

Beta-Carotene Supplementation Positively Affects Selected Blood Metabolites Across Time Around the Onset of Puberty in Goats

CESAR A. MEZA-HERRERA^{1*}, PEDRO PACHECO-ALVAREZ¹, ORNELLA E. CASTRO²,
ULISES MACIAS-CRUZ³, LEONEL AVENDAÑO-REYES³, MIGUEL MELLADO⁴,
FRANCISCO G. VELIZ-DERAS⁴, VIRIDIANA CONTRERAS-VILLARREAL⁴,
JOSE ABAD-ZAVALA⁵, RAFAEL RODRIGUEZ-MARTINEZ⁴,
GERARDO ARELLANO-RODRIGUEZ⁴

¹Regional University Unit of Arid Lands, Chapingo Autonomous University, Bermejillo, Mexico

²Faculty of Agricultural Sciences, National University of Catamarca, San Fernando de Catamarca, Argentina

³Institute of Agricultural Sciences, Baja California Autonomous University, Mexicali, Mexico

⁴Department of Veterinary Sciences and Department of Animal Nutrition, Antonio Narro Agricultural Autonomous University, Torreon, Mexico

⁵Institute of Biotechnology, University of Papaloapan, Tuxtepec, Mexico

*Corresponding author: cmeza2020@hotmail.com

ABSTRACT

Meza-Herrera C.A., Pacheco-Alvarez P., Castro O.E., Macias-Cruz U., Avendaño-Reyes L., Mellado M., Veliz-Deras F.G., Contreras-Villarreal V., Abad-Zavaleta J., Rodriguez-Martinez R., Arellano-Rodriguez G. (2017): **Beta-carotene supplementation positively affects selected blood metabolites across time around the onset of puberty in goats.** Czech J. Anim. Sci., 62, 22–31.

The possible effect of beta-carotene supplementation upon peripubertal changes in serum concentrations across time for total protein (TP), urea (UR), cholesterol (CHOL), and glucose (GLU) around puberty onset was evaluated. The experiment was carried out from June to November and prepubertal goats ($n = 17$, 3 months old, 7/8 Saanen-Alpine, 1/8 Criollo) were randomly assigned to: (1) beta-carotene group (BC) ($n = 9$; 17.3 ± 1.0 kg live weight (LW), 3.3 ± 0.12 body condition score (BCS), oral supplementation with 50 mg beta-carotene per day per goat) and (2) control group (CC) ($n = 8$; 16.1 ± 1.0 kg LW, 3.1 ± 0.12 BCS). Serum blood samples were collected along the experiment to quantify progesterone concentrations (P4) through radioimmunoassay, while TP, UR, CHOL, and GLU through spectrophotometric analyses. No differences ($P > 0.05$) occurred between treatments regarding LW and BCS, and TP (67.6 ± 2.4 g/l), UR (3.8 ± 0.17 mmol/l), GLU (5.06 ± 0.09 mmol/l), and CHOL (1.62 ± 0.07 mmol/l) concentrations. However, while a treatment \times time interaction occurred between treatments for TP, GLU, CHOL ($P < 0.05$) favouring the BC group, an increased serum UR levels occurred in the CC group. Nonetheless, such general serum metabolite profile was related neither to the age

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(215.7 vs 226.5 ± 6.6 days; $P > 0.5$) nor to the percentage (44.4 vs 25.0 ± 17.0%; $P > 0.05$) of goats reaching puberty in the BC and CC groups, respectively. Results should help speed-up goat productivity while may also engender key management applications to different animal industries.

Keywords: reproduction; nutritional supplementation; focus feeding; glucose; cholesterol; pubertal activation

Puberty onset is a complex process regulated by several environmental cues, in which the neuroendocrine system must convey external information to align different internal mechanisms and promote anatomical, physiological, and behavioral changes (Meza-Herrera et al. 2010). The last is accomplished throughout activation of an intricate circuitry which involves diverse endocrinological messengers and neurotransmitters, whose core endeavour is to reinitiate the activation of GnRH neurons at peripubertal stages (Dupont et al. 2014).

Chronological age was originally involved as a key modulator of puberty onset (Meza-Herrera et al. 2010). Later on, other internal signals such as the acquisition of a critical body mass, serum increases in various metabolic hormones (e.g. insulin, IGF-1, T3, and leptin) along with augments in some blood analytes (e.g. glucose, cholesterol), have been closely linked to the reactivation of the hypothalamic–pituitary–gonadal (HPG) axis (Meza-Herrera and Tena-Sempere 2012; Meza-Herrera et al. 2014). Whereas body energy reserve status has been defined as an important modulator of puberty initiation, other studies highlight the rate of muscle and fat accumulation across time in the activation of GnRH neurons (Rosales-Nieto et al. 2013). Interestingly, both approaches denote the pivotal role that, not only the adipose tissue but also the adipocyte-derived molecules adipokines (e.g. leptin, adiponectin, resistin, omentin, vaspin) may exert upon both energy balance and reproductive function (Meza-Herrera and Tena-Sempere 2012).

Beta-carotene (BC), which is present in green plants, has a paramount role in mammals as precursor of vitamin A and retinoid, demonstrating to be a key bioactive micronutrient involved in multiple biological actions while promoting strategic events in a range of physiological processes at cellular and tissue level both in humans and animals (Harrison et al. 2012; Eroglu and Harrison 2013). Although many gene products linked to reproductive performance are known to be modulated by retinoic acid, the product of retinol oxidation (Harrison et al. 2012), other studies have proposed that BC

might act in a direct fashion, independently from vitamin A (Kawashima et al. 2010, 2012; Kramer and Aurich 2010).

Two different types of BC-metabolizing enzymes have been identified in several tissues from different species: (i) BCMO1 (BC-15',15'-monooxygenase-1) which right through a central cleavage converts BC to all-trans retinal, acting at cytosol level, and (ii) BCDO2 (BC-9',10'-dioxygenase) which throughout an eccentric cleavage generates β -ionone and β -apocarotenal, acting at mitochondrial level (Eroglu and Harrison 2013). Besides liver being the main site of BC accumulation (Eroglu and Harrison 2013), the mRNA expression of both types of BC oxygenases has also been detected at hepatocyte level (von Lintig et al. 2005).

Building on such findings and previous studies of our group, we hypothesized that BC supplementation could be associated with changes in the profile of blood metabolites around the onset of puberty in goats. The blood analytes evaluated included total cholesterol (CHOL), glucose (GLU), total protein (TP), and urea (UR). The final seek was to gain insights regarding a possible relationship between BC supplementation and some blood analytes related to lipid, carbohydrate, and protein metabolism.

MATERIAL AND METHODS

General. All the methods and management of the experimental units used in this study were in strict accordance with accepted guidelines for ethical use, care, and welfare of animals in research at international (FASS 2010), national (NAM 2002), and institutional levels (reference number UACH/DGIP/URUZA/11-510-405).

Location, environmental conditions, animals, feeding, and experimental design. This study was conducted at the Southern Goat Research Unit (26°N latitude, 103°W longitude, 1117 m a.s.l.) 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 ± 1.0 kg; body condition score (BCS) 3.31 ± 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 2007). The goats were fed twice a day, with alfalfa hay (14% crude protein (CP), 1.14 Mcal/kg net energy for maintenance (NEM), and corn silage (8.1% CP, 1.62 Mcal/kg, NEM) in the morning (7:00), 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) beta-carotene group (BC) ($n = 9$, LW = 17.3 ± 1.0 kg, BCS 3.34 ± 0.12), and (2) control group (CC) ($n = 8$, LW = 16.1 ± 1.0 kg, BCS 3.17 ± 0.12). The BC group was orally supplemented with 50 mg beta-carotene (Syntex Mexico S.A. de C.V.) per goat per day 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°N latitude) and had free access to water, shades, and minerals during the entire experiment.

Chemical composition of the basal diet was determined from representative samples taken throughout the experimental period (Table 1) and analyzed according to the procedures outlined by AOAC (1990). Allotments of food and BC were individually fed to each goat. Since the basal diets were entirely consumed by all the goats, it may be assumed that each goat received the same BC level from the offered basal diet. Therefore, the only difference in BC consumption between the experimental groups was the oral BC supplementation provided to the BC-supplemented group. Thus, the effect to offer or not supplemental beta-carotene in both experimental groups was evaluated.

Both 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. The health status of all the experimental units was

controlled by an experienced veterinarian during the whole experimental period; no health problems were observed during the trial. Besides, efforts were made to minimize any possible discomfort in the experimental units.

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. 2011); 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 CORVAC[®] vacuum tubes (Kendall Health Care, USA) and allowed to clot at room

Table 1. Composition and nutrient content (feed basis and DM basis) of daily basal diet for peripubertal crossbred female goats ($n = 17$, 3 months old, 7/8 Alpine-Saanen and 1/8 Criollo) supplemented with betacarotene (BC) or non-supplemented (CC) exposed to a naturally decreasing photoperiod (June–November, 26°N latitude)^a

| Item ^b | Feed basis | % DM |
|--|------------|-------|
| Fresh matter (kg) | 1.00 | 0.91 |
| DM (kg) | 0.91 | 90.78 |
| CP (kg) | 0.14 | 15.62 |
| Degradable protein (% CP) | 76.89 | 76.89 |
| Non-degradable protein (% CP) | 23.11 | 23.11 |
| Digestible protein, degradable nitrogen (kg) | 0.08 | 9.12 |
| Digestible protein, fermentable energy (kg) | 0.06 | 6.98 |
| Crude fibre (kg) | 0.29 | 32.32 |
| Starch (kg) | 0.11 | 12.37 |
| Crude fat (kg) | 0.12 | 1.66 |
| Carotene (mg) ^c | 56.58 | 51.48 |
| Betacarotene (mg) ^c | 33.22 | 30.23 |

DM = dry matter, CP = crude protein

^amineral block offered *ad libitum* contained (% w/w): NaCl 95, Fe 0.2, Cu 0.033, I 0.007, Zn 0.005, Co 0.0025

^bcomposition values (% of diet DM) represent values from five samples taken throughout the experimental period and dried in a forced air stove at 60°C until constant weight; DM, CP were determined according to the procedures outlined by AOAC (1990)

^c1 mg carotene = 400 IU vitamin A; IU vitamin A = 1.46 µg betacarotene (NRC 2007)

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temperature for 30 min. The serum was separated by centrifugation (1500 g, 15 min), decanted and collected in duplicate in polypropylene microtubes (Axygen Scientific, USA) and stored at -20°C until the hormonal analysis. The serum progesterone (P4) concentration was determined by radioimmunoassay (RIA) using a commercial RIA kit (Diagnostic Products, 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 P4 serum profiles; for each goat, a serum P4 level ≥ 1 ng/ml in two consecutive samples was considered indicative of ovulation as well as the onset of puberty (Meza-Herrera et al. 2011).

Intermittent blood sampling and blood analytes quantification. Blood samples (10 ml) were collected biweekly by jugular venipuncture from all goats to evaluate blood metabolite concentrations; the analytes were all measured throughout spectrophotometric analyses (Coleman 15 Junior II;

Coleman Instruments Division, PerkinElmer, USA). Serum total protein (TP) concentrations were determined in duplicate by using a commercial kit based on the bicinchononic acid reagent considering the bovine serum albumin 16 as standard and performed as described in the manual kit (Pierce Chemical Co., USA). Serum glucose (GLU) analyses were also conducted in duplicate throughout spectrophotometer techniques, following protocols supplied by the kit manufacturer (Roche Diagnostic Systems, Inc., USA). In addition, urea (UR) and cholesterol (CHOL) analytes were also measured in duplicate; serum UR concentration was quantified using the 640-A kit, based on the urease-18 (Sigma-Aldrich, USA), while serum CHOL concentrations were analyzed using the EnzyChrom™ kit ECCH-100 (Bioassay Systems, USA); assays were carried out following the protocols outlined by the manufacturer. Figure 1 shows a schematic representation of the experimental protocol, considering the birth of the animals, the adaptation period (March–May), the experimental period (June–November), and the intermittent blood sampling (June–November;

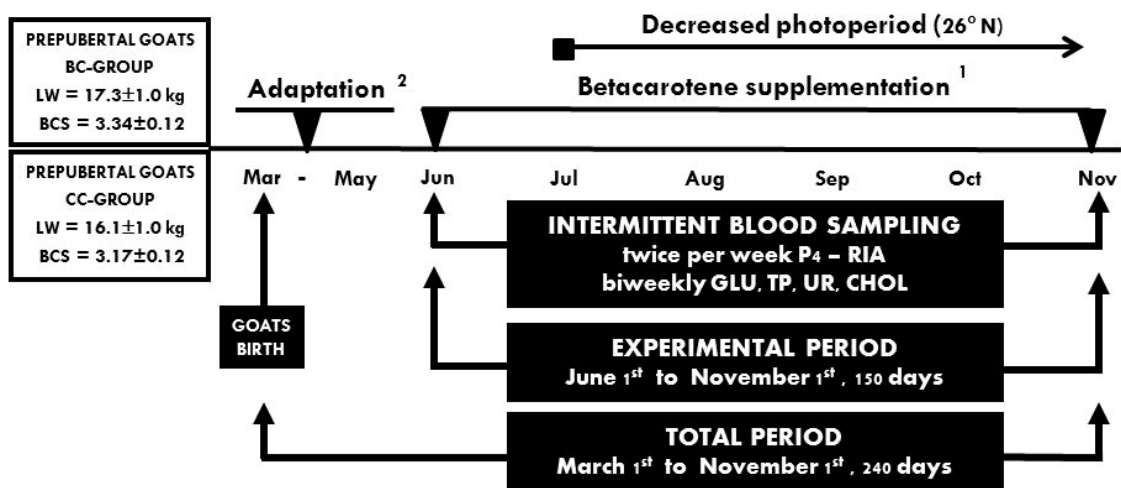


Figure 1. A scheme of the experimental protocol including data on animals' birth, adaptation period (March–May), and 150-day experimental period

Intermittent blood sampling (twice a week) for serum quantification of progesterone (P4), and every two weeks for total protein (TP), urea (UR), glucose (GLU), and cholesterol (CHOL) was performed during the whole experimental period in peripubertal crossbred female goats ($n = 17$, 3 months old, 7/8 Alpine-Saanen and 1/8 Criollo) supplemented with betacarotene (BC) or non-supplemented (CC), and exposed to naturally decreasing photoperiod (June–November, 26°N latitude)

LW = live weight, BCS = body condition score

¹oral supplementation, 50 mg/goat/day

²adaptation period to management and diets (March–May)

progesterone (P4)), and the blood analytes GLU, TP, UR, CHOL throughout the experimental period.

Statistical analyses. The response variables LW, BCS, serum TP, UR, GLU, and CHOL concentrations throughout the experimental period were determined by split-plot ANOVA for repeated measures across time. Previously, all blood analytes were log transformed because they were not normally distributed. The models included treatment in the main plot, which was tested using animal within treatment as the error term. Time and the time \times treatment interaction were included in the subplot and were tested by using the residual mean square (Littell et al. 1998). In the case of a significant treatment effect, mean separations were achieved using the PDIFF option of the GLM Procedure. While age at puberty was compared using ANOVA for Completely Randomized Design, the proportions of either pubertal or non-pubertal goats were compared with a chi-square test. All the analyses were computed using the procedures of the SAS software (Statistical Analysis System, Version 9.1, 2004). Results are expressed as Least Squares Means and standard errors and evaluated at the significance level of $P \leq 0.05$.

RESULTS

Initial LW and BCS were 16.7 ± 1.0 kg and 3.2 ± 0.12 units, with respective values at the end of the experimental period of 23.5 ± 0.8 kg and 3.4 ± 0.11 units. No significant differences between treatments for LW and BCS were observed along the experimental period. In addition, no significant differences were observed regarding the serum concentrations for TP: 67.6 ± 2.4 g/l, UR: 3.8 ± 0.17 mmol/l (22.83 ± 1.05 mg/dl), GLU: 5.06 ± 0.09 mmol/l (91.15 ± 1.77 mg/dl), and CHOL: 1.62 ± 0.07 mmol/l (62.32 ± 2.75 mg/dl). Interestingly, however, while a treatment \times time interaction was observed between treatments for TP, UR, GLU, CHOL across time, such differences favoured the BC group, except the UR-analyte the serum level of which increased in the CC group (Figures 2–5). Yet, such general serum metabolite profile was related neither to the age (215.7 vs 226.5 ± 6.6 days; $P > 0.05$) nor to the percentage (44.4 vs $25.0 \pm 17.0\%$; $P > 0.05$) of goats reaching puberty in the BC and CC groups, respectively. According to the natural photoperiod observed during the experimental period, the longest photoperiod occurred

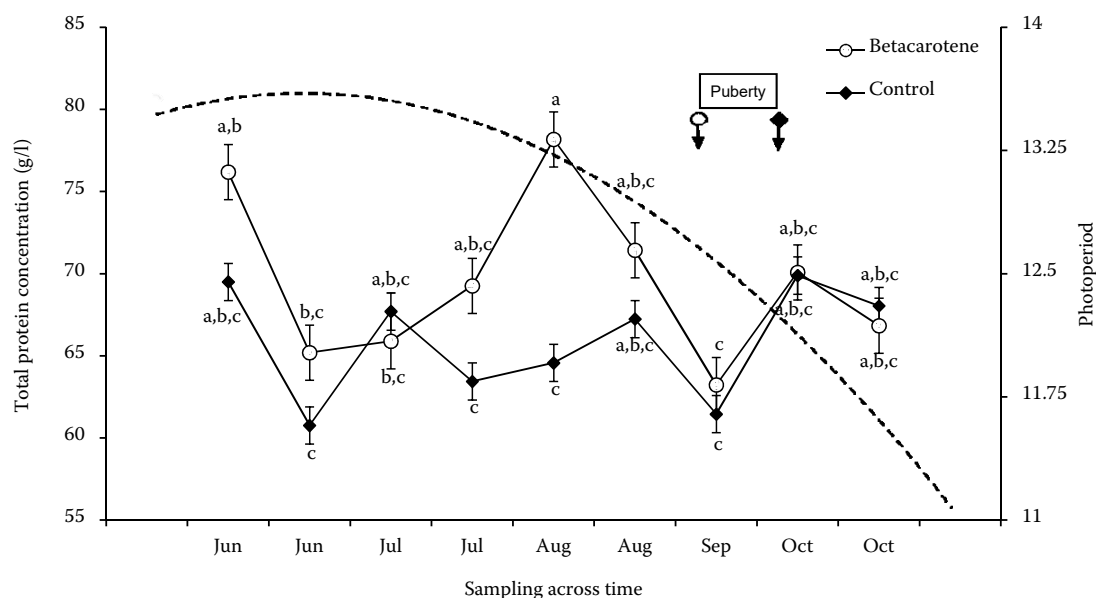


Figure 2. Serum total protein concentrations (g/dl) of experimental animals

Peripubertal crossbred female goats ($n = 17$, 3 months old, 7/8 Alpine-Saanen and 1/8 Criollo) were supplemented with betacarotene (BC) or non-supplemented (CC) and exposed to a naturally decreasing photoperiod (June–November, 26°N)

^{a-c}statistical differences ($P < 0.05$) observed between the BC and CC treatments across the 150-day experimental period; average dates for puberty onset were September 5th (BC group) and September 26th (CC group)

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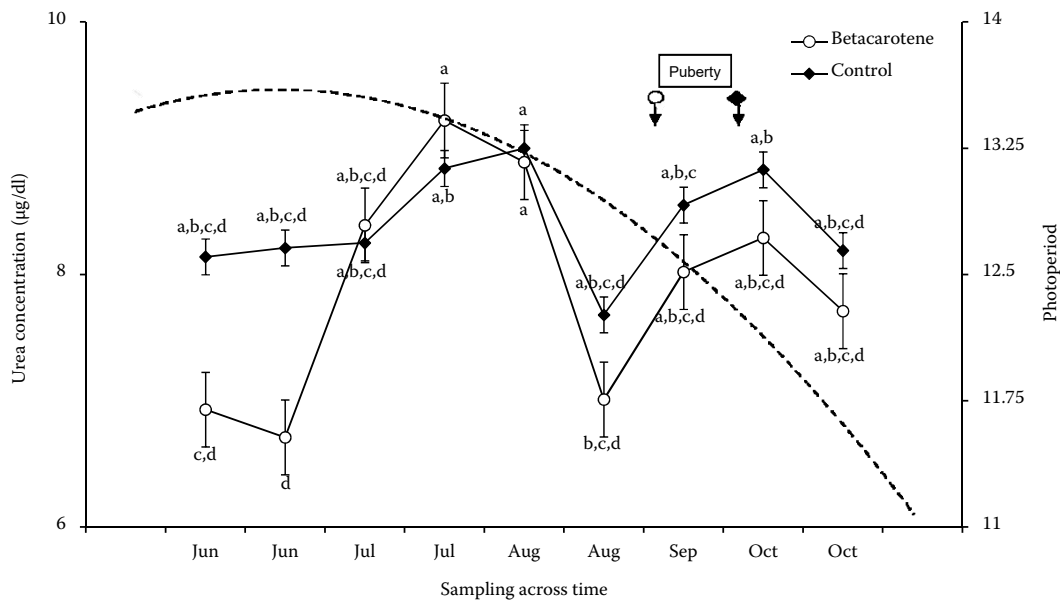


Figure 3. Serum urea concentrations (mg/dl) of experimental animals across time. Peripuberal crossbred female goats ($n = 17$, 3 months old, 7/8 Alpine-Saanen and 1/8 Criollo) were supplemented with betacarotene (BC) or non-supplemented (CC) and exposed to a naturally decreasing photoperiod (June–November, 26°N). ^{a–d}statistical differences ($P < 0.05$) observed between the BC and CC treatments across the 150-day experimental period; average dates for puberty onset were September 5th (BC group) and September 26th (CC group).

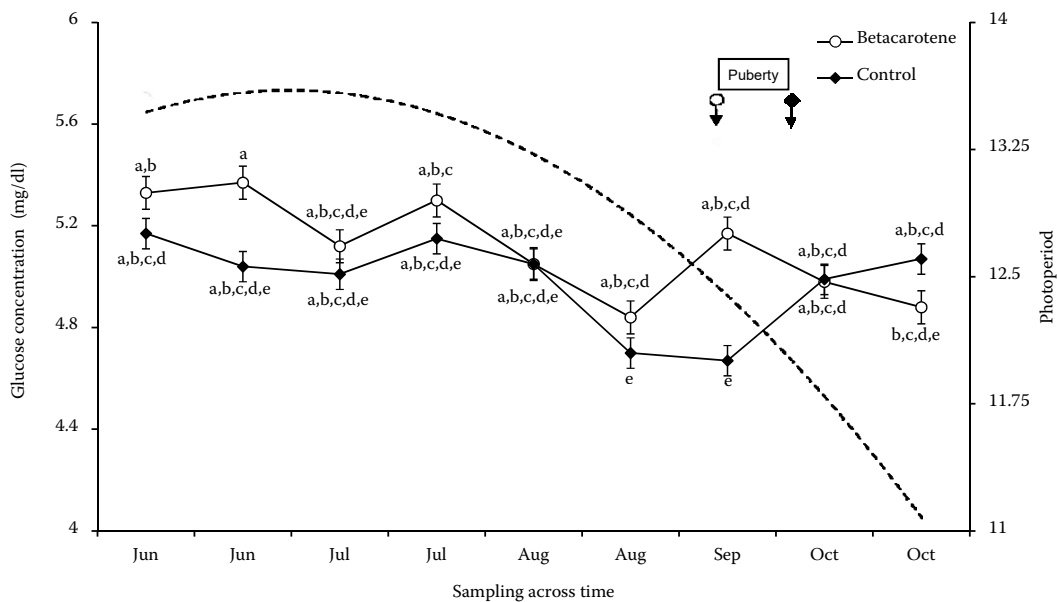


Figure 4. Serum glucose concentrations (mg/dl) of experimental animals across time. Peripuberal crossbred female goats ($n = 17$, 3 months old, 7/8 Alpine-Saanen and 1/8 Criollo) were supplemented with betacarotene (BC) or non-supplemented (CC) and exposed to a naturally decreasing photoperiod (June–November, 26°N). ^{a–e}statistical differences ($P < 0.05$) observed between the BC and CC treatments across time, although especially manifested during the 3/3 of the 150-day experimental period, coinciding with the onset of puberty in the BC group; average dates for puberty onset were September 5th (BC group) and September 26th (CC group).

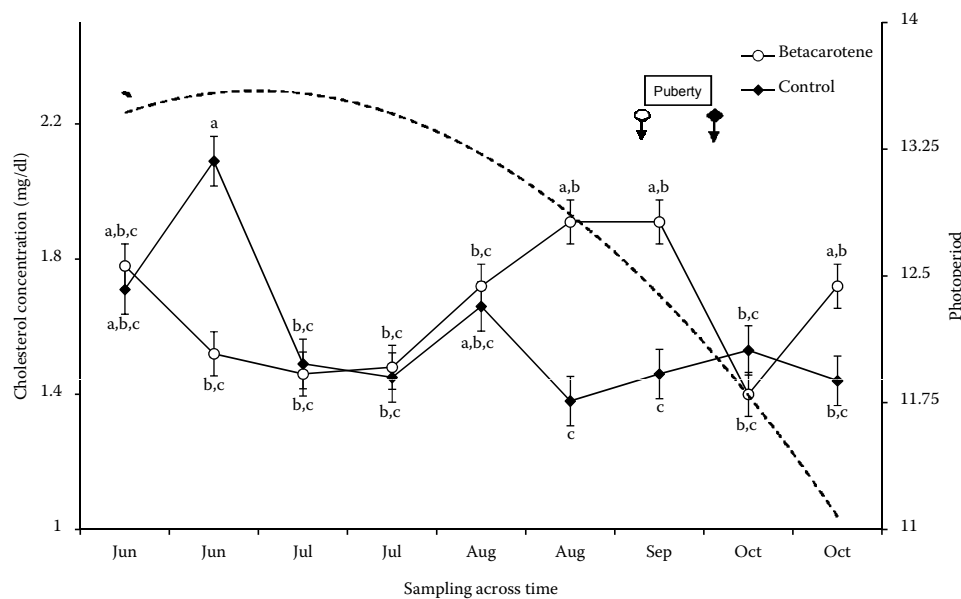


Figure 5. Serum cholesterol concentrations (mg/dl) of experimental animals across time. Peripubertal crossbred female goats ($n = 17$, 3 months old, 7/8 Alpine-Saanen and 1/8 Criollo) were supplemented with betacarotene (BC) or non-supplemented (CC) and exposed to a naturally decreasing photoperiod (June–November, 26°N)

^{a-c}statistical differences ($P < 0.05$) observed between the BC and CC treatments across time, although especially manifested during the 3/3 of the 150-day experimental period, coinciding with the onset of puberty in the BC group; average dates for puberty onset were September 5th (BC group) and September 26th (CC group)

in July (13.57 h) with a gradual decrease until early November (11.3 h). Besides, and as observed in the figures, a negative relationship was perceived between photoperiod and the onset of puberty in both experimental treatments.

DISCUSSION

Our working hypothesis stated that BC administration would promote increases across time in blood metabolites, specifically TP, GLU, and CHOL, while expecting a decrease in UR around the onset of puberty in goats. According to the observed results in our study, such hypothesis is supported by our main outcomes. This was particularly true regarding the blood analytes GLU and CHOL, the main increases of which were observed towards the final part of the experimental period, also coinciding with the onset of puberty in the BC treated group. Despite our fragmentary knowledge regarding the mechanisms modulating the intermediate metabolism (Meza-Herrera and Tena-Sempere 2012), results of our study suggest that such neurophysiologic scenario observed in

the BC-supplemented peripubertal goats may potentially involve BC as an acting molecule involved in the intermediate metabolism, specially upon protein, carbohydrate, and lipid metabolism.

While an optimal intake of BC is hypothesized to have positive effects upon reproductive outcomes both in ruminant (Arellano-Rodriguez et al. 2007, 2009) and monogastric (Krammer and Aurich 2010) species, results have been contradictory, with studies reporting both positive (Kawashima et al. 2009, 2012) and negative (Folman et al. 1987) effects. Nonetheless, a positive relationship between BC supplementation, metabolic and endocrine status as well as reproductive outcomes has been previously proposed. Short-term BC supplementation positively affected ovarian follicular development and ovulation rate in adult goats (Arellano-Rodriguez et al. 2007), increased both corpus luteum diameter as well as progesterone synthesis (Arellano-Rodriguez et al. 2009). Besides, short-term BC supplementation in the adult goat increased ovarian activity and enhanced serum concentrations of insulin (Meza-Herrera et al. 2013a), although without increases in serum luteinizing hormone (LH) concentrations,

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LH-pulse or LH-area under the curve (LH-AUC) (Meza-Herrera et al. 2013b). In addition, long-term BC supplementation in peripubertal goats increased serum insulin (Meza-Herrera et al. 2011) and triiodothyronine (Meza-Herrera et al. 2014).

The precise site of BC action upon the increased release of blood metabolites observed in this study cannot be established without additional research. Yet, a clear relationship has been reported between energy balance, metabolic fuel availability, and reproductive outcomes (Meza-Herrera and Tena-Sempere 2012). As a consequence, changes in the levels not only in metabolic hormones but also in blood metabolites are dreadfully important cues that convey information to the central nervous system (CNS) regarding to the nutritional status of animals (Dupont et al. 2014). In turn, goats have to align such metabolic status to a corresponding extent of reproductive function activating, if it is the case, the hypothalamic-pituitary-gonadal (HPG) axis (Meza-Herrera and Tena-Sempere 2012). Certainly, not only the endocrine but also different neural systems are activated in response to the metabolic status as well as in reply to the circulating levels of specific blood metabolites (Meza-Herrera and Tena-Sempere 2012; Dupont et al. 2014). As stated by Krammer and Aurich (2010), BC has elicited positive effects upon reproductive outcomes in different species, and such BC action has been suggested to be most likely BC specific and independent from its role as a precursor of vitamin A. Regarding the general concentrations of the quantified blood analytes observed in our study, they are within the range with respect to TP, yet urea is slightly above the upper limit, the average concentration of glucose is higher, while the reference value for cholesterol is below the reference values reported in goats: glucose 2.78 to 4.16 mmol/l (50–70 mg/dl), cholesterol 2.08–3.38 mmol/l (80–130 mg/dl), urea 2.09–3.65 mmol/l (12.6–22 mg/dl), and total protein 61.1–70.1 g/l (Kaneko et al. 2008). Besides, the present general concentrations of blood analytes are in close agreement with the average blood metabolite concentrations reported in goats by Ye et al. (2014).

Glucose, as chemical moiety, has been involved as a key regulator of reproductive function. Its serum levels either above or below physiological range generate deleterious effects upon reproductive outcomes (Dupont et al. 2014). A synergic effect of glucose and insulin has been reported at both

CNS and ovarian level (Dupont et al. 2014). Despite most mammal tissues use glucose and fatty acids as energy source, glucose is undoubtedly the principal energy source at ovarian level, denoting that its effects upon reproductive outcomes are mainly related to its sole feature as metabolic fuel (Meza-Herrera and Tena-Sempere 2012). At ovarian level, the follicle seems to possess well defined sensing systems to intuit both glucose levels and nutritional status (Dupont et al. 2014). For that reason, the follicle is able to discern information regarding the glucose level and facilitate, if this is the case, growth and development, throughout modulation in the follicle stimulating hormone (FSH) actions upon the ovarian steroidogenic pathway exerted by the theca-granulosa cell complex. Interestingly, such glucose influence is also extended upon the biological quality and competence of the oocyte (Dupont et al. 2014).

In mammals, significant differences have been described regarding serum cholesterol concentrations at different windows within the prepubertal-to-pubertal transition period as well as upon other reproductive outcomes such as fertility, which has been positively related to metabolic status (Dupont et al. 2014). Liver X receptors α and β (LXR α , LXR β) are nuclear receptors activated by oxysterols, which are oxidized derivatives from cholesterol. They control ovarian endocrine and exocrine function; such physiological role denotes the LXRs as important molecules linking cholesterol and reproductive function (Lobaccaro et al. 2013; Urlep and Rozman 2013).

Serum total proteins, mainly composed by albumin (60%) and globulin (40%), are synthesized in hepatocytes, while gamma globulin is synthesized by plasma cells of the immune system; all of them show a wide variety of physiological functions (Kaneko et al. 2008). Regarding the observed profile of this analyte, quite high values were quantified during the first sampling date, a situation that can be related to a dehydration status which could potentially occur since the weaning of the experimental units took place prior to the onset of the experiment. As suggested by Boldt (2010), albumin possesses excellent binding capacities, especially water, calcium, sodium while it is very important in the transport of fatty acids and hormones, principally steroids. In addition, albumin acts as a free radical scavenger while it is able to bind toxic substances (Arasteh et al. 2014), sug-

gesting a beneficial effect in the animal wellbeing. Serum albumin is also very sensitive to glycation, which involves the attachment of glucose, galactose and fructose, among other sugars, to the free amine groups of albumin; the extent of glycation depends on the glycemic status of individuals (Arasteh et al. 2014). This feature highlights its role as an important glycemic marker under both physiological and pathological scenarios (Koga and Kasayama 2010). According to Rondeau and Bourdon (2011), glucose is a vital nutrient required for cellular oxygen metabolism, hence albumin glycation should have important implications for cellular function. The last is of particular importance in that glucose has been defined as a key energy source to both the reproductive brain and the reproductive gonads (Meza-Herrera and Tena-Sempere 2012; Dupont et al. 2014).

CONCLUSION

The current study is the first report demonstrating that BC supplementation generates serum increases of total protein, glucose, and cholesterol, while decreases urea concentrations across time, around the onset of puberty in the female goat. Because of our fragmentary knowledge, further studies are needed to better understand the precise physiological actions of beta-carotene upon different reproductive outcomes and physiological scenarios; such efforts may also engender important practical applications in other animal industries

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