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Reproductive response of Saanen x Alpina goats to the application of a metabolic reconstituent during estrus synchronization

Respuesta reproductiva de cabras Saanen x Alpina al aplicar un reconstituyente metabólico durante la sincronización del estro



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ABSTRACT

The action of a metabolic treatment was evaluated in response to estrus synchronization in Saanen x Alpina goats. Thirty-six multiparous goats were used and randomly assigned to one of three treatments: T1 (n= 12): CIDR+eCG with metabolic restorative (MR); T2 (n= 12): CIDR+eCG with isotonic solution (ISO); T3 (n= 12): CIDR+eCG (CONTROL). Data were tested to come from a normally distributed population. Response to estrus (RE), the onset of estrus (OE), return to estrus (RET), gestation (GEST), calving (PAR), and fertility (FER) were analyzed using the non-parametric Kruskal-Wallis test; estrus duration (ED), fecundity (FEC) and prolificacy (PROL) through an analysis of variance and Tukey's test (α = 0.05) to compare means between treatments. MR treatment reduced (p <0.05) OE (T1: 20.78 h, T2: 32.54 h, T3: 33.68 h), increased FEC (T1: 1.42, T2: 0.92, T3: 0.92), and increased PROL (T1: 2.1, T2: 1.4, T3: 1.4). The ISO treatment had a similar effect to the CONTROL treatment (p >0.05) in the study variables. The metabolic restorative treatment during estrus synchronization in Saanen x Alpine goats reduces the onset of estrus and increases fertility and prolificacy.

Keywords: Amino acids, fecundity, gonadotropins, progestogens, prolificacy.

RESUMEN

Se evaluó la acción de un reconstituyente metabólico como respuesta al estro de cabras Saanen x Alpina. Se utilizaron 36 cabras multíparas y se asignaron aleatoriamente a uno de tres tratamientos: T1 (n= 12): CIDR+eCG con reconstituyente metabólico (REC); T2 (n= 12): CIDR+eCG con solución isotónica (ISO); T3



(n= 12): CIDR+eCG (TEST). Se evaluó que los datos provinieran de una población distribuida normalmente. La respuesta al estro (RE), inicio del estro (IE), retorno al estro (RET), gestación (GEST), parición (PAR) y fertilidad (FER) se analizaron mediante la prueba no paramétrica de Kruskal-Wallis; la duración del estro (DE), fecundidad (FEC) y prolificidad (PROL) mediante un análisis de varianza y se utilizó la prueba de Tukey (α = 0.05) para comparar medias entre tratamientos. El tratamiento con REC redujo (p <0.05) el IE (T1: 20.78 h, T2: 32.54 h, T3: 33.68 h), aumentó la FEC (T1: 1.42, T2: 0.92, T3: 0.92) e incrementó la PROL (T1: 2.1, T2: 1.4, T3: 1.4). El tratamiento con ISO tuvo efecto similar al tratamiento TEST (p >0.05) en las variables de estudio. El reconstituyente metabólico durante la sincronización del estro en cabras Saanen x Alpina reduce el inicio del estro e incrementa la fecundidad y la prolificidad.

Palabras clave: Aminoácidos, fecundidad, gonadotropinas, progestágenos, prolificidad.

INTRODUCTION

Currently, many strategies are explored to improve the reproductive capacity of production animals, with the objective of satisfying the production needs of meat, milk and skins (Omontese, 2018). In this way, by increasing the physiological reproductive events of females of any animal species, there is an increase in prolificacy, productive variables and consequently a higher economic income for the producer (Dubeuf, 2011). Knowing goat reproductive physiology influences the development and application of practical protocols in reproductive management that improve productive and reproductive efficiency (Hashemi & Safdarian, 2017). Therefore, it is known that when goats do not receive strategic reproductive management, reproductive seasonality becomes productive seasonality; resulting in a problem for producers in the commercial aspect (Escareño *et al.*, 2011).

Reproduction in goats can be controlled with methods that involve the administration of exogenous hormones that modify the estrous cycle; for example, progesterone (P4) or its analogs that simulate the activity of natural P4 produced in the *corpus luteum* (CL) during the luteal phase after ovulation. In the case of prostaglandins (PGF2 α) they are an alternative to control reproduction by eliminating the CL and inducing the next follicular phase with ovulation (Abecia *et al.*, 2012); and administration of equine chorionic gonadotropin (eCG) concurrent with a second injection of PGF2 α or withdrawal of progestogen. It increases the estrous response by stimulation for ovarian follicles to reach the final stage of maturation, in synchronization protocols in a goatherd (Bukar *et al.*, 2012).

Gonadotropin-releasing hormone (GnRH) is the first messenger involved in the restart of reproductive cyclic activity in sheep and goats, and it is controlled by different neuromodulators (Meza-Herrera *et al.*, 2010). This hypothalamic-adenohypophysial endocrine communication can be favored by the action of certain compounds that act as neurotransmitters, whose activity can be increased by supplementation of neuroexcitatory amino acids (AANE; Mahesh & Brann, 2005; Meza-Herrera *et al.*, 2014), such as arginine, glutamate and aspartate (Alvarez-Cardona *et al.*, 2019). It is known that the use of a metabolic restorative containing NSAIDs per 100 mL: L-arginine, 240 mg; aspartic acid, 150 mg and glutamic acid, 150 mg. They exert a response on ovarian activity in



prepubertal ewes (Hernández-Marín *et al.*, 2016) or to improve lambing percentage and fecundity in ewes treated with recombinant bovine somatotropin and a metabolic restorative with a five-day postpartum ovulation induction protocol (Fraire-Cordero *et al.*, 2018). In this regard, the response of a parenteral treatment of a metabolic restorative during combined estrus synchronization in goats has not been determined. Therefore, we hypothesized that administration of an injectable metabolic product that also contains neurostimulatory amino acids (Metabolase[®], Fatro, Bologna, Italy), combined with a hormonal protocol of CIDR for 12 d, and with PGF2 and eCG 48 h before withdrawal, could improve reproductive response in goats.

The objective of the present investigation was to evaluate the action of a metabolic restorative on reproductive variables, in response to estrus synchronization in Saanen x Alpina goats.

MATERIAL AND METHODS

Location of the study area

The research was carried out in the sheep and goat area of the Experimental Farm of the Animal Husbandry Department of the Autonomous University of Chapingo, in Texcoco de Mora, Mexico state, located at 19°29' N and 98°53'W, at an altitude of 2250 m above sea level. All animals were conducted during the experimental development according to Mexican Official Standards NOM-024-ZOO-1995 (SAGARPA, 1995) and NOM-051-ZOO-1995 (SAGARPA, 1995). The experiment was supervised and the protocol authorized by the AG Research Committee (AG03-2021).

Animal characteristics and feeding

Thirty-six multiparous Saanen x Alpina crossbred goats with 54.12 6.34 kg live weight, 28.2 4.18 months of age and body condition of 2.5 units on the scale 1 to 5 where 1: wasted and 5: obese (Gosh *et al.*, 2019) were used. Additionally, 3 entire bucks with 62.27 7.61 kg live weight and 30.6 5.27 months of age were used for estrus detection and controlled natural mating. All animals were kept in pens provided with shade, feeding trough, automatic waterer and dirt floor; where they received 2.5 kg animal⁻¹ day⁻¹ of a feed ration offered from 8:00 to 9:00 am, which was based on a mixture of *Zea mays* (silage, 70%), *Medicago sativa* (fresh, 20%) and commercial concentrate (pellet, 10%).

Experimental protocol and treatments

Goats were randomly assigned to one of three experimental treatments: T1, n=12: estrus synchronization protocol + 100 mL of metabolic restorative (MR); T2, n=12: estrus synchronization protocol + 100 mL of isotonic solution (ISO); T3, n=12: estrus synchronization protocol (CONTROL).



Estrus synchronization in goats consisted of the insertion of intravaginal devices impregnated with progesterone (CIDR, P4: 0.3 g; Zoetis, Mexico) for 12 d; during this period. Each goat was checked twice daily (8:00 and 20:00 h) to verify that the CIDR remained inserted. At 48 h before withdrawal, prostaglandins (PGF₂ α : 5 mg, Dinoprost tromethamine, Lutalyse[®]; Zoetis, Mexico) and equine chorionic gonadotropin (eCG: 200 I.U.; Folligon[®]; Intervet International, Netherlands) were applied intramuscularly. The first hormone to induce some ovarian CL to lysis, and the second, to promote a subsequent elevation in E₂ and LH levels (Fatet *et al.*, 2011), enhance follicular development, obtain the external manifestation of estrus (Abecia *et al.*, 2012) and the presence of an LH peak accompanied by ovulation (Omontese *et al.*, 2014).

The isotonic solution was applied in order to homogenize the experimental conditions with the additional products to the synchronization protocol. Thus, the metabolic reconstituent (MR; Metabolase[®], Schütze-Segen, Italy) or the isotonic solution (ISO; Hartmann® Solution; Finlay, Honduras) were applied twice during estrus synchronization. The first application for treatments T1 and T2 was performed 10 d after inserting the CIDR, and 50 mL were administered intravenously, respectively; and in the second, 50 mL were applied subcutaneously at the time of removal of the CIDR (12 d).

Estrus detection and return

All goats were detected in estrus for 60 min, eight hours after the removal of the CIDR, which was repeated every 8 h (7:00, 15:00 and 23 h) for 64 h. The goats were introduced into the pen, where an apron was placed between the buck's arms, in the sternal region; non-toxic dye was applied with a crayon in order to mark and detect females in estrus. It was determined that a goat was in estrus when it accepted mounting by the male goat, showing total immobility. The goats were then given controlled natural mating immediately after being detected. The onset of estrus (OE) and its duration (ED) were recorded as the elapsed time (h) in which each goat accepted mounting by the buck and showed total immobility during mounting. Evaluation of return to estrus (RET) was performed at 21 d after removal of the CIDR. Estrus synchronization response (ESR) was calculated by expressing the number of goats that showed estrus as a percentage of the total number of goats treated in each treatment.

Diagnosis of gestation

This variable was determined 45 days after insemination by natural mating, using Vet 10 (Mindray) ultrasonography equipment with a 5 MHz linear rectal transducer. Females with or without defined fetal product(s) were recorded as pregnant or empty. Gestation rate (GEST) was recorded as the percentage of goats diagnosed as pregnant divided by the number of goats exposed to the buck that did not return to estrus.



Fertility, prolificacy, and fecundity.

The date of parturition, sex and weight of the offspring(s) at birth were recorded. For each treatment, the time at which each goat gave birth was determined; where the calving rate (PAR) was calculated as the number expressed as a percentage of goats that gave birth with respect to the total number of goats' diagnosed positive for gestation; after parturition. Fertility (FER) was calculated as the number of goats that gave birth divided by the number of goats mated, result multiplied by 100; fecundity (FEC) was the number of kids born divided by the total number of goats treated; and prolificacy (PROL) was the number of kids born divided by the number of goats that gave birth.

Statistical analysis

All data analyses were performed using statistical software (SAS, 2012). A complete design with randomized treatments was used; performing Shapiro & Wilk (1965) test to evaluate that all data came from a normally distributed population. Response to estrus synchronization, return to estrus, gestation, calving and fertility rates, and onset of estrus were analyzed by the Kruskal-Wallis nonparametric test using the NPAR1WAY procedure. To analyze the duration of estrus, fecundity and prolificacy, an analysis of variance was performed and Tukey's test was used to compare the means of their values among treatments at 95% confidence.

RESULTS AND DISCUSSION

No differences (p >0.05) between treatments were observed for the variables RE, ED, RET (Table 1), and for GEST, PAR and FER (Table 2); however, the action of the metabolic restorative during estrus synchronization improved (p <0.05) the response of Saanen x Alpina goats for the variables OE (Table 1), FEC and PROL (Table 2). The response to estrus synchronization was 100% in all three treatments. The response to estrus synchronization of goats treated with the isotonic solution was similar (p >0.05) to that of the control goats, but different from that obtained with the metabolic restorative, which shortened (p <0.05) the onset of estrus. After removing the CIDR from the 36 goats, 12 of them (33.33%) treated with the metabolic restorative started estrus at 20 h; whereas, the remaining 24 goats (66.67%) treated without metabolic restorative, started estrus at 32 h. For the goats treated with the isotonic solution, 25% showed estrus in the first 24 h, 67% showed estrus between 24 and 48 h, and the remaining 8% showed estrus before 64 h after withdrawal of the CIDR. The duration of estrus of the control goats was similar (p > 0.05) to that of the goats treated with the isotonic solution and the metabolic restorative (Table 1).



Table 1. Response to estrus synchronization in Saanen x Alpina goats treated with a metabolic restorative or isotonic solution

Treatments	n	Response to estrus (%)	Estrus onset (h)	Duration of estrus (h)	Return to estrus (%)
T1: Metabolic reconstituent	12	100	20.78 ^b	37.78	33.0
T2: Isotonic solution	12	100	32.54 ^a	37.95	33.0
T3: Control	12	100	33.68 ^a	37.69	33.0
p-value		0.96	0.001	0.76	0.90

^{a, b}. Mean values with different literals in the same column are different (p <0.05)

Table 2. Response to gestation diagnosis and number of offspring born to synchronized Saane	en x
Alpina goats treated with a metabolic restorative or isotonic solution	

Treatments		Goats			Fortility	Fecundity	
	n	Pregnant (n)	Calved (n)	Calves born (n)	(%)	(n)	Prolificacy (n)
T1: Metabolic reconstituent	12	8	8	17	66.7	1.42 ^b	2.1 ^b
T2: Isotonic solution	12	8	8	11	66.7	0.92 ^a	1.4 ^a
T3: Control	12	8	8	11	66.7	0.92 ^a	1.4 ^a
p-value					0.87	0.049	0.049

^{a,b}. Mean values with different literals in the same column are different (p < 0.05)

Currently, estrous synchronization protocols control follicular development and ovulation to improve the response in reproductive variables, mainly by reducing progesterone exposure from 10 to 14 days to a period of 5 to 7 days (short-term protocols; Luo et al., 2019). However, in the present study, the estrus synchronization protocol was designed to control luteal function by exogenously administering progesterone via CIDR for 12 d (long-term protocol), associated with a dose of eCG and PGF2α at the time of CIDR withdrawal, with which all goats in estrus were achieved. This response to estrus coincides with the 100% reported in some studies (Nogeira et al., 2011; Kajaysri & Thammakarn, 2012); but differs with other results (Menchaca et al., 2007; Montes-Quiroz et al., 2017), in goats synchronized with a similar hormonal treatment. The use of progestogens, eCG and prostaglandins offers good response in estrus synchronization (Abecia et al., 2012; Hashemi & Safdarian, 2017; Salleh et al., 2021), because PGF2a or its analogues have luteolytic function, modify ovarian follicle development and cause the dissolution of the corpus luteum to resume ovarian activity (Abecia et al., 2011; Rivas-Muñoz et al., 2021). It is possible to synchronize estrus through luteolysis by injecting PGF2a to induce regression of the functional corpus luteum to stop the luteal phase in goats synchronized with CIDR+eCG, because the presence of a functional corpus luteum inhibits the estrous response (Kajaysri & Thammakarn, 2012).



On the other hand, nutrition is considered to affect reproductive function in domestic ruminants, which influences the onset of ovarian activity in goats (Walkden-Brown *et al.*, 1994), where metabolic energy is the most important factor controlling reproductive success and gonadal hormones affect energy intake, storage and expenditure (Schneider *et al.*, 2012). Taking into account the energy requirements, the relationship between metabolic and reproductive regulatory systems, must be adjusted largely for the probability of success to be reasonable. Therefore, the regulatory processes linking nutrition and reproduction are the same that control energy homeostasis (Martin *et al.*, 2010).

Specific ingredients that make up the diet and products of metabolism may impair hypothalamic-adenohypophyseal-ovarian action by incurring FSH and LH secretion and ovarian activity response (Schneider *et al.*, 2012). Therefore, it is likely that the estrus response of goats in the present study was due more to the hormonal protocol used, as long-term (12 d) progestogen administration can be used with adjunctive treatments such as gonadotropins that promote ovarian follicular growth and ovulation, or prostaglandins that lyse an active *corpus luteum* (O'Brien & Wildeus, 2019). The duration of progestogen is shorter than the luteal phase; thus, estrus and ovulation may delay or inhibit the presence of a functional *corpus luteum* upon withdrawal of progestogen and prostaglandin is required to induce luteolysis (Swelum *et al.*, 2015).

Female reproductive efficiency depends on the ovarian response to hypothalamuspromoted pituitary secretions Meza-Herrera et al., 2010). This endocrine communication is enhanced by the activity of neurotransmitters in the hypothalamic-adenohypophysial axis, whose activity can be increased by supplementation of excitatory amino acids (EAA; Mahesh & Brann, 2005), which propitiate the release of gonadotropins from the adenohypophysis; and thus control physiological events in the gonads (Maia et al., 2017). Most of the available studies on the interactions between nutrition and reproduction in small ruminants are obtained under different environmental conditions, feed resources, available nutrients or supplementation sources that promote reproductive efficiency in goats (Rekik et al., 2007). Therefore, it is possible that some of the components of the metabolic restorative used in the present study, mainly the amino acids L-arginine (0.240 g), aspartic acid (0.150 g) and glutamic acid (0. 150 g) influence ovarian follicular development and generated an increase in the number of dominant follicles. It could produce greater estrogen secretion and consequently, reflected a shorter onset of estrus in response to the neurostimulatory action on the release of GnRH-LH. In addition, it has been reported that other amino acids contained in the metabolic restorative used in the present study, such as glutamine, proline, and glycine, regulate growth, development, lactation and participate in reproductive events (Wu, 2010); or in those related to fertility and neurotransmission (Wu, 2014).



In the present study a reduction in time to onset of estrus was observed in goats treated with the metabolic restorative, this response differs with the results of Pinheiro et al. (2012) who observed that the onset of estrus was significantly equal in goats treated with different dosage of insulin in estrus synchronization with progestogens and PGF2a. In goats and sheep, insulin reduces follicular atresia and increases the number of gonadotropindependent ovarian follicles; furthermore, it has been confirmed that it is possible to manipulate ovulation rate by stimulating the activity of ovarian modulators, such as IGF-I, through changes in the insulin-glucose system (Meza-Herrera et al., 2008). Hernández-Marín et al. (2016) reported in a study in sheep, that treatment with a metabolic restorative, similar to the one used in the present study, the content of L-arginine, aspartic acid and glutamic acid participate as neurostimulatory amino acids with endocrine action on ovarian activity. Besides, it is possible that in the present study the reduction in the onset of estrus in goats responded to the action of the neurostimulatory amino acids contained in the metabolic restorative. The response in reproductive activity in the female can be modified with energy or protein supplementation, either in the neuroendocrine pathways that depend on metabolic hormones or by circulating levels of FSH and LH (Meza-Herrera et al., 2014) or in those involved in GnRH secretion (Mahesh & Brann, 2005; Wu, 2010; Alvarez-Cardona et al., 2019).

Ruiz et al. (2002) synchronized estrus in goats with 325 mg cloprostenol with a protocol of 12 d duration and recorded the onset of estrus at 90.7±11.6 h; whereas, Khanthusaeng et al. (2013) recorded 38.5±1.5 h when synchronizing estrus in goats with CIDR for 14 d plus 300 IU of hCG at the time of CIDR withdrawal. These values differ with the onset of estrus obtained in the present investigation. Differences in the values for the onset of estrus are due to the duration of the hormonal protocol and the dose used, because the synchronization of estrus with intravaginal progesterone-releasing devices (CIDR) and gonadotropins increases the number of recruited follicles. It increases the maximum diameter and the growth rate of large follicles in the first wave of follicular growth (Uribe-Velásquez et al., 2008). On the contrary, when gonadotropins are administered in combination with prostaglandins, it allows longer activity time, responds with better recruitment and maturation of follicles and oocytes (Abecia et al., 2011). Estrus presentation varies from 24 to 96 h and depends on breed, age, reproductive season, presence of the buck and the types of reproductive management protocols to be used (Fatet et al. 2011). In accordance with animal welfare recommendations, pharmaceutical companies and advances in research have developed possible alternatives based on reducing the duration or dosage of hormone treatments (Abecia et al., 2011). Therefore, it is important to consider the appropriate dose for each reproductive purpose, with the objective of using the optimal, but efficient dose to achieve the herd's purposes.



The estrus duration obtained in the present investigation is longer than that reported by Khanthusaeng *et al.* (2013), who obtained 27.0 ± 1.2 h when synchronizing estrus in goats with CIDR for 14 d plus 300 IU of hCG at the time of CIDR withdrawal. CIDRs result in great estrus synchrony when associated with luteolytic agents; in addition to high fertility rates (Fatet *et al.*, 2011; Bukar *et al.*, 2012).

P₄ and its synthetic analogues are efficient in inducing and synchronizing estrus in goats, because the influence of gonadotropins on the ovaries stimulates follicular growth in cyclic or non-cyclic goats, and maximizes estrus occurrence rates (Omontese *et al.*, 2013). The results of the present study suggest that the duration of estrus was obtained on the one hand to the response of the hormonal protocol, by reducing the duration of the luteal phase of the estrous cycle. All this through the action of prostaglandin or by artificially controlling this phase, using intravaginal devices impregnated with P₄; because P₄ and progestogens, associated with gonadotropins and luteolytic agents, achieve results for the induction and synchronization of estrus (Abecia *et al.*, 2012). In this regard, the reported factors that affect the estrous response after applying PGF2α or its analogues are the dose used the interval between the administration of the estrous cycle, the year season and the combination with gonadotropins as hormonal treatment (Fierro *et al.*, 2013).

On the other hand, it is possible that the action of amino acids such as arginine, aspartate and glutamate, contained in the metabolic restorative, could have influenced the duration of estrus in the goats in the present study. It has been reported that methionine supply increases the liver's ability to transport triacylglycerol, which serves as an energy reserve; thus, administration of an energy restorative combined with oral propylene glycol improves reproductive outcomes by providing a glucose substrate (Jeong et al., 2018). Meza-Herrera et al. (2014) concluded that short-term administration of glutamate during the follicular phase increases the number of ovarian follicles with antrum and ovulatory rate in adult cyclic goats, indicating that glutamate is involved in the regulation of gonadotropic release and the ovulatory cycle of the female (Meza-Herrera et al., 2020). However, it is important to know the mechanism by which the profiles of these amino acids affect metabolites, metabolic and reproductive hormones that act as direct signals in ovarian follicles to regulate folliculogenesis and in response to the onset and duration of estrus. For example, an alternative mechanism in response to estrus synchronization is the immediate nutritional effect on ovarian follicular development. It involves direct nutritional actions at the ovarian level (Scaramuzzi et al., 2010); although the influence of short-term nutritional manipulation during the luteal phase on folliculogenesis might not be due to the variation of the amino acid profile at the intrafollicular level, but to the alteration of the amino acid profile in peripheral blood (Nie et al., 2018). Therefore, it is possible that at the ovarian level the amount of P₄ is not affected by energy intake or intake; but to an insufficient response to nutrition by the corpus luteum to secrete P4 required during the



luteal phase of the estrous cycle (Ying *et al.*, 2011). Thus, the incidence of an abnormal corpus luteum may be related to the endogenous P₄ capacity of goats to exhibit a short estrous cycle or in the duration of estrus.

Studies conducted on estrus synchronization in goats report values that differ with the results obtained in the present investigation in fertility (Uribe-Velásquez et al., 2011), fecundity (Hashemi & Safdarian, 2017) and prolificacy (Pérez-Clariget et al. (2012). Reproductive physiology in goats has been investigated extensively, including hypothalamic and adenohypophysial control of the ovary related to sexual behavior and the estrous cycle. In turn, commercial hormonal protocols were designed with the goal of controlling luteal function by administering exogenous progesterone for 10 to 14 days (long-term protocols; Luo et al., 2019). In this sense, it is possible that the duration for 12 d of the hormonal protocol used in the present study will have clustered the estrus and synchronized the timing of ovulation, rather than having influenced the fertility of the goats, because the hormonal treatment was the same for the three experimental treatments. It is possible to describe that the formation and development of ovarian follicles and ovulation generated with the hormonal protocols depend entirely on the combined effects of gonadotropin production and secretion. In addition, the selection of dominant ovarian follicles depends on the level of gonadotropin in blood and the expression of hormone receptors in the ovarian follicles (Graff et al., 2000).

In addition, several factors that influence reproductive response have been reported. Some of these suggest that higher fertility values are obtained with higher number of developed ovarian follicles (Barioglio *et al.*, 1997), by using controlled natural mating instead of artificial insemination (Martínez-Rojero *et al.* 2006), by using different hormonal protocol and using controlled natural mating (Pérez-Clariget *et al.*, 2012) or short hormonal protocols and fixed-time artificial insemination (Menchaca & Rubianes, 2007).

The use of hormonal protocols with CIDR or other progestogens alone or in combination with PGF2a and eCG, are equally efficient in synchronizing estrus in goats; however, although CIDRs contain a less potent hormone (P₄; Bukar *et al.*, 2012) the efficacy of these hormonal protocols can be improved with nutritional management strategies during estrus synchronization. In this regard, it is possible that the fecundity and prolificacy results obtained in the goats in the present study are due to the response of the metabolic restorative treatment during estrus synchronization.



Nutrition response is observed by supplementing the feed ration with neurostimulatory amino acids (Wu, 2010), energy (Schneider *et al.*, 2012), by offering trace elements (Vázquez-Armijo *et al.*, 2011); and that ovarian activity in goats is improved with the application of selenium (Se) individually or attached to amino acids such as cystine or methionine, as a less toxic form (Spallholz, 1994). Therefore, the role of nutrition in ovarian activity is due to alterations in the secretion or release of FSH and LH by the adenohypophysis, by a negative reaction at the ovarian level in response to gonadotropins, or by the same hormonal metabolic or reproductive axis regulation (Rodrigues *et al.*, 2015).

In contrast, even though treatment effects with the metabolic restorative used in the present study, due to its content in neurostimulatory amino acids, on ovarian activity has not been widely reported, particularly on ovarian follicular development and ovulatory rate, but its response on the secretion of some reproductive hormones. For example, arginine promotes LH release in prepubertal females, aspartate increases LH and testosterone release in males, glutamate stimulates the preovulatory LH peak, accelerates the onset of puberty and reproductive behavior (Mahesh & Brann, 2005). The response of some neurostimulatory amino acids have also been reported on reproductive efficiency in goats (Meza-Herrera *et al.*, 2008; Meza-Herrera *et al.*, 2010; Meza-Herrera *et al.*, 2014; Meza-Herrera *et al.*, 2020) and sheep (Hernández-Marín *et al.*, 2016; Fraire-Cordero *et al.*, 2018; Nie *et al.*, 2018). In addition, it is important to consider that this product is also used as a vitamin, bioenergetic, metabolic activator and regulator of protein, carbohydrate and lipid metabolism; therefore, it is possible to understand the action of some of its components and to describe their metabolic or reproductive response during estrus synchronization with CIDR, PGF2 α and eCG.

In addition to hormonal protocols in goats, treatments with oral glycerol during the first six days of embryonic growth enhance embryo survival has been reported, which increases the number of pregnant goats and prolificacy (Aguilar *et al.*, 2016). The maintenance of pregnancy in goats depends on the concentration of P₄ secreted by the corpus luteum, which in turn depends on the balance of luteotropic and luteolytic factors (LH and PGF2 α ; Ford *et al.*, 1996).



It is possible that the difference in fecundity and prolificacy of the goats treated with the metabolic restorative in the present study was due to the amount of usable nutrients, energy or neurostimulatory amino acids supplied during estrus synchronization. This responds to the fact that energy intake or body reserves for energy metabolism are related to the ovulatory rate in the female (Vázquez-Armijo et al., 2011). In this regard, the increasing ovulation rate and prolificacy in small ruminants has been practically achieved by hormonal treatments and by increasing energy intake in the diet (flushing; Aguilar et al., 2016). Therefore, there is a probability that in the present study the goats treated with the metabolic restorative during estrus synchronization, with the 12 d hormonal protocol, presented a hormonal balance of gonadotropins in the reproductive axis, maintained adequate P₄ secretion, and therefore improved the ovulatory rate although the mechanism of action of *flushing* is not fully understood yet. Evidence indicates that its effects occur at the ovarian level, are independent of gonadotropin concentrations, and are related to an increase in blood glucose and insulin concentration Dupont et al., 2014). Estienne et al. (1991) reported that neurostimulatory amino acids control LH secretion through mechanisms that regulate GnRH secretion from the hypothalamus. Thus, LH can reach the luteal tissue and regulate through an indirect effect, the secretion of P4 that possibly describes the hormonal regulation of the corpus luteum and its response in increasing the ovulatory rate and the number of offspring born.

Some factors that cause alterations in reproductive activity during estrus synchronization in goats can be controlled, and in turn evaluated to improve the response in the number of offspring born per goat treated. In this regard, it can be assumed that females with a higher number of offspring born in response to experimental treatment may have the potential to give multiple births in subsequent births. However, in goats, there is a correlation between goat age and prolificacy, thus females older than 2.6 years record prolificacy of 1.65 kids (Haldar et al., 2014). This value is lower than that obtained in the prolificacy of the goats in the present study even though the goats presented an average age of 2.35 years. In addition, other characteristics can also be considered to register differences in prolificacy values, such as reproductive season (Pinheiro et al., 2012), age and body condition (Haldar et al., 2014), duration and dose of hormonal treatments (Abecia et al., 2011), estrus synchronization protocol (Abecia et al., 2012), nutrition (Aguilar et al., 2016), environment (Dubeuf, 2011), among others. On the contrary, sodium chloride has an impact on hormonal control and energy balance in sheep; thus, high concentrations of sodium chloride can change the hormonal response in ewes, such as an alteration in the concentration of progesterone resulting in complicated lambing; which directly affects the reproductive capacity in sheep (Digby et al., 2011).



Based on the above, the knowledge of the action of the energetic and protein metabolites, as well as of the neurostimulatory amino acids, generate management options in a technical and economical way, capable of increasing the reproductive efficiency of a herd, with the advantage of controlling the reproductive events from natural non-hormonal methods, based on reproductive physiology and nutrition. Therefore, to describe the action of the components of the metabolic restorative and its response at the ovarian level and the variables evaluated in the synchronization of estrus in goats further research is suggested.

CONCLUSIONS

The action of a metabolic restorative reduces the onset of estrus, and increases fecundity and prolificacy without affecting the response, duration and return to estrus; as well as fertility in response to estrus synchronization in Saanen x Alpina goats.

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