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# Effects of Supplementary Nutrition in Awassi Ewes on Sexual Behaviors and Reproductive Traits

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## ABSTRACT

**The present study was conducted to improve sexual behavior in Awassi ewes by the nutritional supplement containing multiple nutrients, such as vitamins, minerals, amino acids and sorbitol. In addition, the purpose of this study was to evaluate the effect of this nutritional supplement on litter size in ewes and birth weight of lambs. Twenty six Awassi ewes (50 ± 3.20 kg), aged 2 to 4 years, and three sexually experienced Awassi rams (90 ± 5.30 kg), aged 3 years, were used. The ewes were sorted by age, body weight and litter size of them in previous years and assigned to one of the two groups (experimental; EG and control; CG). Each group consisted of 13 ewes. The daily ration of the ewes in EG was added 30 g/ewe the nutritional supplement for 5 days before mating period and 5 days during last month of gestation. Despite the fact that there were no statistically differences for sexual behaviors, the number of lambs born, lambing percentage and birth weights of lambs between groups ($p > 0.05$), our results showed that the combination of nutrients improving animal health and reproduction had a positive effect on embryonic survival, litter size and birth weight.**

**Key Words: Supplementation of multiple nutrients, sexual behavior, litter size, birth weight.**

## INTRODUCTION

The field of sexual behavior of domestic farm animals is discussed with emphasis on studies of cattle, sheep and goats. Descriptions are provided of behaviors related to the attractivity, proceptivity and receptivity of females, as well as environmental and physiological factors influencing both male and female sexual behavior (Katz and McDonald 1992). Attractivity refers to the female's value as a sexual stimulus whereas proceptivity consists of appetitive activities shown by females. Receptivity includes the behaviors exhibited by the female that allow intravaginal ejaculation (Tilbrook et al. 1990). In most female mammals, expression of these behaviors is restricted to distinct periods around ovulation separated by periods of sexual inactivity (Carter 1992; Pfaff 1999). Three components of sexual behavior (attractivity, proceptivity and receptivity) are important for successful mating to ocur (Beach 1976). The level of proceptive behavior clearly influences the chances of the ewe being mated, and the number of ewes that are mated will influence the fertility of the flock (Tilbrook et al. 1990).

Litter size at birth is an important economic index in multiparous species (Smith and Akinbamijo 2000). The three major variables that contribute to litter size are ovulation rate, embryonic survival, and fetal survival (Martin et al. 2004).

Birth weight is a significant predictor of later health outcomes (Gardner et al. 2007). It is well documented that low birth weight negatively affects lambs' survival (Fogarty et al. 2000).

Various studies have reported that many nutrients play important roles in reproductive performance (Hidiroglou 1979a; Hurley and Doane 1989; Meschy 2000; Smith and Akinbamijo 2000). Some nutrients, such as cobalt, selenium, manganese, zinc and β-carotene, are affected on synthesis of ovarian steroids playing a major role in controlling sexual behavior (Hidiroglou 1979b; Hurley and Doane 1989; Corah and Ives 1991; Basini and Tamanini 2000). Copper, iodine, iron, manganese, selenium, zinc, vitamin A and E have a positive effect on litter size (Hidiroglou 1979b; Harrison et al. 1984; Hambidge et al. 1986; Davis and Mertz 1987; Hurley and Keen 1987; Whaley et al. 1997). Trace minerals (Hostetler et al. 2003; Gardner et al. 2007) and energy (Schwartz et al. 1964; Vaugher et al. 1973; Stafford et al. 2007) affect both fetal growth and lamb birthweight. We suggested that supplementation of multiple nutrients can be more useful than supplementation of a single nutrient for improving reproductive performance.

In ewes, proceptive and receptive behaviors are only expressed for a short period during the estrous cycle, around the time of ovulation (Tilbrook et al. 1990). Our aim was to detect easily and immediately the ewes in estrus in the flock through increasing intensity of sexual behavior in ewes. The present study was conducted to improve sexual behavior in Awassi ewes by the nutritional supplement containing multiple

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nutrients. In addition, the purpose of this study was to evaluate the effect of this nutritional supplement on litter size in ewes and birth weight of lambs.

## MATERIALS AND METHODS

***Study area and animals***

This study was carried out at Uludag University Applied Research Center for Veterinary Faculty Unit in Bursa, where place within the North West Turkey, 40° north latitude, 29° east longitude and at an altitude of 120 m above sea level. All animals were handled according to the EU directive number 86/609/EEC concerning the protection of animals used for experimental and other scientific purposes. Twenty six Awassi ewes with an average initial body weight of 50 ± 3.20 kg, aged 2 to 4 years, and three sexually experienced Awassi rams with an average initial body weight of 90 ± 5.30 kg, aged 3 years, were used. The Awassi breed of sheep is characterized by having a fat tail. This tail can cause a copulation barrier to inexperienced rams that stand directly behind females before mounting. At the time of mating, an experienced Awassi ram stands to the ewe's side and kicks up the fat tail as it mounts the female (Kridli and Al-Yacoub 2006). Inexperienced rams can cause negative effects upon reproductive parameters. Therefore experienced rams were chosen for copulation. The ewes were sorted by age, body weight and litter size of them in previous years and assigned to one of the two groups (control and experimental). Each group consisted of 13 ewes. There were one each ewe aged 4 years, three each ewes aged 3 years, and nine each ewes aged 2 years in each group. The groups were similar with regard to body weight (control; 51.30 ± 3.30 kg, experimental; 50.80 ± 2.40 kg) and litter size of ewes in the previous years (control; 1.46, experimental; 1.46).

***Management and experimental design***

Experiment was conducted between August of 2008 and March of 2009. The ewes were housed in 1.22 * 1.70 m individual pens equipped with a feeder and water pot, with wheat straw bedding, and fed with 1.45 kg/day/ewe oats hay and 200 g/day/ewe sheep pellets for 5 days (August 13 to 17, 2008) before mating period. The daily ration of the ewes in experimental group (EG) was added 30 g/ewe nutritional supplement (Hepato Force®, Evialis International, Cédex, France) in this period. The ewes were not synchronized before mating period. The all ewes were housed in a paddock and fed with 1.45 kg/day/ewe oats hay and 300 g/day/ewe sheep pellets from mating period to last month of gestation. Mating period (the time between first mating and last mating) lasted 30 days (August 21 to September 19, 2008). During last month of gestation, the all ewes were housed again in individual pens and fed with 1.45 kg/day/ewe oats hay and 500 g/day/ewe sheep pellets. The ewes in EG were given additional 30 g/day/ewe nutritional supplement for 5 days during last month of gestation. The rams were housed in a paddock and fed with oats hay ad libitum and 1 kg/day/ram sheep pellets during the study. Water was supplied ad libitum during the study. Nutrient compositon of oats hay and sheep pellets was presented in Table 1. Nutrient compositon of nutritional supplement was listed in Table 2.

**Table 1.** Nutrient composition of the feeds*

| | **Oats hay** | **Sheep pellets****|
|---|---|---|
| Dry matter, % | 95.10 | 92.35 |
| Crude protein, % of DM | 6.36 | 18.75 |
| Ether extract, % of DM | 1.34 | 5.08 |
| Crude fiber, % of DM | 35.5 | 7.42 |
| Ash, % of DM | 7.28 | 8.48 |

* Nutrient analyses of the feeds were performed according to AOAC (1990)
** ProYem, Lactation Period Concentrate Mixture, Matlı Feed Industry, Karacabey/TURKEY

***Behavioral observations***

Ewes and rams were kept together in a paddock for one hour twice a day during mating period. Each ewe was randomly assigned to one of the 3 rams. The ewes to come into estrus in the time of introducing the rams were mated and sexual behaviors of the ewes in estrus were observed. During the observations, soliciting, sniffing scrotum, head-turning, anogenital sniffing, non-firm standing, squatting and tail-fanning were recorded as proceptive behavior, and firm standing was recorded as receptive behavior. All the behavioral traits investigated are described in Table 3. The behaviors of animals were noted on individual checksheets prepared for each ewe. Each ewe was considered to be in estrus when she was directly observed to accept a mount from the ram (Romano et al. 2000).

**Table 2.** Nutrient compositon of nutritional supplement (Hepato Force) (as fed)

| | |
|---|---|
| Dry matter, % | 94.02 |
| Niacin, mg/kg | 2000 |
| B-carotene (active), mg/kg | 30 |
| Betaine, mg/kg | 2000 |
| Choline chloride, mg/kg | 7000 |
| Methionine, mg/kg | 55000 |
| Lysine base, mg/kg | 4400 |
| Sorbitol, mg/kg | 65000 |
| Vitamin A, IU/kg | 8000000 |
| Vitamin D3, IU/kg | 200000 |
| Vitamin E, mg/kg | 8500 |
| Vitamin C, mg/kg | 150 |
| Vitamin B1, mg/kg | 750 |
| Copper (sulphate), | 300 |
| Iron (sulphate), mg/kg | 1500 |
| Manganese (oxide), mg/kg | 4000 |
| Zinc (oxide), mg/kg | 6500 |
| Iodine (iodate), mg/kg | 90 |
| Cobalt (carbonate), mg/kg | 25 |
| Selenium (selenite), mg/kg | 40 |
| Soluble phosphorus, % | 2.5 |
| Magnesium, % | 3 |
| Sodium, % | 3 |
| Calcium, % | 3 |

**Table 3.** Behavioral traits recorded during the observation periods

| | |
|---|---|
| Soliciting | The ewe approaches to the ram, nuzzles the body of him, shows a tendency to stay in the vicinity of the ram and follows him |
| Sniffing scrotum | The ewe sniffs the scrotum and anogenital region of the ram |
| Head-turning | The ewe stands and swings her head to look at the courting ram |
| Anogenital sniffing | The ewe allows the ram to sniff her tail and genitalia |
| Non-firm standing | The ewe stands in front of the ram in response to the courtship of him but does not allow him to mount and she avoids when the ram attempts to mount |
| Squatting | Typical urination posture of the ewe |
| Tail-fanning | Repeated movement of the tail |
| Firm standing | The ewe stands still to receive a mount attempt or mount |

***Litter size and birth weight of lambs***

At lambing time, ewes were segregated into small groups and were closely monitored to facilitate accurate recording of the number of lambs born for a final assessment of litter size. Lambs were weighed into 2 h after lambing and birth weights of lambs were recorded.

***Statistical analysis***

Sexual behaviors and the numbers of lambs born per group were compared by Pearson Chi-Square test. Lambing percentage (percentage of ewes lambing) was analyzed by Fisher Exact test. Birth weights of lambs were analyzed by Independent samples t test. SPSS 15 (2004) computer program package was used for the statistical analyses. Significance was accepted at $p < 0.05$.

## RESULTS AND DISCUSSION

During the study, control (CG) and experimental group (EG) had similar dry matter intakes (Table 4). The ewes in EG consumed the whole of nutritional supplement given for 5 days before mating period and during last month of gestation.

**Table 4.** Dry matter intakes

| | CG* | EG** |
|---|---|---|
| before mating period | 1.357 ± 0.105 kg | 1.349 ± 0.198 kg |
| mating period to last month of gestation | 1.380 ± 0.112 kg | 1.389 ± 0.193 kg |
| last month of gestation | 1.413 ± 0.215 kg | 1.477 ± 0.188 kg |

* Control Group
** Experimental Group

### *Sexual behaviors*

Sexual behaviors data was presented in Table 5. All ewes showed at least one proceptive and receptive behavior in this study. During mating period, two ewes in both CG and EG showed a second estrus but sexual behaviors in second estrus were not recorded. Both the numbers of proceptive (32 and 28 for CG and EG, respectively) and receptive (14 and 15 for CG and EG, respectively) behaviors observed were similar for CG and EG. There were no statistically differences for proceptive and receptive behaviors between CG and EG ($p > 0.05$) (Table 5).

**Table 5.** Behavioral and Reproductive Traits

| Item | | Groups CG* (n=13) | Groups EG** (n=13) | P |
|---|---|---|---|---|
| Sexual Behaviors | Proceptive | 32 | 28 | NS |
| | Receptive | 14 | 15 | NS |
| | Total | 46 | 43 | NS |
| Reproductive Traits | The numbers of lambs | 13 | 15 | NS |
| | Lambing percentages | 84.62 % | 100 % | NS |
| | Birth weights of lambs (kg) | 4.48 ± 0.77 | 4.49± 0.56 | NS |

* Control Group
** Experimental Group
NS: not significant

Ovarian steroids play a major role in controlling sexual behavior (Carter 1992; Pfaff 1999). In domestic ruminants and rodents, an increase in estradiol concentration always precedes the expression of sexual behavior by 1 to 2 days (Morali and Beyer 1979). In ruminants, estradiol is the trigger for both the behavioral and the endocrine changes (Pillon et al. 2003).

Treating with cobalt results in stronger manifestations of estrus (Hidiroglou 1979a). β-carotene and vitamin A play a major role in synthesis of ovarian steroids (Lothammer et al. 1976; Zerobin 1987). Selenium stimulates estradiol production (Basini and Tamanini 2000) and manganese, zinc and vitamin C are involved in steroidogenesis (Hurley and Doane 1989; Corah and Ives 1991; Murray et al. 2001). Selenium acts on granulosa cells by modulating their proliferation and estradiol synthesis (Basini and Tamanini 2000). Suboptimal ovarian activity and depressed estrus are associated with copper (Hidiroglou 1979a).

We hypothesized that these vitamins and minerals, indicated above, might affect sexual behaviors because of their role in synthesis of ovarian steroids. However, the nutritional supplement, used before mating period in this study, containing the nutrients indicated above, did not improve sexual behaviors (Table 5). Influences of the nutrients on sexual behaviors in ruminants have not been adequately investigated and the the relationship between nutrients and sexual behaviors is not clear. Therefore, we could not have a chance of comparing the results of sexual behavior obtained in our study with those in other similar studies. We suggested that effect of supplementation of multiple nutrients on sexual behaviors was not observed because of sexual behaviors expressed for a short period in ewes (Tilbrook et al. 1990) as well as limited number of the ewes in this study. In addition, amount of the nutritional supplement added to ration might be inadequate.

***Litter size and birth weights of lambs***

The number of ewes giving multiple birth (twin) was 2 in both CG and EG. The numbers of lambs born were 13 and 15 in CG and EG, respectively. There was no statistically difference for the number of lambs between groups ($p > 0.05$) (Table 5). Lambing percentages (percentage of ewes lambing) were 84.62 % (11 of 13 ewes in CG) and 100 % (13 of 13 ewes in EG). The difference between groups was not significant ($p > 0.05$) (Table 5). Mean birth weights of lambs were 4.48 ± 0.77 kg and 4.49± 0.56 kg in CG and EG, respectively. There was no statistically difference for mean birth weights of lambs between CG and EG ($p > 0.05$) (Table 5).

Maximising potential litter size is provided by maximising ovulation rate and minimising post-fertilisation wastage by ensuring successful embryonic and fetal development (Martin et al. 2004).

Negative impacts of vitamin E and / or selenium deficiencies have been observed on various components of the reproductive event, including ovulation rate (Harrison et al. 1984). In addition, Mudd and Mackie (1973) demonstrated that selenium and vitamin E increased the number of lambs born per ewe. It is generally known that short-term supplementary lupin grain (Lupinus angustifolius) feeding significantly increases the ovulation rate in ewes (Downing and Scaramuzzi 1991; Downing et al. 1995). The increase in the ovulation rate after lupin feeding is interpreted as a local effect of glucose and glycogenic amino acids, such as methionine (increased availability of energetic substrates for growing and developing follicles) (Downing et al. 1995). Glucose is the major source of energy for the ovary (Rabiee et al. 1997) and hepatic oxidation of sorbitol leads to glucose production (Boyles 1993).

In this study, the number of ewes giving multiple birth (twin) were same in CG and EG, which showed that the nutritional supplement containing vitamin E, selenium, sorbitol as source of glucose and methionine (glycogenic amino acid) did not improve ovulation rate when it was added to ration before mating period. The cause of this result obtained might be insufficient amount of one or more of additional nutrients.

As a result of ultrasound examinations, we observed 2 embryonic deaths in CG during pregnancy. 2 ewes having embryonic death did not come into estrus again. The numerical (non-significant) differences between CG and EG for the number of lambs born and lambing percentages (Table 5) were because of embryonic deaths in CG.

Copper, iodine, iron, manganese, selenium and zinc are known to influence embryonic and fetal survival (Hidiroglou 1979b; Hambidge et al. 1986; Davis and Mertz 1987; Hurley and Keen 1987). Whaley et al. (1997) examined that vitamin A supplementation improved litter size in pigs due to increased embryonic survival.

We observed that the nutritional supplement containing above-mentioned minerals and vitamin A decreased incidence of embryonic death numerically and suggested that significant differences for the number of lambs born and lambing percentages may be obtained in a study conducted by using more ewes than used in current study.

Hemingway et al. (2001) reported that the multi-trace element/vitamin ruminal bolus administered to ewes before mating period significantly increased lambing percentage. The ruminal bolus was composed of copper, selenium, cobalt, iodine, manganese, zinc, vitamin A, $D_3$ and E. The result obtained by Hemingway et al. (2001) supports positive effect of the nutritional supplement containing combination of the minerals and vitamins indicated above on lambing percentage (Table 5).

Koyuncu and Yerlikaya (2007) observed that supplementation with selenium and vitamin E had a positive effect on birth weight. Birth weight was increased with increasing copper concentration in pig (Berger 2004). Trace minerals (copper, iodine, manganese, selenium and zinc) are required for synthesis of many proteins and activate a vast array of enzyme systems (Hostetler et al. 2003). Metalloenzymes, of which trace minerals are constituents, are important in bone formation (Leach 1967), lipid metabolism (Cunnane et al. 1993), glucose utilization (Jovanovic-Peterson and Peterson 1996), DNA synthesis and transport (Townsend et al. 1994) and free radical metabolism (deHaan et al. 1994). Through one or more of these mechanisms, trace minerals may directly affect embryonic and fetal development having a close relationship with birth weight (Hostetler et al. 2003; Gardner et al. 2007). Fructose is the major carbohydrate found in the fetal blood of ruminants and the main source of energy for developing fetus (Schwartz et al. 1964; Vaugher et al. 1973; Stafford et al. 2007). Sorbitol, found in the nutritional supplement, may be converted to blood fructose via enzymatic action in the placenta and fetal liver (Goodwin 1956).

Litter size and gender are affected on birth weight in sheep. Individual birth weights decline as litter size increases (Gardner et al. 2007). Birth weights of male lambs are generally heavier than those of female lambs (Karakus et al. 2008) as found in this study (mean birth weight of male lambs 4.66 kg, mean birth weight of female lambs 4.26 kg). In our study, 69.23 % and 53.33 % of lambs born to the ewes used were male in CG and EG, respectively, while the number of twins was same in CG and EG. Mean birth weights of lambs were similar in CG and EG (Table 5) despite the fact that the number of male lambs born in CG was more than those in EG, which would suggest that the nutritional supplement containing the nutrients indicated above might be effective on birth weight.

## CONCLUSION

We investigated effects of the nutritional supplement, having a wide utilization area such as preventing ketosis and fatty liver, increasing production and improving reproduction, on sexual behavior, litter size and birth weight of lambs in ewes. Our results showed that the nutritional supplement containing many nutrients improving animal health and reproduction has a positive effect on embryonic survival, litter size and birth weight. These findings will be useful to help further explore the frequency, timing, and amount of multiple nutrients supplementation that may alter reproductive performance of ewes. Different results can be attained when this study renew with changing feeding frequency and amount of the nutritional supplement.

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