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Variation of sexual behavior of photo-stimulated bucks during the transition from winter to spring in the semi-arid climate of Mexico

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Abstract Sheep and goat production takes place under unsuitable climate conditions, where animals are more susceptible to high temperatures. The objectives of this study were to determine, i) whether sexual behavior of photo-stimulated bucks varies through 24 h/day in March and April and, ii) whether the environmental temperature and the relative humidity affect their expression. Six bucks were submitted to artificial long days (16 h light and 8 h darkness per day/2.5 months). Bucks were exposed to ovariectomized females once a week during the non-breeding period and sexual behavior was recorded for 15 min at 2 h intervals along 24 h/day. The environmental temperature, relative humidity, temperature-humidity index (THI), and body temperature were recorded in each behavior test. Plasma testosterone, sexual behavior, environmental temperature, relative humidity, and body temperature were analyzed using Generalized Estimation Equations. Plasma testosterone showed a significant difference over the experimental period ($P < 0.001$). The highest frequency of nudging and anogenital sniffing was at 08:00 h ($P < 0.001$); flehmen and penis unsheathed were variable ($P < 0.001$). The highest environmental temperature and the lowest relative humidity were registered from 14:00 to 18:00 h ($P < 0.001$). The highest body temperature was at 18:00 h and the lowest was at 06:00 h ($P < 0.001$). The highest THI >77 was recorded at 16:00 h. In conclusion, photo-stimulated bucks showed a variation of sexual behavior through 24 h/day exposed to ovariectomized females, and these sexual activities were affected by the high environmental temperature and the low relative humidity throughout the study.

Keywords animal behavior, caprine, photoperiod, seasonality, weather

1. Introduction

Small ruminants such as sheep and goats display a seasonal breeding pattern. Reproductive seasonality is regulated by the photoperiod (Delgadillo et al 1999; Rosa and Bryant 2003; Bronson 2009). In bucks, testosterone secretion and sexual behavior vary through the year, during the breeding season they display high levels of testosterone and sexual behavior, whereas during the non-breeding season, those variables are expressed to a minimum or are not observed (Delgadillo et al 2002). However, a strategy to induce sexual activity in bucks during the non-breeding season is the application of a photoperiodic treatment which consists of exposing them to artificially long days for 2.5 months (Rivas-Muñoz et al 2007). As a result of this treatment, photo-stimulated bucks increase plasma testosterone concentration and sexual behavior (Ponce et al 2014).

In the revised literature, sexual behavior of photo-stimulated bucks has been recorded only in two intervals of one hour during the day (from 08:00-09:00 and 18:00-19:00) and for the first three or four days of contact between bucks and anestrous females during the non-breeding season (Bedos et al 2014; Muñoz et al 2016; Fernández et al 2018). For example, Bedos et al (2016) reported sexual behavior of bucks in three different periods, during the breeding season, during the sexual season of photo-induction, and during the non-breeding season, the behavior tests were recorded on the same day (from 08:00-12:00 h) by two different days with an interval of 9 days. Therefore, the sexual activity of these photo-stimulated bucks through 24 h a day assessing longer periods remains unknown.

On the other hand, climate change caused by anthropogenic emissions in the atmosphere increases the risks from droughts, precipitation deficits, and the highest temperature. These activities are related to population development and animal production (IPCC 2018). Climate change affects seasonal reproduction species such as sheep and goats (Bronson 2009), although whether these species will be able to adapt or not to climate change is currently uncertain. Most of the worldwide small ruminant productions occur under unsuitable climate conditions where

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animals are more susceptible to higher environmental temperature (Bronson 2009). The high temperatures have a negative effect on productive and reproductive parameters, as well as on animal welfare (Polsky and von Keyserlingk 2017). The high body temperature is a consequence of a combination of the increase of environmental temperature, relative humidity, and high solar radiation (Collier et al 1982; Al-Dawood 2017). In ruminants, a way to measure the level of heat stress is through the THI (Ravangnolo et al 2000; Hill and Wall 2015). For animals exposed to a THI >71 units, physiological, anatomical, behavioral, and reproductive mechanisms are adversely affected (Du Preez 2000; Al-Dawood 2017).

The hypothesis of this study states that the sexual behavior of photo-stimulated bucks varies during the day and that some environmental descriptors such as the temperature and the relative humidity could affect these behaviors. Therefore, the objectives of this study were to determine, i) whether sexual behavior of photo-stimulated bucks varies through 24 h/day in March and April, and ii) whether the environmental temperature and the relative humidity affect these sexual behaviors.

2. Materials and Methods

2.1. General conditions of the study

The experiment was performed in the Centro de Investigación en Reproducción Caprina of the Universidad Autónoma Agraria Antonio Narro, Torreon, Coahuila, Mexico (25°23'N, 104°47'W, 1200 m above sea level), situated in the Chihuahuan Desert. The climate in this region is semi-arid, which is characterized by having an extreme climate. Data show that extreme maximum temperature (under sheltered) during Spring is 44.8 °C and extreme minimum temperature (under sheltered) during Winter is -1.0 °C (CONAGUA 2013). Mexican creole goats (*Capra hircus*) breed described previously by Escareño Sánchez et al (2011) were used. In this area, goat bucks of this breed display sexual rest from January to May (Delgadillo et al 1999).

2.2. Animals

Eight females underwent ovariectomies of 3.5 years old were used with a body weight (BW) of 34.7 ± 3.01 kg (± SEM) and a body condition score (BCS) of 2.08 ± 0.08. The females were housed in group and were fed with alfalfa hay *ad libitum* (21% crude protein, 2.0 Mcal/kg of ME), and each female received 200 g of commercial concentrate per day (12% crude protein, 1.95 Mcal/kg of EM).

Sexually experienced goat bucks (*n* = 6) of approximately 3 years old were used. The bucks registered a BW of 67.7 ± 3.01 kg and BCS of 2.8 ± 0.10. The bucks were housed in group and were fed with alfalfa hay *ad libitum* (21% crude protein, 2.0 Mcal/kg of ME), each buck received 300 g of commercial concentrate per day (14% crude protein, 2.2 Mcal/kg of EM). Drinking water and mineral salts (12% phosphorus and 11% calcium) were supplied *ad libitum* for bucks and females.

2.3. Photoperiodic treatment of goat bucks

A photoperiod treatment of artificial long days was applied to bucks to induce an increase of testosterone secretion, sexual behavior, odor, and vocalizations during the natural sexual rest (March-April; Delgadillo et al 2002). During the photoperiodic treatment, lamps of 65 W with a light intensity of 300 lx at the level of the buck eyes. The treatment was applied for 2.5 months and consisted of 16 h light and 8 h darkness per day, starting on November 1 and ending on January 15. From January 16, bucks perceived the natural photoperiod (Figure 1). In the present study, a group of bucks without photoperiodic treatment of artificially long days was not included, because previous studies have shown that sexual behavior of untreated bucks is expressed to a minimum or is not observed during the natural sexual rest (Delgadillo et al 2002; Muñoz et al 2016).

2.4. Blood samples and testosterone assay

The bucks were subjected to a blood sampling by jugular venipuncture to determine plasma testosterone concentrations. Each sample was put in a 5 mL tube containing 30 µL of sodium heparin (5000 I.U./mL) as an anticoagulant. Blood samples were taken at 08:00 h every Friday from January 18, after photoperiodic treatment, until May 31 when the experiment ended. Blood samples were centrifuged at 3500 × *g* for 30 min, and plasma was stored in 1.5 mL centrifuge tubes at -15°C until the determination of hormone concentrations. Testosterone concentration was determined by Enzyme-Linked Immunosorbent Assay (ELISA, EIA-1559, DRG International, Inc., USA) according to the technique described and validated for goats by Gholib et al (2016). The sensitivity of the assay was 0.30 ng/mL. Samples were run in a single assay; the intra-assay coefficient of variation was 2.82%.

2.5. Goat buck sexual behavior

Sexual behavior of six bucks was recorded once a week in March and April (Figure 1), for 15 min at 2 h intervals along 24 h/day starting on Friday at 08:00 h and finishing Saturday at 06:00 h. During the darkness period of behavior assessment, artificial light was not used to maintain the experimental conditions. In each behavior test, the bucks were individually exposed to four ovariectomized females. Each group of females was placed in a pen (4 × 5 m). A solid fence separated the pens where every male was under observation during the behavior test with the group of females to avoid any interaction (smelling, seeing, or touching) with the other male. The testing pens were located at least 100 m far away from the housing pen. Females were interchanged each 2 h. At the time of entering the buck into the female pen, their sexual behavior was recorded. Each buck was observed individually recording sexual behavior such as anogenital sniffing, nudging, flehmen (Fernández et al 2018), and penis unsheathed (Ungerfel et al 2014).

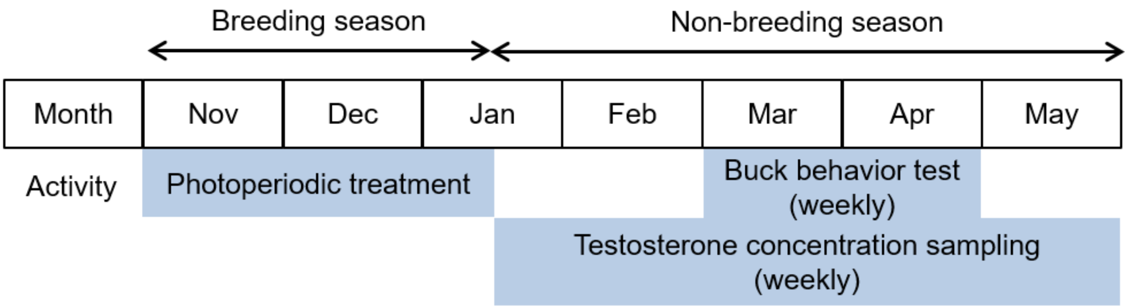

Figure 1 Experimental design depicting the dates when the photoperiodic treatment was applied to bucks, behavior tests and sampling of plasma testosterone. Bucks were exposed to ovariectomized females during non-breeding period. Bucks were submitted to a treatment of artificial long days (16 h of light and 8 h of darkness) from November 1 to January 15. Afterwards, bucks received the natural photoperiod. Buck sexual behavior was recorded weekly from March 1 to April 26. Blood samples were taken weekly from January 18 to May 31 to determine plasma testosterone concentration.

2.6. Environmental temperature, relative humidity, and temperature humidity index

At the time of entering each buck into the female pen, the environmental temperature (°C) and the relative humidity (%) were recorded using a thermo hygrometer (Steren) in each behavior test. The body temperature of each buck was taken rectally (each 2 h) using a digital thermometer (Neutek) when each behavior test ended. Experienced and trained personnel recorded sexual behavior of bucks, environmental temperature, relative humidity and body temperature, for this purpose individual lanterns were used for not disturbing animals.

The THI was calculated using the following equation (Ravangnolo et al 2000):

$$THI = (1.8 \times T + 32) - (0.55 - RH \times 0.0055) \times (1.8 \times T - 26)$$

where T is the environmental temperature (°C) recorded during each behavior test and RH is the relative humidity (%) recorded during each behavior test.

2.7. Ethical note

The protocol of the Official Mexican Rule that provides technical specifications for the production, care and use of laboratory animals (SAGARPA 2001) was followed.

2.8. Statistical analyses

Plasma testosterone concentration, anogenital sniffing, nudging, flehmen, penis unsheathed, and body temperature were carried out using the routines of Generalized Estimation Equations (GEE) implemented in the Generalized Linear Models procedure of SPSS version 20.0 (IBM SPSS 2011). The model included goat buck (as a subject variable) and, hour and day (as a within-subject variable). The Quasi-likelihood under Independence Model Criterion (QIC) and the Corrected Quasi-likelihood under Independence Model Criterion (QICC) were used to determine the best model, as well as, the probability distribution and the correlation matrix for intra-subject dependencies. Based on the results of QIC and QICC, the correlation matrix of first-order autoregressive [AR (1)] was used for plasma testosterone concentration, anogenital sniffing, nudging, and flehmen, whereas for penis unsheathed the unstructured correlation matrix was used. The Log link function for the distribution Gamma was used for plasma testosterone concentration and anogenital sniffing. The Log link function for the Poisson distribution was used for nudging. The Identity link function for the normal distribution was used for the penis unsheathed. The Log link function for negative Binomial was used for flehmen. The parameter estimation method was Fisher's Scoring and the scale estimate with maximum likelihood and the 95% Wald confidence interval for estimated marginal means was obtained. A simple contrast was used to compare the estimated means with a sequential, the Bonferroni test was used as a reference for the level of the first value of the day (January 18 for testosterone or March 1 for other variables) and the hour of the day (08:00). Data are expressed as the Estimated Marginal Means and standard error of the mean (mean ± SEM) and differences were considered significant when $P < 0.05$.

3. Results

3.1. Plasma testosterone profile

Plasma testosterone concentrations differed significantly over the analyzed experimental period ($P < 0.001$; Figure 2). A significant increase of testosterone level was observed the week 4 of February, after six weeks from the end of the photoperiodic treatment (≥ 13 ng/mL), keeping significant higher for the following seven weeks until the week 4 of April (≥ 5 ng/mL) where the testosterone level decreased to those observed the week 1 of January at the beginning of the experiment until the week 5 of May when the study ended (Figure 2).

3.2. Response of bucks to females

The highest frequency for nudging was at 08:00 but decreased along the day; from 20:00 decreased significantly compared to the first measurement ($P < 0.001$; Figure 3A).

Although at 06:00 the frequency of this sexual behavior increased, the analysis detected a significant difference concerning 08:00 ($P < 0.001$; Figure 3A). The results showed that the frequency of nudging increased over time, recording the lowest value at the beginning of the behavior test reaching the highest value on April 19 ($P < 0.001$; Figure 3A).

The results showed that the highest frequency of anogenital sniffing was expressed at 08:00, but decreased along the day from noon until 06:00 ($P < 0.001$; Figure 3B). Furthermore, the analysis detected that the frequency of anogenital sniffing showed a tendency to decrease over time, but from March 29 differ significantly concerning those registered at the beginning of the study ($P < 0.001$; Figure 3B).

The frequency of flehmen was low and did not differ along the day ($P > 0.05$; Figure 3C), although it was not observed at 20:00 ($P < 0.001$; Figure 3C). However, when this behavior was analyzed weekly, the results showed a tendency to increase its intensity over time ($P < 0.001$; Figure 3C), although on March 1 and 15 this behavior was not observed ($P > 0.05$; Figure 3C).

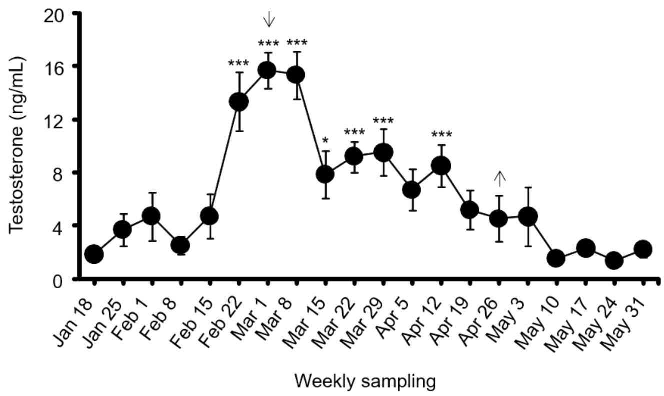

Figure 2 Plasma testosterone profiles (mean ± SEM) in sexually experienced photo-stimulated bucks. The arrows (↓↑) indicate when the bucks were exposed to ovariectomized females weekly during March and April. Bucks were submitted to a treatment of artificial long days (16 h of light and 8 h of darkness) from November 1 to January 15. Afterwards, bucks received the natural photoperiod. Blood samples were taken weekly from January 18 to May 31. *$P < 0.05$, ***$P < 0.001$; sequential Bonferroni test, simple contrast, reference category January 18.

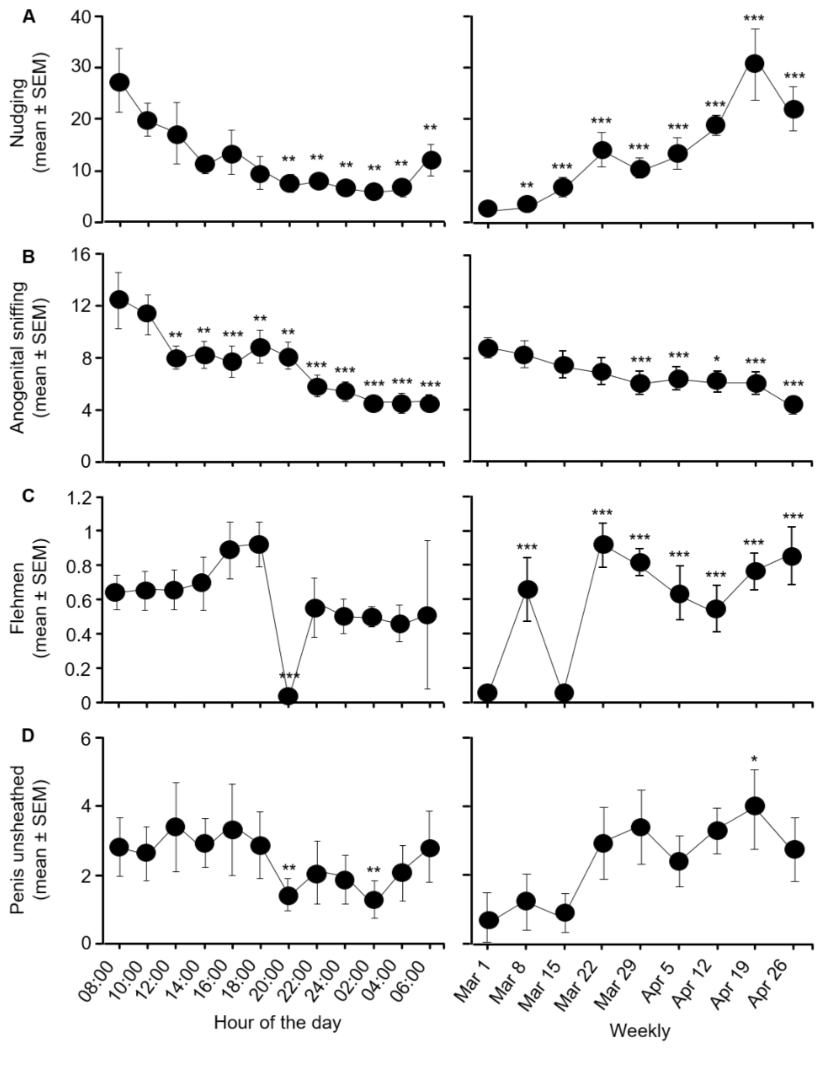

Figure 3 Sexual behavior of photo-stimulated bucks along the day and weekly during March and April. Bucks were submitted to a treatment of artificial long days (16 h of light and 8 h of darkness) from November 1 to January 15. Afterwards, bucks received the natural photoperiod. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$; sequential Bonferroni test, simple contrast, reference category at 08:00 h on March 1.

The frequency of penis unsheathed along the day was variable, although it was significantly lowest at 20:00 and 02:00 ($P < 0.05$; Figure 3D). During the measurement period, the results showed a tendency to increase over time, but on April 19 this sexual behavior was displayed with more frequency concerning those registered at the beginning of the study ($P < 0.001$; Figure 3D). In this study penis unsheathed was displayed by bucks for at least 14 sec.

3.3. Environmental temperature and relative humidity

Along the day the environmental temperature recorded a bell-like profile (Figure 4 top), the same was observed with the relative humidity but with an inverted profile (Figure 4 middle). Figure 4 (top) shows that at 06:00 the lowest environmental temperature was registered; on the contrary, at the same hour, the highest percentage of relative humidity also was registered (Figure 4 middle). The highest values for environmental temperature were registered from 14:00 to 18:00 (Figure 4 top), while at the same hour the lowest percentages of relative humidity were recorded. The Rho Spearman's correlations (Rs) revealed significant associations between environmental temperature and relative humidity (Rs = -0.78; $P < 0.001$).

Besides, when the environmental temperature was analyzed weekly, the lowest value was recorded on March 15 ($P < 0.001$); the environmental temperature dropped drastically due to a cold front compared to the previous week, increasing again the following week. The highest values for environmental temperature were registered on April 26 ($P < 0.001$; Figure 4 top). While for relative humidity the lowest values were registered on March 8 and April 12 (Figure 4 middle).

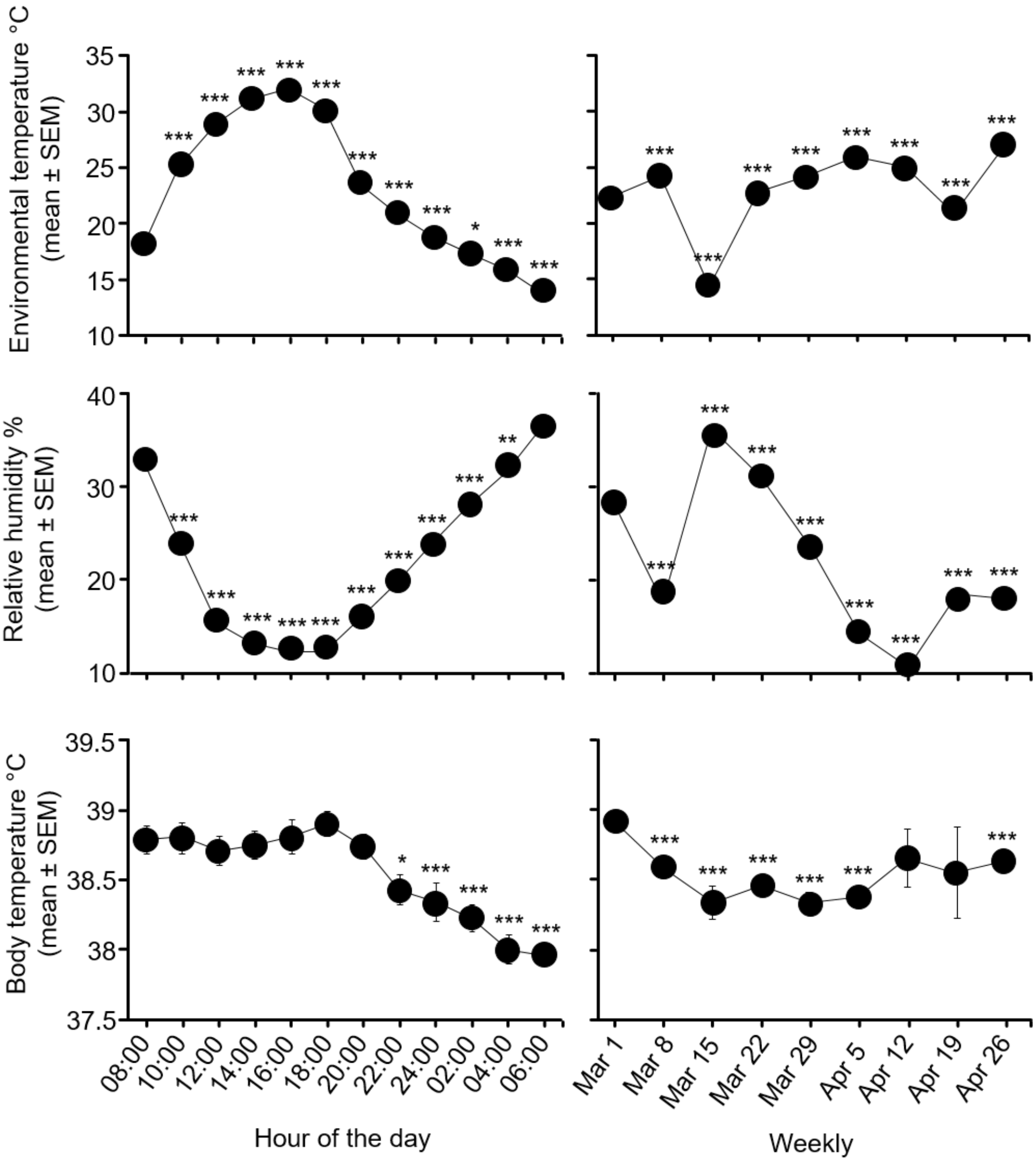

Figure 4 Environmental temperature (°C top), relative humidity (% middle), and body temperature of photo-stimulated bucks (°C bottom) along the day and weekly during March and April. Bucks were submitted to artificial long days (16 h of light and 8 h of darkness) from November 1 to January 15. Afterwards, bucks received the natural photoperiod. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$; sequential Bonferroni test, simple contrast, reference category at 08:00 h on March 1.

3.4. Body temperature from bucks and THI

The body temperature was similar from 08:00-16:00, although at 18:00 the highest value was registered (38.9 ± 0.09°C; Figure 4 bottom). Figure 4 shows that the environmental temperature (top), the body temperature (bottom), and THI (Figure 5) decreased at 20:00. In addition, the lowest values for body temperature were from 22:00-06:00 ($P < 0.001$; Figure 4 bottom). Whereas when the body temperature was analyzed weekly were detected differences between March 1 and the following weeks ($P < 0.001$), except on April 12 and 19 ($P > 0.05$). Figure 5 shows that on March 15 at 14:00 the THI was around 65 units, whereas on April 19 at 18:00 h was 70 units. By contrast on March 1, 8, 22, and 29, and on April 5, 12, and 26 the THI was higher to 71 units between 10:00 and 18:00. The Rho Spearman's correlations revealed significant associations between body temperature and environmental temperature (Rs = 0.47; $P < 0.001$), relative humidity (Rs = -0.28; $P < 0.001$), and THI (Rs = -0.48; $P < 0.001$).

4. Discussion

Our results indicate that photo-stimulated bucks increased plasma testosterone secretion during the natural sexual rest, showing a high variation of sexual behavior through 24 h/day when exposed to ovariectomized females, as well as, throughout the period of study. The results in this study show a relationship among the environmental temperature and the relative humidity and sexual behavior of photo-stimulated bucks. The present results showed a THI >71 units along the day, which indicated that photo-stimulated bucks experienced heat stress. Therefore, the current results are in accordance with the hypothesis stated.

Our results show that photo-stimulated bucks increased plasma testosterone concentration during March and April as was previously reported by Delgadillo et al (2002), in contrast to untreated bucks which do not present high testosterone secretion during the natural sexual rest (Delgadillo et al 2002). Furthermore, photo-stimulated bucks during the same breeding season increased the level of plasma testosterone even without exposing them to females (Ponce et al 2014). The present results show that our photo-stimulated bucks, sexual behaviors such as nudging and anogenital sniffing were negatively affected by the high environmental temperature. These sexual behaviors stimulate female sexual behavior (Fernández et al 2018). In the current study in March and April from 10:00 to 18:00, a THI between 71-77 units was recorded. In fact, in animal production, the thermal-neutral zone is considered with a THI <70, in these conditions the animal feels more comfortable and is more efficient, whereas a THI of 71-78 units indicates that the animals are in a state of alert, and a THI 79-83 indicates a danger category (Du Preez 2000). Likewise, heat stress occurs when these animals are exposed to higher environmental temperatures compared to those recorded in the thermal-neutral zone, this high temperature is the result of an increase in heat production due to increased body temperature (Du Preez 2000). Heat stress is described as the result of continuous environmental forces that act on the animal causing alteration of homeostasis (Al-Dawood 2017). Then, animals exposed to high ambient temperatures activate various physiological mechanisms to compensate for the adverse effects caused by heat stress (Berihulay et al 2019).

Our results show that photo-stimulated bucks displayed the highest frequency of nudging and anogenital sniffing during the mornings at 08:00 as mentioned above when the environmental temperature was lower than 18 °C, and the THI is around 59-66 units. Besides, the present results agreed with a previous study where the sexual behavior of Morada Nova rams was expressed with more intensity during the mornings (Gonçalves dos Santos et al 2015). In the present study, the frequency of sexual behavior is similar to those displayed by sexually experienced photo-stimulated bucks exposed to anestrous females through the male effect (Bedos et al 2014; Fernández et al 2018). Sexual behavior as nudging and anogenital sniffing are essential components to induce sexual activity in seasonal anestrous does (Loya-Carrera et al 2014; Muñoz et al 2016). In this study, sexual behaviors as flehmen and penis unsheathed showed high variability along the day. Perhaps, photo-stimulated bucks showed high variability in the flehmen response due to they did not receive the olfactory signal in urine from ovariectomized females to increase their frequency (Mirto et al 2017). It is worthy to remark another potential factor which could influence flehmen response. This behavior was not observed on March 15, the day when the lowest ambient temperature and the highest relative humidity were registered. Similarly, our results indicate that males showed high variability in the frequency of penis unsheathed observed all day, as also was reported by Ungerfel et al (2014).

In the present study, the highest body temperature was 38.9 ± 0.09 °C, which was recorded at 18:00 h, when the environmental temperature also was the highest 32.0 ± 0.06 °C showing that both THI 71-77 units and high body temperature affected sexual behavior of photo-stimulated bucks. In goats, the normal body temperature is 38.5 °C (Ayo et al 1998), therefore, an increase of 0.4 °C above their normal body temperature negatively affected the expression of sexual behavior of photo-stimulated bucks.

In goats, previous studies indicate that they show tolerance to heat when they are in environmental temperatures from 20 to 35 °C (Lu 1989). In addition, in small ruminants such as sheep and goats, previous studies indicate that the high temperatures in combination with high relative humidity induce heat stress which decreases sexual behavior and, semen and oocyte quality (Lindsay 1969; Al-Tamimi 2007; Marai et al 2008).

In the present study, when the environmental temperature decreased in the evening, the body temperature decreased, and the sexual activity of the photo-stimulated bucks also decreased. Then, this fact can be the response of two causes: firstly, goats as diurnal species they need to rest through the night and, secondly, these bucks need to dissipate the thermal load accumulated during the day (Berihulay et al 2019). Therefore, one of the abilities of ungulates is their thermoregulation as a mechanism of adaptation to high environmental temperatures. Animals have peripheral thermoreceptors that send a signal to the central nervous system which activates the preoptic area of the hypothalamus to activate physiological mechanisms and increase vasodilation, respiratory rate, heart rate, to dissipate the thermal load (Baker 1989; Al-Dawood 2017). Although the environmental temperature is highly variable, some factors are involved such as relative humidity, wind speed, the intensity of sunlight, time of year, body size, age, color, sex, body fat, isolation through hair, and adaptation of animal (Collier et al 1982; Berihulay et al 2019).

Even though goats are heat tolerant as mentioned above, our results show that photo-stimulated bucks males experienced sudden changes in ambient temperature and ambient humidity in March and April, during the transition from Winter to Spring. Then, the present results show that both the environmental temperature as the relative humidity were highly variable throughout the study in conditions of the Chihuahuan Desert from Mexico (CONAGUA 2013). Finally, our findings show that climate affects those species that manifest a seasonal pattern of reproduction as those located in subtropical latitudes (Bronson 2009; IPCC 2018).

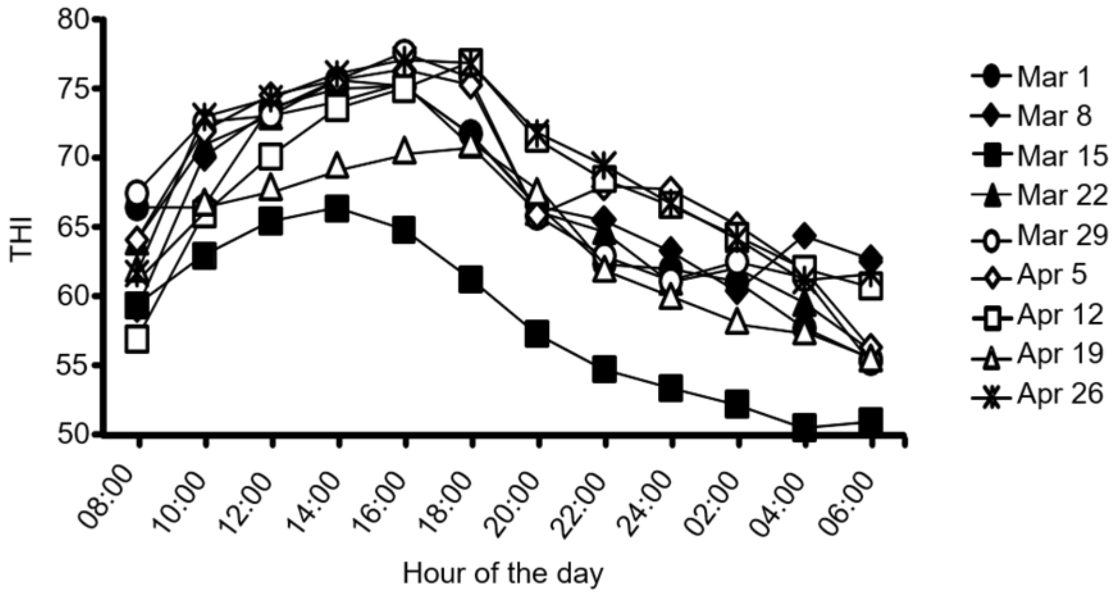

Figure 5 Temperature humidity index (THI) along the day and weekly during March and April. The environmental temperature and relative humidity were recorded in each behavior test in photo-stimulated bucks. Bucks were submitted to a treatment of artificial long days (16 h of light and 8 h of darkness) from November 1 to January 15. Afterwards, bucks received the natural photoperiod.

5. Conclusions

Photo-stimulated goat bucks showed a high variation of sexual behavior during 24 h/day in March and April when exposed to ovariectomized females, and these sexual activities were affected by the high environmental temperature and the low relative humidity throughout the study.

Acknowledgments

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Conflict of Interest

The authors declare that they have no conflict of interest.

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