Bulgarian Journal of Veterinary Medicine, 2023 ONLINE FIRST
ISSN 1311-1477; DOI: 10.15547/bjvm.2023-0111

Original article

THE IMPACT OF PROBIOTIC-BASED FOOT BATHS ON OVINE INTERDIGITAL DERMATITIS

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Summary

Ayalp (Cecen), G., A. E. Borum & E. T. Akyol, 2023. The impact of probiotic-based foot baths on ovine interdigital dermatitis. *Bulg. J. Vet. Med.* (online first).

Ovine interdigital dermatitis (OID) is a mild infection of the skin that can predispose to more serious infections such as foot rot and foot abscess. This study was conducted to investigate the effectiveness of probiotic foot baths in controlling OID. Sheep with signs in the interdigital space during clinical examination were selected (n=19). Swab samples were collected before and after the treatment. Each sheep underwent the foot bath once daily for 5 days, with each session lasting 5 minutes. The bath solution, containing 10^6 CFU of probiotic microorganisms per mL, was prepared with strains including *Lactobacillus acidophilus*, *Lacticaseibacillus rhamnosus, Lacticaseibacillus casei,* and *Bifidobacterium bifidum*. In bacterial isolation, *Staphylococcus aureus* was identified, whereas *Fusobacterium necrophorum* and *Dichelobacter nodosus* were not detected. *Staphylococcus aureus* was isolated and identified at the highest rate and significantly reduced from 68.42% to 5.26%. Bacterial loads were decreased after the probiotic foot bath ($P<0.05$). While the total microbial load before probiotic bath application was 4.693±0.644 (TAMC), 3.969±0.625 (TC) and 3.612±0.644 (EC) log CFU/mL, respective values after application were 2.269±0.739, 1.823±0.783 and 1.538 ± 0.742. Our findings suggest that probiotic foot baths effectively reduce pathogenic microbial loads in sheep feet, demonstrating significant prophylactic potential. The study underscores also the importance of non-antibiotic strategies in managing foot diseases. This highlights the viability of probiotics as an alternative approach, particularly in the context of growing antibiotic resistance and the need for sustainable animal health practices.

Key words: foot baths, foot diseases, ovine interdigital dermatitis, probiotics

INTRODUCTION

Ovine interdigital dermatitis (OID), also known as scald, is clinically indistinguishable from benign foot rot (BFR) and the early stages of virulent foot rot due to its manifestation as an infection and inflammation limited to the interdigital space (Hosie, 2004). The initial infection of the interdigital skin can lead to foot rot, a chronic, infectious, and potentially highly contagious disease of the foot. Foot rot

has a major impact on the health and productivity of sheep globally (Clifton *et al.*, 2022). OID is primarily caused by *Fusobacterium necrophorum* (*F. necrophorum*), a Gram-negative anaerobic bacillus. Exposure to this organism is unavoidable due to its ubiquity in soil and ruminant faeces (Wassink *et al.*, 2000). Damage to the interdigital area predisposes sheep to *F. necrophorum* colonisation. There are various bacterial species on the feet of sheep, both healthy and those affected by foot rot. However, *Dichelobacter nodosus* (*D. nodosus*), another Gram-negative anaerobe, is the primary cause of foot rot (Clifton *et al.*, 2019). In addition to *F. necrophorum* and *D. nodosus*, other bacteria such as *Bacteroides fragilis*, *Prevotella* spp. and *Treponema* spp. may contribute to the pathogenesis of the disease. The involvement of these organisms, isolated from foot rot cases, is subject to ongoing debate (Demirkan *et al.*, 2001).

In foot diseases, swabs are taken to isolate and identify the causative agent. Swab samples taken from animals are prepared and incubated in appropriate environments under aerobic and anaerobic conditions for 1–7 days. For culture, MacConkey agar, blood agar (5% sheep blood), 4% hoof agar, trypticase-arginine-serine agar (TAS) and Wilkins-Chalgren agar etc. are used. Swabs are incubated under aerobic and anaerobic conditions. Reproductive colonies are identified according to their biochemical, staining and morphological characteristics. Additionally, different PCR techniques are applied (Hamil *et al.*, 2020; Duncan *et al*., 2021; Zanolari *et al*., 2021).

Foot bathing represents an effective and practical way to control foot infections in sheep. However, it is crucial to pay special attention to the correct application, maintaining hygiene conditions, the concentration of disinfectants used, and the duration that animals spend in the foot bath (Kimberling *et al.*, 1990; Gelasakis *et al.*, 2019). Common disinfectants include solutions of zinc sulfate (10% to 20%), copper sulfate (5%), and formalin (3% to 5%). However, these disinfectants have certain drawbacks. For instance, sheep have to remain in the zinc sulfate solution for 2 to 30 minutes, instead of walking through it, and there are issues with reduced solubility. The use of copper sulfate poses risk of copper toxicity if ingested by sheep. Furthermore, there is a growing trend to eliminate the use of formalin due to its toxicity and negative impacts on both animal and human health. The use of antibiotic solutions in foot baths can lead to increased antibiotic resistance and poses challenges for appropriate disposal, which is often not feasible (Gelasakis *et al.*, 2019).

In recent years, there has been growing interest in using probiotics for treating inflammatory and allergic conditions, owing to their ability to modulate the immune system at both local and systemic levels (Cinque *et al.*, 2011). Probiotics, defined as live microorganisms that provide health benefits to the host when administered in sufficient amounts (Hill *et al.,* 2014), have been extensively studied in clinical and experimental settings. These studies have documented probiotics' capacity to positively influence not only intestinal function but also skin health, due to their unique properties. Scientific and evidence-based reports support the idea that certain probiotics can modulate cutaneous microflora, lipid barrier, and skin immune system, thereby maintaining skin homeostasis. Topical probiotic formulations have been utilised to prevent and treat various skin problems, including acne, yeast infections, bacterial

infections, and dermatitis (Cinque *et al.*, 2011). Although research on using probiotics for microflora-related skin disorders is still limited, the idea of applying topical probiotics to prevent or treat skin diseases associated with altered microflora is gaining traction (Krutmann, 2009). It is thought that cutaneous dysbiosis may be a precursor to foot rot, suggesting that probiotic culture with established dermatological efficacy could be a promising topical treatment option (Ross *et al.*, 2019).

For the present study, we explored the idea that probiotics with experimentally proven efficacy against skin diseases might serve as viable topical treatment option. With a focus on developing proposed treatment algorithms and supporting their therapeutic potential, our aim was to analyse the effect of probiotic foot baths on the healing process in feet afflicted with interdigital dermatitis.

MATERIALS AND METHODS

Ethical approval

The study was approved by the Balikesir University Animal Experiments Local Ethics Committee (Balikesir, Türkiye) (Decision Date: 24.05.2022 and No: 2022/4-4).

Study design

The study was carried out in the autumn of 2021 and the winter of 2022 at Balikesir University Animal Husbandry Application and Research Centre. Two hundred and eighty feet of 70 crossbreed sheep (Karacabey Merino × Curly), which exhibited hoof deformation but no lameness were examined. Out of these, a total of 19 sheep (aged 2.5 to 4 years) with signs of moisture, hyperaemia, and inflammation in the interdigital space of one or more feet during clinical examination were selected for the study. They were housed in covered pens and allowed daily pasture access. Visual inspections for lameness and physical examinations were performed in these sheep. The foot rot scoring system of Stewart & Claxon (1993) for evaluating the sheep feet was used both before and after the foot bath (Table 1). The day of the clinical examination was accepted as day 0. On this day, foot examinations, hoof trimming, and lesion scoring were performed with the sheep in lateral lying position. To ensure consistency, the same individual (GA) was responsible for assigning scores before and after the foot bath for each sheep.

Sample collection & foot bath regime

In order to determine the microbiological load and infectious etiology on ovine feet, swab samples were collected after roughly cleaning the feet of dirt and debris using a piece of gauze. This cleaning was done in the interdigital region of each foot. The swabs were then placed in Amies Agar Gel with Charcoal Transport Swabs me-

Table 1. Scoring system for foot rot (Stewart & Claxton, 1993)

Score	Description
1	Inflammation on the interdigital skin with erosion of the epithelium
2	Necrotising inflammation on the interdigital skin and part of the axial wall of soft horn
3	Necrotising inflammation and under running of the soft horn of the heel and sole
4	Under running extending to the abaxial edge of the sole
5	Necrotising inflammation of the laminae of the abaxial wall and under running of the hard horn

dium (Thermo Scientific™ TS0002A) and sent to the laboratory for analysis. In total, 76 swabs (one for each foot) were collected from 19 sheep before the treatment (day 0). For the foot bath, the sheep were divided into two groups of 9 and 10, and placed in paddocks of approximately 25 m^2 each with fresh litter. They were kept in these paddocks both during and after the treatment until the follow-up samples were collected (7^{th} day). Under *ad libitum* feeding, no treatment protocol was applied except for the foot bath. Two days following the completion of the 5-day treatment, the sheep feet were re-examined, lesion scoring was repeated, and follow-up samples were collected before releasing the sheep back into the flock on the 7^{th} day.

The effect of probiotic microorganisms was investigated using repeated foot bath applications. Prior to the foot bath, the hooves were not cleaned; only before swab sampling a rough cleaning with a piece of gauze was performed to remove dirt and debris from the interdigital space. Each sheep (covering all 4 feet) underwent the foot bath treatment once daily for 5 days, with each session lasting 5 minutes. The bath pool was placed at the base of a restricter (Fig. 1). Following the foot bath, the sheep were returned to the paddock (Fig. 2). A modified footbath pool was used to fully immerse the hooves. The bath solution, containing 10^6 CFU of probiotic microorganisms per mL, was prepared with strains including *Lactobacillus acidophilus, Lacticaseibacillus rhamnosus, Lacticaseibacillus casei,* and *Bifidobacterium bifidum* (Tulemissova *et al.*, 2020). This solution was formulated using packages containing 5×10^9 CFU probiotic microorganisms: *Lactobacillus acidophilus* (*L. acidophilus)*, *Lacticaseibacillus rhamnosus* (*L. rhamnosus)*, *Lacticaseibacillus casei* (*L. casei),* and *Bifidobacterium bifidum (B. bifidum)* per 1 sachet (7 g) (Prolex, Ledapharma, Kocaeli, Türkiye). To achieve a concentration of 10^6 CFU/mL, the solution was prepared at a concentration of 0.14% (4 sachets per 20 L of water). Sheep were treated with the foot bath each day, and a fresh solution was prepared daily for treatment.

Fig. 1. The bath pool was placed at the base of a restrictor, allowing sheep to comfortably stand with all four feet in the bath.

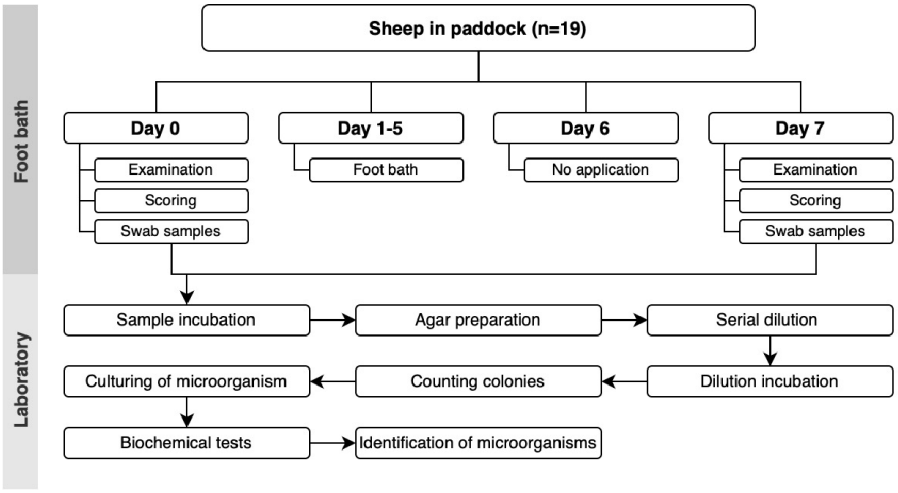

Fig. 2. Experimental design.

Laboratory processing

Agent isolation and identification were made on the swabs to determine the microbial load. The swab samples collected from the animals were prepared and incubated for 1–7 days in appropriate media under both aerobic and anaerobic conditions. Samples were processed in the laboratory within 3 hours of collection. The swabs were initially moistened in sterile brain heart infusion broth (Oxoid CM1135) and then placed in sterile 10 mL tubes containing 1 mL of same medium and vortexed for one minute. Serial dilutions were subsequently prepared using phosphate-buffered saline (PBS). These dilutions were then incubated on various media including MacConkey agar (Oxoid CM0007), blood agar (5% sheep blood) (Oxoid CM0055), and Wilkins-Chalgren agar (Oxoid CM0619) under both aerobic and anaerobic conditions at 37 °C for 24–72 hours. After the incubation, colonies were counted and CFU per mL (log CFU/mL) were calculated. For the ten-fold serial dilutions, 1 mL of homogenised swab sample was transferred into 9 mL of diluent, preparing dilutions in the range of 10^{-1}–10^{-6} (Hamil *et al.*, 2020). From these dilutions, a 0.1 mL aliquot was plated onto various media types for microbial counts (Fig. 2) (Hamil *et al.*, 2020; Marshall *et al.*, 2022).

Total aerobic mesophilic bacteria count (TAMC): A 0.1 mL aliquot from the appropriate dilutions was spread onto plate count agar (Oxoid, CM0325). The inoculated plates were then incubated at 30 °C for 48–72 hours. Following incubation, media with 30 to 300 colonies were counted (Saad *et al.*, 2020; Atlabachew *et al.*, 2021).

Total coliform count (TC): 0.1 mL from the previously prepared serial dilutions was spread on violet red bile agar (VRBA) plates and incubated at 37 °C for 24 hours. All suspicious purple colonies surrounded by purple halos were counted and recorded (Saad *et al.*, 2020).

Total Enterobacter enumeration (TE): Enterobacteriaceae were counted on Mac Conkey agar and incubated at 37 °C for 24 hours (Bouazza *et al.*, 2021; Marshall *et al.*, 2022).

Identification of bacteria

Morphological features: The morphological characteristics of the developing colonies were evaluated by examining the shape, colour and surface of the colonies, their distinctive odour, texture, transparency, haemolysis characteristics on blood agar, and lactose fermentation on MacConkey agar.

Microscopic features: Gram staining was performed on the colonies. In addition, catalase, coagulase, oxidase, triple sugar ıron agar (TSI), urease, indole, methyl red (MR), Voges Proskauer (VP), carbohydrate fermentation, and H_2S production tests were performed on colonies.

Statistical analysis

All statistical analyses were performed using software (SPSS v20, IBM). The distribution of values was assessed for normality using the Shapiro-Wilk normality test. To compare the mean bacterial counts – total aerobic mesophilic bacteria count (TAMC), total coliform count (TC), and *Enterobacter* count (EC) in the samples collected from hooves before and after the probiotic foot bath treatment, the paired samples t-test was utilised. A P-value of less than 0.05 was considered statistically significant for all analyses.

RESULTS

Prior to the treatment, there was no routine hoof trimming or foot bathing practiced on the the farm. Although lameness was not observed in the sheep, almost every sheep had one or more claw deformations (< 25% mildly overgrown hoof wall covering of the sole). The distribution of claw deformation was as follows: both the front and hind hooves (n=12), only the front hooves (n=1), and only the hind hooves (n=5). One sheep had no hoof claw deformation. The lesion scores for

Table 2. The lesion scores of the 19 sheep before and after the treatment

	Front hooves (score)		Hind hooves (score)	
	Before the treatment	After the treatment	Before the treatment	After the treatment
1	0	0	1	0
2	0	0	2	0
3	0	0	1	0
4	1	0	1	0
5	0	0	0	1
6	0	1	1	1
7	0	0	1	0
8	1	0	1	1
9	0	1	0	1
10	0	0	1	1
11	0	0	0	0
12	0	0	0	1
13	0	0	0	0
14	1	0	0	0
15	0	0	1	0
16	1	0	1	0
17	1	0	0	0
18	1	0	0	0
19	2	0	1	0

Fig. 3. Illustration of a moist, hyperaemic, and inflamed interdigital space.

Table 3. Microbial population density (Log CFU/mL) in the samples taken from hooves before (Day 0) and after the probiotic foot bath (Day 7). Data are expressed as mean ± SEM

	TAMC	TC	EC
Before the probiotic foot bath (Day 0, n=76)	4.693 ± 0.644	3.969 ± 0.625	3.612 ± 0.644
After the probiotic foot bath (Day 7, n=76)	2.269 ± 0.739	1.823 ± 0.783	1.538 ± 0.742

TAMC: total aerobic mesophilic bacteria count; TC: total coliform; EC: enterobacteriaceae count

the 19 sheep before the treatment are given in Table 2.

During the clinical examination, no temperature increase was observed in the hooves of any sheep; claw hardness was ideal, and there was no imbibition at the sole. However, slight damage to the white line was detected in three sheep, with the hind claws being more frequently affected: right and left hind medial (n=1), left hind medial (n=1), right hind medial (n=1). The interdigital spaces in the sheep were characteristically moist, hyperaemic, and inflamed (Fig. 3).

In bacterial isolation, *Staphylococcus aureus (S. aureus)* was identified, whereas *F. necrophorum* and *D. nodosus* were not detected. Among the strains from samples taken from animals, *S. aureus* was isolated and identified at the highest rate. *S. aureus* was significantly reduced from 68.42% to 5.26% after the probiotic foot bath. Bacterial loads were compared before and after treatment. As a result of the probiotic foot bath, a statistically significant decrease was found in the number of TAMC, TC and EC in sheep ($P<0.05$) (Table 3).

DISCUSSION

The use of probiotics to alter the gut microbiota has become an accepted concept to improve gut health in humans (Roberfroid *et al.,* 2010). The effect of *Lactobacillaceae* on ovariectomy and lipopolysaccharide (OVX-LPS)-induced gut-bone

dysbiosis in rats was examined. Dairy products fermented with *Limosilactobacillus fermentum* MF27 and/or *Lacticaseibacillus casei* 393 have been shown to selectively modulate the composition of the gut microbiota, improve gut-barrier function, suppress osteoclastogenesis, and therefore increase trabecular bone volume. These findings suggest that the gut-bone axis can be modulated not only by live *Lactobacillaceae* species but also by *Lactobacillus*-fermented dairy products, which may contain metabolites and/or bioactive peptides (Eor *et al.,* 2020). Probiotics isolated from Palmyra palm sugar, which can produce antimicrobial compounds against methicillin-resistant *Staphylococcus aureus* (MRSA) and foodborne pathogens, have been determined to be highly effective (Mitsuwan *et al.,* 2022).

Recent scientific interest has shifted towards the topical application of specific probiotic microorganisms to assess their efficacy in preventing wound inflammation and accelerating the healing process. However, research showing the effects of probiotics on the skin microbiome is still in its early stages (Maguire & Maguire, 2017). There is great scientific interest regarding the role of skin microflora in the process of wound healing. Probiotics shorten the healing time by maintaining microbiota balance (Lolou & Panayiotidis, 2019). In our study, the rate of *S. aureus* was found to be 68.42% before the probiotic foot bath. It seems that *S. aureus* is a predominant infective agent of interdigital region infection in sheep. Studies have shown the antibacterial potential of specific probiotics (*L. acidophilus* and *L. casei*) against MRSA. Three different probiotics (e.g., *Limosilactobacillus reuteri, Lacticaseibacillus rhamnosus* and *Ligilactobacillus salivarius*) were tested against *S. aureus* infection in epidermal keratinocytes. Overall, it was found that *L. reuteri* and *L. rhamnosus* (but not *Ligilactobacillus salivarius*) reduced the ability of the pathogen to induce keratinocyte cell death. To conclude, given that *S. aureus* adheres to the epidermal keratinocyte cells via the α5β1 integrin, it was suggested that both of the protective probiotics reduce keratinocyte cell death by competitively excluding the pathogen from the integrin's binding sites on these skin cells (Lolou & Panayiotidis, 2019). There is evidence from recent studies that *Lactobacillaceae* bacteria and their topical application can help maintain a healthy skin microbiome (Maguire & Maguire, 2017). In particular, *L. acidophilus* positively modulates the epidermal environment through cellular metabolites, antimicrobial peptides, and the immune system (Jeong *et al.*, 2016; Lim *et al.*, 2020). *Lactobacillus casei* has been shown to reduce skin inflammation either by targeting the inhibition of INF-γ or via mechanisms that include the involvement of regulatory CD4+ T cells. In addition, the microorganism has also been shown to increase the production of IL-10, thus further supporting its specific mode of action against skin inflammation (Lolou & Panayiotidis, 2019). In accordance with the literature, the content of the commercial probiotic used in the present study, *Lactobacillaceae* bacteria, was found to help protect skin health as a result of topical application. Especially the use of *L. acidophilus, L. rhamnosus* and *L. casei* in the probiotic foot bath causes decrease in *S. aureus* in line with the literature data.

Another study investigating the foot skin microbiota in cattle with digital dermatitis lesions stated that studies similar to those on the use of probiotics on the skin microbiota in humans may be successful in stopping the development of

digital dermatitis lesions in cattle. It was affirmed that these studies carried out for preventive treatment are promising and can potentially be carried out using a probiotic or prebiotic foot bath (Bay *et al.*, 2023). In the presented study, the protective and therapeutic effects of probiotic foot bath were demonstrated in line with these literature findings. Especially, there was a significant reduction in the isolated *S. aureus* and observable clinical improvement in the interdigital region following the probiotic foot bath. This underscores the potential of probiotic foot baths as an effective treatment strategy in managing similar conditions.

The research evaluating the impact of topical probiotics on foot lesions in farm animals is scarce. In one notable study, the topical application of probiotic powder to early-stage interdigital necrobacillosis in dairy cows was found to be nearly as effective as intramuscular oxytetracycline over a 28-day period (Tulemissova *et al.*, 2020). With the application of probiotics in powder form, antibiotics have been reported to have equivalent therapeutic properties. However, our findings, along with supporting literature, suggest that applying probiotics in a foot bath, rather than the powder form, is a more feasible method for treating herds. This approach allows for more practical and efficient administration of treatment on a herd-wide basis, as opposed to individual treatments.

In cases of foot rot, *F. necrophorum* and *D. nodosus* are the main causative agents and are stated to be present on the skin located in the interdigital spaces of cattle feet (Osova *et al.*, 2018). Additionally, *Porphyromonas levii*, *Porphyromonas asaccharolytica*, *Prevotella intermedia*, *Prevotella melaninogenica*, *S. au reus*, *Escherichia coli,* and *Trueperella pyogenes* can also be isolated (Kontturi *et al.*, 2019). Nayakwadi *et al.* (2014) showed that *F. necrophorum* is the main causative agent in foot rot among small ruminants, with *D. nodosus* not detected in most cases (Nayakwadi *et al.*, 2014). Conversely, another study identified both *D. nodosus* and *F. necrophorum* as leading organisms causing foot rot; along with other Gram-negative and Gram-positive bacteria (Ossova *et al.*, 2018; Kontturi *et al.*, 2019). In our study, neither *D. nodosus* nor *F. necrophorum* were not isolated from the swab samples using microbiological culture methods. This results steered our focus towards evaluating the protective efficacy of a probiotic foot bath as a preventative application against foot rot, rather than as a treatment of foot rot. The use of a probiotic foot bath did not cause any adverse reaction in sheep feet. The healing potential of probiotics observed in the current study is supported by the reduced total microbial loads in the feet treated with probiotic foot bath.

In the present study, a probiotic foot bath solution with a concentration of 10^6 CFU/mL was used. This concentration aligns with the range used in other studies across various species, including cattle, horses, humans, and laboratory rodents. Studies investigated the effects of topical *Lactobacillaceae* family treatments on conditions such as interdigital necrobacillosis, limb wounds, diabetic leg ulcers, and burn wounds with concentration varying between 10^5 and 10^8 CFU (Tulemissova *et al.*, 2020; Wilmink *et al.*, 2020). To illustrate the impact of probiotics on microbial load in tangible way, it's noteworthy that with a total dose of 10^6 CFU, we observed a significant reduction in the microbial load (log CFU/mL). Therefore, a dose of 10^6 CFU was considered sufficient.

In the present study, there are some limitations. Firstly, the relatively small and homogenous sample size from a single institution potentially limits the generalisability of our results. Additionally, the study primarily focuses on short-term outcomes without addressing long-term effects or follow-up, leaving the duration of the treatment's efficacy and potential delayed adverse reactions unexplored. Furthermore, the study does not delve into the potential for resistance development against probiotics, an emerging concern in microbial management. Lastly, the practicality and cost implications of implementing probiotic foot baths on a large scale were not thoroughly evaluated, which is crucial for understanding the feasibility of this treatment approach in real-world settings.

CONCLUSION

Although *D. nodosus* and *F. necrophorum*, which are the most common causes of foot rot in sheep were not isolated in the study, it is thought that other isolated factors may affect the disease score and predispose sheep to interdigital dermatitis and foot rot. The development of non-antibiotic control strategies and evidence-based biosecurity protocols is crucial. Our study results suggest that foot baths with probiotics reduce the microbial pathogen load in feet, and this situation has prophylactic importance. To further understand and optimise the use of probiotic foot baths, new studies involving larger groups of subjects are recommended. The cost-effectiveness and practicality of implementing probiotic foot baths on a larger scale, particularly in diverse farm settings, warrant thorough evoluation. Insights from such research could be instrumental in determining the optimal concentration and application methods of foot bath, investigate the long-term safety and efficacy of these treatments, including any potential for delayed adverse reactions or resistance development.

ACKNOWLEDGEMENTS

The study was funded by Project Number: 2022/074, Balikesir University, Scientific Research Projects Unit. Also, we would like to thank Murad Gurses for his help with data analysis.

REFERENCES

Atlabachew, T. & J. Mamo, 2021. Microbiological quality of meat and swabs from contact surface in butcher shops in Debre Berhan, Ethiopia. *Journal of Food Quality*, **2021**, 7520882.

Bay, V., A. Gillespie, E. Ganda, N. J. Evans, S. D. Carter, L. Lenzi, A. Lucaci, S. Haldenby, M. Barden, B. E. Griffiths, E. Sánchez-Molano, R. Bicalho, G. Banos, A. Darby & G. Oikonomou, 2023. The bovine foot skin microbiota is associated with host genotype and the development of infectious digital dermatitis lesions. *Microbiome*, **11**, 4.

Bouazza, F., R. Hassikou, L. Amallah, J. Ennadir & K. Khedid, 2021. Evaluation of microbiological and hygienic quality of raw sheep milk and detection of antibiotic residues. *Turkish Journal of Physiotherapy and Rehabilitation*, **32**, 21599–21616.

Cinque, B., C. La Torre, E. Melchiorre, G. Marchesani, G. Zoccali, P. Palumbo, L. Di Marzio, A. Masci, L. Mosca, P. Mastromarino, M. Giuliani & M. G. Cifone, 2011. Use of probiotics for dermal applications. In: *Probiotics: Biology, Genetics and Health Aspects,* Vol 21. Microbiology Monographs, ed M. T. Liong, Springer, Berlin, Heidelberg, pp. 221–241.

Clifton, R., K. Giebel, N. L. B. H. Liu, K. J. Purdy & Green L. E., 2019. Sites of persis-

tence of *Fusobacterium necrophorum* and *Dichelobacter nodosus*: A paradigm shift in understanding the epidemiology of footrot in sheep. *Scientific Reports*, **9**, 14429.

Clifton, R., E. M. Monaghan, M. J. Green, K. J. Purdy & L. E. Green, 2022. Differences in composition of interdigital skin microbiota predict sheep and feet that develop footrot. *Scientific Reports*, **12**, 8931.

Demirkan, I., S. D. Carter, C. Winstanley, K. D. Bruce, N. M. McNaır, M. Woodside & C. A. Hart, 2001. Isolation and characterisation of a novel spirochaete from severe virulent ovine foot rot. *Journal of Medical Microbiology*, **50**, 1061–1068.

Duncan, J. S., J. W. Angell, P. Richards, L. Lenzi, G. J. Staton, D. Grove-White, S. Clegg, G. Oikonomou, S. D. Carter & N. J. Evans, 2021. The dysbiosis of ovine foot microbiome during the development and treatment of contagious ovine digital dermatitis. *Animal Microbiome*, **3**,19.

Eor, J. Y., P. L. Tan, Y. J. Son, C. S. Lee & S. H. Kim, 2020. Milk products fermented by Lactobacillus strains modulate the gut-bone axis in an ovariectomised murine model. *International Journal of Dairy Technology*, **73**, 743–756.

Gelasakis, A. I., A. I. Kalogianni & I. Bossis, 2019. Aetiology, risk factors, diagnosis and control of foot-related lameness in dairy sheep. *Animals*, **9**, 509.

Hamil, L. E., D. D. Smeak, V. A. Johnson & S. W. Dow, 2020. Pretreatment aerobic bacterial swab cultures to predict infection in acute open traumatic wounds: A prospective clinical study of 64 dogs. *Veterinary Surgery,* **49**, 914–922.

Hill, C., F. Guarner, G. Reid, G. R. Gibson, D. J. Merenstein, B. Pot, L. Morelli, R. B. Canani, H. J. Flint, S. Salminen, P. C. Calder & M. E. Sanders, 2014. Expert consensus document. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nature Reviews Gastroenterology & Hepatology*, **11**, 506–514.

Hosie, B., 2004. Footrot and lameness in sheep. *The Veterinary Record*, **154**, 37–38.

Jeong, J. H., C. Y. Lee & D. K. Chung, 2016. Probiotic lactic acid bacteria and skin health. *Critical Reviews in Food Science and Nutrition*, **56**, 2331–2337.

Kimberling, C. V. & R. P. Ellis, 1990. Advances in the control of foot rot in sheep. *Veterinary Clinics of North America: Food Animal Practice*, **6**, 671–681.

Kontturi, M., R. Junni, H. Simojoki, E. Malinen, E. Seuna, K. Klitgaard, M. Kujala-Wirth, T. Soveri & S. Pelkonen, 2019. Bacterial species associated with interdigital phlegmon outbreaks in Finnish dairy herds. *BMC Veterinary Research*, **15**, 44.

Krutmann, J., 2009. Pre- and probiotics for human skin. *Journal of Dermatological Science*, **54**, 1–5.

Lim, H. Y., D. Jeong, S. H. Park, K. K. Shin, Y. H. Hong, E. Kim, Y. G. Yu, T. R. Kim, H. Kim, J. Lee & J. Y. Cho, 2020. Anti-wrinkle and antimelanogenesis effects of tyndallized *Lactobacillus acidophilus* KCCM12625P. *International Journal of Molecular Sciences*, **21**, 1620.

Lolou, V. & M. I. Panayiotidis, 2019. Functional role of probiotics and prebiotics on skin health and disease. *Fermentation*, **5**, 41.

Maguire, M. & G. Maguire, 2017. The role of microbiota, and probiotics and prebiotics in skin health. *Archives of Dermatological Research*, **309**, 411–421.

Marshall, H. J., A. M. Blanchard, K. R. Kelly, J. N. Goh, A. D. Williams, L. King, F. Lovatt, P. L. Davies & S. Tötemeyer, 2022. The impact of glutaraldehyde based footbaths on *Dichelobacter nodosus* prevalence and the antimicrobial resistant community of the ovine interdigital skin. *Veterinary Microbiology*, **272**, 109459.

Mitsuwan, W., P. Sornsenee & C. Romyasamit, 2022. *Lacticaseibacillus* spp.; Probiotic candidates from Palmyra palm sugar

possesses antimicrobial and anti-biofilm activities against methicillin-resistant *Staphylococcus aureus*. *Veterinary World*, **15**, 299–308.

Nayakwadi, S., V. K. Gupta, N. Sharma, A. K. Mishra, V. K. Singh, R. S. Pawaiya, K. Gururaj, D. K. Sharma, A. Kumar, S. Paul, N. Kumar & S. V. Singh, 2014. Rapid detection of *Fusobacterium necrophorum* as a main causative agent of foot rot in small ruminants by polymerase chain reaction assay. *Advances in Animal and Veterinary Sciences*, **2**, 302–304.

Osova, A., I. Segurado Benito Pilipcincova, J. Kiraly, M. Dolnik & P. Mudron, 2018. Assessment of two different methods for sampling and detection of *Dichelobacter nodosus* and *Fusobacterium necrophorum* in dairy cows in Eastern Slovakia. *Journal of Applied Animal Research*, **46**, 1452–1456.

Roberfroid, M., G. R. Gibson, L. Hoyles, A. L. McCartney, R. Rastall, I. Rowland, D. Wolvers, B. Watzl, H. Szajewska, B. Stahl, F. Guarner, F. Respondek, K. Whelan, V. Coxam, M. J. Davicco, L. Léotoing, Y. Wittrant, N. M. Delzenne, P. D. Cani, A. M. Neyrinck & A. Meheust, 2010. Prebiotic effects: Metabolic and health benefits. *British Journal of Nutrition*, **104**, 1–63.

Ross, A. A., A. Rodrigues Hoffmann & J. D. Neufeld, 2019. The skin microbiome of vertebrates. *Microbiome*, **7**, 79.

Saad, S. M., F. S. Hassanin, A. M. Salem & E. A. E. Saleh, 2020. Efficiency of some organic acids as decontaminants in sheep carcasses. *Benha Veterinary Medical Journal*, **38**, 116–119.

Stewart, D., & P. Claxton, 1993. Ovine footrot: Clinical diagnosis and bacteriology. In: *Australian Standard Diagnostic Techniques for Animal Diseases*, eds L. A. Corner & T. J. Bagust, CSIRO, pp. 1–27.

Tulemissova, Z. K., M. A. Torehanov, R. Z. Myktybayeva, A. S. Ibazhanova, D. M. Khussainov, Z. M. Batanova & S. S. Usmangaliyeva, 2020. Comparison of probiotic *Lactobacillus acidophilus* and oxytetracycline for the treatment of early stage ınterdigital necrobacillosis in dairy cows. *World's The Veterinary Journal*, **10**, 375–379.

Wassink, G. J., L. E. Green, R. Grogono-Thomas & L. J. Moore, 2004. Risk factors associated with the prevalence of interdigital dermatitis in sheep from 1999 to 2000. *Veterinary Record*, **154**, 551–555.

Wilmink, J. M., S. Ladefoged, A. Jongbloets & J. C. M. Vernooij, 2020. The evaluation of the effect of probiotics on the healing of equine distal limb wounds. *PLoS One*, **15**, e0236761.

Zanolari, P., S. Dürr, J. Jores, A. Steiner & P. Kuhnert, 2021. Ovine footrot: A review of current knowledge. *The Veterinary Journal*, **271**, 105647.

Paper received 11.09.2023; accepted for publication 30.11.2023

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