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RESEARCH ARTICLE

The Use of Probiotic Footbaths for the Treatment of Ovine Interdigital Dermatitis

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Abstract

Ovine interdigital dermatitis (OID) is a mild infection of the skin that may lead to more serious infections, such as foot rot and foot abscess. The objective of this study was to investigate the efficacy of probiotic foot baths in controlling ovine interdigital dermatitis (OID). Prior to and following the administration of the treatment, swab samples were obtained from sheep exhibiting symptoms of OID. Each sheep underwent a single daily foot bath session for a period of five days, with each session lasting five minutes. The solution, comprising of *Lactobacillus acidophilus*, *Lacticaseibacillus rhamnosus*, *Lacticaseibacillus casei* and *Bifidobacterium bifidum*, was prepared with a probiotic microorganism concentration of 10^6 CFU per millilitre. The most prevalent bacterial strain was *Staphylococcus aureus* (36.62%), while *Fusobacterium necrophorum* (1.91%) was isolated and identified at the lowest frequency. Prior to the application of the probiotic solution, the total microbial load was 4.693±0.644 (TAMC), 3.969±0.625 (TC) and 3.612±0.644 (EC) log CFU/mL. Following the application of the probiotic foot bath, the corresponding values were 2.269±0.739, 1.823±0.783 and 1.538±0.742 ($P<0.05$). The results show that probiotic foot baths are effective for reducing pathogenic microbial loads in sheep feet. The study emphasises the importance of non-antibiotic strategies in the management of foot diseases and demonstrates the potential of probiotics as an alternative approach.

Keywords: Foot baths, Foot diseases, Ovine interdigital dermatitis, Probiotics

Introduction

Ovine interdigital dermatitis (OID), also referred to as 'scald', is a mild infection of the skin between the hooves of sheep [1,2]. Uncomplicated and non-progressive OID is caused by a superficial infection of the skin with *Fusobacterium necrophorum*. This can potentially facilitate the development of more severe infections, such as footrot and foot infection. It is inevitable that exposure to this organism will occur, given its ubiquity in soil and ruminant faeces [3]. The damage to the interdigital area renders sheep susceptible to colonisation by *F. necrophorum*. Subsequent infection with *Dichelobacter nodosus* is a probable consequence of OID. Footrot is known to develop following infection with *D. nodosus*. The global impact of footrot on the health and productivity of sheep is significant [2,4].

A variety of bacterial species are present on the feet of sheep, both in healthy individuals and those affected by footrot [4]. Bacteria such as *Staphylococcus* and *Streptococcus* are typically present in the interdigital area and on the surface of the skin, and these opportunistic bacteria have a secondary effect on the development of the disease [5]. In addition to *F. necrophorum* and *D. nodosus*, other bacteria, including *Bacteroides fragilis*, *Prevotella spp*. and *Treponema spp*., may also play a role in the pathogenesis of the disease. The involvement of these organisms, which have been isolated from footrot cases, is still a matter of ongoing debate [6].

The practice of footbathing has been demonstrated to be an efficacious and pragmatic method for the management of foot infections in sheep. However, these disinfectants have disadvantages, including adverse effects on both animal and human health, and problems with efficacy and solubility. 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 [7].

In recent years, there has been a notable increase in the utilisation of probiotics in a multitude of physiological and

pathological cases. The outcomes have been favourable in a considerable number of instances, indicating the potential efficacy of this approach. This is largely attributed to their capacity to regulate the immune system at both local and systemic levels [8]. Probiotics are defined as live microorganisms that provide health benefits to the host when administered in sufficient amounts [9]. Extensive research has been conducted on probiotics in both clinical and experimental settings. These studies have documented the capacity of probiotics to exert a beneficial influence on not only intestinal function but also on skin health, due to their unique properties. A body of scientific evidence supports the hypothesis that specific probiotics can influence the cutaneous microflora, lipid barrier, and cutaneous immune system, thereby maintaining skin homeostasis. Topical probiotic formulations have been employed for the prevention and treatment of various dermatological conditions, including acne, yeast infections, bacterial infections, and dermatitis [8]. Despite the paucity of research in this area, the concept of utilising topical probiotics to prevent or treat dermatological conditions associated with altered microflora is gaining traction [10]. It is hypothesised 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 [11].

The present study aimed to investigate the potential of probiotics with demonstrated efficacy in the treatment of skin diseases as a viable topical therapeutic option. The objective was to develop proposed treatment algorithms and assess their therapeutic potential, with a particular focus on the effect of probiotic foot baths on the healing process in cases of interdigital dermatitis.

Materials and Methods

Ethical Statement

The study was approved by the Balıkesir University Animal Experiments Local Ethics Committee (Balıkesir, Türkiye) on 24 May 2022 (decision number 2022/4-4).

Study Design

The study was conducted in autumn 2021 and winter 2022. Crossbred sheep in Balıkesir University Livestock Application and Research Centre and private sheep farms located in villages of central Balıkesir were examined for foot diseases. Sheep were housed in closed pens and allowed daily access to pasture. Visual and physical examinations were performed for lameness in all sheep. A total of 71 crossbred ewes (Karacabey Merino x Curly), aged between 2.5 and 4 years, showing signs of moisture, hyperaemia and inflammation in the interdigital space of one or more feet during clinical examination were included in the study. Each foot of every sheep was examined visually and scored according to the scoring system developed by Stewart and Claxton (1993). The severity of the lesions was quantified on a scale of 1 to 5, with 1 indicating minimal disease progression and 5 indicating severe disease progression and extensive hoof capsule involvement [12].

The day of clinical examination was considered as day 0. On this day, foot examinations, hoof trimming and lesion scoring were performed with the ewes lying on their side. To ensure consistency, the same person (GA) performed the scoring before and after the foot bath for each sheep. Although sampling, isolation and identification studies were carried out from a total of 71 animals, only two farms approved the 5-day probiotic bath application. Therefore, the number of animals that could be administered probiotics in the study was 19.

Sample Collection & Footbath Regime

To determine the microbiological load and infectious etiology on the feet of sheep, a piece of gauze was used to roughly clean the interdigital area of dirt and debris and swab samples were collected. Swab samples were collected from all four feet of each animal prior to and following bathing, and subsequently analysed for bacterial loads.

Swabs were coated with Amies Agar Gel with Charcoal Transport Swabs medium (Thermo Scientific™ TS0002A) and sent to the laboratory for analysis. A total of 76 swabs (one for each foot) were collected from 19 sheep before treatment (day 0). The sheep were divided into two groups of 9 and 10 sheep and housed in cleanly littered paddocks of approximately 25 m^2 each and fed ad libitum. The sheep were kept in these paddocks both during and after treatment until follow-up samples were collected (day 7) and received no treatment other than foot bathing. Two days after the completion of the 5-day treatment, foot examinations were performed again, lesion scoring was repeated and follow-up samples were collected before release to the flock on day 7.

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. Foot baths were prepared again for each group. 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 colony-forming unit (CFU) of probiotic microorganisms per mL, was prepared with strains including *Lactobacillus acidophilus, Lacticaseibacillus*

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

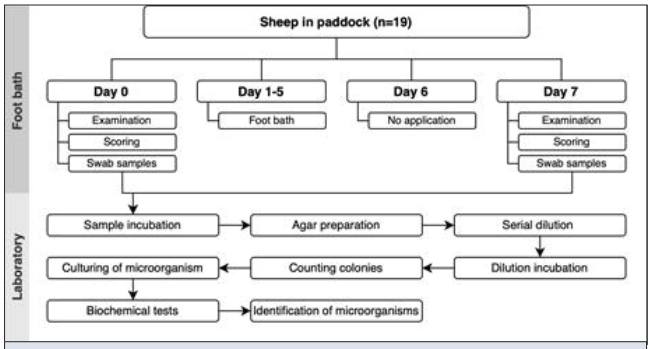

Fig 2. Experimental design

rhamnosus, Lacticaseibacillus casei, and *Bifidobacterium bifidum* [13]. This solution was formulated using packages containing $5x10^9$ CFU probiotic microorganisms: *Lactobacillus acidophilus*, *Lacticaseibacillus rhamnosus, Lacticaseibacillus casei,* and *Bifidobacterium 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.

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 h 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 h. 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} [14]. From these dilutions, a 0.1 mL aliquot was plated onto various media types for microbial counts [14,15] *(Fig. 2)*.

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 h. Following incubation, media with 30 to 300 colonies were counted [16,17].

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 h. All suspicious purple colonies surrounded by purple halos were counted and recorded [16].

Total Enterobacter enumeration (TE): Enterobacteriaceae were counted on Mac Conkey agar and incubated at 37°C for 24 h [15,18].

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, Tsu-Triple Sugar Iron Agar (TSI), urease, indole, Metil Red (MR), Voges Proskauer (VP), carbohydrate fermentation, and H_2S production tests were performed on the colonies.

Polymerase Chain Reaction (PCR)

Suspected anaerobic colonies were subjected to polymerase chain reaction (PCR) analysis. For *D. nodosus*, the 16S rRNA gene was amplified using the primers 5'-CGGGGTTTATGTAGCTTTGC-3' and 5'-TCGGTTACCGAGTATTTCTACCCAACACCT-3'. For *F. necrophorum*, the lktA gene was amplified using the primers 5'-AATCGGAGTAGTAGTAGGTTCTG-3' and 5'-CTTTGGTAACTGCCACTGC-3'. These agents are the most frequently isolated anaerobic bacteria from cases of ovine digital dermatitis. The HiGenoMB kit (HiMedia) was employed for the purposes of DNA isolation and extraction, which was conducted in accordance with the instructions provided by the manufacturer. The amplification process was conducted at a temperature of 94°C for a duration of 10 min, followed by 30 cycles of 30 sec at 94°C, 30 sec at 58°C, and 1 min at 72°C. Subsequently, the PCR products were subjected to electrophoresis in a 1.5% agarose gel, stained with OrisafeDNA (Sugenomics), and visualised under ultraviolet (UV) illumination. The temperature was maintained at 72°C for 5 min.

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

The study included 284 feet from 71 crossbred sheep. Deformities (slight overgrowth of the hoof wall covering of the sole) were observed in both the front and rear hooves of 30 sheep. During clinical examination, no increase in hoof temperature was observed in any sheep; claw hardness was normal and there was no imbibition (bleeding foci) on the sole. The lameness status, claw deformities and lesion scores of the sheep examined are given in *Table 1*.

Table 1. *Lameness status, hoof deformations and lesion scoring of the examined sheep*

Examined Sheep		**Sheep (n)**	**Ratio (%)**
Sheep with lameness		33	46.47
Claw deformation	No deformation	27	38.02
	Only on the forelegs	3	4.22
	Rear legs only	11	15.49
	Both front and rear legs	30	42.25
Lesion score	0	15	21.12
	1	22	30.98
	2	22	30.98
	3	6	8.45
	4	4	5.63
	5	2	2.81

Table 2. *Isolation results of the samples*

Total Number of Bacteria Isolated	**Microorganism Type**
115 (36.62%)	*Staphylococcus aureus*
92 (29.29%)	*Escherichia coli*
91 (28.98%)	*Bacillus* spp.
10 (3.18%)	*Trueperella pyogenes*
6 (1.91%)	*Fusobacterium necrophorum*

Table 3. *Microorganisms isolated according to assigned scores*

Score	**Isolated Microorganisms**
0	*Escherichia coli* *Bacillus* spp. *Staphylococcus aureus* *Yeast*
1	*Escherichia coli* *Bacillus* spp. *Staphylococcus aureus* *Yeast*
2	*Escherichia coli* *Bacillus* spp. *Staphylococcus aureus* *Yeast*
3	*Escherichia coli* *Bacillus spp.* *Staphylococcus aureus* *Trueperella pyogenes*
4	*Escherichia coli* *Bacillus* spp. *Staphylococcus aureus* *Trueperella pyogenes* *Fusobacterium necrophorum*
5	*Escherichia coli* *Staphylococcus aureus*

Isolations and identifications were made from samples taken from the fore and hind feet. In total, 314 bacterial strains were isolated. The most common bacterial strain was *Staphylococcus aureus* [115 (36.62%)], while *F. necrophorum* [6 (1.91%)] was isolated and identified with the lowest frequency. The table of isolated and

Table 4. *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*

Microbial Population Density	TAMC	TC	EC
Before the probiotic foot bath (Day 0, n=19)	4.693±0.644	3.969±0.625	3.612±0.644
After the probiotic foot bath (Day 7, n=19)	2.269±0.739	1.823±0.783	1.538±0.742

TAMC: *Total Aerobic Mesophilic Bacteria Count;* ***TC:*** *Total Coliform;* ***EC:*** *Enterobacteriaceae Count*

Table 5. *The lesion scores of the 19 sheep before and after the treatment*

Sheep	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

identified pathogens is presented below *(Table 2, Table 3)*.

In the bacterial isolation of the samples taken from 19 sheep that could be treated with probiotic foot bath, *S. aureus* was identified at the highest rate, while *F. necrophorum* and *D. nodosus* were not found. The *S. aureus* rate decreased significantly from 68.42% to 5.26% after the probiotic foot bath. In sheep where bacterial loads were compared before and after treatment, a statistically significant decrease in TAMC, TC and EC counts was observed ($P<0.05$). This was supported by the decrease in total microbial load and a lower score in the probiotic treated feet *(Table 4, Table 5)*.

Furthermore, a higher bacterial load was observed in feet with deformity and clinically high scores in the swab samples. This finding was found to be statistically significant.

Discussion

The use of probiotics to alter the gut microbiota has become an accepted concept for improving human gut health [19]. The effect of *Lactobacillaceae* on ovariectomy and lipopolysaccharide (OVX-LPS)-induced gut-bone dysbiosis in rats was investigated. Dairy products fermented with *Limosilactobacillus fermentum* MF27 and/or *L. casei* 393 were shown to selectively modulate the composition of the gut microbiota, improve gut barrier function, suppress osteoclastogenesis and thereby 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 [20]. Probiotics isolated from

Palmyra palm sugar, which can produce antimicrobial compounds against methicillin-resistant *Staph. aureus* (MRSA) and foodborne pathogens, have been found to be highly effective [21].

Recent scientific interest has focused on the topical application of specific probiotic microorganisms to assess their efficacy in preventing wound inflammation and accelerating the healing process. However, research into the effects of probiotics on the skin microbiome is still in its early stages [22]. There is considerable scientific interest in the role of skin microflora in the wound healing process. Probiotics reduce healing time by maintaining the balance of the microbiota [23]. In our study, the rate of *S. aureus* before the probiotic foot bath was 68.42%. It appears that *S. aureus* is a predominant pathogen in interdigital infections in sheep. Studies have demonstrated the antibacterial potential of specific probiotics *(L. acidophilus* and *L. casei)* against MRSA. Three different probiotics (e.g. *Limosilactobacillus reuteri*, *L. 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 *L. salivarius)* reduced the ability of the pathogen to induce keratinocyte cell death. Given that *S. aureus* adheres to epidermal keratinocytes via the alpha5β1 integrin, it has been suggested that both protective probiotics reduce keratinocyte cell death by competitively excluding the pathogen from the integrin binding sites on these skin cells [23]. There is evidence from recent studies that *Lactobacillaceae* bacteria and their topical application can help maintain a healthy skin microbiome [22]. In particular, *L. acidophilus* positively modulates the epidermal environment via cellular metabolites, antimicrobial peptides and the immune system [24,25]. *L. casei* has been shown to reduce skin inflammation either by inhibiting INF-γ or by mechanisms involving regulatory CD4+ T cells. In addition, the microorganism has also been shown to increase the production of IL-10, further supporting its specific mode of action against skin inflammation [23]. Consistent with the literature, the content of the commercial probiotic used in the present study, *Lactobacillaceae* bacteria, was found to contribute to the protection of skin health as a result of topical application. In particular, the use of *L. acidophilus*, *L. rhamnosus* and *L. casei* in the probiotic footbath resulted in a reduction of *S. aureus*, in line with literature data [26].

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 human skin microbiota may be successful in preventing the development of digital dermatitis lesions in cattle. It has been confirmed that these studies conducted for preventive treatment are promising and can potentially be carried out using a probiotic or prebiotic foot bath [26]. In the present study, the protective and therapeutic effects of probiotic footbath were demonstrated in line with these literature findings. In particular, there was a significant reduction in isolated *S. aureus* and an observable clinical improvement in the interdigital region following the probiotic foot bath. This highlights the potential of probiotic foot baths as an effective treatment strategy in the management of similar conditions.

There is a paucity of research evaluating the effect of topical probiotics on foot lesions in livestock. In one notable study, topical probiotic powder was found to be almost as effective as intramuscular oxytetracycline over a 28-day period for early stage interdigital necrobacillosis in dairy cows [13]. The use of powdered probiotics has been reported to have equivalent therapeutic properties to antibiotics. However, our results and the supporting literature suggest that the application of probiotics in a foot bath, rather than in powder form, is a more practical method of treating flocks. This approach allows for more practical and efficient administration of treatment on a flock-wide basis, as opposed to individual treatments.

In cases of foot rot, *F. necrophorum* and *D. nodosus* are the main causative agents and are reported to be present on the skin in the interdigital spaces of bovine feet [27]. In addition, *Porphyromonas levii*, *Porphyromonas asaccharolytica*, *Prevotella intermedia*, *Prevotella melaninogenica*, *S. aureus*, *E. coli* and *Trueperella pyogenes (T. pyogenes)* can also be isolated [28]. Nayakwadi et al.[29] showed that *F. necrophorum* was the major causative agent of foot rot in small ruminants, while *D. nodosus* was not detected in most cases. Conversely, another study identified both *D. nodosu*s and *F. necrophorum* as leading organisms causing foot rot, along with other Gram-negative and Gram-positive bacteria [28-30]. In our study, *E. coli*, *Bacillus spp*. and *S. aureus* were isolated from sheep with healthy/dry feet (score 0) and 1 and 2 scores according to culture results. In addition to these bacteria, *T. pyogenes* was isolated from feet with scores of 3 and 4. In cases with a lesion score of 4, *F. necrophorum* was isolated in addition to these bacteria. These findings are consistent with those of other studies. Eradication of *F. necrophorum* is challenging, particularly given its ability to persist in the environment through faecal shedding. *D. nodosus* was thought to persist in the environment for only a few hours to a few days. In contrast to *D. nodosus*, *F. necrophorum* is an opportunistic pathogen that causes necrotic lesions at various anatomical sites and in many host species. Furthermore, our study also gave positive results for the isolation of *F. necrophorum*.

In the present study, a probiotic foot bath solution with a concentration of 10^6 CFU/mL was used. This concentration is consistent with the range used in other

studies in various species, including cattle, horses, humans and laboratory rodents. Studies have investigated the effects of topical treatments of the *Lactobacillaceae* family on conditions such as interdigital necrobacillosis, limb wounds, diabetic leg ulcers and burn wounds, with concentrations varying between 10^5 and 10^8 CFU [13,27]. To illustrate the effect of probiotics on microbial load, it's worth noting that we observed a significant reduction in microbial load (log CFU/mL) with a total dose of 10^6 CFU. A dose of 10^6 CFU was therefore considered sufficient. 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 load in the feet treated with the probiotic foot bath. These results led us to focus on evaluating the protective efficacy of a probiotic foot bath as a preventive application against foot rot, rather than as a treatment for foot rot.

It should be noted that this study is not without limitations. The study primarily focuses on short-term outcomes, without addressing long-term effects or follow-up. As a result, the duration of treatment efficacy and potential delayed adverse reactions remain unexplored. Moreover, the study does not investigate the potential for the development of resistance to probiotics, which is an emerging concern in microbial management. Additionally, the practicality and cost implications of implementing probiotic foot baths on a large scale have not been comprehensively evaluated, which is crucial for understanding the feasibility of this treatment approach in real-world settings. Further studies are necessary to address these limitations.

Declarations

Availability of Data and Materials: The corresponding author can provide the datasets of this research upon reasonable request.

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Ethical Statement: The study was approved by the Balıkesir University Animal Experiments Local Ethics Committee (Balıkesir, Türkiye) on 24 May 2022 (decision number 2022/4-4).

Conflict of Interests: The authors declare that they have no conflicts of interest.

Declaration of Generative Artificial Intelligence: The authors declare that the article and/or tables and figures were not written/created by AI and AI-assisted Technologies.

Author Contributions: G.A., the clinical examination on the animals, followed by scoring and sample collection. A.E.B., collected the samples and performed the laboratory analyses. E.T.A., participated in the fieldwork component of the study. Editing, all authors read and approved the final manuscript.

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