



## Investigating the Effectiveness of Nutrition on the Sexual and Breeding Behaviors in Ghezel Sheep

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### ABSTRACT

This study aims at investigating the effectiveness of nutritional supplements containing vitamins, minerals, and ammonia on the sexual and breeding behaviors in Ghezel sheep. To this end, 28 three and half-year old ewes ( $50\pm 3$  kilograms) with the record of giving birth to two lambs and 4 three-year old ( $90\pm 3.5$  kilograms) rams were used. The ewes were divided into two groups of A (i.e., treatment) and B (i.e., control) with 14 ewes in each group. The treatment group received 40 grams of the compound 7 days before and 7 days after sexual intercourse. The results demonstrate that the treatment group showed sexual receptivity behavior earlier than the control group. Also, such a behavior in the treatment group was accomplished in a shorter period of time than the control group. Moreover, in the treatment group, the breeding rate was demonstrated to be higher than the control group. Nevertheless, the measurement of estrogen, calcium, and phosphate in three different stages showed no significant differences between the two groups. The receptivity power (i.e., the number of successful jumping permission) and the new-born lambs' weight were not reported to be significant. The current study demonstrated that livestock supplements (nutrients) improve the livestock's health and sexual behavior. In addition, such supplements have a positive influence on breeding rate.

**Key words:** Livestock Compound, Sexual Behavior, Reproductive Behavior, Ghezel Sheep

### INTRODUCTION

Reproduction and breeding are considered as one of the most prominent economic characteristics in livestock breeding and as one of the most important determining factors in livestock nurturing efficiency, survival, as well as genetic advancement. As high-fat, colored-wool, heavy-weight breeds, Ghezel sheep are meaty-milky ecotype whose lambs increase their weight 200 grams a day; also, in average ewes

produce 70 kilograms of milk in a lactation period (Tavakkolian, 2000). Studies carried out on the sexual behaviors of livestock are mainly focused on cattle, goats, and sheep. Sexual behavior is mainly related to the females' power of attraction and receptivity (Çağdaş Kara et al., 2010) by which both environmental and physiological factors can affect both males and females (Katz & McDonald, 1992). The sexual attraction of female livestock is pertained to the males' attraction and stimulation upon the female breeds; moreover, the females' sexual receptivity is related to the females' sexual permission to the males for jumping and successful mating (Whaley et al., 1997). The reproductive hormones of both sexes cause sexual behaviors; hence, both males and females attract each other and mate (Margaret et al., 2002). In most mammals, sexual behavior occurs during a limited amount of time and near the period of egg-planting which is thoroughly separated from the inactive period of sexual behavior (Smith & Akinbamijo, 2000). Both attraction and receptivity are considered as two requirements for successful mating. A plethora of studies demonstrate that nutritional materials play a significant role in livestock sexual behavior (Pfaff, 1999). Many nutrients such as Cobalt, Selenium, Manganese, Zinc, Beta-Carotene influence the synthesis of ovarian steroids which play a dramatic role in livestock's sexual behavior (Harrison, 1984; Hidiroglou, 1979b). Also, ovarian activity with reference to ruminants can be affected by mineral deficiency. Elements such as calcium and phosphorus are essential for growth and reproduction. Calcium-dependent mechanisms involve biosynthesis of steroids in the adrenal glands and ovaries. Furthermore, Calcium may affect the stimulation or application of cholesterol in mitochondria or may influence the conversion of perigonole into progesterone in steroidogenesis. Also, stimulating the release of LH from pituitary cells by GnRH require the mechanisms which are specifically dependent on calcium. Besides, the calcium ion is required for insulin secretion (Hurley & Doane, 1989). McClure (1994) reported that phosphorus is probably the most important element affecting the blood in terms of fertility and reproductive performance of livestock. To this end, the current study aims to investigate the improvement of livestock sexual and breeding behaviors through using nutritional supplements.

## MATERIALS AND MTHODS

The present study was carried out from August to October 2012 in the research center of Ghezel sheep located in the city of Miandoab, Orumiyeh, Iran. For this study, 28 three and half-year old ewes ( $50\pm 3$  kilograms) with the record of giving birth to two lambs and the body condition score of approximately 3 within the same environmental conditions were assigned to two groups of 14. Also, 4 three-year old ( $90\pm 3.5$  kilograms) rams were put to two groups of two. The release time of the rams was from 08:00 A.M. to 12:00 P.M. and 04:00 P.M. to 08:00 P.M.; also, the ewes were selected randomly and were released within the groups.

The observations involved direct observation as well as camera recording and reviewing of livestock sexual behavior. The sheep in each group were fed 4 times daily, with the mixed ration of TMR (i.e., the ratio of 25 percent of Concentrate and 75 percent of alfalfa and straw). The sheep in group A received 40 grams of nutrient supplements along with the above-mentioned combinations as can be seen in table 1 plus basic food ration. While group B (i.e., the control group) received only the basic food ration. The rations were formulated based on NRC table (1985). The sheep were synchronized in terms of sexual receptivity in 14 days by CIDR made by New Zealand Ltd (No. 39020401-0606) and 48 hours after sexual receptivity synchronization with CIDR, the rams were set free in the flock. The experiment was carried out one week before and one week after inoculation the sheep sexual intercourse.

Sexual receptivity observation in the sheep was recorded in both groups from the initial to the final contact which involved successful sexual jumping culminating in the ejaculation of the male's penis into the female animal. Also, the breeding percentage and birth weight were measured. The sheep samples of

blood were tested in three stages, namely the initial experiment, 48 hours, and 24 hours after CIDR taken from the jugular vein of the ewes' neck. The blood serum samples were separated using a centrifuge with 3000 rpm for 15 minutes and were stored in the freezer with  $-20^{\circ}\text{C}$  until the analysis of microtubes. The levels of hormones and metabolites were measured using ELISA and spectrophotometric methods. The estrogen-measuring kits were made by DRG German Company (ELA-1561); also, the calcium and phosphorus metabolites were produced by the Bio- Chemistry of Tehran, Iran.

**Table 1. Nutrient Composition of Livestock Supplement**

Nutrient Materials	Amount	Nutrient Materials	Amount
Vitamin A, IU/kg	500,000	Zinc (oxide), mg/kg	3,000
Vitamin D3, IU/kg	100,000	Iodine (iodate), mg/kg	100
Vitamin E, mg/kg	100	Cobalt (carbonate), mg/kg	100
Carrier mg/kg	Up to 1 kg	Selenium (selenite), mg/kg	40
Antioxidants, mg	3,000	phosphorus, mg/kg	25,000
Copper (sulphate), mg/kg	300	Magnesium, mg/kg	30,000
Iron (sulphate), mg/kg	3,000	Sodium, mg/kg	60,000
Manganese (oxide), mg/kg	2,000	mg/kg Calcium,	180,000

Made by Science Laboratories, Ghazvin, Iran

### Statistical analysis

For the analysis of data, the researcher has made use of SAS and the procedures of Freq. and GLM. Also, the means were compared by Duncan test.

## RESULTS AND DISCUSSION

### Dry Matter Intake

As shown in Table 2, the dry matter intake in both groups was similar in different stages. However, it is evident that the dry matter intake was reduced in the period of sexual receptivity. Such results are in line with Daghighkia et al.'s (2011) study on Markhaz goats and Daghighkia et al.'s (2014) study on Afshar goats. Such reduction appeared in both groups similarly.

**Table 2. Dry Matter Intake in Treatment Groups (Kg/Livestock/Day)**

DM intake(kg)	Experiment Initialization	7th Day of Experiment (sexual receptivity time)	14th Day (Final) of Experiment
Control Group	1.52	1.44	1.53
Treatment Group	1.49	1.46	1.56

### Reproductive performance

Multiple offspring's, breeding percentage, fertility, and twin-breeding rate are illustrated in Table 3. The use of livestock supplements improves the reproductive performance of sheep and has a significant influence on breeding rate ( $\chi^2= 172.43$ ,  $p <0.006$ ). Hence, the results of the current study correspond with Hemingway et al.'s (2001) study who reported that utilizing the tablets containing the minerals and vitamins before sexual intercourse increases the rates of breeding and twin-breeding rates. Moreover, Çağdaş Kara et al. (2010) have shown that the use of nutritional supplements containing minerals and vitamins increase the number of offsprings. A plethora of studies demonstrate that the utilization of vitamin E along with Selenium for ewes increase the rate of twin-breeding and improves their reproductive performance (Hartley & Grant, 1961; Hartley, 1963; Andrews et al., 1968). Generally, it is evident that feeding the livestock with soybean (Jahani-Moghadam et al., 2008) and with short-term Egyptian lupine grain increases the rate of egg-laying through increasing glucose and amino acids. Amino acids such as methionine increase the availability of energetic substrates for the growth and development of Follicle (Downing et al., 1995). The maximum number of offspring's depends on three conditions: the period of egg-laying rate at the highest point, after fertilization and the formation of embryo, as well as taking care of the embryo (Martin et al., 2004). Therefore, the stages after fertilization play an important role in the number of offsprings. In addition, copper, iodine, iron, manganese, selenium, and zinc influence the rate of embryo survival (Hidiroglou, 1979b; Hambidge et al., 1986; Davis & Mertz, 1987; Hurley & Keen, 1987); hence, the findings of this study are in line with the above-mentioned researches. In the treatment group, the birth rate of lambs is higher than the control group; however, the difference is not significant as indicated in Table 3. Hence, the results of the present study are consistent with the findings of Çağdaş Kara et al.'s (2010) study. The combination of minerals (i.e., copper, iodine, manganese, selenium, and zinc) is vital for the synthesis of many proteins and regulating the enzyme systems (Hostetler et al., 2003). Minerals may influence the growth and development of embryo directly and may affect the birth weight of offsprings. Since the increase in the number of embryos in each female cause reduction in the birth weight of offspring's (Gardner et al., 2007), the weight of such offsprings is not significant due to the high frequency of twin-breeding in the treatment group.

**Table3. Effects of Livestock Supplements Treatment on Sheep Reproductive Traits**

Group	No. of offspring's	Breeding Percentage	Fertility Percentage	Twin-Breeding	Birth Weight (Kg)
Control Group	14	100	92.86	7.14	4.36±0.073
Treatment Group	17	121.4	100	21.43	4.46±0.085

Table 3 illustrates the amount of estrogen, calcium, and phosphorus serum levels at different time intervals and shows that there exists no significant difference between the groups in any of the stages. Nevertheless, at the time intervals of Pro-Estrus and Estrus (the 2nd and 3rd stages of blood-sampling), the amount of estrogen, calcium, and phosphorus levels was higher in the treatment group than the control group. Since vitamin A plays a vital role in steroid-making (Pillon et al., 2003) and selenium has a dramatic impact on the duplication and proliferation of granulosa cells as well as the propagation of steroids (Basini & Tamanini, 2000) and manganese, vitamin C, as well as zinc also play important roles on steroidogenesis (Murray et al., 2001), this study's findings are consistent with Seifi et al.'s (2005) research on cattle which showed that the concentration of calcium and phosphorus in cattle blood was higher in fertile than the non-fertile cattle ( $p <0.05$ ). Hurley and Doane (1989) reported direct correlation between calcium intake and pregnancy rates. Mechanisms which are dependent on calcium play a vital role in biosynthesis of steroids and secretion of the adrenal glands and ovaries. Calcium can, also, be significant in terms of the reception or application of cholesterol through mitochondria or the stimulation

of pregnenolone conversion into progesterone in steroid-making (Seifi et al., 2005). Moreover, the stimulation of GnRH and the release of LH from the anterior pituitary are associated with calcium-dependent mechanisms; hence, in the absence or blocking of calcium LH is not released (Hurley & Doane, 1989). Phosphorus is regarded as one of the components of nucleic acids, nucleotides and a number of proteins. Furthermore, it is considered as a means of energy transmission, a metabolism of normal phospholipids, and a main part of many coenzymes (Seifi et al., 2005). The presence of phosphorus in the synthesis of phospholipids and CAMP can project the most important effect on reproduction. The proteins which are related to the CAMP can have a prominent role in the mediation of hormone operation (Hurley & Doane, 1989).

**Table 4. Effects of Animal Supplements Treatment on Serum Hormones and Metabolites**

	Blood-Sampling Time	Control Group	Treatment Group	SEM	P-value
Estrogen	1	30.8	29.2	2.56	Ns
	2	41	49.4	4.16	Ns
	3	61.8	69.4	5.64	Ns
Calcium	1	13.37	13.94	1.19	Ns
	2	16.28	20.97	1.88	Ns
	3	12.57	13.32	1.8	Ns
Phosphorous	1	5.04	5.62	0.46	Ns
	2	4.26	4.97	0.30	Ns
	3	3.98	4.75	0.41	Ns

Table 4 illustrates the effect of livestock supplements on some of the sexual behaviors of sheep. As can be observed, the sheep in the treatment group showed sexual receptivity earlier than the control group ( $p < 0.05$ ). Also, the total amount of time regarding the sexual receptivity in the treatment group was lower than its counterpart group. Besides, the sexual receptivity of ewes in the treatment group was reported to be higher than the control group; however, such a difference was not significant. Ovarian steroids have a significant role in the control of estrous behavior (Carter 1992; Pfaff 1999) in domestic animals. Also, for rodents, an increase in estradiol show sexual receptivity behavior in one or two days (Morali & Beyer 1979). Moreover, for ruminants, estradiol causes sexual receptivity behavior and endocrine changes (Pillon et al., 2003). The treatment with cobalt, also, causes sexual receptivity behavior (Hidiroglou, 1979a).

**Table 5. Effects of Livestock Supplements Treatment on Sexual Behavior**

	Control Group	Treatment Group	SEM	P-value
The Initial Period of Sexual Receptivity <sup>1</sup>	33.09	31.79	0.29	*
The Total Hours of Sexual Receptivity <sup>1</sup>	37.69	36.36	0.36	*
Sexual Receptivity	21	23	-	Ns

<sup>1</sup> The Number of Hours after CIDR-Taking

## CONCLUSIONS

The utilization of nutrient combinations before the sexual intercourse improves both the sexual receptivity and the reproductive performance of sheep. Since vitamins and minerals influence the synthesis of steroids, we assume that such supplements play a significant role on the sexual behavior of sheep through

influencing on the synthesis of steroids. Also, we conclude that livestock supplements along with nutrients have a positive influence on breeding rate which is due to an increase in follicle growth rate and egg-laying.

## REFERENCES

- Andrews E. D., Hartley W. J., & Grant A. B. (1968). Selenium responsive diseases of animals in New Zealand. *N. Z. Vet. J.*, 16, 3-17.
- Basini G. & Tamanini C. (2000). Selenium stimulates estradiol production in bovine granulosa cells: Possible involvement of nitric oxide. *Domest. Anim. Endoc.*, 18, 1-17.
- Çağdaş, K., Orman, A., Topal E., & Çarkungöz, E. (2010). Effects of supplementary nutrition in Awassi ewes on sexual behaviors and reproductive traits. *J. Biol. Enviro. Sci.*, 4(10), 15-21.
- Carter, C. S. (1992). Neuroendocrinology of sexual behavior in the female. In: *Behavioral Endocrinology*, (Eds.: J. B. Becker, S. M. Breedlove, & D. Crews). MIT Press, Cambridge, pp. 71- 96.
- Daghighkia H., Mohamadi Chapdareh, W., Hossein Khani A., Moghaddam G., Rashid A., Sadri H., & Alijani, S. (2011). Effects of flushing and hormonal treatment on reproductive performance of Iranian Markhoz goats. *J. Anim. Physiol. Anim. Nut.* doi: 10.1111/j.1439-0396.2011.01234.x.
- Davis, G. K. & Mertz, W. (1987). Copper. In: *Trace Elements in Human and Animal Nutrition*, 5th ed., (Ed.: W. Mertz). Academic Press, San Diego, pp. 301-364.
- Gardner, D. S., Buttery P. J., Daniel, Z., & Symonds, M. E. (2007). Factors affecting birth weight in sheep: Maternal environment. *J. Reprod. Fertil.*, 133: 297-307.
- Hambidge, K. M., Casey, C. E., & Krebs, N. F. (1986). Zinc. In: *Trace Elements in Human and Animal Nutrition*, 4th ed., (Ed.: W. Mertz). Academic Press, San Diego, pp. 1-37.
- Harrison, J. H., Hancock, D. D., & Conrad, H. R. (1984). Vitamin E and selenium for reproduction of the dairy cow. *J. Dair. Sci.*, 67, 123-132.
- Hartley, E. D., Grant, A. B. (1961). A review of selenium responsive diseases in New Zealand livestock. *Fed. Proc.*, 20, 679-688.
- Hartley, W. J. (1963). *Selenium and ewe fertility. Proc. N. Z. Soc. Anim. Prod.*, 23, 20-27.
- Hemingway, R. G. (1998). Supplying trace elements to cattle. *Feed mix.*, 6 (4), 8-10.
- Hidiroglou, M. (1979a). Trace element deficiencies and fertility in ruminants: A review. *J. of Dairy Sci.*, 62, 1195-1206.
- Hidiroglou, M. (1979b). Manganese in ruminant nutrition. *Can J. Anim. Sci.*, 59, 217-236.
- Hostetler, C. E., Kincaid, R. L., & Miranda, M. A. (2003). The role of essential trace elements in embryonic and fetal development in livestock. *The Vet J.*, 166, 125-139.

- Hurley, L. S. & Keen, C. L. (1987). Manganese. In: *Trace elements in human and animal nutrition*, 5th ed., (Ed.: W. Mertz). Academic Press, San Diego, pp. 185–223.
- Hurley, W. L. & Doane, R. M. (1989). Recent development in the role of vitamins and minerals in reproduction. *J. Dair. Sci.*, 72, 784–804.
- Jahani-Moghadam, M., Amanlou, H, & Nikkhah, A. (2008). Metabolic and productive response to ruminal protein degradability in early lactation cows fed untreated or xylose-treated soybean meal-based diets. *J. Anim. Physiol. Anim. Nut.* doi: 10.1111/j.1439-0396.2008.00867.x.
- Katz, L. S. & McDonald, T. J. (1992). Sexual behavior of farm animals. *Theriogenology*, 38, 239–253.
- Margaret, M., McCarthy, M., Jill, B., & Becker, R. (2002). Neuroendocrinology of sexual behavior in the femals. *J. Dair. Sci.*, pp117- 140.
- Martin, G. B., Milton, J. T. B., Davidson, R. H., Banchemo Hunzicker, G. E., Lindsay, D. R., & Blache, D. (2004). Natural methods for increasing reproductive efficiency in small ruminants. *Anim Rep Sci.*, 82–83, 231–246.
- McClure, T. J. (1994). *Nutritional and metabolic infertility in the cows* (1st Ed.) Cab. International: London, P. 47.
- Meschy, F. (2000). Recent progress in the assessment of mineral requirements of goats. *Livest Prod Sci.*, 64, 9-14.
- Morali, G. & Beyer, C. (1979). Neuroendocrine control of mammalian estrous behavior. In: *Endocrine control of sexual behavior*, (C. Beyer Ed.). New York: Raven Press, pp. 33–75.
- Murray, A. A., Molinek, M. D., Baker, S. J., Kojima, F. N., Smith, M. F., Hillier, S. G., & Spears, N. (2001). Role of ascorbic acid in promoting follicle integrity and survival in intact mouse ovarian follicles in vitro. *J. Reprod. Fertil.*, 121, 89–96.
- Pfaff, D. W. (1999). Hormone-Controlled Drives. In: *Drive: Neurobiological and molecular mechanisms of sexual motivation*, (Ed. D.W. Pfaff). MIT Press, Cambridge, pp. 48–51.
- Pillon, D., Caraty, A., Fabre-Nys, C., & Bruneau, G. (2003). Short-term effect of oestradiol on neurokinin BMRNA expression in the infundibular nucleus of ewes. *J. Neuroendocrinol.*, 15, 749–753.
- Seifi, H. A., Farzaneh, N., & Mohri, M. (2005). Relationships between fertility, serum calcium and inorganic phosphorus in dairy cows. *Iranian Journal of Veterinary Research, university of Shiraz*, 6 (2), 12.
- Smith, O. B. & Akinbamijo, O. O. (2000). Micronutrients and reproduction in farm animals. *Anim Rep Sci.* 60–61,549–560.
- Tavakkolian, J. (2000). An introduction to genetic resources of native farm animal in Iran. *Dep of Ani Scie, Anim. Sci. Rese. Ins, Karaj, Iran.*

Tilbrook, A. J., Hemsworth, P. H., Topp, J. S., & Cameron, A. W. N. (1990). Parallel changes in the proceptive and receptive behaviour of the ewe. *Appl. Anim. Beh. Sci.*, 27, 73–92.

Whaley, S. L., Hedgpeth, V. S., & Britt, J. S. (1997). Evidence that injection of vitamin A before mating may improve embryo survival in gilts fed normal or high energy diets. *J. Anim. Sci.*, 75, 1071–1077.