



The Effect of Vitamin and Mineral Supplementation in Different Forms on Placenta and Birth Weight and Reproductive Performance in Kangal Sheep

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Received: 16.05.2023

Accepted: 13.07.2023

ABSTRACT

The objective of this study was to evaluate the out-of-season reproductive performance and lamb birth weight of Kangal ewes orally administered a bolus of vitamin and mineral premix or mineral premix given as injection with at 40-45 days postpartum during the anestrus period. In total, 78 primiparous Kangal ewes were randomly allocated to three experimental groups. Estrus induction protocol was performed and a ram was introduced to the ewes at the 80th days postpartum. Ewes in the group 1 a dose of 2 mL of injectable mineral solution at 40-45 days before oestrous synchronisation, was administered at once (n = 25). As for group 2, at 40-45 days before oestrous synchronisation, a mineral bolus was given orally once (n = 27). To the ewes in the group 3 a dose of 2 mL of physiological saline was given once to the animals (n = 26) simultaneously with group 1 and group 2. Reproductive parameters such as estrus and pregnancy rates; single, twin, triplet, and multiple pregnancy rates; litter size; embryonic mortality; fecundity; and dystocia rates were evaluated. In addition, the placentas were weighed to evaluate the effect of mineral supplements on placentation. There were no significant differences between the groups in terms of parameters above (p>0.05). However, the rate of dystocia was significantly lower in group 2 compared to Groups 1 and 3 (p<0.05). In conclusion, as a result, it was determined that slow-releasing boluses could not produce efficacy during the throughout pregnancy.

Keywords: Mineral, Postpartum, Pregnancy, Reproduction, Sheep.

ÖZ

Kangal Koyunlarında Farklı Formlarda Verilen Vitamin ve Mineral Desteğinin Plasenta ve Doğum Ağırlığı ve Reprodüktif Performansa Etkisi

Bu çalışmanın amacı, doğumdan sonraki 40-45 günde anöstrus döneminde oral olarak verilen bolus vitamin ve mineral premiks veya enjeksiyonluk olarak uygulanan mineral premiksin Kangal koyunlarının sezon dışı üreme performanslarını ve kuzu doğum ağırlıklarını değerlendirmektir. Toplamda 78 adet primipar Kangal koyunu rastgele üç gruba ayrıldı. Hayvanlar senkronize edildi ve postpartum 80. günde koç katımı yapıldı. Grup 1'deki koyunlara östrus senkronizasyonundan 40-45 gün önce 2 mL enjekte edilebilir mineral solüsyonu tek doz olarak uygulandı (n=25). Grup 2'ye ise, östrus senkronizasyonundan 40-45 gün önce oral olarak bir kez mineral bolus verildi (n=27). Grup 3'teki koyunlara grup 1 ve grup 2 ile eş zamanlı olarak hayvanlara (n = 26) bir kez 2 mL fizyolojik tuzlu su verildi. Östrus oranları gibi üreme parametreleri; gebelik oranları; tek, ikiz, üçüz ve çoğul gebelik oranları; bir batında yavru sayısı; embriyonik ölüm; doğurganlık; ve güç doğum oranları değerlendirildi. Ek olarak, mineral takviyelerinin plasentasyon üzerindeki etkisini değerlendirmek için plasentalar tartıldı. Yukarıdaki parametreler açısından gruplar arasında anlamlı fark yoktu (p>0.05). Ancak, güç doğum oranı Grup 2'de Grup 1 ve Grup 3'e göre anlamlı olarak daha düşüktü (p<0.05). Sonuç olarak, uzun salınımlı bolusların tüm gebelik boyunca etkinlik oluşturmadığı belirlendi.

Anahtar Kelimeler: Gebelik, Koyun, Mineral, Postpartum, Üreme.

INTRODUCTION

Sheep farming contributes significantly to the economic existence of many small low-input households that form part of their social culture, particularly in developing countries (Kosgey et al. 2006). Sheep are seasonal polyestrous animals that give birth once a year and

undergo prolonged anoestrous periods. To improve the economic contribution of sheep, efforts should be made to boost their reproductive efficiency by utilising straightforward and affordable solutions (Asaduzzaman et al. 2021). Reproduction management is the most important factor that determines the sustainability of sheep farms (Sharkey et al. 2001). Although various



methods have been used to successfully control reproduction in different regions of the world during several breeding seasons and in numerous breeds of sheep, a management strategy for the most productive reproduction has not yet been identified (Yu et al. 2018). Therefore, studies aimed at increasing reproductive success are needed (Abecia et al. 2012).

Minerals, such as phosphorus (P), calcium (Ca), magnesium (Mg), iodine (I), manganese (Mn), copper (Cu), selenium (Se), and zinc (Zn) are responsible for successful sheep reproduction. Vitamins and minerals are known to be insufficient in pastures where sheep are grazed, which negatively affects their reproductive performance (Garg et al. 2003; Robinson et al. 2006). Trace elements and vitamins may have significant effects on fertility and reproductive performance in sheep (Robinson et al. 2006). Several vitamins (such as A and E), which are essential components of biological processes, including fertility and embryonic development, can improve reproductive performance, reduce oxidative stress from mating and pregnancy, and maintain fertility in sheep (Kamiloglu et al. 2017). Deficiencies in many vitamins and minerals, such as I, Cu, Mg, Mn, Se, and vitamins A and E cause calm oestrous, anovulation, abortion, and the birth of difficult-to-live lambs (Smith and Sherman 2009). Inadequate intake of trace minerals may impair reproductive function (Hostetler et al. 2003). Microminerals play an important role in the stability of secondary molecules and the intracellular system that protects the cells from free radicals. Since microminerals are components of hormones and changes in the plasma levels of these minerals can affect hormone synthesis and reproduction, microminerals may have an impact on endocrine activity (Kumar et al. 2011). The reproductive performance of sheep fed in pastures, such as oocyte development, oestrous, ovulation, implantation, and embryonic and foetal development processes, is generally insufficient without the reinforcement of premix, block, bolus, and injectable minerals (Vázquez-Armijo et al. 2011).

Vitamin and mineral mixtures are being studied using different synchronisation protocols aimed at increasing the reproductive performance of sheep in and out of season (Awawdeh et al. 2019; Kuru et al. 2020; Robinson et al. 2006). However, no studies have investigated the effectiveness of different mineral and vitamin mixes on reproductive data and lamb parameters in Kangal sheep during the postpartum period. The purpose of this study was to investigate the effect of vitamin-trace element mixtures in injectable and bolus forms on reproductive parameters (oestrous rate, pregnancy rate, number of pregnancies, fertility, and dystocia rate) in Kangal sheep subjected to postpartum oestrous synchronisation during the nonbreeding season.

MATERIAL AND METHODS

Location

The study was carried out in a sheep farm between February-July with the following coordinates: latitude: 39.83371433796894, longitude: 36.34688098838113, and altitude: 1290 m in Sivas Province, Türkiye. During the study, the weather conditions in the current location were as follows: in April, temperature, relative humidity, and rainfall were 12±3 °C, 62%, and 76 mm (annual average 11%), respectively, while in June these data were 20±2 °C, 66%, and 66.62 mm (annual average 9 %), respectively.

Animals and treatment schedule

This study was approved by the Sivas Cumhuriyet University Animal Experiments Ethics Committee

(Approval No: 65202830-050.04.04-702 numbered and 10.12.2022 dated).

The material used in this study consisted of 78 healthy primiparous Kangal sheep (2-2.5-year-old). 10 Kangal rams between the ages of 4 and 6, which had proven their fertility were used. At the beginning of the study, the average live weight of Kangal sheep was 53±5 kg and the body condition score (BCS) was between 2.5 and 3.25, while the live weight of the Kangal rams was 102±5 kg and BCS was between 3.0 and 3.5. From the beginning to the end of the study, pasture flora was used as feed. Rams were fed 250 g of barley flakes daily in addition to the grazing pasture.

Kangal sheep breeders have abandoned milk production in recent years. The milk of the sheep constituting the study material was used only to feed the offspring. Estrous stimulation was performed after weaning. In conclusion, the milk yield was not monitored in this study. When the existing maternal ewes reached 40–45 days postpartum, each group was further divided into three groups containing 25, 27, and 26 ewes each. Exogenous mineral applications were performed as described below during the animal division of the groups.

From an injectable mineral mixture (Activate, Alke, Turkey), which included 2.5 mg copper equivalents of copper gluconate, 1.25 mg sodium selenite, 5 mg manganese equivalents of manganese gluconate and 5 mg zinc equivalents of zinc gluconate, a 2 mL dose was administered intramuscularly to maternal ewes in Group 1.

Kangal ewes in Group 2 were administered a bolus (TMR Nutrition Min Vit Sheep Bolus, Biochem Turkey) using a swallowing applicator. The bolus contains the following: vitamin A (5.000.000 IU/gr) 135 mg, vitamin D₃ (5.000.000 IU/gr) 1240 mg, (3b302) cobalt hydroxide carbonate 2.596 mg, vitamin E (50%) 4063 mg, magnesium citrate 12.023 mg, manganese (manganese sulfate) 15.575 mg, 3b202 iodine (calcium iodine) 15.575 mg, carbon oxide 24.339 mg, omega 3 67.720 mg, zinc (zinc oxide) 167.000 mg, dicalcium phosphate 179.000 mg, magnesium oxide 223.800 mg, and beeswax 284.000 mg.

The ewes in Group 3 were injected 2 mL of saline intramuscularly.

When the animals in all groups reached 65–70 days postpartum, they were separated and weaned from their lambs. Complete involution of the udder lasted for 15 days after weaning. An estrus induction protocol was performed to the all ewes in three groups. Induction protocol is displayed in figure 1. Pregnancy was confirmed by ultrasonography twice: 30 days (45th day) and 60 days (75th day) after ram introduction. The births were monitored and recorded. The lambs were weighed after weaning by their mothers for the first half hour. The lambs were monitored for 28 days to determine their viability. Births were considered dystocia if they were delivered in a longer time than required for any reason (foetal or maternal) and only with intervention (Jacobson et al. 2020). During the first 28 days after birth (in the neonatal stage), lochia and body temperature were controlled throughout the day to detect infectious diseases. Lochia, foul odour, serosanguinous discharge, and high fever were considered positive signs of infection.

In the postpartum period, the effects of exogenous mineral supplements on reproductive parameters such as oestrous rate, pregnancy rate, twin pregnancy rate, triplet pregnancy rate, multiple pregnancy rate, lambing rate, embryonic mortality rate, fecundity, and dystocia rate, as well as lamb live birth weight, placental weight, and lamb viability were assessed (Table 1 and Table 2).

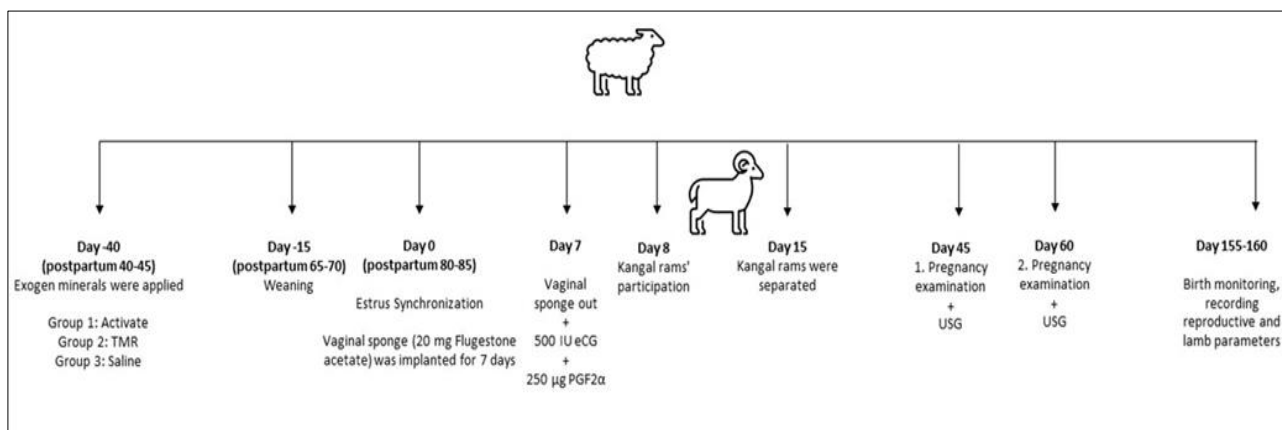


Figure 1: The synchronization protocol and schedule of minerals (Injectible and oral bolus) administered to the ewes (group 1 n=25, group 2 n=27, group 3 n=26).

Table 1: The effect of different treatments on the reproductive performance of Kangal sheep.

Reproductive parameters	Groups			p value
	Group 1 (n = 25)	Group 2 (n = 27)	Group 3 (n = 26)	
Estrous rate	21 (84 %)	23 (85.2 %)	18 (69.2 %)	> 0.05
Pregnancy rate	17 (68 %)	19 (70.4 %)	15 (61.5 %)	> 0.05
Twin pregnancy rate	8 (32 %)	11 (40.7 %)	7 (26.9 %)	> 0.05
Triplet pregnancy rate	2 (8 %)	2 (7.4 %)	1 (3.8 %)	> 0.05
Multiple pregnancy rate	10 (40 %)	13 (48.1 %)	8 (30.8 %)	> 0.05
Lambing rate	17/17 (1)	19/19 (1)	13/15 (0.87)	> 0.05
Embryonic mortality rate	0 (0 %)	0 (0 %)	2 (7.7 %)	> 0.05
Fecundity rate	29/17 (1.71)	34/19 (1.79)	22/15 (1.47)	> 0.05
Dystocia rate	5/17 ^b (29.4 %)	2/19 ^a (10.5 %)	3/13 ^b (23.1 %)	0.018

p<0.05 is statistically significance according to one-way ANOVA and post hoc Duncan test. Data expressed as mean ± SD. Uncalculated: Statistical analysis could not be performed since there was one birth of 3.

Table 2: Effect of bolus and injectable mineral mixture on some lamb birth parameters in Kangal sheep.

Lamb parameters	Groups			p value
	Group 1 (n = 25)	Group 2 (n = 27)	Group 3 (n = 26)	
Lamb weight (single) (kg)	5.02 ± 1.24	4.99 ± 0.98	4.97 ± 0.29	> 0.05
Lamb weight (twin) (kg)	4.16 ± 0.59	4.49 ± 0.15	4.05 ± 0.20	> 0.05
Lamb weight (triplet) (kg)	3.13 ± 0.11	3.94 ± 0.37	uncalculated	uncalculated
Placenta weight (g)	429.14 ± 28.19	420.83 ± 16.10	418.80 ± 18.44	> 0.05

p<0.05 is statistically significance according to one-way ANOVA and post hoc Duncan test. Data expressed as mean±SD. Uncalculated: Statistical analysis could not be performed since there was one birth of 3.

Pregnancy examination

Pregnancy was examined twice: as first pregnancy examination via transrectal ultrasonographic method 1 month (45th day) after participation of the ram, and second pregnancy examination via transabdominal ultrasonographic method 2 months later (75th day). If an animal was pregnant at the first control but not at the second control, early embryonic death was considered. To

determine pregnancies and offspring counts in the early period, a B-mode, linear-array ultrasonography device (Mindray DP50/Vet/US) containing a 5.0-7.5-MHz rectal probe was used in the supine position to determine embryonic and fetal losses, or transabdominally in the following days of pregnancy through the rectal route.

Statistical Analysis

Data were analysed using SPSS version 26 (IBM Corp., Armonk, NY, USA). Reproductive parameters (oestrus rate, pregnancy rate, twin pregnancy rate, triplet pregnancy rate, multiple pregnancy rate, number of pregnancies, embryonic mortality rate, fecundity, and dystocia rate) were analysed using the chi-square test. Lamb parameters (Lamb weight of single, twin, and triple, and placental weight) were analysed by one-way ANOVA and post hoc Duncan's test, and the results were expressed as mean±standard deviation (SD). Statistical significance was set at $p < 0.05$.

RESULTS

No infection was found in any animal in any group based on measurements of lochia and body temperature taken during the first 5 days following delivery. The first 28 days after birth showed no lamb mortality in any of the three groups.

Table 1 shows the reproductive parameters. There were no significant differences in the estrous rate, pregnancy rate, twin pregnancy rate, triplet pregnancy rate, multiple pregnancy rate, lambing rate, embryonic mortality rate, or fecundity between the groups ($p > 0.05$). The rate of dystocia in the sheep in Group 2 treated with TMR was significantly lower than that in Group 1 treated with Activate and the control group treated with saline ($p < 0.05$). The parameters related to the lambs are listed in Table 2. There were no significant differences between the groups in terms of lamb and placental weights ($p > 0.05$).

DISCUSSION AND CONCLUSION

To the best of our knowledge, no study has investigated the effects of mineral supplementation in different forms (injectable and bolus) on reproductive and lamb parameters before oestrus synchronisation (progesterone-containing sponge + eCG + PGF2 α) in postpartum Kangal sheep during the non-breeding season. Previous studies (Karadas 2014; Kivrak et al. 2022) have reported that there is a trace element deficiency in the rangelands of Central Anatolia where the present study was conducted. Trace element deficiency has been observed in previous studies on pastures of the Central Anatolia region (Alper and Taşova 2019; Karadas 2014; Kivrak et al. 2022). Therefore, neither macro- nor trace-mineral analyses of the rangeland forage were performed in our study.

In the previous study in which oestrus synchronisation was performed using a progestogen sponge and eCG + PGF2 α outside of breeding season on Kangal sheep, the oestrus rate as 71.19%, and the pregnancy rate was 31.03% (Gonzalez-Bulnes et al. 2020). In our study, we found the oestrus rate to be 84% in the group administered Activate, 85.2% in the group administered TMR, and 69.2% in the control group administered physiological saline. In addition, the pregnancy rate in our study was 68% in the group administered Activate, 70.4% in the group administered TMR, and 61.5% in the group administered saline. Although there was no significant difference, the rate of twin, triplet, and total multiple pregnancies in Kangal ewes in the groups that received TMR and Activate was quantitatively higher than that in the control group. In the TMR group, these values were higher compared to the out-of-season pregnancy rates obtained with different estrous synchronisations, as reported in a previously published meta-analysis

(Cizmeci et al. 2022). In a previous study, which also included findings supporting our study, it was reported that the percentage of oestrous and multiple pregnancy rates were quantitatively high, and the pregnancy rate was significantly higher in Pırlak sheep administered a mineral mixture of soft capsules (Toryum) (Kuru et al. 2020). However, in another study, the proportion of twins born from ewes administered the bolus was reported to be significantly higher than that in untreated ewes (Hemingway et al. 2001). Lactation-related changes in the concentrations of macro and trace elements in the blood of sheep during the postpartum period have been reported previously (Antunović et al. 2021). In this study, the blood Ca concentration was lowest in the early lactation period, higher in the late period, and highest in the middle period. As lactation progresses, there is a significant increase in the concentration of Mg, Co, and Cd in the blood and a significant decrease in the concentration of Na, Fe, Cu, Zn, Mo, and Se (Antunović et al. 2021). Trace element deficits have been found to have a deleterious impact on reproductive efficiency in sheep and goat (Vázquez-Armijo et al. 2011). In our study, we compensated for the trace element deficiency in the postpartum period and increased the oestrous and pregnancy rates quantitatively in the non-breeding season.

Trace element supplementation (Cu, Mn, Zn, Fe, Co, and Se) increases lambing rates under deficient conditions, but only Se provides good evidence that embryo development during implantation is impacted (Gürdoğan et al. 2006). In a previous study, ewes receiving a bolus had a higher lambing percentage than those administered Cu or copper oxide injections and the control group (Hemingway et al. 2001). In our study, this was 1 in the Activate group, 1 in the TMR group, and 0.87 in the control group. The quantity of mineral combinations, particularly Se, enhanced the lambing rate.

Foetal mortality, embryonic loss, and embryonic implantation are caused by low levels of Se, Zn, and Cu (Vázquez-Armijo et al. 2011). Previous research has shown the possibility of ruminal boluses with higher bioavailability improving reproductive efficiency, embryo number, embryo quality (Kuru et al. 2020; Mitchell et al. 2007), and multiple births (Hemingway et al. 2001). Research on Kangal sheep demonstrated no embryonic mortality in the control group when progesterone sponge + eCG + PGF2 α were used in synchronisation (Cizmeci et al. 2022). In our study, embryonic mortality rates were 0% in the Activate group, 0% in the TMR group, and 7.7% in the control group. Because of the content of the mineral mixture, embryonic mortality was not observed in the Kangal sheep treated in our study.

Most sheep breeds exhibit seasonal polyestrous behaviour; thus, exogenous hormone treatments are required to induce oestrus outside of the breeding season. Increased litter size and financial gain are key goals in this situation. Supplementation of ewes with Se and I before mating can increase fecundity by decreasing perinatal mortality and increasing lambing percentage (Grace and Knowles 2012).

Koyuncu and Yerlikaya (2007) reported that Se (1.31) and Se + Vit E (1.48) supplementation significantly increased the fecundity rate compared to the control group (1.15). Although vitamin E and Se supplementation in Awassi ewes did not cause a statistical difference in fecundity, it was reported to be higher than that in the control group (Awawdeh et al. 2019). In our study, the

fecundity rate was found to be 1.71 in the group receiving Activate, 1.79 in the group receiving TMR, and 1.47 in the control group, which is consistent with the findings of this study.

Dystocia and other reproductive problems have been linked to deficiencies in Ca, Mg, P, Cu, Se, Zn, and Mn, among other minerals (Molefe and Mwanza 2020). Ovarian function can be affected by a change in the Ca:P ratio by inhibiting the pituitary gland, resulting in a prolonged first oestrous and ovulation, delayed uterine involution, increased frequency of dystocia, placental retention, and uterine prolapse (Kumar 2003). In high-yielding dairy cows under heat stress, bolus mineral administration reduced the incidence of dystocia numerically but not statistically. However, the relationship between sheep dystocia and the prevalence of trace mineral deficiencies remains largely unknown. The dystocia rates in our study were as follows: Activate group (29.4%); TMR group (10.5%); and control group (23.1%). This finding suggests that addressing trace mineral imbalance that develops during the postpartum period may decrease the incidence of dystocia.

A previous study reported that the bolus had no effect on lamb birth weights compared to the non-treatment group (Garín et al. 2003). In another study, Zn, Se, and Co as slow-release ruminal bolus supplements in advanced pregnant Mehraban ewes were reported to increase lamb birth weights compared with the non-treatment group (Aliarabi et al. 2019). In contrast, pre-breeding vitamin E and Se injections do not affect the birth weight of lambs of Mehraban sheep (Farahavar et al. 2020). In our study, no statistically significant differences were found between the injectable and bolus mineral mixes in terms of lamb birth weights. This may be related to the duration of the mineral supplementation.

Nutritional imbalances, such as vitamin E and selenium deficiency, have been observed to decrease placental size and reduce foetal development and birth weight in surviving lambs (Freer and Dove 2002). According to a recent study, Se and vitamin E deficiencies may not be severe enough to prevent the placenta from growing normally or reduce the birth weight of lambs (Farahavar et al. 2020). A previous sheep study reported no effect of maternal Se diet on total placental weight (348.8 ± 13.8 g) or cotyledon weight (96.1 ± 4.9 g) (Vonnahme et al. 2010). In our study, there was no difference in the placental weights between the treated and non-treated groups, which is consistent with the results of these studies.

Our study has a few limitations. The injectable mineral combination was first administered as a single dosage. Second, the mineral combinations were tested only during the postpartum period. Oestrous synchronisation and testing at different stages of pregnancy should be conducted in future studies. Third, the number of animals in the study groups should be increased in future studies. Finally, blood will be collected from the animals in the group, and the hormonal and biochemical parameters will be monitored in future studies.

In this study, the effectiveness of injectable and bolus mineral mixes on reproductive and lamb-related parameters before oestrous synchronisation in postpartum Kangal sheep during the non-breeding season was evaluated. In this study, TMR administered as a bolus decreased the rate of dystocia and caused numerical improvements in other reproductive parameters, with Activate as an injectable mineral. Further studies with

wider participation are required to determine the effectiveness of these mineral mixes in Kangal sheep.

CONFLICTS OF INTEREST

The authors report no conflicts of interest.

AUTHOR CONTRIBUTIONS

Idea / Concept: AT
Supervision / Consultancy: AT
Data Collection and / or Processing: AT, ME
Analysis and / or Interpretation: MBK
Writing the Article: ME
Critical Review: AT, MBK

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