

El suplemento alimenticio mejora las tasas de ovulación y gestación en las cabras manejadas en condiciones extensivas y expuestas al efecto macho

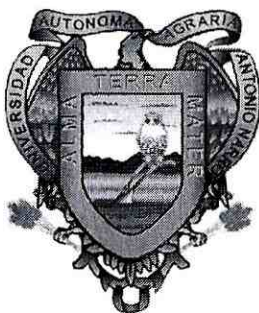
GONZALO FITZ RODRÍGUEZ

TESIS

PRESENTADA COMO REQUISITO PARCIAL

PARA OBTENER EL GRADO DE

DOCTOR EN CIENCIAS AGRARIAS



UNIVERSIDAD AUTÓNOMA AGRARIA

ANTONIO NARRO

UNIDAD LAGUNA

DIRECCIÓN DE POSGRADO

Director de tesis: Dr. José Alberto Delgadillo Sánchez

Torreón, Coahuila, México

FEBRERO 2009

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Elaborada bajo la supervisión del comité particular de asesoría y aprobada como requisito parcial para optar por el grado de:

DOCTOR EN CIENCIAS AGRARIAS

COMITÉ PARTICULAR

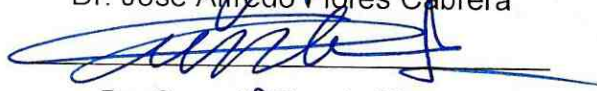
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DEDICATORIA

Con mucho cariño para: Rosy, Gonzalo, Airy Michelle y mi gran familia.

De manera especial para el Dr. Efrén Fitz Rodríguez, quien me apoyo y aconsejo durante este proyecto.

AGRADECIMIENTOS

Mi agradecimiento especial al Dr. José Alberto Delgadillo Sánchez, quien guió mi camino por el fascinante mundo de la reproducción caprina y que además durante mi estancia en Postgrado me invito a conocer personas y lugares interesantes, quienes me han brindado su apoyo y compartido de sus experiencias en esta área del conocimiento. Del INRA-Francia los doctores: Pascal Poindron, Benoît Malpoux, Bernard Leboeuf. De la Facultad de Veterinaria en Montevideo Uruguay Rodolfo Ungerfeld y su equipo de trabajo. Al equipo de casa los doctores: Gerardo Duarte, Alfredo Flores, Jesús Vielma, Horacio Hernández y a los estudiantes de la UAAAN-UL de antes y ahora que han colaborado conmigo. A la Universidad Autónoma Agraria Antonio Narro y al Departamento de Postgrado de la Unidad Laguna por las facilidades otorgadas para la realización de los estudios de Doctorado y al Consejo Nacional de Ciencia y Tecnología (CONACyt) por el financiamiento otorgado para la realización de este proyecto.

Muchas Muchas Muchas Gracias...

“Las cosas simples son las más extraordinarias y solo los conocedores
consiguen verlas”

Compendio

El suplemento alimenticio mejora las tasas de ovulación y gestación en las cabras manejadas en condiciones extensivas y expuestas al efecto macho

POR

GONZALO FITZ RODRÍGUEZ

DOCTOR EN CIENCIAS AGRARIAS

UNIVERSIDAD AUTÓNOMA AGRARIA ANTONIO NARRO

Unidad Laguna

Director de Tesis: Dr. José Alberto Delgadillo Sánchez

Torreón, Coahuila, México, Febrero de 2009

En las últimas décadas, la caprinocultura se ha convertido en la fuerza transformadora social y económica de las comunidades rurales alrededor del mundo. Los productos y subproductos caprinos han contribuido con la economía de las comunidades rurales y se ha desarrollado la producción, industrialización, y mercadeo de los productos caprinos. Dada su importancia, las instituciones gubernamentales y educativas, así como las asociaciones de caprinocultores, han promovido que se genere investigación e innovación tecnológica que permitan que los animales produzcan y se reproduzcan fuera de la estación natural de reproducción. En efecto, la producción caprina

depende de la eficiencia reproductiva de los animales (Martin y Kadokawa, 2006). Por ello, la estacionalidad reproductiva que manifiestan algunas razas caprinas es una limitante de su producción (Martin y Kadokawa, 2006; Duarte *et al.*, 2008). La inducción de la ovulación de las hembras durante el periodo de anestro estacional es primordial para evitar la estacionalidad de la producción y poner a la disponibilidad de los consumidores los productos caprinos durante todo el año. La inducción de la actividad sexual de las hembras durante el anestro estacional puede hacerse mediante el efecto macho (Ungerfeld *et al.*, 2004; Pellicer-Rubio *et al.*, 2008). Esta técnica es simple de utilizar, ya que el estro y la ovulación se estimulan al poner las hembras en contacto con los machos (Walkden-Brown *et al.*, 1999; Delgadillo *et al.*, 2006).

En las latitudes subtropicales en general, y en la Comarca Lagunera en el norte de México en particular, la mayoría de los caprinos son manejados en condiciones extensivas y sometidos a variaciones importantes de la disponibilidad alimenticia, lo que disminuye considerablemente la respuesta estral y ovulatoria de las hembras sometidas al efecto macho (Mellado y Hernández, 1996). En efecto, más del 80% de las cabras locales de la Comarca Lagunera manejadas de manera intensiva y bien alimentadas, manifiestan un estro y ovulan al ser expuestas a los machos, mientras que menos del 30% lo hacen cuando están en condiciones extensivas y subalimentadas (Flores *et al.*, 2000; Mellado *et al.*, 2000). La prolificidad también es afectada por el nivel de alimentación de las hembras, la cual se asocia generalmente al sistema de explotación. En las hembras bien alimentadas, la prolificidad es de 2.0 y en las subalimentadas de 1.6 (Fitz-Rodríguez, 2004). La diferencia en la prolificidad

puede estar relacionada con una tasa ovulatoria inferior, o con un incremento en la mortalidad embrionaria provocadas por la subalimentación de las hembras explotadas en condiciones extensivas (Atti *et al.*, 2004; Martin *et al.*, 2004a). Sin embargo, en las hembras subalimentadas, un suplemento alimenticio puede incrementar las tasas ovulatoria y de gestación (Atti *et al.*, 2004; Martin *et al.*, 2004b; Rhind, 2004). En efecto, un complemento alimenticio 7 días antes de exponer las cabras al efecto macho incrementa la tasa ovulatoria (1.6 ± 0.2) cuando se compara con las hembras no complementadas (1.0 ± 0.2 ; De Santiago-Miramontes *et al.*, 2008). Sin embargo, este incremento de la tasa ovulatoria ocurre solamente en la primera ovulación inducida por el macho, la cual es seguida de un ciclo ovulatorio de corta duración, por lo que las hembras no pueden quedar gestantes (Chemineau *et al.*, 2006; De Santiago-Miramontes *et al.*, 2008). La subnutrición provoca también una pérdida embrionaria en los primeros 30 días de gestación, probablemente por cambios en el ambiente uterino (Mani *et al.*, 1992; Abecia *et al.*, 2006). El efecto negativo de la subalimentación sobre la gestación puede atenuarse con un complemento alimenticio (Rassu *et al.*, 2004). Sin embargo, en caprinos no se ha determinado con precisión el momento y la duración del suplemento alimenticio en las hembras expuestas al efecto macho para incrementar las tasa de gestación al ser expuestas a los machos (Martin *et al.*, 2004b).

Se efectuaron dos estudios para determinar si un suplemento alimenticio incrementa las tasas de ovulación y de gestación en las hembras mantenidas en condiciones extensivas y expuestas al efecto macho.

Estudio 1. El objetivo de este estudio fue determinar si un suplemento alimenticio de 7 días al momento de exponer las hembras a los machos sexualmente activos incrementa la tasa ovulatoria en la segunda ovulación en las cabras manejadas de manera extensiva. Se utilizaron cabras multíparas anovulatorias las cuales se dividieron en dos grupos homogéneos (n=27 cada uno) de acuerdo a su peso y condición corporal. Ambos grupos fueron manejados en condiciones extensivas y pastaban de 0900 a 1800. Durante la noche las hembras se alojaron en corrales abiertos con los machos (n=2 por grupo). Un grupo no recibió suplemento alimenticio (grupo control), mientras que a cada hembra del otro grupo (suplementado) se le ofreció un suplemento alimenticio diariamente que consistió en 260 g de maíz roado, 110 g de pasta de soya y 900 g de heno de alfalfa durante 7 días. El suplemento alimenticio se ofreció a las 0800 (50% antes del pastoreo) y 1800 (50% después del pastoreo) a partir de la introducción de los machos (24 de marzo) inducidos a una intensa actividad sexual al someterlos a 2.5 meses de días largos a partir del 1 de noviembre. La tasa ovulatoria se determinó por la presencia de los cuerpos lúteos mediante ultrasonografía transrectal los días 7 y 19 después de la exposición a los machos. La conducta estral se registró dos veces al día durante todo el estudio. La tasa ovulatoria en los primeros 5 días después de la introducción de los machos no fue diferente ($P>0.05$) entre el grupo suplementado (1.2 ± 0.1) y el grupo control (1.1 ± 0.1). En cambio, en la segunda ovulación inducida por el macho ocurrida entre los días 6 y 14 después de la introducción de los machos la tasa ovulatoria fue superior ($P<0.05$) en las hembras suplementadas (2 ± 0.1) que en el grupo control (1.6 ± 0.1). Los

porcentajes de estros diarios y acumulados no fueron diferentes ($P>0.05$) entre los grupos. Estos resultados demuestran que 7 días de suplemento alimenticio iniciando al momento de la introducción de los machos incrementa la tasa ovulatoria en la segunda ovulación inducida por el macho en hembras caprinas expuestas al efecto macho bajo condiciones extensivas.

Estudio 2. El objetivo de este estudio fue determinar si un suplemento alimenticio después del segundo estro inducido por el macho mejora la tasa de gestación de las cabras sometidas al efecto macho y manejadas de manera extensiva. Se utilizaron cabras multíparas anovulatorias que se dividieron en 4 grupos homogéneos ($n=23$ cada uno) de acuerdo a su peso y condición corporal. Los grupos se manejaron en condiciones extensivas y pastaban de 0900 a 1800. Durante la noche las hembras se alojaron en corrales abiertos con los machos ($n=2$ por grupo). Un grupo no recibió ningún suplemento alimenticio (grupo control), mientras que a cada hembra de los otros 3 grupos se les ofreció un suplemento alimenticio que consistió en 1100 g de heno de alfalfa, 150 g de maíz roado y 100 g de pasta de soya por un periodo de 7, 14 y 28 días iniciando 9 días después de la introducción de los machos. El suplemento alimenticio se ofreció individualmente a las 0800. El 6 de abril, las hembras se expusieron a los machos sexualmente activos ($n=2$ por grupo). La actividad estral se registró dos veces al día durante 15 días. La tasa de gestación se determinó por ultrasonografía transrectal 45 días después de detectado el segundo estro inducido por el macho. Ninguna diferencia ($P>0.05$) existió entre los grupos referente a los porcentajes de hembras detectadas en estro durante

el estudio, ni en el intervalo entre la introducción de los machos en los grupos de hembras y el inicio de la actividad estral. La proporción de hembras gestantes en el grupo suplementado durante 28 días fue superior (82.6%; $P < 0.05$) que en el grupo no suplementado (52.1%), pero no difirió ($P > 0.05$) del grupo suplementado durante 14 días (86.9%), y existió una tendencia ($P = 0.10$) a ser superior que en el grupo suplementado durante 7 días (65.2%). La proporción de hembras gestantes en el grupo suplementado durante 14 días fue superior ($P < 0.05$) que las proporciones registradas en los grupos suplementado durante 7 días y en el no suplementado. No hubo diferencia ($P > 0.05$) entre el grupos suplementado por 7 días y el no suplementado. Estos resultados demuestran que un suplemento alimenticio durante 14 ó 28 días después del segundo estro inducido por macho, incrementa la tasa de gestación en cabras expuestas al efecto macho bajo condiciones extensivas.

Palabras clave: eficiencia reproductiva, bioestimulación, nutrición, prolificidad, fertilidad

Summary

Nutritional supplementation improves ovulation and pregnancy rates in female goats managed under natural grazing conditions and exposed to the male effect

By

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Torreón, Coahuila, México, February 2009

In the last decades, goat production has become a social and economic activity of rural communities around the world. Goat products and sub-products contribute to the economy of rural communities. The production, industrialization, and marketing of goat products have been further developed. Given their importance, government agencies, educational institutions and the associations of goat producers have promoted the development of technological innovation and funded research that stimulates the production of goat products and reproduction of goats outside the natural breeding season. Indeed, goat production depends on the reproductive efficiency of the animal (Martin and

Kadokawa, 2006). For this reason, the reproductive seasonality of some breeds is an obstacle for their production performance (Martin and Kadokawa, 2006; Duarte et al., 2008). Induction of anoestrus in the females during the seasonal anoestrus is fundamental to avoid the seasonality of production and to make available goat products throughout the year. The induction of the sexual activity of does during seasonal anoestrus can be achieved with the male effect (Ungerfeld *et al.*, 2004; Pellicer-Rubio *et al.*, 2008). This technique is simple to use, since estrus and ovulation are stimulated when the females are exposed to the males (Walkden-Brown *et al.*, 1999; Delgadillo *et al.*, 2006). In subtropical latitudes generally, and in particular in the La Laguna Region in the northern part of Mexico, most of the goat production is managed under extensive conditions and subjected to considerable variations in the nutritional content of available food, which considerably diminishes the estral and ovulatory responses of the females exposed to the male effect (Mellado and Hernandez, 1996). Indeed, more than 80% of the goats produced locally in the Laguna Region under intensive condition and well fed, show an estrous and ovulate when exposed to the males. In contrast only 30% of females show this response under extensive production and with limited food (Flores *et al.*, 2000; Mellado *et al.*, 2000). Prolificacy is also affected by the feeding level of the females, which is associated generally with the management system. Prolificacy in well fed females results in the production of 2.0 kids per female *versus* 1.6 kids in undernourished females (Fitz-Rodriguez, 2004). This difference of prolificacy can be related to a lower ovulation rate, or an increased embryonic mortality, which resulting from the under-nourishment of the females under extensive

conditions (Atti *et al.*, 2004; Martin *et al.*, 2004a). Nevertheless, in undernourished females, nutritional supplementation can increase the ovulation and gestation rates (Atti *et al.*, 2004; Martin *et al.*, 2004b; Rhind, 2004). Indeed, a nutritional complement given 7 days before the goats are exposed to the male effect increases the ovulation rate ($1,6 \pm 0,2$) when compared to non-supplemented females ($1,0 \pm 0,2$; De Santiago-Miramontes *et al.*, 2008). However, this increased of in the ovulation rate happens only at the first ovulation induced by the male, which is followed by a short ovulatory cycle, and as a result the females cannot get pregnant (Chemineau *et al.*, 2006; De Santiago-Miramontes *et al.*, 2008). Sub-nutrition also causes embryonic losses during the first 30 days of gestation, probably due to the changes in the uterine environment (Mani *et al.*, 1992; Abecia *et al.*, 2006). The negative effect of under-nutrition on gestation can be attenuated with a nutritional supplementation (Rassu *et al.*, 2004). Nevertheless, the precise moment and the duration of nutritional supplementation of females exposed to the male effect to increase gestation rate has not been determined (Martin *et al.*, 2004b). Two studies were conducted to determine if nutritional supplementation increases ovulation and gestation rates in female goats maintained in extensive conditions and exposed to the male effect.

Experiment 1. The objective of our first study was to determine if nutritional supplementation for 7 days starting at the introduction of the male increased ovulation rate at the second male-induced ovulation in female goats managed under natural grazing conditions. Multiparous anovulatory female goats were

divided in two homogenous groups (n=27 each) according to their weight and corporal condition. Both groups were handled in extensive conditions and grazed from 0900 to 1800. During the night, the females were kept in open pens with the males (n=2 by group). One group did not receive feed supplementation (non-supplemented group), the other group grazing natural vegetation and received in addition daily supplementation with 260 g of rolled corn, 110 g soy bean and 900 g of alfalfa hay per animal (supplemented group). The nutritional supplementation started on the day the males were introduced (March 24th) and continued for 7 days. The supplement was offered individually twice a day at 0800 (50% before grazing) and at 1800 (50% after grazing). Males were treated with 2.5 months of long days (16h of light by day) from November 1 to stimulate their sexual activity in non-breeding season. The ovulation rate was estimated by the number of corpora lutea detected by transrectal ultrasonography. All females were assessed twice, at days 7 and 19 after introduction of the bucks. The ovulation rate in the first 5 days after the introduction of the males was not different ($P > 0.05$) between the supplemented group (1.2 ± 0.1) and non-supplemented (1.1 ± 0.1). However at the second male-induced ovulation that was detected between days 6 and 14 after male introduction ovulation rate was greater ($P < 0.05$) in supplemented (2.0 ± 0.1) than in non-supplemented (1.6 ± 0.1) females. These results demonstrate that nutritional supplementation of anestrus does for 7 days starting at the time when sexually active males were introduced to them, increased ovulation rate at the second male-induced ovulation under grazing conditions.

Experiment 2. The objective of the second study was to determine if nutritional supplementation starting after the second ovulation induced by the male would improve gestation rate in does exposed to males under grazing conditions. Anovulatory multiparous female goats were used; does were divided into four groups (n = 23 each) balanced for body condition score (1.7 ± 0.6) and body weight (37 ± 0.4 kg). One group did not receive feed supplementation (non-supplemented group), while the other three groups were supplemented daily with a mixture of 1100 g of alfalfa hay (18% CP), 150 g of rolled corn (8.6% CP) and 100 g of soy bean (49% CP) per animal for periods of 7, 14 and 28 days starting the day after the second ovulation induced by the male (starting 9 days after exposure to males). The nutritional supplementation was offered individually at 0800. The 6th of April, the females were exposed to the sexually active males (n=2 by group). Estrous behavior was recorded twice daily throughout the experiment. Pregnancy was determinate by transrectal ultrasonography, 45 days after the second male-induced ovulation. Percentages of does displaying estrus behavior after 15 days of exposure to bucks was similar ($P > 0.05$) among groups. The interval between the day of introduction of the males and the onset of estrous behavior was similar ($P > 0.05$) between groups. The proportion of females having shorter than typical-length estrous cycles (<17 days) and the duration of these short estrous cycles did not differ ($P > 0.05$) between groups. The proportion of pregnant does in the group supplemented for 28 days was greater ($P < 0.05$) than in the non-supplemented group, but did not differ ($P > 0.05$) from the group supplemented fed for 14 days, while it tended to be greater ($P = 0.10$) than in the 7-days supplemented group.

The proportion of pregnant does in the 14-day supplemented group was greater ($P < 0.05$) than in the 7-days supplemented and the non supplemented groups but these two later groups did not differ ($P > 0.05$). These results demonstrate that nutritional supplementation for 14 or 28 days starting at the time of the second-male induced ovulation improve gestation rate in goats exposed to the male effect under grazing conditions.

Key words: reproductive efficiency, biostimulation, nutrition, prolificacy, fertility,

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1. Introducción

En los últimos años, la caprinocultura se ha convertido en una alternativa de bienestar en las comunidades rurales al producir alimento de alto valor nutricional en condiciones en las que otras especies no podrían subsistir. Debido a ello se ha promovido la investigación e innovación tecnológica que permitan una mayor productividad de los hatos caprinos. Efectivamente, en años recientes se han incrementado las publicaciones científicas sobre la inducción de la actividad sexual de las hembras de pequeños rumiantes que manifiestan una estacionalidad reproductiva utilizando el efecto macho (Delgadillo *et al.*, 2003; Delgadillo *et al.*, 2006; Ungerfeld 2007; Pellicer-Rubio *et al.*, 2008). Esta técnica permite inducir el estro y la ovulación de las hembras al ponerlas en contacto con los machos. Sin embargo, la subalimentación disminuye considerablemente la respuesta estral y ovulatoria de las hembras expuestas a los machos, así como la fertilidad y prolificidad (Mellado y Hernández, 1996). Esto sugiere que la reproducción, y por ende la producción de las cabras, depende de una interacción entre la estacionalidad de las hembras, las relaciones socio-sexuales y la nutrición. Por ello se realizaron dos estudios para determinar si un suplemento alimenticio al momento de exponer las hembras a los machos incrementa la tasa ovulatoria en la segunda ovulación inducida por el macho, y si un suplemento alimenticio después del segundo estro detectado mejora la tasa de gestación de las cabras sometidas al efecto macho y explotadas de manera extensiva.

2. Revisión de literatura

1. Estacionalidad reproductiva en ovinos y caprinos

La estacionalidad reproductiva de las especies es una estrategia que permite que los partos ocurran en el momento más óptimo del año para favorecer la sobrevivencia de las crías (Ortavant *et al.*, 1985). En las hembras de razas ovinas y caprinas originarias de zonas templadas, y en algunas originarias o adaptadas a zonas subtropicales, la estación sexual se desarrolla durante el otoño y el invierno (Karsch *et al.*, 1984; Restall, 1992; Amoah *et al.*, 1996; Rivera *et al.*, 2003; Duarte *et al.*, 2008). En las hembras el periodo de anestro está asociado con la ausencia de estros y ovulaciones. Por el contrario, la estación sexual se caracteriza por la sucesión de ciclos estrales y ováricos de 21 ± 3 días de duración. En las cabras del subtrópico mexicano (26° N), por ejemplo, el anestro ocurre en primavera-verano y el periodo de actividad sexual se observa en otoño-invierno (Duarte *et al.*, 2008). En los machos originarios de zonas templadas, la estación sexual se desarrolla en el otoño-invierno (Lincoln y Short, 1980; Ortavant *et al.*, 1985), mientras que en los machos de latitudes subtropicales, ésta ocurre en primavera y verano (Delgadillo *et al.*, 1999). En ambos sexos, la estacionalidad reproductiva es controlada por las variaciones de la duración del día (el fotoperiodo), el cual sincroniza un ritmo endógeno de reproducción (Thiéry *et al.*, 2002; Malpoux, 2006). En condiciones artificiales los días cortos estimulan la actividad sexual, y los días largos la inhiben (Lincoln y Short, 1980; Delgadillo *et al.*, 1991; 1992; 2004; Lincoln y Short, 1980).

2. Efecto macho

La estacionalidad reproductiva es una limitante para la producción ovina y caprina, porque ésta se vuelve también estacional. Este patrón reproductivo estacional provoca que se concentre la producción en algunos meses del año, provocando variaciones en los precios de la leche y la carne (Chemineau *et al.*, 2007). Existen diferentes técnicas para inducir la actividad sexual de las hembras caprinas y ovinas durante los periodos de anestro. Una de ellas es el uso combinado de hormonas exógenas como el acetato de flurogestona (FGA) o el acetato de medroxiprogesterona (MAP), y la gonadotropina coriónica equina (eCG; Wildeus, 2000; Whitley y Jackson, 2004; Fonseca *et al.*, 2005; López-Sebastian *et al.*, 2007; Husein y Ababneh 2008; Karaca *et al.*, 2008). La melatonina también permite inducir el estro y la ovulación durante el periodo de anestro (Chemineau *et al.*, 1988; Chemineau *et al.*, 1992; Chemineau *et al.*, 1996; Abecia *et al.*, 2007). Otra alternativa es la manipulación de las relaciones socio-sexuales para estimular la actividad sexual durante el anestro, particularmente el efecto macho (Delgadillo *et al.*, 2002; Ungerfeld *et al.*, 2004; Delgadillo *et al.*, 2006; Ungerfeld, 2007; Delgadillo *et al.*, 2008). Esta técnica ha sido ampliamente descrita en diferentes especies como las cabras (Ott *et al.*, 1980; Chemineau, 1987; Delgadillo *et al.*, 2006; Pellicer-Rubio *et al.*, 2008), las ovejas (Martin *et al.*, 1986; Rosa y Bryant, 2002; Ungerfeld *et al.*, 2004), las gacelas (Skinner *et al.*, 2002), las vacas (Chenowet, 1983) y las cerdas (Kemp *et al.*, 2005).

El evento endocrino que ocurre inmediatamente después de exponer las hembras ovinas y caprinas a los machos es un aumento en la pulsatilidad de la hormona luteinizante (LH) de las hembras, secreción que culmina con el pico preovulatorio de LH y la ovulación en los primeros 3-5 días (Martin *et al.*, 1986; Vielma, 2006; Ichimaru *et al.*, 2008). La mayoría de las hembras que son estimuladas mediante el efecto macho presentan un ciclo ovárico de corta duración, que en promedio dura de 5 a 7 días (Chemineau *et al.*, 2006). En las cabras, después de este ciclo corto, se produce otra ovulación que se acompaña en un 95% de ellas de un estro seguido de una fase lútea de duración normal. En esta segunda ovulación inducida por el macho, las hembras pueden quedar gestantes (Chemineau, 1987; Flores *et al.*, 2000).

2.1 Factores que afectan la respuesta sexual de las hembras al efecto macho

2.1.1 Machos

2.1.1.1 Intensidad del comportamiento sexual

La libido es descrita comúnmente como el conjunto de conductas sexuales mostradas por los machos, es decir, la disposición y habilidad de éste para cortejar y montar a la hembra (Chenoweth, 1981). En el caso de los machos cabríos, la conducta sexual está representada por elementos motores del comportamiento sexual como el automarcaje, los olfateos ano-genitales, el

flehmen, las aproximaciones, los intentos de monta y las montas con penetración (Price *et al.*, 1986; Fabre-Nys, 2000). Perkins y Fitzgerald (1994) exploraron la idea de que la intensidad de la conducta sexual desplegada por los machos hacia las hembras, incrementa la intensidad del estímulo y consecuentemente mejora la respuesta estral y ovulatoria de éstas. Estos autores compararon machos que exhibían altos y bajos niveles de conducta sexual y encontraron que los machos con alta actividad inducen un mayor número de hembras al estro (95%) que los machos con baja libido (78%). Estudios realizados con machos cabríos inducidos a una intensa actividad sexual durante el periodo de reposo, al someterlos a 2.5 meses de días largos, demuestran que estos machos estimulan la actividad sexual de un mayor número de hembras anéstricas que los machos no tratados (Flores *et al.*, 2000; Delgadillo *et al.*, 2002; Fitz-Rodríguez, 2004). En efecto, en un grupo de hembras en anestro, el 95% respondieron a la introducción de machos sexualmente activos, contra el 10% registrado en las hembras expuestas a machos en reposo sexual (Delgadillo *et al.*, 2002). Estos resultados demuestran que la intensidad de la conducta sexual desplegada por el macho es un factor importante en la efectividad del efecto macho.

2.1.1.2 Nivel de alimentación: machos bien alimentados y subalimentados

En las zonas subtropicales, la nutrición es un regulador importante en la función reproductiva de algunas razas de ovinos y caprinos originarias o

adaptadas a estas latitudes (Blache *et al.*, 2000). En los machos cabríos, la subalimentación puede reducir la libido, el olor, el volumen del eyaculado, el número de espermatozoides por eyaculado, el porcentaje de espermatozoides vivos, y la motilidad espermática (Walkden-Brown y Restall, 1996). Una buena nutrición puede inducir una rápida respuesta endocrina y testicular, por lo que una sobrealimentación de 6 semanas antes de la monta, mejora las variables antes mencionadas, adelantando el inicio de la estación sexual en los machos cashmere Australianos (Walkden-Brown *et al.*, 1994; Martin y Walkden-Brown, 1995). Asimismo, los machos alimentados con una dieta de alta calidad durante 16 meses inducen, a través del efecto macho, la ovulación en un mayor número de hembras, que los machos expuestos a dietas de baja calidad durante el mismo tiempo (Walkden-Brown *et al.*, 1993). Estos resultados indican que el nivel de alimentación de los machos influye en la eficiencia de éstos para estimular la actividad sexual de las hembras a través del efecto macho.

2.2.2 Hembras

2.2.2.1 Nivel de alimentación: hembras bien alimentadas y subalimentadas

La nutrición juega también un papel importante en la respuesta sexual de las hembras expuestas al efecto macho. La proporción de hembras que despliegan una conducta estral y ovulatoria en respuesta a los machos es más

alta en hembras bien alimentadas que las que están subalimentadas (Khaldi, 1984; Henniawati y Fletcher, 1986; Wright *et al.*, 1990; Kusina *et al.*, 2001). El intervalo entre la introducción de los machos y el inicio de la actividad estral, es más prolongado en las hembras subalimentadas (5 días), que tienen una baja condición corporal, que en las hembras bien alimentadas, que tienen una alta condición corporal (2 días; Mellado *et al.*, 1994). La subalimentación también afecta la tasa ovulatoria de las hembras expuestas al efecto macho. Lassoued *et al.* (2004) reportaron que las ovejas D'Man con un mejor nivel de alimentación antes del contacto con los machos, presentan una mayor tasa de ovulación (2.3) que las sometidas a un menor nivel alimenticio (1.8).

2.2.2.2 El suplemento alimenticio y la respuesta estral y ovulatoria de las hembras expuestas al efecto macho

Los sistemas de producción pecuaria han implementado varias estrategias de suplemento alimenticio para maximizar el rendimiento reproductivo de los hatos. El flushing, es decir, el aporte de un suplemento alto en energía y proteína por un periodo corto mejora la respuesta sexual y reproductiva de las hembras expuestas al efecto macho. Existen tres efectos del suplemento alimenticio sobre el peso corporal y la tasa ovulatoria (Figura 1). En el efecto "agudo" se observa un incremento de la tasa ovulatoria en ausencia de un cambio detectable en el peso corporal. En el efecto "dinámico"

se observa un incremento en la tasa ovulatoria acompañado de un incremento en el peso corporal. En el efecto "estático" no se produce un de la tasa ovulatoria ni de peso corporal, porque las hembras tienen un alto peso corporal (Scaramuzzi *et al.*, 2006; Goodman y Inskeep, 2006). Aunque los efectos benéficos de un suplemento alimenticio son principalmente sobre la tasa de ovulación, éste también mejora la respuesta estral y la tasa de gestación en las hembras expuestas al efecto macho (McWilliam *et al.*, 2004; De Santiago-Miramontes *et al.*, 2008).

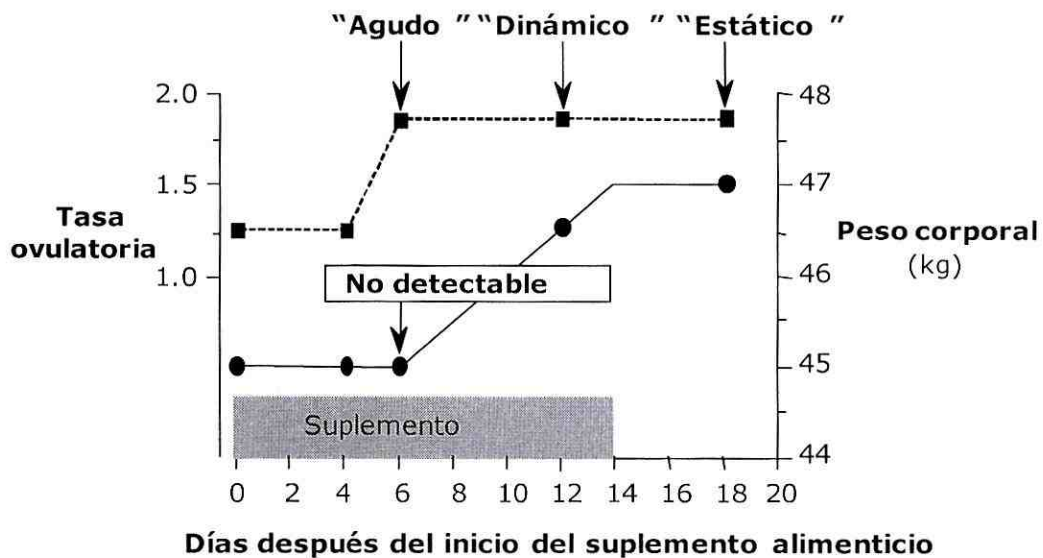


Figura 1. Efectos del suplemento alimenticio sobre el peso corporal (●) y la tasa ovulatoria (■) en ovejas (Scaramuzzi *et al.*, 2006).

Por ejemplo, el porcentaje de cabras en estro durante los primeros 5 días de contacto con los machos fue superior (92%) en las hembras que recibieron un suplemento alimenticio 7 días antes del efecto macho, que las hembras no suplementadas (60%). Asimismo, la tasa ovulatoria en el mismo periodo fue mayor en las cabras suplementadas (1.6) que en las control (1.0; De Santiago-Miramontes *et al.*, 2008). Sin embargo, este efecto positivo del suplemento alimenticio no persistió en la segunda ovulación, cuando la mayoría de las cabras pueden quedar gestantes. En ovejas, un suplemento alimenticio 14 días antes o iniciando 12 días después del efecto macho incrementa también la tasa ovulatoria (Molle *et al.*, 1995; Nottle *et al.*, 1997; Scaramuzzi *et al.*, 2006). De igual manera, el suplemento alimenticio en ovejas mantenidas en forrajes de pobre calidad durante unas semanas que preceden al contacto con los machos y durante la monta, incrementa la frecuencia de partos gemelares (Coop, 1966; Dunn y Moos, 1992; Lassoued *et al.*, 2004).

3. El suplemento alimenticio y la tasa de gestación

La influencia del estado nutricional de las hembras en la sobrevivencia embrionaria fue descrita ampliamente en vacas y ovejas (Robinson, 1986, 1990; Robinson *et al.*, 2002). De manera general, las hembras con bajo peso corporal tienen una mayor incidencia de mortalidad embrionaria que aquellas que tienen un alto peso corporal (Dunn y Moos, 1992). Esta diferencia se debe, probablemente, porque la subnutrición disminuye la calidad del ovocito

(O'Callaghan *et al.*, 2000) causando la pérdida embrionaria en los primeros 30 días de gestación (Mani *et al.*, 1992; Abecia *et al.*, 2006; Martin y Kadoka 2006; Robinson *et al.*, 2006; Blache *et al.*, 2007). Sin embargo, en hembras subalimentadas, un suplemento alimenticio incrementa la tasa de gestación (Kleemann y Cutten, 1978; Rhind *et al.*, 1989; Rassu *et al.*, 2004). En efecto, las ovejas de raza Karayaka bien alimentadas presentan mejores tasas de desarrollo embrionario que las hembras subalimentadas (Ocak *et al.*, 2006). Asimismo, las ovejas de raza Sarda explotadas en pastoreo y suplementadas con soya durante 14 días antes de la monta, y dos días después de ésta tienen una menor tasa de pérdida embrionaria (3%) que en aquellas hembras suplementadas únicamente durante 7 días antes de la monta (28%; Molle *et al.*, 1997). Esto sugiere que la duración del suplemento alimenticio y el momento en que éste se otorga, tienen una influencia directa sobre el rendimiento reproductivo (Resse *et al.*, 1990; Molle *et al.*, 1995, 1997; Nottle *et al.*, 1997; El-Hag *et al.*, 1998). La condición corporal, la cual es el reflejo de la alimentación previa a la que fueron sometidos los animales, afecta también de manera importante la tasa de sobrevivencia embrionaria (Molle *et al.*, 1995). Estos datos sugieren que un suplemento alimenticio mejora la gestación. Sin embargo, el momento óptimo y la duración del suplemento alimenticio en relación al efecto macho no se han determinado con precisión en las cabras (Martin *et al.*, 2004b).

En el subtrópico mexicano como en otras latitudes subtropicales, la mayoría de los caprinos son manejados de manera extensiva y consumen en 8 ó 9 horas por día sólo la vegetación nativa disponible (Sáenz-Escárcega *et al.*,

1991). Por tal motivo, estos animales están sujetos a importantes variaciones estacionales de la disponibilidad y calidad del alimento. En el subtrópico mexicano, el periodo seco se extiende de noviembre a mayo, lo cual produce una pronunciada escasez de alimento en dicho periodo (Sáenz-Escárcega *et al.*, 1991), provocando un bajo peso corporal en los caprinos explotados de manera extensiva. Este periodo coincide con el anestro estacional de las hembras, el cual se desarrolla de marzo a agosto (Duarte *et al.*, 2008). Las cabras que paren al ser expuestas a machos en reposo sexual es del 47%, y la prolificidad de 1.2 cabritos/hembra (Mellado y Hernández, 1996). En cambio, cuando las hembras son expuestas a machos sexualmente activos, la proporción de éstas que exhibieron un comportamiento estral fue similar (>90%) al observado en las hembras bien alimentadas mantenidas en confinamiento (Rivas-Muñoz *et al.*, 2007). Sin embargo, la prolificidad fue menor en las hembras en condiciones extensivas (1.6 cabritos/hembra) que en aquellas mantenidas en confinamiento (2.0 cabritos/hembra; Fitz-Rodríguez, 2004). Esta diferencia pudo deberse a una tasa ovulatoria más baja en la segunda ovulación inducida por el macho en las hembras subalimentadas, o a un incremento de la mortalidad embrionaria (Abecia *et al.*, 2006). Por tal motivo se efectuaron dos estudios para determinar si un suplemento alimenticio incrementa las tasas de ovulación y gestación en las hembras mantenidas en condiciones extensivas y expuestas al efecto macho.

3. Objetivos

1. Determinar si 7 días de suplemento alimenticio iniciando al momento de la introducción de los machos con los grupos de hembras, incrementa la tasa ovulatoria en la segunda ovulación inducida por el macho en cabras mantenidas en condiciones extensivas.

2. Determinar si un suplemento alimenticio al momento de iniciar el segundo estro inducido por el macho, incrementa la tasa de gestación de las cabras mantenidas bajo condiciones extensivas.

4. Hipótesis

1. Un suplemento alimenticio de 7 días iniciando al momento de la introducción de los machos con los grupos de hembras, incrementa la tasa ovulatoria de las cabras mantenidas en condiciones extensivas.

2. Un suplemento alimenticio al momento de iniciar el segundo estro inducido por el macho, incrementa la tasa de gestación de las cabras mantenidas bajo condiciones extensivas.

5. Artículo

Los resultados de los dos estudios se publicaron en un solo artículo.

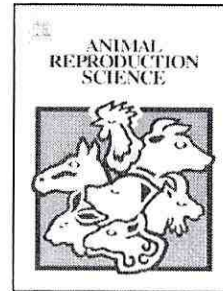
Artículo en prensa: Animal Reproduction Science

Nutritional supplementation improves ovulation and pregnancy rates in female goats managed under natural grazing conditions and exposed to the male effect

Accepted Manuscript

Title: Nutritional supplementation improves ovulation and pregnancy rates in female goats managed under natural grazing conditions and exposed to the male effect

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PII: S0378-4320(09)00005-0
DOI: doi:10.1016/j.anireprosci.2009.01.004
Reference: ANIREP 3774

To appear in: *Animal Reproduction Science*

Received date: 31-10-2008
Revised date: 31-12-2008
Accepted date: 14-1-2009

Please cite this article as: Fitz-Rodríguez, G., De Santiago-Miramontes, M.A., Scaramuzzi, R.J., Malpaux, B., Delgadillo, J.A., Nutritional supplementation improves ovulation and pregnancy rates in female goats managed under natural grazing conditions and exposed to the male effect, *Animal Reproduction Science* (2008), doi:10.1016/j.anireprosci.2009.01.004

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1 Nutritional supplementation improves ovulation and pregnancy rates in female goats managed
2 under natural grazing conditions and exposed to the male effect

3

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14

15 **Abstract**

16 Two experiments were conducted to determine if nutritional supplementation improved
17 ovulation and pregnancy rates in female goats managed under grazing conditions and submitted
18 to the male effect. In Experiment 1, one group of does did not receive nutritional
19 supplementation, while the other group was supplemented daily for 7 days starting at the time
20 when the males were introduced to the females. The ovulation rate at the second male-induced
21 ovulation was greater ($P<0.05$) in supplemented (2.0 ± 0.1) than in non-supplemented (1.6 ± 0.1)
22 does. For Experiment 2, female goats were supplemented for 0, 7, 14 or 28 days, starting 9 days
23 following buck introduction. The proportion of does that were pregnant in the group
24 supplemented for 28 days was greater ($P<0.05$) than in the non-supplemented group, but did not
25 differ from 14-day and the 7-day supplemented groups. The proportion of pregnant does was
26 greater ($P<0.05$) in the group supplemented for 14 days compared to the group supplemented for
27 7 days and the non-supplemented group. These latter two groups did not differ ($P>0.05$). In
28 conclusion, feed supplementation for 7 days, starting at the time when males were introduced
29 increased ovulation rate and feed supplementation for 14 or 28 days starting 9 days after males
30 were introduced improved pregnancy rates in goats managed under grazing conditions and
31 exposed to males.

32
33 *Keywords:* Goats - Does; Flushing; Ovulatory activity; Estrous behavior; Reproductive
34 performance

35

36

37

38

39 **1. Introduction**

40 Induction of ovulation in anovulatory females following exposure to ram or buck is called
41 the male effect (Ungerfeld et al., 2004; Delgadillo et al., 2006; Pellicer-Rubio et al., 2008).
42 Ovulation and pregnancy rates of ewes and does exposed to males can be influenced by several
43 factors including the nutritional status of the females. Generally, ovulation and pregnancy rates
44 are reduced in under-nourished females (Henniawati and Fletcher, 1986; Mani et al., 1992;
45 Abecia et al., 2006). However, ovulation rate can be increased by a feed supplementation. Indeed,
46 in ewes fed supplemented diets for 14 days before or commencing 12 days after the male effect
47 had increased ovulation rates (Molle et al., 1995; Nottle et al., 1997; Scaramuzzi et al., 2006). In
48 goats, fed supplemented diets 7 days before the male effect, there was an increased ovulation rate
49 at the first male-induced ovulation but the stimulatory effect did not persist into the second male-
50 induced ovulation (De Santiago-Miramontes et al., 2008). In addition, under-nutrition is one of
51 the many factors suggested as a cause for embryo loss up to 30 days of pregnancy, probably
52 because of changes in the uterine environment (Mani et al., 1992; Abecia et al., 2006; Martin and
53 Kadokawa, 2006). In under-nourished females, a feed supplementation improves pregnancy rates
54 (Kleemann and Cutten, 1978; Rhind et al., 1989; Rassu et al., 2004). However, the optimum
55 timing and duration of feed supplementation in relation to the “male effect” has not been
56 precisely determined for either sheep or goats (Martin et al., 2004). In many sub-tropical
57 latitudes, such as northern Mexico, does are managed under extensive conditions, grazing with
58 access to only unimproved natural vegetation, without nutritional supplementation. In these
59 goats, the anestrous period of does lasts from March to August (Duarte et al., 2008), coinciding
60 with the dry season, and as a consequence, with lesser body condition scores (Sáenz-Escárcega et
61 al., 1991; De Santiago-Miramontes et al., 2008). When does managed under grazing conditions

3

62 were exposed to sexually active bucks, during the anestrus period, the proportion of females
63 displaying estrous behavior was no different from well-nourished females maintained under
64 confined conditions (Rivas-Muñoz et al., 2007), but prolificacy was less (Fitz-Rodríguez, 2004).
65 This difference could be due to a lesser ovulation rate at the second male-induced ovulation when
66 most of does would have become pregnant, or to increased embryo mortality associated with the
67 under-nourished state of the does (Rhind et al., 1989; Robinson et al., 2002; Abecia et al., 2006).
68 Therefore, the aim of the present study was to determine i) if nutritional supplementation for 7
69 days starting at the male introduction increased ovulation rate at the second male-induced
70 ovulation in female goats managed under natural grazing conditions and ii) if nutritional
71 supplementation starting after the second male-induced ovulation would improve pregnancy
72 rates.

73

74 **2. Materials and methods**

75 *2.1. General*

76 Procedures used in this experiment were in accordance with the “Guide for the Care and
77 Use of Agricultural Animals in Agricultural Research and Teaching” (FAAS, 1999). The current
78 study was conducted in the Laguna region in the State of Coahuila, Mexico (Latitude, 26°23’ N
79 and Longitude, 104°47’ W). The climatic characteristics of this region were previously described
80 (Delgadillo et al., 1999). Briefly, this area is characterized by a dry climate with an average
81 annual rainfall of 266 mm (range: 163 to 504 mm) generally occurring between June and
82 September with a wide inter-year variability. Annual temperature average is 20.3° C, ranging
83 from 36.6° C between May and August and 5.7° C between December and January. Most of does
84 were allowed to graze natural vegetation from 0900 to 1800 without supplementation. At night,

85 does were kept in outdoor pens. Does are in anestrus between March and August (Duarte et al.,
86 2008) and bucks are in non-breeding between January and May (Delgadillo et al., 1999).

87

88 2.2. Experiment 1

89 2.2.1. Males

90 Four adult male goats were treated with 2.5 months of long days (16 h of light by day)
91 from November 1 to stimulate their sexual activity in the non-breeding season (Delgadillo et al.,
92 2002). Males were fed alfalfa hay (18% CP) *ad libitum* and 300 g/d of commercial concentrate
93 (14% CP; 1.7 Mcal/kg) per buck, with free access to mineral blocks and water. Bucks were
94 between 2 to 4 years old at the beginning of the study.

95

96 2.2.2. Females

97 Multiparous anovulatory female goats (n = 54) were used in the present experiment. Does
98 had given birth between November and December the previous year, and their kids were weaned
99 at approximately 30 days of age. All females were hand-milked once a day during the experiment
100 and isolated from all male goats between December 15 and March 24 when they were exposed to
101 the males. Prior to introduction of males (21, 14 and 7 days), transrectal ultrasonography was
102 performed to determine the ovarian status, using an Aloka SSD-500 machine connected to a 7.5
103 MHz linear probe. Absence of corpus luteum was used as evidence of anovulation. All does
104 grazed natural vegetation in a range setting between 0900 and 1800. At night, the does were
105 housed with the four bucks in two pens. On March 22, the female goats were divided into two
106 groups balanced for body weight and body condition score (n = 27 each): (i) grazing natural
107 vegetation (non-supplemented group; body condition score 1.3 ± 0.1 ; body weight 35.9 ± 1.5 kg)
108 and (ii) grazing natural vegetation plus daily supplementation with 260 g of rolled corn (8.6%

109 CP), 110 g of soy bean (49% CP) and 900 g of alfalfa hay (18% CP) per animal (supplemented
110 group; body condition score 1.3 ± 0.1 ; body weight 34.7 ± 1.0 kg). The nutritional
111 supplementation started on the day the males were introduced (March 24th) and continued for 7
112 days. The supplement was offered individually twice a day at 0800 (50% before grazing) and at
113 1800 (50% after grazing).

114

115 2.2.3. *Male effect*

116 On March 24 at 1800 (day 0), females were exposed to sexually active bucks ($n =$
117 2/group). Males remained in the shaded pen during day whereas the females were allowed to
118 graze on an open range. Females were, therefore, exposed to males between 1800 and 0900 daily
119 (Rivas-Muñoz et al., 2007). Males remained with females for 15 days and the pairs of bucks were
120 alternated daily between groups.

121

122 2.2.4. *Measurements*

123 Ovulation rate was estimated by number of corpora lutea detected by transrectal
124 ultrasonography (Schrick et al., 1993). All females were assessed twice, at days 7 and 19 after
125 exposure to bucks. Estrous behavior was recorded twice daily (0800 and 1800) throughout the
126 experiment (Chemineau et al., 1992). The interval between the introduction of the males and the
127 onset of estrous behavior was recorded to the nearest half day and the length of the estrous cycle
128 following the introduction of males was also recorded to the nearest half day.

129

130 2.3. *Experiment 2*

131 2.3.1. *Males*

132 Sexual activity was induced in eight adult male goats during the non-breeding season as
133 described for Experiment 1 (Delgadillo et al., 2002). Bucks were between 3 and 5 years old at the
134 beginning of the study.

135

136 2.3.2. Females

137 Anovulatory multiparous female goats ($n = 92$) were used in this experiment. Does had
138 given birth between November and December of the previous year, and their kids had been
139 weaned at approximately 30 days of age. All females were hand-milked once a day during the
140 experiment and isolated from all male goats between December 15 and April 6 when they were
141 exposed to males. Before introduction of males (23, 16 and 9 days), transrectal ultrasonography
142 was performed to determine the ovarian status as described in Experiment 1. All does grazed
143 natural vegetation on an open range between 0900 and 1800. On March 24, females were divided
144 into four groups ($n = 23$ each) balanced for body condition score (1.7 ± 0.6) and body weight (37
145 ± 0.4 kg). One group did not receive feed supplementation (non-supplemented group), while the
146 other three groups were supplemented daily with a mixture of 1100 g of alfalfa hay (18% CP),
147 150 g of rolled corn (8.6% CP) and 100 g of soy bean (49% CP) per animal for periods of 7, 14
148 and 28 days starting the day after the second male-induced ovulation (starting 9 days after
149 exposure to males). The nutritional supplementation was offered individually at 0800.

150

151 2.3.3. Male effect

152 On April 6 at 1800 (day 0), females were exposed to sexually active bucks ($n = 2/\text{group}$).
153 Males remained in the shaded pens during the day whereas the female were allowed to graze on a
154 range setting. As in Experiment 1, females were exposed to males between 1800 and 0900 daily

155 (Rivas-Muñoz et al., 2007). Males remained with the females for 15 days and each pair of bucks
156 was alternated daily among groups.

157

158 2.3.4. *Measurements*

159 Pregnancy was determined by transrectal ultrasonography using an Aloka SSD-500
160 machine connected to a 7.5 MHz linear probe, 45 days after the second male-induced ovulation
161 (Schrick et al., 1993; Rubianes et al., 1997). Estrous behavior was recorded twice daily (0800 and
162 1800) throughout the experiment. The interval between introduction of the males and the onset of
163 estrous behavior was determined to the nearest half day (Chemineau et al., 1992).

164

165 2.3.5. *Statistical analyses*

166 Ovulation rates were compared using the Mann-Whitney U test. The proportions of does
167 showing estrus and ovulating each day, the proportions of does displaying shorter than typical
168 length estrous cycles and the proportion of pregnant does were all compared using the χ^2 test.
169 The interval between the introduction of males and onset of estrous behavior and the duration of
170 short estrous cycle were analyzed by a one-way ANOVA. Data are reported as mean \pm SEM.
171 Analyses were computed using SYSTAT 10 (Evanston, IL, USA, 2000).

172

173 3. Results

174 3.1. *Experiment 1*

175 3.1.1. *Ovulatory response*

176 The ovulation rate at the second male-induced ovulation that was detected between Days
177 6 and 14 after male introduction was greater ($P < 0.05$) in supplemented (2.0 ± 0.1) than in non-

178 supplemented (1.6 ± 0.1) females, but did not differ ($P>0.05$) over the first 5 days following
179 introduction of the males (1.2 ± 0.1 in both groups). Over the first 5 days of exposure to bucks,
180 the proportion of does that had ovulations was greater ($P<0.05$) in supplemented compared with
181 non-supplemented does. In contrast, between days 6 and 15 following the introduction of bucks,
182 the proportion of does that had ovulations was not affected ($P>0.05$) by nutritional
183 supplementation (Table 1).

184

185 3.1.2. Estrus response

186 The daily and cumulative percentages of does displaying estrous behavior are depicted in
187 Figure 1 and did not differ ($P>0.05$) between supplemented and non-supplemented groups at any
188 point of the study. The data for estrus response of females are included in Table 1. The
189 proportions of does showing estrous behavior associated with ovulation over the entire study
190 were not different ($P>0.05$) in supplemented and non-supplemented groups. The interval
191 between the introduction of the males and the onset of estrous behavior did not differ between
192 groups (supplemented: 2.2 ± 0.2 days; non-supplemented: 2.1 ± 0.2 days). The proportion of
193 females with short estrous cycles was not different between supplemented (59%) and non-
194 supplemented (56%) groups. The duration of short estrous cycles did not differ ($P>0.05$) between
195 groups (supplemented: 4.7 ± 0.2 days; non-supplemented: 5.0 ± 0.2 days).

196

197 3.2. Experiment 2

198 3.2.1. Estrus Response

199 Data for percentages of does displaying estrus after 15 days of exposure to bucks are
200 included in Table 2. The interval between the day of introduction of the males and the onset of
201 estrous behavior was similar ($P>0.05$) among groups. The proportion of females having shorter

202 than typical length estrous cycles and the duration of these short estrous cycles did not differ
203 ($P>0.05$) between groups.

204 3.2.2. Pregnant does

205 The proportion of pregnant does in the 28-day supplemented group was greater ($P<0.05$)
206 than in the non-supplemented group, but did not differ ($P>0.05$) from the 14-day group and
207 tended to be greater ($P=0.10$) than the 7-day supplemented group. The proportion of pregnant
208 does in the 14-day supplemented group was greater ($P<0.05$) than in the 7-day and non-
209 supplemented groups but these two later groups did not differ ($P>0.05$; Figure 2).

210

211 4. Discussion

212 A period for 7 days of nutritional supplementation of anestrus does starting at the time
213 that sexually active males were introduced to them increased ovulation rate at the second male-
214 induced ovulation and feed supplementation for 14 or 28 days commencing 9 days after males
215 were introduced increased the proportion of does that were determined pregnant 45 days after the
216 second male-induced ovulation. In Experiment 1, the ovulation rate at the second male-induced
217 ovulation was greater in nutritionally supplemented does compared to non-supplemented does. In
218 addition, the percentage of does that had ovulations over the first 5 days following the
219 introduction of bucks was greater in the supplemented group. These data agree with Nottle et al.
220 (1997) who reported that Merino ewes fed a daily nutritional supplement of lupin grain between
221 12 and 26 days following the introduction of rams had increased ovulation rate at the second
222 male induced-ovulation. Results of the present study also agree with those of De Santiago-
223 Miramontes et al. (2008) who reported that in anestrus does managed under grazing conditions,
224 fed supplemented diets for 7 days before exposure to sexually active bucks increased the

225 percentage of does having ovulations as well as ovulation rate. Overall, data from the current
226 study suggests that 7 days of feed supplementation increased the ovulation rate of does exposed
227 to sexually active males. The increased ovulation rate in nutritionally supplemented does could
228 be because nutritional supplementation stimulated the growth of more ovulatory follicles and
229 decreased follicular atresia (Muñoz-Gutiérrez et al., 2002; Viñoles et al., 2005). Similar results
230 were reported in does supplemented 7 days prior to male introduction. The ovulation rate at the
231 first ovulation after male introduction was increased, but this effect was not observed at the
232 second male-induced ovulation (De Santiago-Miramontes et al., 2008). These results indicate that
233 ovulation rate of the first or second-induced male ovulations can be modified by short-term feed
234 supplementation.

235 In the present study, a feed supplementation also increased the percentage of does having
236 ovulations over the first 5 days after exposure to males, but without affecting ovulation rate. In
237 ewes, feed supplementation for 5 days with lupin grain stimulated folliculogenesis, and increased
238 the number of follicles capable of ovulation (Rhind et al., 1998). The increased proportion of
239 does having ovulations in the current study is probably the result of short-term nutritional
240 supplementation promoting the growth of ovulatory follicles (Muñoz-Gutiérrez et al., 2002).
241 However, in the present study most females had ovulations within the first 5 days following the
242 introduction of males, which may explain lack of improved ovulation. In contrast to ovulation,
243 the proportions of females displaying estrous behavior and having short estrous cycles were
244 similar in supplemented and non-supplemented groups. These results are not consistent with
245 those reported by De Santiago-Miramontes et al. (2008). These authors reported feed
246 supplementation for 7 days prior to introduction of males increased the proportion of females
247 showing estrous behavior and the proportion with short estrous cycles at the first male-induced

248 ovulation. These differences are probably related to differences in the timing of nutritional
249 supplementation.

250 In Experiment 2, the proportion of pregnant does in the groups supplemented for 14 or 28
251 days did not differ, but these were both greater than in the non-supplemented group. These results
252 agree with those reported for other species such as pigs, cattle and sheep where feed
253 supplementation after mating increased pregnancy rates and prolificacy (Jindal et al., 1996;
254 Boland et al., 2001; McWilliam et al., 2004). Results of the present study clearly show that feed
255 supplementation for 14 or 28 days increased the pregnancy rate in does managed under extensive
256 conditions. In the present study, pregnancy rates in does supplemented for 14 and 28 days were
257 greater than in the non-supplemented group. This difference was not due to different responses to
258 the male effect, because the pattern of estrous behavior was similar among supplemented and
259 non-supplemented groups. This suggests that the improvement in pregnancy rates were a direct
260 effect of feed supplementation around the time of embryo implantation. It is generally accepted
261 that feed supplementation during the month after conception increases pregnancy rates (Rassu et
262 al., 2004). Kakar et al. (2005) found that ewes were able to respond to acute changes in nutrition
263 immediately following ovulation, with beneficial changes in embryo quality and development.
264 However the time and duration of a feed supplementation had not been precisely defined (Martin
265 et al., 2004). The present study demonstrates that feed supplementation for at least 14 days
266 starting at the second male-induced estrus improved pregnancy rates in does induced to have
267 ovulations by exposure to sexually active bucks. This increase is probably due to a combination
268 of positive influences of nutritional supplementation on the uterine environment, embryo
269 development and implantation of the conceptus. Indeed, in ewe maternal under-nutrition results
270 in retarded embryonic development at 8 to 11 days after mating (Abecia et al., 1995) reducing the
271 day-14 pregnancy rates (Rhind et al., 1989). In goats exposed to males, pregnancy rates and

272 prolificacy were less in under-nourished does compared to well-nourished does (Mellado et al.,
273 1996; Fitz-Rodríguez, 2004) and may be attributed to lesser ovulation rates and increased of
274 embryo mortality in under-nourished females (Henniawati and Fletcher, 1986; Mani et al., 1992;
275 Abecia et al., 2006).

276

277 **5. Conclusion**

278 It is concluded that anestrous does managed under natural grazing conditions in an arid
279 sub-tropical environment responded to 7 days of nutritional supplementation starting at the time
280 that males are introduced with an increased ovulation rate at the second male-induced ovulation
281 in anestrous female goats. Furthermore, nutritional supplementation for 14 or 28 days starting at
282 the time of the second-male induced ovulation improved pregnancy rates in these goats induced
283 to have ovulations when in anestrous after being exposed to males.

284

285 **Acknowledgements**

286 The authors are grateful to Juan Manuel de Arco and Juan Antonio Rodríguez Ramírez
287 for providing the female goats used in this study and to all members of the Centro de
288 Investigación en Reproducción Caprina (CIRCA) of the Universidad Autónoma Agraria Antonio
289 Narro, for their technical assistance. The secretarial assistance of Dolores López Magaña is
290 gratefully acknowledged. G. Fitz-Rodríguez and M.A. De Santiago-Miramontes were supported
291 by CONACYT scholarships during their doctoral studies.

292

293

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399

400 *Figure legends*

401 Fig. 1. Daily (top) and cumulative (bottom) percentages of female goats displaying estrous
402 behavior following exposure to sexually active bucks over a 15-day period. The non-
403 supplemented (\circ ; $n = 27$) and supplemented (\bullet ; $n = 27$) groups grazed natural vegetation in a
404 range setting between 0900 and 1600. At night, the does from each group were housed with two
405 bucks in open pens. The period of nutritional supplementation was for 7 days commencing on the
406 day that the bucks were introduced. Day 0 is the first day of exposure to bucks.

407

408 Fig. 2. Pregnancy rates of female goats exposed to sexually active bucks over a 15-day period.
409 Female goats grazed natural vegetation in an open range setting from 0900 to 1800. At night,
410 does were housed with bucks in four open pens ($n = 2$ bucks per group). Control group did not
411 receive nutritional supplementation. The other three groups received nutritional supplementation

18

412 for periods of 7, 14 or 28 days starting on the day of the second male-induced ovulation. Bars

413 with different letters are different ($P < 0.05$).

414

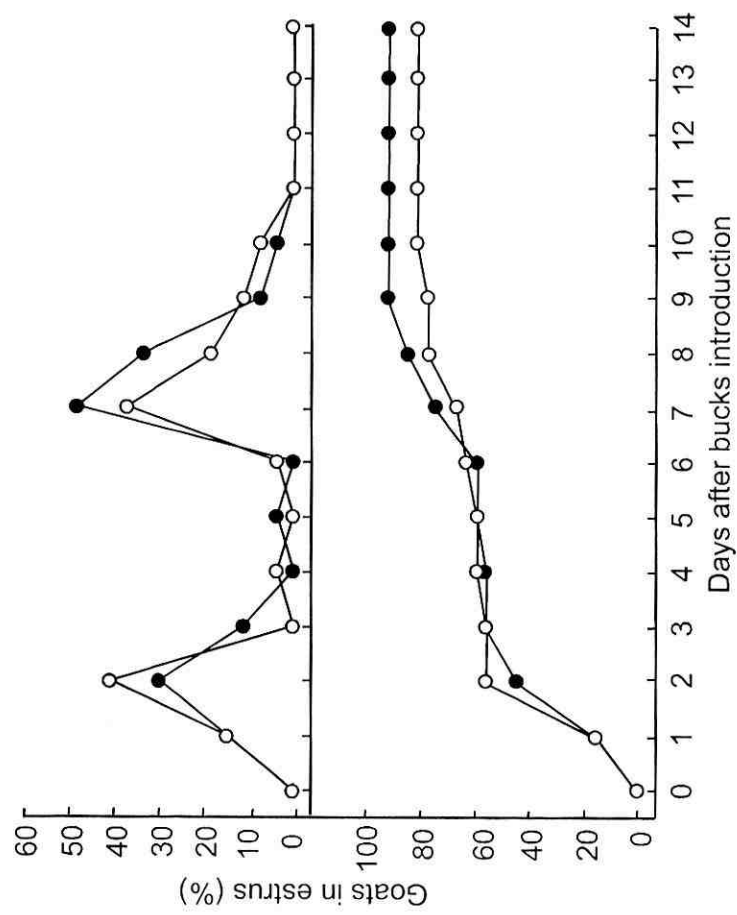


Figure 1. Fitz-Rodriguez et al

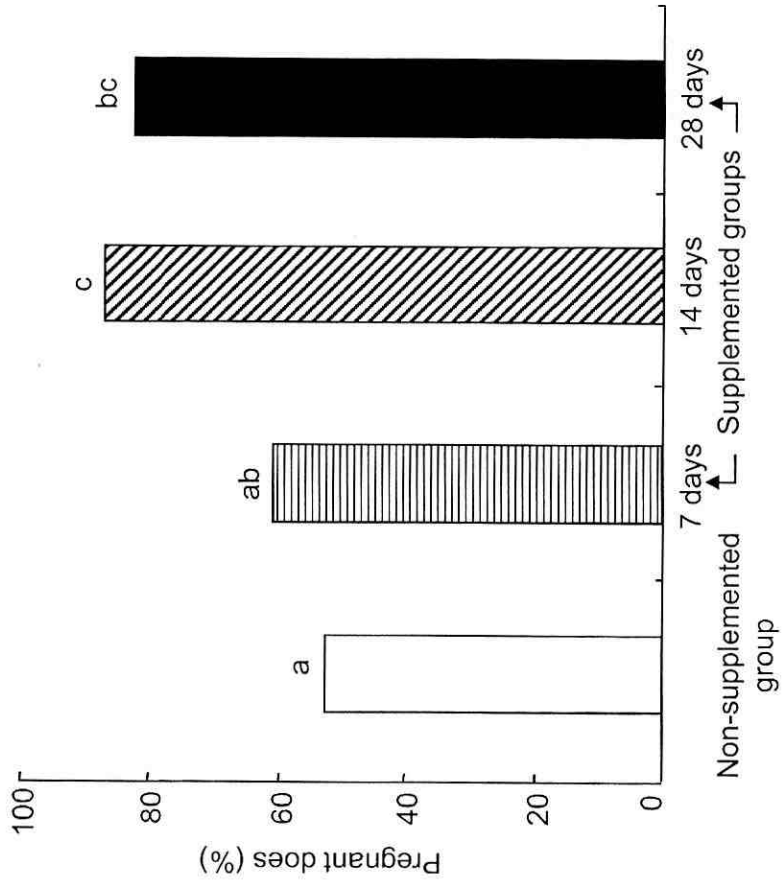


Figure 2. Fitz-Rodríguez *et al*

Table 1. Ovarian and estrus responses of anovulatory goats following the introduction of bucks. Supplemented does were given 7 days of nutritional supplementation (n = 27) starting on the day the bucks were introduced. Non-supplemented does received no supplementation (n = 27).

	0-5 days		6-14 days		0-14 days	
	Non-supplemented group	Supplemented group	Non-supplemented group	Supplemented group	Non-supplemented group	Supplemented group
Does that exhibited estrus (%)	56 (15/27)	60 (16/27)	78 (21/27)	93 (25/27)	81 (22/27)	93 (25/27)
Does that exhibited estrus and ovulated (%)	37 (10/27)	56 (15/27)	74 (20/27)	89 (24/27)	79 (21/27)	89 (24/27)
Does that ovulated (%)	48 (13/27)	74 (20/27)*	85 (23/27)	96 (26/27)	89 (24/27)	96 (26/27)

Does of both groups were allowed to graze natural vegetation on an open range from 0900 to 1800 daily. At night, does were housed with sexually active bucks in open pens (n = 2 bucks/group). *P<0.05.

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Table 2. Estrus responses of four groups (n = 23 each) of anovulatory goats following the introduction of bucks. One group did not receive supplementation. The other three groups received daily supplementation for 7, 14 or 28 days starting 9 days after bucks were introduced to does

Groups	Non-supplemented group		Supplemented groups	
	7 days	14 days	28 days	28 days
Percentage in estrus between 0-14 days (%)	86 (20/23)	96 (22/23)	96 (22/23)	100 (23/23)
Interval (mean \pm SEM) from introduction of bucks to first estrus (days)	2.7 \pm 0.2	2.5 \pm 0.6	2.1 \pm 0.2	2.1 \pm 0.2
Percentage of females goats with short estrous cycles (%)	43 (10/23)	39 (9/23)	52 (12/23)	47 (11/23)
Length (mean \pm SEM) of short estrous cycles (days)	5.1 \pm 0.2	5.5 \pm 0.3	4.8 \pm 0.21	5.6 \pm 0.3

Does of four groups were allowed to graze natural vegetation on an open range from 0900 to 1800 daily. At night, does were housed with sexually active bucks in open pens (n = 2 bucks/group). Differences (P>0.05) between groups were not detected.

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