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(Organizadores)



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EVALUATION OF THE SEASON OF THE YEAR ON THE CONDITIONED SEXUAL BEHAVIOR IN RAMS

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ABSTRACT: The aim of this study was to compare how season of the year affects sexual behavior in rams, trained to mount a dummy. Two specimens per breed were included in a study to analyze their sexual behavior throughout the year (spring, summer, autumn and winter).

Animals were trained for conditioned mounting and then, their activity was monitored by video recorder and ethograms on weekly bases (52 records / ram / year). To test the hypothesis that reproductive behavior differences occur among ram breeds during the different seasons of the year, a descriptive statistical analysis of states and events of conditioned mounting was carried out, through analysis of variance (ANOVA) and Tukey test. Results showed no differences in sexual behavior during semen collection among breeds throughout the year. In average, animals displayed the state of flehmen in 16.3 seconds and the copulation in 71.1 seconds. While the average frequency for events such as olfaction, kicking and mounting attempts was 2.1, 4.3 and 2.1 times per mating event, respectively. When considering the season of year, significant differences ($P < 0.05$) were found among the time invested by rams to complete copulation state, for the olfaction period and for the number of kicking events, while no differences ($P > 0.05$) were observed for the flehmen state or for the mounting event. All the animals used in this study were subjected to classical conditioning and were sexually active throughout the year. Animals had best reproductive behavior patterns during winter, while no differences among rams were observed in sexual activity during the other

three seasons of the year. Our results confirm previous reports indicating that rams have best reproductive performance during winter, however reproductive behavior patterns observed in five breeds of rams during spring, summer and autumn, also confirm that rams can be used for semen collection and processing purposes throughout the year.

KEYWORDS: Ethogram, sexual behavior, states, events, rams.

RESUMO: O objetivo deste estudo foi comparar como a estação do ano afeta o comportamento sexual em carneiros, treinados para montar um boneco. Dois espécimes por raça foram incluídos em um estudo para analisar seu comportamento sexual ao longo do ano (primavera, verão, outono e inverno). Os animais foram treinados para montagem condicionada e, em seguida, suas atividades foram monitoradas por gravador de vídeo e etogramas em bases semanais (52 registros / ram / ano). Para testar a hipótese de que diferenças de comportamento reprodutivo ocorrem entre raças de raças durante as diferentes estações do ano, foi realizada uma análise estatística descritiva dos estados e eventos de montagem condicionada, através da análise de variância (ANOVA) e teste de Tukey. Os resultados não mostraram diferenças no comportamento sexual durante a coleta de sêmen entre as raças ao longo do ano. Em média, os animais exibiram o estado de flehmen em 16,3 segundos e a cópula em 71,1 segundos. Enquanto a frequência média para eventos como olfação, tentativas de chute e montagem foi de 2,1, 4,3 e 2,1 vezes por evento de acasalamento, respectivamente. Ao considerar a estação do ano, foram encontradas diferenças significativas ($P < 0,05$) entre o tempo investido pelos carneiros para completar o estado de cópula, para o período de olfação e para o número de chutes, enquanto não foram observadas diferenças ($P > 0,05$) para o estado flehmen ou para o evento de montagem. Todos os animais utilizados neste estudo foram submetidos a condicionamento clássico e foram sexualmente ativos ao longo do ano. Os animais apresentaram melhores padrões de comportamento reprodutivo durante o inverno, enquanto não foram observadas diferenças entre os carneiros na atividade sexual durante as outras três estações do ano. Nossos resultados confirmam relatórios anteriores indicando que os carneiros têm melhor desempenho reprodutivo durante o inverno, no entanto, os padrões de comportamento reprodutivo observados em cinco raças de carneiros durante a primavera, o verão e o outono também confirmam que os carneiros podem ser usados para fins de coleta e processamento de sêmen ao longo do ano.

PALAVRAS - CHAVE: Etograma, comportamento sexual, estados, eventos, carneiros.

1 | INTRODUCTION

Most accepted contemporary evolutionary theories propose that reproductive behavior has the purpose of preserving species and promoting genetic variability that allows species to adapt to the ever-changing environment (Ferreira, 2009). However, reproductive environment for farm animals has been deeply modified by human intervention, because animals are selected for characteristics other than reproductive potential and submitted

to artificial reproduction. Therefore it is important to study how artificial reproduction is affecting reproductive performance in animals. In the case of sheep, different factors may affect natural sexual behavior, such as their physical conformation, gregarious instinct, the male/female interaction according to the type of production system, the dominance/subordination relationship given by physical characteristics associated to body weight (head, horns and body size of males), agonistic behavior (social behavior associated with dominance) and libido. Additionally, there are some breed associated factors, such as; seasonality, endocrine regulation and age at puberty, that may also deeply affect sexual behavior of the species (Hernández, 2011).

Studies about rams sexual behavior in natural mating, indicate that breeding behavior can be considered from the moment the ram tracks and/or approaches a particular female, and it ends with copulation and ejaculation (Odagiri *et al.*, 1995). Eight pre-copulation breeding behavior units can be studied in rams: tracking or approaching, chin's relaxation, flehmen sign, nasal contact, bump, rubbing the female's flank, raising a front limb, and mounting and breeding attempts. The flehmen and the snuzzle-vulva contact are thought to be more associated with olfactory perception and the female's physiological state identification (Orihuela, 2014).

Natural mounting has been extensively studied for sexual behavior of rams. However, semen collection for artificial insemination used to maximize the male's performance and for the optimization of production oriented genetic programs (animal selection), has been studied less extensively. The semen collection routine requires that males are conditioned to mount a dummy and ejaculate on an artificial vagina. Therefore, a sheep artificial breeding program requires qualified personnel, with deep knowledge about ram's reproductive behavior and breeding habilitation strategies, capable of identifying problems associated to libido or stereotypes generated by the monotony related to the semen collection room (García, 2004).

Rams are reproductively seasonal, therefore for artificial reproduction programs, semen collection is affected by the season of the year. However, comparative seasonal semen collection performance associated to different economically important ram breeds in Mexico, either collected with dummy or anestrous females, has not been sufficiently studied. Therefore, the objective of the present study was to evaluate, through a behavioral catalog (ethogram), the comparative sexual behavior in rams, trained for semen collection. Experimental procedure considered the variability of reproductive behavior observed during the four seasons of the year (spring, summer, autumn and winter).

2 | MATERIAL AND METHODS

Ten rams (two specimens from each breed: Dorper, Dorset, Hampshire, Katahdin and Suffolk) ranging between 1 and 8 years of age, trained for semen collection with artificial

vagina, were used for artificial semen collection either using a dummy or when necessary an ewe. The animals were housed in the Center for Ovine Genetic Improvement (CeMeGO) of the Faculty of Veterinary Medicine and Zootechnics of the Autonomous University of the State of Mexico, Mexico, housed in individual pens, fed twice a day with a balanced diet containing 14% protein and 2.9 Mcal Em / Kg. M.S. Animals had access to water *ad-libitum* through an automatic water dispenser.

The study was conducted in a 12 months (April 2017 to April 2018) period. Initially, rams were used to create a behavioral catalog. Behavior of each animal was recorded in video for one hour, twice a week for four weeks, while animals were presented to the dummy until, and until semen collection using artificial vagina was completed. When a total of 80 hours video was completed, an analysis of all different conducts was registered in a global record. Subsequently, most frequent ram conducts were described and used in the behavioral catalog (Appendix 1) and the design of the ethogram for ram reproductive behavior. Video and ethogram recordings were conducted once a week for each ram, obtaining a total of 52 records / ram / year, including the four seasons of the year (spring, summer, autumn and winter). All animals work and records were made between 8:00 - 10:00 a.m., to prevent behavior variables due to schedule changes.

After preparation of rams for semen collection, each animal was introduced independently in the breeding area, and induced to mount the dummy. Rams were always handled by the same qualified person, to prevent behavior variables associated to the handler. Once inside the breeding room the ram was released, and from that moment on, behavioral states and events were registered in the behavioral record. Video and ethogram were continued until ram's ejaculation, during semen collection process (video 1, video 2). Animals were allowed to remain 30 seconds next to the collection dummy, when recording was considered completed, then the animal was returned to his pen. Ethogram was divided in two sections: a) states (sexual behavioral patterns such as copulation and flehmen) were registered in seconds the ram spent in such activity, and b) events, considered the number of olfaction, kicking and mounting attempts related to the semen collection process.

2.1 Statistical analysis

A descriptive statistical analysis of behavioral states and events occurred during de semen collection process categorized by the season of the year was carried out. Analysis of variance (ANOVA) and Tukey test were used to identify differences in States durations and number of Events among breeds or seasons of the year. JMP 9.0 software from SAS, was used for the analysis.

3 | RESULTS

For all animals included in the study, annual averages for flehmen and copulation

state were 16.3 (± 10.1) and 71.1 (± 48.2) seconds, respectively. While olfaction, kicking and mounting Events were observed in an average of 2.1 (± 1.1), 4.3 (± 4.4) and 2.1 (± 1.8) seconds per recorded episode, respectively. Table 1 shows the general descriptive statistics of conditioned sexual behavior in rams, indicating the average, standard deviation (SD), ranges and coefficient of variation (CV, %) of the States and Events of rams' sexual behavior.

Concept	Age	States (seconds)		Events (frequency)		
		Flehmen	Copulation	Olfaction	Kicking	Mounting
Average \pm SD	5.4 \pm 1.6	16.3 \pm 10.1	71.1 \pm 48.2	2.1 \pm 1.1	4.3 \pm 4.4	2.1 \pm 1.8
Range	2 - 8	3 - 49	16 - 331	1 - 8	1 - 26	1 - 10
C.V (%)	29.4	61.9	67.8	49.8	93.5	83.9
n	479	110	469	431	266	450

Table 1. General descriptive statistics of conditioned sexual behavior in rams (States and Events)

Seasonal effect on artificial breeding performance in rams

When comparing sexual behavior among seasons (Table 2), significant statistical differences were found in the state of copulation and in the olfaction and kicking events ($P < 0.05$), while for the state of flehmen and the mounting event, no statistical differences were found ($P > 0.05$). However, a tendency for state of flehmen to last more in autumn, followed by winter was observed. Regarding the copulation, it presented longer episodes in the winter ($P < 0.05$), while in spring, summer and autumn showed no statistical differences ($P > 0.05$).

Season	States (seconds)				Events (frequency)					
	Flehmen		Copulation		Olfaction		Kicking		Mount	
	n	LSM \pm SE	N	LSM \pm SE	n	LSM \pm SE	N	LSM \pm SE	n	LSM \pm SE
Spring	18	14.4 \pm 2.2	80	59.9 \pm 5.1 ^b	79	2.5 \pm 0.11 ^a	45	2.8 \pm 0.60 ^b	79	2.0 \pm 0.18
Summer	31	14.6 \pm 1.6	115	58.7 \pm 4.3 ^b	112	2.1 \pm 0.10 ^{ab}	74	2.7 \pm 0.50 ^b	113	2.0 \pm 0.15
Autumn	29	18.9 \pm 1.8	119	62.8 \pm 4.2 ^b	112	1.8 \pm 0.10 ^b	67	3.0 \pm 0.51 ^b	118	2.0 \pm 0.15
Winter	32	16.5 \pm 1.6	155	88.7 \pm 3.7 ^a	128	2.1 \pm 0.09 ^{ab}	80	6.6 \pm 0.49 ^a	140	2.3 \pm 0.14
P Value		0.223		0.0001		0.0003		0.0001		0.538

Table 2. Statistical analysis of conditioned sexual behavior in rams (states and events) according to the season of year

Different superscript literals in the same column indicate significant differences (a, b, c) ($P < 0.05$).

The olfaction event occurred with more frequency in the spring than in any other season, it was statistically different from autumn, which was the season with lowest frequency ($p < 0.05$). When comparing olfaction frequency for Spring Summer and Winter, no differences were observed ($P > 0.05$). The kicking event was observed with highest frequency during the winter period ($P < 0.05$). The other seasons showed no differences among them ($P > 0.05$). No significant differences ($P > 0.05$) were found for the mounting event throughout the year.

A result that should be considered with some limitation in this study due to the low number of sires per breed is that for the mounting Event, Suffolk and Dorper had highestest number of episodes ($P < 0.05$) in comparison with all other breeds, while Dorset, Hampshire and Katahdin did not show significant differences ($P > 0.05$) among them.

4 | DISCUSSION

Changes in light periods of exposure have an impact in melatonin secretion level, which in turn has an impact on gonadotropins and other hormones such as testosterone. These hormonal changes are responsible for the seasonality of sexual behavior observed in rams, which are characterized for a reduced activity during spring and early summer, when the day light length increases (Orihuela, 2014). In the present report, we found no significant differences ($P > 0.05$) throughout the year in the mounting Event or in the "flehmen" State. Rams did not lose interest in sexual activity throughout the year and remained active in exploring the physiological condition of the ewe and were responsive to estrous cycle.

Perkins and Roselli (2007) reported that in most breeds, rams are seasonal breeders and therefore show better libido during the short light days period of the year, when ewes resume ovarian activity and the consequent secretion of pheromones. On the other hand, Aguirre *et al.* (2005) showed that the magnitude of the seasonal effect was not enough to prevent rams from being used as reproducers throughout the year. Findings of the present report support Aguirre and co-workers' findings, because we observed that rams display copulation state throughout the year, although they showed longer episodes. Furthermore, the olfaction event recorded was as frequent in the spring as in winter ($P > 0.05$), presenting the same interest in olfaction of the female for the mount, regardless of whether ewe was or not in estrus. Additionally, the kicking event tended to be more frequent in winter, however no statistical differences with the other seasons of the year were registered, showing that some behavioral patterns typical of courtship can be found throughout the year.

5 | CONCLUSIONS

The rams used in this investigation were included in a classic conditioning artificial semen collection program, where the animals were trained to mount a dummy or on an anestrus ewe and collected with an artificial vagina. These rams showed better sexual behavior patterns during winter than in any other season of the year. Some behavioral differences were found among breeds, where the Katahdin and Dorset showed better performance in terms of the courtship and semen collection service. These result may be useful as additional selection criteria of rams used for semen collection and processing in artificial insemination facilities.

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APPENDIX 1. BEHAVIORAL CATALOG

States	Description
Flehmen	Retraction movement in the lips in some mammals that consists in collecting odorous stimuli through the vomeronasal organ, linked to estrus.
Copulation	Set of behavioral guidelines that range from when the male enters the area where the female dummy is for the mount; It is positioned on one side of it, passes in front or failing to surround it, in addition to a constant tongue, either directed to the air or directed to some part of the body of the female, before and after mount.
Events	Description
Olfaction	Procedure aimed at the female to detect odors through chemical-sensory perception by the nasal contact, this can be done in the lateral portions of the female or in her posterior train.
Kicking	Movement consisting of raising and lowering one of its previous limbs, keeping it rigid and making a "knock" on the lateral or posterior portion of the female.
Mount	It is defined as mount the sexual union of a male with a female to fertilize it. Procedure in which the male mounts the female with one or several attempts, until ending with the ejaculate in the artificial vagina.

