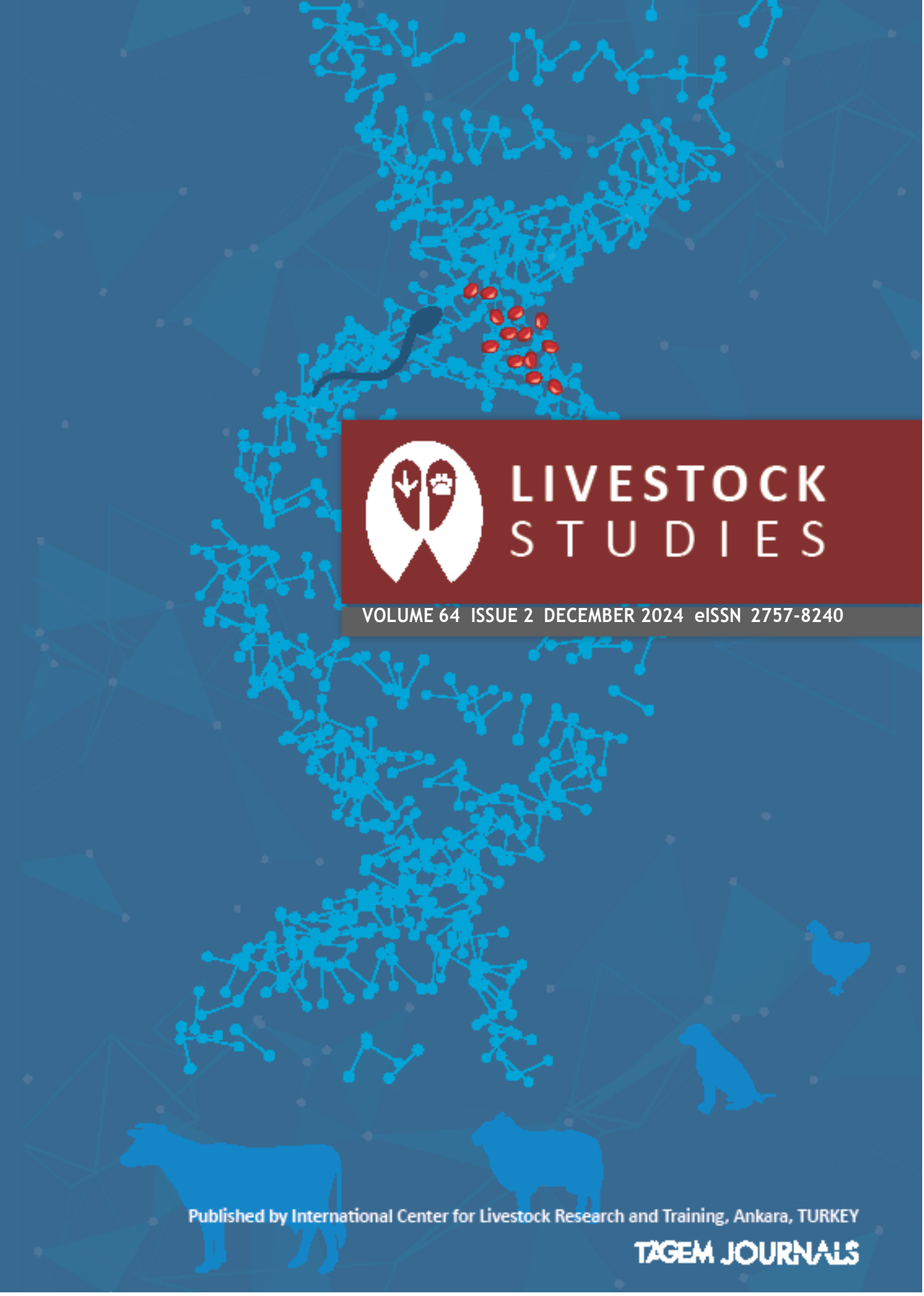

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LIVESTOCK STUDIES

RESEARCH ARTICLE

The Effects of Biscuit on Fermentation Characteristics, Aerobic Stability and In Vitro Organic Matter Digestibility of Alfalfa Silage

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Silage

Abstract

This study was conducted to determine the effects of addition of expired biscuit to alfalfa silage on silage quality, fermentation characteristics, aerobic stability and *in vitro* organic matter digestion. In the study, the silage group without additives constituted the control group, while the silages prepared by adding 1% biscuit, 2% biscuit and 4% biscuit constituted the experimental groups. Silages were opened after 70 days ensiling. There were significant differences among groups for dry matter (DM), ash, crude protein (CP), neutral detergent fiber (NDF), pH, carbon dioxide (CO_2) formation, *in vitro* organic matter digestion (IVOMD), metabolizable energy (ME) and in vitro methane gas (CH4) values of silages. The silage pH range of all silage groups was 5.12-5.82. When the CO_2 formation amounts of the silage groups were compared with the control group silages, it was determined that the amount of CO_2 released in the silages with increasing amounts of biscuits decreased ($P<0.05$) and IVOMD, ME and CH4 values increased ($P<0.05$). As a result, biscuits past their expiration date can be used as a silage additive in the ensiling of alfalfa. It was determined that it was appropriate to add 4% of expired biscuit waste in the ensiling of alfalfa.

Introduction

Animals meet their green fodder needs from meadows and pastures in certain periods of the year according to the specific vegetation conditions of each region. The productivity of animals is high in these 150-200 day periods and decreases in other periods. It is possible to minimize the difference in yield between seasons by providing sufficient quantity and quality of roughages rich in sap to animals outside these periods (Acar and Bostan, 2016).

Alfalfa has an important place in the rations of dairy cows, especially due to its contribution to the amount of cellulose and protein in rations. In order to ensure high milk yield, high quality alfalfa is primarily needed in enterprises. In dry climates, alfalfa can be preserved by drying. In humid climates, however, alfalfa harvest carries a significant risk of rain during drying in the field (Aksu and Yakışır, 2019). Therefore, the necessity of silage production arises in the green storage of green forages. Preservation of green forages by making silage during the seasons when they are abundant can ensure

that the productivity of animals is at the same level throughout the year (Çetiner and Polat, 2022).

Successful ensiling of alfalfa without additives is difficult (Aksu *et al*., 2017). The high buffering capacity, low water-soluble carbohydrate content and low dry matter content of legumes make their ensiling difficult. In recent years, there have been studies in which various silage additives have been used to obtain high quality legume green grass silages by eliminating these negativities. These additives are inoculants to improve fermentation in alfalfa silage (Koç *et al*., 2017), sugar-based fermentable liquids (Denek *et al*., 2011), carbohydrate sources (Şakalar and Kamalak, 2016) and fermentation- limiting organic acid additives (Ke *et al*., 2017).

Due to the insufficiency of feed resources, the use of many industrial wastes as an alternative feed source in animal nutrition has come to mind. Foods that are used in human nutrition and whose shelf life has expired, cannot be offered for human consumption due to problems that occur during their production (spillage, damage to packaging, etc.), and problems arising during transportation and storage lead to the formation of significant amounts of food waste. This situation causes great economic losses for food production companies. When these foods are utilized in animal husbandry enterprises, both economic and environmental damage is prevented. Pasta, biscuits, cakes, wafer crumbs, instant soups, puddings, etc. are used as animal feed in many farms (Korkmaz and Önenç, 2017). However, there are few studies on their use in animal nutrition (Çotuk and Önenç, 2016; Aydın, 2023).

This study was conducted to determine the effects of adding expired biscuits to alfalfa silage (0%, 1%, 2% and 4%) on silage quality, fermentation properties, aerobic stability and *in vitro* organic matter digestion.

Materials and Method

In this study, alfalfa (Medicago sativa L.) plant was used as silage raw material. The alfalfa was obtained from a private enterprise during the flowering period. The alfalfa was withered and chopped in the silage machine to approximately 1.5-2.0 cm in size. The control group used in the study consisted of alfalfa without additives (0%), while experimental groups were formed with 1%, 2%, 4% biscuit waste additions. Expired sweet biscuits were obtained from a supermarket. Biscuits were used by grinding. The control and each experimental group were pressed into 1,5 liter glass jars as 5 replicates. Silages were opened after 70 days of fermentation at room temperature in a dark environment. Silages were opened after 70 days of fermentation in a dark environment. After the top 3-5 cm of the jars were discarded, 100 ml of pure water was added to 25 g of silage samples taken homogeneously and the pH value of the disintegrated silage liquid was quickly measured with a laboratory pH meter in just a few minutes and recorded. Aerobic stability test (determination of CO_2 production values) analysis of silage samples was performed according to the method reported by Ashbell *et al*. (1991). After the silages were opened, they were subjected to an aerobic stability test for 5 days. The pH of the silage samples on the 5th day of aerobic stability was measured and their CO_2 production was determined. In the research, abrasion-resistant, gas-tight 1.5 L polyethylene (PET) bottles were used to perform the aerobic stability test. To create a test unit, the pet bottle was cut into two: 1L and 0.5L. 1 cm diameter hole was drilled in the lid of the 1L PET bottle to ensure air circulation. Then, 0.5 L was placed on the cut section. Fresh silage samples between 250-300 g were placed on the upper part of the unit without compressing, and 100 ml of 20% potassium hydroxide (KOH) solution was placed on the lower part of the unit. The prepared unit in question was kept for 5 days. In this way, CO_2 gas, which is formed in silage samples as a result of aerobic activity and is 1.5 times denser than air, settled at the bottom and was kept at the bottom. 10 ml of the solution was taken and titrated with 1N 37% hydrochloric acid solution. The amount of HCl spent between pH 8.1-3.6 was determined and the amount of CO2 gas was calculated according to the equation below.

CO_2 (g/kg DM) = 0.044xTxV / (AxFMxDM)

T= Amount of 1 N HCl (37%) acid consumed in titration (ml).

V= Total volume of 20% KOH solution (ml).

A= Amount of KOH added to the bottom of the apparatus (ml).

FM= Weight of fresh material (kg).

DM= Dry matter amount of fresh material (g/kg).

Dry matter (DM), ash and crude protein (CP) analyses of the silages and raw materials (alfalfa and biscuit) obtained in the study were performed according to AOAC (2005), while ADF and NDF analyses were performed according to Van Soest *et al*. (1991).

In vitro organic matter digestibility (IVOMD), metabolizable energy (ME) and methane gas (CH4) contents of silages were determined according to the method reported by Menke *et al.* (1988). The IVOMD and ME values were calculated by using the production amounts of the gases produced by the silages at the end of 24 hours using the equation reported by Menke *et al.* (1979).

IVOMD (%) = 14.88 + 0.889xGP + 0.45xCP + 0.0651xCA

ME (MJ/kg DM) = 2.20 + 0.136xGP + 0.057xHP

GP = Net amount of gas released after 24 hours of incubation (ml).

CP= Crude protein content of feed (%, DM).

CA= Crude ash content of feed (%, DM).

The data obtained at the end of the research were evaluated by one-way analysis of variance (One Way Anova) and Duncan multiple comparison test was used to compare group means (Soysal, 1998). For this purpose, SPSS (2008) package program used.

RESULTS AND DISCUSSION

The nutrient analysis results of the alfalfa plant and biscuit used in the research are presented in Table 1.

The nutrient contents of alfalfa silages prepared by adding biscuit waste at different levels (1%, 2% and 4%) are given in Table 2.

In this study, when Table 2 was analyzed, it was found that the differences between the groups were statistically significant (P<0.05) in DM, CA, CP and NDF values of silages.

When the DM contents of the silages prepared by adding different ratios of biscuit to alfalfa plants were analyzed, an increase in the DM levels was observed in parallel with the increase in biscuit addition compared to the control group. This increase in DM level suggested that this increase was due to the high level of biscuit DM (90.12%). Similarly, Aydın (2023) investigated the effect of the addition of wafer waste as a readily soluble carbohydrate source to alfalfa silage on silage quality, fermentation characteristics, *in vitro* organic matter digestion and CH4 values and reported that there was a parallel increase in DM in parallel with the increase in the addition of wafer waste and that this increase was realized depending on the DM content (98.75%) of the wafer waste. Canbolat *et al*. (2013) reported that the addition of gladichia fruit, which was used as a carbohydrate source in alfalfa silage, increased the DM values of alfalfa silages, and this increase was due to the differences in DM between fresh alfalfa and gladichia fruit (88.60%) in the study they conducted to determine the effects of gladichia fruit on fermentation, aerobic stability, *in vitro* gas production and microbiological properties of silages made under laboratory conditions.

When the ash values were analyzed, a decrease was observed due to the addition of biscuit. This decrease was realized due to the low ash level of biscuit (2.18% DM). Şerbetçi (2020) conducted a study to determine the effects of waste wafer addition on fermentation, aerobic stability and *in vitro* digestibility of alfalfa silages and observed that the ash content decreased with the increase in the wafer level added to alfalfa. He stated that the decrease in ash content was due to the low ash content of the wafer used as an additive.

When the CP values of the silages obtained were analyzed, there was a decrease due to the addition of biscuit compared to the control group (P<0.05). This decrease was realized due to the low CP (3.2% DM) content of biscuit. Similarly, it was reported that the addition of wafer waste decreased the CP values, and this decrease was due to the low CP content of wafer waste (Aydın, 2023).

The difference between the ADF values of the silages was found statistically insignificant (P>0.05). When NDF values were analyzed, biscuit addition decreased NDF values (P<0.05). The NDF values obtained in this study are in accordance with the report that adding carbohydrate source to silages accelerates the lactic acid bacteria activities in the environment, causing the breakdown of cell wall components and

Table 1. Crude nutrient analysis results of alfalfa and biscuit.

Silage material	DM	Ash	CP	ADF	NDF
Alfalfa	19.94	12.03	14.72	44.28	55.85
Additive					
Biscuit	90.12	2.18	3.2	3.14	7.48

* DM: Dry matter, %; CP: Crude protein, % DM; ADF: acid detergent fiber, % DM; NDF: Neutral detergent fiber, % DM.

Table 2: Nutrient composition of silage groups prepared in the study.

GROUPS	DM	Ash	CP	ADF	NDF
Control	20.405^{b}	13.213^{a}	14.648^{a}	44.355	56.630^{a}
1% Biscuit	20.295^{b}	12.274^{b}	14.583^{ab}	44.327	56.587^{a}
2% Biscuit	20.016^{b}	12.033^{bc}	14.483^{b}	43.852	53.049^{b}
4% Biscuit	22.337^{a}	11.763^{c}	14.250^{c}	43.450	52.500^{b}
SEM	0.287	0.169	0.048	0.227	0.623
P	**<0.001**	**<0.001**	**<0.001**	0.495	**<0.001**

a,b,c: Values with different letters in the same column were found different (P<0.05); **DM:** Dry matter, %; **CP:** Crude protein, DM%; **ADF:** Acid detergent fiber, %DM; **NDF:** Neutral detergent fiber, %DM**; SEM:** Standard Error of Mean.

Table 3: The effect on pH, CO_2 values and fermentation properties of silages prepared by adding biscuit to alfalfa plant.

GROUPS	pH	CO_2	IVOMD	ME	CH_4
Control	5.82[a]	8.877[a]	53.736[b]	7.986[b]	15.187[b]
1% Biscuit	5.53[b]	7.398[b]	53.609[b]	7.943[b]	16.533[a]
2% Biscuit	5.51[b]	6.943[b]	54.103[b]	7.979[b]	16.800[a]
4% Biscuit	5.12[c]	4.301[c]	56.223[a]	8.420[a]	17.133[a]
SEM	0.075	0.512	0.343	0.062	0.241
p	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**

[a,b,c:] Values with different letters in the same column were found different (P<0.05); **CO_2** : Carbon dioxide formation g/kg DM, **IVOMD:** *In vitro* organic matter digestibility %**, ME:** Metabolizable energy MJ/kg DM, **CH_4 :** *In vitro* methane gas (%)**, SEM**: Standard Error of Mean.

activating the proliferation of some anaerobic bacteria, increasing the breakdown of NDF, ADF and hemicellulose in silage (Bolsen *et al*., 1996).

When the pH values of the silages were analyzed, a decrease was observed in all experimental groups compared to the control group, but the lowest pH value (5.12) was obtained from the 4% biscuit supplemented group (P<0.05). With the addition of biscuit to alfalfa silage, there was an increase in DM and a tendency to decrease in silage pH. As the easily fermentable carbohydrate content increases in the ensiled material, the ideal acidic environment required for a good silage is formed. Therefore, the decrease in silage pH with the addition of biscuit to alfalfa silage is a necessary condition (Kılıç, 1986). After seventy days of fermentation, the silages were subjected to a five-day aerobic stability test and CO2 formation was examined. Biscuit supplementation increased lactic acid, pH decreased when the environment became acidic and accordingly CO2 output was less. Dry matter losses are directly related to increased CO2 production as a result of respiration (Kurtoğlu, 2011). The high DM in the same group supports this situation.

When IVOMD, ME value and CH4 values of silages were analyzed, IVOMD, ME value and CH4 values increased in all experimental groups compared to the control group (P<0.05). The highest values were in the group with 4% biscuit supplementation. The decrease in plant cell wall components of forages such as ADF and NDF, which are difficult to dissolve in rumen, parallel to the increase in biscuit supplementation increased the IVOMD and *in vitro* gas production of silages. The ME contents of alfalfa silages varied between 7.986 and 8.420 MJ/kg DM and the highest ME value was determined in alfalfa silage with 4% biscuit supplementation. The increase in the ME content of silages and the increase in IVOMD and CH4 gas production due to the increase in the level of biscuit added to alfalfa can be attributed to the decrease in NDF and ADF levels (Canbolat *et al*., 2010). ME contents of alfalfa silages were found to be compatible with the results of Getachew *et al*. (2002).

CONCLUSION

In this study, it was concluded that biscuits past their expiration date can be used as a silage additive in the ensiling of alfalfa. It was determined that it was appropriate to add 4% of expired biscuit waste in the ensiling of alfalfa when ensiling alfalfa. In future studies, it can be supported by both *in vitro* and *in vivo* digestion experiments and studies on biscuit additive levels.

Conflict of Interest

The authors declare there is no conflict of interest.

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LIVESTOCK STUDIES

RESEARCH ARTICLE

The Effects of Different Doses of eCG Injection on Reproductive Parameters in Nulliparous Merino Ewes During the Non-Breeding Season

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Abstract

In this study, the effects of adding 400, 500, and 600 IU eCG to progesterone treatment on rate of estrus, conception, pregnancy, fetal mortality, lambing, multiple birth and litter size in nulliparous Merino ewes the out of breeding season were investigated. A total of 90 nulliparous Merino ewes were utilized in the study. On day 0, a vaginal sponge containing 60 mg medroxyprogesterone acetate was inserted for a seven-day period. Ewes were randomly divided into three groups at the time of sponge removal. Group I (G-400), comprising 30 ewes, received 400 IU of eCG. Group II (G-500) received 500 IU of eCG (n=30), while Group III (G-600) received 600 IU of eCG (n=30). The statistical analysis revealed that the conception rates between the groups were statistically higher in the G-600 group than in the G-400 and G-500 groups (G-400 vs. G-600, P=0.091; G-500 vs. G-600, P=0.080). Pregnancy rates between the groups were found to be statistically higher in the G-600 group than in the G-400 group (P<0.021). The administration of a high dose of eCG (600 IU) in conjunction with progesterone synchronization yielded favorable outcomes with respect to reproductive parameters, particularly an elevated conception and pregnancy rates.

Introduction

In sheep breeding, the primary objectives are to achieve greater efficiency without increasing costs and to enhance reproductive performance (Özyurtlu and Bademkıran, 2010). The profitability of sheep farming is closely linked to the number of lambs produced annually (Bulbul *et al*., 2014). Exogenous hormones are the leading biotechnological products used in animal husbandry, especially to improve fertility. During the breeding season, progesterone and and prostaglandin F2 alpha (PGF2α) can be used for estrus synchronization and equine chorionic gonadotropin (eCG) to induce ovulation in sheep farming (Köse *et al*., 2022). The protocols entail the administration of progesterone to the ewe, whether short- or long-term, with the objective of stimulating the luteal phase of the cycle. Once this period has elapsed, the source of the progesterone is removed and eCG is applied (Cline *et al*., 2001).

eCG is a glycoprotein hormone secreted by trophoblastic epithelial cells of fetal origin that form the equine endometrial cups (Manteca Vilanova *et al*., 2019). eCG has been observed to exhibit high affinity for follicle-stimulating hormone (FSH) and luteinizing hormone (LH)-related receptors. This affinity has been associated with increased estradiol before ovulation (Rostami *et al*., 2011) and increased progesterone after ovulation in dairy cows (Rowe *et al*., 2019). In these treatments, the estrus response and onset of estrus may be affected by factors such as the dose and the time of administration of eCG, breed and season, body condition and geographical region (Quintero-Elisea *et al*., 2022).

In estrous synchronization protocols, low doses of eCG generally reduce the time to onset of estrus.

Additionally, evidence indicates that low doses of eCG typically enhance the number and size of large follicles, though it may exert minimal influence on the ovulation rate (Moakhar *et al*., 2012). The effect of low-dose eCG in non-breeding season ewes is of great interest. The effect of eCG on the dynamics of the follicular wave in ewes during the non-breeding season is well known (Roshan *et al*., 2023). A deeper understanding of this mechanism could facilitate the development of more effective methods for regulating sheep breeding (Barrett *et al*., 2004).

The number of progesterone and eCG-based studies on nulliparous ewes is insufficient, and the available information is limited (Santos-Jimenez *et al*., 2022). In studies conducted out of the breeding season, there are reports indicating that multiparous ewes have show a better response to these hormones compared to nulliparous ewes (Santos-Jimenez *et al*., 2022), as well as studies reporting no significant difference (Ungerfeld and Rubianes, 1999). In a study in ewe lambs, higher pregnancy and conception rates were found in the groups using eCG compared to the control group (Dias *et al*., 2020). It can be posited that the utilisation of eCG in ewe lambs may facilitate the attainment of a greater number of lambs.

It is thought that increasing the dose of eCG may induce estrus by stimulating follicular development and improve some reproductive parameters. It is hypothesized that the response to progesterone and different doses of eCG may vary non-breeding season in nulliparous Merino ewes. In this study, the effects of adding 400, 500, and 600 IU eCG to progesterone treatment on rate of estrus, conception, pregnancy, fetal mortality, lambing, multiple birth and litter size in nulliparous Merino ewes out of the breeding season were investigated.

Materials and Methods

The present study was conducted with approval from Selçuk University Animal Experiments Local Ethics Committee, Konya, Türkiye (2024/084).

Animals

This study was conducted in a commercial sheep farm (Lat: 37° 86’ 44.06» N, Long: 34° 16’ 33.55» E and Alt: 1.020 m) in Konya province, Türkiye during the non-breeding season in 2024. A total of 96 nulliparous (1 year old, 45-50 kg) Merino ewes, clinically healthy with no abnormalities in the perineum, were used. Six ewes whose sponge had been fell were excluded from the study. The study continued with 90 ewes. The ewes were permitted to graze on pasture for a duration of 12 hours each day and were not provided with any compound feed. They had ad libitum access to water. Prior to mating, the animals were not subjected to any nutritional flushing.

Treatment groups, synchronization and mating protocols

On day 0, a vaginal sponge containing 60 mg of medroxyprogesterone acetate (Esponjavet®, Hipra, Spain) was inserted for a seven-day period. On day 7, the sponges were removed. The ewes were randomly divided into 3 groups at the time the sponges were removed and 400 IU of eCG (Oviser®, Hipra, Spain) was injected to ewes in Group 1 (G-400) (n=30), 500 IU of eCG to ewes in Group 2 (G-500) (n=30), and 600 IU of eCG was injected to the ewes in Group 3 (G-600) (n=30).

A teaser ram was employed twice daily for 1-hour sessions over 36 hours following the removal of sponges to detect estrus. Ewes identified as being in estrus were hand-mated with one of the proven fertile Merino rams, maintaining a ewe-to-ram ratio of 7:1.

Ultrasonography and calculation of reproductive parameters

In all ewes, transabdominal ultrasound examination (Hitachi EUB-405, Japan, 3.5 MHz convex probe) was performed to diagnose pregnancy on day 45 post-mating. Pregnancy (single/twin) were diagnosed when embryos were seen on ultrasound examination. The litter size was determined at parturition.

The rate of estrus, conception, pregnancy, fetal mortality, lambing, multiple birth and litter size were calculated as reproductive parameters as follows;

$$Estrus\ rate = \frac{the\ number\ of\ ewes\ showing\ estrus\ behaviours}{the\ number\ of\ ewes\ receiving\ intravaginal\ sponge}$$

$$Conception\ rate = \frac{the\ number\ of\ pregnant\ ewes}{the\ number\ of\ mated\ ewes}\ x\ 100$$

$$Pregnancy\ rate = \frac{the\ number\ of\ pregnant\ ewes}{the\ number\ of\ ewes\ receiving\ intravaginal\ sponge}$$

$$Fetal\ mortality\ rate = \frac{number\ of\ detected\ death\ fetus}{the\ number\ of\ pregnant\ ewes}\ x\ 100$$

$$Lambing\ rate = \frac{the\ number\ of\ lambing\ ewes}{the\ number\ of\ pregnant\ ewes}\ x\ 100$$

$$Multiple\ birth\ rate = \frac{the\ number\ of\ multiple\ lambing\ ewes}{the\ number\ of\ pregnant\ ewes\ in\ each\ group}\ x\ 100$$

$$Litter\ size = \frac{the\ number\ of\ total\ lambs}{the\ number\ of\ lambing\ ewes}\ x\ 100$$

Statistical analysis

Statistical analyses were performed using SAS (Version 8.0). Reproductive parameters were assessed using the Chi-squared test, Fisher’s exact test (Selvi, 2024), and PROC GENMOD procedure. The results were reported as percentages. Statistical significance was defined as $p < 0.05$, and tendency was considered for $0.05 < p \leq 0.10$.

Results

Results for the rate of estrus, conception, pregnancy, fetal mortality, lambing, multiple birth and litter size are given in Table 1.

In the statistical analysis, differences between the groups in rate of estrus, fetal mortality, lambing, multiple birth and litter size were found to be insignificant (P > 0.05). Conception rates between groups were found to be higher in the G-600 group than in the G-400 and G-500 groups (G-400 vs G-600 P=0.091; G-500 vs G-600 P=0.080). Pregnancy rates between the groups were found to be statistically higher in the G-600 group than in the G-400 group (P<0.05).

Discussion

One method for increasing productivity in sheep is to obtain offspring during the anestrous period in Turkiye. During the non-breeding season, progestagens and eCG hormones are often used to stimulate functional activity in the ovaries. (Kaçar *et al*., 2008). eCG is known to be the most commonly used hormone to improve fertility in reproduction (Moakhar *et al*., 2012). The effectiveness of eCG is affected by many factors such as injection method, dose and source of eCG and season (Ali, 2007).

The objective of the presented study was to investigate the effects of different doses of eCG on reproductive parameters in nulliparous Merino ewes that had been synchronized with progesterone during the non-breeding season. In ewes, it has been reported that short term intravaginal progestagen treatment successfully induce and synchronize estrus in both breeding and non-breeding seasons (Ahmed Amer and Maher Hazzaa, 2009; Ataman *et al*., 2006). A study conducted non-breeding season demonstrated that the administration of eCG in conjunction with short-term progesterone resulted in a high estrus rate in both nulliparous and multiparous ewes (Ungerfeld and Rubianes, 1999). Santos-Jiménez (2022) observed that the estrus rate was higher in multiparous ewes than in nulliparous ewes during the non-breeding season. This result was attributed to the silent estrus of nulliparous ewes.

In some studies, conducted during the non-breeding season, estrus rates were observed to be high in ewes that had been synchronized with progesterone-based eCG applications at varying doses (Efe, 2010; Ungerfeld and Rubianes, 1999). The administration of 300, 500, and 700 IU of eCG following the intravaginal sponge application for 14 days during the seasonal anoestrus period resulted in estrus rates of 69.66% in all groups of Algerian Rembi sheep. (Bacha *et al*., 2014). Aköz *et al*. (2006) injected 300, 500 and 700 IU eCG after using intravaginal sponge containing 30 mg and 40 mg fluorogesterone acetate (FGA) in Merino x Akkaraman crossbred ewes during non-breeding season, and found the estrus rates to be 100%, 93.3% and 100% in the 30 mg FGA group, and 93.3%, 92.8% and 100% in the 40 mg FGA group, respectively. As with the aforementioned studies, it can be stated that the administration of varying doses of eCG does not result in statistically significant alterations in estrus rates, as observed in the presented study. It has been reported that eCG exhibits both FSH and LH activity and that these hormones are necessary for peri-ovulatory maturation of mammalian follicles (Murphy, 2018). Additionally, some studies have reported that eCG

Table 1. Reproductive parameters of nulliparous ewes.

	G-400 (*n*=30)	**G-500 (*n*=30)**	**G-600 (*n*=30)**	***P***
Estrus rate (%)	73.3 (22/30)	90.0 (27/30)	90.0 (27/30)	0.135
Conception rate (%)	59.1^{c} (13/22)	53.3^{c} (16/27)	81.5^{d} (22/27)	**
Pregnancy rate (%)	43.3^{a} (13/30)	53.3^{ab} (16/30)	73.3^{b} (22/30)	*
Fetal mortality rate (%)	7.6 (1/13)	18.8 (3/16)	13.6 (3/22)	0.679
Lambing rate (%)	92.3 (12/13)	81.2 (13/16)	86.3 (19/22)	0.679
Multiple birth rate (%)	16.7 (2/12)	23.1 (3/13)	42.1 (8/19)	0.261
Number of Lambs	14	16	28	
Single	10	10	11	
Twin	2 (4)	3 (6)	7 (14)	
Triplets	-	-	1 (3)	
Litter Size	1.17 (14/12)	1.23 (16/13)	1.47 (28/19)	0.244

Notes: Values with different superscripts (a, b) in the same row are significant differences among the groups (P < 0.05)
Values with different superscripts (c, d) in the same row are tendency differences (0.05 < P ≤ 0.1) among the groups.
* Pregnancy rate P value *G-400 vs G-600 P=0.021*
**Conception rate P value *G-400 vs G-600 P=0.091*; *G-500 vs G-600 P=0.080*
The numbers in parentheses are the formulation of the reproductive parameter.

causes an increase in progesterone concentrations (Baruselli *et al*., 2009). The findings of this study indicate that the administration of 600 IU eCG may enhance luteal activity, thereby improving pregnancy rates. While numerical differences were observed in certain examined characteristics, no statistically significant differences were identified. Takcı *et al*. (2023) reported that different eCG doses did not have a significant effect on reproductive parameters in primiparous ewes during the early anoestrus period. Köse *et al*. (2022) reported that different doses (400 and 600 IU) of eCG did not result in any differences in reproductive performance in ewes during the breeding season. Efe (2010) stated that although the use of 500 - 600 and 700 IU eCG after progesterone during the anoestrus period in ewes caused higher pregnancy rates than the control group (without eCG) and the dose difference did not affect the pregnancy rates. In a study conducted on Merino ewes during the non-breeding season, Doğanay (2011) used 400 and 600 IU eCG in addition to 14-day intravaginal progesterone application and found no statistical difference in pregnancy rates. Koyuncu *et al*. (2001) stated that the use of 0, 500 and 700 IU eCG in ewes synchronized with progesterone and found the litter size to be 1.21, 1.58 and 1.96, respectively on Kıvırcık ewes during the breeding season. In studies on the effects of eCG on reproductive parameters in ewes, it is seen that many factors are effective and therefore the results of the studies are not consistent (Köse *et al*., 2016). In order to eliminate this confusion in pregnancy rates, the optimum eCG dose needs to be determined. (Sharif *et al*., 2023). For this purpose, Sharif *et al*. (2023) used 300 and 600 IU eCG excluding the control group (without eCG) in Beetal goats at the beginning of the breeding season, and reported an increase in pregnancy rates in the group receiving 600 IU eCG compared to the other groups. Moaktar *et al*. (2012) in their study on Chall sheep during the natural breeding season, used 0, 450, 550, 650, 750, and 850 IU eCG and as a result, obtained higher pregnancy rates in the groups that received 550 and 650 IU eCG. Based on the studies conducted, it has been observed that different reproductive outcomes occur during breeding and non-breeding season. Many authors have argued that these results are due to the different responses of sheep breeds to eCG doses (Quintero-Elisea *et al*., 2022; Roshan *et al*., 2023). In our study, the Merino ewes used are capable of exhibiting reproductive activity for most of the year and have good fertility rates and, it is thought that the reproductive outcomes obtained, compared to other studies, to be due to the breed-specific characteristics.

Conclusion

In conclusion, it was determined that high dose eCG (600 IU) used in addition to synchronization with intravaginal progesterone during non-breeding season in nulliparous Merino ewes had positive results on reproductive parameters, especially causing an increase in pregnancy and conception rates.

Conflict of Interest

The authors declare that there is no conflict of interest.

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LIVESTOCK STUDIES

REVIEW ARTICLE

The Role and Importance of Acupuncture in Domestic Animals

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Abstract

This review provides a concise overview of acupuncture in veterinary medicine, tracing its roots from ancient Chinese practices to its integration into global veterinary care. It also examines the mechanism of acupuncture, focusing on the balance of vital energy (qi) along the meridians. The historical journey and applications of acupuncture in various animal species, pain management, neurological disorders, cancer support and more are discussed. In summary, the review highlights the importance and utility of acupuncture in veterinary medicine worldwide.

Introduction

Originating from Chinese medicine, acupuncture, and age-old therapeutic practice has become an invaluable tool in veterinary care for domestic animals Jishun and Mittelman (2014). This holistic method entails delicately inserting fine needles into specific points on an animal's body, promoting energy flow and facilitating the healing process Koh and Harrison (2023). Scientific and clinical endeavors over the past two decades have affirmed acupuncture's therapeutic value in treating animal diseases. A growing number of veterinarians aspire to integrate acupuncture therapy into their regular practices (Schwartz, 1992; Schoen, 1994; Guo and Ma, 2019; Yu *et al.*, 2020; Yu and Kim, 2023). Despite the absence of rigorous controls and statistical analysis in many published reports on acupuncture, several clinical studies offer valuable insights for routine practice. Notable Chinese Yu *et al.* (2020) and Western Jefferson (2020) publications have documented therapy methods and theories in veterinary acupuncture. This comprehensive review delves into acupuncture's extensive history, diverse mechanisms, and broad applications, emphasizing its ongoing relevance and evolution in veterinary medicine worldwide. This review aims to outline clinical indications demonstrating positive responses to veterinary acupuncture.

History of Acupuncture

The origins of acupuncture are intertwined with the philosophical and medical beliefs of traditional Chinese culture. The earliest recorded evidence of acupuncture dates to around 100 BCE in texts such as the Huangdi

Neijing (Yellow Emperor's Inner Canon), a foundational work of traditional Chinese medicine (Schwartz, 1992; Schoen, 1994; Jishun and Mittelman, 2014; Guo and Ma, 2019; Jefferson, 2020; Yu *et al.*, 2020; Koh and Harrison, 2023; Yu and Kim, 2023). Acupuncture techniques, originally developed by the Chinese, constitute an integral component of Traditional Chinese Medicine (TCM) for humans and TCVM for animals. Contemporary acupuncture can be categorized as either traditional Chinese medical acupuncture or Western medical acupuncture, the latter also referred to as transpositional acupuncture in veterinary practice Pyne and Shenker (2008). During the 1960s, there was a growing Western fascination with acupuncture medicine, leading to increased interest in TCM and TCVM. This curiosity spurred veterinary professionals to explore incorporating acupuncture into their practices, giving rise to the Western veterinary approach known as "transpositional" acupuncture Robinson (2007). Historical Chinese veterinary acupuncture point body maps were initially limited to cows, pigs, horses, and poultry, providing imprecise locations for a restricted number of acupuncture points. In the 1970s, North American veterinarians sought collaboration with human acupuncturists worldwide to develop point maps specifically for dogs and cats. They utilized human point system maps as a foundation but encountered challenges due to variations in posture and anatomy across animal species. Notably, the presence of a tail introduced an additional area for acupuncture stimulation, which lacked a corresponding site on the human body Koski (2011).

Mechanism of Acupuncture

The conceptual framework behind acupuncture is based on the idea of vital energy, known as "Qi" (pronounced chee), flowing through the body along specific pathways called meridians Pyne and Shenker (2008). The balance and harmonious flow of qi, which travels through the body's meridians, are considered essential for maintaining good health, while disruptions or blockages in this flow can lead to illness or pain (Acupuncture, 2024; Singapore Paincare TCM Wellness, 2024). Though the theories underlying traditional Chinese and Western veterinary medical acupuncture differ, both methods rely on the selection of effective acupuncture points, as a prerequisite for successful treatment Zang Hee *et al.* (2006). The Great Compendium of Acupuncture and Moxibustion and Huangdi's Canon of Medicine, which are recognized as the founding works of acupuncture, are where the concept of acupuncture points originated (Haltrecht, 1995; Li *et al.*, 2015; Cui *et al.*, 2022) Contemporary research on acupuncture's mechanism of action utilizes advanced neuroimaging techniques, including functional positron emission tomography, magnetic resonance imaging, magnetoencephalography, and electroencephalography. These sophisticated imaging modalities offer a secure and efficient means of observing brain activity, facilitating the identification, and mapping of neural correlates associated with acupuncture (Dhond *et al.,* 2007, 2008; Cho *et al.*, 2010). In veterinary medicine, the three most commonly employed acupuncture techniques are dry needling, aqua-acupuncture, and electro-acupuncture.

Aqua-acupuncture is a stimulation technique in which a liquid agent, often vitamin B12, is injected into an acupuncture point Chen *et al.* (2014). Unlike traditional acupuncture, where needles are left in place for a period, the stimulation in aqua-acupuncture is achieved through the changes in spatial configuration at the acupuncture point caused by the injected liquid Zhang *et al.* (2007). Electroacupuncture, on the other hand, combines manual acupuncture with electrostimulation DeBord *et al.* (2023). The effects of electroacupuncture are influenced by the frequency of the electrical impulses, provided that the waveform and all other parameters remain constant CMMI (2023). However, acupuncture, as a practice, entails the insertion of slender needles into specific points along these meridians to either stimulate or restore the balance of qi. Traditionally, the identification of these acupuncture points involves careful observation of the body and its responses to various stimuli. Classical acupuncture theory posits that disturbances in visceral conditions and organs are manifested at specific points, either on the skin surface or beneath it Li *et al.* (2015). An acupuncture points is regarded as a perforation in the skin that establishes communication with internal organs through a channel or meridian Haltrecht (1995). When assessing an illness, one must examine obvious signs such as tongue appearance, mental attitude, urine and feces characteristics and odor, palpate trigger points or painful locations on the body, and perform a thorough assessment of the body's general state Koski (2011). Various methods can be employed to stimulate acupuncture points, such as inserting fine filiform needles, applying heat (moxibustion), or pressure (acupressure), utilizing laser light, friction, and employing cupping Huntingford and Petty (2022). In ancient Chinese literature, there is acknowledgment of 361 classic acupuncture points associated with meridians, along with over 2000 extra-meridian acupuncture points. 309 of these acupuncture points are found on or close to nerves, according to recent research, and 286 of these points are encircled by tiny nerve bundles close to large blood arteries. (Chan, 1984; Zang Hee *et al.*, 2006). In addition, Acupuncture's physiological effects are tied to neuromodulation through direct nerve stimulation Dung *et al.* (2004). When tissue is needled at an acupuncture point, it activates the peripheral nervous system, prompting responses from the peripheral, central and autonomic nervous systems (Zang Hee *et al.*, 2006; Lindley and Cummings, 2008; Gaynor and Muir, 2015). Acupuncture induces changes in

neurohumoral and neuroendocrine factors, thereby altering pain transmission and augmenting the body's internal pain control mechanisms Gaynor and Muir (2015). The precise mechanisms elucidating the effects of acupuncture are still a subject of debate, but analgesia a key focus in literature has been extensively studied among the diverse effects exerted by acupuncture on the body and an increasing body of evidence suggests that acupuncture analgesia has physiological, anatomical, and neurochemical foundations Zhao (2008). Some studies suggest that acupuncture triggers the release of various bioactive chemicals encompass opioids at both spinal and supraspinal levels and serotonin and norepinephrine at the spinal level (Chang *et al.,* 2004; Kim *et al.,* 2005; Zhang, 2014). Recent animal studies have also indicated that a portion of electroacupuncture analgesia is not inhibited by naloxone (an opioid antagonist) Schoen (1986). It was found that median nerve stimulation releases an endogenous neuropeptide (orexin) from the hypothalamus to inhibit pain responses in mice through an endocannabinoid (an endogenous lipid functioning like chemicals from cannabis) that reduces the inhibitory (GABAergic) control in a midbrain pain-control region (the periaqueductal gray) Chen *et al.* (2018). The exploration of non-opioid mechanisms of acupuncture holds the potential to unveil new pharmacological targets for future pain treatment.

Acupuncture Points

In order to overcome the challenge of accurately transposing human acupuncture sites to animals, a modern method entails pinpointing the locations of peripheral nerves, neurovascular structures, and relationships with the central nervous system associated with human acupuncture points Koski (2011). Ongoing research, particularly at institutions like the Colorado State University of Veterinary Medicine and the University of Veterinary Medicine in Florida, is focused on developing precise transposition point location maps specifically tailored for dogs, cats, and horses (Deriu *et al.,* 2002; Xie and Wedemeyer, 2012).

Acupuncture points, classified into Four Types (Table 1), demonstrate elevated electrical conductance, reduced impedance, and enhanced capacitance in comparison to the surrounding tissue. A significant number of these points are located in areas where pain and muscle dysfunctions lead to the development of myofascial trigger points Schoen (1986).

By creating linkages between acupuncture sites throughout the body, activating these points aims to promote a balanced flow of Qi via the meridians.

The term "Zang" refers to the organs that are considered "solid" and yin in nature – the Heart, Liver, Spleen, Lung, and Kidney. In contrast, "Fu" refers to the "hollow" yang organs – the Small Intestine, Large Intestine, Gall Bladder, Urinary Bladder, Stomach and San Jiao (Lozano, 2013; Zhaoguo *et al.,* 2019).

Named after the Zhang Fu organs, there are twelve regular or major meridians (Lung Meridian (LU), Large Intestine Meridian (LI), Stomach Meridian (ST), Spleen Meridian (SP), Heart Meridian (HT), Small Intestine Meridian (SI), Bladder Meridian (BL), Kidney Meridian (KI), Pericardium Meridian (PC), Triple Warmer Meridian (TW), Gallbladder Meridian (GB), Liver Meridian (LV)) Xie (2007). These 12 meridians represent a conceptual grouping rather than literal anatomical structures. These organs, attributed to Western names, collectively play a role in generating and regulating Qi, illustrating interconnected functions governing Qi production and circulation Wright (2019). Meridians like Bladder and Gall Bladder have acupuncture points designated with numerical values along their trajectories. Additionally, there are eight Extra Meridians (Governing Vessel (GV), Conception Vessel (CV), Penetrating Vessel (PV or Chong Mai), Girdle Vessel (GV or Dai Mai), Yin Motility Vessel (Yin Qiao Mai), Yang Motility Vessel (Yang Qiao Mai), Yin Linking Vessel (Yin Wei Mai), Yang Linking Vessel (Yang Wei Mai)), each serving specific functions (Xie, 2007; Sudhakaran, 2013). These Extra Meridians contribute significantly to preserving bodily equilibrium by managing excess pathogenic Qi, regulating metabolism, and supporting the balance of blood, fluids, and Qi Harrison and Churgin (2022). Acupuncture can be used to treat a variety of common

Table 1. Acupuncture point types.

Type of Point	Location of Point
Type I	Located in areas where nerves enter muscles 67% of all acupuncture points are motor points
Type II	Located on superficial nerves in the sagittal plane on the dorsal and ventral midlines.
Type III	Located at high density loci of superficial nerves and nerve plexuses
Type IV	Located at musculotendinous junctions where the Golgi tendon organs are located.

(WHO, 2013)

illnesses and animals. Table 2 shows these ailments along with possible acupuncture point types. Figure 1 1-4 illustrate acupuncture points locations in for tranquilization, cardiovascular issues, liver function, and gastrointestinal health, respectively.

Acupuncture Around the World

Acupuncture gained recognition beyond China, spreading to other parts of Asia, and eventually reaching Europe and the Americas. The transmission of acupuncture knowledge occurred through both written texts and oral traditions. However, its acceptance in Western medicine was initially met with skepticism. For many current healthcare practitioners, embracing the traditional Chinese theory and its intricate yet elegant metaphysical concepts prove to be exceptionally challenging. This difficulty arises from the stark contrast between traditional Chinese medicine and mainstream Western medicine, which is rooted in contemporary scientific disciplines such as biochemistry, anatomy, physiology, and pharmacology Cheng (2009). In the 20th century, interest in acupuncture increased globally, and efforts were

Table 2. Animals and common conditions treatable with acupuncture, along with corresponding types of points for each condition.

Species	Common Conditions	Potential Main Points
Avian	Wing arthritis	LI11, HT3, SI4, BL11, BL 23
Avian	Limb arthritis	BL11, BL 23, BL40, BL60, KID3
Avian	Appetite stimulation	ST36, ST40, ST45
Avian	Feather plucking	HT7, LI11, ST36, and points for pain near picking
Avian	Pododermatitis	LIV8, GB34
Carnivore	Arthritis front limb	LI10, LI11, LI15, TH14
Carnivore	Arthritis hind limb	ST36, BL54, GB29, GB30, BL39, BL40, BL60, KID3
Carnivore	Spinal arthritis	Likely under anesthesia, points cranial and caudal to lesion, BL11, BL23, BL39, BL40, KID10, BL60, KID3
Hoofstock	Arthritis front limb	LI10, LI11, LI15, TH14
Hoofstock	Arthritis hind limb	ST36, BL54, GB29, GB30, BL39, BL40, BL60
Hoofstock	Gastrointestinal	LI10, LI11, ST36, ST40, BL20, BL21, BL25 (depends on height of animal as to success of obtaining all BL points)

(Chen *et al.,* 2014)

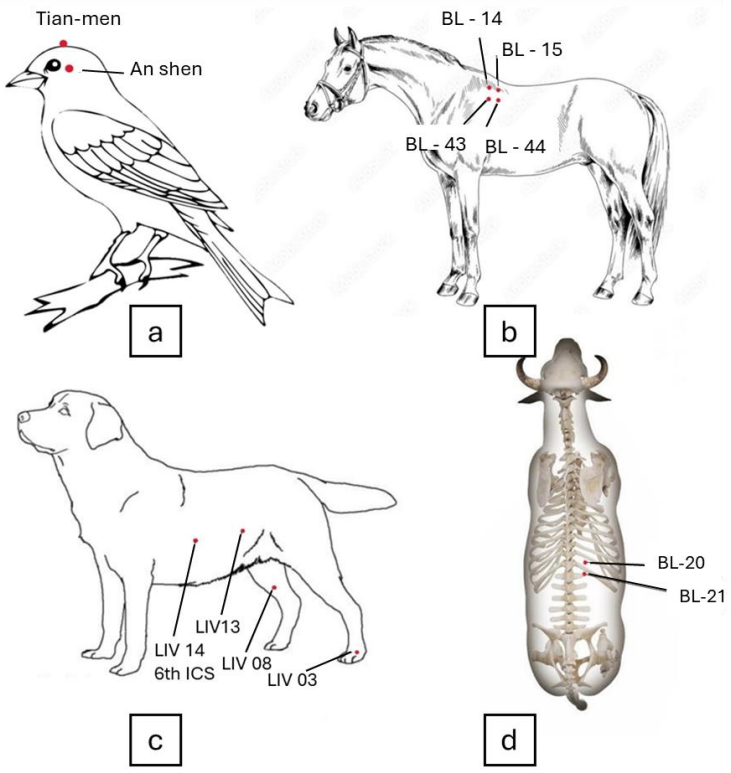

Figure 1. a) Tranquilization acupuncture points, b) Acupuncture points for cardiovascular problems, c) Liver acupuncture points, d) Gastrointestinal system acupuncture points

made to integrate it into Western medical practices. The World Health Organization recognized the potential benefits of acupuncture in treating a variety of health conditions (WHO, 2019; WHO, 2021). In the late 20th and early 21st centuries, acupuncture gained popularity as a complementary and alternative therapy in many parts of the world. According to a survey conducted by the World Federation of Acupuncture and Moxibustion Societies, Chinese acupuncture had been adopted in various forms in 183 out of 202 countries and regions worldwide by 2013 WHO (2013).

China boasts an array of over 80 distinct acupuncture techniques, and additional methods are utilized in countries such as The Americas, Europe, Japan, Korea, and Vietnam. While sharing a common diagnostic and therapeutic approach, there is no singular "traditional Chinese acupuncture," similar to the diversity observed in the interpretation and practice of Western medicine globally, despite shared principles. Acupuncture, too, exhibits variations in its application and interpretation Zang Hee *et al.* (2006). Western acupuncture medicine is rooted in anatomy and physiology, with a specific focus on concepts such as neuroanatomy and trigger points. The Western medical approach provides a clear bridge for medical professionals trained in the West, enabling the smooth integration of acupuncture into their treatment protocols.

Acupuncture finds various applications, with pain management standing out as a prominent use (Wright, 2019; Dewey and Xie, 2021), particularly in addressing conditions like arthritis Yu and Kim (2023), joint injuries, and post-surgical discomfort in animals Dewey and Xie (2021). Musculoskeletal issues Zhang and Wang (2020), such as lameness and muscle strains in domestic pets and working farm animals, neurological disorders Guo and Ma (2019), or gastrointestinal problems Li *et al.* (2022). In the realm of behavioral concerns, acupuncture is employed to manage anxiety, aggression, and stress-related behaviors (Koski, 2011; Mayo, 2013). This approach is not limited to specific animal types, as it is explored for its potential benefits in both small and large animals Schoen (1994). Acupuncture is considered a versatile tool in veterinary care, contributing to overall well-being. Table 3 shows the percentage of veterinarians using acupuncture in different countries throughout the world.

Acupuncture Applications in Horses

Acupuncture has become an integral component of equine medicine, offering a comprehensive approach to addressing a spectrum of health concerns in horses. Foremost among its applications is the effective management of pain such as back pain (Martin and Klide, 1987; Xie *et al.,* 2005; Rungsri *et al.,* 2009; Varhus and Xie, 2019) and the treatment of other conditions in horses such as Laminitis and navicular disease (Lancaster and Bowker, 2012; Faramarzi *et al.,* 2017), cervical stiffness Pasteur (2021), reproductive disorders and mastitis (Jerng *et al.,* 2014; He *et al.,* 2015), stress response Villas-Boas *et al.* (2015), emergency resuscitation Juffe *et al.* (2004), metabolic capacity Angeli and Luna (2008), laryngeal hemiplegia Kim and Xie (2009) and stem cell Salazar *et al.* (2017).

The utilization of acupuncture in treating horses can be traced back to the period between 475–221 BC. A significant milestone occurred during the Liang Dynasty (502–587 AD) with the publication of the Bole Liaoma Jing (Bole's Equine Therapy Classic), representing an early documentation of acupuncture methods for horses.

Two primary theories guide the mapping of effective acupuncture points in horses. The first, as per the ancient theory (1) outlined by Tangjitjaroen *et al.* (2009), suggests that specific points on the body, where injuries occurred, were found to successfully treat certain chronic diseases in horses. Subsequent treatments of other horses in a similar manner led to the deduction that these points had the potential to heal specific diseases. The second theory (2), described by Schoen (2000), involves the transpositional method. Considering the anatomical variations between the two species, this technique extrapolates

Table 3. Veterinarians employing acupuncture in various countries.

Country	Percentage	Country	Percentage
Australia	1.0-2.5	Japan	<2.5
Austria	<1.0	Mexico	1.0
Belgium	10-20	Netherlands	<0.2
Canada	<0.5	Norway	<0.1
Czechoslovakia	<0.4	South Africa	3.0
Denmark	<1.0	Switzerland	<0.5
Finland	10.0	Taiwan	10.0
France	<3.0	United Kingdom	<0.5
Germany	1.0-5.0	USA	<0.5
Irland	1.0		

(Santos *et al.,* 2022)

the established and well-known human meridians to horses. Despite criticisms regarding the use of comparative anatomy, clinical responses have been observed in cases where compatible anatomical structures exist, such as the lumbosacral space found in both species. This suggests that transpositional points may indeed have clinical effects Júnior *et al*. (2007).

The primary clinical applications of acupuncture in horses revolve around its utility in diagnosing and treating lameness (Schoen, 1993; Emily, 2023). Acupuncture points' exact anatomical location is important since it determines their function. Acupuncture points are thought to be mostly located at the locations where two or more muscles converge, in between fascia, and close to nerve ganglia and peripheral nerve branches that are connected to internal organs Robinson *et al.* (2007). Points related to nervous ganglia are particularly concentrated in the back, running parallel to the spinal cord Chapple (2013). In total, horses are recognized to have 361 acupuncture points Lin (2023).

Acupuncture Applications in Cattle and Sheep

Acupuncture has found widespread application in various ruminants, particularly in dairy and beef cattle, as well as sheep. Its uses span surgical analgesia, reproductive enhancement, lactation improvement, surgical adjunct, wound healing facilitation, immunity promotion, elevation of hematological and biochemical values, stimulation of ruminal motility, and correction of musculoskeletal disorders Acorda (2017).

In the context of reproductive performance, Rayos *et al.* (2001) demonstrated the positive impact of electroacupuncture and aquapuncture, using red pepper decoction, in reducing the calving to estrus interval and improving the overall conception rate in postpartum Holstein-Friesian cows. Additionally, Sumano *et al.* (1993) observed that acupuncture treatment of repeat breeding cattle, experiencing more than three failed inseminations, resulted in a pregnancy rate comparable to cows with normal fertility and no failed examinations. However, in bulls, acupuncture was found to be ineffective in treating semen abnormalities Arlt *et al.* (2006) and reducing libido Arlt and Heuwieser (2009).

In a study involving Friesian x Sahiwal crossbred lactating cows with mastitis, Daga *et al.* (2013) noted that aquapuncture using chili pepper decoction had the potential to decrease mastitis incidence. Both conventional needle acupuncture and aquapuncture were observed to potentially increase milk production. The effects of acupuncture on hematological and biochemical parameters were explored by Singh *et al.* (2008), who found that calves subjected to electroacupuncture exhibited changes indicating an enhanced immune response.

In sheep, the combined electrostimulation of four acupuncture points (GV-20, GB-34, ST-36, and SP-6) with xylazine resulted in effective analgesia, complete muscle relaxation of the abdominal and pelvic regions, increased heart rate, and decreased respiration rate Yadav *et al.* (2007). An analysis comparing four acupuncture point stimulation methods—aquapuncture, conventional needle acupuncture, hypodermic needle acupuncture, and pneumoacupuncture—revealed that hypodermic needle acupuncture and aquapuncture show promise as cost-effective alternatives to conventional acupuncture needles for inducing analgesia in sheep (Acorda *et al.,* 1997; Acorda, 1998, 1999).

Acupuncture Applications in Avian

Acupuncture is not as widely employed in avian species compared to other animals, but when utilized, birds generally exhibit positive responses (McCluggage, 2001; Eckermann-Ross, 2009; Burkett, 2021). Various techniques are applied in bird treatment, such as inserting and retaining needles, inserting and manipulating needles, or simply inserting and removing needles immediately Burkett (2021). Aquapuncture is often preferred in certain species due to anatomical differences that can make accessing specific points challenging or impossible.

Birds are commonly treated for various conditions, including osteoarthritis, paresis, anorexia, egg binding, and pododermatitis West (2011). Feather-destructive behavior, also known as feather plucking, can be addressed alongside other diagnostic and treatment approaches such as behavioral modification, habitat adjustments, and enrichment Burkett (2021).

Acupuncture Applications in Cats and Dogs

Acupuncture for dogs and cats proves to be a versatile and effective therapeutic approach, addressing a diverse range of conditions to enhance their well-being. This treatment method is particularly valuable for pain management, encompassing various issues like arthritis, back pain, hip and shoulder discomfort, knee pain, and conditions such as intervertebral disc disease Jia *et al.* (2023).

A review of older dogs with ruptured cranial cruciate ligaments found that, even in the absence of surgery, stifle function was restored in 6 to 10 months in those dogs treated with acupuncture and Chinese medicine Lee (2019). Ciolanescu (2020) examined 40 dogs with chronic lymphocytic leukaemia in another investigation. While the other half of the dogs received conservative care including joint supplements, exercise therapy, and pain management, the other half of the dogs received acupuncture and TCVM therapy. By week 24, both groups showed progress, but the acupuncture-treated dogs showed quicker and more noticeable outcomes.

Acupuncture extends its benefits to neurological disorders Santos *et al.* (2022), including degenerative myelopathy, seizures, and paralysis Kern and Erb (1987). Acupuncture also plays a significant role in alleviating symptoms related to cancer, either as a sole therapy or in conjunction with chemotherapy, contributing to an improved quality of life Ryu *et al.* (2014). The practice is found effective in addressing gastrointestinal disorders, respiratory problems Schwartz (1992) and internal organ diseases such as kidney, heart, or liver conditions Rose *et al.* (2017). Moreover, acupuncture serves as a valuable tool in managing autoimmune diseases, reproductive or infertility disorders, behavioral issues like anxiety Kontagionis *et al.* (2019) and endocrine disorders like hypothyroidism or Cushing's disease. According to a review conducted by Rose *et al.* (2017), dogs emerged as the predominant subjects in experimental acupuncture trials when compared to cats. There are over 360 recognized acupuncture points located throughout a dog's body (Snow and Zidonis, 2000; Jeong *et al.,* 2013). In cats, these acupressure points are generally found in the same locations as in dogs Snow and Zidonis (2000).

Acupuncture without Needles (Acupressure)

Acupressure, a form of traditional Chinese medicine, stands as an alternative to acupuncture, offering a needle-free approach to stimulate specific points on the body for therapeutic purposes. Unlike acupuncture, which involves the insertion of needles into these points, acupressure utilizes manual pressure applied by fingers, hands, elbows, or various devices to achieve similar therapeutic effects. Acupressure can be administered to specific acupuncture points using firm digital pressure, wooden massage-sticks, or plastic needle-holders, thereby circumventing the use of dry needle techniques (Scognamillo-Szabó and Bechara, 2010; Rogers, 2012). Applying daily acupressure on specific Diagonal Mirror Points in horse treatment, together with physical therapy aimed at pain sites, enhances, and supplements the clinical results obtained from weekly veterinary acupuncture sessions Rogers (2012).

Adverse Effects of Acupuncture in Veterinary

Acupuncture, especially when carried out by veterinarians with specialized training, has demonstrated its safety, with an exceptionally low incidence of side effects and adverse events White (2004). The risk of unintentional injury to vital organs or vessels is low if practitioners are diligent in identifying anatomical landmarks Robinson (2022). Severe reactions, possibly indicating nerve involvement, necessitate immediate needle withdrawal. It's noteworthy that horses undergoing acupuncture treatment may display adverse reactions, posing risks to both practitioners and handlers. While there is a potential for needle ingestion by patients, there have been no documented cases of associated injuries Robinson (2022). In contrast to human cases reporting adverse reactions like syncope, skin infections, and hepatitis, such incidents appear to be rare in veterinary patients. After a comprehensive analysis of 12 prospective human studies totaling more than a million treatments, the risk of a major adverse event from acupuncture was calculated to be 0.05 times per 10,000 treatments White (2004). This aligns with the NIH Consensus Statement, underscoring acupuncture's advantage in having a substantially lower incidence of adverse effects compared to many drugs or conventional procedures for similar conditions NIHCC (1998).

Conclusion

In conclusion, this comprehensive review sheds light on the evolution, mechanisms, and global applications of acupuncture in veterinary medicine. From its ancient roots in Chinese medicine to its integration into diverse veterinary practices worldwide, acupuncture has proven to be a valuable therapeutic tool for a range of animal species. The emphasis on its role in TCVM and the exploration of its mechanism, rooted in balancing vital energy (qi) along meridians, provide valuable insights. The review underscores acupuncture's versatility in addressing various health concerns in animals, including pain management, neurological disorders, and cancer support. The global acceptance of acupuncture highlights its continued relevance and evolution in veterinary care. The narrative concludes by introducing acupressure as a needle-free alternative, offering a nuanced perspective on holistic veterinary practices. Thanks to this method, many diseases and disorders that cause significant financial and sentimental losses for breeders can be prevented.

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LIVESTOCK STUDIES

RESEARCH ARTICLE

The Effect of Live Weight and Body Condition Scores of Akkaraman and Lalahan Sheep During Mating and Lambing Periods on Lamb Birth Weights

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Abstract

This study examines the effects of body weight and body condition scores (BCS) during mating and lambing periods of Akkaraman and Lalahan (KıvırcıkxAkkaraman G1) sheep breeds on birth weights of the lambs. The study was conducted with a total of 100 lambs born in February-March 2021, comprising 29 Akkaraman and 71 Lalahan lambs. The average live weights during the mating season were 59.17±1.20 kg and 54.07±0.77 kg; the average postpartum live weights were 61.58±1.43 kg and 59.05±0.92 kg (P>0.05); and the average BCS were 2.69±0.08 and 2.82±0.05 (P>0.05) in Akkaraman and Lalahan genotype (P<0.01), respectively. The birth weights of lambs were 4.95±0.14 kg and 4.75±0.09 kg in Akkaraman and Lalahan genotype (P>0.05), 4.92±0.07 kg and 4.25±0.18 kg in single and twin lambs (P<0.001), 4.89±0.11 kg and 4.74±0.10 kg in male and female lambs (P>0.05), respectively. Analysis using the CART algorithm revealed that the birth weight of lambs from ewes with a BCS of 2.5 or below and those with a BCS of 3 or above was 4.72 kg and 4.88 kg (P<0.05), respectively. The results indicate that Akkaraman lambs have slightly higher birth weights, and lamb birth type and ewe BCS have more pronounced effects on lamb birth weight.

Introduction

Sheep farming holds a significant place in animal production in Türkiye, accounting for approximately 60% of domesticated animals (FAO, 2022). Sheep are fed through natural grazing, supported by stubble and cereal stubble left fallow during the summer months (Sezenler *et al.*, 2011). Most of the sheep raised in Türkiye are native breeds, with Akkaraman breed, which constitutes around 40-45% of the small ruminant population, being the most common in Central Anatolia (Şahin, 2023; Sakar, 2024). Akkaraman sheep, a fat-tailed breed, has adapted to the region's harsh climate conditions and is raised for both meat and milk production. Lalahan sheep (Kıvırcık:0.75 x Akkaraman:0.25) is a genotype developed at Lalahan International Center for Livestock Research and Training (Ankara) to obtain a new genotype suitable for the steppe region conditions for lamb meat production (Erol *et al.*, 2017).

In most sheep production systems under natural grazing conditions, sheep mobilize their body reserves to overcome periods of feed scarcity. Therefore, simple and reliable methods are vital for assessing the nutritional status of animals in the flock and

determining when and how to provide nutritional support (Sezenler *et al.*, 2011). One of these methods is body condition scoring (BCS). In sheep farming, knowing the body condition (thin, ideal, or fat) of sheep at different stages of the production cycle is of great importance (Koyuncu *et al.*, 2018).

In sheep farming dominated by extensive conditions, body condition score (BCS) and its application are important for achieving desired performance during specific physiological periods (Sezenler *et al.*, 2011). There is an optimal BCS for each stage of the production cycle in the flock (Koyuncu *et al.*, 2018). Sheep with different BCS at stages such as mating, pregnancy, lambing, and lactation should be subjected to special feeding regimens according to these scores (Şireli, 2019). Body condition during mating and lambing directly affects the performance and productivity of both ewes and lambs (Karakuş and Atmaca, 2016).

The aim of this study is to examine the effects of live weight and body condition score (BCS) during mating and lambing periods on tbirth weights of lambs in Akkaraman and Lalahan sheep breeds. Within the scope of the study, the live weights and BCS of sheep from different age groups during mating and lambing periods were recorded, and the relationship of these data with the birth weights of lambs was analysed.

Materials and Methods

Animal Material

The study was conducted at International Center for Livestock Research and Training (ICLRT). The animal material consisted of 29 Akkaraman and 71 Lalahan (Kıvırcık x Akkaraman) lambs born in February-March 2021.

The animals are taken to pasture for approximately 6 months during the summer. During this period, no additional feed is provided. In the winter, the animals are given 40% roughage and 60% concentrate feed. The ration used for feeding contains 2.250 kcal/kg of Metabolizable Energy and 12% Crude Protein (115 g of crude protein/kg). The daily dry matter requirement for the sheep is determined to be 2.5 kg. Concentrate feed supplementation begins two weeks before the mating season. During last 3 weeks of pregnancy, 700 g/day/head of concentrate feed is provided, and at the start of lactation, 400 g/day/head is provided, with roughage mixtures of alfalfa hay and barley straw. Feeding is conducted twice a day, at 08:30 in the morning and 16:30 in the evening.

Mating took place over a 6-week period from September 8 to October 23. For hand-mating, every morning between 08:00 and 09:00 during the season, an experienced detection ram was introduced into the flock of approximately 40 ewes to identify those in estrus. The ewes in estrus were mated on the same day, both in the morning and afternoon, with rams previously assigned to them. Mating weight, mating date, and ear tag numbers of both ewes and rams were recorded. Internal parasite treatment was applied at the first mating date, and mating ewes were marked with red paint.

Live weight and BCS records

In the study, the mating and lambing dates of the ewes were routinely recorded. Subsequently, within the scope of the study, the live weights of the ewes during the mating and postpartum periods, as well as the birth weights of the lambs, were measured using a 0.20 g precision scale (Iconix FX41). Additionally, body condition scores (BCS) were taken from the ewes after they gave birth.

To determine the BCS of the mating ewes, a scale ranging from 1 to 5 with 0.5 intervals was used, as recommended by Sarı *et al.* (2013) and Koyuncu *et al.* (2018) (Figure 1). When recording the BCS values of the ewes postpartum, two assessors scored simultaneously. In cases where there was a discrepancy in the independently assigned BCS values, scoring continued until a consensus was reached between the assessors.

Figure 1. Determination zones of body condition score in sheep (Koyuncu *et al.*, 2018).

Statistical Analysis

The age of the ewes has been divided into four groups: 2, 3, 4, and 5 years and older (5+). The live weights during the mating period and postpartum live weights of the ewes were grouped based on the frequency of the distribution range, taking into account the class intervals. Information regarding the groupings is provided in Table 1.

Table 1. Categorization of dam age, mating live weight, and postnatal live weight values

Feature	Group	n
Dam age	2	23
	3	30
	4	25
	5+	22
Mating Live Weight (kg)	41-50 (Light)	30
	51-57 (Middle)	34
	58-77 (Heavy)	36
Postnatal Live Weight (kg)	43-58 (Light)	40
	59-65 (Middle)	33
	66-82 (Heavy)	27

The effects of ewe age, ewe mating period live weight, ewe postpartum live weight, and ewe BCS, along with breed, birth type, and sex, on lamb birth weight were examined using a General Linear Model (GLM). Correlation analyses were performed by breed to determine the relationships among the traits examined for both ewes and lambs. Following the correlation analysis, regression analysis was conducted to identify the priorities and levels of influence of the factors on ewe BCS and lamb birth weight. The decision tree method (CART - Classification and Regression Tree) was utilized to analyse the effective factors on ewe BCS and lamb birth weight. In the decision trees, some statistical approaches were employed to place the data at the tree's nodes.

In this study, the regression tree model was pruned using a maximum depth parameter of 5, and for the analysis of ewe BCS, all minimum conditions were defined as 20 at the upper node and 10 at the lower nodes. In examining lamb birth weights, the minimum conditions for Akkaraman breed were set to 4 at the upper node and 2 at the lower nodes, while for Lalahan genotype, the upper nodes were set to 20 and the lower nodes to 10. The relationship between the predicted values and the actual values in the CART algorithm was examined using correlation analysis. All analyses were performed using IBM SPSS Statistics for Windows, v 25.0 (Armonk, NY: IBM Corp.).

Results

In the study, the values obtained for the mating period and postpartum live weights and postpartum BCS of Akkaraman and Lalahan sheep, categorized by breed and age groups, are presented in Table 2. The average live weight during the mating period was found to be 59.17±1.20 kg for Akkaraman ewes and 54.07±0.77 kg for Lalahan genotype ewes, with this difference being statistically significant (P<0.01). The postpartum live weight was determined to be

Table 2. Least square means and standard error values of live weights at the mating and postpartum periods and BCS at the postpartum period in sheep

Feature	n	Mating Live Weight (kg)	Postnatal Live Weight (kg)	BCS
Genotype		0.001	0.142	0.230
Akkaraman	29	59.17±1.20	61.58±1.43	2.69±0.08
Lalahan	71	54.07±0.77	59.05±0.92	2.82±0.05
Dam Age		0.001	0.001	0.010
2	23	51.09±1.48b	53.94±1.77b	2.57±0.11b
3	30	57.90±1.42a	63.84±1.70a	3.04±0.10a
4	25	57.57±1.46a	61.28±1.75a	2.73±0.10ab
5+	22	59.91±1.33a	62.20±1.59a	2.68±0.09ab
Breed * Dam Age				
Akkaraman		0.007	0.002	0.053
2	6	53.33±2.55b	53.90±3.05b	2.50±0.19ab
3	6	61.85±2.55a	66.91±3.05a	3.16±0.19a
4	6	57.43±2.50a	58.31±3.05a	2.33±0.19b
5+	11	64.07±1.88a	67.20±2.25a	2.77±0.14ab
Lalahan		0.001	0.001	0.003
2	17	48.84±1.51b	53.98±1.81c	2.64±0.10b
3	24	53.95±1.27ab	60.76±1.52ab	2.91±0.09ab
4	19	57.72±1.43a	64.24±1.71a	3.13±0.10a
5+	11	55.76±1.88a	57.20±2.25bc	2.59±0.13b

a,b,c The difference between groups with different letters in the columns is significant.

61.58±1.43 kg for the Akkaraman breed and 59.05±0.92 kg for the Lalahan genotype, with the differences between groups being no significant. The BCS were found to be 2.69±0.08 for Akkaraman ewes and 2.82±0.05 for Lalahan genotype ewes, with the differences being statistically no significant. The effect of ewe age on live weight during the mating period, postpartum live weight, and BCS was found to be significant (P<0.01).

The birth weights of lambs obtained from Akkaraman breed and Lalahan genotype examined in the study are presented in Table 3. While the effects of breed, sex, and dam age on lamb birth weight were found to be statistically no significant, the effect of birth type was found to be significant (P<0.001).

Table 3. Least square means and standard error values of lamb birth weights

Feature	n	Birth Weight (kg)
Genotype		0.225
Akkaraman	29	4.95±0.14
Lalahan	71	4.75±0.09
Birth Type		0.001
Single	84	4.92±0.07[a]
Twin	16	4.25±0.18[b]
Sex		0.325
Male	48	4.89±0.11
Female	52	4.74±0.10
Dam age		0.112
2	23	4.69±0.15
3	30	5.05±0.13
4	25	4.62±0.15
5+	22	4.82±0.16

The relationships between the ewe's live weight during the mating period, live weight at lambing, body condition score (BCS), and lamb birth weight are presented in Table 4. Ewes' live weights during the mating period and post-lambing show significant, positive correlations with lamb birth weight. Additionally, the ewes' BCS also has a significant relationship with these variables; however, these relationships are generally weak to moderate.

By utilizing the classification and regression trees' ability to determine the class of a variable without any assumptions about the independent variable, the regression decision tree method was used to identify factors influencing ewe BCS. Using the CART algorithm, which operates as binary node splitting, both genotypes were analysed together. The ewe BCS was used as the root node (node 0), with sub-nodes (nodes 1 and 2) defining subgroups and terminal nodes. The CART outputs for this characteristic are presented in Figure 2.

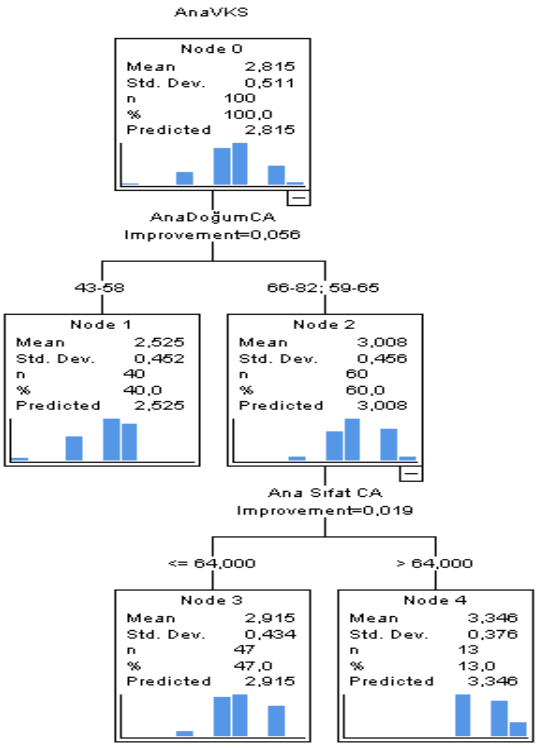

Figure 2. Determination of factors affecting BCS using CART method

The classification of factors affecting live birth weight in lambs using the CART method is presented in Figure 3. According to the CART results, when both genotypes were evaluated together, birth type was identified as the most significant factor influencing lamb birth weight. The average live birth weight of lambs was found to be 4.92 kg for single births and 4.25 kg for twin births.

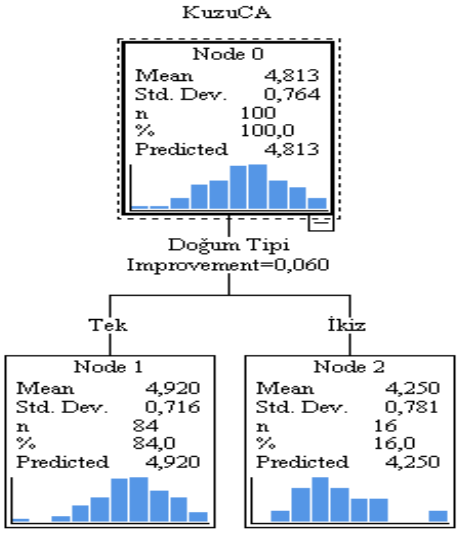

Figure 3. Determination of factors affecting lamb live birth weight using CART method

Table 4. Correlations between ewe live weight during the mating period, live weight post-lambing, body condition score (BCS), and lamb birth weight

Feature	Mating Live Weight	Postnatal Live Weight	Dam BCS	Lamb Birth Weight
Mating Live Weight	1,000			
Postnatal Live Weight	0,653***	1,000		
Dam BCS	0,294**	0,453***	1,000	
Lamb Birth Weight	0,200*	0,247*	0,266**	1,000

Significance levels: *:<0.05; **:<0.01; ***:<0.001

Discussion

Dam BCS

The study observed that breed and age had a significant effect on the ewes' live weight during the mating period, post-lambing weight, and BCS (Table 2). Akkaraman ewes were generally heavier and had lower BCS compared to Lalahan ewes. As age increased, there was generally an increase in live weight for both breeds, along with an increase in BCS. However, some fluctuations in BCS could occur with advancing age. These results indicate that breed and age should be considered in sheep breeding and management. Differences in breed and age are important for determining sheep nutrition and health management strategies. In this study, ewes' body weights at the start of breeding were found to be lower than their post-lambing weights. Similar results were reported by Sezenler *et al.* (2011), who also found that Kıvırcık ewes were heavier during breeding and lambing periods compared to Gökçeada and Sakız ewes. Şireli (2019) determined the average birth weight of İvesi ewes to be 53.80 kg, with twin giving birth ewes having the highest average birth weight at 54.88 kg. Schreurs *et al.* (2010) noted that live weight in the late pregnancy period has a small but positive effect on lamb birth weight, and suggested that body weight and condition during pregnancy and mating periods could influence lamb birth weight. Considering that birth weights were recorded post-lambing, Akkaraman lambs may have been born at higher live weights compared to Lalahan lambs, which could indicate lower post-gestation weight gain. Given that studies on Lalahan genotype were conducted in the early stages of genotype stabilization, it can be said that the desired progress has been achieved over the years with the influence of breeding efforts. The results obtained from the breed x age interaction in both Akkaraman and Lalahan genotypes reflect the sheep's health and nutritional status, and these fluctuations should be considered. Age and breed differences should be particularly taken into account for nutrition strategies and health management.

Birth Weight

The average live birth weight for Akkaraman lambs was determined to be 4.95±0.14 kg, while for Lalahan genotype lambs, it was 4.75±0.09 kg (Table 3). The close birth weight values of Lalahan genotype lambs to those of Akkaraman breed suggest that over the years, the characteristics of the genotype have stabilized, and desired progress has been achieved due to breeding efforts. Although it is generally known that lamb birth weight is influenced by genotype, the lack of significant difference can be considered natural given that Lalahan genotype is a hybrid genotype containing Akkaraman genes. The results are consistent with the findings of Tekin *et al.* (2015) for Akkaraman lambs, while the birth weight of Lalahan lambs was found to be higher than that reported by Mundan and Özbeyaz (2004). Since studies on the birth weight of Lalahan lambs were conducted during the genotype's developmental phase and stabilization efforts are ongoing, progress may have been made. Considering that the Lalahan ewes used in this study were randomly selected from the conservation flock, the progress observed can be regarded as normal. While it is generally understood that genotype affects live birth weight in lambs, the lack of significant difference can be seen as natural due to the hybrid nature of the Lalahan genotype and its inclusion of Akkaraman genes. This finding aligns with the results of Tekin *et al.* (2005) and Kandemir *et al.* (2013), but differs from those of Mundan and Özbeyaz (2004). The primary reason for this discrepancy may be attributed to the fact that the study by Mundan and Özbeyaz (2004) was one of the first on Lalahan genotype, while in the period between that study and the current one, genotype stabilization may have occurred.

When examining the effect of birth type on lamb birth weight, it was observed that single-born lambs were heavier than twin-born lambs (P=0.001). The results obtained in the present study are consistent with the findings of Tekin *et al.* (2005), Kandemir *et al.* (2013), Yavuz (2015), Şireli (2019), and Kutlu *et al.* (2022). Since it is physiologically known that single lambs have an advantage over twins during the maternal development period, the similarity between this study's findings and the literature can be considered expected. When examining the differences in lamb birth weight by sex, the difference between sexes was found to be statistically no significant (P=0.325). The results obtained in this study differ from the findings of Tekin *et al.* (2005), Şireli (2019), and Kutlu *et al.* (2022). This difference can be regarded as normal, considering the distribution of lamb sexes, the occurrence of single and twin births, and the fact that the ewes were selected from conservation flocks. It should be considered that the increase in lamb birth weight may have statistically reduced the significance of sex differences. Regarding the effect of dam age on lamb birth weight, the highest values (5.05±0.13 kg) were found in lambs born from three-year-old ewes, though the differences were no significant. This finding is in line with the results of Tekin *et al.* (2005), Kandemir *et al.* (2013), Yavuz (2015), Şireli (2019), and Kutlu *et al.* (2022). Although no statistically significant, numerical differences were observed within the age groups themselves.

Correlation

The correlation analysis results obtained in this study indicate that the live weight and BCS of ewes during the mating and lambing periods have a significant impact on lamb birth weight (Table 4). Considering that the sire line of Lalahan genotype, which was obtained through backcrossing, is the Kıvırcık breed-characterized by lower live weight and BCS compared to Akkaraman breed-it can be inferred that the dam's physical development might be more limited. The significant association of lamb birth weight with the ewe's live weight and BCS during mating and post-lambing periods suggests that the dam's overall condition and nutritional status have an important effect on lamb birth weight. A low positive correlation was determined between lamb live birth weight and dam BCS (r=0.266, p=0.007), which is similar in significance to the findings of Sezenler *et al.* (2008), Kandemir *et al.* (2013), Koyuncu *et al.* (2018), and Şireli *et al.* (2019), though lower in correlation degree. In the cited studies, a moderate to strong relationship between lamb birth weight and dam BCS was observed, but the use of different genotypes in the present study likely accounts for this variation. The strongest correlation was found between live weight during mating and post-lambing periods (r=0.653), indicating that ewes entering the mating period in good condition tend to remain in good condition post-lambing. These results demonstrate that the ewe's nutritional status and body condition can affect lamb birth weight.

Regression Tree

Dam BCS is influenced by both dam live weight at birth and dam live weight during mating (Figure 2). Ewes with a live birth weight below 58 kg generally have a lower BCS. Ewes with a live birth weight above 58 kg tend to have higher BCS, and this group is further differentiated based on dam live weight during mating. Ewes with a mating live weight above 64 kg exhibit the highest average BCS. This indicates that the BCS of ewes is associated with their live weights both post-lambing and during the mating period. These results show that the nutrition and care of ewes have a significant impact on BCS. The study demonstrates that the birth type is an effective factor on lamb birth weight as shown by the regression tree model (Figure 3). The birth weight of single-born lambs is higher compared to that of twin-born lambs. The "Improvement" value is shown to be 0.060, indicating the amount of improvement contributed by the "birth type" to the model. It was determined that birth type accounts for 10% of the variance in lamb live birth weight, while other factors have a much greater impact (R^2=0.104). When considering lamb live weight, the maternal environment, particularly the structure of the uterus and fetal circulation, develops a physiological system based on a single fetus in singleton pregnancies, while in multiple pregnancies, this network is increased, which can be seen as a division of maternal nutritional resources. In this context, it is inevitable that the birth weight of multiple pregnancies is lower than that of single pregnancies. However, when considering the main effects, factors such as genotype, age, live weight, and BCS should be evaluated in relation to their physiological effects on the formation of maternal environmental conditions.

Conclusion

This study investigated the effects of ewes' live weight and body condition scores, during the mating and lambing periods on lamb birth weights. The results showed that older ewes with higher BCS gave birth to heavier lambs. While birth type had a significant effect on lamb birth weights, the effects of other factors (breed, sex, dam age) were found to be statistically no significant. Additionally, it was confirmed through correlation analysis that good nutrition and health status of ewes during pregnancy positively influence lamb birth weight. These findings emphasize the importance of appropriate breeding practices before and during pregnancy in sheep farming.

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Conflicts

The authors declare that there is no conflict of interest.

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RESEARCH ARTICLE

The effects of supplementation of milk with sodium butyrate on calf performance, some blood parameters and fecal *Escherichia coli (E. coli)* presence

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Abstract

The present study was conducted to determine the effects of supplementation of milk with sodium butyrate (SB), on calf performance, some blood parameters and *Escherichia coli (E.coli)* presence in feces. 10 male and 10 female Holstein calves of 7 days of age and 40-45 kg live weight were selected for the trial which lasted 50 days. The milk given to the trial group in the morning feeding was supplemented with SB at a dosage of 3 g/day from day 7 to 21 and 5 g/day from day 21 to 49. Water was provided *ad libitum*. On days 7 and 50, blood samples were drawn from 6 randomly selected calves from each group for Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), β-Hydroxybutyric Acid (BHBA), Immunoglobulin A (IgA) and Growth Hormone (GH) determinations. Fecal samples were also collected for *E.coli* counts. At the end of the study, it was observed that SB supplementation had a positive effect on IgA and GH throughout the trial, as well as on GCAA from day 21 to 35 ($p<0.05$). Whereas BHBA, AST and ALT concentrations, body weight, feed consumption and feed conversion parameters remained unaffected ($p>0.05$). *E.coli* analysis in feces, revealed %33.33 less pressure in trial group calves.

Introduction

Calves undergo significant physiological and metabolic changes in the time frame from birth to weaning. During this period, also referred to as pre-ruminant stage, calves are similar to monogastric animals in many aspects of digestion and metabolism (Heinrichs, 2005). The feature that distinguishes calves from monogastric is the presence of rumen, reticulum and omasum in their digestive system (Quigley *et al.*, 1991). However, these organs are not functional in the early stages of life and do not participate in the digestive process as they have not completed their development yet (Jiao *et al.*, 2015). The rumen constitutes %25 of the total stomach in newborn calves (Sato *et al.*, 2010) and its development begins around 2-3 weeks after birth, continuing until 6 months of age. For the stimulation of rumen development, inoculation and establishment of the anaerobic microbial ecosystem, initiation of solid feed intake and subsequent fermentation processes and absorption mechanisms are essential (Baldwin *et al.*, 2004). If volatile fatty acids (VFAs) can be produced in the rumen by means of adequate forage intake, the calves can use the energy obtained therefrom for organ and digestive system development. Besides, the growth in number and height of papillae as the rumen develops, increases the total absorption surface. Therefore, nutrient absorption through the rumen wall is enhanced (Govil *et al.*, 2017). Of the rumen VFAs, butyric acid (BA) provides the energy necessary for the thickening of the rumen wall, papilla formation and increased capillary development (Suarez *et al.*, 2007), whilst acetic and propionic acids provide the energy required for the growth of the calf. The effect of milk and milk replacers (MRF) on the rumen development of calves is limited, paving the way to digestive problems, metabolic acidosis or villus atrophy (Heinrichs, 2005; Berends *et al.*, 2012).

As excessive milk and milk replacer feeding causes a decrease in solid feed intake, VFA production gets impaired, negatively affecting the development of the anterior stomach, post-weaning feed intake and body weight gain (BWG) (Khan et al., 2007b; Laborde, 2008).

In recent years, alternative organic acid (OA)-derived feed additives are being developed with a view to improving gastrointestinal flora in animals. Although they come under the Generally Recognized as Safe (GRAS) category, palatability issues (Moss and Newbold, 2002), inconsistent responses on ruminal pH and cost considerations limit their use (Newbold and Rode, 2006). One of these acids, the BA, has been the subject of considerable research work in the last few years. Butyric acid (systematic name: butanoic acid, chemical formula: CH3 (CH2) 2 COOH) is found in all biological fluids and tissues as a natural component of cellular metabolism and has a pKa value of 4.82. BA is present in the digestive tract contents, milk, and also in the perspiration and feces of most mammals, and in the initial stages of life it is supplied first by colostrum, then by milk (Ceballos *et al.*, 2009; Garcia *et al.*, 2014). BA used for feed additive purposes consists of water-soluble and odorless sodium (Na), potassium (K), magnesium (Mg) and calcium (Ca) butyrate, usually supplied in powder or protected form (Gorka *et al.*, 2009; Nazari *et al.*, 2012). These forms have the advantage of being odorless and having less solid and volatile properties which facilitate their use (Fernández-Rubio *et al.*, 2009; Guilloteau *et al.*, 2010). Third generation butyrate are produced with a specific oil coating process to protect the active ingredient, which at the same time may eliminate the odor problem and help reduce the level of pathogenic bacteria, especially *Salmonella* (Fernández-Rubio *et al.*, 2009). Studies on bacterial colonization of the intestine during the calf period have focused on *E. coli* which has been identified as the most common pathogen implicated in diarrhea cases (Chanter *et al.*, 1984; Janke *et al.*, 1989). Frequent diarrhea problems can be significantly reduced by preventing the attachment of pathogens such as *E. coli* to the intestinal wall (Diebold and Eidelsburger, 2006).

Sodium butyrate (SB) supplementation has been reported to affect calf performance and health status positively by stimulating intestinal cell proliferation, villus development and digestive enzyme activity (Guilloteau *et al.*, 2010; Valentine, 2016). In a study, it was observed that the addition of %0.3 SB to the milk replacer (MRF) of 46-day-old calves did not alter the ruminal flora and pH but improved the reticulo-rumen epithelial structure and increased its mass (Gorka *et al.*, 2009). The addition of SB to the milk replacer fed to newborn calves had positive effects on the development of rumen papillae (Gorka *et al.*, 2009; Ślusarczyk *et al.*, 2010), butyrate added to milk and calf starter feed (CSF) improved calf health by reducing diarrhea in the early weeks of life (Gorka et al, 2009), accelerates the maturation process of the intestinal mucosa (Kotunia *et al.*, 2004) and delays gastric evacuation (Guilloteau *et al.*, 1981; Zabielski *et al.*, 1998). It has also been reported that SB can be an effective growth promoter when added to the MRF and CSF of calves aged 3-26 days at an inclusion rate of %0.3 of dry matter (DM) (Guilloteau *et al.*, 2009b). Furthermore, there are also studies indicating a more efficient feeding and rumen development with the addition of SB to CSF (Gorka *et al.*, 2009; Gorka *et al.*, 2011a). It was reported that the growth performance of animals increased with the addition of butyrate to the feed, the most effective SB dose being %1 to 4 of DM, and that the protection of the butyrate molecule by microencapsulation in a lipid matrix to ensure slow release increases the effectiveness of the molecule, by preventing its rapid metabolism in the stomach and increasing its availability in the upper sections of the small intestine (Claus *et al.*, 2007; Gorka *et al.*, 2009).

The purpose of this study was to determine the effects of adding SB, a form of BA protected with Na salt, to the milk on calf performance, some blood parameters and fecal *E. coli* presence.

Materials and Methods

Ethic approval

This study was approved by Animal Experiments Local Ethics Committee of Ankara University with the letter of consent dated 05/07/2017, protocol number 2017-14-115. The trial was conducted at a commercial dairy farm in Adana, Turkey (Sarıçam Alibaba Süt Sığırı İşletmesi, 37°07'04.1"N 35°35'49.6"E), between August the 5th and October the 1st, 2017.

Animals, feeds and feed additive

A total of 20 Holstein calves, 10 males and 10 females with an average body weight (BW) of 40-45 kg at 7 days of age, were used in the study. A period when high calving frequency was expected in the farm was chosen for the trial and births were monitored. Calves were fed in the infirmary during the first week of life to obtain consistent animal

material. Immediately after birth, calves were weighed individually to record their birth weights. Calves were weighed again on day 7, just before they were placed in individual hutches, to record their initial trial weights. Attention was paid to ensure that the distribution of male and female calves in the groups and their 7th day body weights were close to each other.

Sodium butyrate in powder form, soluble in milk and MRFs, protected with sodium salts of distilled palm fatty acids (SB %70, palm fatty acid %30) was used as trial material. Dry matter (DM) and ether extract (EE) of regular and SB supplemented milks were determined by gravimetrically while crude protein (CP) levels were measured by Kjeldahl method (Table 1) (Akyıldız, 1984).

Calf feeding and vaccination program

Calves were allowed to receive colostrum for the first two days of life. Subsequently, each calf was given a total of 5 L of milk from day 2 to 25, and 6 L from day 25 to 49. This amount was bottle fed in two accurately measured and controlled feedings at 7:30 AM and 5 PM. The animals were divided in two groups of 10 mixed sex calves each: one control, and the other trial (SB supplementation) group. Calves in the trial group received 3 g/day SB from day 7 to 21 and 5 g/day from day 21 to 49, added into the morning milk. Butyric acids were dosed by weighing on scales with a sensitivity of 0.1 gr and added in the milk of the trial group calves. To ensure homogeneous dispersion and maximum dissolution, SB supplemented bottles were shaken well before feeding. On the seventh day the calves were removed from the infirmary and transferred to individual calf hutches, where, alongside milk, CSF, Alfalfa hay and water were offered free choice according to the trial design. Drinking water was refreshed 3 times during the day, considering local temperatures and relative humidity levels. Ademin (vitamin) and Yeldif (B group vitamins and selenium) injections were administered on days 3 and 53, as well as Pasteurella vaccine on days 15 and 45 days, as per the routine practice of the farm.

Performance parameters

Body weights (BW) were recorded individually by weighing at birth, day 7 and thereafter every two weeks until the end of the trial. Calves were weighed before the morning feeding and at the same hours. Body weight gain (BWG) was determined over the difference between weighing and then daily body weight gain (DBWG) was calculated by division. Individual daily solid feed intake of the calves was calculated by weighing the feeds before distribution in the morning and subtracting the leftover feed collected the next morning. Feed conversion ratio (FCR) was calculated using the aforementioned data. Performance data set was thus compiled.

Blood parameters

Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), β-Hydroxybutyric Acid (BHBA), Immunoglobulin A (IgA) and Growth Hormone (GH) determinations were performed on the blood samples drawn on days 7 and 50, from 6 calves randomly selected from each group (12 in total). To ensure that SB is fully transferred to blood circulation, the samples were taken 4 hours after the milk feeding from the vena jugularis by means of a catheter. Serum was separated from the blood samples by centrifuge for 5 minutes at 5000 rpm in

Table 1. Chemical compositions of feeds and milk fed to the calves

	DM (%)	CP (%)	EE (%)	CC (%)	ADF (%)	NDF (%)	Starch (%)	Sugar (%)	ME (kcal/kg)
Feed									
AH	94.64	14.3	2.4	20.5	33.7	46.0	1.1	2.4	1927
CSF	88.00	23.58	1.25	9.75	10.65	26.27	30.60	5.0	2437
Milk									
Regular	11.63	3.18	3.40						
+3 gr SB	11.67	3.20	3.40						
+5 gr SB	11.75	3.19	3.40						

DM: Dry matter, CP: Crude protein, EE: Ether extract, CC: Crude cellulose, ADF: Acid detergent fiber, NDF: Nort detergent fiber, ME: Metabolizable energy, AH: Alfalfa hay, CSF: Calf starter feed, SB: Sodium butyrate

a NUVE NF 200 bench-top device. Separated sera were then placed in Eppendorf tubes using Finnpipet. On the sera thus obtained, GH was determined by Ferritin Electro-chemiluminescence immunoassay (ECLIA) method using a Roche E-170 device with Roche diagnostic kit; IgA by NEF (nephelometric) method (Aksu *et al.*, 2006), whilst ALT and AST were determined spectrophotometrically using commercial kits (Herbos Dijagnostika).

Fecal *E. coli* presence

Escherichia coli presence in feces was determined on fecal samples collected on days 7 and 50 from a total of 12 calves randomly selected from the trial (n=6) and control (n=6) groups. Fecal samples were taken rectally to evade the risk of contamination of the samples. The samples were placed in gel tubes and stored in deep freezer to prevent increases or decreases in the bacterial load of the feces over time. The samples were subjected to microbiological analysis by culture method and *E. coli* presence was determined as positive or negative (present - absent).

Statistical methods

The data obtained in the trial are descriptive statistics related to the variables studied in the trial and control groups of 10 subjects each. The correspondence of the data to a normal distribution (goodnes-of-fit) was evaluated with Saphiro Wilk's test. It was observed that the data structure was not distributed normally. Change over time was analyzed with Wilcoxon Sign test (Wayne, 1987).

Results and Discussion

Performance parameters

The average BW, BWG, DBWG, feed intake (AH, CSF, TF) and FCR results of the groups are given below (Tables 2, 3 and 4). As shown in Tables 2 and 3, the supplementation of SB to milk did not create any difference in BW, BWG and DBWG ($p>0.05$), except for the period from day 21 to 35, during which BWG and DBWG increased with SB addition in milk ($p<0.05$). Alfalfa hay (AH), calf starter feed (CSF), total feed (TF = AH + CSF) intakes and feed conversion ratio (FCR) remained unchanged ($p>0.05$) (Table 4). Until day 21 calves did not demonstrate any propensity to consume the AH offered free choice.

In earlier studies of similar nature, it was reported that SB supplementation generally increased (Gorka *et al.*, 2011; Nazari *et al.*, 2012; Zahao *et al.*, 2013; Serbester *et al.*, 2014), decreased (Gorka *et al.*, 2009; Ślusarczyk *et al.*, 2010; Araujo *et al.*, 2015; Wanat *et al.*, 2015) or, as is the case in this study, did not alter the performance parameters (Kato *et al.*, 2011; Guerrero, 2015; Hiltz and Laarman, 2019). There are also studies evaluating calcium as a butyrate source, which report no advantage in performance parameters over control (Serbester *et al.*, 2014), improved performance (Nazari *et al.*, 2012) or, increased average daily gain and feed efficiency although feed intake (CSF, alfalfa) remained unaffected (Davarmanesh *et al.*, 2015). There is even a study recommending SB over calcium butyrate (CaB) as it improved performance parameters (Serbester *et al.*, 2014).

According to this, the discrepancies with their results can be attributed to the supplementation of SB in MRF or CSF instead of milk unlike the present study, different SB feeding concentrations and protocols, differences in the amount of SB per calf, not including forage in the calculations, not offering forage to calves, differences in the ingredients and chemical composition of calf starter feed or its presentation (restricted or ad libitum), differences in weaning age and breed differences.

Blood parameters

The averages of serum BHBA, IgA, AST, ALT and GH results obtained from blood samples taken from

Table 2. Effects of sodium butyrate supplementation on body weight of calves

Day						
Group	n	0.day	7. day	21. day	35. day	49. da
BW, kg						
Control	10	38.80 ± 0.61	42.61 ± 0.98	47.31 ± 0.83	56.13 ± 0.89	65.81 ± 1.20
Trial	10	38.64 ± 0.69	41.68 ± 0.93	48.21 ± 1.45	59.48 ± 2.11	69.65 ± 2.77

BW: Body weight

Table 3. Effects of sodium butyrate supplementation on body weight and daily weight gain of calves

	Weight gain, kg			
	BW		**DBW**	
Day	Control	Trial	Control	Trial
0-7	3.81 ± 0.63	3.28 ± 0.49	0.54±0.09	0.43±0.07
7-21	4.70 ± 0.44	5.72 ± 0.62	0.34±0.03	0.47±0.07
21-35	8.82 ± 0.53b	11.27 ± 1.02a	0.63±0.04b	0.80±0.07a
35-49	9.68 ± 0.66	10.17 ± 0.87	0.69±0.05	0.73±0.06
7-49	23.20 ± 1.07	27.97 ± 2.11	0.55±0.03	0.67±0.05
0-49	27.01 ± 1.02	31.01 ± 2.41	0.55±0.02	0.63±0.05

a,b: Means within a row with different letters differ significantly ($p<0.05$).
BW: Body weight; DBW: Daily body weight

Table 4. Effects of sodium butyrate supplementation on feed intake and feed conversion ratios of calves

	AH		**CSF**		**TF**		**FCR**	
Day	Control	Trial	Control	Trial	Control	Trial	Control	Trial
7-21	-	-	1.04 ± 0.10	1.22 ± 0.27	1.04 ± 0.10	1.22 ± 0.27	2.00 ± 0.49	2.27 ± 0.43
21-35	0.89±0.2	1.31± 0.19	3.27± 0.48	4.11± 0.55	3.89 ± 0.56	5.29 ± 0.67	4.28 ± 0.71	4.47 ± 0.27
35-49	1.10± 0.11	1.53 ± 0.19	5.88± 0.92	7.62± 1.26	6.98 ± 1.00	10.56 ± 2.4	7.72 ± 1.31	9.97 ± 2.15
7-49	1.73± 0.25	4.18 ± 1.52	10.20± 1.41	12.90± 1.91	11.93± 1.57	17.09± 3.17	4.65 ± 0.45	5.28 ±0.55

AH: Alfalfa hay, CSF: Calf starter feed, TF: Total feed, FCR: Feed conversion ratio

both groups at the beginning (day 7) and end (day 50) of the trial are presented in Table 5.

Sodium butyrate supplementation did not lead to any difference in serum BHBA levels between the groups ($p>0.05$), however BHBA tended to increase over time in both control and trial groups ($p<0.05$). The finding of the present study that SB supplementation did not create any difference in BHBA between the groups was found to be consistent with the results of some studies (Ferreira and Bittar, 2011, SB in commercial starter feed at 150 g/kg DM; Guerrero, 2015, in MRF; Araujo et al., 2015 and Hiltz and Laarman 2019, 2.5%/BW in colostrum). The fact that in the present study, unlike others, SB was evaluated independently from CSF consumption, renders its results unique in this respect. On the other hand, the finding that BHBA concentrations of calves increased with the addition of SB (0, 0.03, 0.06 and 0.09 g isobutyrate/kg BW/calf) pre- and post-weaning (Wang et al., 2016) is inconsistent with the results of the present study, in which serum BHBA increased over time in both control and trial groups. The difference from Wang et al., (2016) is presumed to be arising from the difference in SB concentrations.

While no difference was found between the groups in serum IgA levels over the 7th day samples ($p>0.05$), serum IgA increased with SB supplementation in the trial group ($p<0.05$) by day 50. In a study, SB supplementation (15, 30 and 45 g/day) did not alter serum IgA, IgG and IgM concentrations, but improved performance and antioxidant capacity in pre-weaned calves (Wenhui et al., 2020). In that study as well, SB supplementation did not result in a difference compared to the control group, but increased serum IgA in the trial group ($p<0.05$). In their studies, the authors added SB to the milk, as in the present trial, but they tested higher levels of SB supplementation. Nevertheless, the findings of those studies were in line with the findings of the present trial. The report that the addition of sodium butyrate (%1 of DM) increased serum IgA, IgG and IgM levels ($p<0.05$) (Zhao et al., 2013) also supports the serum IgA results of the present study.

Table 5. Effects of sodium butyrate supplementation on some blood parameters in calves

Parameter	Days	n	Control	Trial	P
BHBA (mmol/l)	7	6	0.12 ± 0.01aA	0.11 ± 0.07aA	0.935
	50	6	0.24 ± 0.03bA	0.26 ± 0.02bA	0.470
P			0.027	0.027	
IgA (mg/dl)	7	6	5.89 ± 0.25aA	5.15 ± 0.28aA	0.092
	50	6	5.35 ± 0.45aA	6.12 ± 0.27bA	0.200
P			0.345	0.028	
AST (IU/l)	7	6	48.66 ± 8.75aA	42.83 ± 4.86 aA	0.749
	50	6	32.00 ± 2.98aA	35.50 ± 5.61aA	0.629
P			0.116	0.293	
ALT (IU/l)	7	6	9.00 ± 1.03aA	7.16 ± 1.51aA	0.226
	50	6	8.00 ± 1.15aA	7.00 ± 1.78aA	0.688
P			0.357	0.750	
GH (ng/ml)	7	6	6.23 ± 0.50aA	5.88 ± 0.15aA	0.748
	50	6	6.94 ± 0.85aA	9.64 ± 0.60bB	0.025
P			0.500	0.028	

a,b: Means within a column with different letters differ significantly ($p<0.05$). A, B: Means within a row with different letters differ significantly ($p<0.05$). BHBA: Beta hydroxy butyric acid, IgA: Immunoglobulin A, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, GH: Growth hormone

It was reported that the addition of SB to colostrum (at %2.5 of BW) decreased IgG absorption due to forming bonds with IgG (Hiltz and Laarman, 2019). On the basis of that study, it would be more appropriate to add SB to milk rather than colostrum in order to positively affect immunoglobulin absorption and reinforce the immune system of calves.

It has been concluded that addition of SB to milk had no effect on serum AST and ALT levels ($p>0.05$). In a study conducted to determine the effects of essential oils on calf performance and blood parameters, it was found that essential oils improved butyrate concentration in calves but did not lead to any increase in AST and ALT levels (Vakili et al., 2013). The data obtained in the present trial(AST 35.50 and ALT 7.00IU/L) are similar to the results of the above-mentioned study.

It was reported that ALT and AST (IU/L) in a healthy calf respectively ranged between 3.3-12.1 and 0.0-60.0 at 2 weeks of age and between 4.3-19.5 and 19.0-178 at 5 weeks of age (Yu et al.,2019). The figures of the present study were found to vary within the reference values (Huang et al., 2006; Hsueh et al., 2011; Yu et al., 2019).

However, the 7th day AST value (48.66IU/l) of the control group found in the present study was slightly above the reference range of 5-40 IU/L for maximum AST of Huang *et al.*, (2006) and Hsueh *et al.*, (2011). This can be explained by the presence of *E. coli* pressure in the control group, which will be described below. In the context of the scientific literature, the fact that the AST and ALT values found in this study were within the reference ranges may be interpreted as an indication that the calves were healthy during the trial. The ALT and AST values determined in this study are in congruence with the reports that ALT and AST are lower ($p\leq0.05$) in calves with respiratory problems(Almujalli *et al.*, 2015), ALT is generally higher than AST in liver diseases (Kaneko *et al.*, 1997), and serum AST and Alanine Aminotransferase concentrations are higher in calves with diarrhea compared to healthy ones

(Sing and Sodhi, 1992; Albayrak and Kabu, 2016).

Growth hormone level did not change with age in the control group but increased in the SB supplemented trial group ($p<0.05$). The increase in GH level with the addition of SB to milk found in the present study was consistent with Wang *et al.*, (2016), reporting that isobutyrate (0, 0.03, 0.06 and 0.09 g/kg BW/calf) added to calf feeds before and after weaning increased GH level ($p<0.05$), and similar to the GH data reported by Kato *et al.*, (2011), for SB and control group calves. However, the drop in GH concentration in the SB group following the first feeding observed in Kato *et al.*, (2011), was not detected in the present study. The authors attributed this decrease to the ability of SB to alter plasma hormone concentration and suppress cellular calcium ion concentration when administered via jugular vein or directly into the rumen. In the present study, blood samples were taken 4 hours after milk feeding to ensure that SB was fully absorbed into the blood circulation, which is presumed to be the reason of the divergence from the aforementioned study. The report that the basal serum GH level varies between 11 - 16 µg/L in the first day of life, increasing 7-folds within a few hours following colostrum feeding, and serum GH levels vary between 10-20 µg/L depending on age and level of animal development (Kühne *et al.*, 2000) supports the results of the present study. It has also been reported that butyrate exerts positive effects on growth, digestibility and utilization efficiency of feeds by means of modulating mucosal epithelial cells and defense systems (barrier function, immune system and antimicrobial effect), cell proliferation, differentiation and function in the digestive system, in both sick and healthy animals (Pouillart, 1998; Partanen ve Mroz, 1999; Manzanilla *et al.*, 2006; Mazzoni *et al.*, 2008). The results of the present study are also backed by the reports that calves receiving SB supplementation displayed a much faster growth (Ślusarczyk *et al.*, 2010), stronger immune functions (Zhao *et al.*, 2013), and that performance and growth were positively affected depending on the amount of bacteria associated with intestinal health in the digestive system (O'Hara *et al.*, 2018).

Fecal *E.coli* presence

The results of *E. coli* presence/absence analysis of fecal samples taken from the groups on days 7 and 50 are given in Table 6. At the start of the trial on the 7th day of life, no *E. coli* was found, neither in the control nor in trial groups, while on day %50, %33 less *E. coli* pressure was found in the feces of the calves receiving SB supplementation.

This finding is aligned with the reports that the addition of %0.3 SB to MRF and CSF decreases the pH in abomasal fluid ($p=0.02$) (Gorka et al, 2009), the growth of numerous microorganisms such as *E. coli*, Salmonella and Clostridium stops at pH levels below 5, and at the same time a barrier against pathogens is formed in the ileum and large intestine due to low pH, that this decrease in pH alters cell integrity and enzyme activity, thus inhibiting the intraluminal microbial growth of pathogens in the stomach (Kluge et al, 2004) and duodenum (Hebeler *et al.*, 2000) by the use of OA (Abhishek and Biswadeep, 2014), and that calves supplemented with SB in MRF and CSF had a lower incidence of diarrhea, requiring less treatment and electrolyte administration than the control group (Gorka *et al.*, 2009; $p=0.01$, Gorka *et al.*, 2011a; $p=0.08$).

It was also observed in this study that calves receiving SB supplemented milk consumed it readily without refusal. This finding is in parallel with the reports indicating that the unpleasant odor problem has been eliminated in third generation butyrate produced by a specific oil coating process which protect the active ingredient (Fernández-Rubio *et al.*, 2009) and that therefore such products can be recommended (Guillotetau *et al.*, 2010).

Table 6. E. coli presence in the groups

***E coli* presence**			
	n	Day	*E. coli**
Control	6	7.	- - - - - -
	6	50.	++- - - -
Trial	6	7.	- - - - - -
	6	50.	- - - - - -

*+ (positive): indicates *E. Coli* presence

Conclusion

As a result, sodium butyrate (SB) supplementation to milk did not affect calves' BW, FCR parameters and CSF, AH, TF (AH + CSF) consumptions in this study. However, SB supplementation did increase daily body weight gain between days 21-35. SB supplementation had positive effects on serum IgA and growth hormone levels of calves, but did not change BHBA, AST and ALT. Fecal *E.coli*presence/absence analysis revealed that SB supplementation reduced *E.coli* pressure by %33.33.

In conclusion, SB supplementation in milk had positive effects on immunity, growth performance and manure *E. coli* pressure in pre-weaned calves. It is

a well-known fact that a healthy calf performance in early life stages will be carried over to post-weaning growth and productivity later on. Therefore, it may be recommended to add SB to the milk fed to calves during the pre-weaning period, in which foundations of a healthy and robust herd are laid.

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Conflicts

No potential conflict of interest was reported by the authors.

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