

ABSTRACT

Ten male Merghoz goats with initial live weights of 29.2 ± 1.2 kg (Mean \pm SE) were used to investigate the effect of natural changes in photoperiod on dry matter intake (DMI) and reproductive activity during a 365 day study. The bucks were housed in individual pens, under natural daylength condition at west of Iran, Kermanshah (34° 18' N and 47° 3' E and 1420 m above sea level). A diet was offered with an estimated concentration of metabolizable energy and crude protein of 7.9 MJ and 98.8 g per kg dry matter (DM) respectively. Live weight, DMI, and additive daily gain (ADG) of individual animals were recorded weekly. The serum testosterone concentrations were determined in blood samples collected at monthly intervals. During the observation period, semen was collected monthly with the aid of an electro-ejaculator and examined microscopically immediately after collection. The physical parameters of semen and the semen index were recorded. The sexual behavior and testicular size of goats were assessed monthly throughout the study period. The highest DMI was observed during summer (64.3 ± 0.9 g/kg BW^{0.75}) and spring (62.2 ± 2.9 g/kg $BW^{0.75}$) however, the lowest was recorded in the winter season (54.0±0.9 g/kg $BW^{0.75}$). The seasonal values of ADG (g/d) ranked spring > summer > winter > autumn. Bucks demonstrated the highest sexual behavior in late summer and, thereafter during autumn, using the lower (P<0.05) number of mounts per ejaculation and a higher frequency (P < 0.05) to sniff, approach, and vocalize a doe with estrous status. This was coincided with a higher (P<0.05) level of plasma testosterone (8-10.1 ng/mL) and the best semen quality (semen index: 744-989×10⁶/mL) and quantity (semen volume: 1.1-1.2 mL per ejaculate), higher testis length (13.3-14.7 cm) and width (6.0-6.1 cm) and scrotal circumference (32.2-35.5 cm). It is concluded that, in Merghoz bucks, the seasonal pattern of DMI and reproductive activity may be attributed to each other as well as photoperiod.

KEY WORDS dry matter intake, Merghoz buck, natural photoperiod, reproductive activity, sexual behavior.

INTRODUCTION

It is well known that the productivity of livestock can be affected by the seasonality of reproduction and feed intake. Several studies reported a decline in reproductive activities and, quality and quantity of semen during the non-breeding season in male goats (Barkawi et al. 2006; Talebi et al. 2009; Zarazaga et al. 2009). Similarly, seasonal change in feed intake has been reported previously in several species (Rhind et al. 2002). It has been demonstrated that the photoperiod is the major factor for seasonality of reproduction and feed intake especially at higher latitudes (Delgadillo et al. 1993; Rhind et al. 2002). Nonetheless, other parameters such as management, environmental factors (temperature and humidity), availability of food, and social interactions can also influence the seasonal pattern of reproduction and

feed intake (Rhind *et al.* 2002; Walkden-Brown *et al.* 1993; Mani *et al.* 1996). Studies demonstrated that the seasonality of feed intake is a function of physiological changes and do not simply reflect a seasonal reduction in food availability (Barry *et al.* 1991; Iason *et al.* 2000). In fact, this phenomenon is considered to have evolved as an adaptation to regular seasonal reductions in food availability, so that the animal's drive to search for food is reduced when supply is reduced and less energy is expended in foraging (Kay and Staines, 1981).

Merghoz goat is reared in west of Iran as a seasonal breeder goat. In this particular area, the majority of goats are maintained in extensive or semi-extensive systems, and subject to seasonal variations in food availability, hence, it is often thought that the nutrition and photoperiod are responsible for the seasonal reproductive pattern.

Very recently, we have demonstrated that the seasonal variation of hair follicles activity, fibre growth and fibre diameter produced by Merghoz goats are associated with fluctuation of DMI (Mirmahmoudi *et al.* 2011). However, there is no information on concurrent study of seasonal changes of DMI and reproductive activity of this goat breed. This could be of value in a number of ways. For instance, if there is a concurrent seasonal pattern of DMI and reproductive activity, then efficiency of food given to animal could vary during the year, and most appropriate diets or supplementation strategies will differ among seasons.

Hence this study was conducted to investigate a) whether or not there is a true seasonal pattern of DMI in the Merghoz bucks independent of natural variations in food availability and b) whether or not this seasonality is simultaneous with the natural changes of reproductive activity.

MATERIALS AND METHODS

Location and duration

The study was conducted on the Animal Farm, Faculty of Agriculture, Razi University, Kermanshah, Iran (34° 18' N and 47° 3' E and 1420 m above sea level) for a period of one year (started June 21 and ended June 21). The year was divided into the four geographical seasons based on the solar calendar, with winter (from 21 December to 21 March), spring (from 21 March to 21 June), summer (from 21 June to 21 September) and autumn (from 21 September to 21 December). Climatologically, the data for this location during the experimental period are summarized in Table 1. The meteorological station was about 3 km far away from the farm.

Animals

Ten male healthy Merghoz buck aged about 21 months with initial live weight of 29.2 ± 1.2 (Mean \pm SE) kg were ran-

domly selected from the herd maintained at Faculty of Agriculture, Razi University, Iran. All animals were housed in individual pens (dimension: 2×1.5 m) and were subjected to natural lighting via windows and skylights. A forty five day period has been given to animals for adaptation before onset of the experiment. A general management program for de-worming, disease prevention, and hoof trimming was followed throughout. All animals had free access to fresh water and mineral blocks containing trace elements and vitamins.

Measurements

DMI, ADG and live weight

All bucks were fed a diet consisting of 80% dehydrated alfalfa hay and 20% concentrate (based on barley, minerals and vitamins), providing 7.9 MJ and 98.8 g per kg DM ME energy and crude protein, respectively. They were offered an amount of feed according to their live weight, to meet the estimated requirements for a rate of increase in live weight (50 g per day) and 2 kg mohair production per year (NRC, 1996). The diet was offered daily in two equal portions at 09.00 h and 16.00 h.

The daily food refusals for each individual goat were collected, weighed and a sample (200 g) was taken to the laboratory for estimation of dry matter and subsequently ADG (g/day).

Live weight and DMI of each individual buck were recorded weekly. The amount of feed offered to each goat was adjusted following weekly measurement of live weight.

Blood sampling and hormone assay

The monthly blood samples were collected from the jugular vein of goats into 10 mL vacutainer tubes (Prand, Japan) during the entire observation period. Sera were separated after centrifugation at $2500 \times \text{g}$ for 20 min and stored at -20 °C, until testosterone estimation, using coated tube kits (Demeditec Diagnostic GmbH, Germany). The sensitivity of the assay for serum testosterone was 0.083 ng/mL. The intra- and inter-assay coefficients of variation were 4.16 and 9.94%, respectively.

Semen collection and evaluation

The semen were collected at monthly intervals from individual buck using an electro-ejaculator (Model 66000-D, Nasco, WI, USA)-with a series of short electrical stimuli (approximately 5 s) being administered at 20 s intervals (Buckrell *et al.* 1994; Belibasaki and Kouimtzis, 2000). Fresh semen samples were evaluated immediately according to Evans and Maxwell (1987). The semen volume was measured as per graduated collection vial and a pH-meter (Testo-230-GmBH, Germany) was used to record the seminal pH.

Table 1 Climatological data for the experimental period, obtained from the Meteorological Laboratory of the Kermanshah province

	Ambient to	emperature (°C)		Relative h	numidity (%)		Daylength (h) season
	Mean	Min	Max	Mean	Min	Max	
Summer	29.7	22.5	33.7	25.4	22.0	32.5	13: 33
Autumn	15.1	5.6	24.3	56.9	25.0	87.0	10: 44
Winter	9.4	0.0	15.4	62.5	44.0	82.5	10:40
Spring	20.7	11.6	30.3	43.7	23.0	65.5	13: 24

Subsequently, individual semen sample was microscopically analyzed for sperm concentration, motility and integrity. Sperm concentration was determined using a haemocytometer following dilution of an aliquot of semen with 0.05% formalin or normal saline (1:400). The overall number of sperm per ejaculate were then calculated (volume×density). For the assessment of the gross and sperm progressive motility, semen diluted with 0.1 M sodium citrate solution (pH 6.7-6.9) was transferred onto a warm glass slide (37 °C), mounted with a cover slip and examined under a light microscope (400×magnification) on a scale of 0-5 for gross motility (0: no discernable motion; 1) weak undulated or oscillatory motion; 2) slow progression, including a stop and start motion; 3) steady progressive motion; 4) rapid progressive motion and 5) very rapid and vigorous motion) and on a continuous scale of 0-100% for progressive motility (Evans and Maxwell, 1987). Semen smears were prepared for evaluation of morphological sperm integrity after staining with an eosin-nigrosin stain. Sperm were categorized as intact or abnormal cells, according to Barth and Oko (1989). The proportion of live sperm was determined by counting the unstained sperm and a semen index (semen volume×sperm concentration/mL×live sperm %×progressive motility %) was calculated, as a semen quality indicator.

Assessment of sexual behavior and testicular size

In a monthly schedule, all bucks were examined in the presence of one sexually receptive female goat for estimating the expression of different sexual behaviors viz. anogenital sniffing, approaching, vocalization, number of mounts (per ejaculation), and ejaculation latency. Concurrently, scrotal circumference was measured using a tape measure, and the combined testes width, and testis length in the scrotum determined with aid of a caliper.

Statistical analysis

Data on the sperm volume, total sperm concentration per ejaculate, sperm and serum testosterone concentration, semen index, semen pH, sexual behavior, and scrotal circumference were tested for homogeneity of variance. Data found not to be homogeneous were modified by application of a square root or log₁₀ transformation where appropriate. All data were subjected to repeated measures ANOVA using Proc Mixed of the SAS (SAS, 1996). Live weight was included in the model as the covariate. For this purpose, the AR (1) covariance structure was selected, based on the Schwarz Bayesian criterion. Least squares means and standard errors are quoted in the text.

RESULTS AND DISCUSSION

Effect of season on DMI, ADG and live weight

The seasonal and monthly changes of DMI, ADG, and live weight are presented in Table 2 and Figure 1. There was observed a natural seasonal change in DMI, ranging from 54.0 g in winter to 64.3 g in summer, with the annual mean of 59.7 g.

Although there was a gradual increase in live weight of the goats from summer until spring, the changes in the DMI and ADG were declined from late summer until mid-winter and thereafter increased until late spring which was coincident with the natural changes of daylength (Table 2 and Figure 1).

Table 2 Least-squares mean (±SE) of live weight (kg), average daily gain (ADG) (g/day), dry matter intake (DMI) (g/kg BW0.75), ejaculate volume (mL), sperm concentration (×109/mL), total spermatozoa (×106/mL), serum testosterone (ng/mL) and serum testosterone concentration of Merghoz bucks in different seasons

14	Season						
Item	Summer	Autumn	Winter	Spring			
Live weight (kg)	$30.7{\pm}0.2^{d}$	33.3±0.3°	35.4±0.3 ^b	38.5±0.2ª			
ADG (g/day)	$39.9{\pm}1.4^{\rm b}$	23.0 ± 2.2^{d}	30.1±1.9 ^c	$48.4{\pm}1.7^{a}$			
DMI (g/kg BW ^{0.75})	64.3±0.9 ^a	58.2 ± 0.8^{b}	$54.0\pm0.9^{\circ}$	62.2 ± 2.9^{a}			
Ejaculation volume (mL)	1.1 ± 0.03^{a}	$1.2{\pm}0.06^{a}$	0.7 ± 0.03^{b}	0.7 ± 0.03^{b}			
Sperm concentration (×10 ⁹ /ML)	$0.9{\pm}0.1^{b}$	1.0±0.1 ^b	1.2±0.1ª	1.3±0.2 ^a			
Total spermatozoa (×10 ⁶ /mL)	1010 ± 0.6^{a}	1162±113 ^a	859±83.9 ^b	877 ± 138^{b}			
Serum testosterone (ng/mL)	$10.1{\pm}1.1^{a}$	$8.0{\pm}1.2^{a}$	$2.6{\pm}0.4^{b}$	$2.9.0{\pm}0.4^{b}$			

The means within the same row with at least one common letter, do not have significant difference (P>0.05).

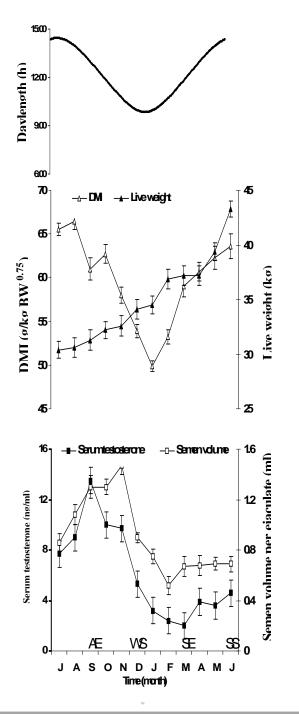


Figure 1 The monthly changes of serum testosterone concentration (ng/mL), semen volume (mL), DMI (g/kg $BW^{0.75}$) and live weight (kg) (least-square Means±SE), and daylength (h)

AE: autumn equinox; WS: winter solstice; SE: spring equinox and SS: summer solstice

Effect of season on semen quality and quantity, and serum testosterone concentration

The seasonal changes in semen quality and quantity, and serum testosterone concentration of Merghoz buck are presented in Table 2 and 3. Semen volume per ejaculation, sperm concentration, total spermatozoa, serum testosterone, gross motility, progressive motility, live sperm, and semen index were significantly (P<0.05) higher in the seasons of summer and autumn than those recorded in the winter and spring seasons (Table 2 and 3).

In contrast, the greatest sperm abnormality was observed in the winter and spring seasons. The highest and lowest values for semen pH were observed in the seasons of spring and autumn, respectively (Table 3). The monthly pattern of serum testosterone concentration and semen volume per ejaculation are presented in Fig. 1. Concurrent with gradual decline in the natural daylength from September (Autumn Equinox) until early winter (Winter Solstice), both serum testosterone concentration and ejaculate volume were also decreased and thereafter moderately increased until late spring, at which daylength was the maximum; summer solstice (Figure 1).

Effect of season on testis length and width, scrotal circumference and the sexual behaviors

The seasonal changes of testis length and width, scrotal circumference, and expression of sexual behaviors in Merghoz buck are presented in Table 4. The testis length was measured as maximum in the autumn season, and the highest testis width was measured in summer and autumn seasons (Table 4). The seasonal values of scrotal circumference ranked autumn > summer > winter > spring. The shortest latency to ejaculation was recorded during the autumn season and the longest during the spring season (Table 3). Similarly, the lowest and highest values for mounting (per ejaculation) were observed in the autumn and spring, respectively (Table 3). Moreover, the bucks tended to sniff, approach, and vocalize toward sexually receptive doe with higher frequency rates during the autumn and summer in comparison with the seasons of winter and spring (Table 3).

In the current study, live weight of the bucks increased gradually during the study. This is most likely the consequence of growth of the bucks, which were only 21 months old at the beginning of the study. Several parameters including cold and heat stress can influence nutrient requirements of goats (Sahlu *et al.* 2004).

While we fed goats to gain 50 g/day, the lower attained growth rate of 35.4 g/day may reflect either a higher maintenance energy requirement of these bucks perhaps related to cold stress or to higher rates of fat deposition than expected. We do not have data on either of these aspects of energy balance.

Sharma *et al.* (1998) suggested that overall feeding pattern in goats is related to photoperiod, with larger, more frequent meals during the daylight hours. It has been also shown that some breeds of sheep exhibit clear seasonal cycles of feed intake when penned and offered standard diets to appetite (Gordon, 1964; Blaxter *et al.* 1982).

 Table 3
 Least-squares mean (\pm SE) of gross motility (1-5), progressive motility (%), live sperm (%), abnormal sperm (%), semen pH and semen index (×106/mL) of Merghoz bucks in different seasons

Item	Season						
Item	Summer	Autumn	Winter	Spring			
Gross motility (1-5)	4.3±0.1 ^a	$4.4{\pm}0.2^{a}$	$4.0{\pm}0.2^{b}$	3.3±0.3 ^b			
Progressive motility (%)	$81.1{\pm}1.9^{a}$	$82.7{\pm}1.6^{a}$	70.3±3.5 ^b	69.1 ± 4.4^{b}			
Live sperm (%)	87.9 ± 1.2^{a}	90.2±0.8ª	83.3±1.5 ^b	79.4±3.5 ^b			
Abnormal sperm (%)	$9.0{\pm}0.4^{\rm b}$	$4.9{\pm}0.4^{\circ}$	10.9 ± 0.8^{a}	12.0±0.4ª			
Semen pH	7.1±0.05 ^c	7.0 ± 0.03^{d}	7.2±0.06 ^b	7.3±0.06 ^a			
Semen index ($\times 10^{6}$ /mL)	744 ± 54.3^{b}	989±106 ^a	503±52.4°	600±136 ^c			
he means within the same row with at least on	e common letter, do not have significan	t difference (P>0.05).					

Table 4 Least-squares mean (±SE) of testis length (cm), testis width (cm), scrotal circumference (cm), ejaculation latency (sec), and sexual behaviors

expression	(0-10)	of M	erghoz	bucks	in	different	seas	sons	\$

14	Season						
Item	Summer	Autumn	Winter	Spring			
Testis length (cm)	13.3±0.2 ^b	14.7±0.1ª	13.1±0.2 ^b	13.0±0.2 ^b			
Testis width (cm)	$6.0{\pm}0.1^{a}$	6.1±0.1 ^a	5.3±0.1 ^b	5.2±0.1°			
Scrotal circumference (cm)	32.2±0.1 ^b	35.2±0.2ª	29.2±0.3°	29.3±0.2°			
Ejaculation latency (sec)	$67 \pm 5.8^{\circ}$	31±7.2 ^d	92±8.1 ^b	124±10.3ª			
Number of mounts per ejaculation	$1.4{\pm}0.05^{\circ}$	$1.1{\pm}0.08^{d}$	1.9±0.03 ^b	2.6±0.05 ^a			
Frequency of:							
Anogenital sniffing	20.4 ± 6.3^{b}	48.5 ± 8.4^{a}	7.0±3.6°	4.2±2.1°			
Approach	17.2±4.8 ^b	$49.4{\pm}8.7^{a}$	6.2±2.3°	$4.9{\pm}2.2^{\circ}$			
Vocalization	16.3±4.6 ^b	34.9 ± 7.3^{a}	4.2±2.1°	$3.9{\pm}1.9^{d}$			

The means within the same row with at least one common letter, do not have significant difference (P>0.05).

This may perhaps be regarded as reflecting the nutrients demands arising not only from seasonal reproductive activities but also from photoperiodic growth cycles (Forbes, 1982). In this study, despite with the gradual increase in live weight, there was a distinct decline in DMI in autumn and winter, and thereafter, a linear increase from midwinter until late spring. These suggest a concurrent pattern of DMI in Merghoz bucks and seasonal changes of daylength, which is independent of food availability. The results of the present study show that male Merghoz goats in west of Iran at latitude of 34° 18' N exhibit a large seasonal variation in sexual activity. Testosterone secretion and the testicular size (testis length and width), the good predictors of sperm production at the testis level (Walkden-Brown et al. 1994; Delgadillo et al. 1995) exhibited significant changes during the study, with the highest values during the months of September, October, and November. Breed and individual differences in scrotal circumference and testis length and width have been found in bucks (Pandey et al. 1985; Noran et al. 1998). Similar to Rayini (Zamiri and Heidari, 2006), British (Ahmad and Noakes, 1995) and Damascus (Al-Ghalban et al. 2004) bucks, the largest value for scrotal circumference of Merghoz bucks was observed in late summer and during autumn. Figure 1 shows that the maximum testosterone secretion was occurred about 60 days earlier than the maximum semen production. This is totally understandable because a previous testosterone secretion and growth of testicular structures are the prerequisite for high semen production (Barkawi et al. 2006).

Changes in testis size can to some extent be explained by variations in body weight, however, it has been reported that seasonality may have a greater influence on testis size than body weight (Delgadillo *et al.* 1991; Ahmad and Noakes, 1995).

On the other hand, Howland *et al.* (1985) reported no seasonal effect on testis size of Pygmy bucks in Canada, although peripheral testosterone concentration was highest in autumn. In the yearling British goats (51° 46′ N), the increase in peripheral testosterone concentration and testis size preceded the improvement in libido by a month (Ahmad and Noakes, 1995). In the current investigation, despite with the gradual increase of body weight throughout the year, a seasonal pattern of reproduction has occurred, which explain that in the Merghoz bucks; the seasonality has more influence on reproductive activity rather than the body weight.

In this study, the semen parameters recorded, were similar to those reported by Barkawi *et al.* (2006) on Zaraibi goats. The ejaculation volume was the highest in autumn and summer and decreased to a minimum in spring and winter. Different patterns of seasonality in ejaculation volumes have been reported (Karagiannidis *et al.* 2000; Al-Ghalban *et al.* 2004).

The sperm concentration of Merghoz bucks investigated in this experiment followed a trend opposite to that of the ejaculation volume, reflecting seasonal variations in the secretion and release of the seminal plasma by the accessory glands. The glands were more active when the concentration of testosterone is high during the breeding season and quiescent when the testosterone levels are low during the non-breeding season (Corteel, 1977).

The total sperm output (volume×concentration) followed a pattern similar to that of the ejaculate volume-being higher during summer and autumn. The high sperm output produced in autumn season by Merghoz bucks is in agreement with the results of Barkawi *et al.* (2006) and Karagiannidis *et al.* (2000). Mass and progressive sperm motility was lower during spring and winter in this study, which is consistent with Karagiannidis *et al.* (2000) and Barkawi *et al.* (2006). The percentage live sperm was highest during autumn and summer, while dead sperm values were highest during winter and spring. The same trend has been reported by Barkawi *et al.* (2006). In the present study, the lowest percentage of abnormal sperm was observed in autumn, which is in accordance with the results reported by Karagiannidis *et al.* (2000).

Semen pH values in the present study were slightly alkaline and above those reported for Rayini goats (Zamiri and Heidari, 2006), and Zaraibi goats (Barkawi *et al.* 2006). It was the highest in spring and the lowest in spring season. Overall, the semen index which is considered as semen quality was the highest in autumn and summer and the lowest in the winter and spring.

Additionally, Merghoz bucks exhibited a seasonal cycle of sexual interest and libido in association with perception of doe estrous status. This was underlined by shorter ejaculation latency, lower mounts per ejaculation and high frequency rates of sniffing, overlapping, and vocalizing toward sexually active female goat from late summer and during autumn. Similar seasonal pattern of sexual behaviors were reported by Delgadillo et al. (1999) on male Creole goats. Altogether, these data indicate the existence of a well-defined breeding season (late summer and during autumn) characterized by high testicular size, testosterone secretion, semen quality and quantity, and expression of sexual behaviors. Since all these changes in reproductive activity as well as DMI were observed despite the animals receiving a constant diet throughout the experiment, these data indicate that the season has a strong influence on the reproductive physiology of Merghoz bucks, independently of the availability of food resources.

CONCLUSION

In conclusion, the findings of this study demonstrate that male Merghoz goats reared in west of Iran, that were fed constantly throughout the year, exhibit large seasonal variations in sexual activity, with an intense of sexual activity occurring from September to December. Therefore, food availability does not appear to be the major factor influencing sexual activity. Moreover, these bucks exhibited a real seasonal cycle of dry matter intake which itself may had an indirect influence on the seasonal pattern of reproductive activity and vice versa. However, both seasonal patterns of dry matter intake and reproductive activity are most probably affected and synchronized by the direct impact of the photoperiod.

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