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Original Research Article

Betacarotene supplementation increases ovulation rate without an increment in LH secretion in cyclic goats

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ABSTRACT

This study aimed to evaluate the effects of betacarotene (BC) supplementation on ovulation rate (OR) and luteinizing hormone (LH) secretion in adult goats during the breeding season. Additionally, total ovarian activity (TOA) comprising the total number of ultrasonographically detectable antral follicles (AF) and corpora lutea (OR) was also assessed. In early October, adult goats [$n = 22$, 3.5 years of age, 7/8 Sannen-Alpine; 26°N, 103°W at 1117 m.a.s.l.] were randomly assigned to: (i) BC group (BCG), orally supplemented with 50 mg of BC/goat/day [$n = 10$; live weight (LW) = 45.9 ± 2.0 kg, body condition score (BCS; range: 0-emaciated to 5-obese) = 3.0 ± 0.1], and (ii) control group (CONT) [$n = 12$; LW = 46.2 ± 2.0 kg, BCS = 3.0 ± 0.1]. All animals received a basal diet of alfalfa hay, corn silage and corn grain, with free access to water and mineral salts. The whole experimental period spanned 34 days before and 17 days after ovulation. On day 23 of the experiment, estrus was synchronized with progestin-releasing intravaginal sponges; 36 h prior to estrus, an intensive blood sampling (every 15 min for 6 h) was performed to determine mean LH concentrations, pulsatility (LH-PULSE) and area under the curve (LH-AUC) for serial LH concentrations. Afterwards, by the end of the luteal phase (i.e., 17 days after the onset of estrus), an ultrasonographic scanning was performed to evaluate OR and TOA [AF + OR]. The average LW and BCS did not differ ($p > 0.05$) during the experimental period. BC-supplemented goats showed an increase in OR (3.4 ± 0.2 versus 2.8 ± 0.2; $p < 0.05$) and exhibited lower ($p < 0.05$) serum LH concentrations, LH-AUC and LH-PULSE compared to CONT. A positive correlation was recorded between OR and LW ($r^2 = 0.42$, $p < 0.05$) and BCS ($r^2 = 0.47$, $p < 0.05$). In addition, AF (5.0 ± 0.6 versus 3.4 ± 0.6) and TOA (8.4 ± 0.6 versus 6.2 ± 0.6) were greater ($p < 0.05$) in the BC-supplemented group than CONT. Supplementation with BC enhanced ovarian follicular development and ovulation rate in adult female goats under decreased photoperiods through LHRH-independant pathways or direct effects of BC on ovarian function.

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1. Introduction

One of the major functions of the ovary is to produce mature oocytes, a process that depends on an extremely well coordinated interactions among several components of the ovarian follicle, i.e., the oocyte, theca and granulosa cells [1–3]. Follicular growth and oocyte maturation are primarily dependent upon the hypopyseal gonadotropins (FSH and LH) as well as some other, intraovarian factors (e.g., IGF-1) [2–4]. The function of the hypothalamic–pituitary–gonadal (HPG) axis is modulated by an array of neuronal inputs, governed by either photoperiodic or thermoperiodic cues, which ultimately modulate the neuronal activity of the GnRH-releasing terminals in the hypothalamus and also have an impact on energy homeostasis. These cues provide the basis for the environmental control of the: (1) onset of reproductive function at puberty; (2) recurrent reproductive cycles observed in adult females of non-seasonal breeders; and (3) intermittent reproductive cycles observed in seasonally breeding species [5,6]. In all of the above scenarios, nutritional status and/or nutritional supplementation strongly affect the functioning of the HPG axis [4,7–10].

Betacarotene (BC), a bioactive component of green plants, is a precursor of vitamin A and retinoids, while an important chemical for both the human beings and animals [11,12]. BC has been involved in multiple actions at cellular and tissue levels that promote key events in an ample range of biological processes [13,14]. In addition to being a precursor of retinol, BC exerts biological effects similar to those of vitamin E by acting as a potent scavenger of free radicals, especially the singlet-state oxygen, and therefore as a potent antioxidant, just as other carotenoids [11–14]. An optimal BC intake has been shown to have positive effects on ruminant reproductive outcomes [15], although there have been contradictory reports of either negative effects [16], a lack of effect [17], or positive effects of BC on reproductive [18–20] and metabolic [21] processes. Even though many gene products linked to reproductive performance are known to be modulated by retinoic acid, the product of retinol oxidation [12,14], some studies have proposed that BC may act independently of vitamin A, particularly in increasing both follicular and luteal steroidogenesis in ruminant species [19,20,22,23]. Despite the positive action of BC on ovarian steroidogenesis, studies of the possible role of BC on LH secretion and ovarian function are scarce. This study examined the effect of BC supplementation on ovulation rate and pulsatile LH secretion in adult female goats.

2. Materials and methods

2.1. Location, environmental conditions and animal management

The present study was carried out at the Regional University Unit on Arid Lands, Chapingo Autonomous University (URUZA-UACH), the Southern Goat Research Unit (latitude: 26°N, longitude: 103°W; 1117 m.a.s.l.). Adult goats [$n = 22$, mean live weight (LW) = 45.35 ± 1.35 kg, age: 3.5 years, 7/8 Sannen-Alpine] were employed in this study conducted under short-day photoperiodic conditions during October and November (i.e., natural breeding season at 26°N). Both the LW and body condition score (BCS) were recorded weekly prior to feeding. BCS was determined on a five point scale (from 1 = emaciated to 5 = obese) [24] by an experienced technician. All the methods used in this study were in accordance with accepted international guidelines [25].

2.2. Experimental design and treatment groups

In early October, goats were randomly placed in individual pens and allocated to two experimental groups: (1) betacarotene group (BCG; $n = 10$, LW = 45.9 ± 2.0 kg, BCS = 3.0 ± 0.1) and (2) control group (CONT; $n = 12$; LW = 46.2 ± 2.0 kg, BCS = 3.0 ± 0.1), with no differences ($p > 0.05$) for LW and BCS between experimental groups. Goats in BCG, were orally supplemented with betacarotene (50 mg/goat/day, mixed with mineral salts) (Syntex-Roche de Mexico; Guadalajara, Jalisco, Mexico) during the entire experimental period, which lasted from 34 days before estrus to 17 days post-estrus. Both groups received a basal diet of alfalfa hay (14% crude protein (CP), 4.7 net energy for maintenance (NEm) MJ/kg), corn silage (8.1% CP, 6.7 NEm MJ/kg) and corn grain (11.2% CP, 9.9 NEm MJ/kg) in a mixed-ration twice a day (0700 and 1600; 1 kg/goat/day) formulated to cover their nutritional requirements [26]. Animals had free access to water, shaded areas and mineral salts. Composition values of the ingredients of the basal diets as dry matter basis (DM%) were obtained from representative samples taken throughout the experimental period and analyzed according the previously described procedures [27] (Table 1).

2.3. Estrous synchronization, blood sampling, and LH measurements

On day 23 of the experimental period, estrus was synchronized with intravaginal sponges containing 45 mg of fluorogestone acetate (Chronogest®; Intervet International B.V., Boxmeer, Holland) left in place for 10 days; 9 days after insertion of the

Table 1 – Chemical composition of alfalfa hay, corn silage and corn grain samples which conformed the basal diet of adult crossbred goats ($n = 22$) under natural photoperiodic conditions (October–November, 26°N).[a]

Item	Alfalfa hay (%)	Corn silage (%)	Corn grain (%)
Nutrient composition[b]			
Dry matter[c]	92.0	35.8	85.3
Crude protein[c]	15.8	8.5	9.5
Neutral detergent fiber[c]	59.9	40.6	9.9
Acid detergent fiber[c]	42.1	25.0	4.0

[a] Mineral block offered ad libitum contained (%, w/w): NaCl 95; Fe 0.2; Cu 0.033; I 0.007; Zn 0.005; Co 0.0025.
[b] Composition values (% of diet DM) represent values from five samples taken throughout the experimental period. Samples dried in a forced air stove at 60 °C until constant weight.
[c] Determined according to the procedures outlined by AOAC (1990).