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Occurrence of some pathogenic bacteria in cattle feed samples before and after invasion by European starlings

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Abstract: This study was carried out to investigate the species identification and enumeration of some pathogenic microorganisms before and after feeding the full ration contaminated by predatory starling bird herds on 5 different days in the dairy cattle farm in Aksaray. At the end of the study, the average number of *E.coli* obtained from the feed samples taken before the arrival of starlings was 6.46 log cfu/g, while the number of *E.coli* obtained from the feed taken after starling birds flocked to the feed was found as 6.80 log cfu/g. While the average *Campylobacter* spp. number was 5.50 log cfu/g, this value was found as 5.66 log cfu/g after starlings swooped down on the feed. As the average *Yersinia* spp. number was 5.04 log cfu/g, this value was 5.78 log cfu/g after the starlings flocked to the feed. *Salmonella* was found in 3 samples taken from the feed samples taken after the starlings came to the farm only on the 5th day in 5 different days when they attacked the cattle feed. Considering the current results, necessary precautions should be taken against starlings that cause contamination in farms.

Key words: *Campylobacter*, cattle feed, *Salmonella*, starling, *Yersinia*.

1. Introduction

Starlings are omnivorous, that is, animals that can eat feeds of both vegetable and animal origin. In this respect, it is an animal that can feed on insects and can also feed on seeds and fruits. These birds, which generally seem to be beneficial to farmers by eating snails, worms, spiders, mosquitoes, moths, dragonflies, grasshoppers, bees, ants, and similar insects, have become famous for their damage to fruits and grains[1]. So much so that a flock of starlings consisting of around 1000 birds can consume 16–18 kg of feed per day[2]. In addition to the damages they cause to fruits and grains, starlings are carriers of some pathogenic (disease causing) microorganisms, and in this way, they infect animals and humans with various diseases [1]. Therefore, starlings, an invasive and predatory species, should be fought. Pimentel et al. [2] reported that the loss of starlings from agricultural activities is $800 million annually in the US. For example, the market value of the death of 10,000 pigs in Nebraska due to the disease caused by starlings is around $1,000,000 [3].

According to Lee [4], starlings infect some pathogenic bacteria in addition to the common damage they cause to animals' feeds such as grain and pellets. The same researcher reported that since starlings can easily enter the farm, they pose a threat to biosecurity and can carry pathogens such as *Salmonella*. Indeed, winged salmonellosis (primarily *Salmonella enterica*) has been reported in starlings. This disease can be passed on to humans, chickens and livestock, meaning it is zoonotic. Also, starlings are heavily infected with *Mycobacterium paratuberculosis*. As a result, Johne's disease (also known as paratuberculosis, a bacterial disease characterized by chronic weakening and diarrhea) can be observed in cattle. *E. coli*, which produces dysentery toxin, is another disease agent that can be passed from starlings to cattle. The annual cost of this disease to the cattle industry is over 267 million dollars [3]. The researchers reported whether starlings could act as a source of human or livestock infection and concluded that starlings produced a distinct *Campylobacter* genotype population that was largely host-specific[3]. The same

[1] Anonymous (2018). Common starling in 2018 [online]. Website: https://wikivisually.com/wiki/Common_%20starlin [accessed 03 September 2018].

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[3] Shipton J, Shipton P, Forbes D. Starling Infestations on the Somerset Levels and Their Impact on Dairy Farming in 2012 [online]. Website https://dairy.ahdb.org.uk/non_umbraco/download aspx?media=12993 [accessed September 2018].

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researchers noted that large flocks could potentially lead to large-scale fecal contamination. Carlson et al. [5] also reported that starlings are associated with an increased risk of *Salmonella enterica* contamination in cattle feed as a result of their studies on starlings in the feed-lot cattle breeding system in the USA.

This study aims to contribute literature originally by examining the level of microbial contamination in feed caused by starlings that have been proven to have negative effects on dairy cattle feed in different countries and leads to huge economic losses also in Turkey and by identifying some pathogens such as *L. monocytogenes*, *E. coli*, *Salmonella* spp., *Vibrio* spp., *Campylobacter* spp., *Streptococcus* spp. and *Yersinia* spp. that have not been examined in the feed before in this country.

2. Material and methods

2.1. Material

The main material of the study was the full ration thought to be contaminated by predatory starlings in a dairy cattle farm in Aksaray province.

2.2. Collection of feed samples

Feed samples were collected on five separate days from designated a dairy cattle farm between December and January during the winter season when starlings are known to have difficulty finding food and invade cattle farms for feed. Immediately after distribution, feed samples were taken from five different parts of the feeder, and five more samples were taken from the same points one to one and a half hour after the flocking started. This process continued in this manner for five days. Ten samples were taken daily, five before and five after, and pathogenic microorganisms were investigated in 50 samples in total. Feed samples were taken into sterile stomacher bags without any contamination and brought to laboratory conditions and placed in a freezer at –86 °C.

2.3. Microbiological analysis of feed samples

On each day of analysis, three samples were analyzed, both in and out of starlings. All feed samples were preenriched before being taken for microbiological analysis. For this process, the feed samples were weighed in 25 g of presterilized 225 mL enrichment liquids to be used for preenrichment and left to stand. At the end of this incubation, serial dilutions were made by taking 1 mL each into sterile dilution fluids obtained by using Ringer tablet (Merck KGaA, Darmstadt, Germany) from enrichment liquids for solid media. Appropriate aliquots of each dilution were pipetted into the Petri dishes.

2.3.1. Total mesophilic aerobic bacteria enumeration

1 mL each of the feed samples kept for preenrichment in Buffered Peptone Water (Merck KGaA, Darmstadt, Germany) for 12 h at 35 °C was taken and diluted serially in 9 mL ringer solutions sterilized previously. 0.1 mL of inoculum of appropriate dilution was spread onto the Petri dishes containing Plate Count Agar (Merck KGaA, Darmstadt, Germany). Petri dishes were incubated aerobically at 30 °C for 24–48 h and all colonies observed on the agar at the end of incubation were counted [6].

2.3.2. *Streptococcus spp.* enumeration

For *Streptococcus spp.* load, appropriate dilutions were prepared with preenriched samples in Buffered Peptone Water (Merck KGaA, Darmstadt, Germany) for 12 h at 35 °C. 0.1 mL of inoculum of each dilution was spread onto the Petri dishes containing M17 Agar (Merck KGaA, Darmstadt, Germany). Petri dishes were incubated aerobically at 30 °C for 24–48 h and the white colonies on the agar were counted at the end of incubation.

2.3.3. *Listeria monocytogenes* enumeration

1 mL each of the feed samples kept for preenrichment in Buffered Peptone Water (Merck KGaA, Darmstadt, Germany) for 12 h at 35 °C was taken and diluted serially in presterilized 9 mL ringer solutions. Appropriate dilutions (1 mL) of Compact Dry LS (Nissui Pharmaceutical Co., Ltd, Tokyo, Japan) were pipetted into ready-made media. Petri dishes were incubated aerobically at 35 °C for 24 h and 1–2 mm diameter and blue colonies were counted at the end of the incubation [7].

2.3.4. *Escherichia coli* enumeration

1 mL each of the feed samples kept for preenrichment in Buffered Peptone Water (Merck KGaA, Darmstadt, Germany) for 12 h at 35 °C was taken and diluted serially in 9 mL ringer solutions previously sterilized. 1 mL of inoculum of each dilution was pipetted into ready-made media Compact Dry EC (Nissui Pharmaceutical Co., Ltd, Tokyo, Japan). The Petri dishes were incubated at 35 °C for 24 h using the anaerobic environment provided by Anaerocult A (Merck KGaA, Darmstadt, Germany). The blue colonies were counted as *E.coli* [7].

2.3.5. *Vibrio spp.* enumeration

For *Vibrio* spp. counts, appropriate dilutions were prepared with preenriched samples in Alkaline Peptone Water (Merck KGaA, Darmstadt, Germany) for 6 h at 37 °C. Accordingly, 0.1 mL of aliquots of each dilution was spread onto the Petri dishes containing CHROMagar Vibrio and aerobic incubation at 37 °C for 24 h was used. Results were given after biochemical confirmation tests were applied to lilac, beige and turquoise colonies [8]. All results were reported as log CFU/g.

2.3.6. *Campylobacter spp.* enumeration

1 mL each of the feed samples kept for preenrichment in Buffered Peptone Water (Merck KGaA, Darmstadt, Germany) for 8 h at 35 °C was diluted serially in presterilized 9 mL ringer solutions. 0.1 mL of inoculum of each dilution was spread onto the Petri dishes containing

Campylobacter Selective Agar LAB112 supplemented with X112 (LABM, UK) and Horse Blood Lysed (Liofilmchem, Italy). Petri dishes were incubated at 37 °C for 48 h using the microaerophilic media provided by Anaerocult C (Merck KGaA, Darmstadt, Germany). The biochemical verification tests were applied to white and beige colonies at the end of the incubation and the results were given [9].

2.3.7. *Yersinia spp.* enumeration

1 mL each of the feed samples kept for preenrichment in Buffered Peptone Water (Merck KGaA, Darmstadt, Germany) for 8 h at 35 °C was diluted serially in presterilized 9 mL ringer solutions. 0.1 mL of aliquots of each dilution was spread onto the Petri dishes containing Yersinia Selective Agar CIN (Oxoid, UK) with Yersinia Selective supplement (Oxoid, UK). Petri dishes were incubated aerobically at 32 °C for 24 h and red bull's-eye surrounded by a transparent border colony was counted at the end of the incubation. Results were given after applying biochemical verification tests to these colonies [10].

2.3.8. *Salmonella* enumeration

The feed samples kept for preenrichment in Buffered Peptone Water (Merck KGaA, Darmstadt, Germany) for 8 h at 35 °C were then taken to Rappaport Vasiliadis Soy Broth (Oxoid, UK) for selective enrichment and kept for one night. At the end of this period, 1 mL was taken and serially diluted in 9 mL ringer solutions which were sterilized beforehand. 1 mL of the aliquots was pipetted into XLD Agar (Oxoid, UK). Petri dishes were incubated aerobically at 35 °C for 24 h and at the end of the incubation red colonies with black centers were collected. Results were given after applying biochemical verification tests to these colonies [11].

2.4. Statistical analysis

Pathogen differences in ration samples before and after feeding were checked by paired t-test.

3.Results and discussion

3.1. *Escherichia coli* counts of feed samples

The results regarding the number of *E. coli* are presented in Table 1. The average number of *E. coli* obtained from the feed samples taken before the arrival of starlings to the farm where the study was conducted was 6.47 log cfu/g, while the number of *E. coli* obtained from the feed taken after starlings flocked to the feed was found as 6.81 log cfu/g. However, this 0.34 log cfu/g increase feed was statistically insignificant.

This increment in the number of *E. coli* after the arrival of starlings is an expected situation. A previous study hold starlings responsible for the transmission and spread of the *E. coli* to cattle [12]. Cernicchiaro et al. [13] also found a direct relationship between the frequency of *E. coli* O157: H7 isolated from the feces of cattle and the density of starlings in their study on dairy farms in Ohio and confirmed the hypothesis that these birds carry pathogens to dairy farms. One of the evidence of the spread by birds has been shown as the identification of the same serotype *E. coli* O157: H7 in birds 50–100 km away from the cattle feeding units [14]. As a result of previous studies in animal farms, it was suspected that livestock were consuming starlings' stools with feed, and it was thought that farm animals that consumed the feed were contaminated in this way, however, in recent studies published on wild bird-livestock interaction in concentrated animal feeding units (CAFOs), it was stated that in addition to the damage of their own feces, starlings also mechanically move cattle feces on their feet and feathers into cattle feed and water sources, or they carried this bacteria mechanically when they come to the farms from their night perches and *E. coli* contamination in feeds occurred mostly like this [12]. Again, in studies conducted in Turkey, Şahin and Sarı [15] found *E. coli* in 72.7% of the feed samples in their study on mixed feeds in Elazığ region, while Aslantaş [16] reported that 16.1% of the mixed feeds in the Kars region had *E. coli*. In the present study, the fact that *E. coli* counts before and after birds arrived is statistically insignificant is consistent with the study of [17]. The researchers stated that there was no statistical difference between the numbers of ciproflaxacine-resistant *E. coli* number counted after the arrival of starlings to the farms and before they came, and even reported that there was a negative correlation between the number of *E. coli*, another antibiotic resistant to cefotaxime, and the arrival of these birds to the farms.

3.2. *Campylobacter* spp. counts of feed samples

The results regarding the number of *Campylobacter* spp. are presented in Table 2. While the average count of *Campylobacter* spp. obtained from the feed before the starlings arrived on 5 different days in the farm where the study was conducted was 5.50 log cfu/g, *Campylobacter* spp. number was found as 5.66 log cfu/g after starlings flocked on the feed. This 0.16 log cfu/g was statistically insignificant.

In a previous study, Daniels et al. [18] reported that in addition to pathogens such as *Salmonella* spp. and *E. coli* O157: H7, *Campylobacter* spp. was detected in the feces of starlings and that the consumption of feed contaminated with bird feces by livestock may lead to possible infection of cattle. Similarly, Sanad et al. [19] also reported that they genotypically isolated the same *Campylobacter* strains from the feces of cattle and starlings in dairy farms. Carlson et al. [12] and Corn et al. [20] also reported that they suspected starlings to be responsible for the spread of *Campylobacter* strains to cattle farms and their transmission to cattle. As expected in the present study and as in the literature, there was an increase in the number of *Campylobacter* spp. in the cattle compound feed after the arrival of starlings. However, this increase was found lower than expected. The

Table 1. *E. coli* counts in mixed feed samples taken detected before and after invasion.

Days	Before invasion *E. coli* (log cfu/g)			Days	After invasion *E. coli* (log cfu/g)		
Day 1	5.79	6.41	4.84	**Day 1**	6.09	6.19	7.30
Day 2	7.00	6.71	7.19	**Day 2**	7.09	6.95	7.16
Day 3	6.23	5.73	7.32	**Day 3**	6.48	6.33	7.03
Day 4	5.69	7.14	6.13	**Day 4**	6.53	7.40	6.87
Day 5	8.07	6.70	6.03	**Day 5**	7.55	5.65	7.51
Average	6.47 ± 0.21			**Average**	6.81 ± 0.14		

Table 2. *Campylobacter* counts in mixed feed samples taken detected before and after invasion.

Days	Before invasion *Campylobacter spp.* (log cfu/g)			Days	After invasion *Campylobacter spp.* (log cfu/g)		
Day 1	5.50	4.51	4.90	**Day 1**	6.11	5.00	6.14
Day 2	5.19	5.00	3.82	**Day 2**	5.91	5.76	4.76
Day 3	5.97	6.07	5.92	**Day 3**	5.78	5.90	5.07
Day 4	5.63	6.74	6.38	**Day 4**	6.53	5.44	5.84
Day 5	5.28	5.31	6.27	**Day 5**	5.72	5.45	5.52
Average	5.50 ± 0.19			**Average**	5.66 ± 0.12		

reason for this is can be thought that not all starlings are the carrier of *Campylobacter* spp. Accordingly, Waldenström et al. [21] reported that only 21.6% of starlings examined in Sweden were *Campylobacter* spp. positive, and French et al. [22] stated that 30.6% of the starling birds they studied on carried *Campylobacter* spp.

3.3. *Yersinia* spp. counts of feed samples

The results regarding the number of *Yersinia* spp. are given in Table 3. While the average *Yersinia* spp. count obtained from the feed on 5 different days before the starlings arrived at the farm where the study was conducted was 5.04 log cfu/g, the number was 5.78 log cfu/g after starlings flocked on feed. This 0.74 log cfu/g increase in feed after the arrival of starlings was statistically insignificant.

It has long been known that migratory birds are an important source and carrier of *Yersinia* species, especially *Yersinia pseudotuberculosis* and *Yersinia enterocolitica* play a role in the transmission of this bacterium to humans and other animals [23]. For example; Hamasaki et al. [24] detected *Yersinia* in total 11 birds in 5 different bird species among a total of 586 birds from 15 different bird species in Japan on which they studied *Yersinia* spp. Similarly, Kato et al. [25] examined a total of 500 birds from 9 different species in Japan and found *Yersinia* strains in 34 of them and reported that *Yersinia* spp. was isolated in 6 (10.5%) of the 57 gray starling birds they examined. Odyniec et al. [26] determined *Yersinia* spp. varying between 1.4% and 5.00% separately in bird species in their study carried out with 894 samples from a total of 447 different wild birds and obtained 20 different *Yersinia* isolates belonging to *Y. kristensenii, Y. frederiksenii, Y. enterocolitica, Y. intermedia* species. Based on these examples, it is clear that starlings can be carriers of *Yersinia*, but the rate at which the birds coming to the farms being carriers was not determined in this study. The difference of 0.74 log cfu/g in our results about *Yersinia* spp. in the cattle mixed feed samples taken before the starlings came to the farm and those taken supports previous studies on this subject.

3.4. *Salmonella* spp. counts of feed samples

Results related to the count of *Salmonella* are summarized in Table 4. *Salmonella* was found in 3 samples taken from the feed samples taken only on the 5th day on the farm where the study was conducted after the starlings came to the farm and attacked the cattle feed. The amount of *Salmonella* in 3 different feed samples was determined as 4.30, 3.77, and 3.54 log cfu/g, and the average was calculated as 3.87 log cfu/g *Salmonella* was not detected in any of the samples taken before the attack of starling birds on the mentioned day and in any of the samples taken for the other 4 days.

Table 3. *Yersinia* spp. counts in mixed feed samples taken detected before and after invasion.

Days	Before invasion *Yersinia spp.* (log cfu/g)			Days	After invasion *Yersinia spp.* (log cfu/g)		
Day 1	5.74	6.54	6.14	**Day 1**	7.30	7.21	7.42
Day 2	4.47	4.00	3.90	**Day 2**	5.95	6.34	4.30
Day 3	3.60	2.86	4.43	**Day 3**	4.36	4.19	4.90
Day 4	7.39	6.79	6.79	**Day 4**	6.49	6.83	7.16
Day 5	4.43	4.96	3.56	**Day 5**	4.09	6.04	4.16
Average	5.04 ± 0.36			**Average**	5.78 ± 0.33		

Table 4. *Salmonella* spp. counts in mixed feed samples taken detected before and after invasion.

Days	Before invasion *Salmonella spp.* (log cfu/g)			Days	After invasion *Salmonella spp.* (log cfu/g)		
Day 1	-	-	-	**Day 1**	-	-	-
Day 2	-	-	-	**Day 2**	-	-	-
Day 3	-	-	-	**Day 3**	-	-	-
Day 4	-	-	-	**Day 4**	-	-	-
Day 5	-	-	-	**Day 5**	4.30	3.77	3.54
Average	-			**Average**	3.87 ± 0.26		

These results seem to be compatible with the literature, in a similar way, Gaukler et al. [27], investigating the *Salmonella* carriage of starlings caught in and around cattle farms in Kansas, stated that when they examined the feces of these birds, only 3 of 434 feces were *Salmonella* positive. In other words, the rate of *Salmonella* positive starling was found as 0.7% in this study. Similarly, Medhanie et al. [28] found *Salmonella* in 2 birds in their study, in which they examined 179 starling feces, that is, 1.12% of common starlings were found to be *Salmonella* positive. In studies on feed in our country, Baran et al. [29] examined 60 mixed feeds and reported that *Salmonella* species were found in 3.33% of all mixed feeds. Based on findings of study conducted by Carlson et al. [12], they reported that starlings or other wild birds actually contaminate the feed and water resources in the farm mechanically, by contact, with *Salmonella* rather than being a gastrointestinal vector in actually contaminating cattle feed, not by the way their feces contaminate animal feed. The figures given above do not mean that *Salmonella* carriage in starlings is not important. The number of starlings examined in these studies is quite limited, however, thousands of different starlings attack the farms depending on the location, and even the fact that 1% or 2% of them carry *Salmonella* is enough for the livestock to contaminate this bacterium and this situation has serious consequences. In the present study, a total of 30 different feed samples were examined in terms of *Salmonella* on five different days, and *Salmonella* was found in only three of them, on the same day and the treatment. Hence, this study supports the studies in the literature that reveal the potential of starlings in terms of *Salmonella* carriage.

3.5. *Listeria monocytogenes* counts of feed samples

The results obtained in terms of *L. monocytogenes* counts are given in Table 5. Before starlings arrived, the average number of *L. monocytogenes* obtained from the feed was 4.21 log cfu/g, while the number of *L. monocytogenes* obtained after starlings flocked to the feed was 3.55 log cfu/g. The difference of 0.65 log cfu/g was not found statistically significant.

In this context, Sauders and Wiedmann [30] reported that *Listeria* species were found in these animal feeds, which were mostly sold in pellet form and subjected to heat treatment. Also, Müller [31] reported that *L. monocytogenes* was found at the rate of 40% in silages. In a study conducted on forages in Turkey Baran et al.

Table 5. *L. monocytogenes* counts in mixed feed samples taken detected before and after invasion.

Days	Before invasion *L.monocytogenes* (log cfu/g)			Days	After invasion *L.monocytogenes* (log cfu/g)		
Day 1	4.62	4.17	4.59	**Day 1**	4.47	4.30	4.17
Day 2	4.11	3.95	4.80	**Day 2**	3.00	3.01	3.08
Day 3	4.77	4.10	4.69	**Day 3**	3.19	3.91	2.36
Day 4	3.97	3.91	4.50	**Day 4**	4.69	4.50	4.36
Day 5	3.75	3.38	3.77	**Day 5**	2.30	3.60	2.38
Average	4.21 ± 0.11			**Average**	3.55 ± 0.21		

[29] reported that 46.66% of the fattening feeds and 26.6% of the mixed feeds examined in Diyarbakır region were *L. monocytogenes* positive. In previous studies, *L. monocytogenes* was considered among the pathogenic bacteria that migratory birds could carry and cause public health problems. For example, Ryser and Marth [32] reported that 33% of healthy birds carry *L. monocytogenes* without showing any symptoms, and that the birds are most likely infected with this bacterium with *Listeria*-contaminated soil, feces, or beaks from the dead animal. Similarly, in a study conducted in the Helsinki region, Danish researchers collected a total of 212 wild bird feces and detected *L. monocytogenes* in 36% of them [33]. In short, it has been assumed that *L. monocytogenes*, which already has a high probability of existing in animal feed, can also be transmitted to livestock by wild birds that infest the feed. However, in the present study, no positive result was obtained in terms of *L. monocytogenes*, indicating that starlings contaminated animal feed with this bacterium. One result that may support the result is the study by Yoshıda et al. [34], in which they examined the intestinal contents of a total of 996 different birds from 18 species, including 60 gray starlings in Japan, 13.4% of these wild birds were Listeria positive but *L. monocytogenes* was not detected in any of the 60 starlings examined.

3.6. Total mesophilic aerobic bacteria (TMAB) counts of feed samples

The results obtained with the total number of mesophilic aerobic bacteria are presented in Table 6. While the average total mesophilic aerobe bacteria count obtained from the feed on 5 different days was 10.04 log cfu/g before starlings arrived at the farm where the study was conducted, the total number of mesophilic aerobe bacteria obtained after the starling flocked to the feed was 9.47 log cfu/g. The 0.57 log cfu/g decrease in between was found statistically insignificant.

The total number of mesophilic aerobe bacteria is regarded as one of the important criteria in determining the general microbiological quality of animal feeds. It is also a parameter that shows the efficiency of the process applied to the feed, harvesting, transportation, and the hygienic conditions of the environment where the feed is produced. Looking at the number of mesophilic aerobic bacteria in animal feed in the form of cereal grains, Kukier and Kwiatek [6] reported that the count was 6 log cfu/g at the most. Accordingly, Wojdat et al. [35] examined the feeds of livestock in Poland in terms of microbiological criteria and found a total bacteria count above 10^7 cfu/g in only one feed sample of 65 mixed feed samples, between 10^6–10^7 in four mixed feeds and 10^5–10^6 in 20 feeds. In Turkey, Erdogan and Aslantas [36], who conducted research in mixed feed samples earlier, reported that there was an average $1.7.10^5$–$1.6.10^7$ cfu/g total bacteria in 50 mixed feed samples in Antakya region. In the present study, when the total numbers of mesophilic aerobe bacteria in the feed samples were examined before and after the starlings flocked to the feed, it was seen that starlings did not have any negative effect on the cattle feed in the farms in terms of this parameter.

3.7. *Streptococcus* spp. counts of feed samples

The results obtained with *Streptococcus* spp. counts are presented in Table 7. The average *Streptococcus* spp. numbers obtained from feed before starlings arrived at the farm where the study was conducted was 9.06 log cfu/g and number obtained after starlings flocked to feed was found as 8.66 log cfu/g. The 0.4 log cfu/g decrease in between was found statistically insignificant.

Numerous *Streptococcus* spp. strains zoonosically affect fish, birds, and many mammal species such as horse, pig and cattle, and also cause negative economic effects. The most important disease caused by *Streptococcus* strains in various animals is known as mastitis and it is known that *S. uberis, S. agalactiae*, *S. dysgalactiae* and *S. zooepidermicus*, which mostly live commensally in the animal mucous membrane, are Streptococci, mainly responsible for this disease [37]. There are also studies showing that domestic

Table 6. TMAB counts in mixed feed samples taken detected before and after invasion.

Days	Before invasion *TMAB* counts (log cfu/g)			Days	After invasion *TMAB* counts (log cfu/g)		
Day 1	10.24	10.12	10.16	**Day 1**	10.10	9.99	10.40
Day 2	10.28	10.33	9.93	**Day 2**	9.14	9.27	8.86
Day 3	9.31	9.27	9.77	**Day 3**	8.72	8.88	10.20
Day 4	10.00	10.06	10.05	**Day 4**	9.58	9.21	9.43
Day 5	9.94	10.70	10.47	**Day 5**	9.60	8.85	9.88
Average	10.04 ± 0.099			**Average**	9.47 ± 0.141		

Table 7. *Streptococcus* spp. counts in mixed feed samples taken detected before and after invasion.

Days	Before invasion *Streptococcus* spp. (log cfu/g)			Days	After invasion *Streptococcus* spp. (log cfu/g)		
Day 1	7.63	7.37	7.76	**Day 1**	7.55	7.40	7.73
Day 2	9.31	9.34	9.20	**Day 2**	9.25	9.17	8.92
Day 3	9.25	9.04	8.85	**Day 3**	8.20	8.73	9.35
Day 4	9.25	9.43	9.13	**Day 4**	9.00	8.84	8.97
Day 5	9.82	9.85	10.60	**Day 5**	9.01	9.05	8.77
Average	9.06 ± 0.224			**Average**	8.66 ± 0.163		

or wild birds can carry Streptococci. For example; Crispo et al. [38] revealed 95 cases in their study investigating the Streptococci cases in commercial and noncommercial birds between 2000–2017 in California and stated that they isolated *S. bovis*, *S. lutetiensis*, *S. gallolyticus* and *S. pluranimalium* species from birds. In the present study, it was emphasized that starlings can also carry Streptococci to cattle feed and *Streptococcus* counts were also made before and after starlings infested in animal feed. However, there was no statistical difference. This study did not provide any data to contribute to the thesis that Streptococci can be carried into feed by starlings.

3.8. *Vibrio* spp. counts of feed samples

The results obtained with *Vibrio* spp. count are presented in Table 8. The average *Vibrio* spp. counts obtained from the feed before starlings arrived at the farm were found as 4.66 log cfu/g while *Vibrio* spp. count was 4.66 log cfu/g after starlings flocked to the feed. This 0.89 log cfu/g increase was found statistically insignificant. *Vibrio* is a genus that can be found everywhere in nature, but more commonly isolated from aquatic and marine habitats. There is a belief that the spread of *Vibrio* strains by migratory waterfowls that land on rivers, lakes and seas is a public health problem. For example; Laviad-Shitrit et al. [39] reported that *Vibrio* species were detected in many bird species in Israel and that migratory waterfowl has great potential to spread these pathogens, especially through migrations southward in autumn and north in spring. In this regard, Smibert [40] detected *Vibrio* in 52% of birds as a result of autopsies he performed on 25 sparrows, crows and starlings. However, there is no study on *Vibrio* contamination in livestock feeds. *Vibrio* analysis was also carried out in this study on the possibility that migratory birds may be contaminated with *Vibrio* from the water sources where they meet their water need and carry these bacteria species to the cattle feed they infest. Indeed, there was a remarkable increase of 0.89 log cfu/g in terms of *Vibrio* count among the feed samples taken before and after starlings arrived. The emergence *Vibrio* in feeds before the birds flock is attributed to the frequent entry of these birds into the environments where the feed is present during the preparation of the feed, that is, before the livestock eat, and the potential of these bacteria to be found in the water sources used by the animals in the farms.

4. Conclusion

The results about the microbiological status of the feed samples taken from a farm that was infested with starlings especially in winter in Aksaray province are given above.

Table 8. *Vibrio* spp. counts in mixed feed samples taken detected before and after invasion.

Days	Before invasion *Vibrio* spp. (log cfu/g)			Days	After invasion *Vibrio* spp. (log cfu/g)		
Day 1	3.86	3.69	3.87	**Day 1**	5.24	5.64	4.85
Day 2	4.18	4.44	3.97	**Day 2**	4.39	3.77	4.62
Day 3	4.17	4.25	4.82	**Day 3**	4.19	4.49	4.38
Day 4	2.00	2.69	2.00	**Day 4**	3.59	3.50	3.50
Day 5	3.76	5.04	3.85	**Day 5**	6.36	4.90	4.95
Average	3.77 ± 0.23			**Average**	4.56 ± 0.21		

One of the striking points about the results is that the microbial quality of the feed is far below the desired level, even in samples taken before the starling infestation. There have been noticeable increases especially in the number of *E. coli, Yersinia, Vibrio*, and *Campylobacter* after the arrival of starlings to cattle feed and this state is mostly supported by studies in the literature, but the figures in feeds taken prior to the daily bird infestation are surprising. According to the information obtained from the farms, the idea that starlings only deal with feed after the feed is in front of the animal is wrong, because the birds intensely enter the compartments where the feed is stored and prepared before and during the preparation phase. Birds frequently enter and exit both the feeding area and the parts where the livestock drink water in the farms, as reported in the literature, for example, they touch the feces of the farm animal and carry the microorganism to another place and the water source that the animals drink, and then to the feed again through their beak or body. When all these factors come together, especially in the autumn and winter months, when the food resources in nature decrease, the microbial load in the feed of farm animals increases in the farms they infest. There is no doubt that this may cause various diseases and symptoms in the winter months when livestock are already more susceptible to infection. Overall, measures should be taken to keep starlings, which cause great financial damage due to the diseases they transmit, away from the business.

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Conflict of interest
The authors have no conflict of interest.

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