzalez de Bulnes *et al*., 2000) and cattle (Haliloglu *et al*., 2002; Trojačanec *et al*., 2012). This positive correlation is ascribed to the fact that CL is responsible for P4 synthesis and increase in CL size is associated with increase in CL cells responsible for P4 synthesis and thus high P4 concentration. However, in the study of Arellano-Rodriguez *et al*. (2009), no significant correlation between CL numbers and P4 synthesis were found in goats.

It is well established in goats that sex and litter size can affect birth weight. Male kids are heavier compared to female kids at birth and the birth weight decreases with increase in litter size regardless of sex (Todaro *et al*., 2004; Lehloenya *et al*., 2005; Bushara *et al*., 2013; Patel & Pandey, 2013). This is consistent with our present finding that recorded the male kids to be heavier at birth compared to female kids. The reason behind male kids being heavier than

female kids has always attributed to the anabolic effect of male sex hormones during pre-natal growth and uterine environment (Bushara *et al*., 2013). The typical sexual dimorphism of mammals are due to the anabolic effects of androgens on male skeletal muscle, and that is why in most species, males are larger, with a greater proportion of muscle than females (Hossner, 2005).

In goats, the birth weight of kids can be affected by the litter size. It has been noted that birth weight decreases with an increase in litter size (Husain *et al*., 1996; Mohammed & Amin, 1997; Lehloenya *et al*., 2005). Lehloenya *et al*. (2005) reported that birth weight from singles, twins, triplets and quadruplets significantly differed from each other. Similarly, birth weight for singles was higher than that of twins (Gebrelul *et al*., 1994; Mioč *et al*., 2011). The present study found that the birth weight for singles, twins and triplets were significantly higher than quadruplets and quintuplet. It has been noted that, as the number of foetuses increases, the number of caruncles attached to each foetus decreases and this reduces the feed supply to the foetus, as a result the birth weight of the lambs is reduced (Robinson *et al*., 1977; Das *et al*., 1996; Bushara *et al*., 2013).

5.2 Effect of β-carotene supplementation on the milk yield and components of Saanen goats

β-carotene is a provitamin A that has been implicated to have antioxidant activity, and as a result, it has been reported to play important role in reproduction, immune and mammary functions. The present study hypothesised that β-carotene supplementation during drying off period would have an influence on milk yield, milk composition and quality of Saanen goats. However, β-carotene supplementation during drying off period did not improved milk yield, milk composition and quality of Saanen goats.

There are limited studies in goats related to the effect of β-carotene on milk yield, milk composition and quality. Therefore, we are unable to compare or contrast the present findings with the previous work conducted in goats. However, similar studies have been conducted in other ruminant species. For milk yield, Brozos (2006) reported that β-carotene supplementation had not altered milk yield in sheep. Similarly, supplementing cattle with β-carotene did not improved milk yield (Bindas *et al*., 1984; Rakes *et al*., 1985; Wang *et al*., 1987; De Ondarza *et al*., 2009; De Ondarza & Engstrom, 2009; Kaewlamun, 2010). These studies are consistent with the finding of the present study with regard to milk yield. Contrarily, some studies reported that β-carotene supplementation had a positive effect on

milk production in heat stress cows (Aréchiga *et al*., 1998; Chawla & Kaur, 2004). The inconsistency from these studies on the effect of supplemental β-carotene on milk yield can be attributed to variations in β-carotene concentrations in the diet, the blood concentration of β-carotene, the level, time and the duration of supplementation (Kaewlamun, 2010). It is well known that sheep and goats are efficient converters of dietary β-carotene in the intestine (McDonald, 2000). Therefore, lack of effect of β-carotene supplementation on the milk yield from the present study may be attributed to absence or insufficient concentration of β-carotene in goat milk which made it unable to exert its influence on the quantity of milk produced.

In cows conflicting results have been documented concerning the influence of β-carotene on milk components. Similar to the present study, it has been noted that β-carotene supplementation had no positive effect on milk fat, protein and lactose (Kaewlamun, 2010; Machpesh, 2013). In addition, De Ondarza *et al*. (2009) also reported lack of beneficial effect of β-carotene on milk protein. Conversely, it had been noted that β-carotene supplementation can increase milk fat % in cows (De Ondarza *et al*., 2009). But Oldham *et al*. (1992), reported lower fat % in cows supplemented with β-carotene.

In agreement with the present study, supplemental β-carotene had not influenced milk SCC in cows (Wang *et al*., 1987; Oldham *et al*., 1992; De Ondarza *et al*., 2009; Kaewlamun, 2010). Conversely, in other studies β-carotene supplementation had reduced milk SCC in cows (Rakes *et al*., 1985; Wang *et al*., 1988b). Failure of β-carotene to improve milk SCC in the present study may be attributed to the fact that goats did not absorb and transport enough β-carotene to the mammary tissues to exert its antioxidant activity. This may be associated to the lower doses of β-carotene supplemented in the present study.

Regarding the effect of milk type on milk components, normally after kidding, goat secrets milk from the mammary gland during the first 5 days called the colostrum. Colostrum is different from normal milk in terms of their composition (Yang *et al*., 2009). Goat colostrum is significantly richer in fat, protein and SCC but lower in lactose compared to milk (Akingbade *et al*., 2003; Ontsouka *et al*., 2011; Mahmoud *et al*., 2012; Sánchez-Macías *et al*., 2014). These results agree with the findings of the present study. During the transitional period from colostrum to milk, the quantity of milk increases gradually and is closely associated with high demand for water in milk synthesis. High lactose production in milk has been attributed to cause water influx in milk through osmotic effects (Sánchez-Macías *et al*.,

2014). In contrast, the total milk protein concentration decreases in part due to dilution resulting from increased milk production (Ontsouka *et al*., 2011). High SCC is mostly attributed to mastitis, however, there were no cases of mastitis in the goats during the present study period. It has been reported that the number of SCC are high immediately after calving but drops rapidly during the first week of lactation (Woloszyn, 2007). Therefore, the high SCC in colostrum in the present study could be of physiological nature, probably due to cells penetration through leaky tight junctions between the mammary epithelial cells (Nguyen & Neville, 1998) and high immunoglobulins content in the colostrum (Woloszyn, 2007).

CHAPTER SIX

6. CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

β-carotene supplementation and oestrous synchronisation had no significant effect on body weight, oestrous response, onset and duration of oestrus, number of follicles, size of largest follicle, size of corpus luteum, oestradiol-17β concentration, gestation length, birth weight and the litter size. However, β-carotene supplementation increased progesterone (P4) concentration and glutathione peroxidase (GPx) activity. There is a significantly positive correlation between the CL size and P4 concentration regardless of β-carotene supplementation. Male introduction at CIDR removal resulted in higher conception rate than injection with eCG. β-carotene supplementation during drying off period did not affect milk yield, protein, fat, lactose and SCC in Saanen goats. Goat colostrum was very rich in fat, protein and SCC components but low in lactose compared to normal milk during early stage of lactation. Therefore, it can be concluded that, β-carotene supplementation during the breeding period may play a beneficial role during embryo implantation and development as a result of increased P4 concentration and GPx activity. Incorporation of the male effect in progesterone synchronised oestrus can improved the conception rate. Male introduction can be used as a co-treatment to replace eCG in order to reduce multiple hormonal use for oestrous synchronisation in Saanen goats primed with progesterone. Supplementation of goats with 50 mg/day β-carotene during the drying off period had no beneficial effect on the milk yield and components of Saanen goats.

6.2 Recommendations

Based on the present study, the following are recommended:

- Further studies should be conducted evaluating the effect of different levels of β-carotene on reproductive and productive performance of goats.
- Future studies should determine the blood levels of β-carotene and vitamin A to correlate them with reproductive and productive performance of the animal.
- Concentration of β-carotene in animal tissues can also be affected by season, as such future studies should also be conducted in different seasons of the year.

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