Received: 30 November 2023 | Accepted: 19 January 2024

DOI: 10.1111/rda.14539

Reproduction in Domestic Animals WILEY

ORIGINAL ARTICLE

The effect of alfalfa supplementation during the peripartum period on udder oedema and fertility in dairy sheep

Gokhan Bozkurt[1] | Feyyaz Kaya[2] | Ibrahim Tasal[1] | Ramazan Yildiz[3]

[1]Department of Obstetrics and Gynecology, Faculty of Veterinary Medicine, Burdur Mehmet Akif Ersoy University, Burdur, Turkey

[2]Department of Internal Medicine, Faculty of Veterinary Medicine, Balikesir University, Balikesir, Turkey

[3]Department of Internal Medicine, Faculty of Veterinary Medicine, Burdur Mehmet Akif Ersoy University, Burdur, Turkey

Correspondence
Gokhan Bozkurt, Department of Obstetrics and Gynecology, Faculty of Veterinary Medicine, Burdur Mehmet Akif Ersoy University, Burdur, Turkey.
Email: gbozkurt@mehmetakif.edu.tr

Abstract

In recent years, there has been a trend towards hyperoestrogenic feeding methods in field conditions, particularly in dairy small ruminants. This study aimed to investigate the effect of alfalfa supplemented ration in the prepartum period on the occurrence of udder oedema in dairy ewes and its impact on fertility problems (dystocia, vaginal prolapse, uterine prolapse, retained placenta) and diseases (mastitis, udder dermatitis) occurring in the postpartum period. In addition, it aimed to follow up the data on maternal rejection behaviours and litter viability, which are believed to affect the future productivity and fertility of the herd. The ewes were divided into two groups: the oedema group ($n=20$) and the control group ($n=23$). For trial purposes in the oedema group, primiparous animals were provided with alfalfa supplementation. The control group consisted of ewes fed the same ration but without alfalfa supplementation. Ingredient analysis of the rations was performed in the groups. In biochemical analyses, the oedema group demonstrated significantly higher levels of serum urea ($p<.05$), Ca ($p<.000$) and Ca/P ratio ($p<.01$) compared to the control group. Clinical mastitis (0–7 days, $p=.051$) and (8–30 days, $p=.149$) were reported at a higher rate in the oedema group compared to the control group. Udder dermatitis cases were more common in the oedema group ($p=.005$). The mother's rejection of the lamb was higher in the oedema group. Dystocia was determined to be 30% in the oedema group ($p=.023$). In conclusion, applying hyperoestrogenic feeding strategies during the periparturient period firstly exacerbates physiological udder oedema in ewes, thus predisposing them to various herd fertility problems. This feeding strategy, frequently encountered in field conditions, seriously harms the physiological fertility process.

KEYWORDS
dystocia, feeding, fertility, mammary, mastitis, oedema

1 | INTRODUCTION

Udder oedema, characterized by the accumulation of lymphatic fluid in the interstitial space of the mammary gland and surrounding tissues, is a periparturient disorder (Kojouri et al., 2015; Morrison et al., 2018). While udder oedema is more prevalent in dairy cows compared to other species, it is relatively rare in small ruminants. Reported cases in both species are not linked to herd problems (Atif et al., 2020; Aziz & Kamal, 2018; Morrison et al., 2018; Okkema & Grandin, 2021). Despite its prevalence in dairy herds,

the exact aetiology of this disorder remains unknown (Okkema & Grandin, 2021). Physiological udder oedema is widely recognized as a metabolic disorder (Kojouri et al., 2015) and has been associated with various factors. These factors include an excessive intake of sodium (Na) and potassium (K) ions in the diet, older age at first parturition (Malven et al., 1983), above-ideal body condition score, genetic characteristics, physiological changes during udder development (Tyler & Ensminger, 2006), animal transfer or crowding stress (Fustini et al., 2017), oxidative stress and heat stress (Tao & Dahl, 2013).

Feeding management is known as one of the key factors contributing to the aetiology of udder oedema (Kojouri et al., 2015). In particular, the inclusion of phyto-oestrogenic plants like alfalfa in the diet during the late pregnancy period may play a role in the development of udder oedema. Phytoestrogens are plant-derived compounds that are present in various animal food sources. While phytoestrogens are associated with potential health benefits, they also have adverse effects on the reproductive health of both males and females (Adnan et al., 2022). Furthermore, Malven et al. (1983) found a positive association between prepartum oedema, as well as plasma levels of oestrone and oestradiol-17α. Similarly, Erb (1977) suggested that endogenous hormonal factors may contribute to the onset of udder oedema during the peripartum period.

Research has indicated that udder oedema has several negative impacts on dairy herds. First, it is associated with a decrease in milk production (Melendez et al., 2006). Additionally, it leads to an increase in dystocia (Melendez et al., 2006), mastitis (Bačić et al., 2007) and udder dermatitis (Beattie & Taylor, 2000), potentially resulting in permanent damage to the mammary tissues (Dentine & McDaniel, 1984). In this respect, Okkema et al. (2023) observed that udder oedema leads to an increase in kick and kick-off behaviours in affected animals.

The existing literature contains numerous studies examining udder oedema during the periparturient period in dairy cows. Yet, there is a lack of sufficient research on udder oedema and its efficiency on reproductive performance in small ruminants. The present study aimed to investigate the effect of alfalfa supplemented ration in the prepartum period, on the occurrence of udder oedema in dairy ewes and its effect on fertility problems (dystocia, vaginal prolapse, uterine prolapse and retained placenta) and postpartum diseases (mastitis and udder dermatitis). In addition, it aimed to follow up the data on maternal rejection behaviours and litter viability, which are believed to affect the future productivity and fertility of the herd.

2 | MATERIALS AND METHODS

The experimental procedures were approved by the Committee of Animal Experiments of Burdur Mehmet Akif Ersoy University (Approval number: 23/113-1149).

2.1 | Animals and management

There was a total of 353 sheep in the herd, with 210 being multiparous, 43 primiparous and 100 nulliparous. All animals in the herd were subject to a regular vaccination protocol. As part of preventive medicine, vaccinations for *pasteurellosis* (Ovilis Ovipast®, MSD), mastitis (Vimco®, HIPRA), *Mycoplasma agalactia* (Agalaxipra®, HIPRA), Clostridial infections (Ultrachoice-8®, Zoetis), *Escherichia coli* bacterin (VBR-K99®, AtaFen) were administered annually. Sheep received parasite treatment twice a year. The mating of all animals was synchronized in the previous breeding season. The herd was not subjected to crowding stress and the animals were housed in facilities that met the specific welfare standards for the species. In the previous season, the average milk yield of the herd was approximately 2.3 L, with a lactation period of approximately 190 days. The animals were grouped based on the number of births and milk yields in the herd, following management rules.

2.2 | Experimental design and collection of blood samples

The study involved a total of 43 pregnant Lacaune ewes, all of which were primiparous. The ewes were divided into two groups: the oedema group ($n=20$) and the control group ($n=23$). In the oedema group, all animals exhibited varying degrees of udder oedema. Ewes were randomly selected from the herd and none of the animals in the control group showed any signs of physiological or pathological udder oedema. All animals in both groups were in good clinical health and had no previous history of udder infection. For trial purposes, alfalfa supplementation was given to some primiparous animals with high milk yield, with a known daily intake of 500 g of alfalfa. The ewes consumed the supplemented ration for at least 1 month up until 1 week before parturition. The control group consisted of ewes in different paddocks in the herd, fed the same ration, except excluding alfalfa supplementation. Table 1 shows the daily rations of the two different groups. All ewes in the udder oedema group began to develop udder oedema approximately 3 weeks before the expected date of birth.

Figure 1 shows the appearance of the mammary structures of the animals. The diagnosis of udder oedema was confirmed when the indentation made by finger pressure on the mammary skin remained for more than 3 s (Figure 2), following the criteria established by Melendez et al. (2006). During the study, several periparturient diseases such as mastitis and dermatitis, physiological process disorders including vaginal prolapse, uterine prolapse, retained placenta and dystocia, as well as maternal behaviour disorders such as mother's rejection of the lamb, were observed in the herd. A total of 8 mL of whole blood samples were collected from all groups 7 days (±1 day) before parturition. The samples were kept at room temperature for 20 min and then centrifuged at

TABLE 1 Composition of the oedema and control group ration (as dry matter basis).

	Daily amount (kg)	Nutrients/unit	Requirement	Ration
Oedema group feeds				
Barley hay	1.00	Dry matters/kg	1.62	1.91
Alfalfa hay, blooming stage	0.50	Metabolic energy/Mcal	3.11	4.20
Barley, grain, steam, crushed	0.25	Crude protein/g	136.95	240.76
Maize, grain, crushed	0.18	Metabolic protein/g		168.53
Sunflower meal, hulled, % 28	0.06	Rumen degradable protein/g		162.27
Cottonseed meal, solvent, % 41	0.04	Rumen undegradable protein/g		46.63
Vitamin–mineral mix	0.10	Ca/g	5.58	9.50
		P/g	3.87	6.06
		Ca/P	1.44	1.57
Control group feeds				
Barley hay	1.00	Dry matters kg	1.62	1.46
Barley, grain, steam, crushed	0.25	Metabolic energy/Mcal	3.11	3.26
Maize, grain, crushed	0.18	Crude protein/g	136.95	155.26
Sunflower meal, hulled, % 28	0.06	Metabolic protein/g		108.68
Cottonseed meal, solvent, % 41	0.04	Rumen degradable protein/g		98.82
Vitamin–mineral mix	0.10	Rumen undegradable protein/g		30.88
		Ca/g	5.58	3.16
		P/g	3.87	4.89
		Ca/P	1.44	0.65

FIGURE 1 Udder structures of ewes in the oedema group.

3000 rpm for 10 min. This allowed for the separation of blood serums, which were promptly stored at −25°C until further analysis. On the same day, alfalfa was removed from the ration of the ewes in the oedema group.

2.3 | Treatment protocol for udder oedema

After the collection of blood samples in the prepartum period, alfalfa was removed from the ration. The oedematous udders were treated with hot–cold compresses during this period. The animals were provided with short-distance walking and exercises. Regular monitoring and close observation of the animals were conducted, and any signs of pain were noted. Animals with a postpartum oedema score of two or higher were medically treated for physiological mammary oedema in accordance with the guidelines established by Ghodasara et al. (2012). Treatment was administered for any complications associated with udder oedema, such as clinical mastitis, metritis, dystocia and uterine or vaginal prolapse, among others. Clinical mastitis was diagnosed through a clinical examination of the udder and milk (Mørk et al., 2007). Milk samples were collected from animals with signs of inflammation in the udder and treated based on antibiotic sensitivity.

2.4 | Biochemical analysis and oedema scores

Biochemical analysis was conducted to measure the values of urea, Mg, Ca, P, beta hydroxybutyric acid (BHBA), triglyceride, total protein, gamma-glutamyl transferase (GGT), aspartate aminotransferase (AST), total cholesterol and albumin. These measurements were performed by the Abbott Architect Clinical Chemistry Analyser (model no: C8000, serial number: C802239) using the spectrophotometric method. Udder oedema was assessed using a grading system with scores of 0, 1, 2 and 3, indicating the absence, slight, moderate and severe presence of oedema respectively (Morrison et al., 2018).

The scoring criteria were as follows:

Score 1: Medial suspensory ligament visible, irregular udder half.
Score 2: Medial suspensory ligament partially visible, oedema partially extended to the navel and vulva.

FIGURE 2 Fingerprint and teat plugs in oedema groups. Fingerprint diagnosis before (left) and after (right). Teat plug appearance on teats.

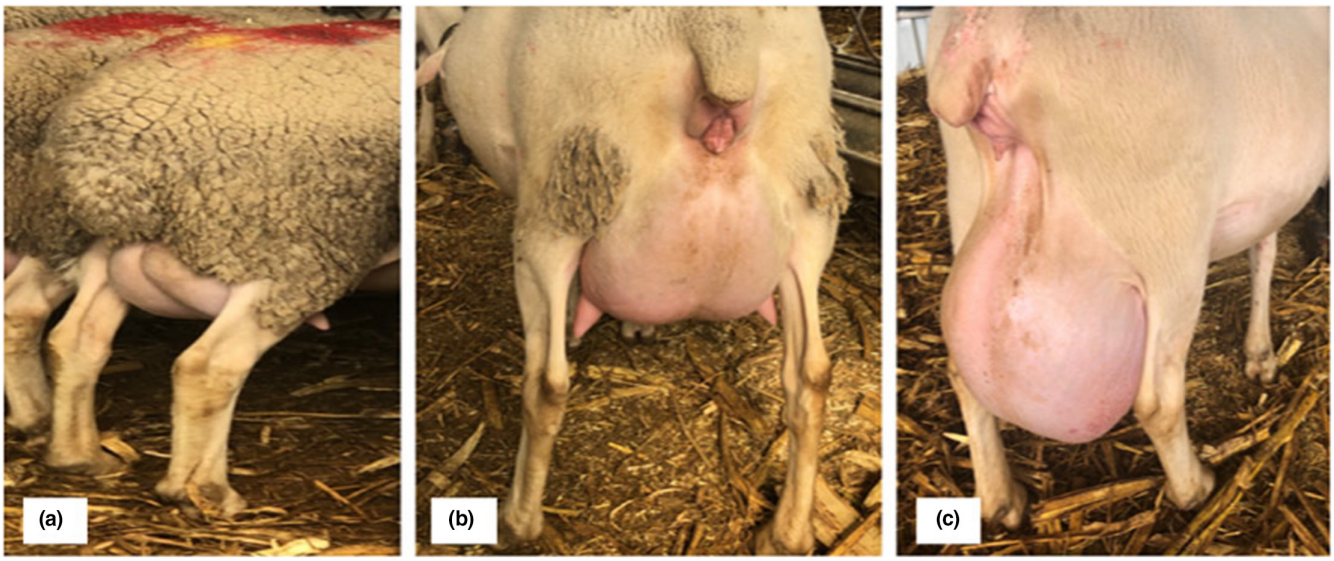

FIGURE 3 Evaluation of udder scores. Oedema score 1 (a), oedema score 2 (b) and oedema score 3 (c).

Score 3: Medial suspensory ligament partially not visible, oedema completely extended to the navel and vulva (Figure 3). In the study, udder oedema scoring was conducted for the first time at the time of blood collection and again at the time of parturition. After parturition, daily scoring of udder oedema was performed. The duration required for the complete resolution of udder oedema was recorded in days. Animals in need of treatment and those that showed spontaneous recovery were identified and monitored throughout the study.

2.5 | Statistical analysis

The statistical analyses were conducted using the SPSS 25.0 software. The normality of the data was assessed using the Shapiro–Wilk test. For variables with a normal distribution, a *t*-test was used to compare groups. For variables with a non-normal distribution, the Mann–Whitney *U*-test was employed. The correlation analysis was performed using the Spearman correlation analysis method. The results were presented as the mean ± standard error of the mean. The association between diseases or disorders and udder oedema was examined using a chi-square test. Statistical significance was defined as $p < 0.05$. All graphs were generated using the GraphPad Prism 9.0 software.

3 | RESULTS

The results of the biochemical analyses are presented in Table 2. Significant differences were found between the oedema and control groups in terms of urea levels ($p < 0.05$), BHBA levels ($p < 0.05$), total protein ($p < 0.01$), Ca levels ($p < 0.000$) and the Ca/P ratio ($p < 0.01$). Scatter dot plots depicting the results of the biochemical analyses are shown in Figure 4. The correlation analysis results of the biochemical parameters in the oedema group are provided in Table 3. Based on the udder oedema scoring, the oedema score in the oedema group was 2.150 ± 0.196 1 week before parturition and 2.158 ± 0.175 during parturition. Animals with a postpartum oedema score of ≥2 received treatment for physiological udder oedema. One ewe in the oedema group was excluded from the study due to severe acute septic mastitis 1 day after parturition. In the control group, the udder oedema score was zero 1 week before parturition and 0.174 ± 0.808 at parturition. No treatment was administered for oedema in the control group after parturition. Among the control group, four ewes developed udder oedema with

a score of 1 after parturition, which resolved spontaneously within 1–2 days. The scores for udder oedema recovery day interval and the number of associated diseases (mastitis, dermatitis) and/or disorders (rejection of kids, dystocia) in the groups are presented in Tables 4–6. Additionally, metritis (one ewe), retained placenta (one ewe) and uterine prolapse (one ewe) were observed in the oedema group, although they were not directly related to udder oedema during this period. Retained placenta was observed in only one animal in the control group.

TABLE 2 Biochemical analysis results.

Parameter	Oedema group	Control group	*p*-Value
Urea	77.5 ± 7.21	57.73 ± 5.19	.041*
Mg	2.3 ± 0.16	2.09 ± 0.19	.095
BHBA	0.84 ± 0.14	0.62 ± 0.12	.044*
Triglyceride	21.29 ± 1.44	22.01 ± 1.34	.706
Total protein	5.85 ± 0.15	6.56 ± 0.16	.003**
P	6.53 ± 0.6	6.65 ± 0.55	.883
Ca	15.63 ± 0.79	11.73 ± 0.44	.000***
GGT	128.5 ± 5.36	113.47 ± 8.67	.163
AST	101.57 ± 12.72	77.75 ± 6.28	.214
Total cholesterol	60.51 ± 3.51	70.55 ± 5.57	.279
Albumin	2.84 ± 0.2	3.26 ± 0.18	.084
Globulin	3.00 ± 0.18	3.29 ± 0.2	.312
Alb/Glob	1.1 ± 0.15	1.15 ± 0.12	.609
Ca/Phos	2.74 ± 0.24	1.98 ± 0.13	.007*

Note: There are significant differences between groups containing different letters.
*$p < .05$. **$p < .01$. ***$p < .001$.

4 | DISCUSSION

Udder oedema can have a negative impact on animal production by predisposing them to various diseases (Bačić et al., 2007; Melendez et al., 2006). While udder oedema is more commonly observed in dairy cows, sporadic cases can also occur in small ruminants (Mills, 1983). Capillary permeability plays a crucial role in the movement of fluids. Increased blood flow leads to higher hydrostatic pressure, which can result in the leakage of fluid and blood proteins into the interstitial tissues (Tyler & Ensminger, 2006). Challis (1971) reported a 10-fold increase in maternal circulating oestrogen levels on the day before parturition. Additionally, Rosenfeld et al. (1976) demonstrated that oestradiol promotes increased blood flow in reproductive organs and mammary tissue. Therefore, the increased blood flow to mammary tissue may contribute to decreased serum total protein levels, alongside udder oedema. This finding is consistent with both previous findings and the present study. Kojouri et al. (2015) observed significantly higher serum total protein and triglyceride levels in healthy heifers compared to heifers with udder oedema. Although the present study revealed elevated triglyceride levels in the control group compared to the oedema group, there were no significant differences between the groups. These findings suggest that the results of the current study align with previous reports. Torell et al. (1974) reported elevated blood urea levels in sheep with increased consumption of alfalfa. Similarly, Turner et al. (2005) found that kids fed an alfalfa-based diet exhibited significantly higher blood urea levels compared to those who did not consume alfalfa. In our study, the animals in the oedema group consumed an alfalfa-added diet, whereas the animals in the control group did not. Therefore, the difference in serum urea levels between the groups is consistent with previous findings. Dhiman et al. (1993) observed higher plasma levels of BHBA and NEFA in cows fed a protein-based

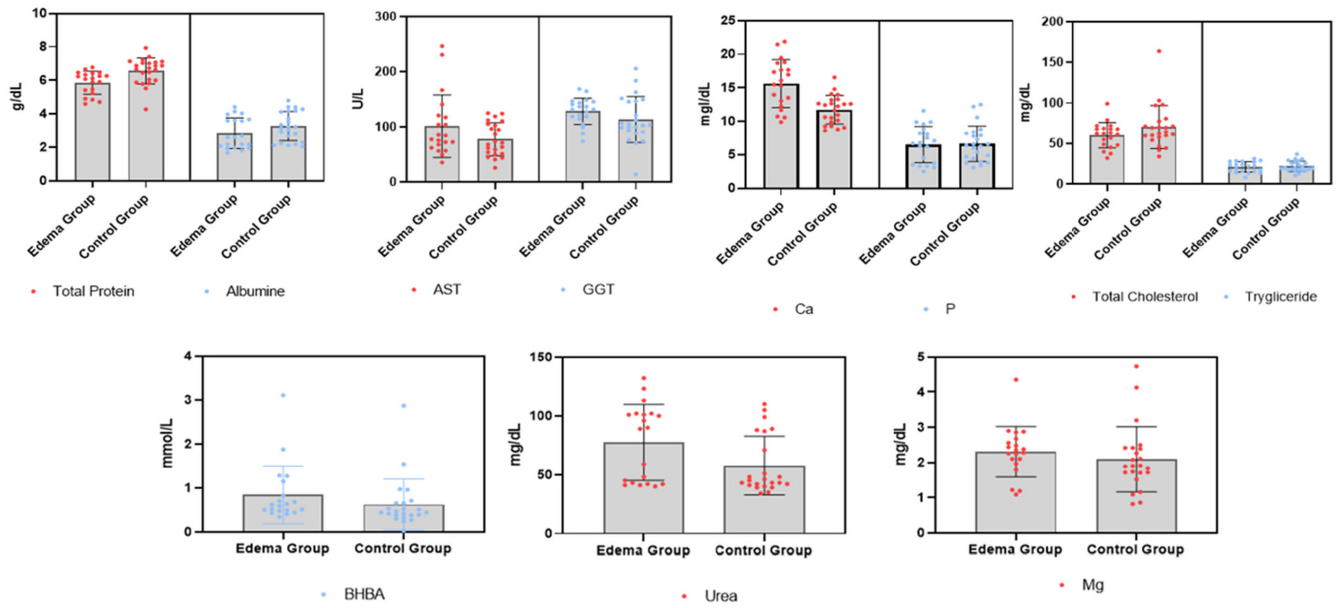

FIGURE 4 Dot plot and mean values of biochemical parameters in the groups.

TABLE 3 Correlation analysis results of the biochemical parameters of the oedema group.

Parameter	Oedema	Urea	Mg	Triglyceride	Total protein	P	Ca	GGT	AST	Total cholesterol	Albumin	BHBA	Ca/P
Oedema	1	−.405 .076	.301 .196	.186 .433	−.270 .249	−651** .002	.034 .887	.372 .106	−.442 .051	.340 .143	−.529* .017	.264 .261	.861** .000
Urea	−.405 0.76	1	−.037 .876	−.213 .367	.226 .338	.653 .002	.452* .046	−.106 .657	.510* .021	.023 .922	.703 .001	.017 .945	−.508* .022
Mg	.301 .196	−.037 .876	1	−.122 609	−.090 .707	.037 .879	.555 .011	.083 .728	−.036 880	.156 .512	−.123 .604	−.326 .160	.191 .420
Triglyceride	.186 .433	−.213 .367	−.122 .609	1	−.153 .519	−.141 .552	−.248 .292	.020 .932	−.183 .441	.317 .173	−.062 .796	.111 .640	.041 .865
Total protein	−.270 .249	.226 .338	−.90 .707	−.153 .519	1	.502* .024	.152 .523	−.176 .458	.335 .149	−.164 .490	.602** .005	.132 .578	−.433 .056
P	−.651** .002	.653** .002	.037 .879	−.141 .552	.502* .024	1	.455* .044	−.297 .203	.463* .040	−.034 .887	.787** .000	−.002 .992	−.871** .000
Ca	.034 .887	.452* .046	.555* .011	−.248 .292	.152 .523	.455* .044	1	−.078 .745	.042 .860	.253 .283	.423 .063	−.110 .645	−.050 .835
GGT	.372 .106	−.106 .657	.083 .728	.020 .932	−.176 .458	−.297 .203	−.078 .745	1	−.071 .767	.425 .062	−.134 .572	−.004 .987	.288 .219
AST	−.442 .051	.510* .021	−.036 .880	−.183 .441	.335 .149	.463* .040	.042 .860	−.071 .767	1	−.144 .546	.400 .081	.156 .512	−.572** .008
Total cholesterol	.340 .143	.023 .922	.156 .512	.317 .173	−.164 .490	−.034 .887	.253 .283	.425 .062	−.144 .546	1	.016 .947	.008 .975	.186 .431
Albumin	−.529* .017	.703** .001	−.123 .604	−.062 .796	.602** .005	.787** .000	.423 .063	−.134 .572	.400 .081	.016 .947	1	.168 .480	−.634** .003
BHBA	.264 .261	.017 .945	−.326 .160	.111 .640	.132 .578	−.002 .992	−.110 .645	−.004 .987	.156 .512	.008 .975	.168 .480	1	.086 .719
Ca/P	.861** 000	−.508 .022	.191 .420	.041 .865	−.433 .056	.871** .000	−.050 .835	.288 .219	−572** .008	.186 .431	−.634** .003	.086 .719	1

Note: Correlation is significant at the *: .05. Correlation is significant at the **: 0.

TABLE 4 Scores of the udder oedema and the number of associated diseases and/or disorders in the oedema group.

ID	−7 day score	0 day score	Recovery day interval	Clinical mastitis 0–7 (day)	Clinical mastitis 8–30 (day)	Udder dermatitis	Kids rejection	Dystocia
NO1	2	2	6	+	−	+	+	−
NO2	3	2	4	−	−	−	+	+
NO3	1	1	4	−	+	+	+	−
NO4	1	1	2	−	−	−	+	−
NO5	1	2	1	−	−	−	−	+
NO6	1	1	1	−	−	−	−	−
NO7	3	3	8	+	−	−	+	−
NO8	1	2	2	−	+	+	+	+
NO9	1	2	2	−	−	−	−	−
NO10	3	−	−	+	−	−	−	−
NO11	3	3	7	+	−	−	+	−
NO12	2	3	8	−	−	−	−	+
NO13	3	2	2	−	−	+	+	−
NO14	2	2	1	−	−	−	−	−
NO15	2	1	3	−	+	+	+	−
NO16	3	3	8	−	−	−	+	+
NO17	2	3	5	+	−	−	+	−
NO18	3	3	5	−	+	−	+	−
NO19	3	2	8	+	−	−	+	+
NO20	3	3	7	−	+	+	+	−
Number	2.1 ± 0.2	2.2 ± 0.2	4.4 ± 0.6	5 ewes	5 ewes	6 ewes	14 ewes	6 ewes

Note: −7 days: 7 days before parturition, 0 days: parturition day.

diet compared to those fed a glucose-added diet. Similarly, Nemati et al. (2016) reported significantly increased blood BHBA levels in calves fed an alfalfa hay-based diet compared to control calves. In this context, our results align with previous studies.

The present study showed significant differences between the groups in terms of serum Ca levels and the Ca/P ratio. These findings contrast with the results reported by Kojouri et al. (2015), who found significantly lower Ca levels in heifers with udder oedema. Yet, alfalfa is known to contain significant amounts of Ca (Plaza et al., 2003; Soto-Zarazua et al., 2017). The differences in the ration of ingredients between the studies may explain the disparities in the results. Furthermore, Hicks and Pauli (1976) reported significantly lower serum Mg levels in cows with chronic udder oedema during the summer compared to healthy cows. They also observed a significant negative correlation between chronic udder oedema and serum Mg levels. Yet, the present study found no significant difference in serum Mg levels between the groups and no significant correlation between the severity of oedema and serum Mg levels. This suggests that serum Mg levels may not play a critical role in the development of udder oedema in the oedema group. Therefore, the findings of the present study suggest that serum Mg levels may not be a critical factor in the development of udder oedema in the studied group.

Given that there was no significant difference in serum albumin levels between the groups, hypoalbuminaemia cannot be the primary cause of udder oedema. Yet, it is worth noting that alfalfa, a commonly used phytoestrogen source in ruminant rations (Bora & Sharma, 2011; Dhiman et al., 1993; Turner et al., 2005), may have played a role in the development of udder oedema. Numerous studies have highlighted the adverse effects of phytoestrogens on various systems, including the reproductive system and mammary tissue (Adams, 1995; Adnan et al., 2022; Barnes et al., 2007). Additionally, Malven et al. (1983) found a positive correlation between the severity of udder oedema and plasma levels of oestrone and oestradiol-17α in heifers. Therefore, it is plausible to suggest that the phytoestrogens derived from alfalfa may have contributed to the occurrence of udder oedema in the oedema group and our findings are consistent with previous reports.

The present study indicated significant negative correlations between serum levels of P and albumin and the severity of oedema. Additionally, there was a significant positive correlation between the Ca/P ratio and the severity of oedema. In contrast, Kojouri et al. (2015) did not provide information on the correlations between these parameters. Furthermore, there was no significant correlation between Mg and other parameters, which differs from the findings of Kojouri et al. (2015). It is important to highlight that the Ca/P ratio showed a strong and positive correlation, while albumin levels exhibited a relatively weak and negative correlation with the severity of oedema. These findings suggest that the severity and occurrence of oedema may be primarily influenced by differences in feeding management between the groups.

TABLE 5 Scores of the udder oedema and the number of associated diseases and/or disorders in the control group.

ID	−7 day score	0 day score	Recovery day interval	Clinical mastitis 0–7 (day)	Clinical mastitis 8–30 (day)	Udder dermatitis	Kids rejection	Dystocia
NO21	0	0	0	−	−	−	−	−
NO22	0	0	0	−	−	−	−	−
NO23	0	0	0	+	−	−	−	−
NO24	0	1	1	−	−	−	−	−
NO25	0	0	0	−	−	−	−	−
NO26	0	0	0	−	−	−	−	−
NO27	0	0	0	−	−	−	−	−
NO28	0	0	0	−	−	−	−	−
NO29	0	0	0	−	−	−	−	−
NO30	0	1	1	−	+	−	+	−
NO31	0	0	0	−	−	−	+	−
NO32	0	0	0	−	−	−	−	−
NO33	0	0	0	−	−	−	−	−
NO34	0	0	0	−	+	−	−	−
NO35	0	0	0	−	−	−	−	−
NO36	0	1	2	−	−	−	−	−
NO37	0	0	0	−	−	−	−	−
NO38	0	0	0	−	−	−	−	−
NO39	0	0	0	−	−	−	−	−
NO40	0	0	0	−	−	−	−	−
NO41	0	0	0	−	−	−	−	−
NO42	0	0	0	−	−	−	−	+
NO43	0	1	1	−	−	−	−	−
Number	0	0.17 ± 0.08	0.22 ± 0.11	1 ewe	2 ewes	−	2 ewes	1 ewes

Note: −7 days: 7 days before parturition, 0 days: parturition day.

TABLE 6 Diseases or disorders associated with udder oedema (%).

	Clinical mastitis (0–7 days)	Clinical Mastitis (8–30 days)	Udder dermatitis	Kids rejection	Dystocia	Survival rate
Oedema group	25	25	30[a]	70[a]	30[a]	83
Control group	4.34	8.70	0[b]	8.70[b]	4.34[b]	90
p-Value	.051	.149	.005	.000	.023	.157

Note: There are significant differences between groups containing different letters in the same column.

Predisposing causes or the severity of udder oedema include genetics, steroid status, obesity, lack of exercise, excessive energy intake and excessive Na or K intake. In studies conducted on cows, the effects of increasing age at first calving, gender of offspring, season and milk yield on udder oedema were determined (de Oliveira et al., 2022; Goff, 2006; Kojouri et al., 2015; Morrison et al., 2018; Okkema & Grandin, 2021). In the current study, the fact that all animals were primiparous, in the same period of pregnancy, in similar body conditions, and of the same age and breed minimized the effects on mammary oedema and its severity. Although ration intakes were observed for all animals, oedema scores may have differed due to partial variations in the amount of food consumed within the herd and individual factors.

Udder oedema can have a detrimental effect on animal productivity due to its association with increased susceptibility to various diseases (Bačić et al., 2007; Melendez et al., 2006). One of the significant complications of udder oedema is its predisposition to mastitis. The development of udder oedema during the prepartum period may lead to the opening of the teat canal (Kojouri et al., 2015). Morrison et al. (2018) reported that udder oedema is correlated with a higher incidence of mastitis during early lactation in dairy cows. Consistent with previous studies, mastitis was more frequently observed ($p = 0.051$) in the oedema group compared to the control group during the early postpartum period (0–7 days). Therefore, mastitis occurring within the first 0–7 days postpartum is predominantly associated with the opening of the teat canal during the dry period, facilitating the entry of microorganisms.

Udder oedema can lead to constriction of the mammary duct system (Marnet & McKusick, 2001). This condition can be associated

with pain and stress. The experience of pain or stimuli during milking can trigger a stress response in animals, which can subsequently affect milk secretion. The presence of adrenaline can reduce the effectiveness of oxytocin, a hormone responsible for milk letdown, making it challenging for milk to be released from the udder (Barowicz, 1979; Bobić et al., 2011). The present study hypothesized that clinical mastitis occurring between 8 and 30 days postpartum is primarily a result of secondary effects associated with pain, stress and difficulties in milking the udder. In addition, during this period, most animals with mammary oedema exhibited udder dermatitis, a skin condition characterized by inflammation and irritation.

Furthermore, Okkema et al. (2023) found that udder oedema negatively affects parlour behaviour, leading to an increased rate of kicking and kick-off behaviours. This finding aligns with the results of previous studies. The observed behavioural changes in ewes during milking and caring for their lambs can be attributed to various factors. The rejection of lambs during the first 7 days can be attributed to the stress and pain experienced by the ewes, as the lambs demand more milk. Similarly, the difficulty in milking in the subsequent days may be attributed to the pain caused by udder dermatitis. These factors can lead to behavioural changes and discomfort in the affected ewes.

Atif et al. (2020) conducted a study on goats with udder oedema, investigating the risk factors associated with this condition. The results revealed a higher incidence of dystocia ($p < 0.001$) and retained placenta ($p < 0.015$) in the group with udder oedema. These findings align with the present study, which also observed a higher rate of dystocia ($p = 0.023$) in the oedema group. The high rate of dystocia in this study may be attributed to the early induction of labour by the hyperoestrogenic ration. In addition, pain due to oedema in the udder during this period may have negatively affected labour. The other factor related to dystocia rates between the groups can be attributed to the number of offspring per pregnancy. Yet, the lack of a significant difference between the groups contradicts this thesis. In addition, in the current study, only observed one animal in the oedema group. This finding does not fully support the previous study. In this study, retained placenta might have been caused by the hyperoestrogenic effect due to the increased amount of alfalfa in the ration.

Arsenault et al. (2008) evaluated the survival rates of lambs from sheep with clinical and subclinical mastitis, investigating the effects of this condition on various parameters. They showed that many multifactorial effects change the survival rate. In this study, the survival rate was 83% in the oedema group, while it was 90% in the control group. In the current study, it is not possible to fully explain what causes the effect on survival rate due to the presence of multiple factors such as udder oedema, dermatitis, dystocia and clinical mastitis. Yet, this negatively affects the future fertility of the herd and causes economic damage to the farm management.

In cattle, it is typically observed that calves suckle on all four teats in approximately 67% of mother–offspring interactions. In sheep, the bonding between ewes and lambs occurs within the first 4 h after birth, during the sensitive period (de Passillé & Rushen, 2006; Hogan et al., 2022). Oxytocin is believed to play a crucial role in facilitating social bonding through tactile contact (Goncalves et al., 2008). The present study hypothesized that the primary reason for lamb rejection is the presence of mammary oedema and the resulting lack of milk. Additionally, the release of oxytocin may be disrupted due to the pain experienced by the mother when the lambs attempt to suckle on the oedematous udder. This may contribute to the rejection of the lambs by the ewes.

The limitation of this study is that although it is known that ration is effective in the mechanism of oedema formation, alfalfa is a phyto-oestrogenic plant. Determining the blood oestrogen level could have further strengthened the results of the study.

5 | CONCLUSION

In conclusion, it was observed that hyperoestrogenic feeding strategies applied during the periparturient period firstly exacerbate physiological udder oedema in ewes and predispose them to mastitis, udder dermatitis and various herd fertility problems. It was thought that this feeding strategy, frequently encountered in field conditions, seriously harms the physiological fertility process.

AUTHOR CONTRIBUTIONS

Gokhan Bozkurt and Feyyaz Kaya were responsible for all aspects of planning and conducting the study and collecting the data. Gokhan Bozkurt, Feyyaz Kaya and Ramazan Yildiz analysed the results and wrote the manuscript. Ibrahim Tasal and Ramazan Yildiz reviewed the manuscript. All authors approved the final manuscript.

ACKNOWLEDGEMENTS

We would like to thank Serkan AKPINAR (ProYem—Animal Feeding and Nutrition Technologies) for sharing his valuable scientific suggestions with us.

CONFLICT OF INTEREST STATEMENT

None of the authors have any conflict of interest to declare.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Gokhan Bozkurt https://orcid.org/0000-0003-1837-4492
Feyyaz Kaya https://orcid.org/0000-0001-8820-1509
Ibrahim Tasal https://orcid.org/0000-0003-4632-3115
Ramazan Yildiz https://orcid.org/0000-0001-5772-0891

REFERENCES

Adams, N. R. (1995). Detection of the effects of phytoestrogens on sheep and cattle. *Journal of Animal Science*, *73*, 1509–1515.
Adnan, M. R., Lee, C. H., & Mishra, B. (2022). Adverse effects of phytoestrogens on mammalian reproductive health. *Veterinary Medicine and Animal Science*, *10*, 1.

Arsenault, J., Dubreuil, P., Higgins, R., & Bélanger, D. (2008). Risk factors and impacts of clinical and subclinical mastitis in commercial meat-producing sheep flocks in Quebec, Canada. *Preventive Veterinary Medicine*, *87*(3–4), 373–393.

Atif, M., Avais, M., Khan, J. A., Khan, M. U. R., Asif, M., Munawar, J., & Amjad, S. (2020). Determination of risk factors associated with postparturient udder edema in dairy goats. *LGU Journal of Life Sciences*, *4*(3), 246–257.

Aziz, P. R., & Kamal, K. K. (2018). Udder edema in a doe: A case report. *International Journal of Chemistry Studies*, *6*, 2762–2763.

Bačić, G., Karadjole, T., Mačešić, N., & Karadjole, M. (2007). A brief review of etiology and nutritional prevention of metabolic disorders in dairy cattle. *Veterinary Archives*, *77*, 567–577.

Barnes, J., Anderson, L. A., & Philipson, J. D. (2007). *Herbal medicines* (3rd ed.). Pharmaceutical Press.

Barowicz, T. (1979). Inhibitory effect of adrenaline on oxytocin release in the ewe during the milk-ejection reflex. *Journal of Dairy Research*, *46*(1), 41–46.

Beattie, K. G., & Taylor, D. J. (2000). An investigation into intertrigo (necrotic dermatitis or 'foul udder') in dairy cows. *Cattle Practice*, *8*, 377–380.

Bobić, T., Mijić, P., Knežević, I., Šperanda, M., Antunović, B., Baban, M., Sakač, M. E., Frizon, E., & Koturić, T. (2011). The impact of environmental factors on the milk ejection and stress of dairy cows. *Biotechnology in Animal Husbandry*, *27*(3), 919–927.

Bora, K. S., & Sharma, A. (2011). Phytochemical and pharmacological potential of *Medicago sativa*: A review. *Pharmaceutical Biology*, *49*, 211–220.

Challis, J. R. G. (1971). Sharp increase in free circulating estrogens immediately before parturition in sheep. *Nature*, *229*, 208.

de Oliveira, W. A., de Oliveira Gonçalves, J., Batista, A. C. F., Silva, J. F. M., Pereira, R. V. G., & Silva, L. V. (2022). Classification of udder edema in cattle: Systematic review. *Brazilian Journal of Development*, *8*(3), 16989–16997.

de Passillé, A. M. B., & Rushen, J. (2006). Calves' behaviour during nursing is affected by feeding motivation and milk availability. *Applied Animal Behaviour Science*, *101*, 264–275.

Dentine, M. R., & McDaniel, B. T. (1984). Associations of subjective udder edema scores and descriptive trait codes for udder types. *Journal of Dairy Science*, *67*, 208–215.

Dhiman, T. R., Cadorniga, C., & Satter, L. D. (1993). Protein and energy supplementation of high alfalfa silage diets during early lactations. *Journal of Dairy Science*, *76*, 1945–1959.

Erb, R. E. (1977). Hormonal control of mammogenesis and onset of lactation in cows—A review. *Journal of Dairy Science*, *60*, 155–169.

Fustini, M., Galeati, G., Gabai, G., Mammi, L. E., Bucci, D., Baratta, M., Accorsi, P. A., & Formigoni, A. (2017). Overstocking dairy cows during the dry period affects dehydroepiandrosterone and cortisol secretion. *Journal of Dairy Science*, *100*, 620–628.

Ghodasara, S. N., Savsani, H. H., & Vataliya, P. H. (2012). Therapeutic management of periparturient udder edema in Jaffrabadi buffaloes and Gir cows. *Buffalo Bulletin*, *31*, 111–113.

Goff, J. P. (2006). Major advances in our understanding of nutritional influences on bovine health. *Journal of Dairy Science*, *89*(4), 1292–1301.

Goncalves, L., Dafre, A. L., Carobrez, S. G., & Gasparotto, O. C. (2008). A temporal analysis of the relationships between social stress, humoral immune response, and glutathione-related antioxidant defenses. *Behavioural Brain Research*, *192*, 226–231.

Hicks, J. D., & Pauli, J. V. (1976). Chronic udder edema: Clinical aspects of the syndrome and its connection with hypomagnesaemia and anemia. *New Zealand Veterinary Journal*, *24*(10), 225–228.

Hogan, L. A., McGowan, M. R., Johnston, S. D., Lisle, A. T., & Schooley, K. (2022). Suckling behaviour of beef calves during the first five days postpartum. *Ruminants*, *2*, 22–340.

Kojouri, G. A., Mosavi Pouryeganeh, M., Nekouei, S., & Nazifi, S. (2015). Udder edema and association with some serum biochemical measurands and dietary factors in first calving cows. *Iran Journal of Veterinary Research*, *16*, 345–349.

Malven, P. V., Erb, R. E., D'Amico, M. F., Stewart, T. S., & Chew, B. P. (1983). Factors associated with edema of the mammary gland in primigravid dairy heifers. *Journal of Dairy Science*, *66*, 246–252.

Marnet, P. G., & McKusick, B. C. (2001). Regulation of milk ejection and milkability in small ruminants. *Livestock Production Science*, *70*(1–2), 125–133.

Melendez, P., Hofer, C. C., & Donovan, G. A. (2006). Risk factors for udder edema and its association with lactation performance on primiparous Holstein cows in a large Florida herd, U.S.A. *Preventive Veterinary Medicine*, *76*, 211–221.

Mills, J. A. (1983). Treatment of afebrile postparturient udder edema in goats. *Canadian Veterinary Journal*, *24*(2), 62.

Mørk, T., Waage, S., Tollersrud, T., Kvitle, B., & Sviland, S. (2007). Clinical mastitis in ewes; bacteriology, epidemiology and clinical features. *Acta Veterinaria Scandinavica*, *49*, 1–8.

Morrison, E. I., DeVries, T. J., & LeBlanc, S. J. (2018). Short communication: Associations of udder edema with health, milk yield, and reproduction in dairy cows in early lactation. *Journal of Dairy Science*, *101*, 9521–9526.

Nemati, M., Amanlou, H., Khorvash, M., Mirzaei, M., Moshiri, B., & Ghaffari, M. H. (2016). Effect of different alfalfa hay levels on growth performance, rumen fermentation, and structural growth of Holstein dairy calves. *Journal of Animal Science*, *94*, 1141–1148.

Okkema, C., Eilertson, K., & Grandin, T. (2023). The effects of udder edema on parlor behavior in first and second lactation Holstein dairy cows. *Journal of Dairy Science*, *106*(11), 7924–7931.

Okkema, C., & Grandin, T. (2021). Graduate student literature review: Udder edema in dairy cattle—A possible emerging animal welfare issue. *Journal of Dairy Science*, *104*, 7334–7341.

Plaza, L., de Ancos, B., & Cano, M. P. (2003). Nutritional and health-related compounds in sprouts and seeds of soybean (Glycine max), wheat (*Triticum aestivum* L), and alfalfa (*Medicago sativa*) treated by a new drying method. *European Food Research and Technology*, *216*, 138–144.

Rosenfeld, C. R., Morriss, F. H., Jr., Battaglia, F. C., Makowski, E. L., & Meschia, G. M. (1976). Effect of estradiol-17β on blood flow to reproductive and nonreproductive tissues in pregnant ewes. *American Journal of Obstetrics and Gynecology*, *124*, 618–629.

Soto-Zarazua, M. G., Bah, M., Costa, A. S. G., Rodrigues, F., Pimenthel, F. B., Rojas-Molina, I., Rojas, A., & Oliveira, M. B. P. P. (2017). Nutraceutical potential of new alfalfa (*Medicago sativa*) ingredients for beverage preparations. *Journal of Medicinal Food*, *20*(1), 1–8.

Tao, S., & Dahl, G. E. (2013). Heat stress effects during late gestation on dry cows and their calves. *Journal of Dairy Science*, *96*, 4079–4093.

Torell, D. T., Hume, I. D., & Weir, W. C. (1974). Factors affecting blood urea nitrogen and its use as an index of the nutritional status of sheep. *Journal of Animal Science*, *39*, 435–440.

Turner, K. E., Wildeus, S., & Collins, J. R. (2005). Intake, performance, and blood parameters in young goats offered high forage diets of lespedeza or alfalfa hay. *Small Ruminant Research*, *59*, 15–23.

Tyler, H. D., & Ensminger, M. E. (2006). *Dairy cattle science* (4th ed.). Pearson Prentice Hall.

How to cite this article: Bozkurt, G., Kaya, F., Tasal, I., & Yildiz, R. (2024). The effect of alfalfa supplementation during the peripartum period on udder oedema and fertility in dairy sheep. *Reproduction in Domestic Animals*, *59*, e14539. https://doi.org/10.1111/rda.14539