UNIVERSIDAD AUTÓNOMA CHAPINGO
UNIDAD REGIONAL UNIVERSITARIA DE ZONAS ÁRIDAS

UNIVERSIDAD DE CÓRDOBA
INSTITUTO DE ESTUDIOS DE POSGRADO

THE KEY ROLE OF TARGETED BETACAROTENE SUPPLEMENTATION ON ENDOCRINE AND REPRODUCTIVE PERFORMANCE IN GOATS OF THE MEXICAN ARID REGIONS

EL PAPEL CENTRAL DE LA SUPLEMENTACIÓN DIRIGIDA CON BETACAROTENO EN EL COMPORTAMIENTO ENDOCRINO Y REPRODUCTIVO EN CAPRINOS DE LAS REGIONES ÁRIDAS DE MÉXICO

THESIS IN JOINT SUPERVISION TO OBTAIN THE DOUBLE DEGREE OF DOCTOR ON SCIENCE:

DOCTOR EN CIENCIAS EN RECURSOS NATURALES Y MEDIO AMBIENTE DE ZONAS ÁRIDAS
(UACH-URUZA, MEXICO)

DOCTOR EN RECURSOS NATURALES Y GESTION SOSTENIBLE
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By:

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Bermejillo, Durango, Mexico, February, 2021

THE KEY ROLE OF TARGETED BETACAROTENE SUPPLEMENTATION ON ENDOCRINE AND REPRODUCTIVE PERFORMANCE IN GOATS OF THE MEXICAN ARID REGIONS

Thesis carried out by **NOÉ MISAEL LÓPEZ FLORES** under the supervision of the indicated Advisory Committee, approved by the same and accepted as a partial requirement to obtain the degree of:

DOCTOR EN CIENCIAS EN RECURSOS NATURALES Y MEDIO AMBIENTE EN ZONAS ÁRIDAS

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UNIVERSIDAD Ð CÓRDOBA

PROGRAMA DE DOCTORADO EN RECURSOS NATURALES Y GESTIÓN SOSTENIBLE

TESIS DOCTORAL

El papel central de la suplementación dirigida con betacaroteno en el comportamiento endocrino y reproductivo en caprinos de las regiones áridas de México.

Presentado por **NOÉ MISAEL LÓPEZ FLORES** en satisfacción de los requisitos necesarios para la obtención del grado de **DOCTOR EN RECURSOS NATURALES Y GESTIÓN SOSTENIBLE.**

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Report for thesis presented by Noé Misael López Flores

Dear sirs, Lima, 26 of January 2021

I would like to thank you for inviting me to act as an external evaluator. Having researched goat husbandry in northern Mexico for several years myself, I personally find the topic of the thesis very exciting and highly relevant to the goat sector.

The thesis entitled "THE KEY ROLE OF TARGETED BETACAROTENE SUPPLEMENTATION ON ENDOCRINE AND REPRODUCTIVE PERFORMANCE IN GOATS OF THE MEXICAN ARID REGIONS" is organized in six chapters. After a general introduction, objectives and hypothesis of the research are defined. Chapter IV is dedicated to an extensive literature review with general information on the socio-economic importance of goat production and more specific information on nutritional and reproductive aspects as limiting factors for a higher productivity. The core of the thesis are two publications in internationally recognized journals.

The first article "The key role of targeted betacarotene supplementation on endocrine and reproductive outcomes in goats: Follicular development, ovulation rate and the GH-IGF-1 axis" has been published in Small Ruminant Research (Q2). The second article "Precision Betacarotene supplementation enhanced ovarian function and the LH release pattern in yearling crossbred anestrous goats" has been published in the journal Animals (Q1). In both publications the candidate Noé Misael López Flores is listed as first author.

The two publications in high-quality journals show that the research on supplementation of goats on their productive performance is of high relevance and is recognized by the international community.

The thesis concludes with general conclusions and provides an outlook on further research opportunities.

The work meets the international requirements for a doctoral thesis.

Therefore, my decision is:

definitively approved - aprobada definitivamente

If you have any further questions, please do not hesitate to contact me for further information.

With best regards

Maria Wurzinger

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LIST OF ABBREVIATIONS

AF	Antral Follicle
AUC	Area Under Curve
BC	Betacarotene
BCS	Body Condition Score
CH_4	Methane gas
CL	Corpus Luteum
CRD-ANOVA	Completely Randomized Designs-Analysis of Variance
CV	Coefficient of Variation
ERFα, β	Estrogen-Induced Transactivation of the Estrogen-Receptor
FAO	Food and Agriculture Organization
FAOSTAT	Food and Agriculture Organization Statistical
FSH	Follicle Stimulation Hormone
GH	Growth Hormone
GnRH	Gonadotropin Releasing Hormone
GSH	Glutathione
HPG-axis	Hypothalamus-Pituitary-Gonads-axis
IGF-1	Insulin-Like Growth Factor 1
IU	International Unit
LH	Luteinizing Hormone
LW	Live Weight
MDA	Malondialdehyde
NAM	National Academy of Medicine
NEm	Neta Energy Metabolizable
NRC	National Research Council
OR	Ovulation Rate
$PGF_2\alpha$	Prostaglandin $F_2\alpha$
PROC GLM	Procedure Generalized Linear Model
RA	Retinoic Acid
RIA	Radioimmunoassay

ROS	Reactive Oxygen Species
SAS	Statistical Analysis Software
SE	Standard Error
SOD	Superoxidase Dismutase
TOA	Total Ovarian Activity
UNEP	United Nations Environment Programme
PU	Production Units

DEDICATED TO

To God:

For having accompanied me on my journey of investigation.

To my Son:

For putting up with the days of stress and pressure, for accompanying me on my waking days.

To my wife:

For your support during this doctoral route.

To my parents:

For encouraging me to continue, to improve myself professionally and personally, patience and advice.

To my sisters and brother:

For understanding me in the absence of family meetings.

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BIOGRAPHICAL DATA

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Scientific development: Reviser in three Licentiate Thesis, in the livestock scientific area. Author of two scientific articles on goat supplementation and Co-author of two scientific articles on ruminant nutrition.

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RESUMEN GENERAL

El papel central de la suplementación dirigida con betacaroteno en el comportamiento endocrino y reproductivo en caprinos de las regiones áridas de México

López-Flores, Noé, M.[1], Meza-Herrera, Cesar, A.[2], Pérez-Marín, Carlos, C.[2].

En América, México es líder en inventario y nivel de producción de leche caprina; la Comarca Lagunera, situada en el norte árido (26° N), es la principal región productora de leche caprina en México. Se desarrollaron dos experimentos (Exp1 y Exp2) para evaluar el posible efecto de la suplementación de betacaroteno (BC) a nivel endocrino y reproductivo. El Exp1, consideró cabras adultas (n=22, 45.35 ± 1.35 kg., 3.5 años, Saanen-Alpino x Criollo) en la época natural de empadre (oct-nov) evaluando los niveles séricos de la hormona del crecimiento (GH), el factor de crecimiento análogo a insulina tipo 1 (IGF-1), y en la actividad ovárica total (AOT; folículos antrales + cuerpos lúteos). El Exp2 consideró cabras jóvenes (n=22, anéstricas, 29.17 ± 1.02 kg, 1 año de edad, Alpino-Saanen-Nubian x Criollo), en la época no-reproductiva (abr-mayo), evaluando la pulsatilidad de la hormona luteinizante (LH) y la AOT. En el Exp1, el grupo-BC mostró un aumento en la AOT respecto al grupo control (CONT) (8.4 vs 6.2 unidades), paralelo a una reducción en los niveles medios de GH en el grupo-BC (10.0 vs 14.3 ng/mL), sin diferencias en los niveles de IGF-1 (254.6 ng/mL). En el Exp2, además de un incremento en la AOT en el grupo-BC (3.44 vs 1.87 unidades), se observó una mayor amplitud en el pulso de LH (0.55 vs 0.24 ng/mL). Los resultados demuestran por primera vez al BC como una molécula reguladora no solo de la actividad ovárica tanto en época reproductiva como en el anestro, sino como un modulador del patrón de secreción de GH en hembras adultas y LH en primalas, en genotipos con un alto encaste hacia razas lecheras con una marcada reproducción estacional. Futuras investigaciones deberán definir el probable sitio de acción del BC en la función del eje hipotalámico-hipofisiario-gonadal caprino.

Palabras clave: Cabras, Betacaroteno, Endocrino y Reproductivo.

ABSTRACT

The key role of targeted betacarotene supplementation on endocrine and reproductive performance in goats of the Mexican arid regions

López-Flores, Noé, M.[1], Meza-Herrera, Cesar, A.[2], Pérez-Marín, Carlos, C.[2].

In the Americas, Mexico leads the census and the production of goat milk; the Comarca Lagunera, located in the arid northern (26° N), is the main goat milk production region in Mexico. Two experiments (Exp1 and Exp2) were carried out to evaluate the possible effect of betacarotene (BC) supplementation at endocrine and reproductive level. In the Exp1, adult goats (n=22, 45.35 ± 1.35 kg, 3.5 years-old, 7/8 Saanen-Alpine x Criollo), during the natural breeding season (Oct-Nov) it was evaluated the serum levels of growth hormone (GH), the insulin-like growth factor type-1 and upon the total ovarian activity (AOT; antral follicles + corpus luteum). In from October to November (during the natural breeding season). The Exp2 considered young goats (n=22, anestrous, 29.17 ± 1.02 kg, 1 year-old, Alpine-Saanen-Nubian x Criollo) during the non-reproductive season (Apr-May), evaluating the pulsatility of the luteinizing hormone (LH) and the AOT. In the Exp1, the BC-group depicted an augmented AOT regarding the control groups (CONT) (8.4 vs 6.2 units), parallel to a reduction in the serum average levels of GH in the BC-group (10.0 vs 14.3 ng/mL), with no differences in the IGF-1 levels (254.6 ng/mL). In the Exp2, besides to an increased AOT in the BC-group (3.44 vs 1.87 units), it was observed a grater LH pulse amplitude (0.55 vs 0.24 ng/mL). These results demonstrate for the first time to betacarotene not only as AOT-regulating molecule but also as a modulator of the release pattern of GH in adult goats as well as LH in yearling goats with genotypes with a high degree towards highly marked seasonal reproduction. Future research should define the probable site of BC-action regarding the function of the hypothalamic-hypophyseal-gonadal axis in goats.

Keywords: Goats, Betacarotene, Endocrine and Reproductive.

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I. INTRODUCTION

In Mexico, there are 494,000 goat production units and they are supposed to be the primary or complementary production activity for approximately 1.5 million Mexicans (Arechiga et al., 2008). About 8.7 million goats are registered in Mexico, producing 167 tons of milk (1.1% world production) and 48 tons of meat (0.89% world production) (Andrade-Montemayor, 2017). The typical production systems of the arid and semi-arid zones of Mexico maintain about 64% of the goats, while the remaining 36% are found in the country's temperate zone. The afore mentioned arid and semi-arid zones, almost 60% of the national goat inventory, are located in the Comarca Lagunera, between Coahuila and Durango, considered the largest milk producing zone in the country. Goat farming is considered as a natural resource with high social importance among the inhabitants of rural areas (Arechiga et al., 2008). Reproduction in this species is restricted as a consequence of the low reproductive effectiveness, delayed onset of puberty, long anestrous periods, low fertility and prolificacy (mainly in marginalized areas), and high aridity and food shortages. These condition can also be found in the Comarca Lagunera (Vergara, 2014). Reproductive performance is one of the most important economic variables in modern goat production (Haliloglu et al., 2002).

Goat farming is one of the most important activities for the subsistence of the peasants both at national and regional level. This practice faces a marked limitation due, as before mentioned, to the scarcity of forage during the dry season, which forces the herd to consume plants or forages with low nutritional content, causing weight loss, and high content of secondary metabolites (López-Flores et al., 2018a). Nutritional signals act as direct regulators of seasonal reproduction and they also govern fertility in cycling animals (Meza-Herrera, 2012). Under semi-arid conditions in northern Mexico, goats satisfy their nutritional needs mainly through the consumption of available vegetation; however, the forages do not maintain a sufficient nutritional level for all the year and, only during summer months, the animals consume food enough to cover their nutritional requirements. If nutritional requirements are not covered, animals use their body reserves, with the consequent loss of weight and body condition, affecting on their productive and reproductive performance

(Rosales-Nieto et al., 2006). Nutritional deleterious effects are linked to reproductive failures in males and females, and this impact is really important since all the flock are usually affected (Campos and Hernandez, 2008).

The nutritional level affects the processes involved in the follicular development and ovulatory rate of ruminants, particularly through changes in live weight (LW) and body condition (BC), (Meza-Herrera et al., 2004; Scaramuzzi et al., 2006). These nutritional effects are mediated by changes in metabolic hormone levels and the growth factor superfamily (Meza-Herrera et al., 2006). Growth hormones (GH) serum concentrations fluctuate in response to the nutritional status and they can depress the synthesis and secretion of gonadotropins (FSH and LH) and also the reproductive efficiency (Scaramuzzi et al., 2006; Guerra-Garcia et al., 2009).

The improvement of the ovulatory and reproductive rates after nutritional supplementation can be achieved by increasing live weight and body condition (Meza-Herrera et al., 2017). In this regard, nutrition influences on the reproductive function throughout three different effects: a) static effect, which is achieved in the long term by heavier females; b) dynamic effect, generated by increased feeding for 3-4 weeks (Scaramuzzi et al., 2006); and c) acute effect, generated without promoting changes in the female's live weight, also called immediate effect by providing nutritional supplements for <10 days (Martín et al., 2004).

Small herbivorous ungulates are seasonal polyestrous breeders, i.e. they show estrous cycles only during an established season over the year, and their reproductive activity is intimately related to the rhythm of meat and milk production (Vergara, 2014). Reproductive events are closely aligned with metabolic state and food availability, nutritional level, food supplementation and body reserves (Meza-Herrera and Tena-Sempere, 2012). Nutritional supplementation is mainly carried out when animals are exposed to corporal condition loss during periods with deficient forage resources, in order to reforce the endocrine or metabolic signals involved in some reproductive purposes (Lopez-Flores, 2016). The management of supplementary nutritional components, as vitamin A or its precursor betacarotene, promotes a wide range of biological processes, such as cell development, differentiation and morphogenesis through retinoic acid (RA) (Amann et al., 2011).

Betacarotene (BC) belongs to the family of carotenoids. These phytochemical pigments are naturally synthesized in many fruits and vegetables, plants, algae and photosynthetic bacteria (Eggersdorfer & Wyss, 2018). In mammals, carotenoids are mainly metabolized in the liver; cattle and horses have the highest BC content in the liver, followed by goats (3.4 µg/g of tissue) (Darwish et al., 2016). From a reproductive standpoint, BC supplementation has been associated with increased steroidogenesis in both corpus luteum and follicular tissue (Lopez-Flores et al., 2018a). The fundamental endocrine actions of pituitary gonadotropins (LH, FSH) and other intra-ovarian molecules that act in an autocrine or paracrine manner (i.e., IGF-1 and 2) on both follicular growth and oocyte maturation have also been established (Meza-Herrera et al., 2010). In addition, the communication between the hypothalamic-pituitary-gonadal axis (HPG), is closely modulated by an extremely complex arrangement of neural inputs governed not only by photo- and thermo-periodic keys, but also by the metabolic state in animals (Meza-Herrera et al., 2011). In addition, BC has been shown to downregulate estrogen-induced transactivation of the estrogen receptor (Hirch et al., 2007), demonstrating its potential role as a HPG axis-modulating molecule. In addition, nutritional supplementation with a mixture of BC, polyphenolic compounds, probiotics and n-3 and n-6 polyunsaturated fatty acids was shown to regulate genes involved in the activation of gonadotropic cells and in the GnRH regulation (Haliloglu et al., 2002). While the participation of BC has been proposed as a possible modulator on the HPG axis (Salem et al., 2015), previous studies of our group demonstrated an interesting BC role as a modulator not only for serum insulin (Meza-Herrera et al, 2011), triiodothyronine (Meza-Herrera et al., 2014) and GH-IGF-1 system (López-Flores et al., 2018b), but also for some selected blood metabolites (Meza-Herrera et al., 2017). Based on previous findings, we hypothesized that BC supplementation might exert a positive effect on adult female goats during natural breeding season and on young female goats out of season, affecting on the ovarian function and also on the serum levels of GH, IGF-1 and LH release pattern.

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II. OBJECTIVES

Doctorado en ciencias en recursos naturales y medio ambiente en zonas áridas
Doctorado en recursos naturales y gestión sostenible

2.1. General objective

- To evaluate the effect of supplementation on the reproductive efficiency of female goats reared in the arid regions of Comarca Lagunera, Mexico.

2.2. Specific objectives

- To evaluate the effect of BC supplementation on ovarian function in adult female goats, as well as to obtain possible evidence of BC influences on serum GH and IGF-1 levels.

- To evaluate the possible effect of BC supplementation on ovarian function and relation to the pattern of LH release in yearling anestrous goats.

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III. HYPOTHESIS

H1. Betacarotene supplementation will generate an increment in ovarian function and GH/IGF-1 release pattern in adult female goats.

H2. Betacarotene supplementation will reactivate and enhance ovarian function, and the LH release pattern in yearling anestrous goats.

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IV. STATE OF THE ART

Doctorado en ciencias en recursos naturales y medio ambiente en zonas áridas
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4.1. Situation of goat farming in the world

The earliest evidence of goats dates back to the Neolithic period; moreover, cave paintings found in the Sacred Mountains of Southwest Asia suggest a relationship between humans and goats for a long time, as far as 8,000 years B.C. Despite the evidence, the domestication of the goat species is still not entirely clear and, although it is considered that goats were domesticated thousands of years ago, nowadays it has not evolved as much as other ruminants who were also domesticated. For this reason, the goat retains certain anatomical, physiological and behavioral characteristics of its primitive ancestors (Mellado, 1985).

In the last decades, the world goat population has perceived a remarkable increment, reaching a number over one billion heads, most of them located in developing countries (FAOSTAT, 2014). The indigenous breeds are undoubtedly the most widespread breeds in these areas, being the American continent a good example; only in Mexico and Brazil there are about 20 million goats (Pares-Casanova, 2016).

Table 1. Shows de continental distribution of goats (FAO, 2019).

Country	Goat Population (%)
• Asian	60
• Africa	33.1
• America	4.3
• Europe	2.2
• Oceania	0.4

China is the most representative country, with around 167 million goats, followed by India with around 130 million; these two Asian countries represent 29.22% of the world total (FAO, 2014). It is worth mentioning that 94% of the world's goat population is located in developed countries and 55% of this is in arid regions (Arechiga et al., 2008). In addition, goat production is the main source for milk and meat production for many subsistence farmers and 5.6 million tons of meat were worldwide produced during 2016, representing 1.7% of total production (FAO, 2016). However, in the last decades, high genetic merit breeds have been exported to many developing countries in order to carry out crossbreeding with local breeds, increasing the milk production and becoming cost-efficient. Saanen, Anglo Nubian, Toggenburg, Alpine and West African Dwarf breeds have been used for improving the genetics of dairy goats (FAO, 2018).

4.2. Goat farming in Mexico

Goats were introduced in Mexico by the Spaniards, and genotypic and phenotypic studies indicate a high influence of Navarra and Andalusia breeds on the original goats that arrived to Mexico. These animals are now adapted to the Mexican environment and they are particularly resistant to drought and fodder shortages. Nowadays, goat farming is a profitable livestock production, which offers incomes to many marginal families. Since the beginning of the 20th century, this type of farming has been tacked as a family activity in Mexico, demonstrating high business impact in different regions of the country due to their ability for milk production and transformation (Guerrero, 2016).

Different ecosystems existing in Mexico reveal a huge biodiversity in this country. Herbivorous ungulates with a great capacity of adaptation are distributed over this territory, which it is mainly occupied by xerophilous scrubs in arid and semi-arid climates, as well as tropical deciduous forests and, on a smaller scale, the grasslands. These ecosystems occupy 47.34% of Mexico and the majority of goat’s population is distributed in this area; Puebla, Oaxaca and Guerrero on the south stand out, and San Luis Potosi, Coahuila, Durango and Zacatecas on the center-north area. In these states, as in other developing countries, small ruminants and especially goats are managed by small producers. Of the national goat population, 80% is governed by an extensive production system, coupled with the existence of as many variants as geographical areas or cultures in the country. In addition, an incalculable wealth of germplasm has been developed thanks to the selection of the breed groups themselves, which leads to two interesting situations (Alejandre-Ortiz et al., 2016). On the one hand, this type of livestock is considered by some to be an unproductive activity because it does not contribute more than 1% of the value of the country's livestock production (SAGARPA-SIAP, 2014). However, another goat sector has committed to the objective of "making it profitable" by implementing a series of monitoring measures that only a small portion of producers can comply with, specifically the large dairy producers in the central zone of the country (El Bajio), and the northern zone (Comarca Lagunera). Another issue, the gradual destruction of ecosystems is a serious problem, which become extremely fragile, requiring land management for their conservation; in this context, goats are usually considered a threat, rather than an integral part of the system, however, they are an essential part of this puzzle. Producers who practice subsistence livestock farming will hardly become entrepreneurs, since it will be difficult for most to radically change their ancestral practices (Alejandre-Ortiz et al., 2016).

The Northern arid and semiarid region produces 42% of meat and 72% of milk of the total annual production per year, and Durango and Coahuila (the Comarca Lagunera), Zacatecas and Nuevo Leon are the most relevant states involved. Goat meat is the largest production, which is consumed almost entirely in the same region. In the same way, the Comarca Lagunera, located in the Mexican arid area, is an important goat production region, but there are also more than 400,000 cows (SAGARPA-SIAP, 2014). The goats currently raised in the Comarca Lagunera are descendants of Murciano-Granadina and Malagueña Spanish breeds which, in turn, were crossbred with other as Alpine, Saanen, Toggenbourg and Anglo-Nubian during the last 60 years, improving the milk and meat production. These mixed goat breeds are currently the predominant population in the Comarca Lagunera and they are called "criollos" or "locals" (Merlos et al., 2008). In the Comarca Lagunera, 90% of the goats are a mix of breeds with high dairy background, called "mosaico lagunero", and they are managed under extensive production systems (Meza-Herrera et al., 2014). These animals are fed only on the natural pastures and do not receive nutritional supplementation. The availability of forage species that are consumed by goats suffers a drastic decrease during the period from November to March (Saenz-Escarcega et al., 1991).

Goats reared under extensive systems in the Comarca Lagunera are grazed during the morning and return in the afternoon. At night, they are housed in open facilities and, in general, females and males remain together throughout the year. Kids are suckled and later, they are weaned and sold at around 4-6 weeks-old. However, the milk sale is considered as the activity that generates the greatest incomes for producers (Sáenz-Escárcega et al., 1991). In this region, goats generate profits higher than 781 million MXN or around 39 million USD due to the milk production and commercialization (SAGARPA-SIAP, 2014). In the same course, the Comarca Lagunera is the region producing the highest goat milk yield, with nearly 60 million liters of milk per year (SAGARPA-SIAP, 2013), which represents approximately 36% of the total production in the country.

4.3. Environmental impact of goat farming

Extensive goat production systems impact on the ecology due to the uncontrolled grazing, which conduct to the desertification, contributing to a worrying climate change. Awareness campaigns are nowadays aimed at producers, in order to highlight the importance of conserving these ecosystems without undermining the productive activities (Alejandre-Ortiz et al., 2016).

In recent years, the negative impact of livestock farming on the environment has been emphasized. The report "*Livestock's Long Shadow*", published by FAO in 2006, brought together a large body of evidence showing the detrimental environmental effect of livestock and livestock-related anthropogenic activities (Steinfeld et al., 2006). Most of the summer pastures in Mexico are managed by "ejidos" or shared lands. An "ejido" is a legal entity, in which a community of people (not businesses) owns a large land area whose objective is the exploitation and integral use ("supposed") of its natural and human resources through personal work. In the "ejido", grazing lands are managed under two forms of land tenure: common and parcel (private property) uses. In the first one, "ejidatarios" with livestock can graze their animals without restrictions, and in the second one, the use of the land is exclusive to a rancher with exclusive use rights for that area. Grazing lands have different degrees of deterioration attributed to the relationship between the density of animals and the capacity of the pastures to serve as food and to resist trampling (Barrera et al., 2018).

At present, thanks to the deepening in the study of the characteristics of the genus Capra, it has been proposed that the goat operates from chaos to order and not that due to its rusticity it generates disturbances in different habitats.

Its role as the cause of soil erosion has been questioned and it has been suggested by many people that the real predator is the human being, who, due to irrational attitudes and a lack of knowledge, places this species in ecozones where no other species can develop, thus generating disturbances or overgrazing. The goat, thanks to its great rusticity, survives in such places consuming the scarce vegetal component that this habitat offers. This can be seen in two ways, that domestic animals live in human society as useful animals, or that society believes that natural resources were created for them. Bedotti (2008) mentions that the goat, more than any other species in animal production, should be considered as part of a complex in which it is in permanent interaction with man and the environment in which it is raised, especially if one thinks of intervention policies in the systems in which this species plays such an important role.

Goat is a multifunctional animal, having unique characteristics, which allow them to carry out a socio-economic role in extreme marginal areas. They can also be integrated into controlled livestock systems, exercising a preventive role in the control of forest fires, opening up grazing areas closed by bushes and even allowing a more harmonious and sustainable management with the environment. Landau et al. (2000) mention that the demands faced by goats to find sustenance and to interrelate with soils, climates and people even more adapt to each of these changing systems, life for herbivorous ungulates extends between the limits of order and chaos.

Bedotti (2008) affirms that the goat, more than any other species in animal production, should be considered as a part of a complex where man and environment are continuously interacting, mainly in case of intervention policies.

Having reviewed some unique characteristics of this species that have developed through its survival, we can therefore consider that the goat is a multifunctional animal, but above all, it is instrumental in implementing lines of research on animal production and above all, producing a cultural change in those who have seen the goat as a problem and not as an answer.

Documentation reported by Rodríguez-Florentino (undated) informs about the main countries in the world generating methane emissions from enteric fermentation (produced by cattle, buffaloes, sheep and goats and pigs). The first contributing country is sub-Saharan Africa with 2.30 million tons (Tg) of CH4 year^{-1} by dairy cattle, and 1.82 Tg CH4 year^{-1} by sheep and goats with, followed by Asia with 0.84 and 0.88 Tg CH4 year^{-1}, respectively. Central and South America together generate 3.36 Tg CH4 year^{-1} by dairy cattle and 0.58 Tg CH4 year^{-1}, among others. According to the livestock production systems, the extensive system is positioned as the second issuer of methane gas per year with a total of 29.58 Tg and being in first place the mixed system with a total of 55.02 Tg CH4 year^{-1}.

4.3.1. Social impact of goat farming

The goat sector is underdeveloped in the country due to a series of weaknesses of different nature, specifically focused on the socio-cultural aspect, which limits its development. Most Mexicans reject this species because they consider that they cause too much work with minimum earnings and it is an activity carried out by low social status population (*chiveros*), (Rubio & Perez-Eguia, 2014). It is very important to identify the problems that are determining this decline and to propose the most favorable policy for the development of the goat farming. In general, goat products have a high value when they reach the final consumer, which is not reflected in the income or standard of living of primary producers, indicating a latent potential development (Guerrero-Cruz, 2010). Although there is a very close relationship between goats and poverty, they cannot be considered as synonymous terms, although these are beliefs or thoughts that are very culturally rooted in many societies and therefore, they should be reconsidered (López-Flores, 2018b).

In the last 16 years, the production units (UP) dedicated to goat farming have decreased by more than half. It is estimated that goat farming generates more than 19 million workdays per year and it provides salary for low-income producers in marginalized areas (Alejandre-Ortiz et al., 2016).

Farming based on traditional breeding management pursues to save money and to practice the self-consumption. It is currently known as the "social sector", which has great importance not only from the economic point of view, but also socially by providing work for families and fostering rural roots

(Dorantes-Coronado et al., 2014). However, in Mexico, as Cavallotti reported, "livestock farming has historically been subject to a kind of stigmatization, mainly in the social sciences due to its association with extensive livestock farming linked mostly to land dispossession and environmental degradation" (Cavallotti, 2017). In particular, goat farming is one of the sectors that has received attention from governments and international organizations; the academic sector has however shown little interest on goat farming and on the social groups that practice it (Anzaldo-Montoya, 2020). Consequently, the importance of goat production in the social sector or in traditional systems is not only limited to technical innovations, since the interaction with social and economic factors are really diverse and important, as already mentioned (Dorantes-Coronado et al., 2014).

Recently, within the framework of the discussion on the global environmental crisis, grazing livestock (goats, camels, reindeer, llamas, etc.) emerged as a relevant issue in the public agenda of international organizations. Pastoralist groups that for years had been kept away from studies, programs and funding, are now a focus of attention within the global climate change agenda. The report "*A case of benign neglect: knowledge gaps about sustainability in pastoralism and rangelands*", published in 2019 by the United Nations Environment Programme (UNEP), states that "*pastoralism (goats, camels, reindeer, llamas, etc.) is a fundamental activity in the protection of ecosystems in natural grazing lands*". On the other hand, it recognizes that "*it is an activity anchored to the identities and traditional knowledge of diverse social groups, which contributes to the food security of millions of people around the world*" (Anzaldo-Montoya, 2020).

4.3.2. Economic impact of goat farming

Mexico possesses great biodiversity thanks to the presence of different ecosystems. Herbivorous ungulates occupy around 47.34% of the surface of this country, being small goat farms highly represented in the southern and center-northern areas (Alejandre-Ortiz et al., 2016). Goat farming is considered a natural resource of high social importance and considerable among rural dwellers, without whose support they would have practically no other resource on which to depend (Arechiga et al., 2008). Reproductive indexes are limited as a consequence of low reproductive effectiveness, delayed onset of puberty, prolonged anestrus periods or low fertility and prolificacy, due particularly to high aridity and scarcity of food in this marginalized areas, as the Comarca Lagunera (Vergara, 2014).

In Mexico, there are 494,000 goat production units and approximately 1.5 million Mexicans who have as their primary or complementary productive activity goat farming. Goat production is associated mostly with lower income rural population strata located in characteristic zones of arid and semi-arid areas of the country, 80% of which are subsistence production systems (Andrade-Montemayor, 2017).

Regional production systems are heterogeneous, with technological and health gaps and little or no organization and integration. Thus, goat farming generates 48,000 tons of meat and 167,000 tons of milk annually (SAGARPA-SIAP, 2012). The states with the largest goat population are Puebla with 15.4%, Oaxaca with 12%, San Luis Potosí with 10.5%, Guerrero with 7.9% and Zacatecas with 6.1% of the national population. Among the most milk-producing states, Coahuila stands out with 37.2% of the national total, Durango with 21%, Guanajuato 16.8%, Nuevo León 9.9%, Jalisco 3.7% and Zacatecas 3.2% (Arechiga et al., 2008).

Annual per capita consumption of goat meat is 0.4 kg, of which 2.1% is imported meat. It is important to mention that a high percentage of goats are slaughtered and consumed by the farmer himself, so the information that exists may not be as accurate (Arechiga et al., 2008).

An example of the low level of diversification in the goat world commercialization chain is the fact that the main commercial product is generally live animals (domestic markets). As for goat dairy products, cheese is the main product marketed, with the United States, Canada, Mexico and Japan being the main importers. France is the country with the largest and best goat dairy technology and its products with designation of origin are highly recognized in the world market (PlanNet Finance, 2011).

Most economic analysis studying extensive livestock do not consider the cost of feed in grazing, i.e. the cost of the pasture as a source of food is not taken into account, which causes overestimate the profitability of these production systems. Costs are an important tool for planning and controlling the activities of any production system. The estimation of production costs allows the generation of information for making decisions in aspects such as investment in technological innovations and changes in the production processes that influence the economic profitability and competitiveness of the company (Barrera et al., 2018).

The Mexican agrifood system has been undergoing transformations as a result of a series of factors that have affected it, such as economic liberalization, the industrialization of agriculture and changes in consumption patterns. These situations have caused our agricultural production to face an increasingly intense competition, mainly due to the increase of imported products based on subsidized schemes and in general, with superior quality standards (Ducoing-Watty, undated). Due to its socioeconomic importance at a national level, goat production in Mexico represents an important option for sustainable development for the rural population. However, there are currently a series of challenges to be faced by producers, marketers, technicians and researchers in order to make this activity a generalized element of increased quality of life for goat producers. Goat production should be considered as having a great productive, economic, social, cultural and ecological importance; this activity offers multiple and diverse contributions to society, mainly in the marginalized regions and social sectors, where in addition to the food and raw materials it produces, it is an important source of self-employment and income. Undoubtedly, goat farming in Mexico is a great challenge for scholars of the conservation of local domestic animal biodiversity and sustainable development (Alejandre-Ortiz et al., 2016).

4.4. Reproductive aspects in the female goat

The goat is a seasonal polyestrous animal, with cyclic estrous only during a specific season over the year. When the species is managed without any reproductive manipulation scheme, the reproductive season becomes a productive season, which presents serious marketing problems for producers who are generally immersed in a market that demands product throughout the year and curiously increases its demand during the season that corresponds to the lowest natural production (Vergara, 2014).

Reproduction in goats is conditioned by a series of environmental factors. Photoperiod, stress, nutrition, pheromonal factors and socio-sexual cues are determining factors in the reproductive process. Females detect the prevalent photoperiod and condition their estrus response through the manifestation of their reproductive activity (estrus season and ovulation rate). In this way, a strategy is established that will ensure the success of conception, pregnancy and lactation (Cueto et al., 2000).

The goat presents a gestation period of 5 months and its sexual behavior is usually expressed during the end of the summer and during the autumn, when daylight hours are diminishing (decreasing photoperiod) (Cueto et al., 2000). During the breeding season, goat shows estrus every 19 to 21 days. Sometimes, they present infertile estrus and short estrous cycles (5 to 7 days) at the beginning of the reproductive season and after the males are incorporated. In addition, the corpus luteum formed at the beginning of the breeding season has a short half-live, producing low progesterone levels (Chemineau et al., 1984). This is a characteristic of the goat species and has been reported by several authors in different dairy, meat and mohair breeds (Gibbons et al., 1994). Reproductive activity is manifested at the onset of puberty and is closely related to live weight and/or metabolic status, occurring at highly variable ages (Meza-Herrera et al., 2010b). Pubertal females present estrus with ovulations when they reach 45 to 65% of their adult weight. In the Saanen and Alpine breeds, the reference weight is 31 to 32 kg and in the Angora breed, 25 to 26 kg (Cueto et al., 2000).

4.4.1. Reproductive seasonality

Reproductive seasonality is an adaptive strategy by which wild and some domestic animals reduce the effects of annual variations in temperature and food availability (Karsch et al., 1984). In these species, the reproductive activity takes place during a specific period and then, births occur at the most appropriate time of the year and newborns will find the most favorable environment for development and growth. Seasonal species adapt their reproductive activity according to the gestation length in order parturition will be coinciding with spring season (Paleta, 2015).

Seasonality is maintained in most of sheep and goat breeds originating from medium and high latitudes (> 35°); (Malpaux et al., 1996), in which the period of reproductive activity corresponds to autumn-winter (short or decreasing days), while the period of reproductive inactivity appears in spring and summer (long or increasing days). In Mexico, female goats have a period of reproductive activity between August and January (Zarazaga et al., 2005) and male goats have a significant reduction in libido during the spring, regardless of the level of feeding (Zarazaga et al., 2009).

The environment defines the lifestyle and activity of the animals through three important components that interact with each other: the basic intensity of stress, the time scale magnitude of fluctuations, and the availability of energy and resources, causing living organisms to develop responses on different time scales: evolutionary and behavioral strategies accumulated over many years, adaptive strategies on a circa-annual, lunar or solar basis, and circadian and circa-annual strategies (Gonzalez-Bulnes et al., 2011).

One or several environmental factors influence on the gonadotropic neuroendocrine activity along the annual reproductive cycle in small ruminants, being the duration of light hours per day or photoperiod the most repeatable factor. This is considered as the main external factor that determines the reproductive activity period (Santa Maria *et al*., 1990). As a consequence, the reproductive seasonality produces important variations throughout the year in reference to the meat and milk production, causing important oscillation in their prices (Celi, 2012).

Nutritional signals are also direct regulators of seasonal reproduction and a main regulator of fertility in cycling animals (Meza-Herrera, 2012). Under semi-arid conditions in northern Mexico, goats satisfy their nutritional needs mainly through the consumption of available vegetation. However, forages frequently do not have nutrients enough to cover these needs, and only during summer months they can consume the required nutrients in order to cover or exceed their necessities. In case of deficient nutrition, animals must use their body reserves, with the consequent loss of weight and body condition, which could negatively effect on their productive and reproductive performance (Rosales-Nieto et al., 2006).

4.4.2. Seasonal Anestrous

Seasonal anestrus is the consequence of a decrease in the activity of the hypothalamus-pituitary axis, noting a reduction in the frequency of GnRH pulses and, consequently, also the secretion of pituitary hormones. The photoperiodic message acts on the hypothalamus and on the neurosecretory cells (in the central nervous system) to regulate the reproductive function. The hypothalamus regulates the secretion of GnRH through the retraction of the ovarian steroids, which translates into a seasonal variation of the LH pulses, so that the frequency of the pulses increases during the short days and decreases in the long ones. The effects of the photoperiod on LH secretion and negative steroid feedback can be simulated using pinealectomized sheep and using serial melatonin infusions (Paleta, 2015).

In the case of the Comarca Lagunera, the period of anestrus in the females and sexual rest in the males coincide with the drought period and, consequently, with a dramatic decrease in the quantity and quality of forage, suggesting that the absence of sexual activity is partially caused by underfeeding (Vergara, 2014). Anestrus is a physiological event, sometimes associated with pre-puberty period, gestation or post-partum, but it is also associated to environmental and management factors (Lopez-Sebastian, 1999).

4.4.3. Influence of photoperiod in the female goat to migrate from the anestrous period to another of sexual activity.

The domestication in some mammals has allowed us to understand reproductive variations; such characteristics were inherited from the wild ancestors (Urviola and Riveros, 2017).

Photoperiod could exert a positive effect on goats, resulting in ovulations two months after the end of the summer solstice. Then, goats show cyclic reproductive activity during more than half of a year. The light stimulus, directed towards the retina, is transported to the central nervous system and pineal gland. This acts as a neuroendocrine transmitter, secreting melatonin under fewer light hours. Melatonin affects the production of hypothalamic GnRH, which initiates of gonadal action (Matamoros and Salinas, 2017). The released steroids act on the folliculogenesis process, sexual drive and development of secondary sexual characteristics. In addition, they promote a negative or positive feedback on the hypothalamus-pituitary axis, preventing an unblocking of the system.

As mentioned, in seasonal species such as goats, cyclicity is not perennial for a whole year, thus defining an anestrus phase and an activity phase. The neuroendocrine action during these two seasons is very noticeable. Ovulation occurs in normal estrus cycles and, after the luteinization, the corpus luteum is formed, secreting progesterone during approximately 16 days. Then, luteolysis is initiated and corpus luteum is destroyed, beginning a new estrus cycle. The follicular growth promotes the increment of estradiol 17β, which induce the estrus signs during the heat or standing estrus.

On the other hand, the anovulation season is carried out by an almost complete absence of cycles. A low frequency of LH pulse is observed in the female goats, less than 2 pulses per 6 hours, and endogenous progesterone is not produced since no CL is formed. The low activity of the luteinizing hormone during anestrus is driven by the negative feedback of 17β-estradiol on the hypothalamus-pituitary axis (Chemineau and Delgadillo, 1994).

A central factor that translate exogenous and endogenous signals is the so-called "LH-pulse generator". This neuroendocrine processing gives rise to the pulsed mode of gonadotropin secretion and serves to modulate the reproductive action of light signals. Previous studies in sheep have shown that the “LH pulse generator” produces GnRH release from the hypothalamus to be later discharged into the pituitary gland. Due to the effects influenced by the days of less light hours, there is an elevated production of LH pulses that gives the strength to provoke bursts of GnRH discharge from the hypothalamic neurons. The LH pulse frequency will be maintained to complete the preovulatory process, as long as the progesterone does not manifest itself. It is worth mentioning that the influence of progesterone is so important since the reproductive system depends on it, exerting a negative feedback from the somatotropic axis by decreasing the frequency and increasing the amplitude of the LH pulses, suppressing follicular growth and blocking ovulation (Lozano-Gonzalez et al., 2012).

The core of this model is that the transitions between the reproductive stage and estrus are a consequence of LH pulses (Karsch, 1984; Meza-Herrera, 2017). Therefore, these annual variations are due to the synthesis and secretion of melatonin plus a set of neurotransmitters (Urviola and Riveros, 2017).

It is interesting to clarify those neuroendocrine events involved in the seasonal reproduction and why female moves from the anestrus to the reproductive activity. And, it should be also considering the mechanisms or effects that they exert on the hypothalamus-pituitary axis, which modulate the estrous cycle. The key control seems to lie within the system governing the tonic LH secretion, specifically whether or not estradiol can suppress the neural processes that occur due to this secretion. Therefore, it is stated that photoperiod modulates the annual reproductive cycle and thus triggers a hormone cascade, from the peak of the hypothalamus.

During anestrus, the LH generating pulse becomes extremely sensitive to inhibition by estradiol, so much so that the basal estradiol level is powerful enough to cause a suppressive effect. The hypothalamic frequencies of GnRH are very diminished by the low pulses of LH not having supported the effects of the basal estradiol. Redundantly the anestrus to estrus transitions are more than the consequence of the LH pulse generator frequencies (Karsch, 1984; Meza-Herrera, 2017).

4.5. Nutrition-reproduction interaction

Considering the critical role that the nutritional status plays in the reproductive events, nutritional management is essential to optimize reproductive performance in many species. The endocrine system is one of the key mediators between external environmental changes and internal responses, with proven impacts not only on reproductive anatomy and physiology, but also, and consequently, on reproductive behavior and efficiency (Meza-Herrera & Tena-Sempere, 2012). In general, species have adjusting their reproductive cycles to the available food resources, and animal physiology (specifically in females) has been adapted to meet the nutritional requirements related to reproduction. Females tend to accumulate corporal reserves for using in case of negative energy balance, such as occur during lactation, or for improving the reproductive cycles (Carrion & Medel, 2002). In any of the above scenarios, nutritional status is closely related to the function of the hypothalamic-pituitary-gonadal axis (Meza-Herrera & Tena-Sempere, 2012). Under this context, our research group has studied (Meza-Herrera et al., 2014), the possible effects associated to the glutamate and beta-carotene supplementation in goats, aimed at endocrine-reproductive outcomes. Therefore, taking as a background the positive results of the BC-based supplementation, it was decided to continue with this line of research, taking as an experimental unit the adult and young female goat respectively and in different seasonal periods.

4.5.1. Importance of Betacarotene as a modulator of the reproductive function in ruminants

Betacarotene is the main precursor of vitamin A and can be present in forages. This essential vitamin can be found in forage, it is really important for life, since in some mammals it is not produced per se. In the ruminants, the fat participates in the transport of betacarotene by the lymphatic system, and later it is transformed into vitamin A, which it is stored in the liver (Aguiar & Rojas, 2015). It should be noted that some carotenoids can be transformed into vitamin A, depending on the concentrations of beta-ionone, which come precisely from the natural degradation of carotenoids to be subsequently split by animals and obtain as a product retinol (Carranco et al., 2011). Reports indicate that one milligram of beta-carotene is transformed by the intestinal mucosa into an activity equal to 400 IU of vitamin A. Numerous researches have demonstrated the effects of betacaroten in the reproductive function (Arellano et al., 2007; Arellano et al., 2008; López-Flores et al., 2018 and López-Flores et al., 2020). With a focused and regulated influence on the ovary, and acting the betacarotene as a positive modulator in the activity and hormonal development of the follicle and corpus luteum (CL). Enriquez (2016) suggested that "in the cells of the intestinal mucosa, specific amino acids transfer the betacarotene to the liver, where it is carried to high density lipoproteins. Betacarotene, plus cholesterol and accompanied by vitamin E, cross the follicular barrier and are internalized in the follicular fluid. Right there, by the action of the enzyme carotenase, betacarotene is transformed into vitamin. Finally, it will activate the enzymatic complex of cholesterol for the metabolism of progesterone and proteolytic enzymes which will help the follicle to break down for ovulation. It is worth mentioning that vitamin A is also very important to prevent weak births, or in the worst case, miscarriages or uterine atony. Fortunately, herbivorous ungulates are capable of storing vitamin A in considerable proportions in the liver, which is used in times of shortage (Hazard, 2017). It has been observed that cattle, followed by horses, obtain the highest values in hepatic substrate of betacarotene, the goats continuing with (3, 4 g/g of tissue), specifically (Darwish et al., 2016).

The contribution of complementary nutritional substances to the diet is considered essential to achieve reproductive performance goals in many species. One of the contributions to reproductive activity is the modulation of hormone groups and metabolic events, which takes the starting point is the gonadotropin-releasing hormone, forming the link between the brain and the reproductive system. In turn, the production of gonadotropins is released by luteinizing hormone and follicle-stimulating hormone. The nutrition factor is intimately related to the function with the hypothalamic-pituitary-gonadal axis, since they establish a link between the energetic balance, the availability of the metabolic fuel and the reproductive outcomes. The supplementation, besides regulating the hormonal system, is the key to modulate animals that present cyclicity and efficiency in reproduction and production outcomes (Meza-Herrera & Tena-Sempere, 2012). In addition, beta-carotene has played a role as an antioxidant by repressing oxygen free radicals that harm in steroidogenesis, triggering this increases protein, total cholesterol glucose and decreasing urea in goats (Dominic & Khoboso, 2020).

Meza-Herrera et al. (2017), reported that betacarotene has contributed as a major signaling molecule for the activation of the HHG axis and a modulating role in insulin, triiodothyronine and positive effects on blood metabolites.

The use of complementary nutritional substances, and their effects on the reproductive function can be attained through three strategies: the static, dynamic and acute effects. These effects will modulate a large part of the physiological system of animals (Scaramuzzi et al., 2011). On the other hand, undernourished animals present a late puberty, due to weak pulsations of the

GnRH hormone, which in turn triggers a decrease in the pulsatile LH secretion (Mojapelo & Lehloenya, 2019).

INSTITUTO DE ESTUDIOS DE POSGRADO

THESIS IN JOINT SUPERVISION TO OBTAIN THE DOUBLE DEGREE
OF DOCTOR SCIENCE:

THE KEY ROLE OF TARGETED BETACAROTENE SUPPLEMENTATION ON ENDOCRINE AND REPRODUCTIVE PERFORMANCE IN GOATS OF THE MEXICAN ARID REGIONS

EL PAPEL CENTRAL DE LA SUPLEMENTACIÓN DIRIGIDA CON
BETACAROTENO EN EL COMPORTAMIENTO ENDOCRINO Y
REPRODUCTIVO EN CAPRINOS DE LAS REGIONES
ÁRIDAS DE MÉXICO

V. ARTICLES

Doctorado en ciencias en recursos naturales y medio ambiente en zonas áridas
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5.1. The key role of targeted betacarotene supplementation on endocrine and reproductive outcomes in goats: Follicular development, ovulation rate and the GH-IGF-1 axis.

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The key role of targeted betacarotene supplementation on endocrine and reproductive outcomes in goats: Follicular development, ovulation rate and the GH-IGF-1 axis

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Targeted supplementation

ABSTRACT

The possible effects of betacarotene (BC) supplementation on the secretion pattern of growth hormone (GH) and insulin-like growth factor-1 (IGF-1), and their possible relationship with total ovarian activity (TOA), was evaluated in adult goats during the breeding season. In October, goats [$n = 22$, 3.5 y. old, 7/8 Sannen-Alpine] were randomly assigned to: a) Betacarotene group [BC, $n = 10$; 45.9 ± 1.97 kg live weight (LW), 3.04 ± 0.08 units, body condition score (BCS), supplemented with 50 mg of BC goat day^{-1}], and b) Control group [CONT, $n = 12$; 46.2 ± 2.04 kg LW, 3.0 ± 0.08 units, BCS]. An ultrasonographic scan was performed to evaluate corpus luteum number (OR) and antral follicle number (AF); TOA = OR + AF. Average LW and BCS did not differ ($p > 0.05$) during the experimental period, yet BC-goats reflected an increased OR (3.4 ± 0.2 vs. 2.8 ± 0.2), AF (5.0 ± 0.6 vs. 3.4 ± 0.6) and TOA (8.4 ± 0.5 vs. 6.2 ± 0.6). Regarding the endocrine profile, the lowest ($p < 0.05$) serum GH average concentrations (10.0 vs. 14.3 ± 1.0 ng mL^{-1}; $p = 0.01$) and GH-AUC (3670.4 vs. 5235.7 ± 369.8 units; $p = 0.01$), were observed in the BC-supplemented group. Neither serum IGF-1 concentrations (254.6 ± 28.9 ng mL^{-1} $p > 0.05$) nor GH-PULSE (1.4 ± 0.5 pulses 6 h^{-1} $p > 0.05$) differed between treatments. We document a potential role of BC as modulator of somatotrophic function, decreasing mean serum concentration and the area under the curve of GH, while also noting a positive action upon ovarian function with increases in ovulation rate and antral follicular development; such outcomes may embrace not only physiologic significance but also potential translational applications.

1. Introduction

Reproductive and productive success is closely aligned to metabolic status and food availability (Scaramuzzi et al., 2011; Meza-Herrera and Tena-Sempere, 2012). Supplementation of either vitamin A or its precursor betacarotene (BC) promotes an ample range of biological processes such as cellular development, differentiation and morphogenesis through the action of retinoic acid (RA) (Amann et al., 2011). BC is a potent scavenger of free radicals, especially singlet oxygen (Schweigert et al., 2003). Since RA interacts with nuclear receptors, it has the ability to modulate many gene products linked to reproductive performance (Schweigert et al., 2003; Amann et al., 2011).

In herbivorous ungulates, the largest BC accumulation occurs in the liver with cattle and horses reflecting the highest BC liver content, followed by goats, buffalo and sheep; goats' BC liver concentration is around 3.4 μg/g tissue (Darwish et al., 2016). Even though an optimal intake of BC is hypothesized to affect ruminant reproduction, both negative (Folman et al., 1987) and positive effects have been reported (Kawashima et al., 2009). BC supplementation has been linked to an increased steroidogenesis in both luteal and follicular tissues (Haliloglu et al., 2002; Kawashima et al., 2009, 2010). In addition, BC appears to be a modulating molecule involved in the intermediate metabolism

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(Attia, 2009), while also inhibiting the estrogen-induced transactivation of the estrogen receptor (EREα, β) (Hirch et al., 2007), delineating an interesting role of BC as a modulating molecule for the hypothalamic-pituitary-gonadal (HPG) axis.

In addition, while the gonadotropic system is the key driver of reproductive function (Meza-Herrera, 2008; Scaramuzzi et al., 2011), the somatotropic system (GH/IGF-1) modulates not only metabolic processes but also reproductive function. Since GH and IGF-1 receptors are present on the pituitary gonadotrophs, the ovary, the granulosa cells and the oocytes (Bartke et al., 2013), a possible role emerges for the GH/IGF-1 system as a modulator of the HPG axis in females. GH actions along the reproductive tract may promote effects in a dichotomic fashion, in that stimulation of both synthesis and release of GH could relate to inhibition of the hypothalamic discharge of GnRH (Scaramuzzi et al., 2011). The actions of GH may be direct in target organs or could be mediated by IGF-1 released from hepatocytes in response to GH stimulation (Bartke et al., 2013).

Whereas BC has been found to be an important signaling molecule for positive activation of the HPG axis (Haliloglu et al., 2002; Kawashima et al., 2010; Salem et al., 2015), along with its role as an insulin (Meza-Herrera et al., 2011) and triiodothyronine (Meza-Herrera et al., 2014) modulating molecule, BC also has demonstrated a positive action upon selected blood metabolites (Meza-Herrera et al., 2017). Yet the possible effect of BC supplementation upon the GH/IGF-1 system, modulating in turn the HPG axis, has been elusive. The aim of this study was to evaluate the effect of BC supplementation upon ovarian function in adult female goats as well as to obtain possible evidence of BC influences on serum levels of GH and IGF-1.

2. Materials and methods

The methods of this study and the 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 Federation Animal Science Society, 2010) and national (NAM National Academy of Medicine, 2002) levels, with institutional approval reference number UACH-DGIP-REBIZA/11-510-405.

2.1. Location, animals and feeding

The study was carried out at the Regional University Unit on Arid Lands, Chapingo Autonomous University (URUZA-UACH; 26° N, 103° W, at 1,117 m) in northern Mexico. Adult goats [n = 22, LW = 45.35 ± 1.35 kg, 3.5 years old, 7/8 Sannen-Alpine] were fed twice per day to meet their net energy requirements for maintenance (NEm) (NRC National Research and Council, 2007), with alfalfa hay [14% CP, 4.77 net energy for maintenance (NEm MJ kg^{-1})] and corn silage [8.1% CP, 6.78 NEm MJ kg^{-1}] in the morning (0700) and corn grain (11.2% CP, 9.9 NEm MJ kg^{-1}) in the afternoon (1800). Goats had free access to water, shade and mineral salts during the entire experimental period, from October to November. Both LW and BCS were recorded weekly prior to feeding. BCS was determined in all animals by palpation of the transverse and vertical processes of the goats lumbar vertebrae (L2 through L5) on a five- point scale (1 = emaciated, 5 = obese; Aumont et al., 1994) by the same experienced technician.

2.2. Experimental treatments

In early October, goats were randomly distributed in individual pens to form two experimental groups: a) Betacarotene [BC, n = 10; 45.9 ± 1.97 kg live weight (LW), 3.04 ± 0.08 units, body condition score (BCS)], and b) Control, [CONT, n = 12; 46.2 ± 2.04 kg LW, 3.0 ± 0.08 units BCS]. Goats in the BC group were orally supplemented with betacarotene (50 mg $goat^{-1}$ day^{-1}) (Syntex-Roche, Guadalajara Jalisco, Mexico) during the entire experimental period (52 d). Both groups received the same base diet in a mixed-ration offering of 1.0 kg $goat^{-1}$ day^{-1}. Since at the offered level the base diet was completely consumed and because both experimental groups consumed the same quantity of the base diet per goat, the expected intake of dietary BC per experimental unit between treatments was assumed to be the same. The possible effect of consuming versus not consuming supplemental betacarotene by the experimental groups was thereby evaluated.

2.3. Estrus synchronization, blood sampling & quantification of the somatotropic axis hormones

During the second half of October, estrus was synchronized by using intravaginal sponges containing 45 mg of fluorogestone acetate (Chronogest®, Intervet International B.V., Boxmeer, Holland) left in place for 10 d. 9 d after the insertion of the sponge (day −3; day 0 = estrus), goats received a single i.m. dose of 1 mL of a prostaglandin $F_{2\alpha}$ analogue (0.075 mg $goat^{-1}$ of cloprostenol; Prosolvin-C®, Intervet International B. V., Boxmeer-Holland). Thereafter, on day −2, sponges were removed, and 24 h later (day −1) five goats from each group were randomly selected to perform an intensive blood sampling. Blood samples (10 mL) were collected by jugular venopuncture every 15 min for 6-h, starting 3 h after the morning feeding. The intensive blood sampling included 25 samples per goat, 125 samples per treatment, for a total of 250 serum samples per treatment.

Blood samples were collected into sterile vacuum tubes (Corvac, Kendall Health care, St. Louis, MO) and allowed to clot at room temperature for 30 min. Serum was separated by centrifugation (1500 x g, 15 min), decanted and transferred in duplicate to polypropylene micro tubes (Axygen Scientific, Union City, CA, USA) and stored at −20 °C until hormonal analysis. Peripheral serum GH and IGF-1 were determined in duplicate by radioimmunoassay (RIA). Concentrations of GH were determined in all samples in a single RIA assay (Hoefler and Hallford, 1987); intra-assay CV was 9.4%, and a detection limit of 0.2 ng mL^{-1}. The area under the curve (AUC) for GH was calculated using a trapezoidal summation procedure, while the pulsatility was characterized using the Cluster Pulse Analysis Program considering a 16.2% CV, a 0.95 S.D., and a detection limit of 0.3 ng mL^{-1} (Veldhuis and Johnson, 1986). Because the release of IGF-1 is non-episodic, samples collected at two hour intervals were considered to evaluate serum IGF-1 concentrations by double antibody RIA previously described (Berrie et al., 1995), using primary antisera and purified standard and iodination preparations supplied by the National Hormone and Peptide Program (Harbor-UCLA Medical Center, Torrance, CA, USA). An assay of total IGF-1 was conducted after acid-ethanol inactivation of binding proteins and resulted in intra- and inter-assay CV of 12 and 15, respectively, and a detection limit of 0.2 ng mL^{-1}.

2.4. Ultrasonographic scanning of ovarian function: follicular growth & ovulation rate

To evaluate ovarian activity, on day 17 post-estrus, towards the end of the luteal phase, an ultrasonographic scan was performed by a single skilled operator using a 7.5 MHz linear-array transducer for veterinarian use (Toshiba Medical Systems, Ltd, Crawley, UK). The total number of antral follicles (AF) and corpus luteum (OR) observed in each ovary were recorded and the structures were measured according to procedures previously outlined (Dickie et al., 1999). Ovaries were visualized at an image magnification of 1.5×, and number and size of antral follicles (> 5 mm) and corpus luteum, were registered. The total ovarian activity (TOA) was defined as the sum of AF and OR recorded in each animal (Fig. 1).

2.5. Statistical analyses

The ovarian variables AF, OR, TOA and GH-AUC were compared considering a CRD-ANOVA. Due to the non-parametric nature of GH

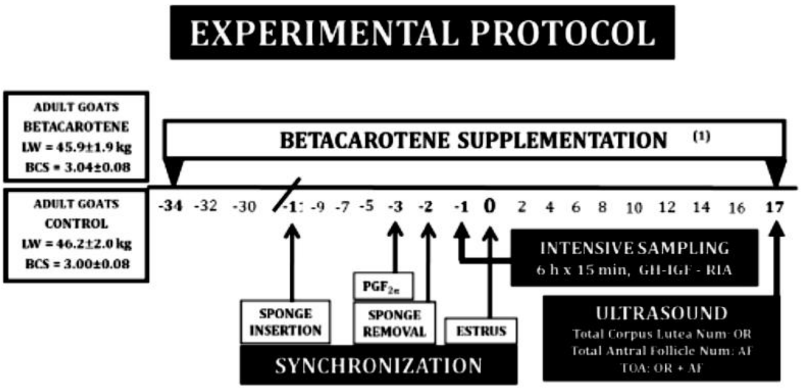

Fig. 1. A schematic representation of the procedure for estrous cycle synchronization, betacarotene supplementation, intensive blood sampling and ultrasound scanning in adult crossbred goats (n = 22), Control (CONT) or supplemented with Betacarotene under natural photoperiodic conditions in northern Mexico (October-November, 26° LN). Note: Once synchronized with the use of intravaginal sponges containing 45 mg of fluorogestone acetate (d–10), goats received a single i.m. dose of 1 mL of a prostaglandin $F_{2\alpha}$ analogue (d −3); on day −2, sponges were removed; and 24 h later (d–1) an intensive blood sampling (every 15 min × 6 h) for GH and IFG-1 serum quantification was performed 36 h prior to the estrus day (d 0). On day 17 post-estrus, an ultrasonographic scan was performed to relate the GH and IGF-1 secretion pattern and ovulation rate (OR), measured as number of corpora lutea present in each ovary; additionally, the antral follicle number (AF) was also recorded to define total ovarian activity (TOA = OR + AF).

(1) Oral supplementation, 50 mg/goat/day

pulse frequency, GH-PULSE was analyzed using the Kruskal–Wallis test. LW and BCS as well as serum GH and IGF-1 concentrations across time were determined by split-plot analysis of variance for repeated measures. The models included treatment in the main plot, which was tested using goat within treatment as the error term. Time and the time x treatment interaction were included in the subplot and were tested by using the residual mean square (Littell et al., 1998). When significant F values were observed, mean separation was conducted using comparisons generated from the least-square mean procedure (PDIFF option) from the PROC GLM. All statistical analyses were computed using the procedures of SAS (SAS Inst. Inc. Cary, NC, USA). Since no treatment by time interactions were observed regarding either GH or IGF-1, the overall averages per treatment across time per hormone are described. Persońs correlations were used to evaluate the association among LW, BCS and the OR. Reported values are defined as least-square mean ± SE; the most conservative SE is presented.

3. Results

Least-square mean for live weight (LW, kg), body condition score (BCS, units), antral follicles (AF, units), corpus luteum number (OR, units), total ovarian activity (TOA = AF + OR) and serum concentrations of growth hormone (GH, ng mL^{-1}) and insulin-like growth factor-1 (IGF-1, ng mL^{-1}) are shown in Table 1. Average LW and BCS at the beginning and the end of the study were 45.35 ± 1.4 kg, 2.97 ± 0.08 units, and 45.05 ± 1.4 kg, 3.27 ± 0.08 units, respectively. Hence, no differences were found, neither at the beginning ($p > 0.05$) nor during the entire ($p > 0.05$) experimental period between groups. Yet, increases in ovulation rate (3.4 vs. 2.8 ± 0.2 units; $p = 0.05$), number of antral follicles (5.0 vs. 3.4 ± 0.6 units; $p = 0.05$) and total ovarian activity (8.4 vs. 6.2 ± 0.6 units; $p = 0.05$) were observed in the BC-supplemented group. Interestingly, the lowest values of serum GH average concentrations (10.0 vs. 14.3 ± 1.0 ng mL^{-1}; $p = 0.01$) and GH-AUC (3670.4 vs. 5235.7 ± 369.8 units; $p = 0.05$) were observed in the BC-supplemented group. However, neither differences for serum IGF-1 concentrations (254.6 ± 28.9 ng mL^{-1} $p > 0.05$) nor for GH-PULSE (1.4 ± 0.5 pulses 6 h^{-1} $p > 0.05$), occurred between treatments (Table 1). In addition, a positive correlation between live weight ($r = 0.42$; $p = 0.04$) and body condition score ($r = 0.47$; $p = 0.02$) with respect to ovulation rate was observed in our study.

Table 1

Least-square means for live weigth (LW, kg), body condition score (BCS, units), antral follicles (AF, units), total corpus luteum (OR, units) and total ovarian activity (TOA, AF + OR, units) at ultrasound evaluation as well as serum growth hormone concentrations (GH, ng mL^{-1}), GH area under the curve (GH-AUC, arbitrary units), GH pulsatitlity (GH-PULSE, units every 6 h) and insulin-like growth factors (IGF-1, ng mL^{-1}) in adult crossbred goats (n = 22), Control (CONT) or supplemented with Betacarotene (BC) under natural photoperiodic conditions (October–November, 26° LN).

	Tratments			
	BC	CONT	p-value	SE[a]
LW, (kg)	45.8	46.3	0.80	1.46
BCS, (units)	3.25	3.30	0.80	0.08
AF, (units)	5.0	3.4	0.05	0.6
OR, (units)	3.4	2.8	0.05	0.2
TOA (AF + OR)	8.4	6.2	0.05	0.6
GH, (ng mL^{-1})	10.0	14.3	0.01	1.0
GH-AUC, (units)	3670.4	5235.7	0.01	369.8
GH-PULSE (units)	1.6	1.2	0.62	0.6
IGF-1, (ng mL^{-1})	261.1	248.2	0.75	028.9

[a] SE, most conservative standard error is presented.

4. Discussion

Current results support our working hypothesis in that BC-supplementation improved ovarian activity in adult goats with increases in both ovulation rate and antral follicle number, which paralleled decreases in GH serum concentration, yet without differences in serum IGF-1 between treatments. Interestingly, since BC-supplementation continued up to d-17 post-estrus, the observed effect of such dietary supplementation upon antral follicle number can be defined as an "acute effect" of BC administration on the growth of the AF population, suggesting a physiologic ovarian scenario prone to an increased follicular steroidogenesis. Our findings suggest a potential positve role of BC supplementation during the aromatization process of the antral follicles, a steroidogentic scenario previously observed (Salem et al., 2015). Moreover, an intrincate association has been proposed between serum antioxidants and endogenous hormones, supporting the hypothesis that concentrations of serum vitamins affect steroidogenesis even after adjustment for oxidative stress (Mumford et al., 2016). Undoubtedly, the precise site of action engaged by BC-supplementation throughout the HPG axis cannot be established without further research.

In addition, changes in metabolic status are strongly related to fluctuations in both live weight and body condition score (Meza-Herrera et al., 2007, 2008; Scaramuzzi et al., 2011). Yet no differences ($P > 0.05$) occurred regarding LW or BCS between treatments. Thus, other endocrinological or metabolic pathways should be involved in the different ovarian outcome observed. Fluctuations in blood concentrations of metabolic hormones are important signals that inform the nutritional status of mammals (Meza-Herrera et al., 2011, 2014). A possible explanation is that the response to supplemental feeding alters the glucose-insulin system (Scaramuzzi et al., 2011), leptin or IGF-I (Gamez-Vazquez et al., 2008; Guerra-García et al., 2009), and probably other reproductive and metabolic hormones (Meza-Herrera et al., 2004; Scaramuzzi et al., 2011) as well as genomic cues (Meza-Herrera et al., 2010a,b).

In ruminants, different studies have shown a direct relationship between serum BC concentration, ovarian function and reproductive performance. In beef cattle, BC supplementation affected the size of corpora lutea and the level of progesterone secretion (Haliloglu et al., 2002). Also, in adult goats, short-term BC supplementation positively affected ovarian follicular development and ovulation rate under short-day photoperiods (Arellano-Rodriguez et al., 2007), as well as ovarian and luteal function and progesterone secretion (Arellano-Rodriguez et al., 2009). Also, a positive relationship between serum BC concentrations and ovarian activity during the first follicular wave was reported in bovine animals (Kawashima et al., 2009), while a positive relationship among BC supplementation, serum retinol concentration, serum gammaglutamyl transpeptidase concentration and luteal activity occurred in dairy cattle (Kawashima et al., 2010).

In addition, in peri-puberal female goats, long-term BC supplementation positively affected the release pattern over time of the metabolic hormone triiodothyronine (Meza-Herrera et al., 2014). BC supplementation also promoted increases in serum estradiol concentrations and estrus percentage in sheep (Salem et al., 2015). Increases in litter size and number of piglets born alive were observed in BC-supplemented sows (i.m.), suggesting that this scenario was most likely BC-specific and independent from the BC role as a vitamin A precursor (Krammer and Aurich, 2010), an important finding that also supports our results. In the ovary, nutrition stimulates follicular growth associated with both systemic and intra-follicular alterations in the insulin-glucose and IGF-1-leptin systems (Scaramuzzi et al., 2011). Interestingly, no significant associations between intake of BC from food, supplements or both upon IGF-1 or IGFBP-3 concentrations were observed in humans (Tran et al., 2006).

Finally, and considering a translational perspective, since GH promotes cell proliferation and angiogenesis while inhibiting apoptosis, the GH/IGF-1 system has been associated in mammals with the development and/or progression of several types of cancers (Kopchick et al., 2014). Moreover, elevated GH levels have been related to signs of premature aging and reduction of lifespan. Although such premature deaths have been diagnosed as multifactorial in origin, tissue-specific pathological organ damage has been constantly present, with an augmented incidence of tumors (Bartke et al., 2013; Kopchick et al., 2014). Acting through autocrine-paracrine-endocrine routes, increased GH levels have been related to oncogenesis; thus, an attempt to reduce the GH/IGF-1 action under some pathological scenarios could theoretically provide protection from certain types of cancers (Pollack et al., 2001; Kopchick et al., 2014). These findings, merged with our results, open interesting possibilities in the search of possible translational applications considering the GH-lowering effect of BC-supplementation observed in our study; further research considering this approach poses an interesting assignment.

To conclude, our data document that betacarotene supplementation generated an increase in ovarian activity and ovulation rate considering the adult goat as a model. Notably, this physiologic scenario involved a decrease in serum GH, yet without effect on IGF-1 levels. Our study unveils for the first time a potential role of BC as a somatotrophe-modulating molecule. The precise site of BC action throughout the HPG axis in females awaits to be established, this being an important line of investigation for the animal industry and with potential clinical translational implications.

Conflict of interest

The authors declare that there are no conflicts of interest that would prejudice the impartiality of this scientific work.

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References

Amann, P.M., Eichmuler, S.B., Schmidt, J., Bazhin, A.V., 2011. Regulation of gene expression by retinoids. Curr. Med. Chem. 18, 1405–1412.

Arellano-Rodriguez, G., Meza-Herrera, C.A., Rodriguez-Martinez, R., Velazquez-Mendez, G., Mellado, M., Salinas, H., Perez-Razo, M.A., Sanchez, F., 2007. Short-term beta-carotene supplementation positively affects ovarian follicular development and ovulation rate in goats. J. Appl. Anim. Res. 32, 177–180.

Arellano-Rodriguez, G., Meza-Herrera, C.A., Rodriguez-Martinez, R., Dionisio-Tapia, R., Hallford, D.M., Mellado, M., Gonzalez-Bulnes, A., 2009. Short-term intake of β-carotene-supplemented diets enhances ovarian function and progesterone synthesis in goats. J. Anim. Physiol. Anim. Nutr. 93, 710–715.

Attia, A.A., 2009. Histological and electron microscopic studies of the effect of betacarotene on the pancreas of streptozotocin (STZ)-induced diabetic rats. Pak. J. Biol. Sci. 12, 301–314.

Aumont, G., Poisot, F., Saminadin, G., Borel, H., Alexandre, G., 1994. Body condition score and adipose cell size determination for in vivo assessment of body composition and post-mortem predictors of carcass components in creole goats. Small Rumin. Res. 15, 77–85.

Bartke, A.L., Sun, L.Y., Longo, V., 2013. Somatotropic signaling: trade-offs between growth, reproductive development and longevity. Physiol. Rev. 93, 571–598.

Berrie, R.A., Hallford, D.M., Galyean, M.L., 1995. Effect of zinc source and level on performance carcass characteristics, and metabolic hormone concentrations of growing and finishing lambs. Prof. Anim. Sci. 11, 149-156.

Darwish, W.S., Ikenaka, Y., Morsdy, A.E., Eldesorky, K.I., Nakayama, S., Mizukawa, N., Ishizuka, M., 2016. β-Carotene and retinol contents in the meat of herbivorous ungulates with special reference to their public health importance. J. Vet. Med. Sci. 78, 351–354.

Dickie, A.M., Paterson, C., Anderson, L.M., Boyd, J.S., 1999. Determination of corpora lutea numbers in Booroola-Texel ewes using transrectal ultrasound. Theriogenology 51, 1209–1224.

FASS Federation Animal Science Society, 2010. Guide for the care and use of agricultural animals in agricultural research and teaching. Federation Animal Science Society. Savoy, IL, USA.

Folman, Y., Ascarelli, I., Kraus, D., Barash, H., 1987. Adverse effect of beta-carotene in diet on fertility of dairy cows. Dairy Sci. 70, 357–366.

Gamez-Vazquez, H.G., Rosales-Nieto, C.A., Bañuelos-Valenzuela, R., Urrutia-Morales, J., Diaz-Gomez, M.O., Silva-Ramos, J.M., Meza-Herrera, C.A., 2008. Body condition score positively influence plasma leptin concentrations in criollo goats. J. Anim. Vet. Adv. 7, 1237–1240.

Guerra-García, M., Meza-Herrera, C.A., Sanchez-Torres-Esqueda, M.T., Gallegos-Sanchez, J., Torres-Hernandez, G., Pro-Martinez, A., 2009. IGF-1 and ovarian activity of goats in divergent body condition and supplemented with non-degradable ruminal protein. Agrociencia 43, 241–247.

Haliloglu, S., Baspinar, N., Serpek, B., Erdem, H., Bulut, Z., 2002. Vitamin A and beta-carotene levels in plasma: corpus luteum and follicular fluid of cyclic and pregnant cattle. Reprod. Dom. Anim. 37, 96–99.

Hirch, K., Atzmon, A., Danielenko, M., Sharoni, Y., 2007. Lycopene and other carotenoids inhibit estrogenic activity of 17beta-estradiol and genistein in cancer cells. Breast Cancer Res. Treat. 104, 221–230.

Hoefler, W., Hallford, D.M., 1987. Influence of suckling status and types of birth on serum hormone profiles and return to estrus in early postpartum spring lambing ewes.

Theriogenology 27, 887–895.

Kawashima, C., Kida, K., Schweigert, F.J., Miyamoto, A., 2009. Relationship between plasma betacarotene concentrations during the peripartum period and ovulation in the first follicular wave postpartum in dairy cows. Anim. Reprod. Sci. 111, 105–111.

Kawashima, C., Nagashima, S., Sawada, K., Schweigert, F.J., Miyamoto, A., Kida, K., 2010. Effect of B-carotene supply during close-up dry period on the onset of first postpartum luteal activity in dairy cows. Reprod. Dom. Anim. 45, e282–e287.

Kopchick, J.J., List, E.O., Kelder, B., Gosney, E.S., Berryman, D.E., 2014. Evaluation of growth hormone (GH) action in mice: discovery of GH receptor antagonists and clinical indications. Mol. Cell Endocrinol. 380, 34–35.

Krammer, G., Aurich, J., 2010. Effect of intramuscularly administered betacarotene on reproductive performance in sows. Berl. Munch. Tierarztl. Wochenschr. 123, 496–499.

Littell, C.R., Henry, P.R., Ammerman, C.B., 1998. Statistical analysis of repeated measures data using SAS procedures. J. Anim. Sci. 76, 1216–1231.

Meza-Herrera, C.A., Tena-Sempere, M., 2012. Interface between nutrition and reproduction: the very basis of production. In: Astiz, S., Gonzalez-Bulnes, A. (Eds.), Animal Reproduction in Livestock, Encyclopedia of Life Support Systems (EOLSS), UNESCO. Eolss Publishers, Oxford, UK http://www.eolss.net.

Meza-Herrera, C.A., Sanchez, J.M., Chavez-Perches, J.G., Salinas, H., Mellado, M., 2004. Protein supplementation, body condition and ovarian activity in goats: preovulatory serum profile of insulin. South Afr. J. Anim. Sci. 34 (Suppl. 1), 223–226.

Meza-Herrera, C.A., Ross, T., Hallford, D.M., Hawkins, D., Gonzalez-Bulnes, A., 2007. Effects of body condition and protein supplementation on LH secretion and luteal function in sheep. Reprod. Dom. Anim. 42, 461–465.

Meza-Herrera, C.A., Hallford, D.M., Ortiz, J.A., Cuevas, R.A., Sanchez, J.M., Salinas, H., Mellado, M., Gonzalez-Bulnes, A., 2008. Body condition and protein supplementation positively affect periovulatory ovarian activity by non-LH mediated pathways in goats. Anim. Reprod. Sci. 106, 412–420.

Meza-Herrera, C.A., Gonzalez-Bulnes, A., Kridli, R., Mellado, M., Arechiga-Flores, C.F., Salinas, H., Luginbuhl, J.M., 2010a. Neuroendocrine, metabolic and genomic cues signaling the onset of puberty in females. Reprod. Dom. Anim. 45, e495–e502.

Meza-Herrera, C.A., Veliz-Deras, F.G., Wurzinger, M., Lopez-Ariza, B., Arellano-Rodriguez, G., Rodriguez-Martinez, R., 2010b. The kiss-1, kisspeptin, gpr-54 complex: a critical modulator of GnRH neurons during pubertal activation. J. Appl. Biomed. 8, 1–9.

Meza-Herrera, C.A., Hernandez-Valenzuela, L.C., Gonzalez-Bulnes, A., Tena-Sempere, M., Abad-Zavaleta, J., Salinas-Gonzalez, H., Mellado, M., Veliz-Deras, F., 2011. Long-term betacarotene-supplementation enhances serum insulin concentrations without effect on the onset of puberty in the female goat. Reprod. Biol. 11, 236–249.

Meza-Herrera, C.A., Reyes-Ávila, J.M., Tena-Sempere, M., Veliz-Deras, F.G., Macias-Cruz, U., Rodriguez-Martinez, R., Arellano-Rodriguez, G., 2014. Long-term betacarotene supplementation positively affects serum triiodothyronine concentrations around puberty onset in female goats. Small Rumin. Res. 116, 176–182.

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.

Meza-Herrera, C.A., 2008. Regluatory mechanisms of puberty in female goats: recent concepts. Trop. Subtrop. Agroecosyst. 9, 29–38.

Mumford, S.L., Browne, R.W., Schliep, K.C., Schmelzer, J., Plowden, T.C., Michels, K.A., Sjaarda, L.A., Zarek, S.M., Perkins, N.J., Messer, L.C., Radin, R.G., Wactawski-Wende, J., Schisterman, E.F., 2016. Serum antioxidants are associated with serum reproductive hormones and ovulation among healthy woman. J. Nutr. 146, 98–106.

NAM National Academy of Medicine, 2002. Guide for the Care and Use of Laboratory Animals. Co-produced by the National Academy of Medicine-Mexico and the Association for Assessment and Accreditation of Laboratory Animal Care International, 1st edition. Harlan Mexico, DF, Mexico.

NRC National Research and Council, 2007. Nutrient Requirements of Small Ruminants: Sheep, Goats, Cervids and New World Camelids. National Academy Press, Washington DC, USA.

Pollack, M., Boluin, M.J., Zhang, J.C., Kopchick, J.J., 2001. Reduced mammary carcinogenesis in transgenic mice expressing a growth hormone antagonist. Br. J. Cancer 85, 428–430.

Salem, A.A., El-Shahawy, N.A., Soliman, I.A., 2015. Effect of beta-carotene injection on estrus, vitamin A and estradiol-17-β concentrations in pubertal Farafra ewe lambs. Egypt J. Anim. Prod. 52, 123-128.

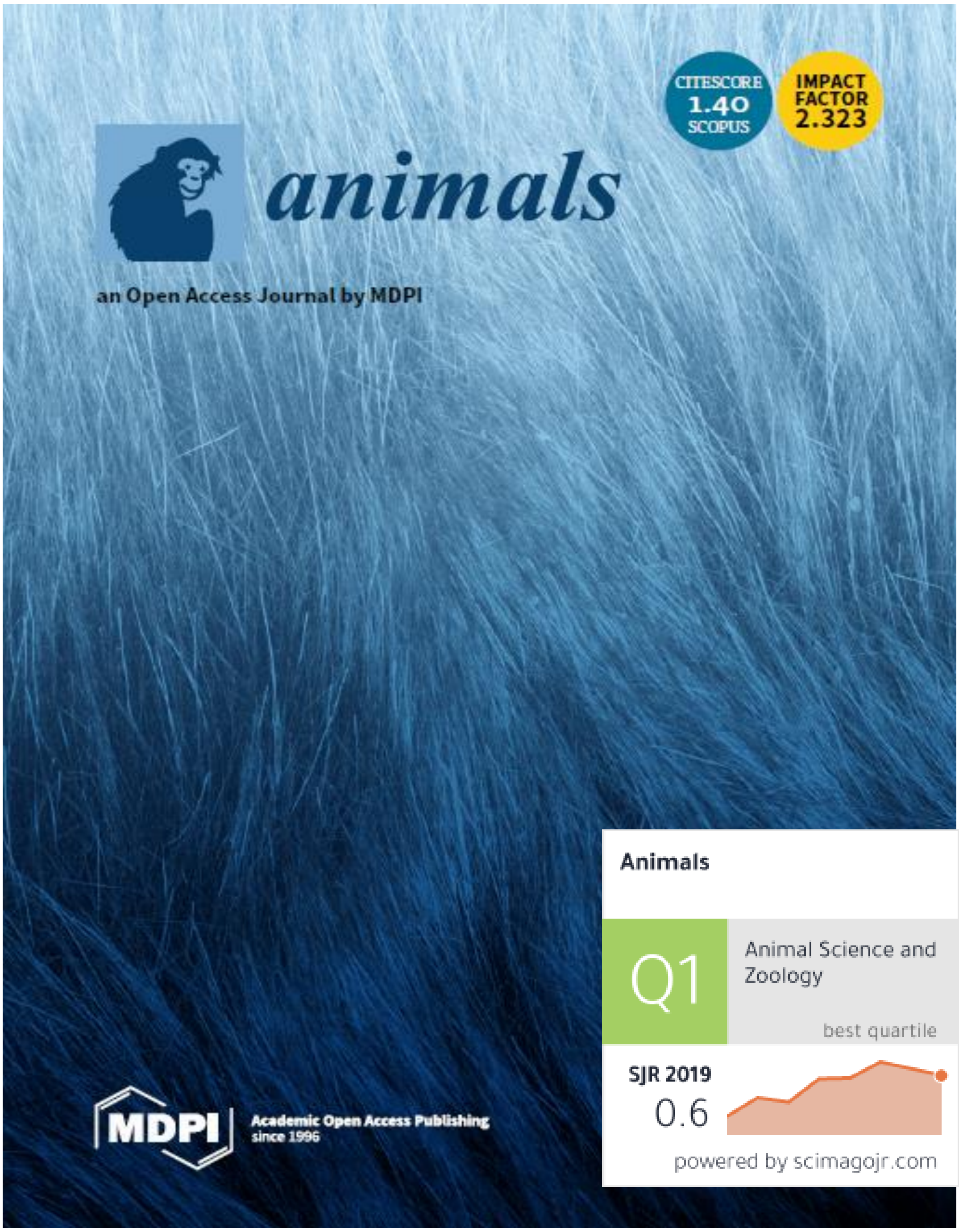
Scaramuzzi, R.J., Baird, D.T., Campbell, B.K., Driancourt, M.A., Dupont, J., Fortune, J.E., Gilchrist, R.B., Martin, G.B., McNatty, K.P., McNeilly, A.S., Monget, P., Monniaux, D., Vinoles, C., Webb, R., 2011. Regulation of folliculogenesis and the determination of ovulation rate in ruminants. Reprod. Fert. Dev. 23, 444–467.

Schweigert, F.J., Steihage, n.B., Raila, J., Seimann, A., Peet, D., Buscher, U., 2003. Concentrations of carotenoids, retinol and α-tocopherol in plasma and follicular fluid of woman undergoing IVF. Hum. Reprod. 18, 1259–1264.

Tran, C.D., Diorio, C., Berube, S., Pollak, M., Brisson, J., 2006. Relation of insulin-like growth factor (IGF) I and IGF-binding protein 3 concentrations with intakes of fruit, vegetables and antioxidants. Am. J. Clin. Nutr. 84, 1518–1526.

Veldhuis, J.D., Johnson, M.L., 1986. Cluster analysis: a simple versatile and robust algorithm for endocrine pulse detection. Am. J. Physiol. 250, E486–E493.

5.2 Precision Betacarotene supplementation enhanced ovarian function and the LH release pattern in yearling crossbred anestrous goats.



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Article

Precision Betacarotene Supplementation Enhanced Ovarian Function and the LH Release Pattern in Yearling Crossbred Anestrous Goats

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Simple Summary: The potential out-of-season supplementation effect of beta-carotene (BETA) upon both ovarian function and the luteinizing hormone LH release pattern in anestrous yearling goats was evaluated. Oral BETA-supply to goats with an enlarged gene-pool of dairy goat breeds, influenced in a positive fashion, the out-of-season ovarian outcomes escorted by changes in the release pattern of LH regarding the Control group. Results denote a likely role of BETA not only as modulator ovarian function measured as ovulation rate and antral follicular number but also involved as modifier of the LH-release pattern. The obtained outcomes would be central in the proposal of out-of-season reproductive schemes to enhance the ovarian outcomes in crossbred dairy goats.

Abstract: The possible out-of-season effect of beta-carotene supplementation on ovulation rate (OR), antral follicles (AFN), and total ovarian activity (TOA = OR + AFN) as related to the LH release pattern in yearling anestrous goats was evaluated. In late April, Alpine-Saanen-Nubian × Criollo goats ($n = 22$, 26 N) were randomly allotted to: (1) Beta-carotene (BETA; $n = 10$, orally supplemented with 50 mg/goat/d; 36.4 ± 1.07 kg live weight (LW), 3.5 ± 0.20 units, body condition score (BCS) or (2) Non-supplemented (CONT; $n = 12$, 35.2 ± 1.07 kg LW, 3.4 ± 0.2 units BCS). Upon estrus synchronization, an intensive blood sampling (6 h × 15 min) was accomplished in May for LH quantifications; response variables included (pulsatility-PULSE, time to first pulse-TTFP, amplitude-AMPL, nadir-NAD and area under the curve-AUC). Thereafter, an ultrasonography scanning was completed to assess OR and AFN. The Munro algorithm was used to quantify LH pulsatility; if significant effects of time, treatment or interaction were identified, data were compared across time. Neither LW nor BCS ($p > 0.05$) or even the LH ($p > 0.05$); PULSE (4.1 ± 0.9 pulses/6 h), NAD (0.47 ± 0.13 ng) and AUC (51.7 ± 18.6 units) differed between treatments. Nonetheless, OR (1.57 vs. 0.87 ± 0.18 units) and TOA (3.44 vs. 1.87 ± 0.45 units) escorted by a reduced TTFP (33 vs. 126 ± 31.9 min) and an increased AMPL (0.55 vs. 0.24 ± 0.9 ng), favored to the BETA supplemented group ($p < 0.05$), possibly through a GnRH-LH enhanced pathway and(or) a direct effect at ovarian level. Results are relevant to speed-up the out-of-season reproductive outcomes in goats while may embrace translational applications.

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Keywords: goats; beta-carotene; targeted supplementation; reproductive efficiency

1. Introduction

Beta-carotene (BETA) belongs to the family of carotenoids, phytochemical pigments naturally synthesized in fruits, vegetables, plants, algae, and photosynthetic bacteria [1]. In mammals, carotenoids are mainly metabolized at hepatic level; bovine and equids have the uppermost BETA hepatic content, followed by caprine (3.4 μg/g tissue) [2]. Specifically, BETA is a precursor of retinol (Vitamin A), a fat-soluble vitamin, involved in cellular division and differentiation, bone development and reproductive function [1,2]. While BETA-supply has demonstrated to protect both cells and cellular components against oxygen free radicals through its antioxidant function [3], it has been proposed that BETA is able to act independently as vitamin-A precursor [4]. Nonetheless, inconsistent results regarding the effect of BETA-supplementation upon reproductive function have been described, with negative effects [5], positive effects [6] and even no effects [7]. From a reproductive stand point, BETA-supplementation has been associated with increased steroidogenesis in both the corpus luteum and the follicular tissue [6,8–11].

Besides, the fundamental endocrine actions of the hypophyseal gonadotropins (LH, FSH) and other intra-ovarian molecules acting either in an autocrine or paracrine fashion (i.e., IGF-1 and 2) upon both follicular growth and oocyte maturation, have been also established [12–14]. Moreover, the communication among the hypothalamic-pituitary-gonadal (HPG) axis is tightly controlled by an extremely complex arrangement of neuronal inputs ruled not only by photoperiodic, thermoperiodic and nutrigenomic hints but also by the metabolic status of the reproductive animal [15,16]. Furthermore, BETA has shown to down-regulate the estrogen-induced transactivation of the estrogen receptor [17] supporting the possible role of BETA as an HPG-modulating molecule.

The ovary has as primordial task to produce mature oocytes; to accomplish that, an impeccable and well harmonized dialogue among the main ovarian cellular groups (i.e., theca, granulosa and the oocyte) must occur [18–20]. Tonic secretion of pituitary LH is the result of a well-coordinated interaction between a brain stimulus and an inhibitory feedback from the gonads; unquestionably, the GnRH pulse generator is the initiator of the LH pulse [20,21]. The GnRH pulse generator has been located within the arcuate nucleus and seems to be composed by a complex array of kisspeptin neurons and their projections merging with GnRH dendrons in the median eminence, suggesting that such complex of arcuate/infundibular kisspeptin neurons are, per se, the GnRH pulse generator in mammals [22]. Interestingly, nutritional supplementation with a mixture of BETA, polyphenolic compounds, and probiotics demonstrated to up-regulate genes involved in both the activation of cellular gonadotropes and up-regulation of GnRH genes [23]. While the involvement nutritional supplementation as a modulator of the HPG axis has been proposed [8–16,20,23–25], previous studies of our group have demonstrated an interesting role of BETA as a modulator not only of serum insulin [18], triiodothyronine [26], as well as the GH-IGF-1 system [6] but also upon some selected blood metabolites [27]. Building on these previous findings, we hypothesized an out-of-season positive effect of BETA supply on both ovarian function and the releasing pattern of LH across time in yearling goats; this study attempts to solve such inquires.

2. Material and Methods

2.1. General

All the procedures, methods and managing of the trial units used in this research were done in strict agreement with recognized recommendations for ethical use, care and welfare of animals in research at worldwide [28] and nationwide [29] levels, with institutional approval reference number UACH-DGIP-REBIZA-IBIODEZA/18-086-C-80.

2.2. *Location, Environmental Conditions, Animals, and Their Management*

The present study was carried out at the Chapingo Autonomous University, Regional University Unit on Arid Lands, (UACH-URUZA; 26° N, 103° W; 1117 m). Yearling anestrous Alpine-Saanen-Nubian x Criollo goats (*n* = 22, live weight (LW) = 29.17 ± 1.02 kg, 3.45 ± 1.02 units, body condition score (BCS)) were used under a long-day photoperiodic conditions during April and May (i.e., natural anestrous season at 26° N). In a weekly periodicity, LW and BCS were recorded previous to feeding; BCS was determined on a five-point scale (from 1 = emaciated to 5 = obese) by an experienced technician.

2.3. *Experimental Design and Treatment Groups*

In late April, animals were randomly located in individual pens to form two experimental groups: (i). beta-carotene (BETA; *n* = 10, LW = 29.1 ± 1.02 kg, BCS = 3.4 ± 0.2 units) and (ii). Control (CONT; *n* = 12; LW = 29.2 ± 1.07 kg, BCS = 3.5 ± 0.2), with similar LW and BCS between treatment groups. The BETA-goats were orally supplemented with beta-carotene (50 mg/goat/day, mixed with mineral salts) (Syntex-Roche de Mexico; Guadalajara, Jalisco, Mexico) during the entire experimental period, which lasted from 34 d pre- to 17 d, post-estrus. The experimental groups received a basal diet twice per day (0700 and 1600) 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, balanced to cover their net energy requests for maintenance (Table 1) [30]. Both groups had free access to water and shaded areas. Composition values of the components of the basal diet (Dry Matter (DM)% basis) were gotten from representative samples taken thru the experimental period and analyzed based on formerly defined techniques [31]. Allowances of the basal diet and BETA were individually offered to each goat. Because the basal diet was completely consumed by all goats, it is assumed that every experimental unit consumed the same BETA shares from the basal diet. Consequently, the only difference in BETA intake between treatments was the beta-carotene supply provided to the BETA-group. Hence, the effect to offer or not supplemental BETA in both treatment groups was assessed. The central actions executed during the experimental period are depicted in Figure 1.

Table 1. Chemical composition of alfalfa hay, corn silage and corn grain samples which conformed the basal diet of yearling crossbred goats (*n* = 22) under natural photoperiodic (April–May, 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 (%, weight/weight): NaCl 95; Fe 0.2; Cu 0.033; I 0.007; Zn 0.005; Co 0.0025, [b] Composition values (% of diet Dry Matter basis) 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 [31].

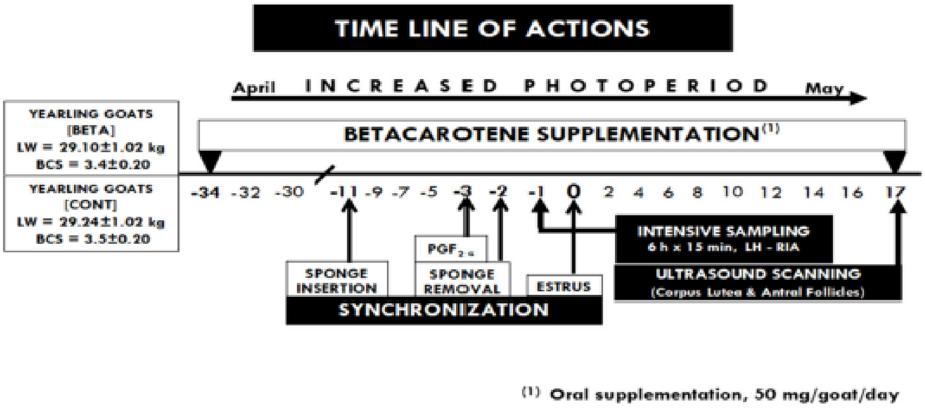

Figure 1. A graphic illustration of the experimental protocol, including the duration of long-term beta-carotene supplementation and estrous synchronization (for more details, see the text). An intensive blood sampling (every 15 min for 6 h) for luteinizing hormone (LH) measurements was performed 36 h prior to the estrus day (day 0). Thereafter, an ultrasonography scanning was performed on day 17 post-estrus to relate the LH secretion pattern and total ovarian activity (TOA = corpus lutea + antral follicles), measured as number of the observed structures present in each ovary on day 17 post-estrus, in yearling crossbred goats (*n* = 22) supplemented with beta-carotene or serving as controls and kept under natural photoperiodic conditions (April–May, 26° N).

2.4. *Estrus Synchronization, Blood Sampling, and LH Quantification*

Estrus was synchronized (day 23) 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 sponges (day −3; day 0 = estrus), goats received a single i.m. dose of 1 mL of prostaglandin $F_{2\alpha}$ analogue (0.075 mg of D-cloprostenol/goat; Prosolvin-C®, Intervet International B.V., Boxmeer, Holland). Thereafter, on day −2, sponges were removed and 24 h later (day −1) five goats from each group were randomly selected to undergo an intensive blood sampling. Blood samples (10 mL) were collected every 15 min for 6 h, starting 3 h after the morning feeding, by jugular venipuncture into sterile vacuum tubes (Corvac; Kendall Health Care, St. Louis, MO, USA) and allowed to clot at room temperature for 30 min. Serum was separated by centrifugation (1500× *g*, 15 min), decanted and transferred to polypropylen microtubes (Axygen Scientific, Union City, CA, USA) for storage at −20 °C until hormonal analysis. Peripheral serum LH concentrations were determined in duplicate in a single radioimmunoassay (RIA) as previously described [32]. The value of an intra-assay coefficient of variation (CV) for LH quantification was 10% and the assay detection limit was 0.2 ng/mL; the Munro algorithm was used to detect LH pulses [33]. The LH basal levels were quantified as the average of the lowest points along the sampling period as previously defined [34,35].

2.5. *Ultrasonographic Scanning of Ovarian Activity*

On day 17 post-estrus, thru the end of the luteal phase in middle May, an ultrasonographic scanning was performed to evaluate the ovarian activity, by a qualified operative, using a 7.5-MHz linear-array transducer (Toshiba Medical Systems Ltd., Crawley, UK). Each ovary was scanned and the corpus luteum number (OR) and the antral follicle (AF) number were recorded as previously outlined [36]. Finally, we define to total ovarian activity (TOA) as the sum of AF and OR recorded in each animal within experimental group.

2.6. *Statistical Analyses*

Live weight, body condition score and serum LH concentrations across time were evaluated by split-plot ANOVA for repeated measures in the same animal. Treatments were included in the main plot, and tested using animal within treatment as the error term. The components within the subplot included Time and time × treatment, and were tested by the residual mean square [37]. When significant F values were observed, mean separation was done using the LSMEANS-PDIFF option of the PROC GLM. The ovarian variables and LH-AUC were compared by ANOVA-CRD; due to the non-parametric nature of LH pulse frequency, LH-PULSE was analyzed using the Kruskall-Wallis test. The response variables were evaluated for normality using the Shapiro–Wilk test for normality and transformed by log 10 transformation to overcome skewness in the data for basal and mean LH concentrations and LH pulse amplitude. Where a significant effect of time, treatment or interaction was detected, data were compared across time. The mentioned statistical analyses were solved by means of the GLM procedures of SAS (SAS Inst. Inc. V9.1, Cary, NC, USA). Pearson's correlations were used to evaluate the associations among LW, BCS and the number of luteal structures. Non-transformed data are presented for ease of interpretation and expressed as least-square means ± standard error (SE); the most conservative SE is presented.

3. Results

Both LW and BCS at the beginning or end of the experiment were 29.17 ± 1.02 kg and 3.4 ± 0.17 or 35.81 ± 1.07 kg and 3.4 ± 0.2, respectively. No differences between groups for both variables occurred either at the beginning ($p > 0.05$) or during the entire ($p > 0.05$) experimental period. Besides, differences ($p < 0.05$) also occurred between treatments concerning the LH release pattern (Figure 2). While no differences for PULSE (4.1 ± 0.9 pulses/6 h), NAD (0.47 ± 0.13 ng) and AUC (51.7 ± 18.6 units) occurred between groups, both a reduced TTFP (33.0 vs. 126.0 ± 31.2 min) as well as an increased AMPL (0.55 vs. 0.24 ± 0.7 ng) favored to the BETA-supplemented goats. Moreover, regarding to the ovarian outcomes, both OR (1.57 vs. 0.87 ± 0.18 units) and TOA (3.44 vs. 1.87 ± 0.45 units), favored to the BETA supplemented group ($p < 0.05$) (Table 2). In addition, positive correlations were detected between OR regarding LW ($r^2 = 0.52$; $p < 0.05$), BCS ($r^2 = 0.57$, $p < 0.05$), AF ($r^2 = 0.89$, $p = 0.01$), OR ($r^2 = 0.65$, $p = 0.03$) as well as between AMPL and OR ($r^2 = 0.55$, $p = 0.06$).

Table 2. Least square means regarding live weight and body condition score at the onset of treatments (live weight (LW)-initial, kg and body condition score (BCS)-initial, units) and at the ultrasound scanning (LW-ultrasound, kg and BCS-ultrasound, units), ovulation rate (OR, units), total ovarian activity (TOA = OR + antral follicles, AF), and luteinizing hormone (LH) profile across time (pulsatility, time to first pulse, amplitude, nadir, and area under the curve) in cross-bred (Alpine–Saanen–Nubian × Criollo; $n = 22$) yearling goats supplemented with betacarotene (BETA) and non-supplemented (CONT) during the anestrous season (April–May) under semiarid conditions in Northern Mexico (26° N) (1).

Variables	BETA	CONT	S.E. (2)
Live weight-initial, kg	29.10 ^{a}	29.24 ^{a}	1.02
Body condition score-initial, units	3.4 ^{a}	3.5 ^{a}	0.17
Live weight-ultrasound, kg	36.42 ^{a}	35.20 ^{a}	1.07
Body condition score-ultrasound, units	3.5 ^{a}	3.4 ^{a}	0.20
Ovulation rate, units	**1.55** ^{a}	**0.87** ^{b}	0.18
Total ovarian activity, OR + AF, units	**3.44** ^{a}	**1.87** ^{b}	0.45
LH pulsatility, pulses/6 h, units	4.61 ^{a}	4.02^{a}	0.79
LH time to first pulse, min	**33.0** ^{a}	**126.0** ^{b}	31.28
LH nadir, ng	1.18 ^{a}	1.30 ^{a}	0.39
LH amplitude, ng	**0.55** ^{a}	**0.24** ^{b}	0.07
LH area under the curve, arbitrary units	36.5 ^{a}	66.9 ^{a}	4.47

(1) Yearling goats were weighed and body conditioned at the onset of the experimental period (early April) and at the ultrasonographic scanning (middle May). Goats confronted an increased natural photoperiod (April–May; anestrous season). While no differences among treatments occurred regarding neither LW nor BCS at either stage of the experimental period, differences were observed regarding the LH release pattern as well as OR and TOA, favoring to the BETA supplemented group ($p < 0.05$). (2) Most conservative standard error is presented. $^{a, b}$ Different superscripts within variable, show differences ($p < 0.05$).

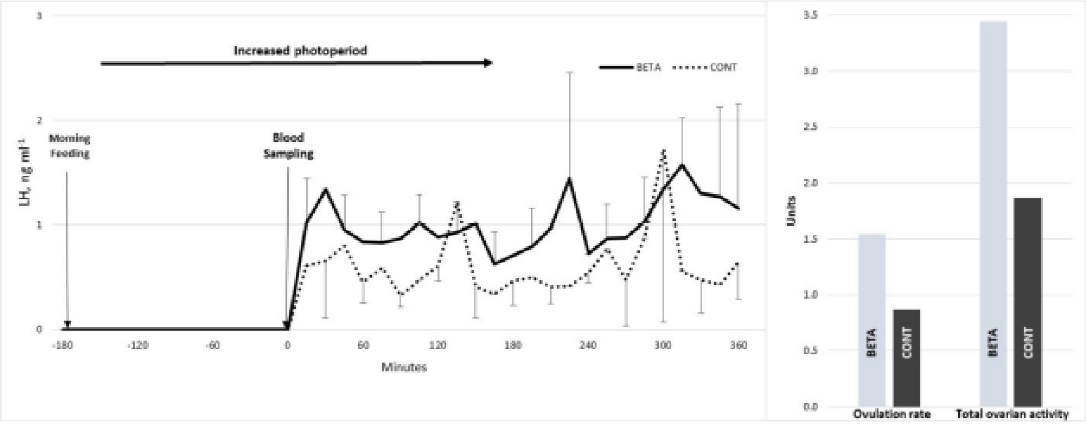

Figure 2. Serum luteinizing hormone concentrations (LH; ng/mL) across time (left panel) and ovulation rate (OR, units), and total ovarian activity (OR + antral follicles, units) (right panel) in crossbred (Alpine–Saanen–Nubian × Criollo; *n* = 22) yearling goats supplemented with beta-carotene (BETA) and non-supplemented (CONT) during the anestrous season (April–May) under semiarid conditions in Northern Mexico (26° N).

4. Discussion

The observed results of the study support our working hypothesis in that BETA-supply enhanced ovarian function in yearling goats with escalations in ovulation rate, antral follicle population, and total ovarian; such ovarian activity was escorted with changes in the LH-release pattern (time to first LH pulse and LH amplitude). However, no differences regarding LW and BCS, as well as pulsatility, nadir, and area under the curve of LH occurred between experimental groups. Such a physiological scenario suggests that BETA supply may had exerted a direct action at the ovarian level, acting throughout a GnRH-LH dependent pathway, likely acting throughout the "dynamic effect" of BETA supply. Remarkably, as the BETA-supply was constant up to 17 d post-estrus, such BETA-supply which positively affected the antral follicle number, can be demarcated as an "acute effect" of BETA-supplementation upon the growth of the antral follicle population. Consequently, it can be proposed that BETA supply generated a physiologic ovarian setting that promoted an increased follicular steroidogenesis and growth, positively acting upon the ovarian physiology throughout both the dynamic and the acute effect of BETA-supplementation, escorted by specific changes in the LH-release pattern.

In domestic livestock, nutritional supplementation modulates not only the timing and functionality of endocrine systems but also regulates seasonal shifts in reproductive activity, while imposing diverse reproductive outcomes (i.e., ovulation rate and fertility) [12–16,19,20,38,39]. For such reasons, nutritional supplementation is an interesting clean, green, and ethical management alternative with respect to the use of diverse hormonal treatments to enhance reproductive efficiency. To better understand our results, it is required to consider that nutritional supplementation (inputs) affects reproductive responses (outputs) through changes in the metabolic status. The last not only in the long term ("static effect") but also in the short-to-middle term ("dynamic effect") through an increased feeding over 3–4 weeks before mating; the heavier the females, the greater the ovulation rates as compared to their lighter counterparts. Moreover, enhanced reproductive outcomes can be also obtained by supplying a nutritional boost in a very short period of time (i.e., less than 5 to 7 days), without any perceptible effect upon live weight; the last has been referred to as "immediate nutrient effect", "acute effect", or "focus supplementation" [13,19,20,39–41]. Diverse studies have proposed that the stimulating effects of nutritional supplementation on follicular development can be linked to the effect of such increased nutritional supply not only upon LH concentrations but also with respect to the LH release pattern, which may directly act upon the ovarian tissue. Certainly, malnourished animals reach puberty at later stages or chronological age due to a decrement in GnRH pulsatility, which in turn leads to corresponding decreases in LH pulses [42]. Furthermore, changes in specific nutritional compounds and different biomolecules, such as BETA, may support increases not only in follicular growth in an FSH-dependent process but also upon increases in the LH-release pattern and then in ovulation rate [12–16,18–20,43]; the last physiological and neuroendocrine scenario possibly occurred in the BETA-group. The effect of nutritional supplementation on reproduction is clearly complex. The static effect has been associated with the body condition status (i.e., adiposity); while increases in body condition upsurges adiposity, such an augment in energy-reserves increases, in turn, follicular growth and ovulation rate. Besides to the previously mentioned FSH and LH involvement, the systemic and/or intra-follicular actions of the insulin-glucose and the IGF-1-leptin systems, should be also involved [12,13,20,39].

Going back to our results, the observed differences in the LH release pattern and the increased ovarian activity observed in the BETA group, suggest not only the activation of the GnRH-LH pathway but also a direct BETA effect upon ovarian tissue. Moreover, a potential positive role of BETA-supply along the aromatization progression in the antral follicles population can be suggested; a steroidogenic setting formerly proposed [24]. When evaluating the BETA uptake by ovarian and uterine tissues and its possible influence upon steroidogenesis during the estrous cycle, not only an increased plasma concentration of estradiol occurred between days 0 to 4 post-ovulation but also an augmented total uterine protein concentration favored to the BETA supplemented females [44]. Such findings point out to BETA as an enhancer not only of the production of healthier follicles and oocytes but also in the concentrations of estradiol, progesterone and uterine proteins, providing an ideal ovarian performance as well as an improved uterine milieu prone to an enhanced embryo implantation and development.

In addition, increases of intra-ovarian reactive oxygen species (ROS) are aligned to increases in maternal age [45]. Such a physiological-chronological scenario highlights the key role of BETA not only as a very effective quencher of ROS, but also as an important molecule which enhances both ovarian development and steroidogenesis. In an elegant study, the possible effect of BETA on oocyte maturation under oxidative stress and the involved underlying mechanisms was explored [46]; while ROS inhibited oocyte development/maturation and parthenogenetic activation, BETA actions vanished such deleterious mechanisms, enhancing ovarian activation and development. Additionally, the protective BETA effects against the oxidative damage in ovarian tissue facing ischemia throughout different expression patterns of the antioxidant enzymes superoxidase dismutase (SOD) and glutathione (GSH), plus the malondialdehyde (MDA) level, which is the end result of lipid peroxidation, have been proposed [47]. Moreover, BETA supply not only restored actin expression, cortical granule-free domain formation, mitochondria homogeneous distribution and nuclear maturation [47] but also reduced both ROS formation and cell apoptosis [1]; such results point out to BETA as an improver of both ovarian function and oocyte quality. Unquestionably, the specific site of action of BETA-supply thru the HPG axis will not be recognized without additional enquiries. In this respect, it has been proposed that serum vitamin concentrations affect steroidogenesis even after adjustment for oxidative stress, the last throughout an intricate association among beta-carotene-like antioxidants and diverse endogenous metabolic hormones [48].

5. Conclusions

Our main research outcomes establish that beta-carotene supply enhanced out-of-season follicular development and ovulation rate, escorted by changes in the LH-release pattern in yearling anestrous goats, with non-differences regarding live weight and body condition score. Whether the effects of BETA-supplementation were exerted by differences in the LH-release pattern, or directly exerted upon the ovarian physiology or even mediated through an up-regulation of the ovarian LH-receptors remains as a pending research assignment. Supplementary research designed to expounding the precise endocrine or metabolic effects of BETA along the HPG axis would contribute to a better understating regarding the positive out-of-season ovarian outcomes observed in the yearling BETA-supplemented anestrous goats, notably with a high degree of genes from very seasonal reproduction breeds.

The observed research outcomes from our study should aid to speed-up the ovarian responses in anestrous goats while may also embrace interesting translational applications aiming to suppress metabolic-physiologic dysfunctions in aging human oocytes.

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Ethics Statement: All procedures and methods used in this study regarding the use and care of animals were carried-out in accordance with accepted international animal use and care guidelines.

References

1. Eggersdorfer, M.; Wyss, A. Carotenoids in human nutrition and health. *Arch. Biochem. Biophys.* **2018**, *652*, 18–26. [CrossRef] [PubMed]
2. Darwish, W.S.; Ikenaka, Y.; Morsdy, A.E.; Eldesorky, K.I.; Nakayama, S.; Mizukawa, N.; Ishizuka, M. β-Carotene and retinol contents in the meat of herbivorous ungulates with special reference to their public health importance. *J. Vet. Med. Sci.* **2016**, *78*, 351–354. [CrossRef]
3. Oliveira, R.C.; Guerreiro, B.M.; Morais, N.N., Jr.; Araujo, R.L.; Pereira, R.A.N.; Pereira, M.N. Supplementation of prepartum dairy cows with B-carotene. *J. Dairy Sci.* **2015**, *98*, 6304–6314. [CrossRef] [PubMed]
4. Krammer, G.; Aurich, J. Effect of intramuscularly administered betacarotene on reproductive performance in snows. *Berl. Munch. Tierarztl.* **2010**, *123*, 496–499.
5. Folman, Y.; Ascarelli, I.; Kraus, D.; Barash, H. Adverse effect of beta-carotene in diet on fertility of dairy cows. *J. Dairy Sci.* **1987**, *70*, 357–366. [CrossRef]
6. Lopez-Flores, N.M.; Meza-Herrera, C.A.; Galan-Soldevilla, C.; Bautista-Rodriguez, D.A.; Veliz-Deras, F.G.; Arellano-Rodriguez, G.; Garcia-De la Peña, C.; Rosales-Nieto, C.A.; Macias-Cruz, U. The key role of targeted betacarotene supplementation on endocrine and reproductive outcomes in goats: Follicular development, ovulation rate and the GH-IGF-1 axis. *Small Rumin. Res.* **2018**, *163*, 29–33. [CrossRef]
7. Kaewlamun, W.; Okouyi, M.; Humblot, P.; Techakumphu, M.; Ponter, A.A. Does supplementing dairy cows with β-carotene during the dry period affect postpartum ovarian activity, progesterone, and cervical and uterine involution? *Theriogenology* **2011**, *75*, 1029–1038. [CrossRef]
8. Kawashima, C.; Nagashima, S.; Sawada, K.; Schweigert, F.J.; Miyamoto, A.; Kida, K. Effect of B-carotene supply during close-up dry period on the onset of first postpartum luteal activity in dairy cows. *Reprod. Dom. Anim.* **2010**, *45*, e282–e287. [CrossRef]
9. Kawashima, C.; Kida, K.; Schweigert, F.J.; Miyamoto, A. Relationship between plasma betacarotene concentrations during the peripartum period and ovulation in the first follicular wave postpartum in dairy cows. *Anim. Reprod. Sci.* **2009**, *111*, 105–111. [CrossRef]
10. Arellano-Rodriguez, G.; Meza-Herrera, C.A.; Rodriguez-Martinez, R.; Dionisio-Tapia, R.; Hallford, D.M.; Mellado, M.; Gonzalez-Bulnes, A. Short-term intake of β-carotene-supplemented diets enhances ovarian function and progesterone synthesis in goats. *J. Anim. Physiol. Anim. Nutr.* **2009**, *93*, 710–715. [CrossRef]
11. Arellano-Rodriguez, G.; Meza-Herrera, C.A.; Rodriguez-Martinez, R.; Velazquez-Mendez, G.; Mellado, M.; Salinas, H.; Perez-Razo, M.A.; Sanchez, F. Short-term betacarotene supplementation positively affects ovarian follicular development and ovulation rate in goats. *J. Appl. Anim. Res.* **2007**, *32*, 177–180. [CrossRef]

12. Scaramuzzi, R.J.; Campbell, B.K.; Dowing, J.K.; Kendall, N.R.; Khalid, M.; Muñoz-Gutierrez, M. A review of the effects of supplementary nutrition in the ewe on the concentrations of reproductive and metabolic hormones and the mechanism that regulate foliculogenesis and ovulation rate. *Reprod. Nutr. Dev.* **2006**, *46*, 339–354. [CrossRef] [PubMed]
13. Scaramuzzi, R.J.; Baird, D.T.; Campbell, B.K.; Driancourt, M.A.; Dupont, J.; Fortune, J.E.; Gilchrist, R.B.; Martin, G.B.; McNatty, K.P.; McNeilly, A.S.; et al. Regulation of folliculogenesis and the determination of ovulation rate in ruminants. *Reprod. Fertil. Dev.* **2011**, *23*, 444–467. [CrossRef]
14. Meza-Herrera, C.A.; Veliz-Deras, F.G.; Wurzinger, M.; Lopez-Ariza, B.; Arellano-Rodriguez, G.; Rodriguez-Martinez, R. The kiss-1, kisspeptin, gpr-54 complex: A critical modulator of GnRH neurons during pubertal activation. *J. Appl. Biomed.* **2010**, *8*, 1–9. [CrossRef]
15. Meza-Herrera, C.A.; Hallford, D.M.; Ortiz, J.A.; Cuevas, R.A.; Sanchez, J.M.; Salinas, H.; Mellado, M.; Gonzalez-Bulnes, A. Body condition and protein supplementation positively affect periovulatory ovarian activity by non-LH mediated pathways in goats. *Anim. Reprod. Sci.* **2008**, *106*, 412–420. [CrossRef]
16. Meza-Herrera, C.A.; Torres-Moreno, M.; Lopez-Medrano, J.I.; Gonzalez-Bulnes, A.; Veliz, F.G.; Mellado, M.; Wurzinger, M.; Soto-Sanchez, M.J.; Calderón-Leyva, M.G. Glutamate supply positively affects serum release of triiodothyronine and insulin across time without increases of glucose during the onset of puberty in the female goat. *Anim. Reprod. Sci.* **2011**, *125*, 74–80. [CrossRef] [PubMed]
17. Hirch, K.; Atzmon, A.; Danielenko, M.; Sharoni, Y. Lycopene and other carotenoids inhibit estrogenic activity of 17beta-estradiol and genistein in cancer cells. *Breast Cancer Res. Treat.* **2007**, *104*, 221–230. [CrossRef] [PubMed]
18. Meza-Herrera, C.A.; Vargas-Beltrán, F.; Tena-Sempere, M.; Gonzalez-Bulnes, A.; Macias-Cruz, U.; Veliz-Deras, F.G. Short-term betacarotene supplementation positively affects ovarian activity and serum insulin concentrations in a goat model. *J. Endocrinol. Investig.* **2013**, *36*, 185–189.
19. Meza-Herrera, C.A. Puberty, Kisspeptin and Glutamate: A Ceaseless Golden Braid. In *Advances in Medicine and Biology*; Berhardt, L.V., Ed.; Nova Science Publishers Inc.: Hauppauge, NY, USA, 2012; Volume 52, pp. 97–124.
20. Meza-Herrera, C.A.; Tena-Sempere, M. Interface between Nutrition and Reproduction. In *Animal Reproduction in Livestock—Encyclopedia of Life Support Systems*; Astiz, S., Gonzalez, A., Eds.; Eolss Publishers: Oxford, UK, 2012.
21. Herbison, A.E. The gonadoprin-releasing hormone pulse generator. *Endocrinology* **2018**, *159*, 3723–3736. [CrossRef] [PubMed]
22. Oszkiel, H.; Wilczak, J.; Jank, M. Biologically active substances-enriched diet regulates gonadotrope cell activation pathway in liver of adult and old rats. *Genes Nutr.* **2014**, *9*, 427. [CrossRef]
23. Haliloglu, S.; Baspinar, N.; Serpek, B.; Erdem, H.; Bulut, Z. Vitamin A and betacarotene levels in plasma: Corpus luteum and follicular fluid of cyclic and pregnant cattle. *Reprod. Dom. Anim.* **2002**, *37*, 96–99. [CrossRef] [PubMed]
24. Salem, A.A.; El-Shahawy, N.A.; Soliman, I.A. Effect of beta-carotene injection on estrus, vitamin A and estradiol-17-β concentrations in pubertal Farafra ewe lambs. *Egypt J. Anim. Prod.* **2015**, *52*, 123–128.
25. Meza-Herrera, C.A.; Calderon-Leyva, G.; Soto-Sanchez, M.J.; Serradilla, J.M.; Garcia-Martinez, A.; Mellado, M.; Veliz-Deras, F.G. Glutamate supply positively affects cholesterol concentrations without increases in total protein and urea around the onset of puberty in goats. *Anim. Reprod. Sci.* **2014**, *147*, 106–111. [CrossRef] [PubMed]
26. Meza-Herrera, C.A.; Reyes-Avila, J.M.; Tena-Sempere, M.; Veliz-Deras, F.G.; Macias-Cruz, U.; Rodriguez-Martinez, R.; Arellano-Rodriguez, G. Long-Term Betacarotene supplementation positively affects serum triiodothyronine concentrations around puberty onset in female goats. *Small Rumin. Res.* **2014**, *116*, 176–182. [CrossRef]
27. 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.; et al. Beta-carotene supplementation positively affects selected blood metabolites across time around the onset of puberty in goats. *Czech J. Anim. Sci.* **2017**, *62*, 22–31. [CrossRef]
28. FASS. *Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching*, 3rd ed.; Federation Animal Science Society: Champaing, IL, USA, 2010; p. 177.

29. NAM-National Academy of Medicine. *Guide for the Care and Use of Laboratory Animals. Co-Produced by the National Academy of Medicine—Mexico and the Association for Assessment and Accreditation of Laboratory Animal Care International*, 1st ed.; Harlan: Mexico City, Mexico, 2002.
30. NRC—National Research Council. *Nutrient Requirements of Goats: Angora, Dairy and Meat Goats in Temperature and Tropical Countries*; National Academy Press: Washington, DC, USA, 1998.
31. Association of Official Analytical Chemists (AOAC). *Official Methods of Analysis*, 15th ed.; AOAC: Arlington, VA, USA, 1990.
32. Hoefler, H.; Hallford, D.M. Influence of suckling status and type of birth on serum hormone profiles and return to estrus early pospartum spring lambing ewes. *Theriogenology* **1987**, *27*, 887–892. [CrossRef]
33. Merriam, G.R.; Wachter, K.W. Algorithms for the study of episodic hormone secretion. *Am. J. Physiol.* **1982**, *243*, E310–E318. [CrossRef]
34. Martin, G.B.; Oldham, C.M.; Lindsay, D.R. Increased plasma LH levels in seasonally anovular Merino ewes following the introduction of rams. *Anim. Reprod. Sci.* **1980**, *3*, 125–132. [CrossRef]
35. Martin, G.B.; Scaramuzzi, R.J.; Oldham, C.M.; Lindsay, D.R. Effects of progesterone on the responses of Merino ewes to the introduction of rams during anoestrus. *Aust. J. Biol. Sci.* **1983**, *36*, 369–378. [CrossRef]
36. Dickie, A.M.; Paterson, C.; Anderson, L.M.; Boyd, J.S. Determination of corpora lutea numbers in Booroola—Texel ewes using transrectal ultrasound. *Theriogenology* **1999**, *51*, 1209–1224. [CrossRef]
37. Littell, C.R.; Henry, P.R.; Ammermam, C.B. Statistical analysis of repeated measures data using SAS procedures. *J. Anim. Sci.* **1998**, *76*, 1216–1231. [CrossRef] [PubMed]
38. Meza-Herrera, C.A.; Ross, T.; Hallford, D.; Hawkins, D.; Gonzalez-Bulnes, A. Effects of body condition and protein supplementation on LH secretion and luteal function in sheep. *Reprod. Domest. Anim.* **2007**, *42*, 461–465. [CrossRef] [PubMed]
39. Gonzalez-Bulnes, A.; Meza-Herrera, C.A.; Rekik, M.; Ben Salem, H.; Kridli, R.T. Limiting Factors and Strategies for Improving Reproductive Outputs of Small Ruminant Reared in Semiarid Environments. In *Semi-Arid Environments: Agriculture, Water Supply and Vegetation*; Degenovine, K.M., Ed.; Nova Science Publishers Inc.: Hauppauge, NY, USA, 2011; pp. 41–60.
40. Viñoles, C.; Forsberg, M.; Martin, G.B.; Cajarville, C.; Repetto, J.; Meikle, A. Short-term nutritional supplementation of ewes in low body condition affects follicle development due to an increase in glucose and metabolic hormones. *Reproduction* **2005**, *129*, 299–309. [CrossRef] [PubMed]
41. Wang, J.Y.; Hafi, C.B.; Larson, L.L. Endocrine responses and estrous activity in Holstein heifers fed supplemental betacarotene. *Theriogenology* **1988**, *29*, 731–742. [CrossRef]
42. Mojapelo, M.M.; Lehloenya, K.C. Effect of selenium supplementation on attainment of puberty in Saanen male goat kids. *Theriogenology* **2019**, *138*, 9–15. [CrossRef] [PubMed]
43. Nogueira, D.M.; Cavalieri, J.; Fitzpatrick, L.A.; Gummow, B.; Blache, D.; Parker, A.J. Effect of hormonal synchronization and/or short-term supplementation with maize on follicular dynamics and hormone profiles in goats during the non-breeding season. *Anim. Reprod. Sci.* **2016**, *171*, 87–97. [CrossRef]
44. Chew, B.P.; Wong, T.S.; Michal, J.J. Uptake of orally administered B-carotene by blood plasma, leukocytes and lipoproteins in calves. *J. Anim. Sci.* **1993**, *71*, 730–739. [CrossRef]
45. Perkins, A.T.; Greig, M.M.; Sontakke, A.A.; Peloquin, A.S.; McPeek, M.A.; Bickel, S.E. Increased levels of superoxide dismutase suppress meiotic segregation errors in aging oocytes. *Chromosoma* **2019**, *128*, 215–222. [CrossRef]
46. Yu, S.; Zhao, Y.; Feng, Y.; Zhang, H.; Li, L.; Shen, W.; Zhao, M.; Min, L. β-carotene improves oocyte development and maturation under oxidative stress in vitro. *In Vitro Cell Dev. Biol. Anim.* **2019**, *55*, 548–558. [CrossRef]
47. Aksak-Karamese, S.; Toktay, E.; Unal, D.; Selli, J.; Karamese, M.; Malkoc, I. The protective effects of beta-carotene against ischemia/reperfusion injury in rat ovarian tissue. *Acta Histochem.* **2015**, *117*, 790–797. [CrossRef]
48. Mumford, S.L.; Browne, R.W.; Schliep, K.C.; Schmelzer, J.; Plowden, T.C.; Michels, K.A.; Sjaarda, L.A.; Zarek, S.M.; Perkins, N.J.; Messer, L.C.; et al. Serum antioxidants are associated with serum reproductive hormones and ovulation among healthy woman. *J. Nutr.* **2016**, *146*, 98–106. [CrossRef] [PubMed]

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THESIS IN JOINT SUPERVISION TO OBTAIN THE DOUBLE DEGREE OF DOCTOR SCIENCE:

THE KEY ROLE OF TARGETED BETACAROTENE SUPPLEMENTATION ON ENDOCRINE AND REPRODUCTIVE PERFROMANCE IN GOATS OF THE MEXICAN ARID REGIONS

EL PAPEL CENTRAL DE LA SUPLEMENTACIÓN DIRIGIDA CON BETACAROTENO EN EL COMPORTAMIENTO ENDOCRINO Y REPRODUCTIVO EN CAPRINOS DE LAS REGIONES ÁRIDAS DE MÉXICO

VI. GENERAL CONCLUSIONS

Doctorado en ciencias en recursos naturales y medio ambiente en zonas áridas
Doctorado en recursos naturales y gestión sostenible

Taking into account a translational perspective, since GH promotes cell proliferation and angiogenesis while inhibiting apoptosis, the GH/IGF-1 system has been implicated in mammals with the development and/or progression of several types of cancer (Kopchik et al., 2014).

On the other hand, GH levels have been associated with signs of premature aging with an extreme reduction in life expectancy. Although these premature deaths have been diagnosed as multifactorial in origin, organ-specific pathological damage has been consistently present with an increased incidence of tumors (Bartke et al., 2013; Kopchik et al., 2014). Furthermore, acting through autocrine/paracrine/endocrine pathways, increased GH levels have been linked to oncogenesis; therefore, any attempt to reduce the action of GH/IGF-1 in some pathological scenarios could provide outstanding protection against certain types of cancer (Pollack et al., 2001; Kopchik et al., 2014). These findings merged with our results offer interesting possibilities in the search for possible translational applications, considering the GH-reducing effect of BC supplementation observed in our study; further research considering this approach emerges as an interesting subject.

Besides, data found by our research group demonstrate that beta-carotene supplementation produces an increase in ovarian activity and ovulation rate, which could be used as a model for adult goats, in particular such physiological scenario invoked the participation of a decrease in serum GH, however, without effects on IGF-1 levels. It remains to be determined if such a decrease in GH secretion could directly affect the hypothalamic centers that modulate the pulsatile release of GnRH or indirectly through peripheral signals that reflect actions of GH/IGF-1 at the ovarian level. However, one study reveals for the first time, a potential role of BC as a somatotropin-modulating molecule. However, the precise site of action of BC in the whole HPG axis in females is expected to be established; being the last one important for the animal industry and with possible implications of translation from a clinical point of view.

To conclude, the results of our two studies developed in this thesis, support the fact that betacarotene would improve the follicular development during the natural breeding season in adult animals as well as the out-off-season ovarian function, inducing changes in the pattern of LH release in yearling anestrus goats, without differences in live weight and body condition score. The question remains whether the effects of BC supplementation were exerted by deviations in the LH release pattern, or whether they were exerted directly on the ovarian physiology or even mediated through increased regulation of LH ovarian receptors. Further research designed to expose the precise endocrine or metabolic effects of BC along the HPG axis would contribute to a better understanding of the positive out-of-season ovarian outcomes observed in BC-supplemented one-year-old anestrus goats, particularly with a high degree of genes from highly seasonal goat breeds.

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VII. GRAPHICAL ABSTRACT

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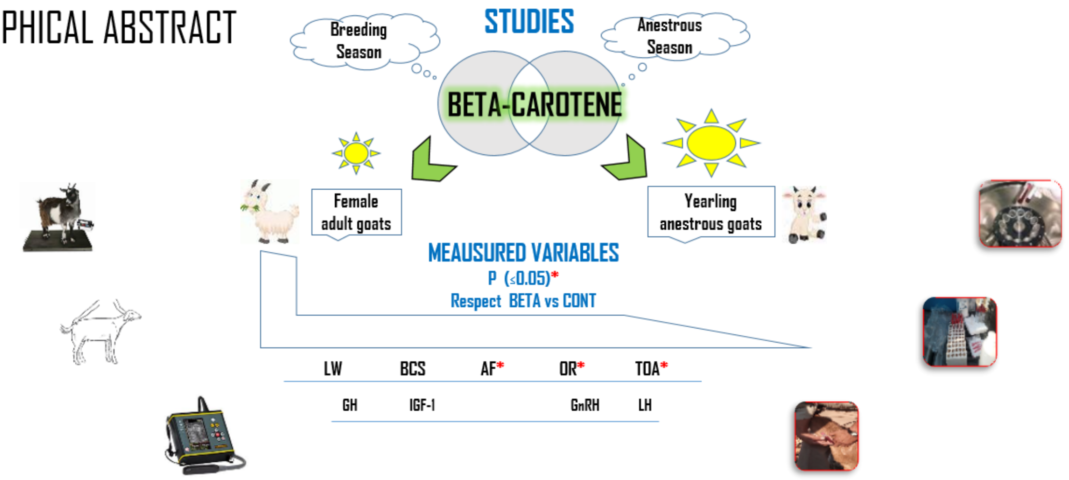
GRAPHICAL ABSTRACT



STUDIES

Breeding Season

Anestrous Season

BETA-CAROTENE

Female adult goats

Yearling anestrous goats

MEAUSURED VARIABLES

P (≤0.05)*

Respect BETA vs CONT

LW BCS AF* OR* TOA*

GH IGF-I GnRH LH

HIGHLIGHTS OF THE STUDIES

- ✓ BETA supplementation generated an increase in ovarian activity and ovulation rate.
- ✓ Showing effect not only during the breeding season with adult goats, but also in the anestrus season with yearling goats.
- ✓ Escorted by a decrease in GH taking into account the adult female & upon the LH release pattern in yearling anestrous goats.
- ✓ The last should speed up the reproductive efficiency in goat production systems under arid and semiarid conditions.

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VIII. REFERENCES

Aguiar-Zalzano, E. & Rojas-Bourrillon, A. 2015. Variaciones de Betacaroteno en sangre de vacas lecheras durante el periodo post parto. Nutrición Animal Tropical. 9 (2): Pp. 91-104.

Alejandre, O. M. E., Rubio, T, E., Pérez, E. E., Zaragoza, M. L. y Rodríguez, G. G. 2016. Los recursos caprinos de México. Biodiversidad caprina Iberoamericana. Universidad Cooperativa de Colombia. Pp. 95-107.

Amann, P. M., Eichmuler, S.B., Schmidt, J. and Bazhin, A.V., 2011. Regulation of gene expression by retinoids. Current Medicinal Chemistry. 18. Pp. 1405–1412.

Andrade-Montemayor, H. M. 2017. Producción de caprino en México. Facultad de Ciencias Naturales. Lic. Medicina Veterinaria y Zootecnia. Universidad Autónoma de Querétaro. Ejemplar dedicado al VIII Foro Nacional del Caprino. 18. Pp. 24.

Anzaldo, M. M. 2020. Entre la vulnerabilidad y la invisibilidad científica. Estudio sobre los aportes de las ciencias sociales a la investigación sobre la ganadería caprina en México. Revista de Alimentación Contemporánea y Desarrollo Regional. Vol. 30. 55. Pp. 1-27. http://dx.doi.org/10.24836/es.v30i55.915

Aréchiga, C., Aguilera, J., Rincón, R., Méndez de Lara, S., Bañuelos, V y Meza-Herrera, C. 2008. Situación actual y perspectivas de la producción caprina ante el reto de la globalización. *Tropical and Subtropical Agroecosystems,* 9. Pp. 1-14. Recuperado de http://www.redalyc.org/articulo.oa?id=93911227001

Arellano, R. G., Meza-Herrera, C. A., Rodríguez, M. R., Dionisio, T. R., Hallford, D. M., Mellado, M. & González, B. A. 2008. Short-term intake of Betacarotene supplemented diets enhances ovarian function and progesterone synthesis in goats. Animal Physiology and Animal Nutrition. https://doi.org/10.1111/j.1439-0396.2008.00859.x

Arellano, R. G., Meza-Herrera, C. A., Rodríguez, M. R., Velázquez, M. G., Mellado, M., Salinas, H., Pérez, R. M. A. & Sánchez, F. 2007. Short-term betacarotene supplementation positively affects ovarian follicular development and ovulation rate in goats. J. Applied Animal Research. 32. Pp. 177-180 https://doi.org/10.1080/09712119.2007.9706872

Barrera, P. O. T., Sagarnaga, V. L. M., Salas, G. J. M., Leos, R. J. A. y Santos, L. R. 2018. Viabilidad económica y financiera de la ganadería caprina extensiva en San Luis Potosí, México. Mundo Agrario, 19(40), e077. https://doi.org/10.24215/15155994e077

Bartke, A.L., Sun, L. Y., Longo, V., 2013. Somatotropic signaling: trade-offs between growth, reproductive development and longevity. Physiological Reviews. 93, Pp. 571–598. https://doi.org/10.1152/physrev.00006.2012

Bedotti, D. 2008. El rol social del ganado caprino. Conferencia plenaria 31. Congreso de la Asociación Argentina de Producción Animal. Potrero de los Funes. San Luis. Pp.15-17.

Campos, G. R. y Hernández, E. A. 2008. Un enfoque hacia el entendimiento de los mecanismos fisiológicos, nutricionales y bioquímicos de las limitantes reproductivas de origen nutricional. Repositorio. Facultad de Ciencias Agropecuarias. Departamento de Ciencia animal. Universidad de Colombia. Pp. 1-57.

Carranco, J. M. E., Calvo, C. M. C. y Pérez, G. R. 2011. Carotenoides y su función antioxidante: Revisión. Archivos latinoamericanos de nutrición. Órgano oficial de la sociedad latinoamericana de nutrición. Vol. 6. No. 3. Pp. 233-241.

Carrión, D. y Medel, P. 2002. Interacción nutrición reproducción en ganado porcino. XVII Curso de Especialización FEDNA. Madrid, 05-14-17. España. Pp. 27-70.

Cavalloti, B. A., Valverde, B. R., Vargas, A. C. y Juárez, J. R. (Eds.). 2017. *Globalización, seguridad alimentaria y ganadería familiar*. México: Universidad Autónoma Chapingo. ISBN 978-607-711-407-9

Celi-Mariátegui, I. 2012. Estudio de los mecanismos implicados en la estacionalidad reproductiva de caprinos mediterráneos: papel de los opioides, las catecolaminas y la serotonina. (Tesis doctoral, Universidad de Huelva) Recuperado de http://rabida.uhu.es/dspace/bitstream/handle/10272/6156/Estudio_mecanismos_implicados. pdf?sequence=2

Chemineau, P. 1984. “Buck effect” in tropical goats. En: M., Courot. (Ed.) The male in the farm animal reproduction. Boston, EUA: Martinus Nijhoff Publishers. Pp.310-315

Chemineau, P. y Delgadillo, J. A. 1994. Neuroendocrinología de la reproducción del caprino. Revista Científica-Universidad Del Zulia. Facultad de ciencias veterinarias. División de Investigación Universidad Del Zulia. 1(2): Pp. 85-101. hal-02706435f

Cueto, M. I., Gibbons, A. E. y Abad, M. 2000. Reproducción en caprinos. EEA Bariloche: Centro regional Patagonia Norte. Recuperado de: http://www.produccion-animal.com.ar/produccion_caprina/inseminacion_transferencia_caprino/56-reproduccion.pdf

Darwish, W. S., Ikenaka, Y., Morsdy, A. E., Eldesorky, K. I., Nakayama, S., Mizukawa, N. and Ishizuka, M. 2016. Β-Carotene and retinol contents in the meat of herbivorous ungulates with special reference to their public health importance. Journal of Veterinary Medical Science, 78, Pp. 351–354. https://doi.org/10.1292/jvms.15-0287

Dominic, L. M. and Khoboso, C. L. 2020. Β- carotene supplementation increases progesterone concentration and glutathione peroxidase activity following anternative progesterone primed oestrous synchronization protocol in goats. Americal Journal of Animal and Veterinary Science. 15 (3): Pp. 211-219.

Dorantes-Coronado, E. J., Hernández, M. J., Rebollar, R. S. y Rojo, R. R. 2014. Caracterización Socioeconómica del Sistema de Producción Caprina en el sur del Estado de México. Metodologías y aplicaciones para la producción ganadera del trópico seco en el sur del estado de México. Pp. 1-24.

Ducoing, W. A. E, recuperado el 3 de agosto de 2020. Situación de la Caprinocultura en México. Pp. 1-6.

Eggersdorfer, M. and Wyss, A. 2018. Carotenoids in human nutrition and health. Archives of Biochemistry and Biophysics, 652, Pp. 18–26. https://doi.org/10.1016/j.abb.2018.06.001

Enriquez, A. 2016. Papel del betacaroteno en la nutrición del ganado lechero. Pp. 1-7.

FAO. Organización de las Naciones Unidas para la Alimentación y la Agricultura. 2019. FAOSTAT. Recuperado de: http://www.fao.org/faostat/es/#data

FAO. Organización de las Naciones Unidas para la Alimentación y la Agricultura. 2016. Portal lácteo. Recuperado de: http://www.fao.org/dairy-production-products/production/productiondairy-animals/productiondairy-animalssmall ruminants/es/

FAO. Organización de las Naciones Unidas para la Alimentación y la Agricultura. 2018. FAOSTAT. Recuperado de: http://www.fao.org/faostat/es/#data/QL

Gibbons, A., Willems, P., González, R., Cueto, M. y García, J. 1994. Actividad sexual de la cabra de raza Angora por efecto macho temporario o permanente. *Revista Argentina de Producción Anim*al, *14*, Pp. 209-214.

González-Bulnes, A.; Meza-Herrera, C. A.; Rekik, M.; Ben Salem, H.; and Kridli. R. T. 2011. Limiting factors and Strategies for Improve Reproductive Outputs of Small Ruminants Reared in Semiarid Enviroments. In: Semi-Arid Enviroments Agriculture, Water Supply and Vegetation. Degenovine K. N. (ed.) Nova Science Publishers, Inc. USA. Chapter II, Pp. 22. ISBN: 978-1-61761-541-2

Guerra-García, M., Meza-Herrera, C. A., Sánchez-Torres-Esqueda, M. T., Gallegos-Sánchez, J., Torres-Hernández, G. y Pro-Martínez, A. 2009. IGF-1 and ovarian activity of goats in divergent body condition and supplemented with non-degradable ruminal protein. *Agrociencia* 43. Pp. 241–247.

Guerrero C. M. M. 2016. La Caprinocultura en México, una Estrategia de Desarrollo. Revista Universitaria Digital de Ciencias Sociales (RUDICS). México.

Guerrero, C. M. M. 2010. La caprinocultura en México, una estrategia de desarrollo. Revista Universitaria Digital de Ciencias Sociales. Universidad Nacional Autónoma de México.

Haliloglu, S., Baspinar, N., Serpek, B., Erdem, H., Bulut, Z., 2002. Vitamin A and betacarotene levels in plasma: corpus luteum and follicular fluid of cyclic and pregnant cattle. Reproductions in Domestic Animals. 37. Pp. 96–99. https://doi.org/10.1046/j.1439-0531.2002.00338.x

Hazard, T. S. 2017. Importancia de la nutrición en la reproducción de vacas lecheras. INIA, Carillanca. Pp. 1-7.

Hirch, K., Atzmon, A., Danielenko, M. and Sharoni, Y. 2007. Lycopene and other carotenoids inhibit estrogenic activity of 17beta-estradiol and genistein in cancer cells. Breast Cancer Research and Treatment. 104, Pp. 221–230. https://doi.org/10.1007/s10549-006-9405-7

Karsh, F. J. 1984. The hypothalamus and anterior pituitary gland, En: C. R., Austin y R. V., Short. (Eds.), *Reproduction in Mammals*. England: Cambridge University Press. Pp. 1-20.

Laundau, S., Provenza, F. and Silanikove, N. 2000. VII International Conference on Goats, tour France May, 2000.

López-Flores, N. M. 2016. Sustitución de sorgo grano con grano seco de destileria sobre la fermentación ruminal y nitrogeno ureico en cabras alimentadas con soca de sorgo. Tesis Maestría. Universidad Juárez del Estado de Durango. Facultadad de Agricultura y Zootecnia. División de Estudios de Posgrado. México. Pp. 1-50.

López-Flores, N. M. 2018b. Epistemología de la Zootecnia y Obstáculos Epistemológicos. Posgrado en Recursos Naturales y Medio Ambiente en Zonas Áridas. Doctorante. Ensayo de la materia Epistemología de la ciencia. México. Pp. 1-10.

López-Flores, N. M., Meza-Herrera, C. A., Galán, S. C., Bautista, R. D. A., Véliz, D. F. G., Arellano, R. G., García de la Peña, C., Rosales-Nieto, C. A. & Macías, C. U. 2018a. The Key rolo of targeted betacarotene supplementation on endocrine and reproductive outcomes in goats. Follicular development, ovulation rate, and the GH-IGF-1 axis. Small Ruminant Research. 163. Pp. 29-33. https://doi.org/10.1016/j.smallrumres.2017.09.009

López-Flores, N. M., Meza-Herrera, C. A., Pérez, M. C., Blache, D., Arellano, R. G., Zuñiga, G. S., Navarrete, M. C., García de la Peña, C., Rosales-Nieto, C. A. & Véliz, D. F. G. 2020. Precision betacarotene supplementation enhanced ovarian function and the LH release pattern in yearling crossbred anestrous goats. Animals. https://doi.org/10.3390/ani10040659

López-Sebastián, A. 1999. Manejo reproductivo en pequeños rumiantes. I Curso International de Fisiología de la reproducción de Rumiantes. Colegio de Postgraduados, México. Pp. 95-105.

Lozano-González, J. F., Uribe-Velázquez, L. F. y Osorio, J. H. 2012. Control hormonal de la reproducción en hembras ovinas. Veterinaria y Zootecnia. Vol. 6. No. 2. Pp. 1-14.

Malpaux B., Viguié C., Skinner D. C., Thiéry J. C., Pelletier J., Chemineau P. 1996. Seasonal breeding in sheep: Mechanism of action of melatonin. Animal Reproductive Science, 42: Pp. 109-117. https://doi.org/10.1016/0378-4320(96)01505-9

Martin, G. B., Rodger, J. & Blache, D. 2004. Nutritional and environmental effects on reproduction in small ruminants. Reproduction, Fertility and Development. 16. Pp. 491-501. https://doi.org/10.1071/RD04035

Matamoros, R. y Salinas, P. 2017. Fundamentos de fisiología y endocrinología reproductiva en animales domésticos. Fisiología animal. Universidad Santo Tomás. Pp. 1-302.

Mellado, B. M. (1985). Producción de caprinos en pastoreo. México: trillas.

Merlos, M., Martínez, R. D., Torres, G., Mastache, A. A. y Gallegos, J. 2008. Evaluación de características productivas en cabritos Boer x Local, Nubia x Local y locales en el trópico seco de Guerrero, México. Veterinaria México, 39(3), Pp.323-333.

Meza-Herrera C. A., Pacheco-Alvarez P., Castro O. E., Macías-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 Journal of Animal Science. 62, 22-31. https://doi.org/10.17221/1/2016-CJAS

Meza-Herrera, C. A. 2012. Puberty, kisspectin and glutamate: A ceasles Golden braid. In: Advances in Medicine and Biology, Ed: Benhardt, L. V. Vol. 52 Chapter 3. Nova Science Publishers NY, USA. Pp. 97-124.

Meza-Herrera, C. A., González-Bulnes, A., Kridli, R. T., Mellado, M., Arechiga-Flores, C. F., Salinas, H., y Luginbuhl, J. M. 2010a. Neuroendocrine, metabolic and genomic cues signalling the onset of puberty in females. Reproduction in Domestic Animals. 45(6). https://doi.org/10.1111/j.1439-0531.2009.01355.x

Meza-Herrera, C. A., Martinez, L., Arechiga, C., Bañuelos, R., Rincon, R. M., Urrutia, J., Salinas, H. and Mellado, M. 2006. Circannual identification and quantification of constitutive heat shock proteins (HSP 70) in goats. Journal of Applied Animal Research. 29. Pp. 9-12. https://doi.org/10.1080/09712119.2006.9706560

Meza-Herrera, C. A., Reyes-Avila, J. M., Tena-Sempere, M., Veliz-Deras, F. G., Macias-Cruz, U., Rodriguez-Martinez, R. and Arellano-Rodriguez, G. 2014. Long-Term Betacarotene supplementation positively affects serum triiodothyronine concentrations around puberty onset in female goats. Small Ruminant Research. 116, Pp. 176–182. https://doi.org/10.1016/j.smallrumres.2013.10.017

Meza-Herrera, C. A., Sanchez, J.M., Chavez-Perches, J.G., Salinas, H., Mellado, M., 2004. Protein supplementation, body condition and ovarian activity in goats: preovulatory serum profile of insulin. South Afr. J. Anim. Sci. 34 (Suppl. 1), 223–226.

Meza-Herrera, C. A., Tena-Sempere, M., 2012. Interface between nutrition and reproduction: the very basis of production, In: Astiz S, Gonzalez-Bulnes A (Eds.), Animal Reproduction in Livestock, Encyclopedia of Life Support Systems (EOLSS), UNESCO, Eolss Publishers, Oxford, UK, [http://www.eolss.net].

Meza-Herrera, C. A., Torres-Moreno, M., Lopez-Medrano, J. I., Gonzalez-Bulnes, A., Veliz, F. G., Mellado, M., Wurzinger, M., Soto-Sanchez, M. J. and Calderón-Leyva, M. G. 2011. Glutamate supply positively effects serum release of triiodothyronine and insulin across time without increases of glucose during the onset of puberty in the female goat. Animal Reproduction Science. 125, Pp. 74–80. https://doi.org/10.1016/j.anireprosci.2011.03.011

Meza-Herrera, C. A., Veliz-Deras, F. G., Wurzinger, M., Lopez-Ariza, B., Arellano-Rodriguez, G. and Rodriguez-Martinez, R. 2010b**.** The kiss-1, kisspeptin, gpr-54 complex: A critical modulator of GnRH neurons during pubertal activation. Journal of Applied Biomedicine. 8, Pp. 1–9. https://doi.org/10.2478/v10136-009-0001-0

Mojapelo, M. M. and Lehloenya, K. C. 2019. Effect of selenium supplementation on attainment of puberty in Saanen male goat kids. Theriogenology. 138. Pp. 9.15. https://doi.org/10.1016/j.theriogenology.2019.06.044

Paleta, O. A. 2015. Suplementación de glutamato y reactivación ovárica en cabras primalas durante la época de anestro estacional: efectos sobre los niveles séricos de triyodotironina. Master en Zootecnia y Gestión Sostenible: Ganadería Ecológica e Integrada. Universidad de Córdoba. Pp. 1-67.

Parés-Casanova, Pere M. 2016. Biodiversidad caprina en España.

PlaNet Finance. 2011. Caracterización del sector caprino argentino.

Rosales-Nieto, C. A., Urrutia, M. J., Gámez, V. H., Díaz, G. M. O. y Rámirez, A. B. M. 2006. Influencia del nivel de la alimentación en la actividad reproductiva en cabras criollas durante la estación reproductiva. Técnica pecuaria en México. Vol. 44. No. 3. Pp. 399-406.

Rubio, T. E. y Pérez-Eguía, E. 2014. Importancia Económica del Ganado Caprino en el Estado de Chihuahua. Pp. 1-15.

Sáenz-Escárcega, P., Hoyos, F. G., Salinas, G. H., Espinoza, A. J., Guerrero, B. A. y Contreras, G, E. (1991). Establecimiento de módulos caprinos con productores cooperantes. En: S., Flores. (Ed.), Evaluación de Módulos Caprinos en la Comarca Lagunera. Matamoros, Coahuila, México. Pp. 24-34.

SAGARPA-SIAP (Sistema de Información Agroalimentaria y Pesquera). 2014. Anuario estadístico de la producción agropecuaria. México.

SAGARPA-SIAP (Sistema de Información Agroalimentaria y Pesquera). (2013). Anuario estadístico de la producción agropecuaria. México.

SAGARPA-SIAP (Sistema de Información Agroalimentaria y Pesquera). 2012. Programa Nacional Pecuario. México.

Salem, A. A., El-Shahawy, N. A. and Soliman, I. A. 2015. Effect of beta-carotene injection on estrus, vitamin A and estradiol-17-B concentrations in pubertal Farafra ewe lambs. Egyptian Journal Animal Production. 52, Pp. 123–128.

Santa María A., Cox J., Muñoz E., Rodríguez R., Caldera L. 1990. Estudio del ciclo sexual, estacionalidad reproductiva y control del estro en la cabra criolla en Chile. Liverstock Reproduction in Latin America. En: Proceedings of the final research co-ordination meeting, Bogotá, 19-23 September 1988, International Atomic Energy Agency, Vienna, Pp. 363-385.

Scaramuzzi, R. J., Baird, D. T., Campbell, B. K., Driancourt, M. A., Dupont, J., Fortune, J. E., Gilchrist, R. B., Martin, G. B., McNatty, K. P., McNeilly, A. S., Monget, P., Monniaux, D., Vinoles, C., Webb, R., 2011. Regulation of folliculogenesis and the determination of ovulation rate in ruminants. Reproduction, Fertility and Development. 23, 444–467. https://doi.org/10.1071/RD09161

Scaramuzzi, R.J.; Campbell, B.K.; Dowing, J.K.; Kendall, N.R.; Khalid, M.; Muñoz-Gutierrez, M. 2006**.** A review of the effects of supplementary nutrition in the ewe on the concentrations of reproductive and metabolic hormones and the mechanism that regulate foliculogenesis and ovulation rate. Reproduction and Nutrition Development. 46, 339–354. https://doi.org/10.1051/rnd:2006016

Steinfeld, H., Gerber, P., Wassenaar, T., Castel, V., Rosales, M., de Haan, C. 2006. Livestock`s long shadow. ISBN.978-92-5-105571-7.

Urviola, G. A. P. y Riveros, J. L. 2017. Factores moduladores de la estacionalidad reproductiva en ungulados. Revista de Investigaciones Altoandinas. Vol. 19. No. 3. Pp. 319-336. http://dx.doi.org/10.18271/ria.2017.297.

Vergara, H. H. P. 2014. Suplementación de glutamato y función reproductiva en cabras primalas durante el periodo de transición al anestro estacional. Tesis Maestro en Ciencias en Recursos Naturales y Medio Ambiente en Zonas Áridas (UACH-URUZA, MÉXICO).Master en Zootecnia y Gestión Sostenible: Ganadería Ecológica e Integrada (UCO-IDEP, ESPAÑA).

Zarazaga L. A., Gatica M.C., Celi I., Guzmán J. L., Malpaux B. 2009. Effect of melatonin implants on sexual activity in Mediterranean goat females without separation from males. Theriogenology, 72: Pp. 910-918. https://doi.org/10.1016/j.theriogenology.2009.05.020

Zarazaga L. A., Guzmán J. L., Domínguez M. C., Pérez M. C., Prieto R. 2005. Effect of plane of nutrition on seasonality of reproduction in Spanish Pyoya goats. Animal Reproduction Science, 87: Pp. 253-267. https://doi.org/10.1016/j.anireprosci.2004.11.004