Small Ruminant Research 116 (2014) 176–182

Contents lists available at ScienceDirect

Small Ruminant Research

journal homepage: www.elsevier.com/locate/smallrumres

# Long-term betacarotene supplementation positively affects serum triiodothyronine concentrations around puberty onset in female goats

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ARTICLE INFO

*Article history:*
Received 25 May 2013
Received in revised form 12 October 2013
Accepted 16 October 2013
Available online 1 November 2013

*Keywords:*
Goats
Betacarotene
Puberty
Progesterone
Triiodothyronine

ABSTRACT

The effect of betacarotene (BC) supplementation on serum triiodothyronine (T3) levels over time in prepubertal goats was evaluated. Goats ($n = 17$; 3 months old; 7/8 Saanen-Alpine; 26° NL) were randomly assigned to one of the following two groups: 1) the betacarotene group, supplemented daily with 50 mg of BC ($n = 9$; live weight [LW]: $17.3 \pm 1.0$ kg; body condition score [BCS]: $3.34 \pm 0.12$), or 2) the control group (CC; $n = 8$; LW: $16.1 \pm 1.0$ kg; BCS = $3.17 \pm 0.12$). The initial mean LW ($16.7 \pm 1.0$ kg) and BCS ($3.31 \pm 0.12$) were similar ($p > 0.05$) in both groups. Whereas BC supplementation did not affect the onset of puberty (215.7 vs. $226.7 \pm 6.6$ days; $p > 0.05$) for the BC and CC, respectively, increases in serum T3 during the second half of the experiment were observed in the BC supplementation group ($p < 0.05$). As the LW and serum T3 levels increased, the natural photoperiod decreased, revealing a negative correlation ($p < 0.05$) between the variables; the observed values were $r = -0.94$ for LW and photoperiod and $r = -0.41$ for T3 and photoperiod. Long-term BC supplementation was not associated with a precocious onset or an increased percentage of goats reaching puberty. Long-term BC supplementation positively affected the release pattern of triiodothyronine over time, suggesting a potential role of BC as a thyroid-activating molecule; these results might possess clinical significance.



## 1. Introduction

The activation of reproductive function in peripubertal stages and the cyclicity of reproductive capacity in adult stages are critical to the survival of a species, and physiological homeostasis dictates the optimal conditions for reproductive success; any disturbance of this balance might affect the function of the gonadotropin releasing hormone (GnRH) neurons (Meza-Herrera, 2008, 2012; Meza-Herrera and Tena-Sempere, 2012). This transit toward complete activation of the hypothalamic-hypophyseal-gonadal (HHG) axis could be compromised by different disruptors, such as signals dictated by stress, nutritional imbalance, body weight decreases and neurological alterations, which, in addition to the photoperiod, might directly influence the HHG axis through modifications of the GnRH secretion pattern (Scaramuzzi et al.,

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http://dx.doi.org/10.1016/j.smallrumres.2013.10.017

2006, 2011; Meza-Herrera, 2008, 2012; Maffucci and Gore, 2009; Meza-Herrera et al., 2004, 2007, 2008, 2010; Urrutia-Morales et al., 2009; Meza-Herrera and Tena-Sempere, 2012). The activity and functionality of this neuronal circuitry is, in turn, controlled through different neurotransmitters and metabolic hormones. Whereas activation of the complex KiSS-1/kisspeptin/GPR54 system augments the glutamatergic neurotransmission, increases in the metabolic status and in the adipocyte-derived hormone leptin are excitatory events, stimulating not only the onset of puberty but also the reproductive cyclicity in adult stages (Maffucci and Gore, 2009; Meza-Herrera et al., 2010; Meza-Herrera, 2012; Meza-Herrera and Tena-Sempere, 2012).

Betacarotene (BC) is an important bioactive substance in green plants that is a precursor of vitamin A and retinoid, which has been defined as an important molecule in humans and animals (Schweigert, 1998). At the cellular and tissue level, BC is involved in multiple actions, such as promoting key events in a range of physiological processes (Schweigert et al., 2002, 2003). Although many gene products linked to reproductive performance are known to be modulated by retinoic acid, the product of retinol oxidation (Schweigert, 1998), other studies have proposed that BC might act independently of vitamin A, particularly in increasing follicular and luteal steroidogenesis in ruminant species (Arellano-Rodriguez et al., 2007, 2009; Haliloglu et al., 2002; Kawashima et al., 2009, 2010, 2012; Meza-Herrera et al., 2013a,b).

Thyroid hormones have been found to be fundamental for the normal development of mammals; they modulate metabolic activity in several tissues, regulate reproductive outcomes, provide neuroprotection and modulate cardiovascular function (Krassas et al., 2010) as well as affecting the establishment of seasonal reproduction (Dardente, 2012). Triiodothyronine (T3) is the main active thyroid hormone, and it binds to several products of two genes, the nuclear thyroid hormone receptors alpha and beta, and regulates gene expression (Braun et al., 2010); T3 has been identified as an important modulator of steroidogenic acute regulatory protein (StAR) expression and gonadal steroidogenesis (Manna et al., 2009). Whereas thyroid hormone receptors have been detected in gonadal tissue, T3 has shown a positive correlation with a number of proliferating Sertoli cells per seminiferous tubule area, as well as a positive relationship to circulating FSH concentrations in sheep (Oluwole et al., 2013). In addition, a positive relationship between serum T3 levels and the onset of puberty in goats has been proposed in males (Gunnarsson et al., 2009) and females (Meza-Herrera et al., 2011a). There is no data demonstrating a possible relationship regarding BC supplementation and serum T3 around the onset of puberty in goats; this study aimed to evaluate such a possible relationship in peripubertal female goats.

## 2. Materials and methods

### 2.1. Location, environmental conditions, animals, feeding and experimental design

This study was conducted at the Southern Goat Research Unit (26° NL, 103° WL, 1117 m) of the Regional University Unit of Arid Lands-Chapingo Autonomous University (URUZA-UACH), Bermejillo, Durango, Mexico. The climate of the area is warm and dry, and the mean annual precipitation and temperature are 217.1 mm and 22.3 °C, respectively. The warmest month is June, with temperatures above 40 °C, whereas the coldest month is January, with the lowest temperature below 0 °C.

Prepubertal female goats ($n = 17$; live weight [LW]: $16.7 \pm 1.0$ kg; body condition score [BCS]: $3.31 \pm 0.11$; 3 months old, 7/8 Saanen-Alpine) were fed a diet of alfalfa hay, corn silage and corn grain to meet 110% of their nutritional maintenance requirements (NRC, 1998). The goats were fed twice a day, with alfalfa hay (14% crude protein; 1.14 Mcal/kg, net energy for maintenance (NEm), and corn silage (8.1% CP; 1.62 Mcal/kg, NEm) in the morning (07:00), and corn grain (11.2% CP; 2.38 Mcal/kg, NEm) in the afternoon (18:00). In early June, the goats were randomly distributed in two groups: 1) the betacarotene group (BC, $n = 9$; LW = $17.3 \pm 1.0$ kg; BCS: $3.34 \pm 0.12$), and 2) the control group (CC; $n = 8$; LW = $16.1 \pm 1.0$ kg; BCS: $3.17 \pm 0.12$). The BC group was supplemented with BC (50 mg/goat/day; orally; Syntex Mexico S.A. de C.V.) during the entire experiment (150 days, from early June to early November). The goats were kept under natural photoperiod conditions from June to November (26° NL) and had free access to water, shade, and mineral salts during the entire experiment.

Allotments of food and betacarotene were individually fed to each goat. The basal diets were entirely consumed by all the goats, and it may be assumed that each goat received the same level of BC in the basal diet. The only difference in the BC consumption between the examined groups was the BC oral supplementation provided to the BC group. The LW and BCS were recorded weekly, always prior to feeding. The BCS was determined by palpation of the goat transverse and vertical processes of the lumbar vertebrae (L2 through L5) as well as upon sternal subcutaneous adipose tissue on a five-point scale (1: emaciated to 5: obese; Aumont et al., 1994) by the same experienced technician. All the methods used in this study were in accordance with accepted international guidelines (FASS, 1999).

### 2.2. Blood sampling, progesterone determination and evaluation of the onset of puberty

The schedules for the blood sampling collection and determination of the onset of puberty have been previously outlined (Meza-Herrera et al., 2011b); the main activities will be briefly considered. From early June to November, blood (10 ml) was collected by jugular venipuncture twice per week, prior to feeding. The blood was collected into sterile vacuum tubes (Corvac, Kendall Health Care, St. Louis, MO) and allowed to clot at room temperature for 30 min. The serum was separated by centrifugation ($1500 \times g$, 15 min), decanted and collected in duplicate in polypropylene microtubes (Axygen Scientific, Union City, CA, USA) and stored at −20 °C until the hormonal analysis. The serum $P_4$ concentration was determined by radioimmunoassay (RIA) using a commercial RIA kit (Diagnostic Products, Los Angeles, CA, USA) validated for ruminant serum (Schneider and Hallford, 1996). The intra- and inter-assay coefficients of variation (CV) were 9.9 and 12.4%, respectively. Whereas the average recovery was 94%, the sensitivity of the assay was 0.1 ng/ml. The onset of puberty was confirmed in both experimental groups based on the $P_4$ serum profiles; for each goat, a serum $P_4$ level $\geq 1$ ng/ml in two consecutives samples were considered indicative of ovulation as well as the onset of puberty (Cushwa et al., 1992).

### 2.3. Intermittent blood sampling and triiodothyronine quantification

Blood (10 ml) was collected monthly by jugular venipuncture from all the goats; because several environmental factors such as temperature, season and circadian rhythm cause fluctuation in thyroid hormone levels, all the samples were collected prior to feeding at 07:00 throughout the experimental period. The serum T3 concentrations were determined in duplicate by solid-phase RIA using components of a commercial kit; the kit utilized antibody-coated tube technology, and the assay was performed without prior extraction of T3 from the serum (Diagnostic Products, Los Angeles, CA, USA (Head et al., 1996)). Whereas the intra- and inter-assay CV values for T3 quantification were 0.55% and 6.98%, the sensitivity of the assay was 0.1 ng $mL^{-1}$. The sources for T3 iodination and the standards were NEX 110 (New England Nuclear, Boston, MA) and T2877 (Sigma, St. Louis, MO), respectively. Fig. 1 shows a schematic representation of the experimental protocol, considering the birth of the animals, the adaptation period (Mar–May), the experimental period (Jun–Nov) and the intermittent blood sampling (Jun–Nov; progesterone (P4), triiodothyronine (T3) throughout the experimental period (Fig. 1).