

**EFFECT OF EXOGENOUS GLUTAMATE SUPPLY ON THE
ONSET OF PUBERTY IN GOATS: I. SERUM LEVELS OF INSULIN**

**[EFECTO DEL SUMINISTRO DE GLUTAMATO EXÓGENO SOBRE EL
INICIO DE LA PUBERTAD EN CABRAS: I. NIVELES SÉRICOS DE
INSULINA]**

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SUMMARY

Glutamate, the main neuroexcitatory amino acid of the central nervous system has a marked stimulatory effect on the reproductive axis in mammals. Precocious puberty occurs in response to glutamate administration in several mammals. The aim of this study was to evaluate the effect of exogenous glutamate supply upon the onset of puberty and possible links to changes in serum insulin levels in prepuberal goats. The study was carried out in northern Mexico at the Southern Goat Research Unit, URUZA-UACH (26° NL, 103° WL, 1,117 m altitude), from June to September. Three-month-old 7/8 Saanen-1/8 Criollo goats (n=18) were fed a diet formulated to met 120% of their nutritional requirements, adjusted for live weight (LW). Both LW and body condition score (BCS) were registered every 15 days prior to feeding. In June, goats were randomly allocated to two experimental groups: 1). Excitatory amino acids (AA, n=10; 16.52±1.04 kg, 3.4±0.12 BCS) and 2). Control, (CC, n=8; 16.1±1.04 kg, 3.1±0.12 BCS). The AA group received an intravenous infusion of 7 mg kg⁻¹ LW of L-glutamate, while the C group received saline. From mid-June to late September, blood samples were obtained from all goats once a week, to asses P₄, by RIA. Goats with serum P₄ levels ≥ 1 ng mL⁻¹ in two consecutive blood samples were considered reproductively active, (onset of puberty). Comparisons between groups for both LW and BCS were made using ANOVA-CRD. Percentage of goats depicting or not ovarian activity was tested with a Xi² analysis. The initial averages for LW and BCS were 16.65±1.04 kg, and 3.31±0.12 units, with no differences (P>0.05) between treatments. Goats in the AA group showed earlier (P<0.05) onset of puberty (6.9±0.8 vs. 7.5±1.0 moths of age) than control goats, and presented a greater (P<0.05) response in ovarian activity (70% vs

25%). The overall average for serum insulin levels (INS) was 1.2 ng mL⁻¹ with no differences between treatments (P>0.50). In addition, serum insulin concentrations were not associated with onset of puberty in the glutamate-supplemented-goats. Therefore, establishment of puberty in goats seems to involve an insulin-independent mechanism for regulating the hypothalamic-hypophyseal-ovarian axis function in peripuberal goats.

Key words: Goats, Glutamate, Progesterone, Puberty, Insulin.

INTRODUCTION

From a neurobiological perspective, LHRH release is critical for the onset of puberty in mammals. The large variability between individuals in the onset and progression of puberty indicates that timing of puberty not only depends on chronological age. Rather, the neurotransmitter and neuromodulatory systems that regulate GnRH secretion and release relays on information about metabolic fuels, body energy reserves and somatic development and, for many species, information about season and social environment (Teresawa and Fernández, 2001; Veldhuis et al., 2006). Clear links exist between metabolic fuel (glucose, pyruvate and lactate) availability and reproductive function (Cheung et al., 1997; Ebling, 2005). In fact, changes in blood levels of metabolic hormones are important signals that inform the nutritional status of mammals (Lindsay et al., 1993). An explanation is that the response to a feed supplementation alters glucose, insulin, leptin or IGF-I and probably others metabolic hormones (Muñoz-Gutierrez et al., 2005; Scaramuzzi et al., 2006).

The excitatory amino acid glutamate functions as the primary mediator of excitatory synaptic transmission in the CNS. Glutamate has also been implicated in a number of physiological processes (Brann 1995; Van den Pol et al., 1990; Rubio et al., 1997). Since glutamate receptors are localized in a variety of hypothalamic nuclei critical for reproduction and neuroendocrine function, it has been hypothesized that excitatory amino acids (EAAs) may play a decisive role in the control of puberty, pulsatility of reproductive hormones, the midcycle surge of gonadotropins, reproductive behavior and stress (Brann and Mahesh, 1997). The aim of this study was to evaluate the effect of administration of excitatory amino acid on the onset of puberty and of serum insulin concentrations in prepuberal goats.

MATERIALS AND METHODS

Experimental area, environmental conditions, animals and feeding

The study was carried out at the Southern Goat Research Unit, URUZA-UACH (26° NL, 103° WL, at 1,117 m). Three-month-old prepuberal crossbred goats (n=18; 7/8 Saanen-1/8 Criollo), were fed a diet to meet 120% of their nutritional requirements adjusted for BW. Both BW, body condition (BCS) were registered every 15 days prior to feeding. Goats were fed twice daily alfalfa hay (14% PC; 1.14 Mcal Kg⁻¹ ENm) in the morning, and corn silage (8.1% PC, 1.62 ENm Mcal kg⁻¹), and corn grain (11.2% PC, 2.38 ENm Mcal kg⁻¹) in the afternoon, under natural photoperiod (June to October).

Preparation of the infusion of L-glutamate and experimental design

A total of 4 g of L-glutamate were dissolved in 50 mL distilled water to get a final solution concentration of 80 mg of L-glutamate. In June, goats were randomly allocated to two experimental groups: 1). Excitatory amino acid (AA, n=10; 16.52±1.04 kg, 3.4±0.12 BCS) and 2). Control, (CC, n=8; 16.1±1.04 kg, 3.1±0.12 BCS). The AA group received an intravenous infusion of 7 mg kg⁻¹ BW of L-glutamate on Monday and Friday throughout the experimental period, while the CC group received saline infusion.

Blood sampling, progesterone quantification and onset of puberty

From mid-June to late October, blood samples (10 mL) were collected once weekly by jugular

venipuncture from all goats. Blood samples were centrifuged (1500 x g, 15 min) and serum was separated in duplicate and stored in propylen microtubes at 4°C until further hormonal analysis. Total samples collected were 200 (AA) and 160 (CC) for a total of 360 original serum samples. Peripheral serum progesterone concentrations were evaluated in blood serum by RIA (Diagnostic Products, Los Angeles, CA, USA). Goats with serum P₄ levels ≥ 1 ng mL⁻¹ in two consecutive samples were considered reproductively active, thus pubertal animals (Cushwa et al., 1992). The endocrine analyses were performed in the Department of Animal Science, New Mexico State University, USA.

Serum insulin quantification and statistical analyses

To evaluate serum insulin levels, blood samples were collected once per month throughout the experimental period. In total 6 samples per goat were collected, for a total of 108 serum samples, which were evaluated for their content of insulin by RIA (Sanson and Hallford, 1984). The intra- and inter-assay CV values were 4.26%, and 4.09% respectively, with a detection limit of 0.1 ng mL⁻¹. Both BW and BCS were compared considering an ANOVA-CRD, serum insulin levels across time were determined by split-plot ANOVA for repeated measures while percentage of goats depicting or not ovarian activity were compared with a chi-square test. All the analyses considered the procedures of SAS (1991).

RESULTS AND DISCUSSION

While the initial average for BW and BSC were 16.65±1.04 kg, and 3.31±0.12 units, respectively, no differences (P>0.05) between experimental groups were observed for these variables along the experimental period (Table 1). An earlier (P<0.05) onset of puberty as well as a greater (P<0.05) percentage of goats depicting ovarian activity were observed in the AA group compared to the C group (Table 1). The overall average for serum insulin was 1.26 ng mL⁻¹ with no differences between treatments (P>0.50) throughout the experimental period (Table 1). Moreover, serum insulin concentrations were not associated with the onset of puberty in the glutamate treated goats. Therefore, onset of puberty seems to involve an insulin-independent mechanism for regulating the hypothalamic-hypophyseal-ovarian axis function in juvenile goats.

Table 1. Least Squares means for body weight (BW, kg), body condition score (BCS, units), percentage of cycling goats (puberty %), and serum insulin concentrations (INS, ng mL⁻¹), in juvenile crossbred goats under natural photoperiod in northern Mexico.

Variables	Glutamate	Control	SE ¹
BW1	16.66 ^a	15.96 ^a	0.936
BCS1	3.42 ^a	3.15 ^a	0.111
BW4	23.75 ^a	22.76 ^a	0.720
BCS4	3.69 ^a	3.38 ^a	0.108
Cycling goats (%)	70.0 ^a	25.0 ^b	46.00
INS ng mL ⁻¹	1.342 ^a	1.182 ^a	0.017

^{ab} Means in the same row with different superscript differ (P<0.05)

¹ SE, standard error of least square means

We hypothesized that administration of glutamate would stimulate the hypothalamic-hypophysial-gonadal axis, leading to an earlier onset of puberty, which would be the result of an increase in serum insulin levels; this hypothesis is partially accepted. The supplementation of excitatory amino acids plays a critical role in the onset of puberty (Urbanski and Ojeda, 1990). These results show that the onset of puberty in goats does not appear to be affected by body weight or body condition score, and disagree with some reports in which puberty was closely related to BW and body fat deposition in mammals (Schneider et al., 2000). Other studies, however, demonstrated that puberty might not occur even with increased levels in body fat and serum leptin (Glass et al., 1979; Bronson, 1987; Hopper et al., 1993; Bronson, 2001). In addition, in animals treated with N-methyl-D-aspartate, an agonist of glutamate, it was observed and increase in LH secretion in a variety of species such as monkeys, sheep and rodent (Plant and Barker-Gibb, 2004).

According to Marshall et al. (1991), glutamate can act as a positive neuromodulator in the response of certain tissues to insulin; therefore, low levels of insulin in the presence of glutamate can potentiate the effect of insulin in several tissues. In addition, glutamate can also regulate metabolic fuels as well as cellular permeability (Hediger and Welbourne, 1999). Therefore, although serum insulin concentrations were not affected by the administration of glutamate, it is possible that the onset of puberty could be controlled by other endocrine system, such as leptin, IGF-I or thyroid hormones. This study clearly demonstrates that exogenous administration of L-glutamate generated an earlier onset of puberty in goats, and that such physiologic scenario seems to involve an insulin-independent mechanism for regulating the hypothalamic-hypophysial-ovarian axis function in peripuberal goats.

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