## **REGULAR ARTICLES**

# Study of male effect on feeding and estrus behavior of Afshari ewes

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Abstract This study was conducted to evaluate the male effect on the manifestation of estrus and feeding behavior of Afshari ewes during their breeding season. The study consists of 48 Afshari ewes, 3 years old, 67±2 kg live weight, body condition score 3, along with 10 Afshari rams. The study was for a period of 6 weeks in a complementary randomized design. Ewes were equally divided into three treatments  $(T_1,$  $T_2$ , and  $T_3$ ) along with a control ( $T_4$ ) with six animals in each group. Variable factors of treatments was the distance of the ram box (from the ewes), which was determined to be the  $T_1$ (0-5 m), T<sub>2</sub> (10–15 m), and T<sub>3</sub> (25–30 m). Exposure of the ewes to the rams resulted in an earlier manifestation of estrus signs (p < 0.05). Moreover, the total recorded estrus signs were significantly affected by the distance from the rams (p < 0.05). The result of this study showed differences in feed intake of the ewes due to the distance from the rams (p < 0.05). In the other words, the distance of the ewes from the rams significantly affected feed intake of the Afshari ewes.

Keywords Afshari ewe · Feed intake · Male effect · Estrus

# Introduction

Among social behaviors of cattle, feeding behavior is the most critical for domesticated animals. In many developing countries, to increase production efficiency, manufacturing systems tend to industrialization and semi- industrialization, because

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industrialization helps in increasing production and benefits (Miranda-de la Lama and Mattiello 2010. Animals behave based on their environment and the extent of composure provided for them (Metz and Wierenga 1997). Factors such as hormone variations (roles of leptin and ghrelin hormones), environmental conditions, and presence or absence of opposite sex can influence dry matter intake. Central neural system plays a key role in regulating hormone secretion. The system itself is affected by hormones and external factors such as light, noise, odor, feeding, environmental temperature, and pheromones. As chemical signals, small amounts of pheromones play a key role in expressing animal behavior such as attracting the opposite sex, mating, determining territory, and distinction and selection of other animals (Okamura and Mori 2005 Ewes feeding behavior is directly influenced by estrogen hormone secretion. This hormone level will be affected by ram's presence, its testosterone, and sexual behaviors (Hawken and Martin 2012). Maximum ovulation and positive reproductive response in female goat occurred when they are among male goats and (or) in separate boxes but close to them (Bedos et al. 2010; Hamada et al. 1996). Pheromones related to the opposite sex are small molecules that are rapidly dispersed in the environment. These molecules end the period of anestrus, thus leading to the resumption of another estrous cycle and fertility (Cohen-tannoudij and Signoret 1987). In mating season, females select superior males depending on the amount of testosterone they produce. Females have potential to identify and distinguish males' behavioral signals (Longpre and Katz 2011). Results from other studies indicate direct relationship between pheromone production in male and blood testosterone level (Okamura and Mori 2005. In female mammals, pheromone is transferred by a secondary smell organ, vomeronasal organ (VNO), which is connected to the nasal cavity by a narrower channel. Pheromones move between the above two parts (secondary smell organ and narrower channel) and induce neural signals. These signals

in central neural system create GnRH/LH signals. Finally, they result in the termination of anestrus season, and the simultaneous occurrence of estrus and effective fertility (Delgadillo et al. 2009; Iwata et al. 2003). Estrus manifestation together with blood testosterone level increase in females which usually accompanies feeding behaviors and results in the reduction of food consumption (Schmidt et al. 1988). Although the mechanism is barely known, however, it seems estrogen reduces feeding by affecting adipocyte tissue metabolism (Forbes 2007). The effect of male on estrus synchronization includes more rapid beginning of sexual cycle in reproduction season and manifestation of further estrus in Markhoz goat. The distance between male and female goats and the amount of pheromone produced have significant effect on female dry matter intake (DMI) during seasonal mating (Mohamadi et al. 2011). Hawken and Martin (2012)) reported that ram excites and increases ewes' sexual activity by increasing the LH in them which finally leads to an increase in estrogen (Fig. 1). So far, no study has been done in determining the effect of distance of pheromone source or effect of male animal on the feeding behavior of the ewes. Hence, this study examines whether the distance between ewes and rams can induce a different DMI in these animals.

#### Materials and methods

This study was carried out using 48 Afshari ewes (3 years of age,  $67\pm2$  kg, body score 3) and 10 rams existing in Kashan Afshari Sheep Breeding Center. This center has an altitude of 1373 m with a mean annual temperature of  $30C^{\circ}$ . The experimental period was 6 weeks (from late August to late September (mating season)). Experiments were conducted at two separate saloons. The first and the main section where test was carried out consisted of seven  $3\times5$  m boxes. The distance between ewes and rams were considered as test treatments, so, five rams were placed in the middle box and ewes were placed

Fig. 1 Impact of male effect on ewes feed intake through hormone mediators (Hawken and Martin 2012 Table 1 Percentage of manifestation of estrus in ewes

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Week	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>2</sub>	$T_4$	$\chi^2$	$p > \chi^2$
1	0	0	0	0	0	_	_
2	33.33	16.67	0	16.67	0	8.38	0.04
3	66.67	58.33	33.33	58.33	25	5.70	0.18
*	100	74.99	33.33	74.99	25	21.23	0.02
4	16.67	16.67	33.33	16.67	25	1.29	0.87
5	16.67	16.67	25	16.67	16.67	0.41	0.99
6	16.67	16.67	25	16.67	33.33	1.98	0.58
Total	149.99	125	116.66	125	100	7.90	0.035

\*The first time that manifestation of estrus in one of the groups reached 100 %

 $T_1$ , 0–5 m away from the rams box;  $T_2$ , 10–15 m away from the rams box;  $T_3$ , 25–30 m away from the rams box;  $T_4$ : ram and ewe mix

in boxes in 0-5 m (T<sub>1</sub>), 10-15 m (T<sub>2</sub>), and 25-30 m (T<sub>3</sub>). Then, for each distance (test treatments), two boxes or replicates were considered with six ewes in each box (total of 36 ewes). Fourth treatment (T<sub>4</sub>) or control group was placed in the second saloon. This saloon included a mix of ewes and rams. Yet, two boxes in the same size with six animals were kept separated from other animals. That is, like T<sub>1</sub>, the distance between them and free rams was practically about 0-5 m. Animals in all test groups received the same basic ration in form of total mixed ration (TMR) and had open access to water. Ration contained hay (14 % protein) 55 % of total ration, wheat straw (15 % of total ration), barley (15 % of total ration), and pellet concentrate (12 % protein (15 % of total ration)). Formulated ration contained 2.41 MGCal/kg metabolic energy, 11.7 % raw proteins, 3.75 g/day calcium, and 1.75 g/day phosphorus for animals. In each treatment, the number of ewes manifesting estrus signs and behaviors were recorded three times every day. Estrus signs included the following: reduction in feeding, standing still, mucus in vulva, and rams' jump on each other and making noises (Hafez and

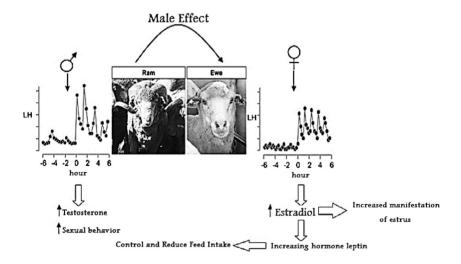
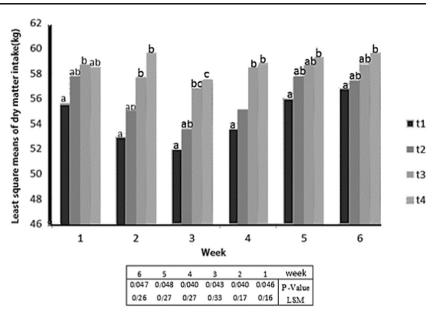


Fig. 2 Least square means of dry matter intake (kg), Numbers or values within columns of each week with different superscripts are different (p < 0.05)



Hafez 2000). Average daily temperature was reduced from the first week of experiment to the end of the test; that is, sixth week (from 30 to 25 °C). It must be noted that the main difference between treatments was the distance between rams and ewes box.

Data analysis was done using SAS 9.1 (SAS Institute Inc. 2003). Ram impact factor (distance from ewes' box) for the induction of sexual cycle and creation of estrus early in reproduction season in each treatment was analyzed using proc Freq of SAS (SAS Institute Inc 2003). Since the attribute under study was the number of estrus animals in each replicate of treatments and respective data was in categorical form, as a result, chi-square test was used for comparing treatments. Accordingly, cross-tabulation was applied to analyze estrus animals' frequencies. Significance level was considered at 0.05. It is noteworthy that since the number of some test units was less than 5, hence, Fisher's exact test was applied to

**Fig. 3** The feed intake and rates of estrus expression in 6-week trial

compare between treatments in various weeks to analyze data related to the manifestation of estrus or anestrus. Since this attribute was a continuous variable, GLM ap-

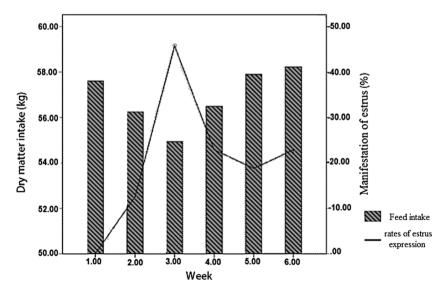
proach with the following statistical model was used for the statistical analysis of records related to dry matter intake (DMI):

$$Y_{ij} = \mu + \text{Treat} + \text{Week}_j + e_j$$

Where:

Yij	Animal performance
$\mu$	Mean society
Treat <sub>i</sub>	The effect of distance between ewe and ram
Week <sub>i</sub>	The effect of test week
e <sub>ij</sub>	Residual effect or error

To compare mean  $T_1$ ,  $T_2$ , and  $T_3$  to  $T_4$ , orthogonal or perpendicular comparisons method was applied.



#### **Results and discussion**

Manifestation of estrus was not observed in any test group during the first week. In the second week, estrus signs appeared. The first signs were shown by the closest group of ewes to rams ( $T_1$ ). The ewes that stay away from rams had reduced manifestation of estrus. That is, in  $T_3$ , no ewes manifested estrus signs. At the same time,  $T_4$  (a mix of ewes and rams) manifested no signs. This phenomenon shows that separating ewes from rams led to stress and manifestation of some behaviors such as changes in dry matter intake behavior.

Results approve the above hypothesis, this is because lack of significant difference between  $T_3$  and  $T_4$  regarding DMI showed that mixing ewes and rams in  $T_4$  reduces tension in these animals in one way or another. It is likely that the effect of male sex is minimized in this situation. Hence, its manifestation in DMI reaches its minimum amount which is similar to  $T_3$ . Till the end of the third week, all ewes in  $T_1$  manifested estrus signs. Yet, as the distance between rams box increased, estrus signs were significantly reduced (p < 0.05). At the same time, there was no significant difference between  $T_3$  and  $T_4$ (the farthest distance from rams and mix of rams and ewes, respectively). It shows that the effect of male sex is small or trivial in 25–30 m. Studying total estrus signs during six weeks showed that  $T_1$  manifested maximum estrus signs (149.99 %) as compared to other groups (Table 1).

These results correlate with results reported by Mohamadi et al. (2011). In their study, total estrus manifestation in a treatment group similar to our study was 131.25 %. The small difference existing in this study—as compared to Mohammadi et al.is probably—resulted from the effect of factors such as species, temperature, and geographical conditions of the place. Perhaps, one of the reasons underlying high estrus signs in  $T_1$ and  $T_2$  includes females' social and behavioral effects as well as male effect. Bedos et al. (2010)), showed that as the first sign of estrus manifests in ewes closest to rams, the effect of the first ewe that manifests estrus—as the excitation and complementary effect—on the manifestation of estrus in other ewes of this group is evident.

Accordingly, it seems that high level of estrus sign manifestation in  $T_1$  resulted from the excitation and complementary effect.

Accordingly,  $T_1$  (mean=54.48 kg) had minimum DMI which increased as the distance from male enhanced (Fig. 2). This difference was significant (p < 0.05). Dry matter intake was also affected by week (Fig. 3). Accordingly, minimum DMI occurred in the third week when peak estrus signs were manifested (Fig. 3). In weeks before and after the third week and concomitant, the smaller manifestation of estrus signs, ewes' dry matter intake increased. Hence, the effect of estrus and its relevant hormones on DMI was crystal clear. Studies showed that estradiol plays a key role in regulating animals' feeding due to being bound to alpha receptor.

Although estradiol has insignificant effect on the frequency of feeding, it reduces DMI at every meal and in general (Fricke et al. 2006). In their studies on Markhoz goat, Mohamadi et al. (2011) achieved similar results. Since many hypothalamus neurons are estrogen receptors, as a result, it is likely that they can affect animal's feeding behavior (Fricke et al. 2006). Hence, the manifestation of estrus signs and reduction of dry matter intake in ewes can be due to the same relationship.

Least mean squares of DMI in test groups for 6 weeks are represented in Fig. 2. In all weeks, T<sub>1</sub> ewes showed the least DMI as compared to other test groups (p < 0.05). Surprisingly, DMI was partially the same for  $T_3$  (25 m from rams' box) and  $T_4$  ewes (Fig. 2). This distance (25–30 m) can probably be introduced as a marginal distance showing minimum effects of male sex. Although no ewes showed estrus signs in the first week (Table 1), least mean squares of DMI showed a significant difference between treatment groups (Fig. 2). Perhaps, it was due to the animals' estrus which did not manifest in the first week. Studies by Feng et al. (2009) showed that estrogen concentration in female rats was at maximum level in proestrus stage. Yet, estrus signs appear in estrus stage. Hence, this hypothesis that the effect of male sex is employed to the opposite sex by male's pheromones is augmented. As a result, aside from the estrus stage where ewes are, high estrogen levels probably show themselves in terms of DMI reduction. Diagram 5 also approves this point. When animals were separated from males (T1, T2, and T3 groups), they were compared to T4 in group. The effects of male sex and separation of two sexes were crystal clear (p < 0.001). In this phenomenon, probably social effects can also be implied as well as the effects of male sex, and complementary and excitation effects of female sex on intensifying estrus, as reported by Bedos et al. (2010). Among social effects, the sense of overcoming fear and relaxation can be implied. This relaxation was in condition where some ewes are near each other and calm. This finally improves their social behaviors status including feeding (Warren 2004; Dwyer 2009). It is likely that the presence of the opposite sexes near each other also leads to relaxation and (or) reduces environmental tensions. It can probably be a reason underlying high DMI in these groups.

**Conflict of interest** The authors declare that they have no conflict of interest.

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