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Influence of spearmint (*Mentha Spicata*) growth performance, hematological and lipid profile of broiler

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ABSTRACT

Introduction: Antibiotics and growth stimulants provide health hazards, prompting a demand for antibiotic-free organic broiler production. Natural plant-based feed additives are both safe and cost-efficient. This study aimed to thoroughly investigate the dietary impact of spearmint in various dosages on broiler production performance, hematobiochemical profile, bacterial load, and cost-effective performance. Materials and methods: In the experiment, a total of 225-day-old Cobb 500 broiler chicks were randomly selected into five experimental groups, each consisting of three replications of 15 birds. Groups T0, T1, T2, T3, and T4 consumed basal feed supplemented with 0 ml, 1 ml, 2 ml, 3 ml, and 4 ml of spearmint juice, and the treatment was given at 7 days to 28 days, respectively. Results: In this study, the T0 and T4 groups' total feed intake was considerably ($P < 0.05$) lower than that of the T1, T2, and T3 groups. Following T1, T3, T4, and T0 groups, group T2 received a 2 ml spearmint treatment, which resulted in a considerably ($P < 0.05$) greater final live weight. The T2 group had a significantly ($P < 0.05$) better feed conversion ratio (FCR) in comparison to the T0, T1, T3, and T4 groups. The weight of the broiler chicken's organs (carcass weight, thigh, breast muscle, drumsticks, wings meat, liver, heart, gizzard, and other organs) and dressing % were significantly affected by any of the treatment groups ($P < 0.05$). Dietary additions affected ($P<0.05$) the hematobiochemical parameters (concentration of haemoglobin, ESR, WBC, RBC, and lipid profile). Compared to the T0 group, the faecal bacterial load was reduced in the T1, T2, T3, and T4 groups. Conclusion: T2 is more cost-effective than other groups because of their faster rate of body growth. The 2% spearmint-treated group had birds with increased body weight, better FCR, and higher feed intake. Overall, 2% spearmint addition proved to be more beneficial than other treatment groups.

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Introduction

Antibiotic Growth Promoters (AGP) will have a significant impact on the livestock and poultry industries. Finding an AGP substitute is necessary to reduce the growth loss. Numerous non-therapeutic options exist, including immune stimulants, enzymes, inorganic acids, probiotics, prebiotics, botanicals, and other management techniques (Banerjee, 1998; Monsoub, 2011). The idea of organic poultry is very

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new and is becoming more and more popular in developed countries. Herbs and spices were added to poultry diets as a non-nutritive feed addition. When these substances are present, the ration's nutritional content is largely supplied to enhance the birds' growth effectiveness, avoid disease, and maximize feed utilization. Among the significant members of the Lamiaceae family are peppermint (Mentha piperita) and spearmint (Mentha spicata) (Zaidi and Dahiya, 2015). According to Sabrina and Metha (1990), active ingredients in herbs help speed up digestion and metabolism. Mentha (mint) mints are fragrant, commonly cultivated, scented plants that are mostly perennial but occasionally annual and may grow in a range of environments (Bbrickell, 2002). The main medicinal properties of mint come from a rich, volatile oil found in the leaves and blossoms. This oil has been shown to contain thymol and highly oxygenated compounds, along with a dihydrocarbon (AOAC.; 1980). It's benefits alcohol, but boiling water is where it really shines. Steams have antispasmodic, choleretic, and carminative properties (Galib et al., 2010). Because it might diminish gastric reflux, which in turn lessens indigestion and colon spasms, mint is usually taken after meals (Spirling and Daniels, 2001). The essential oil is produced from freshly harvested or dried mint leaves using a distillation technique. The essential oil produced was found to have antibacterial, antifungal, antiviral, insecticidal, and antioxidant activities (Singh and Aggarwal, 2013). The essential oil contains high levels of limonene, dihydrocarvone, and 1,8-cineol (Hussain et al., 2010). The characteristic smell of spearmint oil is due to its most abundant ingredient, carvone. According to Abu Isha et al. (2018), there was an improvement in feed conversion rate as a result of increased appetite brought on by the stimulation of gastric and salivary glands by spearmint oil, a decrease in pathogenic bacteria, and improved digestibility. Additionally, there is a suggestion that spearmint oil may stimulate these glands and reduce bacteria, which in turn improves digestibility, FCR, and hematobiochemical profile. Studies in the lab have demonstrated that the growth of Salmonella enteritidis, Candida albican, Staphylococcus aureus, and Escherichia coli is inhibited by the action of peppermint essential oils. Furthermore, feeding spearmint is said to enhance feed intake, which in turn promotes improved chicken growth (Saleh et al., 2014). The mint species has uses in both medicine and commerce. Herbal teas and a variety of meals are flavored and scented with the leaves, stems, and flowers of the Mentha species. Due to inadequate research and information on spearmint in adding poultry ration, that's why this study was conducted. Objective of this study was: 1.To investigate the dietary impact of spearmint in various dosages on broiler production and cost-effective performance. 2. To determine the effect of spearmint on the hematology, lipid profile and bacterial load of broiler.

Material and method

Experimental site

The experiment was carried out at Hajee Mohammad Danesh Science and Technology University's Poultry Farm in Dinajpur from March to April 2022.

Experimental birds

For the experiment, 225-day-old broiler chicks (Cobb 500) were collected from the Kazi Farm hatchery via local traders. The chicks were randomly assigned to five nutritional treatment groups (T0, T1, T2, T3, T4), each of consisted of three replications with 15 birds each. The following are the treatments: T0 = control, T1 = control + 1 ml of spearmint juice per litre of water, T2 = control + 2 ml of spearmint juice per liter of drinking water, T3 = control + 3 ml of spearmint juice per kg of feed, and T4 = control + 4 ml of spearmint juice per kg of feed. When the spearmint was adding the feed or water, mixed all the content carefully. After a week of brooding, the course of treatment was administered across 7 to 28 days, accordingly. On the last day of the experiment, for each replication, 2 birds were slaughtered.

Collection and preparation of spearmint

Spearmint was collected from the botanical garden of Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur-5200, Bangladesh. Fresh leaves were collected (Figure 1), cleaned, and ground before water was added in a 1:1ml ratio. Subsequently, the leaves were blended to make juice, which was then refrigerated at 4 °C to preserve the active components.

Figure 1. Spearmint leaf and leaf juice

Managemental practices

For the experimental trial, commercial feed was used. The feed used in the experiment was bought from a feed store in the town of Dinajpur. In different ages, different feeds are

16-28 days. There were three times morning, noon, and evening were given feed of broilers. Housing (intensive and coop), litter water, lighting and sanitization were all necessities provided. Chicks were immunized against Ranikhet Disease (RD) and Infectious Bursal Disease (IBD) as scheduled. Suitable biosecurity protocols were put in place for the duration of the research.

Table 1. Experimental feed composition (Calculated analysis amount 100kg).

Ingredient	Starter (0-14) days	Grower (15-28) days
Crude protein (%)	22	21
Crude fiber (%)	3	3
Crude fat (%)	5	5-6
Lysine (%)	1.30	1.25
Methionine (%)	0.52	0.48
Calcium (%)	1	0.90
Phosphorus (%)	0.50	0.48
Moisture (%)	11	11
Metabolic Energy.ME (kcal/kg)	3000	3100

Ingredient	Amount (%)
Maize	60.70
Soybean meal	32.24
Soyabean oil	3.0
Dicalcium phosphate	2.20
Ground limestone	0.61
Choline chloride	0.10
DL methionine	0.20
L-lysine	0.15
Salt	0.30
Vitamin –mineral premix*	0.50

* Vitamin-mineral premix contains in the following per kg: vitamin A, 2400000 IU; vitamin D, 1000000 IU; vitamin E, 16000 IU; vitamin K, 800 mg; vitamin B1, 600 mg; vitamin B2 , 1600 mg; vitamin B6 , 1000 mg; vitamin B12, 6 mg; niacin, 8000 mg; folic acid, 400 mg; pantothenic acid, 3000 mg; biotin 40 mg; antioxidant, 3000 mg; cobalt, 80 mg; copper, 2000 mg; iodine, 400; iron, 1200 mg; manganese, 18000 mg; selenium, 60 mg, and zinc, 14000 mg.

Hematological analysis

After 4 weeks, blood samples were collected using a vacutainer tube through the wing vein puncture tubes (BD vacutainer SST Gel-5 ml). They were then allowed to coagulate at room temperature (25 °C) for an hour, which used an ASPO 4 mL clot activator. The serum was extracted from the blood sample by centrifugation at 2000 rpm for 15 minutes. Separated, non-hemolyzed serum samples were kept in clean, dry Eppendorf tubes in the deep freezer (-20 °C) for later use. The analyzes were performed with commercial kits produced by the German cholesterol agent manufacturer Randof (2016). The experiment was conducted using a Merck Microlab300 biochemistry analyzer (India), following the instructions in the manufacturer's booklet. The lipid profiles were determined using biochemical assays.

Collection of fecal sample, storage, transportation, culture and bacterial colony count

On the 28th day, a bacterial colony from one bird was randomly chosen for counting. For bacteriological examination, bird droppings were used to gather feces. Feces were collected and then preserved at 4^{o}C in sealed polythene bags. The dairy microbiology lab received the sample of feces after that. Sample dilution, inoculation, bacterial culture, and ultimately colony count were all meticulously monitored throughout the production of culture medium in the microbiology lab. The colonies are counted after the plate has been incubated under microorganism-appropriate conditions. For the spread, pour, and drop methods, colony counting is self-explanatory: count each colony dot once. A marker can be used to point out each numbered colony on the back of the Petri dish.

Calculation: 1. Total gain in weight = final weight – initial weight. 2. Total feed consumption = total feed offered – total left-over 3. Feed conversion ratio = total feed consumed / total gain in weight . 4. Mortality rate (%) = no. of dead chickens / total no. of birds as a group × 100.

Statistical analysis

The generated data were entered into SPSS version 25 software, which then used one-way ANOVA to analyze them in compliance with the Complete Randomized Design (CRD) principles. Every value was reported as mean ± SEM, and significance was assessed ($P > 0.05$). The Duncan test was used to compare the means of the treatment groups.

Results and Discussion

Dietary effect of spearmint on body weight

The weekly body weight gain in the first and second weeks of age did not differ significantly across treatments ($P > 0.05$). According to similar findings by Amasaib et al. (2013), the addition of spearmint leaf extract in the first and second weeks of age had no effect on broiler chicken weight gain. Only in the third week of life it become clear that the body weight gain in all treatment groups (T1, T2, T3, and T4).It was significantly greater than that of the control group. This could be spearmint-fed groups consumed more feed. Amasaib et al.'s (2013) findings did not support this result. These findings were consistent with those of Galib et al. (2010), who discovered that peppermint addition had no effect on overall body weight but did improve performance over the control group. Demir et al. (2008) revealed similar findings on the effect of spearmint on reducing body weight. The T1, T2, T3, and

Table 2. The effect of spearmint on body weight gram/ broiler.

	Dietary Treatment Groups					
Weeks	**T_0 (control)**	**T_1**	**T_2**	**T_3**	**T_4**	**Level of significant**
Initial body weight	38.5 ± 0.27	38.5 ± 0.13	38.2 ± 0.35	38.2 ± 0.13	38.8 ± 0.18	NS
1st week	173.3 ± 1.16	177.2 ± 1.29	177.3 ± 1.49	173.6 ± 1.23	170.9 ± 1.22	NS
2nd week	272.7 ± 0.54^{a}	285.1 ± 0.57^{c}	288.8 ± 0.80^{d}	279.4 ± 1.55^{b}	284.1 ± 0.49^{c}	*
3rd week	426.1 ± 1.95^{a}	493.0 ± 2.54^{b}	591.1 ± 9.26^{e}	509.4 ± 2.84^{c}	548.8 ± 1.28^{d}	*
4th week	504.7 ± 1.40^{a}	608.7 ± 1.78^{c}	672.7 ± 0.67^{e}	624.4 ± 1.72^{d}	599.7 ± 2.24^{b}	*
Final body weight	1376.9 ± 5.05^{a}	1564.1 ± 6.18^{b}	1729.9 ± 12.22^{d}	1586.9 ± 7.34^{bc}	1603.6 ± 5.23^{c}	*

a, b, c means having different superscript in the same row differed significantly (P<0.05). * = P<0.05 level of significance, NS=Non-significant

(P > 0.05) compared to the T0 group (Table.2), consistent with previous studies by Ocak et al. (2008), Rahman et al. (2017), Abu Isha et al. (2018), and Al-Ankari et al. (2004).

Feed intake

Table. 3 provides information on average weekly feed consumption and total feed use. According to the table, feed intake was highest in the T0 group and lowest in the T4 group during the first week of the bird, but highest in the T1 group and lowest in the T0 group during the second week. Feed consumption peaked in the T2 group in the third and fourth weeks of life and was lowest in the T0 group. Based on the results of the current study, it can be stated that the T2 group had the highest weekly feed intake compared to the control group and that supplementation with spearmint leaf extract at levels of 2 ml improved feed intake. The T2 group consumed the most feed overall per broiler, and the T0 group consumed the least of the various experimental groups. The increase in feed intake shown in this study may have resulted from spearmint's flavoring impact (Deyoe et al., 1962). The results of this study's feed intake were found (P<0.01) to be consistent with those of Amasaib et al. (2013), Ocak et al. (2008), Rahman et al. (2017), Abu Isha et al. (2018), and Al-Ankari et al. (2004).

Feed conversion rate

During the first week of the experiment, the T0 group had the highest weekly feed conversion ratio, and the T2 and T3 groups had the lowest. In the second week, the FCR of the T2 and T4 groups was higher than that of the T0, T1, and T3 groups. It was found that the FCR values for the T0 group were greater and those for the T2 group were better in the third week. The T2 and T4 groups outperformed the T0, T1, and T3 groups in terms of FCR values during the four-week age period. At age 28, broiler feed efficiency was considerably lower and better in T2 (1.35 ± 0.71) than in other treatment groups (P<0.1) (Table.4) These results are consistent with those of Amasaib et al. (2013), Ocak et al. (2008), Rahman et al. (2017), Abu Isha et al. (2018), and Al-Ankari et al. (2004).

Carcass quality

Table. 5 shows that broiler meat yield features following various spearmint treatments. The samples with the highest live weights were T2 (1729.98±12.22) and T4 (1603.67±5.23). The live weights with the lowest values are T0 (1376.91±5.05) and T1 (1564.13±6.18). The samples with the highest carcass weights were T2 (1001.17±3.28) and T4 (955.50±21.27). T1 (963.44±4.44) and T0 (1376.91±5.05) are the carcass weights with the lowest values. Supplementing with 2 ml of spearmint significantly raised the dressing percentage (P < 0.05). These findings are harmonious with those of Ocak et al. (2008), Rahman et al. (2017),

Table 3. Effect of spearmint on feed intake of broiler (gram)

	Dietary Treatment Groups					
Weeks	**T_0 (control)**	**T_1**	**T_2**	**T_3**	**T_4**	**Level of significant**
1st week	192.53±0.72	185.24±2.63	183.59±3.88	179.68±2.31	180.52±0.64	NS
2nd week	372.49±1.23^{a}	384.14±1.87^{c}	381.68±1.48^{bc}	375.99±1.17^{a}	376.81±2.09^{ab}	*
3rd week	682.64±0.80^{a}	729.72±1.93^{b}	795.61±2.63^{e}	745.03±1.01^{c}	764.04±0.84^{d}	*
4th week	882.40±1.70^{a}	983.61±2.63^{d}	971.73±0.67^{c}	964.57±0.74^{b}	882.98±1.40^{a}	*
Total Feed Intake	2130.06±4.45^{a}	2282.71±9.06^{bc}	2332.61±8.66^{d}	2265.27±5.23^{c}	2204.35±4.97^{b}	*

a, b, c means having different superscript in the same row differed significantly (P<0.05). * = P<0.05 level of significance, NS=Non-significant

Table 4. Effect of spearmint on FCR

	Dietary Treatment Groups					
Weeks	**T_0 (control)**	**T_1**	**T_2**	**T_3**	**T_4**	**Level of significant**
1st week	1.11 ± 0.01	1.05 ± 0.01	1.04 ± 0.01	1.03 ± 0.01	1.06 ± 0.01	NS
2nd week	1.37 ± 0.00^{c}	1.35 ± 0.00^{b}	1.32 ± 0.01^{a}	1.35 ± 0.00^{b}	1.33 ± 0.01^{a}	*
3rd week	1.60 ± 0.01^{d}	1.48 ± 0.01^{c}	1.35 ± 0.02^{a}	1.46 ± 0.01^{c}	1.39 ± 0.00^{b}	*
4th week	1.75 ± 0.00^{e}	1.62 ± 0.00^{d}	1.44 ± 0.00^{a}	1.54 ± 0.00^{c}	1.47 ± 0.01^{b}	*
Final FCR	1.55 ± 0.88^{e}	1.46 ± 1.47^{d}	1.35 ± 0.71^{a}	1.43 ± 0.71^{c}	1.37 ± 0.95^{b}	*

a, b, c means having different superscript in the same row differed significantly (P<0.05). * = P<0.05 level of significance, NS=Non-significant

Abu Isha et al. (2018), and Al-Ankari et al. (2004). However, these were dissimilar from Amasaib et al. (2013), who found that adding spearmint to chicken did not significantly raise the dressing percentage. There were notable differences in the percentages of drumstick and breast meat in different diets. As spearmint levels grow, grill meat—especially the drumstick and breast—nearly increases linearly. The findings of the tabulation indicate that the amount of spearmint had a considerable impact on the meat's characteristics. Chopra et al. (1992) reported that the herb with beneficial effects on the digestive system performed better (Brander, 1985). All the meat yield parameters were significantly different (P<0.05).

Serum biochemical profile

Blood biochemical properties in broilers Table 6 displays the effect of spearmint on the lipid profile of broilers. Total cholesterol levels differed significantly (P < 0.01) between treatment groups, with T0 having higher levels and T2 having lower levels. T0 had significantly higher blood triglyceride levels compared to T3, who had lower levels (P<0.01). T3 had a significantly higher high-density lipoprotein HDL value than T0, which had a lower value (P<0.01). Low-density lipoprotein (LDL) levels were significantly higher in T0 and lower in T2. LDL values were lower in the spearmint treatment group compared to the control group. The experimental group that took spearmint supplements had increased blood levels of HDL mg/dl, while spearmint considerably reduced blood LDL levels. This could be because spearmint serves as an antioxidant, inhibiting the oxidation of LDL and cholesterol (Mathur et al., 1996) and slowing the

Table 5. Effect of spearmint on Carcass quality of different dietary groups (gram)

	Dietary Treatment Groups					
Meat yield trait	**T_0 (control)**	**T_1**	**T_2**	**T_3**	**T_4**	**Level of significant**
Live weight	1376.9 ± 5.05^{a}	1564.1 ± 6.18^{b}	1729.9 ± 12.22^{d}	1586.9 ± 7.34^{bc}	1603.6 ± 5.23^{c}	*
Carcass weight	798.9 ± 1.64^{a}	963.4 ± 4.44^{b}	1001.1 ± 3.28^{c}	955.5 ± 21.27^{b}	979.2 ± 9.28^{bc}	*
Breast weight	227.1 ± 0.86^{a}	345.8 ± 2.74^{d}	398.5 ± 1.42^{e}	320.3 ± 0.29^{c}	310.3 ± 2.66^{b}	*
Thigh weight	198.6 ± 1.55^{a}	234.2 ± 0.80^{b}	285.4 ± 0.27^{e}	254.3 ± 2.02^{c}	273.6 ± 2.81^{d}	*
Drum stick meat	86.4 ± 2.80^{a}	120.1 ± 1.43^{b}	131.0 ± 1.19^{c}	119.8 ± 0.70^{b}	124.2 ± 0.70^{b}	*
Wing meat weight	99.2 ± 0.37^{a}	116.8 ± 1.97^{b}	135.0 ± 0.72^{d}	126.7 ± 1.71^{c}	124.3 ± 0.93^{c}	*
Head	30.8 ± 0.34^{a}	34.7 ± 0.55^{c}	41.2 ± 0.28^{b}	35.8 ± 1.00^{b}	35.03 ± 0.73^{b}	*
Gizzard weight	38.1 ± 2.10^{a}	42.0 ± 0.20^{ab}	50.4 ± 1.01^{c}	55.2 ± 1.06^{d}	43.3 ± 1.06^{b}	*
Liver	41.0 ± 1.12^{a}	57.4 ± 0.70^{c}	73.8 ± 0.47^{e}	46.2 ± 1.07^{b}	65.9 ± 2.48^{d}	*
Heart	7.39 ± 0.09^{a}	7.57 ± 0.32^{a}	13.0 ± 0.15^{c}	11.3 ± 0.14^{b}	11.2 ± 0.09^{b}	*
Spleen	2.4 ± 0.01	2.54 ± 0.08	3.50 ± 0.10	3.57 ± 0.05	2.77 ± 0.15	NS
Intestine weight	99.3 ± 1.22^{b}	108.20 ± 1.91^{c}	107.3 ± 0.58^{c}	116.6 ± 2.23^{d}	94.15 ± 0.47^{a}	*
Mortality rate	0.33 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	*

a, b, c means having different superscript in the same row differed significantly (P<0.05). * = P<0.05 level of significance, NS=Non-significant

Table 6. Effect of spearmint on serum biochemical profile

	Dietary Treatment Groups					
Serum biochemical	**T_0 (control)**	**T_1**	**T_2**	**T_3**	**T_4**	**Level of significant**
Total cholesterol	176.4 ± 1.35^{e}	165.0 ± 0.24^{d}	120.5 ± 1.35^{a}	135.9 ± 1.03^{b}	147.7 ± 1.49^{c}	*
Triglyceride	71.2 ± 0.80^{d}	59.5 ± 0.35^{c}	53.9 ± 1.66^{ab}	50.8 ± 0.37^{a}	55.5 ± 2.23^{bc}	*
HDL	35.1 ± 0.87^{a}	42.7 ± 0.61^{c}	39.8 ± 0.17^{b}	46.3 ± 0.72^{d}	47.8 ± 1.18^{d}	*
LDL	103.1 ± 0.53^{e}	96.1 ± 1.25^{d}	55.3 ± 1.00^{a}	91.2 ± 0.85^{c}	63.0 ± 1.38^{b}	*

a, b, c, d, e means having different superscript in the same row differed significantly (P<0.05). * = P<0.05 level of significance, NS=Non-significant

thermogenesis process. The amount of spearmint supplement gradually decreased as it was increased in all treated groups. The findings of this study seem to be closely related to those of Dalal et al. (2018), Alzawqari et al. (2016), and Yeasmin et al. (2023), who discovered that increasing levels of natural supplementation resulted in a reduction in serum cholesterol and achieved the best results with spearmint supplementation. They also observed that blood HDL (mg/dl) levels rose and decreased in response to increased spearmint supplementation amounts. The results seem to corroborate those of Aljumaily et al. (2019), who found that natural organic supplements, such as spearmint, recorded lower triglyceride levels than control; and Dalal et al. (2018), who found that serum triglyceride levels and LDL levels of spearmint supplements were similar to those of the control group. The authors are Saleh et al. (2014) and Abdel-Wareth and Lohakare (2014). Toghyani et al. (2010), Akbari, and Torki (2014) agree that spearmint supplementation affects the lipid profile of the chicken body.

Hematological parameters

Haematological properties Table.7 shows how spearmint affects broiler chicks. All the blood parameters were significant (P<0.05). According to Nobakht and Aghdam Shahriar (2011), Akbari and Torki (2014), and Rahimi et al. (2011), Rahim et al. (2012) found the same hematologically significant results as a supplement of spearmint.

Table 7. Effect of spearmint on hematological parameter

	Dietary Treatment Groups					
Hematological parameter	**T_0 (control)**	**T_1**	**T_2**	**T_3**	**T_4**	**Level of significant**
Hemoglobin (g/dL)	11.7 ± 0.11^{b}	12.3 ± 0.09^{c}	13.3 ± 0.06^{d}	10.8 ± 0.19^{a}	13.6 ± 0.17^{d}	*
ESR	4.0 ± 0.00^{b}	3.27 ± 0.18^{a}	6.27 ± 0.13^{d}	4.47 ± 0.24^{b}	5.13 ± 0.13^{c}	*
Total WBC count/L	9.5×10^{9} ± 0.2^{d}	9.2×10^{9} ± 0.2^{d}	7.8×10^{9}± 0.6^{c}	5.3×10^{9} ± 0.8^{b}	4.9×10^{9} ± 0.6^{a}	*
Neutrophil (%)	7.0 ± 0.12^{b}	5.73 ± 0.18^{a}	8.40 ± 0.20^{c}	7.20 ± 0.31^{b}	5.27 ± 0.13^{a}	*
Lymphocytes (%)	85.7 ± 0.75^{a}	89.07 ± 0.24^{b}	88.07 ± 0.75^{b}	84.2 ± 0.12^{a}	90.73 ± 0.07^{c}	*
Monocyte (%)	3.53 ± 0.27^{ab}	4.27 ± 0.27^{c}	3.20 ± 0.12^{a}	4.07 ± 0.07^{bc}	4.40 ± 0.23^{c}	*
Eosinophil (%)	2.80 ± 0.42^{ab}	3.07 ± 0.07^{ab}	2.40 ± 0.20^{a}	2.27 ± 0.13^{a}	3.53 ± 0.29^{b}	*
PLT (/L)	184 ×10^{9} ± 8.2^{be}	134×10^{9} ± 9c	175×10^{9} ± 6.6^{d}	129×10^{9} ± 2821.2^{b}	123×10^{9} ± 6.82^{a}	*
RBC (1x10^{6}/µL)	1.88 ± 0.08^{a}	2.51 ± 0.05^{c}	2.35 ± 0.01^{bc}	2.59 ± 0.23^{c}	2.06 ± 0.02^{ab}	*
PCV (%)	25.2 ± 0.07^{b}	27.6 ± 0.11^{c}	22.5 ± 0.09^{a}	25.12 ± 0.47^{b}	22.10 ± 1.23^{c}	*
MCV (fL)	122.6 ± 0.07^{c}	121.5 ± 0.07^{b}	118.5 ± 0.06^{a}	123.4 ± 0.04^{d}	121.52 ± 0.32^{b}	*
MCH (pg)	60.3 ± 0.06^{b}	57.7 ± 1.23^{a}	60.9 ± 0.34^{bc}	62.59 ± 0.36^{c}	57.73 ± 0.45^{a}	*
MCHC (g/dL)	49.3 ± 0.06^{b}	49.0 ± 0.07^{b}	51.3 ± 0.05^{d}	50.47 ± 0.02^{c}	47.79 ± 0.40^{a}	*
MPV (fL)	13.2 ± 0.06^{a}	14.2 ± 0.03^{c}	14.5±0.05^{d}	13.05±0.14^{a}	13.61 ± 0.12^{b}	*
PDW (%)	17.8 ± 0.18^{ab}	17.6 ± 0.34^{ab}	18.9±0.17^{c}	17.38±0.23^{a}	18.34 ± 0.08^{bc}	*
PCT (%)	1.39 ± 0.02^{b}	1.85 ± 0.00^{e}	1.65±0.01^{c}	1.74±0.02^{d}	1.26 ± 0.00^{a}	*
RDW SD (fL)	74.0 ± 0.22^{d}	53.4 ± 0.08^{c}	50.38±0.09^{a}	51.82±0.19^{b}	50.16 ± 0.07^{a}	*
RDW CV %	9.4 ± 0.05^{a}	10.5 ± 0.06^{b}	10.19±0.16^{b}	10.22±0.20^{b}	14.35 ± 0.19^{b}	*

a, b, c means having different superscript in the same row differed significantly (P<0.05). * = P<0.05 level of significance, NS=Non-significant

Table 8. Effect of spearmint on bacterial load count in faecal sample of broiler

Parameters		Dietary Treatment Groups					
		T_0 (control)	T_1	T_2	T_3	T_4	Level of significant
Faeces	*E. coli*	272.3 ± 10.34[a]	242 ± 1.56[b]	233.0 ± 14.72[c]	124.5 ± 8.43[d]	87.4 ± 10.48[e]	*
	Salmonella sp.	278.3 ± 17.25[a]	238 ± 1.28[b]	229.6 ± 12.18[c]	121.4 ± 8.21[d]	109.4 ± 4.96[e]	*

a, b, c, d, e means having different superscript in the same row differed significantly (P<0.05). * = P<0.05 level of significance, NS=Non-significant

Table 9. Effect of spearmint on economics of broiler production kept under different treatment groups from day old chick to 28 days of age

Parameters (Tk.)	Dietary Treatment Groups				
	T_0 (control)	T_1	T_2	T_3	T_4
Chick cost	35	35	35	35	35
Litter cost / bird	4.5	4.5	4.5	4.5	4.5
Vaccine and medicine	2	2	2	2	2
Feed cost / broiler production (BDT)	138.4 ± 4.61[a]	148.3 ± 3.28[c]	151.5 ± 5.06[d]	147.22 ± 5.32[c]	143.26 ± 5.14[b]
Dietary treatment cost/broiler production	0.00	2	3	4	5
Miscellaneous cost	5	5	5	5	5
Total cost/broiler	184.9 ± 3.74[a]	196.8 ± 5.66[c]	201.0 ± 6.04[d]	197.7 ± 5.08[c]	194.7 ± 5.00[b]
Average live weight/broiler (gm)	1376.9 ± 5.05[a]	1564.1 ± 6.18[b]	1729.9 ± 12.22[d]	1586.9 ± 7.34[bc]	1603.6 ± 5.23[c]
Sale price Tk./kg	160	160	160	160	160
Sale price / broiler	220.1 ± 15.5[a]	250.2 ± 12.54[b]	276.6 ± 13.54[c]	253.7 ± 16.04[b]	256.4 ± 10.24[b]
Net profit Tk./ broiler	35.2 ± 2.24[a]	53.4 ± 4.58[b]	75.5 ± 5.14[d]	56.0 ± 4.34[b]	61.7 ± 5.29[c]
Profit Tk./kg live weight	25.5 ± 2.74[a]	34.1 ± 2.52[b]	43.7 ± 3.85[c]	35.3 ± 3.68[b]	38.5 ± 3.48[b]
Profit compare Tk. between control	0	18.2 ± 1.5[a]	40.3 ± 2.34[c]	20.8 ± 1.74[a]	26.5 ± 2.64[b]

a, b, c means having different superscript in the same row differed significantly (P<0.05)

Effect of spearmint on bacterial load count

The Table.8 shows the effect of spearmint juice on microbial load count in faecal sample. The E. coli load was significantly (P<0.01) higher in T0 (272.33±10.34), followed by T1 (242 ± 11.56), T2 (233.00±14.72) , T3 (124.50±8.43) and T4 (87.48±10.48)respectively and Salmonella sp. load was also significantly (P<0.01) highest in T0 (278.33 ± 17.25) where as it was 238±10.28, 229.67 ± 12.18 , 121.43 ± 8.21 and 109.45±4.96 in T1, T2 and T3 and T4 respectively. It's possible that intermediate nutrition metabolism is connected to the stabilizing influence on gut flora (Jamroz et al., 2003). In addition, it has been proposed that spearmint oil may activate these glands and decrease bacteria, improving the hematobiochemical profile, digestibility, and FCR. Experimental studies have shown that the activity of peppermint essential oils inhibits the growth of Salmonella enteritidis, Candida albicans, Staphylococcus aureus, and Escherichia coli. Additionally, it has been suggested that giving spearmint to chickens can increase their consumption of feed, which will help their growth (Saleh et al., 2014; Abu Isha et al., 2018).

Cost-effectiveness of broiler production

The cost of producing various treatment groups of broilers is given in Table 9. As shown in Table. 9, the average raising expenses of broilers kept in treatment groups T0, T1, T2, T3, and T4 were 220.16, 250.24, 276.64, 253.76, and 256.48 Taka, respectively. The overall cost of miscellaneous expenses, which included labour, disinfection, and estimated electricity costs, were 5 Tk per broiler. The average live weight/broiler for groups T0, T1, T2, T3, and T4 was 1.376, 1.564, 1.729, 1.586, and 1.603 kg, respectively. The broiler was priced at Tk. 160/kg when sold at live weight. In the T0, T1, T2, T3, and T4 groups, the net profit per kg of live weight was revealed to be taka, 25.21, 34.14, 43.70, 35.33, and 38.50. The amount of spearmint employed in the diet has an impact on the broiler's profit margin. According to Zafar and Fatima (2018) and Yeasmin et al. (2023), poultry production benefits more from organic natural supplements. They have the purpose of reducing feed costs by lowering dose rates without compromising performance because they are more bioavailable and effective. Abdallah et al. (2009) claim that an organic mineral diet benefits the economy. It was discovered that replacing inorganic minerals with organic minerals improved bird performance and chick immune responses.

Conclusions

Therefore, Spearmint supplementation enhanced growth performance, decreased microbial loads, and improved hematobiochemical conditions (P< 0.05). With more 2 ml of spearmint, overall performance and quality were improved. We may conclude that including spearmint in broiler diets could assist in cost-effective and efficient broiler production. As a result, adding 2 ml of spearmint supplement during broiler production may be recommended.

Authors' contributions

This work was carried out in collaboration among all authors. Author MAJ designed the study, collected data, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors TY, US, NA, SHS, MAH, EA and MH managed the literature searches and given proper guideline. MDB and EA were performed microbial load count. All authors read and approved the final manuscript.

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Conflicts of interest

The authors declare that there are no conflicts of interest

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Study of prevalence and efficacy of antibiotics against foot and mouth disease in cattle at Kurigram district in Bangladesh

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ABSTRACT

A study was carried out to investigate the prevalence and efficacy of commercial antibiotics against the FMD virus in cattle in the char areas of the Kurigram district over a period of six months from October 2014 to March 2015. A total of 472 animals were monitored randomly on the basis of age, sex, breed, and season. More or less similar affected rates were recorded in male (12.68%) and female (12.73%) cattle. There was no statistically significant difference in the affected rate of FMD in indigenous cattle by age and sex. The affected rate in animals of different age groups ranged from 16.67% in cattle up to 12 months of age, 13.33% in 13 to 26 months, 12.83% in 27 to 45 months, and 12.22% in 46 to 60 months age groups in cattle. The affected rate of FMD increased gradually from (7.69) % in October to (9.72) % in November to (16.00) % in December with a peak of (18.88) % in January and then gradually decreased to (15.00) % in February to (7.78) % in March. The affected rate on the basis of breed for indigenous animals was (12.30) % and the cross-breed animals were (13.00) %. Some commercial antibacterial drugs were used to evaluate their efficacy against secondary bacterial infections in the foot-and-mouth disease-affected cattle. Efficacy of commercial antibiotic treatment was observed in four groups of animals. The Amoxicillin for group A, combined Penicillin+Streptomycin for group B, Oxytetracycline for group C, and soda, potassium permanganate, suhaga, and honey for group D. Efficacy results of these antibacterial drugs were compared among the treatment groups on the basis of complete recovery from clinical signs and healing of foot lesions in days required. It was observed that the efficacy of the antibiotic above the mentioned treated group treated with the antibiotic Amoxicillin in group A showed statistically significant results to recover from the foot-and-mouth disease than the other groups B, C, and D.

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Introduction

Livestock is an important sub-sector in Bangladesh, considered to the backbone of agriculture. According to the Department of Livestock Service, Bangladesh loses as much as US$125 million annually due to FMD. In Bangladesh, 20 million households keep 23 million of cattle heads under traditional farming system. The density of livestock population per acre of cultivable land is 7.37. Foot-and-mouth disease (FMD) is endemic in Bangladesh and is predominantly due to FMDV serotype O (Rahman et al., 1985). In 2012, FMD outbreaks were identified in five different districts of Bangladesh (Nandi et al., 2015). Foot and Mouth Disease (FMD) is an important viral disease of various animals. Foot and Mouth Disease (FMD) is a contagious viral disease affecting cloven-hoofed animals, causing fever and vesicular eruption in the mouth, muzzle, foot, and other soft areas (Chowdhury et al., 1994). Foot and Mouth Disease (FMD) has constituted a major threat to the health of livestock for at least 450 years worldwide (Brooksby,1958).The

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highest number of outbreaks occurs among beef cattle farms, followed by feedlot farms and dairy farms (Elnekave et al.2016).The economic losses due to FMD occurs in many ways, mainly loss of production, the expense of control, the interference with movement of animal and animal products (milk, meat) at international levels (Ham-m-van et al., 1994) although the disease is not a killing one (the mortality rate in adults 2% and in young stock 20%) but production (meat and milk) and draught power are seriously impaired in acute cases (Blood and radostits, 1989). In 2010 and 2011, incursions of the FMDV O/SEA/Mya-98 strain, normally restricted to countries in mainland Southeast Asia, caused extensive outbreaks across East Asia (Valdazo-González et al.2013).It is a highly infectious febrile disease of cloven hoofed animals, and it is endemic in Indo-Bangladesh sub- continent widespread outbreaks occur throughout the sub-continent in different seasons (Kamaruddin and Pandit, 1988; Sarma and Hazarika, 1996). A number of factors like migratory animals, fodder, wild animals, newly purchased animals, strong wind and rain, flooding necessitating movements of large number of animals and personnel have been recognized to the possible sources of FMD infection (Ray et al., 1989 a,b ; Ekue et al., 1990). Unfortunately, this has not yet been achieved in Bangladesh due to lack of sufficient epidemiological information on the disease. Moreover, the uncontrolled movements of animals, lack of awareness in reporting the disease as well as lack of systematic procedures for mass vaccination of animals complicate the epidemiology of FMD in Bangladesh. This study justifies the economic impact of FMD including how it varies in different settings and how knowledge of this should be used to guide control policy. This included a synthesis of current literature on the subject. To help appreciate the scale of global FMD impact estimates were made of the direct costs of disease and vaccination in endemic countries as well as outbreak costs in free countries. The findings of this study also emphasis the formulation of more effective disease management and control strategies, including appropriate vaccination policies in Bangladesh. A detailed clinico-epidemiological investigation was undertaken with a view to study the following objectives. To determine the affected rate of Foot and Mouth Disease in cattle on the basis of sex, age, month and breed. To evaluate some antibacterial drugs to control secondary infections in clinical cases of Foot and Mouth Disease.

Material and Methods

Study areas

The study was conducted in the Fander char, Messerer char, and Krishnopur char of the Ballaver ghas union of the Nageshwari upazila of the Kurigram district. These chars were under a project named Chars Livelihoods Programme (CLP). These chars are situated on the riverside of Gangadhar. It is 40 kilometres away from Sadar Kurigram, Rangpur, Bangladesh.

Study period and animals

The study was conducted at different chars of the Kurigram district from October 2014 to March 2015. The animals were examined at the household to study the prevalence on the basis of chars, age, sex, month, and breed and to study the efficacy of antibiotics against FMD in cattle. Funder char, Messerer char, and char Krishnopur, respectively, 170, 120, and 182 numbers of the cattle population were recorded in three chars during the study period. Among which, 60 animals were affected with FMD.

Epidemiological study

As a livestock officer for the Chars and Livelihoods Program, researchers frequently observed and inspired beneficiaries while also advising them and providing solutions to animal-related difficulties. As a result, epidemiological data were gathered from that observation and from the beneficiaries through a review of their case histories and a clinical examination. Data on age, gender, and month of occurrence were recorded using procedures specified by Prasad et al. (1981) and Singh et al. (1981). Polymerase chain reaction (PCR) and reverse transcription polymerase chain reaction (RT-PCR) are molecular diagnostics that can detect the presence of FMD in clinical specimens (Dubie & Amare,2020).

Clinical examination

Body Condition Score (BCS), behavior, posture, gait, ulcer in mouth and tongue, salivation, anorexia, red lesion hooves and interdigital space, locomotive disturbance were observed by distant visual examination of the patient. Examination of different physical parts of the body of each of the animal clinically attend at char were done by using various close observation techniques. The general clinical examination was performed to determine the posture, behavior, gait, and physical condition of each of the FMD-affected animals. The inspection and palpation were used to examine the mouth and foot. The tongue was checked for injury, ulceration, vesicles, abnormal mobility, and consistency. The foot of each animal was examined on inspection and palpation to detect any lesion on the interdigital spaces and to find the unwillingness to take weight on the limbs.

Prevalence

Prevalence was calculated as number of cases of disease divided by population at risk and multiple by 100.

$$\text{Prevalence} = \frac{\text{Number of disease cases}}{\text{Population at risk}} \times 100$$

Table1. Sex wise prevalence of Foot-and-mouth disease in cattle in Nageshwari upazila

	Male		Female		Total	
		Affected cases (number and %)	Number of animals examined	Affected cases (number and %)	Number of animals examined	Affected cases (number and %)
Funder	75	10 (13.33 %)	95	13 (13.68 %)	170	23 (13.54 %)
Messerer	50	6 (12.00%)	70	8 (11.43 %)	120	14 (11.66 %)
Krishnopur	80	10 (12.50 %)	102	13 (12.74 %)	182	23 (12.63 %)
Total	205	26 (12.68 %)	267	34 (12.73 %)	472	60 (12.71 %)

Grouping of animals for antibiotic therapy

Antibacterial are known to have no effect on the agent in viral infection. The use should be favored for the prevention of subsequent bacterial infections or when a bacterial infection progresses alongside the viral agent. Group A: Consisting of 15 FMD-affected animals, and each animal was treated with amoxicillin (Moxacil vet) at 8-10 mg/kg body weight, i/m ly sid. Group B: Consisting of 15 FMD-affected animals, and each animal was treated with penicillin + streptomycin (SP vet) combined as 40,000 IU/kg body wt. + 15 mg/ kg body wt. i/m ly sid. Group C: Consisting of 15 FMD-affected animals, and each animal was treated with oxytetracycline (Renamycin) at 5-8 mg/kg body weight i/m ly sid. Group D: Control group consisting of 15 animals, and each animal treated by KMnO4, soda, suhaga (sodium borate), honey, etc. no antibiotics were applied. The therapeutic evaluation was assessed on the basis of days required for complete healing of mouth and foot lesions as described by Rahman et al. (1985).

Monitoring the antibiotic treated results

The Chars Livelihoods Programme selected a Livestock Service Provider (LSP) to assist in every respective char to treat the animals. Sometimes they were provided training about the treatment and the management of animals from the project. Whenever any problem or symptom was observed by beneficiaries, they communicated with LSP, and medicine would be given. Especially the efficacy of different antibiotics against FMD was recorded.

Statistical analysis

The data obtained for different characters were statistically analyzed following the one way ANOVA of SPSS version.25. The significant differences among the treatment means were compared by Duncan's Multiple Range Test (DMRT) at 5% level of probability (Gomez and Gomez, 1984).

Results

Epidemiological investigation

The major outbreak of Foot-and-mouth (FMD) was recorded in the month of November-December 2014 at the Fander char and Messerer char and in the month of January 2015 at the char krishnopur.

The affected rate of FMD on the basis of sex and age is presented in Tables 1 and Figure 1, respectively. It is evident from Figure 1 that all age groups of indigenous cattle are susceptible to FMD. More or less similar affected rates were recorded in male (12.68%) and female (12.73%) cattle (Table 1). The affected rate in animals of different age groups ranged from (14.29) % in cattle up to 12 months of age, (13.33) % in 13 to 36 months, (12.57) % in 37 to 55 months, and (11.87) % in 56 to 72 months age groups in cattle. There was no statistically significant difference in the affected rate of FMD in indigenous cattle by age and sex. This study was conducted over a six-month period only due to the availability of clinical cases under these areas, and the month-wise occurrence of FMD in indigenous cattle is presented in Table 2.

It appears from Table 2 that the affected rate of FMD increased gradually from (7.69) % in October to (9.72) % in November to (16.00) % in December with a peak of (18.88) % in January and then gradually decreased to (15.00) % in February to (7.78) in March.

Table 2. Month-wise prevalence of FMD disease in cattle in the chars of Nageshwari upazila.

Month	Number of animals	Animals affected
October	65	5 (7.69 %)
November	72	7 (9.72 %)
December	75	12 (16.00 %)
January	90	17 (18.88 %)
February	80	12 (15.00 %)
March	90	7 (7.78 %)
Total	472	60 (12.71 %)

Table 3. Breed wise prevalence of FMD in Nageshwari upazila.

Breed name	Number of cattle	Animals affected
Indigenous animals examined	325	40 (12.30 %)
Cross breed animals examined	147	20 (13.00 %)
Total	472	60 (12.71 %)

Clinico-pathological investigation

Most of the cattle affected with foot-and-mouth disease showed high rectal temperature (103 to 105) F accompanied by severe dejection and anorexia, followed by painful stomatitis. The affected animals showed abundant salivation and saliva hanging in long, ropey strings(fig.1A). Vesicles appeared on the buccal mucosa on the tongue and gum, which ruptured to form ulcers (fig.1B). Concurrently with the oral lesions, lesions appeared on the feet, particularly on the clefts and the coronet/interdigital space (fig.1C). The clinical sings of FMD were found more severe in young calves than adults.

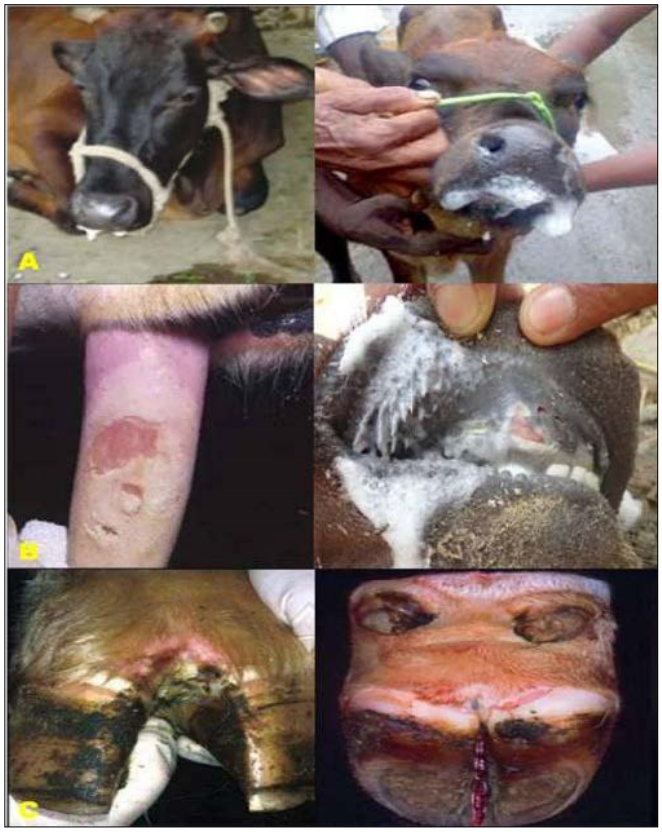

Figure 1. (A) Salivation and saliva hanging, (B) Buccal mucosa, tongue and gum which ruptured to form ulcer, (C) Lesions appeared on the feet (cleft).

Monitoring the therapy result

Some commercial antibacterial drugs available in the Kurigram were used to evaluate their efficacy against secondary bacterial infection in the foot-and-mouth disease affected cattle that is shown in the Table 4.

Group A received amoxicillin per company instructions; Group B received a combination medication such as penicillin + streptomycin per company instructions; Group C received oxytetracycline per company instructions; and Group D received soda, potassium permanganate, suhaga, and honey. Complete recovery from clinical signs and the number of days needed for foot lesions to heal were used to compare the effectiveness outcomes of these antibacterial medications across treatment groups. Antibiotics were shown to be more effective than the group that was treated; foot lesions and clinical symptoms treated with amoxicillin, penicillin + streptomycin, oxytetracycline, and supportive care all recovered in varying amounts of time. Amoxicillin, the antibiotic used to treat foot and mouth illness, produced statistically significant recovery outcomes in group A.

Discussion

Epidemiological investigation

The major outbreaks of FMD were recorded in higher numbers in cattle in the chars of Nageshwari upazila in the month of January 2015 than in any other month of the year. Epidemiological investigation of 472 randomly selected cattle in the outbreak areas revealed that only 60 (12.71%) animals were clinically affected with FMD, of which 2 (3.33%) severely affected calves died. The morbidity, mortality, and case fatality were (12.71%, 0.42%, and 3.33%), respectively. Although the occurrence of FMD outbreaks has been reported to be high in cattle in the district of Tangail (average 8 outbreaks/year), the information on morbidity, mortality, and case fatality rates is lacking in inland literature (Kamaruddin and Pandit, 1988). However, comparatively higher morbidity (48 & 59.5%), mortality (7.2% & 1.8%), and case fatality (12.09%) rates have been reported in Indian cattle due to FMD (Patnaik, 1986; Ray et al., 1989). These variations in morbidity, mortality, and case fatality rates might be due to differences in breed, age, types of virus involved, and annual

Table 4. Grouping of affected animals for therapeutic evaluation of antibacterial drugs against Foot-and-mouth disease in cattle.

Group	Number of animal	Name of antibiotic	Dose and route of administration	Recovery range in days (Mean ± SE)	Level of Significance
A	15	Amoxicillin	8-10mg/kg body wt. i/m ly.	5.2 ± 0.14^{a}	1.01*
B	15	Penicillin+Streptomycin	40,000iu/kg body wt. + 15mg/ kg body wt. i/m ly.	7.1 ± 0.23^{b}	
C	15	Oxytetracycline	5-8 mg /kg body wt. i/m ly.	7.5 ± 0.19^{b}	
D	15	Supportive agents	Soda, suhaga, potassium permanganate and honey	10 ± 0.25^{c}	

One-way ANOVA, Values are expressed as mean ± standard error of means (SEM). NS: Statistically not significant (P>0.05). a b c d means having different superscript in the same row differed significantly (P<0.05), * indicates 5% level of significance.

vaccination programs against FMD. Singh et al. (1981) reported a higher morbidity rate due to FMD in exotic and cross-bred cattle than in indigenous breeds. Blood and Radostits (1989) described only a 2% mortality rate in adult cattle due to FMD and 20% in young stock. It is revealed from the review of literature that types A, O, C, and Asia-1 and subtypes A5 and A22 have been identified in India in association with outbreaks (Jana et al., 2023).

Although the O, A, C, and Asia-1 types of FMD virus have been identified from Bangladesh (Kamaruddin and Pandit, 1988; Rahman et al., 1991), recently subtypes A and A have been identified in association with outbreaks (Chowdhury et al., 1994a, b). These subtypes of the FMD virus might spread from India to Bangladesh through migratory cattle. It may be mentioned here that cattle are not usually allowed to be slaughtered for meat purposes in India due to mainly religious causes (Ghosh, 2019). As a result, a large number of slaughterable cattle are being exported from India to the neighboring countries like Bangladesh. This export and import business of cattle from India to Bangladesh is mainly made by the private sector without considering the health and disease of animals. As a result, different diseases and infections are being introduced in Bangladesh through diseased cattle. Islam investigated (2001) 336 imported Indian cattle and found most of the imported cattle had clinical and subclinical infection, of which 44.34% had clinical infection with FMD. This indicates that the affected rate of FMD is higher in imported Indian cattle (44.34%) than in the local cattle (12.71%) in the Nageshwari chars. Therefore, the importation of Indian cattle should be restricted with necessary quarantine and health examination measures. However, Kamaruddin and Pandit (1988) reported that the occurrence of FMD varied from month to month and season to season, with higher frequency due to the movement of infected animals for different purposes. The insignificantly higher affected rate of FMD has been reported in female than male pigs (Shankar, 1992). However, the severity of infection with mortality was recorded in young stock as had been reported by Blood and Radostits (1989), who reported 2% mortality in adults and 20% in young calves due to FMD. The study found that all age groups of cattle are affected by FMD, with mortality rates recorded in young stock. There was no difference in affected rates between male and female animals, but females had a higher rate. The clinical causes of FMD appeared in all months, with the affected rate increasing from October to January and decreasing from February to March. The disease was more prevalent during winter and monsoon and could be spread through cattle fairs, markets, grazing areas, and the movement of infected animals (Jana et al., 2023). Although the occurrence of FMD has been reported in different species of domestic and wild animals elsewhere (Aslam & Alkheraije, 2023.; Hafez et al., 1993), similar reports have not been documented from Bangladesh. Therefore, the epidemiological role of different animal species in the spread of the infection should be explored in this country.

Clinico-pathological investigation

During the study period, the clinical signs were examined carefully in the affected animal, and the symptoms were recorded. The first clinical symptoms in FMD-affected cattle were salivation, vesicles and ulcers in the mouth, tongue, and gums, and also in the interdigital space of the hoof; lameness, pyrexia, and anorexia. In uncomplicated cases, the oral vesicles and ulcers usually healed within 8 to 10 days either by gradual replacement of the epithelium or after scab formation. In complicated cases, the courses of the disease ranged from 12 to 15 days, and most of the cases are complicated with secondary bacterial infection. The clinical pictures resembled very closely those described by Sard (1978) and Blood and Radostits (1989). However, skin lesions (Sen, 1990), eye and udder lesions with mastitis (Singh et al., 1981, Govindaraj et al., 2021), atypical lesions, and allergic reactions (Jana and Mailty, 1997) reported in the literature have not been observed in this study. Necropsy examination of dead calves revealed atypical lesions of myocardial degeneration, necrosis, and non-suppurative myocarditis, which, though not pathognomonic, are believed to be causes of death (Jones and Hunt, 1983). In addition, there were associated lesions of acute abomastitis, enteritis, and pneumonia.

The effect of antibacterial drugs against FMD

Some commercial antibacterial drugs available in Kurigram were used to evaluate their efficacy against secondary bacterial infections in the foot-and-mouth disease-affected cattle. Efficacy results of antibacterial drugs were compared among the treatment groups on the basis of complete recovery from clinical signs and healing of foot lesions in days required. It was observed that the efficacy of the antibiotic-treated group, that the clinical signs and foot lesions treated with amoxicillin, penicillin + streptomycin, oxytetracycline, and supportive treatment recovered within different days. The treating antibiotic amoxicillin in group A showed statistically significant results in recovering the foot and mouth disease compared to the other groups B, C, and D. The healing of untreated control cases generally required 10 to 15 days, with an average of 12 days, and in some cases, it became more complicated. These results could not be compared due to a lack of similar reports in the available literature. The results indicate that the administration of the drug would be required for rapid healing of FMD lesions and to prevent the spread of the disease to other susceptible animals and neighboring villages. However, Kilner (1994) reported that cattle fed with grain treated with caustic soda and Gangopadhya et al. (1990) and Chowdhury et al. (2020) reported that levamisole and zinc salt have some antiviral activity against FMD virus.

Conclusion

A total of 472 cattle, regardless of sex, different age groups, month, and breed, were investigated carefully, and outbreaks of FMD in those chars' areas revealed that 60 (12.71%) animals were clinically affected with FMD, and the 412 animals were found apparently normal or healthy. The affected rate was also recorded sex-wise, but there was no significant difference in the affected rate between male (12.68%) and female (12.73%) animals. The affected rate was recorded monthwise, and the affected rate was (7.69%)

in October, (9.72%) in November, (16.00%) in December, (18.88%) in January, (15.00%) in February, and (7.78%) in March, respectively. In Bangladesh, November to February is winter season, and peak cold weather is in December-January, and animals are moved to high ground and close herding, so the affected rate is high. The affected rate in Indigenous animals and crossbreed animals was, respectively, 12.30% and 13.00%, which were non-significant. It was observed that the efficacy of antibiotics among the four treated groups with amoxicillin, penicillin + streptomycin, oxytetracycline, and supportive treatment recovered within different days. The treating antibiotic amoxicillin in group A showed statistically significant results in recovering from foot and mouth disease compared to the other groups B, C, and D. So, this study recommends that amoxicillin is better than the combined effect of penicillin and streptomycin for foot and mouth disease. But further research is needed for better recommendations.

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Conflicts of interest

The authors declare that there are no conflicts of interest.

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Organic and inorganic minerals in Sonali chicken diets: Effect on growth performance and meat quality

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Research Article

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ABSTRACT

Background: This study investigated the effects of inorganic minerals and organic trace minerals supplementation on the production and carcass characteristics of Sonali chickens. Materials and methods: A total of 180 Sonali chicks (RIR and Fayoumi cross) were randomly divided into 4 experimental groups, each group occupying 3 replications. Dietary groups were considered as control (T0), organic trace minerals at 100 g/100 kg feed (T1), inorganic minerals at 250 g/100 kg feed (T2), and organic trace minerals at 100 g/100 kg feed + inorganic minerals at 250 g/100 kg feed (T3), respectively. Weekly body weight and feed intake data were taken to 60 days. Results: Initial body weight was not significantly different among the experimental groups ($P > 0.05$). But the final body weight was significantly different among the experimental groups ($P < 0.05$), with the highest value recorded at T3 (857.67 g). Total body weight gain was significantly different among the experimental groups ($P<0.05$) and was the highest value recorded in the T3 group (833.99 g). Total feed intake was non-significant among the experimental groups. Feed conversion ratios differed significantly among the experimental groups ($P<0.05$), with the lowest but best FCR found in the T3 group (1.96). Daily live weight gain was significantly different among experimental groups ($P< 0.05$). Carcass weight, breast, thigh, and drumstick weights were significantly different among the dietary groups ($P< 0.01$). Faecal ash (17.33%) and calcium (2.10%) secretion was lower in the organic trace minerals(T1) group than in the inorganic minerals(T2) group as well as the combined group, but phosphorus secretion (2.65%) was lower in the combined(T3) group than in the organic trace minerals group and the inorganic minerals group. The experimental groups differed considerably ($P <0.01$) in net profit, with T3 having the highest value (39.47 Bangladeshi taka) and T0 having the lowest (17.04 BDT). The benefit over control differed considerably ($P< 0.01$) among the experimental groups, with the highest value in the T3 group (22.43 BDT) and the lowest in the T2 group (6.89 BDT). Conclusion: So, Sonali commercial farmers may combine organic trace minerals and inorganic minerals with basal feeds to increase Sonali chicken production efficiency and profit.

Keywords: organic trace mineral, inorganic mineral, productive performances, carcass characteristics.

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Introduction

Bangladesh's poultry sector, a key part of the country's economy, is a significant contributor to the country's GDP(Gross domestic product). Sonali chicken, a hybrid of Rhode Island Red and Fayoumi chickens, is gaining popularity due to meats unique aroma, taste, and texture. Despite limited herd size and yield, Sonali chickens are two to three times more expensive than commercial chickens. Traders can sell Sonali chicken at a higher price than broiler (Hasan, 2019). Sonali chicken consumption increased 45% in July 2019 compared to 20% in July 2018 (Hasan, 2019). There are two forms of trace elements available for birds: inorganic and organic. Inorganic trace elements such as oxides, chlorides, carbonates, and sulphates have

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long been used in poultry feed (Aksu et al., 2011). The efficacy of minerals is an important issue in modern poultry nutrition. Microelements are essential for normal growth and many metabolic processes in living organisms, as they are catalysts or constituents of the enzymatic systems of many cells (Swiatkiewicz et al., 2014). Inorganic trace minerals (ITM) are unstable and rapidly dissociate in the gastro-intestinal tract and interact with other compounds, leading to their loss before absorption (Aksu et al., 2011). Owing to the low digestibility of ITM, higher levels of dietary ITM are supplemented to meet the requirements of birds (Yan and Waldroup, 2006; Mezes et al., 2012), which eventually increase the cost of production and environmental problems. These problems could be overcome by substituting ITM with organic trace minerals (OTM). OTM is more stable due to its organically bound structure that is better digested and absorbed in the intestine (Sandoval et al., 1998), which in turn increases their bioavailability (Bhoyar, 2015) and consequently reduces the faecal and urinary excretion (Wang et al., 2019). Trace elements such as Cu, Fe, Mn, and Zn are essential for growth and are involved in many digestive, physiological, and biosynthetic processes in the body. Organically complexed zinc has been found to be more bioavailable and more beneficial to birds than the inorganic form (Salim et al., 2011). In poultry farming, these compounds are often used in excess of 2 to 10 times the amounts recommended by the National Research Council to prevent trace element deficiencies and promote bird growth (Esenbu et al., 2008). However, higher concentrations of trace elements, including copper, in diets can affect the bioavailability of other elements (Wapnir, 1998). High levels of confinement result in mineral waste and environmental pollution due to excessive bird excretion (Leeson, 2003). For example, 94% of absorbed zinc is excreted into the environment and can cause phytotoxicity in soil (Zafar et al., 2018). Excretion of phosphorus can lead to eutrophication. Therefore, the purpose of this study was to investigate the effects of organic trace minerals and inorganic minerals on Sonali birds' meat quality and bedding quality in reducing mortality and examining faecal material to determine mineral excretion. Objectives of the experiment was:To determine the effects of organic trace elements and inorganic minerals on the productive performance of sonali chicken. To investigate the effects of organic trace elements and inorganic minerals on the carcass characteristics and assess cost effective benefit of Sonali chicken. To determination the mineral excretion by fecal material examination of sonali chicken.

Materials and Methods

Location of study

The experiment was conducted at a poultry farm in the campus of Hajee mohammad Danesh Science and Technology University, Dinajpur. The experiment was conducted from February to April 2022. The ethical committee of the Institute of Research & Training (IRT), HSTU(HSTU/IRT/112/2021-2022).

Experimental Sonali birds

A total of 180-day-old Sonali chicks were purchased from Polli Chicks Limited in Sadar, Dinajpur, Bangladesh. The experimental bird was healthy and disease-free. Adequate lighting, proper ventilation, and heating were ensured for up to 7 days of brooding and the rest of the days up to 60. There were 4 experimental groups, each group occupying 3 replications.

Design of the experiment

The experiment was conducted in complete randomized design (CRD). The day-old sonali chicks were kept in brooder house for up to 7 days to adapt with the environmental conditions. A total of 180 chicks were randomly divided into 4 experimental groups, each group occupying 03 replications. Dietary groups were considered as control (T0), Organic trace minerals (A-vaila Z/M) at 100 gm/ 100 Kg feed (T1), Inorganic mineral (Rena grower premix) at 250 gm/ 100 Kg feed (T2) and Organic trace minerals (A-vaila Z/M) at 100 gm/ 100 Kg feed+ Inorganic mineral (Rena grower premix) at 250 gm/ 100 Kg feed (T3) respectively. The A -vaila Z/M contains zinc amino acid complex and manganese amino acid (Association of American Feed Control officials, AAFCO), and Rena grower powder contains vitamins & minerals. On the last day of the experiment, for each replication, 2 birds were slaughtered.

Collection of experimental materials

Basal feed was collected from the local market of Dinajpur town. Feed was supplied in two phases as starter diet and finisher diet. Inorganic mineral (Rena Grower premix) and Organic trace minerals (A-vaila Z/ M) were collected from local market of Dinajpur town and stored in a plastic bag on a clean dry place and normal room temperature.

Preparation of the experimental diet

The ration formulation was done for chicken feed. Feed ingredients were mixing properly by using hands for the preparation of basal feed. Digital weight balance was used to weight feed ingredients. The experimental period was divided into three phases (Brooding, Sonali-starter and Sonali–finisher). The sonali chicks were fed ready feed (package commercial feed) in brooding

period, sonali starter for 8 to 20 days and sonali finisher for 21 to 60 days of age. Control group (T0) was supplied formulated feed without organic and inorganic trace minerals. Treatment group (T1) was supplied with 100 g organic trace minerals with 100 kg basal feed, (T2) was supplied with 250 g inorganic minerals with 100 kg basal feed and (T3) was supplied with both 100 g organic trace minerals as well as 250 g inorganic minerals with 100 kg basal feed. Before mixing the trace minerals. This experiment was used to prepare the basal feed by mixing all the ingredients (Table no.1) properly.

Table.1. Composition of basal diets

Ingredient	Starter diet (Days 8 to 20)	Finisher diet (Days 21 to 60)
Maize	58 kg	58 kg
Rice polish	10 kg	10 kg
Soyabean meal	21 kg	20 kg
Soyabean oil	500 g	1 kg
Molasses	500 g	500 g
Fish Meal	6.5 kg	6 kg
Lime Stone	1.5 kg	2 kg
DCP	700 g	1 kg
Methionine	100 g	120 g
Lysine	50 g	80 g
Vitamin Premix	350 g	350 g
SQ Zyme SME	100 g	100 g
Hemeco Sal Dry Powder	200 g	200 g
Turbo Tox	100 g	100 g
Bicarbonate	150 g	150 g
Amprium vet powder	50 g	50 g
Salt	200 g	350 g
Total	100 kg	100 kg

Hemeco Sal Dry Powder = blood meals, Turbo Tox = toxin binder, Amprium vet powder = anti coccidiosis

Managemental practices

Housing, litter, feed (Naurish Feed Co. Ltd. Sonali starter: 1–25, Sonali grower: 25-60 days), water, lighting, sanitization, and vaccination (Table 2) are all necessities. Adequate precautions were implemented throughout the study period.

Performance traits

1. Total gain in weight = final weight – initial weight. 2. Dressing percentage = (dressed weight ÷ body weight) x 100. 3.Total feed consumption = total feed offered – total left-over. 4. Feed efficiency = total feed consumed / total gain in weight. 5. Mortality rate (%) = no. of dead chickens / total no. of birds as a group × 100.

Statistical analysis

Data was analyzed with a one-way ANOVA, using the General Linear Models (GLM) procedure of the SAS (SAS, 1994) with the help of SPSS (Version 25.0). Duncan`s Multiple Range Test option of the SAS was used to separate significant differences between means and data symmetrically distributed. All results were considered significant at the 1 and 5% level of significance ($P<0.05$).

Results

Live weight (g)

At the start of the experiment, the average body weight of the birds did not differ significantly among the treatment group. The live weight of birds in 7th and 14th days of age did not significantly ($P>0.05$) vary among the treatment groups (Table. 3). At 21st day, the body weight was significantly different ($P<0.05$) where the highest values were found in T3 (199.00±2.08) and the lowest value was found in T0 (182.33±2.91). The body weight was significantly ($P<0.01$) among the treatment groups during the 28th, 35th, 42th, 49th, and 56th days of age. Final live weight (60th days of age) was also significantly different ($P< 0.01$) among the dietary groups. Feed supplemented with Organic trace minerals + Inorganic mineral among the birds showed the highest body weight T3 (857.67±4.06) and the lowest body weight was found in control group T0 (741.00±2.08). The T1 group had (806.33±3.84) and T2 had (782.00±4.93).

Live weight gain (g)

The live weight gain of the experiment is presented in Table 4. From the 1st to the 4th weeks of the trial period, this result demonstrated a non-significant difference in the average weight gain of the birds between the treatment groups ($P>0.05$). From the table, it is found that from the 5th week to the 8th week and at the 60th day of sonali, the body weight gain was statistically significant ($P<0.05$). The organic trace minerals + inorganic minerals -supplemented group T3 (833.99±20.66) showed the highest final live weight gain, and control group T0 (717.33±19.4)

Table 2. Vaccination

Name of vaccine	Diseases	Age (Days)	Time	Route
ND+IB	New Castle & Infectious Bronchitis	4th	Evening	Eye drop
IBD	Gumboro	12th	Evening	Eye drop
IBD	Gumboro	17th	Evening	Drinking water
ND+IB	New Castle & Infectious Bronchitis	22th	Evening	Drinking water
ND	New Castle	42th	Evening	Drinking water

Source: Vaccine, prepared by ACI pharmaceutical company was applied as per recommendation of the manufacturer.

Table 3. Effect of dietary supplementation of organic trace minerals and Inorganic mineral on live weight (g) in different treatment groups of Sonali birds.

Live weight (g)/ bird	Treatment groups				Level of significant
	T_0	T_1	T_2	T_3	
Initial body weight	24.67 ± 0.33	24.33 ± 0.33	24.67 ± 0.33	24.67 ± 0.33	NS
7th Days	69.00 ± 0.58	69.00 ± 0.58	69.00 ± 0.58	69.67 ± 0.33	NS
14th Days	122.67 ± 1.76	129.67 ± 1.45	126.67 ± 0.88	131.00 ± 0.58	NS
21st Days	182.33 ± 2.91^{a}	194.33 ± 1.33^{bc}	190.00 ± 1.53^{b}	199.00 ± 2.08^{c}	*
28th Days	282.67 ± 2.33^{a}	301.00 ± 3.46^{bc}	294.00 ± 1.73^{b}	309.33 ± 2.60^{c}	**
35th Days	373.67 ± 1.76^{a}	400.67 ± 5.36^{c}	388.67 ± 1.45^{b}	419.67 ± 4.06^{d}	**
42th Days	476.33 ± 2.40^{a}	512.33 ± 5.33^{c}	494.67 ± 2.85^{b}	541.33 ± 5.90^{d}	**
49th Days	574.67 ± 1.86^{a}	626.67 ± 4.33^{c}	602.67 ± 6.89^{b}	665.67 ± 3.53^{d}	**
56th Days	679.00 ± 3.06^{a}	738.67 ± 4.91^{c}	717.67 ± 6.12^{b}	792.67 ± 2.96^{d}	**
60th Days	741.00 ± 2.08^{a}	806.33 ± 3.84^{c}	782.00 ± 4.93^{b}	857.67 ± 4.06^{d}	**

Values are expressed as mean ± standard error of means (SEM). NS: Statistically not significant (P>0.05). a b c d means having different superscript in the same row differed significantly (P<0.05)*indicates 5% level of significance.**indicates 1% level of significance.

Table 4. Effect of dietary supplementation of organic trace minerals and inorganic minerals on live weight gain (g) in different treatment groups of Sonali birds

Live weight gain (g)/bird	Treatment groups				Level of significant
	T_0	T_1	T_2	T_3	
7th Days	44.33 ± 0.33	44.67 ± 0.33	44.33 ± 0.67	45.00 ± 0.58	NS
14th Days	55.67 ± 2.33	60.67 ± 1.20	57.67 ± 1.33	61.33 ± 0.67	NS
21st Days	63.67 ± 2.67	64.67 ± 1.86	63.33 ± 0.88	68.00 ± 2.00	NS
28th Days	100.33 ± 2.60	106.67 ± 2.40	104.00 ± 1.15	110.33 ± 4.26	NS
35th Days	91.00 ± 3.51^{a}	99.67 ± 3.84^{a}	94.67 ± 1.45^{a}	110.33 ± 1.45^{b}	*
42th Days	102.67 ± 3.53^{a}	111.67 ± 1.45^{b}	105.33 ± 1.45^{ab}	121.67 ± 2.33^{c}	*
49th Days	98.33 ± 2.03^{a}	114.33 ± 2.85^{bc}	110.00 ± 4.58^{b}	124.33 ± 3.48^{c}	*
56th Days	104.33 ± 1.45^{a}	112.00 ± 0.58^{ab}	115.00 ± 1.15^{b}	127.00 ± 4.36^{c}	**
60th Days	60.00 ± 1.00^{a}	64.67 ± 0.88^{b}	63.00 ± 1.00^{b}	67.00 ± 1.53^{c}	*
(1-60th) Days	717.33 ± 19.45^{a}	782.02 ± 15.39^{c}	757.33 ± 13.66^{b}	833.99 ± 20.66^{d}	*

Values are expressed as mean ± standard error of means (SEM). NS: Statistically not significant (P>0.05). a b c d means having different superscript in the same row differed significantly (P<0.05)*indicates 5% level of significance.**indicates 1% level of significance.

Feed intake (g)

The effect of organic trace minerals and inorganic minerals on feed intake of Sonali birds is presented in Table 5. It was observed that at 7th day and 14th day of age, the feed intake was not significantly different (P > 0.05) among the treatment groups. The feed intake from 21st days to 60th days of age significantly differed (P<0.05) among the dietary treatment groups. But the total feed intake of 60 days was statistically non-significant (P>0.05). Total feed intake was lowest in dietary group T0 (1561.99±19.61) and highest in dietary group T3 (1634.32±14.28).

Feed conversion ratio (FCR)

The Feed Conversion Ratio (FCR) of the experimental birds is shown in Table 6. At 7th to 28th days of age, the FCR was non-significant (P > 0.05) in different treatment groups. A significant difference (P<0.05) was found among the treatment groups from 35th to 60th days of age. The lowest FCR was found in dietary treatment group T3(1.96±0.69) and the highest in dietary group T0 (2.18±1.01) at (1–60th) days of age. From the table, it was found that the Organic trace minerals + Inorganic minerals supplemented group (T3) showed the best but lower FCR, and the control diet supplemented group (T0) showed a higher FCR. Organic trace minerals supplemented group T1 had (2.06±1.21) and Inorganic minerals supplemented group T2 had (2.12±1.19).

Table 5. Effect of dietary supplementation of Organic trace minerals and Inorganic minerals on Feed Intake (g) in different treatment groups of Sonali birds

Feed intake (g)/ bird	Treatment groups				
	T_0	T_1	T_2	T_3	Level of significant
7th Days	50.33 ± 0.33	50.33 ± 0.33	50.00 ± 0.58	50.33 ± 0.33	NS
14th Days	95.00 ± 2.65	96.67 ± 1.45	96.33 ± 0.88	97.67 ± 0.33	NS
21st Days	122.00 ± 2.08^{a}	131.00 ± 0.58^{b}	129.67 ± 1.86^{b}	130.33 ± 0.88^{b}	*
28th Days	159.33 ± 1.45^{a}	163.00 ± 2.08^{b}	162.00 ± 1.53^{b}	165.00 ± 1.73^{c}	*
35th Days	188.33 ± 1.20^{a}	191.67 ± 2.91^{b}	195.00 ± 2.52^{bc}	197.00 ± 1.15^{c}	*
42th Days	231.00 ± 5.20^{a}	238.00 ± 3.21^{b}	238.33 ± 1.45^{b}	244.33 ± 1.76^{c}	*
49th Days	271.00 ± 1.15^{ab}	278.00 ± 1.00^{c}	268.67 ± 2.40^{a}	275.00 ± 2.31^{bc}	*
56th Days	285.33 ± 4.10^{a}	295.67 ± 4.48^{b}	298.00 ± 3.46^{b}	303.33 ± 3.93^{c}	*
60th Days	159.67 ± 1.45^{a}	167.33 ± 2.60^{bc}	165.00 ± 1.53^{b}	171.33 ± 1.86^{c}	*
(1-60th) Days	1561.99 ± 19.61	1611.67 ± 18.64	1603 ± 16.21	1634.32 ± 14.28	NS

Values are expressed as mean ± standard error of means (SEM). NS: Statistically not significant (P>0.05). a b c means having different superscript in the same row differed significantly (P<0.05)*indicates 5% level of significance.

Table 6. Effect of dietary supplementation of organic trace minerals and inorganic minerals on feed conversion ratio (FCR) in different treatment groups of sonali birds

Feed conversion ratio	Treatment groups				
	T_0	T_1	T_2	T_3	Level of significant
7th Days	1.14 ± 0.00	1.13 ± 0.01	1.13 ± 0.03	1.12 ± 0.02	NS
14th Days	1.70 ± 0.14	1.59 ± 0.01	1.67 ± 0.03	1.59 ± 0.02	NS
21st Days	1.92 ± 0.09	2.03 ± 0.07	2.05 ± 0.06	1.92 ± 0.07	NS
28th Days	1.61 ± 0.04	1.53 ± 0.03	1.56 ± 0.03	1.50 ± 0.06	NS
35th Days	2.09 ± 0.09^{c}	1.93 ± 0.10^{b}	2.06 ± 0.06^{c}	1.79 ± 0.02^{a}	*
42th Days	2.25 ± 0.08^{c}	2.13 ± 0.02^{ab}	2.26 ± 0.03^{c}	2.01 ± 0.04^{a}	*
49th Days	2.76 ± 0.07^{c}	2.44 ± 0.05^{b}	2.45 ± 0.10^{b}	2.21 ± 0.07^{a}	*
56th Days	2.74 ± 0.00^{c}	2.64 ± 0.05^{b}	2.59 ± 0.01^{b}	2.40 ± 0.09^{a}	*
60th Days	2.66 ± 0.07^{d}	2.59 ± 0.06^{b}	2.62 ± 0.02^{c}	2.56 ± 0.04^{a}	*
(1-60th) Days	2.18 ± 1.01^{d}	2.06 ± 1.21^{b}	2.12 ± 1.19^{c}	1.96 ± 0.69^{a}	*

Values are expressed as mean ± standard error of means (SEM). NS: Statistically not significant (P>0.05). a b c d means having different superscript in the same row differed significantly (P<0.05)*indicates 5% level of significance.

Effect of dietary supplementation of Organic trace minerals and Inorganic minerals on carcass characteristics of sonali birds

Live weight and carcass weight (g)

The effect of organic trace minerals and Inorganic minerals on live weight and carcass weight is shown in table 7. It showed that live weight (g) and carcass weight (g) differed significantly (P<0.01) among the dietary treatment groups. The highest live weight was found in treatment group T3 (857.67±2.52) followed by T1 (806.33±0.88), T2 (782.00±1.15), and T0 (741.00±1.15). The T3 (604.33±1.76) group had significantly (P<0.01) higher carcass weight compared to T0 (487.33±0.88) whereas T1 and T2 had (547.00±1.15) and (525.67±1.20) respectively.

Breast, thigh muscle and drumstick weight, liver, heart, gizzard and wings weight (g)

Breast weight differed significantly (P <0.01) among the dietary treatment groups shown in table 7. The highest breast meat weight was found in T3 (129.00± 2.08) and the lowest in T0 was (96.67 0.88), whereas T1 (115.33± 1.45) and T2 (106.67± 1.45). The thigh meat weight and drumstick weight were also significantly (P 0.01) different among the dietary treatment group presented in Table 6. The highest drumstick + thigh meat weight was in T3 (225.00 ±1.53), and the lowest in control group T0 (184.67± 0.88), whereas T1 (206.67± 0.88), and T2 (197.33±1.20). Table 6 showed that there is no effect of organic trace minerals and Inorganic minerals on liver, heart, gizzard, and wings weight of broiler

Table 7. Effect of dietary supplementation of organic trace minerals and inorganic minerals on carcass characteristics in different treatment groups of Sonali birds

Carcass yield	Treatment groups				
	T_0	T_1	T_2	T_3	Level of significant
Live weight (g)	741.00 ± 1.15^a	806.33 ± 0.88^c	782.00 ± 1.15^b	857.67 ± 2.52^d	**
Carcass weight (g)	487.33 ± 0.88^a	547.00 ± 1.15^c	525.67 ± 1.20^b	604.33 ± 1.76^d	**
Breast (g)	96.67 ± 0.88^a	115.33 ± 1.45^c	106.67 ± 1.45^b	129.00 ± 2.08^d	**
Drumstick+ thigh (g)	184.67 ± 0.88^a	206.67 ± 0.88^c	197.33 ± 1.20^b	225.00 ± 1.53^d	**
Thigh (g)	120.33 ± 0.88^a	134.00 ± 1.15^c	125.00 ± 1.15^b	145.00 ± 0.58^d	**
Drumstick (g)	64.00 ± 0.58^a	77.00 ± 0.58^c	73.33 ± 1.45^b	84.00 ± 0.58^d	**
Liver (g)	28.00 ± 0.58	26.50 ± 0.15	27.00 ± 0.58	26.00 ± 1.15	NS
Heart (g)	7.67 ± 0.09	6.47 ± 0.26	7.37 ± 0.15	6.63 ± 0.22	NS
Gizzard (g)	43.00 ± 0.58	43.00 ± 1.15	40.00 ± 0.58	46.67 ± 0.88	NS
Wings (g)	53.00 ± 0.58	57.00 ± 0.58	52.33 ± 0.88	57.67 ± 1.45	NS

Values are expressed as mean ± standard error of means (SEM). NS: Statistically not significant (P>0.05). a b c d means having different superscript in the same row differed significantly (P<0.05)**indicates 1% level of significance.

Table 8. Effect of dietary supplementation of organic trace minerals and inorganic minerals on fecal materials determination in different treatment groups of sonali birds

Parameters	Treatment groups				
	T_0	T_1	T_2	T_3	Level of significant
Moisture%	13.17 ± 0.21	12.83 ± 0.49	12.17 ± 0.30	13.17 ± 0.45	NS
Dry Matter%	86.83 ± 0.21	87.17 ± 0.49	87.83 ± 0.30	86.83 ± 0.45	NS
Total Ash%	18.16 ± 0.47	17.33 ± 0.35	17.60 ± 0.20	17.50 ± 0.45	NS
Calcium%	2.18 ± 0.02	2.10 ± 0.07	2.17 ± 0.03	2.17 ± 0.03	NS
Phosphorous%	1.87 ± 0.18^a	2.80 ± 0.12^b	3.02 ± 0.09^c	2.65 ± 0.20^b	*

Values are expressed as mean ± standard error of means (SEM). a b c means having different superscript in the same row differed significantly (P<0.05).*indicates 5% level of significance.

pH and drip loss of meat

The average pH from the meat of slaughtered birds was recorded at 4.4 after 1 hour of slaughtering. Average drip loss was recorded at 1 g and 2 g from the experimental meat, which were stored in the refrigerator after 24 hours and 48 hours, respectively.

Fecal materials determination

Faecal material excretion feed supplemented with organic trace minerals and inorganic minerals is shown in table 8. Excretion of moisture, dry matter, total ash, and calcium was not significant among the different treatment groups. But excretion of phosphorus was significantly (P <0.05) higher in the treatment group T2 than other treatment groups.

Cost effectiveness of production

Cost-effective analysis of production performance of sonali birds supplemented with organic trace minerals and inorganic minerals is shown in Table 9. At the end of the experiment, total production cost per bird was found to be significantly (P < 0.05) different among the experimental groups. Total production cost per bird: T0 (153.39±0.47), T1 (156.31±0.13), T2 (155.93±0.06) and T3 (157.79±0.31). Net profit was found to differ significantly (P<0.01) differed among the experimental groups. It showed that net profit per bird (Tk.) in the T3 group had the highest profit (39.47±0.80) and the lowest profit was found in T0 (17.04±0.20) whereas T1 (29.15±0.76) and T2 (23.93±1.17). Benefit over control was found to significantly (P < 0.01) differ among the experimental groups. Benefit over control among T1 group (12.11±0.89), T2 group (6.89±1.33) and T3 group (22.43±0.67) were significantly (P< 0.01) differed from the control group.

Table 9. Effect of dietary supplementation of organic trace minerals and inorganic minerals on cost analysis in different treatment groups of Sonali birds

Carcass yield	Treatment groups				
	T_0	T_1	T_2	T_3	Level of significant
Cost / bird (Tk)	25.00 ± 0.00	25.00 ± 0.00	25.00 ± 0.00	25.00 ± 0.00	NS
Avg. feed consumed (kg/bird)	1.57 ± 0.01	1.61 ± 0.00	1.61 ± 0.00	1.63 ± 0.01	NS
Feed price (Tk/kg)	53.00 ± 0.00	53.00 ± 0.00	53.00 ± 0.00	53.00 ± 0.00	NS
Cost of A-vaila z/m (Tk/ bird)	0.00 ± 0.00^{a}	0.80 ± 0.06^{b}	0.00 ± 0.00^{a}	0.80 ± 0.06^{b}	*
Cost of Rena Grower premix (Tk/ bird)	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.60 ± 0.06^{b}	0.60 ± 0.06^{b}	*
Feed cost (Tk/bird)	83.39 ± 0.47^{a}	85.51 ± 0.18^{bc}	85.33 ± 0.00^{b}	86.39 ± 0.31^{c}	*
Miscellaneous (Tk/ bird)	45.00 ± 0.00	45.00 ± 0.00	45.00 ± 0.00	45.00 ± 0.00	NS
Total cost / sonali (Tk)	153.39 ± 0.47^{a}	156.31 ± 0.13^{b}	155.93 ± 0.06^{b}	157.79 ± 0.31^{c}	*
Average live weight (kg)	0.74 ± 0.00^{a}	0.81 ± 0.00^{c}	0.78 ± 0.00^{b}	0.86 ± 0.00^{d}	**
Sale price / kg live wt (Tk)	230.00 ± 0.00	230.00 ± 0.00	230.00 ± 0.00	230.00 ± 0.00	NS
Sale price /Sonali (Tk)	170.43 ± 0.48^{a}	185.46 ± 0.88^{c}	179.86 ± 1.13^{b}	197.26 ± 0.93^{d}	**
Net profit / Sonali (Tk)	17.04±0.20^{a}	29.15 ± 0.76^{c}	23.93 ± 1.17^{b}	39.47 ± 0.80^{d}	**
Benefit over control / sonali (Tk)	0.00±0.00^{a}	12.11±0.89^{c}	6.89 ± 1.33^{b}	22.43 ± 0.67^{d}	**

Values are expressed as mean ± standard error of means (SEM). NS: Statistically not significant (P>0.05). * Indicates 5% level of significance. **indicates 1% level of significance.

Discussions

Live weight and live weight gain

Organic trace minerals -supplemented group T1 showed a better result than inorganic minerals -supplemented group T2. The results of the present study seem similar to the findings of Vieira et al. (2020), who reported that improvements in broiler growth performance are usually observed at lower levels of organic trace element additions. In a recent study with White Pekin broiler ducks, Attia et al. (2013) evaluated the efficacy of inorganic (ZnO) and organic (Bio-Plex®) Zn sources added to the diets, and the obtained results indicate that BWG and FCR during the entire trial period (1–56 days of age) were better in birds fed a diet supplemented with ZnO. According to El-Samee et al. (2012), organic zinc has a positive effect on body weight in laying quail. In a study of laying hens, an organic source of manganese (manganese proteinate) produced better results than inorganic manganese sulphate in terms of weight gain and egg quality (Yildiz et al., 2011).

Feed intake (g)

The effect of organic trace minerals and inorganic minerals on feed intake of Sonali birds in this study, it was observed that the feed intake from 21st days to 60th days of age significantly differed (P<0.05) among the dietary treatment groups. But the total feed intake was statistically not significant (P>0.05). Total feed intake was lowest in dietary group T0 (1561.99±19.61 g) and highest in dietary group T3 (1634.32±14.28 g). These results agreed with Cao et al. (2000), who reported increased daily feed intake in birds supplemented with organic zinc and other minerals compared to birds supplemented with inorganic zinc and other minerals. Zinc and other mineral deficiency adversely affect protein and carbohydrate metabolism in animals, resulting in reduced feed intake, reduced growth, decreased FCR, abnormal immunological and reproductive processes, and skeletal and skin problems (Underwood and Suttle, 1999).

Feed Conversion Ratio (FCR)

It was found that the organic trace minerals + inorganic minerals supplemented group (T3) showed the best but lower FCR, and the control diet-supplemented group (T0) showed a higher FCR. organic trace minerals supplemented group T1 had (2.06±1.21) and inorganic minerals supplemented group T2 had (2.12±1.19). This result is in agreement with the result obtained by Vieira et al. (2020), who demonstrated that organic chelated trace minerals, even at substantially lower uptake rates, show improved FCR, viability, and growth compared to inorganic trace minerals supplied at equal or higher uptake rates. This is in accordance with similar results previously reported by several

authors (Leeson and Caston, 2008; Bao et al., 2007; Nollet et al., 2007; Abdallah et al., 2009; Gheisari et al., 2010). The highest total feed conversion was observed in birds fed diets with the highest levels of added mineral forms of the element at various times during the experimental period. These results are consistent with a previous report that showed that supplementing a basal diet with Zn from A-vaila®zn, an aminoacid zinc complex, improved feed conversion without altering growth rate (Burrell et al., 2004). Proteinates are mineral-chelated proteins/peptides/amino acids with comparable bioavailability to short peptides and amino acids, making them ideal for creating diets with low trace mineral levels (Klis & Kemme,2002). Organic mineral sources enhance intestinal trace element absorption by reducing interference from insoluble complexes with ionic trace elements (Leeson, 2003).

Carcass yield characteristics

There was no effect of organic trace minerals and inorganic minerals on liver, heart, gizzard, and wings weight of broiler among the different treatment groups. The findings of the present study are in agreement with El-Husseiny et al. (2012), who showed that partial replacement (50%) of inorganic sources of Zn (ZnO), Mn (MnO), and Cu (CuSO4) with organic forms of these trace elements (Zn protein, Mn methionine hydroxy analogueue chelate, and Cu methionine hydroxy analogueue chelate) improved growth performance and carcass characteristics and decreased trace element levels in tibia, liver, and excreta. Manangi et al. (2012) reported that the simultaneous replacement of inorganic Zn, Cu, and Mn sulphates with significantly reduced levels of organic sources of these trace elements (complexed with 2-hydroxy-4-(methylthio) butanoic acid Mintrex) not only adversely affected the performance and slaughter yield but also significantly reduced trace element concentrations in litter. Gheisari et al. (2010) reported that dietary treatments had only a significant ($P<0.05$) effect on carcass yield at 49 d of age. Birds fed Zn, Mn, and Cu in sulphate forms (E treatment) significantly ($P<0.05$) had a higher carcass yield than those fed oxide forms of Zn, Mn, and Cu.

pH and drip loss of meat

The average pH from the meat of slaughtered birds was recorded at 4.4 after 1 hour of slaughtering. Average drip loss was recorded at 1 g and 2 g from the experimental meat, which were stored in the refrigerator after 24 hours and 48 hours, respectively, which was non-significant. These results are in good agreement with Lu et al. (2006), who reported that diets supplemented with organic mineral sources did not affect pH, drip loss, or share forces.

Intestinal material determination

Faecal material excretion feed supplemented with organic trace minerals and inorganic minerals showed the excretion of moisture, dry matter, total ash, and calcium was not significant among the different treatment groups. But excretion of phosphorus was significantly ($P <0.05$) higher in the treatment group T2 than other treatment groups, with lower excretion found in the T0 group. Lower excretion of calcium was found in organic trace minerals supplemented group T1 (2.10±0.07) which gives a better result than other treatment groups. The present study supports the finding of Swiatkiewicz et al. (2014), who stated that the use of organic trace elements can reduce the excretion of minerals in faeces, thereby reducing the negative environmental impact of intensive poultry production. The result also agreed with Zafar and Fatima (2018). Zafar and Fatima (2018) reported that it reduced environmental pollution by reducing trace mineral emissions in bird droppings. The most commonly used and evaluated organic forms in poultry are zinc, copper, and manganese amino acid complexes, proteins, and chelates. Most of the discussed studies have indicated the positive effects of organic minerals over inorganic in both broilers and layers. The main benefit is their lower inclusion rates due to their better absorption and low output in excreta. The low addition of trace elements in this study resulted in lower concentrations of Cu, Zn, and Mn in the faeces and thus lower concentrations in the litter, which have been reported before in other trials (Bao et al., 2007; Leeson and Caston, 2008). According to Wang et al. (2018), mineral removal rates with each organic treatment were generally lower than the commercial ITM treatment. OTM also decreased the concentration of Cu ($P < 0.05$) and Zn in the excreta compared with ITM.

Cost effectiveness of production

The present result showed that net profit significantly ($P< 0.01$) differed among the experimental groups. It showed that net profit per bird (Tk) in the T3 group had the highest profit (39.47±0.80) and the lowest profit was found in T0 (17.04±0.20) whereas T1 (29.15±0.76) and T2 (23.93±1.17). Benefit over control (Tk) among the T1 group (12.11±0.89), T2 group (6.89±1.33) and T3 group (22.43±0.67) were significantly ($P< 0.01$) differed from the control group. The present findings support Zafar and Fatima (2018), who stated This trend towards using organic rather than inorganic sources of minerals in poultry is growing very rapidly. Because they are more bioavailable and efficient, they are said to reduce

feed costs through reduced dose rates without adversely affecting performance. According to Abdallah et al. (2009), the organic mineral diet has had a positive impact on the economy. It concluded that replacing inorganic minerals with organics improves bird performance and chick immune responses.

Conclusions

The results showed significant differences in daily live weight gain, final body weight, feed intake, feed efficiency, and benefits over control. This study finds that sonali chicken farmers in Bangladesh can use organic trace minerals and inorganic minerals alongside basal feed for better production. The study suggests that using organic trace minerals and inorganic minerals combined can improve Sonali chicken production.

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Conflicts of interest

The authors declare that there are no conflicts of interest.

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Use of medicinal plants in the control of fish parasites and problems related to their use in ethnoveterinary treatment-A review

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ABSTRACT

Recently, botanical extracts from temperate and tropical medicinal plants have been shown to manage terrestrial flora diseases and repel aquatic parasites and pathogens. The complex bioactivities of these compounds include alkaloids, flavoids, saponins, tannins, essential oils, and terpenoids. The antimicrobial functions of these phytochemicals depend on the specific environmental conditions at their secretion sites, with longer-lasting compounds to affect infestation cycles at various stages. Other agents can suppress ongoing infections using alternative methods. Examining the effects of phytosociograms in wet environments could yield new antimicrobial solutions with minimal adverse effects compared with synthetic while expanding our knowledge of the capabilities of traditional healers. Some chemicals can eliminate fish parasites, but they only bring benefits if they wipe out all wild fish populations and give rise to aquaculture. In some countries, parasite infestations and fish diseases limit aquaculture production growth. Utilizing herbs with healing properties for fish diseases and parasites is an eco-friendly, cost-efficient, and sustainable aquaculture strategy. The infection rates of fish can be reduced by treating them with certain plant extracts. These species are generally resistant to water-borne chemical pollutants. Despite their rarity, herbal plants and their products significantly aid in combating fish parasites. This review aims to highlight fish health management in aquaculture by emphasizing the traditional medicinal uses of plants to combat fish parasites.

Keywords: active compounds, alkaloids, ethnoveterinary, medicinal plants, parasites

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Introduction

Fisheries systems should adopt new antibiotics and immunoprotectants to address antibiotic resistance and the accumulation of antibiotics in the environment (Mthi et al., 2023). These compounds raise sustainability and environmental concerns. Pollutants can irreversibly change ecosystems (Yasin et al., 2023). Antibiotic resistance can be promoted using antibiotic residues from fish farms (Boti et al., 2023; Melchiorre et al., 2023). Antibiotic use in freshwater habitats alters host-parasite dynamics and increases disease incidence (Salma et al., 2022). Effective management of aqua-chemicals, including those possessing antibiotic properties, in aquaculture significantly decreases environmental and health risks to humans (Hadzevych et al., 2022). In ethnoveterinary practice, fish are treated with herbal remedies from medicinal plants. This method acknowledges and preserves local practices and traditional knowledge. Conventional fish cost-effectiveness infection treatments have driven their increasing adoption (Mariappan et al., 2023). This product boasts low cost, eco-friendliness, and strong consumer protection (Dasgupta, 2023). This method meets human consumption regulations because it does not contain detectable residues (Sophia et al., 2023). Communities' conservation and empowerment depend on preserving and expanding herbal treatments for fish (Radha, 2022). Mbokane and Moyo (2024) noted that although synthetic medications' high costs and inefficiency are notable concerns, the potential development of antibiotic resistance and environmental contamination pose even greater risks. These compounds inhibit bacterial and fungal growth (Hudecová et al., 2023). Aquaculture systems should seek alternative antibiotics and immunoprotectants to address antibiotic resistance and the environmental buildup of antibiotics (Mthi et al., 2023).

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Medicinal plants have been used in aquaculture since ancient times, with contemporary Western medicines based on their chemicals used to control fish parasites (Mariappan et al., 2023; Ranasinghe et al., 2023; Ezenyi et al., 2023). These compounds, including tannins, alkaloids, terpenoids, and flavonoids, act as antimicrobial agents, growth promoters, immune system enhancers, and stress relievers for fish, making them suitable alternatives to antibiotics and vaccines (Varshney et al., 2022). These medicinal plants exhibit promoting, antimicrobial, stress-preventive, appetite-stimulating, and immune-boosting properties, among others (Ranasinghe et al., 2023). Researchers have reported the potential of developing new antibiotics to combat antibiotic resistance and infectious diseases (Praseetha et al., 2023). Through research on medicinal plants, numerous anthelminic plants effective against gastrointestinal nematodes have been discovered (Ranasinghe et al., 2023). According to several studies, plant extracts from Piper betle, Leucas lavandulaefolia, and Moringa oleifera may be effective in treating parasitic conditions caused by fish parasites (Dezfuli and Scholz, 2022). Understanding the biology, ecology, and host interactions of fish parasites is essential for managing the health of aquatic organisms because the importance of ecological functions, intensification of aquaculture, climate change impacts, and growing commercial activities necessitate active attention (Jordan and Kreuels, 2022; Wright et al., 2023). This review aims to highlight fish health management in aquaculture by emphasizing the traditional medicinal uses of plants to combat fish parasites.

Common fish parasites

Fish parasites can be categorized as either external or internal based on location. These parasitic agents, Diphyllobothrium spp., Opisthorchis spp., and Anisakis spp. (Hutson et al., 2019), are the primary representatives of their kind. Examples of external fish parasites inhabiting the skin, gills, and fins include argulus, salminicolids, piscicolid, gyrodactylid, and dactylogyrids (Alhayali et al., 2023). These parasites are classified as fish lice, copepods, fish leeches, and monogeneans. Parasitic worms like nematodes, trematodes, cestodes, and acanthocephalans, inhabit various systems within fish, including their tissues, body cavities, digestive systems, and internal organs (Chong et al., 2023). According to Dykman (2023), such interactions can significantly impact species interactions, community structures, and ecosystem functioning. Their complex life cycles make them resilient against diverse environmental shocks. Several types of parasites, including digenean, cestodes, nematodes, isopods, fish lice, acanthocephalans, and monogenes, such as Dactylogyrus, Ergasilus, and Gyrodactylus, inhabit various fish species. Parasites can harm fish's gills, skin, and eyes, causing respiratory issues, impaired epithelial function, anemia, and elevated mucus production (Gardner et al., 2023).

Common ectoparasites in fish

Copepods: as minute crustaceans that infest fish, engendering diverse impacts. Al-Niaeem et al. (2015) identified six copepod species in Basrah Province: Ergasilus rostralis, E. mossulensis, E. ogawai, Ergasilus sp, Lernaea cyprinacea, and Mugilicola kabatai. Nagasawa (2015) also reported different copepod species, including Caligus fugu, C. lagocephalus, C. lalandei, C. latigenitalis, C. longipedis, C. macarovi, C. orientalis, C. sclerotinosus, C. spinosus, Lepeophtheirus longiventralis, L. paralichthydis, L. salmonis, Alella macrotrachelus, Clavella parva, Parabrachiella hugu, P. seriolae, Peniculus minuticaudae, Acanthochondria priacanthi, and Biacanthus pleuronichthydis, from marine fish in Japan. Among them, five species (C. orientalis, L. longiventralis, L. salmonis, C. parva, and A. priacanthi) are known to parasitize farmed fish in subarctic waters, whereas the remaining species infect farmed fish in temperate waters. At this point, there is a lack of information about copepods from fish raised in subtropical waters. According to Nagasawa (2015), the host fish comprise carangids, sparids, monacanthid, salmonids, scombrids, tetraodontid, pleuronectids, paralichthydis, and trichodontids. The parasitic copepod Helcogrammoides chilensis cohabits with Chilean triplefin fish without adversely affecting their size or health (Palacios-Fuentes et al., 2012). In Pengudang Village's aquatic ecosystems, seven copepod species, namely Callanus spp., Lucicutia spp., Macrosetella spp., Nauplius spp., Oithona spp., Rhincalanus spp., and Scolecithricella spp., were detected. Copepods supply energy to small fish during their larval stage (Sethi et al., 2013). 72 various fish species in Turkey, including wild and farmed carangids, sparids, and salmonids, were identified as hosts to parasitic copepods (Alaş et al., 2015). A total of 25 copepod parasite species have been identified in the gills of 14 different teleost fish species in Algeria (Boualleg et al., 2011). In the Mediterranean Sea, copepods belonging to the Corycaeidae, Calanoidae, Oithonidae, and Oncaeidae families have been found infected with Blastodinium spp., namely B. mangini, B. contortum, and B. spinulosum (Alves-de-Souza et al., 2011). A total of 34 copepod species were reported by Melaku et al. (2022) from South African freshwater habitats. Copepod species data for Ethiopia's freshwater fish populations are missing. Ethiopian

research has primarily focused on breeding valuable fish species. Microalgae, rotifers, copepods, and cladocerans serve as live food for fish larvae (Cumberlidge and Clark, 2012). Researchers have also investigated Ethiopia's freshwater crab population. Mnisi and Dippenaar (2019) and Cumberlidge and Meyer (2010) reported discoveries of the new species Potamonautes kundudo and Potamonautes holthuisi in distinct regions of Indonesia. These discoveries underscore the necessity for further research on copepod species interacting with freshwater fish.

Argulus (Fish lice): Fish lice are crustacean ectoparasites also known as Argulus spp. A. japonicus, A. foliaceus, and A. coregoni are worldwide distributed and harm many fish species (Budijono et al. 2022; Burdukovskaya and Dugarov, 2023). Fish lice (Argulus spp.) attack various fish species, including goldfish (Carassius auratus) (Shukla et al., 2022; Radkhah and Eagderi, 2022), koi carp (Cyprinus carpio) (Budijono et al., 2022), Common carp (Cyprinus carpio L.) (Gallardo-Escárate el al., 2019), and carp (C. carpio) (Nurani et al., 2020; Hunt et al., 2021). Aalberg et al. (2016) and Chang et al. (2023) reported the A. foliaceus in Pike-perch (Sander lucioperca L.), Brook trout (Salvelinus fontinalis M.), and Sea-run Arctic charr (Salvelinus alpinus). Researchers observed coinfection of Pike-perch with A. foliaceus and A. japonicus (Wafer et al., 2015). Argulus spp. were collected from the goldfish's (C. auratus) caudal and anal fins (Koyun, 2011). This crustacean ectoparasites cause significant financial losses in fish farms through their attachment to hosts, feeding, and mass mortality (Misganaw and Getu, 2016; Johnson et al., 2019; Sikkel and Welicky, 2019). Sea lice, including Lepeophtheirus salmonis, Caligus clemensi, and C. rogercresseyi, significantly affect wild and farmed finfish by infecting wild salmon and serving as vessels for fish-infecting viruses (Rochat et al., 2023).

Fish leech: Research on fish leech has been conducted in Australia, New Zealand, and Lake Saint. Clair is from Michigan and Japan, not New South Wales. 14 fish-leeches species unique to Australia and New Zealand have been identified by scientists (Burreson, 2019). The researchers proposed and identified the three leech species, including Actinobdella pediculata, Placobdella montifera, and Myzobdella lugubris, in Lake St. Clair (Schulz et al., 2011). Aloto and Eticha (2018) detected seven leech species, including Crangonobdella maculosa, Johanssonia arctica, Limnotrachelobdella okae, Platybdella olriki, Stibarobdella bimaculata, Taimenobdella amurensis, and Trachelobdella livanovi, in fish in Japan. In various regions, fish leeches pose a threat to many fish species. Researchers identified fish leeches from the species Acipenserobdella volgensis on fish belonging to the families Acipenseridae, Cyprinidae, Salmonidae, and Esocidae (Bolotov et al., 2022). In total, 1.63% of graylings and trouts were found to host Piscicola pojmanskae (Cichocka et al., 2018). In the Southern Ocean, crocodile icefish like Chaenocephalus aceratus, Champsocephalus nunnari, and Chionodraco rastrospinosus (Parker et al., 2020), host trypanosomes spread by leeches, as do South American armored catfish. According to Lemos et al. (2015), these parasites are abundant. In various water environments, several fish species are prone to leech infestations, as indicated by these findings (Pomposini et al., 2019; Parker et al., 2020).

Monogeneans (Platyhelminthes): Fish monogeneans impact various fish species, including common carp (C. carpio), soldier bream (Argyrops filamentosus), common bream, and roach (Dedić et al., 2023; Alghamdi et al., 2023; Vorel et al., 2023). This group includes dactylogyrid/diplectanid, gyrodactylid, capsalid, and polyopisthocotylea parasites. In substantial quantities, these parasites can cause significant fish diseases. Parasites predominantly inhabit external areas of fish, such as the gills, skin, nostrils, mouth, esophagus, cloaca, and urinary tract. Monopisthocotyleans irritate the skin and gills, wheras polyopisthocotylea cause severe blood loss and anemia. Monogenean infections exhibit varying intensities among various fish species and their hybrids. Host-parasite co-evolution determines the number of monogenean species infecting a fish (Mendlová and Šimková, 2014). Fish monogeneans affect many fish types, such as the common carp (C. carpio) (Vorel et al., 2023), soldier bream (Argyrops filamentosus) (Alghamdi et al., 2023), common bream, and roach (Dedić et al., 2023). The monogenean parasite Sparicotyle chrysophrii causes extensive health issues and financial damage in Mediterranean fish farms by attaching to gills and multiplying within sea cages (Mladineo et al., 2024; Riera-Ferrer et al., 2022). Vorel et al. (2023) reported the presence of Eudiplozoon nipponicum in common carp gills and Abdel-Gaber et al. (2023) discovered Haliotrema susanae in soldier bream fish gills. Nitta and Nagasawa (2023) identified Dactylogyrus and Bivaginogyrus species in the gills of freshwater fish from Japan. Dedić et al. (2023) conducted a study focusing on the gills to determine monogenean infection levels between parent fish species and their hybrids. Monogeneans usually infect fish in their gills and skin, but cases of infection in other areas like nostrils, mouth cavities, food pipes, waste openings, and urinary tracts are uncommon (Chong, 2022; Newton and Ritchie, 2022). In fish harboring multiple parasites, monogeneans favor select hosts,

inflicting severe diseases (Félix et al., 2022). The combined presence of multiple monogenean species in a host enhances studies reporting higher infection rates and parasite prevalence (Louizi et al., 2023; Ieshko et al., 2024). Fish monogeneans have a broad host range; therefore, comprehending and managing, these parasitic infections in aquatic environments is crucial (Bakke et al., 2002; Rohde, 2002; Shinn et al., 2023).

Common endoparasites in fish

Digeneans (Trematodes): Digeneans are worms that parasitically infect fish (Pantoja et al., 2022; Yanagi et al., 2022; Allam et al., 2023). Different species of digenean trematodes inhabit various fish species (Romanova et al., 2023; Prasadan et al., 2023). These parasites exhibit intricate life cycles, with mollusks serving as middle hosts and vertebrates serving as end hosts, and include stages such as eggs, miracidia, sporocysts, rediae, cercariae, metacercariae, and adults (Krupenko et al., 2022). Research in Saudi Arabia, Russia, and Brazil has demonstrated the significance of understanding a parasite's genetic structure and distribution. Locating new host species and mapping digenean trematode habitats are essential for controlling and preventing infection in fish populations. The significance of ongoing research is emphasized because these families (Diplostomoidea, Clinostomidae, and Heterophyidae) causing diseases in fish (Pantoja et al., 2022). Certain digenean can infect humans, making them dangerous zoonotic agents. These fish-dwelling trematodes include Tylodelphys clavata, Diplostomum spathaceum, and Paracoenogonimus ovatus. Fish can contract postodiplostomosis or ichthyocotylurosis from trematode infections. In South Georgia, 111 fish from eight species contained harmful digenean trematodes such as Pseudoamphistomum truncatum, Apophallus muehlingi, and Rossicotrema donicum (Romanova et al., 2023). All Notothenia rossii fish were infected by Elytrophalloides oatesi (Zdzitowiecki and White, 1992). 19 fish species in the Taega River were found to host various digenetic larval trematodes, such as Clonorchis sinensis, Cyathocotyle orientalis, and Metagonimus species (Joo et al., 2001). In the intestines of Clarias gariepinus, Orientocreadium batrachoides, Masenia bangweulensis, and digenetic trematodes were found, while Cyanodiplostomum spp. was present in the skin and muscles (Attia et al., 2021). In 2021, research revealed that fish infected can experience inflammation and tissue displacement (Bullard and Overstreet, 2008). Researchers have identified coccidian in 60 families of marine fish, including Eimeria and Goussia species (Saraiva et al., 2023). In the eastern Gulf of Mexico, a new digenean species, Achorovermis testisinuosus, was found living in the heart of the smalltooth sawfish Pristis pectinata (Warren et al., 2020).

Cestodes: Many fish species host cestodes, which are parasitic worms. According to Svensson et al. (2022), three-spined sticklebacks (Gasterosteus aculeatus) infected with the cestode Schistocephalus solidus express reduced antipredator behaviours. Diniz et al. (2021) reported the influence of Grillotia carvajalregorum and Contracaecum helminth larvae on Percophis brasiliensis's serosa, stomach, intestine, liver, and gonads. Polyakova and Gordeev (2020) examined the cestode species Bothriocephalus antarcticus, Parabothriocephalus johnstoni, and Onchobothrium antarcticum in Antarctic and Subantarctic fish. Zuchinalli et al. (2016) identified commercial fish species, such as Oligoplites saurus and, Pterobothrium crassicollis in Brazil. Marine fish species, such as Seriola dumerili, Pseudocarans dentex, Epinephelus haifensis, and Mycteroperca rubra, were found to be infected Callitetrarhynchus gracilis, Callitetrarhynchus speciosus, Protogrillotia zerbiae, and Grillotia brayi (Morsy et al., 2022). Cestodes of Trypanorhyncha order infected various fish species. Parasites negatively impacted marine fish, that leading to customer rejection (Palm et al., 2009; Morsy et al., 2022). The following infected fish species have been identified: gray triggerfish (Balistes carolinensis), mottled grouper (Mycteroperca rubra), common sole (Solea vulgaris), greater amberjack (Seriola dumerili), gulley jack (Pseudocaranx dentex), Haifa grouper (Epinephelus haifensis), and various marine teleosts and elasmobranchs (Morsy et al., 2023; Morsy et al., 2022; Ziarati et al., 2022). Joo et al. (2001) and Saraiva et al. (2023) identified Gymnorhynchus isuri, Pseudotobothrium dipsacum, Heteronybelinia estigmena, Callitetrarhynchus gracilis, Callitetrarhynchus speciosus, Protogrillotia zerbiae, and Grillotia brayi as cestodes found in various fish species. Monitoring and controlling these parasites are crucial for maintaining fish safety and minimizing the possibility of zoonotic diseases transmitted to humans.

Nematodes: Nematodes can negatively impact fish populations, leading to health issues, financial losses, and reducing marketability (Indrayati, 2017). Researchers identified Anisakis simplex, Hysterothylacium aduncum, Hysterothylacium reliquens, Hysterothylacium fabri, and Dichelyne pleuronectidis from various teleost fish, such as snowy grouper (Hyporthodus niveatus), Brazilian flathead (Percophis brasiliensis), European pilchard (Sardina pilchardus), chub mackerel (Scomber japonicas), anchovy (Engraulis encrasicolus), bogue (Boops boops), spinycheek grouper (Epinephelus diacanthus), and orange-spotted grouper (Epinephelus coioides)

(Ramdani et al., 2022; Pereira and González-Solís, 2022; Martin-Carrillo et al., 2022; Wuwei et al., 2023). Nematodes have been discovered in various fish body parts, including the intestine, body cavity, mesenteries, stomach, liver, spleen, gonads, and kidneys (Hussein et al., 2020). Some nematodes like A. simplex and Hysterothylacium spp. affect human health (Saglam, 2013). Studies have shown that nematodes impact fish economics. They can make fish sick, cause economic looses, and change how people view fish as food (Indrayati, 2017). Third-instar larvae of Contracaecum, Terranova, Hysterothylacium deardorffoverstreetorum, and Raphidascaris infect Hyporthodus niveatus (Menezes et al., 2023). According to Diniz et al. (2021), Grillotia carvajalregorum larvae and various nematodes were found in the organs of Percophis brasiliensis. Nematodes from Hysterothylacium and Anisakis infected European pilchards (Fuentes et al., 2022). Scientists identified Anisakis typica and Anisakis pegreffii in chub mackerel, anchovy, and bogue (Aldik et al., 2023). Hysterothylacium spp. nematodes infect Epinephelus diacanthus and Epinephelus coioides (Bannai and Jori, 2022).

Acanthocephalans: Studies identified 13 types of acanthocephalans as fish parasites across diverse oceanic habitats (Polyakova and Gordeev, 2021). In New Zealand, the researchers reported Gorgorhynchoides queenslandensis for the first time, together with at least two new species identified by Bennett et al. (2023). Sclerocollum rubrilabris inhabits the intestines of S. rivulatus. Acanthocephalans help fish cope with toxic metals. A study by Hassanine and Al -Hasawi (2021) revealed that lowering of Cadmium (Cd) and Lead (Pb) levels in fish livers and reductions in liver enzymes, glucose, triglycerides, and urea in fish blood occur. Researchers identified five types of acanthocephalans, including Acanthocephalus johni and Breizacanthus azhari (Hernández-Orts 2019), from Argentina's Patagonian continental shelf and seven species, including Neoechinorhynchus agilis and Longicollum pagrosomi (Panchani, 2021), in the Bizerte lagoon, Tunisia. These parasites infect various fish species, such as Sutorectus tentaculatus, Xenocypris davidi, Acreichthys sp., Clarias batrachus, Hylarana sp., Leiognathus equulus, Anabas testudineus, Heteropneustes fossilis, and Mystus gulio (Smales et al., 2019). These parasites display unique proboscis structures, hook patterns, and host preferences (Perrot -Minnot et al., 2023). The prevalence and modes of infection with acanthocephalans vary among fish species. Some species are more susceptible to specific acanthocephalan species. Fish can acquire acanthocephalans via paratenic transfer or post-cyclic transmission (Dimitrova et al. 2008). The chromosome structures of Pomphorhynchus kashmirensis and Neoechinorhynchus manassasensis from Schizothorax and Cyprinus species were determined (Ahmad et al., 2015). These parasites, known to kill fish, alter blood parameters, and disrupt fish populations (Dezfuli and Scholz, 2022; Öktener and Bănăduc, 2023), are capable of causing mass fish mortalities (Öktener and Bănăduc, 2023). Degradation of water quality, human activities, and environmental isolation can influence parasite population, diversity, and density. Monitoring and safeguarding freshwater ecosystems require a thorough understanding of the interactions of fish parasites with their hosts. In nature, fish and their parasites maintain an equilibrium. Pollution and new fish parasites can adversely affect fish populations and alter fish communities (Pravdová et al., 2023). Parasites significantly impact species interactions, community structures, and ecosystem functions through their reliance on host organisms (Thieltges et al., 2024). Similarly, alterations in host species and quantities can impact the durability of parasites (Dykman, 2023). The examination of fish parasites provides insights into the status of freshwater ecosystem health and pollution levels (Öktener and Bănăduc 2023). Understanding the types and behaviors of fish parasites in their habitats is essential for effectively monitoring environmental changes and managing freshwater ecosystems (Srivastava et al., 2022; Giari et al., 2022).

Medicinal plants and their properties

Parasitic diseases can be effectively treated using medicinal plants. These plants possess antibacterial, antifungal, anticancer, and anti-inflammatory properties (Ahmad and Karmakar, 2023), however, they face challenges in conservation due to habitat loss, uncontrolled wild harvesting, and commercial over-extraction (Sharma et al., 2023). We must intentionally domesticate and cultivate identified plant species (Kumar and Singh, 2023) to maintain a consistent supply (Kumar and Singh, 2023). Experts predict that the global herbal medicine market, driven by medicinal plants, will reach $550 billion by 2030. The global popularity of Ayurvedic medicine, which employs natural herbal products, because of its effectiveness and minimal adverse effects (Obahiagbon and Ogwu, 2023). For thousands of years, plants have been used as a significant medicinal resource (Begum et al., 2023).

Medicinal plants have both medicinal and economic value because they are in high demand in local and international markets (Olsen, 2005; Sher et al., 2014). This knowledge has led people to discover new things and make informed health decisions. Native Americans have an intricate understanding of medicinal plants and

their therapeutic properties. According to Sivaramakrishna et al. (2023), these groups documented the traditional use of these plants. This involves creating medication from various plant parts and addressing various health concerns. Traditional ecological knowledge of edible and medicinal plants influenced indigenous livelihoods. Economic opportunities have been created, and food security has been secured (Mohd Salim et al., 2023). Myths, taboos, and traditional leadership contribute to the conservation of genetic resources (Anand et al., 2023). Indigenous plant-based medical practices vary in their usage, depending on the specific plant and ailment (Kola, 2022).

Active compounds in medicinal plants

Chemical compounds found in medicinal plants affect fish health management (Singh et al., 2022; Garcia-Oliveira et al., 2022; Mariappan et al., 2023). These substances, including phenols, terpenoids, alkaloids, and flavonoids, help fish grow, handle stress, and fight diseases (Faheem et al., 2022). Zhang et al. (2023) and Ahmad et al. (2023) noted that saponins and flavonoids fight inflammation and bacteria; phenolic substances can treat inflammatory conditions (Ramdani et al., 2023). These secondary metabolites in plant extracts noted their use as alternative anthelmintic drugs to treat parasitic diseases in fish without harming the host (Mariappan et al., 2023).

Research is needed to identify appropriate doses of these drugs to reduce parasites and ensure the safety of fish (Bashir et al., 2022). In addition, active ingredients in medicinal plants boost the immune system, enhance immune responses, and improve overall fish health (Pulkkinen et al., 2010). Fish farmers can use medicinal plants and their byproducts as a cheaper and safer option instead of using artificial chemicals, vaccines, and antibiotics. Antioxidants in medicinal plants protect fish against oxidative stress and physical problems. Experts recognize these plant-based substances as safe for fish, humans, and the environment (Singh et al., 2022; Mariappan et al., 2023; Mbokane and Moyo, 2024), and offer a good way to improve fish health and control diseases in fish farming.

Alkaloids: Alkaloids comprise a huge group of organic nitrogen compounds found in nature; scientists have spotted over 20,000 different types. They are weak bases with a positive charge on the nitrogen atom and are found in plants as organic acid salts. These compounds have toxic effects on cells and kill insects, fungi, and bacteria. Their ability to fight parasites in fish health management is well-known (Winzer et al., 2015; Srivasatava, 2022; Alfianna and Situmorang, 2023; Faisal et al., 2023). Plants often contain alkaloids (Tiwari et al., 2023; Ravichandran et al., 2023). Three alkaloids tested for their worm-fighting power against Haemonchus contortus caused 100% paralysis (Espino Ureña et al., 2023). People use alkaloids to treat fish diseases because they relax fish muscles and act like anesthetics. Alfianna and Situmorang (2023) reported that these compounds have narcotic-like effects on parasites and influence the central nervous system, and enhance the fish's immune system. Alkaloids also display interesting biological traits, like anti-inflammatory and anti-cancer effects, and show potential as treatments (Varela et al., 2023).

The specific drug pathways through which alkaloids are used to treat cancer cells have been identified. These routes involve controlling key signaling pathways involved in cell growth, cell cycle, and cancer spread (Mariappan et al., 2023). Alkaloids might be a treatment option for fish diseases because of their possible effects on the immune system, parasites, and central nervous system. Some alkaloids are toxic to fish parasites (Ukwa et al., 2023). The neem tree (Azadirachta indica) contains alkaloids with anti-insect and antiparasitic properties. This explains why people use the neem tree to manage fish in some areas (Rani et al., 2023).

Saponins: The plant families that contain saponins include Leguminosae and Ginseng. They affect the parasites gyrodactylids and monogeneans. Because saponins interfere with the cell membranes of parasites, they aid fish in eliminating them from their bodies, gills, and fins. Fish illness risk is decreases when germs are eliminated (Abdelrahman and Jogaiah, 2020; Nguyen et al., 2020). It has been demonstrated that terpenoids taken from the leaves of Virola surinamensis are effective against Loma salmonae, a parasite that causes kidney illness in salmon and associated species (Štrbac et al., 2022). These findings highlight the importance of investigating the effects of medicinal herbs on fish diseases and parasites. While protecting the environment, the study aims to reduce the number of chemicals used to cure fish.

Saponins are present in various plant parts, including seeds, roots, stems, bark, grains, leaves, and flowers, and have a wide range of biological activities such as immunomodulatory activity, anti-inflammatory activity, and hypoglycemic properties (Mehta et al., 2023; Shen et al., 2023). It is worth noting that Solanum torvum and other plants possess a high amount of the substance saponin, which makes them to have therapeutic value (Ren et al., 2024). According to Shen et al. (2023), plant saponins are involved in activating the growth and development of immune organs in the body, stimulating the activity of immune cells and the

production of cytokines and antigen-specific antibodies, and thus bear an effect of regulating immune response. Gadallah et al. (2024) stated that saponins have been effective in the control of protozoan parasites in aquaculture, including Ichthyophthirius multifiliis and Cryptocaryon irritans. Over the years, saponin extracts in different constituents have addressed numerous fish parasites with P. granatum extract achieving total loss of Neobenedenia girellae at a concentration of 62.5 mg/L (Liu et al., 2021), while Moringa oleifera and Piper betle extracts have offered remediation for Lernaea sp., Argulus sp. and Ergasilus sp. infections (Mariappan et al., 2023). Saponins could be a more eco-friendly option when compared to artificial chemicals employed in aquaculture practices, but negative effects such as cell toxicity and bitterness associated with saponins might limit their application in fish farming (Timilsena et al., 2023).

Flavonols: These bioactive compounds have health benefits, including reduced inflammation, cancer, fungus, infections, and high blood pressure (Nagar et al., 2022; Prasad et al., 2023). It also exhibits antioxidant and germicidal qualities that improve fish health and have a major impact on natural foods, pharmaceuticals, and cosmetics (Barreca et al., 2023). The heterocyclic ring configurations of flavonoids, such as anthocyanins, isoflavone, flavonols, flavanols, flavones, and flavanones, differ from each other (Mancarz et al., 2023). These plant-derived substances support pigmentation, signaling, defense, growth, and UV protection in living world (Rodriguez et al., 2022).

Flavonoids, plant compounds produced through a complex process that can be influenced by flavonoid gene alterations, play protective and preventive roles against numerous diseases. According to Prasad et al. (2023), these compounds affect the NF-κB signaling pathway. Scientists have explored potential health benefits. Flavonoids have been linked to antioxidant, anti-inflammatory, anticancer, and neuroprotective effects according to numerous studies (Crupi et al., 2023; Hussain et al., 2022; Rodriguez et al., 2021). Singh et al. (2023) reported that, these substances lower the risk of long-term health problems, such as type II diabetes, heart disease, and certain cancers. These compounds have antibacterial properties and can aid in the treatment of infectious diseases (Singh et al., 2022). The researchers found that the intervention enhanced both blood fat levels and heart health (Calderaro et al., 2022). Flavonoids act as natural food additives, conferring health benefits (Li et al., 2023).

It has been established in previous studies that flavonoids exert several beneficial effects on fish, especially in zebrafish (Danio rerio) and rainbow trout (Oncorhynchus mykiss). Flavonoids present in the zebrafish studies may also act as a co-agent in the prevention and control of the obesity condition, and in doing so tackle metabolic complications resulting from the deposition of excessive fat. Obesity-related complications are dealt with through pathways that deal with inflammation and lipid metabolism, processes which are key (Montalbano et al., 2021). According to Daya et al. (2021), flavonoids of Leea Indica inhibited orofacial pain in fish to levels similar to those caused by standard pain relief drugs while increasing the mobility of fish that were treated with the flavonoids. It is additionally related to neuroprotection, as well as the regulating effects of neuroinflammation and oxidative stress, both of which are significant in neurodegeneration (Mhalhel et al., 2023).

Flavonoids have a number of benefits concerning their use in aquaculture, but their use has its limitations as well. Phagocytosis, immune response, and antioxidant capacity in fish can be improved with the application of flavonoids, nonetheless, their effectiveness might be affected with different species as well as environmental conditions (Wang et al., 2007; Ponomarev et al., 2020; Shohreh et al., 2023; Affandi and Diniariwisan, 2024). It has been shown that some flavonoids can have positive impacts on growth rates, as for instance, the supplementation of dihydroquercetin to tilapia resulted in a 26% productivity gains (Ponomarev et al., 2020). Evidence exists however suggesting that such compounds may not have such effect on physiologically different fish species (Taştan and Salem, 2021).

Medicinal Plants to Control Fish Parasites

Many of the herbs act as prophylactic agents against different fish parasites. Phytomaterials have been demonstrated to have antiparasitic activity against more than 15 invasive plant species, including Alpinia, Allium sativum, Calotropis procera, Coriander sativum, Datura stramonium, Gymnema sylvestre, Houttuynia, Momordica charantia, Ricinus communis, Solanum xanthocarpum, Aframomum melegueta, Moringa oleifera, Azadirachta indica, Zingiber officinale, and Vitex, among other infected plants. These plants have been poorly researched (Ranasinghe et al., 2023; Kuzminac et al., 2023; Ukwa et al., 2023). They have undertaken in vitro and in vivo experiments against any known or probable parasitic disease related to or instigated by these plants, and the results have been supportive. In addition to having an anti-parasitic effect on Echinococcus granulosus, which causes hydatid disease of echinococcosis disease (Özil, 2023), Allium sativum also fortifies the immune system against the invasive parasite. Studies of garlic (Allium sativum) have

been tested and demonstrated antiparasitic properties. Studies were conducted to determine the effects of trophosts, which are is the vegetative stage of Ichthyophthirius multifiliis, a ciliated protozoan parasite of freshwater fish (Liang et al., 2015; Muahiddah and Diamahesa, 2023).

Reports also indicate that garlic may be more potent against parasites, particularly nematodes, such as Ascaridia sp. found in goldfish (Carassius auratus), and the significant aquarium pathogen Gyrodactylus turnbulli, which invades the guppy (Poecilia reticulata) (Schelkle et al., 2013; Galisteo et al., 2022). The compounds present in garlic, such as ajoene, alliin, and allicin, exhibit bactericidal, virucidal, and parasiticidal activity, as well as antioxidant properties (Valenzuela-Gutiérrez et al., 2021). Studies examined onion (Allium cepa) and its extracts could eliminate various fish parasites. It was found to expel nematode infections (Kouamé et al., 2021; Filgueiras et al., 2023). Research indicated that A. cepa extracts, both crude and ethanol extracts, keep in check Saporlegnia parasitica and Ichthyophthirius multifiliis (Özil, 2023; Elgendy et al., 2023).

Yildiz et al. (2019) found that when adult copepods (Lernantropus kroyeri) were exposed to 100% garlic juice in a cage-cultured European sea bass (Dicentrarchus labrax), every copepod was lethally affected within 5 minutes. It is also known that organosulfur compounds in garlic oil, such as diallyl disulfide and diallyl trisulfide, have strong actions against nematodes, because of their nematicidal properties (Yildiz et al., 2019). Delgado et al. (2023) also showed that garlic enrichment in fish feed contributes to better immunological responses, such as enzyme activity and antibody synthesis, in considerable amounts of mass-reared fish of various species. However, this promising result of using garlic as an anti-parasitic drug should be further investigated in terms of its effect on reducing the number fish parasites, which is certainly an undesired infection (Abdel-Hafez et al., 2014). However, this promising result of using garlic as an anti-parasitic drug should be further investigated in terms of its effect on reducing fish parasites, which surely is an undesired infection (Abdel-Hafez et al., 2014).

Elgendy et al. (2023) reported the nutritional effects of A. cepa-supplemented diets on growth performance and immunity against S. parasitica infection by lowering oxidative stress and fish mortality due to S. parasitica infection and cadmium immunotoxicity in Oreochromis niloticus. Furthermore, A. cepa can help minimize the body burden of cadmium and boost IL-1β and IFNγ expression (Elgendy et al., 2023). Dietary supplementation with A. cepa also has immune-stimulatory effects, and the researchers recommend it as a prophylactic treatment aiming at the management of saprolegniasis and enhancing cadmium's adverse effects (Ahir et al., 2023). Similarly, other studies on the antiparasitic action of A. cepa essential oil were conducted, and its antiparasitic action was studied in greater detail. This essential oil showed 94% efficacy against Ichthyophthirius multifiliis triphones with an exposure time of about sixty minutes at an optimal concentration (Özil, 2023). Rachmawati et al. (2022) reported the significance of the conventional clearness of foods with the addition of extract from garlic on the resistance capacity and survival of Nilem fish (Osteochillus hasselti) against Aeromonas hydrophila bacterium. In the present study, however, consumption of 20 g/kg garlic extract did improve the health of the angled fish, as evidenced by the increased white blood cell counts. Further studies are warranted to evaluate the cost-benefit of including garlic extract in Nile tilapia feed.

Ukwa et al. (2023) evaluated the effectiveness of herbs like Aframomum melegueta, Moringa oleifera, Azadirachta indica, Zingiber officinale, and Allium sativum in treating praziquantel and other herbal treatments against parasites affecting various fish species. Aframomum melegueta either alone or in its blends exhibited replacement efficacy for parasites, especially Electrotaenia spp., with increasing exposure time. Similarly, Azadirachta indica is effective against Tenuisentis spp. and other Acanthocephalan spp. (Ukwa et al., 2024). Azadirachta indica is known to inhibit the development of Argulus spp. (Kumari et al., 2023).

Active plant extracts obtained from Momordica charantia, such as momordicatin, have promising anti-parasitic activities (Phiri et al., 2023). This indicates new medicines against parasitic infections originating from the studied plants and their constituents. Houttuynia cordata and Allium sativum show effects against parasite-compromising plants (Harish et al., 2022; Özil, 2023). Koi Carp treated with Houttuynia extract for Gyrodactylus turnbulli had fewer parasites in total than those without the extract (Mariappan et al., 2023). Houttuynia cordata ethanol extract effectively halted biofilm formation in pathogenic organisms including Fusobacterium nucleatum, Streptococcus mutans, and Candida albicans. It was also responsible for a slight attenuation of tree borne bacteria in the mouth. More importantly, none of these compounds was cytotoxic to gingival fibroblasts challenged with Porphyromonas gingivalis lipopolysaccharide to stimulate interleukin-8 and CCL20 production (Sekita et al., 2016). In addition,

Allium sativum oil extract wiped out many the external parasites of Argulus foliaceous (Radkhah, 2017). Such discoveries are long overdue because of the current scientific trend, which emphasizes the use of traditional plants. This approach not only provides physicians with more options for the management of patients (Ranasinghe et al., 2023) but also improves the efficacy and longevity of of plant-based therapies. It is important to note that several plants, including various parts of these plants, exhibit anti-gut parasite activity both in vitro and in vivo (Kuzminac et al., 2023). Many studies have demonstrated that these specific plants can be used comfortably in combination with or even in the replacement of known anti-parasitic drug therapies. Mentioned above, molecules from herbal sources have also been found to possess the capability to combat infectious agents. Aquatic plants with many of these therapeutic elements, such as natural antibiotics, can cure infectious diseases. However, a more aquatic plants live in-depth, and consideration of targeted medicinal plant compositions and their further elucidation is required. Because there are still no positive results encouraging treating such infections with the plant materials in question, as previously stated, considerable effort will still have to be made (Tiwari et al., 2023; Dar et al., 2023).

Mechanisms of action

Once the host fish consumes compounds from medicinal plants, these compounds are passed to the parasite through the bodily fluids of the host fish, including blood, as mentioned by Mbokane and Moyo (2024). These helpful compounds then interact with enzymes to digest food within the parasite's feeding vacuole (Olanrewaju et al., 2023). This disruption of digestion processes within the food vacuole may lead to starvation of the parasite (Pravdová et al., 2023). Furthermore, the active compounds also affect the parasites during their growth early growth stages, disrupting their life cycles (Mrugała et al., 2023). Research has demonstrated that various herbal remedies can combat fish parasites effectively by impeding the capacity to cause infections effectively. This discovery has implications for crafting preventive and management strategies centered on herbal remedies. These substances may affect parasite cells, leading to dysfunction and deformities in the organelles (Özil, 2023).

Herbal extracts in their natural form have demonstrated success in fighting parasites like Gyrodactylus kobayashii. The use of substances such as dioscin can temporarily remove parasites from fish while altering the surface of their tegument (Dawood et al., 2021). In general, when it comes to how natural remedies from plants function in dealing with parasites in fish, it is by entering their cells and disrupting their structure and normal functions. Alternatively, some medicinal herbs release chemicals, like alkaloids and saponins, which can be harmful to fish, depending on the dosage given. These elements may affect the kidney and blood systems of the fish being treated. Therefore, it is important to research the various types of secondary compounds found in medicinal plants and their impacts on fish, as well as the correct dosages to minimize the risk of overdose and incorrect application (Camilo et al., 2022; Mbokane and Moyo, 2024).

Efficacy and Safety Considerations

Other related studies have shown that medicinal plants are safe for use in aquaculture because they are effective in eradicating parasites in fish. Researchers have found that onion, sage, menthe, garlic, lavender, and oregano essential oils are effective against Ichthyophthirius multifiliisis trophones (Özil, 2023). Mbokane and Moyo (2024) conducted a meta-analysis and revealed the fact that there is evidence to suggest that fish such as carp, trout, African catfish, and tilapia can have their immunity and disease resistance enhanced by the use of medicinal herbs. Some plants that are commonly found in this area are Piper betle, Leucas lavandulaefolia, Moringa oleifera, Morinda citrifolia, Allium sativum, Galla chinensis, Mucuna pruriens, and Carica papaya. The ethanol extracts of Astragalus membranaceus, Thunb (Dryopteris setosa), Gan Cao (Glycyrrhiza uralensis), danshen (Salvia miltiorrhiza), and pomegranate (Punica granatum) have also been proven to be effective in controlling Neobenedenia girellae (Liu et al., 2023).

Medicinal plants are safe, easily available, and cost-effective, and they have the least impact on the environment; therefore, they are an important tool in the treatment of fish infections. When using plant extracts on fish, it is important to be cautious so as not to transfer it to human tissues (Mariappan et al., 2023). A study has shown that Syzygium aromaticum and Punica granatum are effective in treating fish diseases such as saprolegniales (Mostafa and Yassin, 2022). Abou-Taleb et al. (2022) stated that the medicinal plant extracts are not toxic to fish; hence, they may be safe and environmentally friendly agents that can be used in the prevention of diseases. These medicinal plants may be used as natural and non-toxic feed supplements that enhance the immune response and disease tolerance of fish (Muahiddah and Diamahesa, 2023). Medicinal plants have been effective in boosting the immune and disease-resistant status of most commonly cultured freshwater, fish such as Tilapia mozambique (Oreochromis mossambicus), African catfish (Clarias

gariepinus), trout (Oncorhynchus mykiss), and cyprinids (Labeo rohita, Cyprinus carpio, and others) (Mbokane and Moyo, 2024). Mbokane and Moyo (2022) observed that the phytochemicals in papaya leaves increase immunological competencies and possess antibacterial properties in fish. Some studies have proposed that adaptation employing natural immune stimulants, like cyanobacteria, higher plants, and seaweeds, might be effective not only to prevent diseases but also in enhancing overall aquaculture production. Supplementation of fish diets with medicinal herbs increases growth performance, feed conversion ratio, immunity, disease resistance, and reproductive potential in Clarias gariepinus and Oreochromis mossambicus (Mbokane and Moyo, 2022).

Fish health experts and researchers have widely embraced the use of plant extracts as an alternative therapy for fish parasites. Nevertheless, some serious challenges need to be considered when utilizing plant extracts for fish health management. First, one has to analyze whether exposure to the extract is safe and if the extract works (Ribeiro et al., 2023). Secondly, the bioactivity in plant extracts has to be elucidated (Özil, 2023), and the conditions of extraction and storage of the extracts should be regulated. Presumably, for the treatment of fish diseases, it is necessary to use different types of extracts and methods (Mariappan et al., 2023). In addition to, efficiency, factors that have significance for the acceptability of the extract and its compatibility with other agents, as well as the dose and length of therapy, should also be taken into account (Harish et al., 2022). Medicinal plants have also been utilized as antibiotic and immunoprophylactic substitutes in aquaculture practices. Carotenoids, oligosaccharides, and anthocyanins have been applied to enhance the immune status of fish (Plaskova and Mlcek, 2023). The focus of this report is on the use of plant extracts in fish health management; however, some fish species, extract types, and application techniques require careful evaluation before use.

Traditional approaches to administering natural plant products for fish health management are considered to be more human, animal, and environmentally friendly. However, the indigenous knowledge and practices of plant extracts used in fish health management are gradually declining. Further, there is a need to conduct more research studies on this vital area of ethnoveterinary medicine, especially in Sri Lanka, where there has been documented evidence of the use of medicinal rice by indigenous people and other developed practices. To prevent further loss in this area, documentation of Indigenous knowledge of traditional medicines, species used in traditional medicines, and local partnership in the propagation of medicinal plants is also required (Pulkkinen et al., 2010; Kumar et al., 2022; Singh et al., 2022; Mariappan et al., 2023).

It is of great importance for future generations to preserve and utilize the limited traditional knowledge regarding the utilization of medicinal plants for animal health care. The current generation demonstrated to lack of interest in understanding this significant concern, which is ethically unacceptable. Limited by evolutionary constraints, the community cannot acquire complete knowledge and effectively disseminate it. Therefore, the conservation of indigenous knowledge requires proper documentation, identification of plant species, and herbal preparation. However, more scientific research is required to confirm the performance and effectiveness of medicinal plants to enhance the value of traditional fish health management practices (Mariappan et al., 2023). Improving key areas and fundamental components of traditional knowledge regarding medicinal plants in animal care systems will enable the preservation and long-term viability of this knowledge for future use (Chen et al., 2016; Jacob et al., 2024).

Challenges and limitations of ethnoveterinary medicine

Although ethnoveterinary manufacturing validation and standardization are still uncertain in low-income countries (Nodza et al., 2022), its affordability permits its use, even at the excessive costs of allopathic medicines and chemotherapy (Farnsworth, 2021). The widespread adoption of traditional medicinal plants for animal treatment is hindered by the lack of validation and standardization of conventional drug practices, particularly in low-income countries with extremely high livestock disease prevalence (Nwafor and Nwafor, 2022), even though these plants grow abundantly. The changing socio-economic and technological environment surrounding Gashaka Gumti National Park may compromise the preservation of knowledge about ethnoveterinary practices for controlling fish parasites (Dey et al., 2020; Kolarova et al., 2022).

Lack of scientific valid ation: To preserve and potentially utilize ancient healing methods, a merging of traditional knowledge and scientific validation is vital (Ouma, 2022). To prevent resistance, overuse, and contamination from evidence-based fish health treatments, demographic triangles should be defined, and drug delivery should be used carefully (Madrid et al., 2021). Biologists and scholars of conventional medicine learned from ethnoveterinary practices that Sphagnum moss (S. phoenix) effectively treats ulcerative lesions at the base of salmon saddle sores.

These sphagnum mosses, which exhibit antimicrobial properties against wound infections, serve as potential model chemotherapeutic and medicinal plants for disease treatment.

An aquaculture unit's exploration of traditional remedies for diseases could lead to advanced disease management solutions and alternative strategies for mitigating fish ailments (Rakesh et al., 2023). Despite the substantial validation of traditional medicinal plant knowledge for human diseases, there is still a minimal connection between scientific data supporting medicinal plant activities and traditional practices (Mthi et al., 2023). Preserving traditional knowledge within communities where it remains relevant. The integration of non-codified traditional systems of medicine, including local health traditions and ethnomedical practices, is urgently needed (Sukumaran and Keerthi, 2023). Goel and Srikanth (2023) stated that indigenous knowledge systems, which local use to sustainably manage plants, contribute to biodiversity conservation. Kola (2022) advocated conserving and recording indigenous knowledge for future use. Sardar and Giri (2022) conducted research on traditional medicine and plant utilization in the Sundarban mangrove forest and positioned them for future scientific investigations within a Natural Tropical Area (NTA) inhabited by ethnic groups. Due to urbanization and migration, the validation of tribal knowledge and remedies has become challenging (Ouma, 2022). The integration of traditional knowledge with modern scientific validation is essential to conserve and possibly harness ancient remedies.

Ethnoveterinary medicine and ethnopharmacognosy have focused on medicinal plants from diverse cultural backgrounds (Grundmann et al., 2023). Traditional medicinal plants have been validated for treating some human diseases, according to Mthi et al. (2023). Preserving traditional pharmaceutical knowledge from natural heritage areas like the Himalayas is crucial for human health in the face of industrialization and urban development (Chebii et al., 2023). In developing countries, there is a significant need to conserve medicinal plants (Shaheen et al., 2023). Ex-situ conservation of medicinal plants through a global strategy is crucial for preserving traditional medicine and authenticating ethnomedicinal plant information (Clair et al., 2023). Recording traditional plant remedies is indispensable because they represent the ordinary origins of drug production and drug access (Jha and Mughees, 2023). Ex-situ and in-situ methods should be applied to the conservation of medicinal plants for this era (Devi et al., 2023).

Availability and sustainability of medicinal plants: Novra et al. (2023) reported the opportunities that exist in rural economic development through the cultivation of medicinal plants, as they are rich source plants and also due to their abundance in nature; sustainability is economically significant. With the loss of medicinal plant habitats as a result of environmental changes, habitat destruction, and economic demand, many global communities recognize the need to act. Unsustainable harvesting, industrialization, and human lifestyle have all, which have altered the fish scarcity we face today (Shaheen et al., 2023; Shukla, 2023). One of the principal reasons for concern is that there are profound increases in extinction rates among medicinal plants because human interference has a multiple impact, such as habitat destruction and over-exploitation leading to rapid climate change (Novra et al., 2023).

For several medicinal plant resources, a combination of in-situ and ex-situ conservation strategies is needed to achieve sustainable use (Mofokeng et al., 2022; dos Santos et al., 2023). The best way to maintain medicinal plants and ensure their health benefits for future generations is through cultivation, conservation, and biotechnological utilization. To combat the crisis for future generations, strategies like in-situ and ex-situ conservation efforts using conventional cultivation practices as well as sustainable management of resources have been put into place (Ndawonde, 2022; Halder and Jha, 2023). Conservation measures and the adoption of sustainable harvesting methods are the means to avoid overharvesting, thus ensuring that medicinal plants are accessible for medical treatment.

Kola (2022) also agreed that traditional indigenous skills have a high risk of coming under extreme threat due to the reduction in population among Indigenous communities, forced migrations caused by deforestation, and acculturation. Indigenous knowledge systems are important for the sustainable utilization and preservation of medicinal plants in traditional medicinal practices. The conservation of indigenous medicinal practices can place some vulnerable species, like traditional tribal rare plants, at higher risk of extinction (Ouma, 2022). The erosion of traditional knowledge due to changing lifestyles makes documentation and protection more important than a welfare measure (Sukumaran and Keerthi, 2023). In place of viewing nature as a deficient purveyor that must be fixed and set right, conservation might consider how to better manage the world we inherit so it can continue to circulate among options (Anand et al., 2023).

Regulatory and legal considerations: A report by the Department for International Development revealed a significant lack of a regulatory category for traditional remedies in India and rules governing veterinary pharmaceuticals, thereby restricting the development of products accessible to livestock owners with limited resources. Ethnoveterinary practices are widely used in rural areas of Indian states such as Haridwar, Jammu, and Kashmir, as well as in the northern laterite regions of the country. The primary botanical market emphasis of this proposal represents just one of the broader market opportunities for these types of medications: the optimization of the existing inventory of underutilized medicinal plants. The culture conservatory in floristic taxonomy heavily depends on the preservation of indigenous plant conservation efforts. The use of plants as medicine for livestock is especially crucial in areas where veterinary services are scarce or non-existent, with several studies (Sharma et al., 2022; Wani et al., 2022; Mandal, Sand, & Rahaman, 2022; Dutta et al., 2022) supporting this necessity.

As Claire et al. (2023) pointed out, traditional remedies should be placed within a separate regulatory scope, which will lessen the burden of documentation, approval, and control of importation. Such a comprehensive management strategy that incorporates all these aspects without compromising ownership appears to be an ideal short-term strategy. To incorporate herbal and other unconventional therapies into the veterinary profession, policy reforms need to be implemented (Remirez, 2022). It is unlikely that this will happen soon. Prioritize equal treatment for different cultures and simplify regulations to effectively integrate traditional remedies.

The policies that govern ethnovehicle tend to overemphasize pharmaceutical treatment rather than the use of ethnopathic methods (Varshney et al., 2022). Smallholders and traditional medicine practitioners may lack the means to meet such stringent provisions because of restrictions on the accurate dosage of every treatment, including herbal medicine, and the requirement for treatments to be target-specific (Chitra and Arivoli, 2022). There is a demand from regulatory agencies for provisions of both quality and the market for traditional and complementary medicine (Kumar et al., 2022). There is a need for regulations that do not restrict the management of small-scale livestock keeping and allow for the protection of traditional practices in the use of ethnoveterinary medicine (Jarvis, 2022).

Ethno-cure, in a way, blends the modern treatment with the sacred cure. In this regard, traditional healing techniques such as the religious and mystic approaches should have complied because they are part of the cultural heritage that ought to be legally protected and that has health benefits for society (Nirmal et al., 2022). Even though ethnoveterinary approaches, provide effective solutions for animal disease treatment, thus less antibiotic abuse and progression of novel therapies of drug development for humans are possible (Varshney et al., 2022).

Future directions and research opportunities

The future direction of medicinal plant research is to look at complex biological regulation networks through multi-omics studies (Yang et al., 2023). By employing plant tissue culture techniques and elicitors, we can enhance bioactive metabolism production in vitro. Focusing on systematic investigations, spatial and temporal studies, and the exploration of core microbiomes is essential for sustainable agriculture research on medicinal plant microbiomes (Peter and Sharangi, 2022). Biotechnological interventions such as plant tissue culture, genetic modification, and metabolic pathway engineering are transforming medicinal plant research, supporting conservation, and addressing concerns related to habitat destruction and genetic diversity loss (Wang et al., 2022). Through these strategies, it is possible to study plant metabolites using innovative methods, increase bioactive compound yields, and promote eco-friendly medicinal plant applications.

To ensure quality and consistency in developing standardized products, analyses using techniques such as thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), spectrophotometry, and standard samples are paramount, especially with the increased interest in herbal medicinal products (Castka, 2020; Shchepochkina et al., 2020; Kurkin, 2022). It has also been indicated that standard samples for drug standardization ensure the quality of the drugs, especially with the significant rise in the use of herbal medicinal products (Shchepochkina et al., 2020). Initiatives have begun to focus on culturing plants in controlled environments with local substitutes to resolve differences in potency caused by differing environmental factors. Sustainability practices that focus on and support traditional knowledge can support sustainable practices, thereby ensuring that the use of plants as treatment methods are being followed (Aronov et al., 2019). Again, incorporating medicinal plants into fish parasite treatments becomes better, transparent, quality-assured, and conforms to ethical treatment practices.

Traditional healers identifying location-based cures indicate anti-parasitic plants from existing species of plants (Kumar et al., 2019). The understanding of

traditional healers from several locations worldwide has the potential to identify anti-parasitic plants for further drug development (Ranasinghe et al., 2023). Focusing on a plant family known for anti-parasitic properties is potentially a cost-effective approach for drug discovery, whereas molecular breeding and genomic approaches increase the discovery of new targets for medicinal plant treatment. The Eastern Himalayan region, particularly northeast India, can provide ample opportunities (Singh et al., 2019; Adhami et al., 2018). This could allow the discovery of anti-parasitic medications from neglected plant species. Exploration of untapped medicinal plants: Aquatic animals from the 21st century provide important sources of premium animal protein. The expansion of aquaculture promotes the creation of nutritionally complete, cost-effective, and ecological aquatic feeds (Kumar et al., 2024). Biotechnological tools are revolutionizing fish production, increasing the nutritional value of fish products, providing food security with premium animal proteins, and having potential industrial applications (Glencross et al., 2023; Cropotova et al., 2023). The growing global need for protein sources has led to increased interest in the sustainable use of underutilized seafood resources (Han et al., 2022). It accounts for 15% of all animal protein consumed worldwide and exceeds 50% in some underdeveloped countries (Issifu et al., 2022). Aquaculture plays an important role in meeting food needs while supporting sustainable food systems (Cropotova et al., 2023). Environmental concerns, including resource overuse and greenhouse gas emissions, limit the sustainability of global aquaculture (Jiang et al., 2022). Addressing sustainability in aquaculture requires cross-sectoral governance and policy interventions (Viji et al., 2018). Aquaculture can sustain growth and ensure global food security through sustainable practices and innovative solutions (Pradeepkiran, 2019).

Botanical antiparasitics are reported to control gastrointestinal parasites in fish and other organisms and represent a new chemotherapy for parasitic infections (Saxena, 2023). Ultrasonically assisted extraction (UAE) and microwave-assisted extraction (MAE) are the two most advanced modern extraction techniques that can be used to efficiently isolate bioactive molecules (Dar et al., 2023). Many plants have been used for years to treat parasites in humans and other animals, but few have been extensively studied and documented for use in fish. More than 1,500 European plant species have been used in traditional herbal medicine, but many treatments are reserved only for local herbalists, and their studies are limited. It would be possible to study thousands of plants to identify other anti-parasite plants, but little research has been conducted in this area. Many recent studies have emphasized the need to study medicinal plants for antibacterial, antifungal, and antiprotozoal activities that could lead to the treatment of human diseases (Jamil et al., 2022; Ranasinghe et al., 2023; Suaza-Gaviria et al., 2023).

In addition to being useful for ecosystem management, traditional ecological knowledge (TEK) also contributes to the global conservation of environmental ecosystems, particularly through practices transmitted by traditional fishers (Hartel et al., 2023). Indigenous knowledge effectively contributes to the conservation of genetic resources of wild fish and increases the productivity of aquaculture (Obiero et al., 2023). The traditional knowledge of fishing communities in the management of marine resources highlights the importance of customary practices, including fishing rights and maritime tenure, for effective fisheries management and the valorization of traditional knowledge (de Sousa et al., 2022). Medicinal plants are gradually replacing antibiotics in aquaculture because of their safety and effectiveness in boosting immunity (Lako et al., 2023). Community service projects in aquaculture have demonstrated the effectiveness of herbal probiotics, while some medicinal plants, such as garlic, ginger, turmeric, and green tea, have antioxidant and immune properties that support fish health and ecological aquaculture practices (Soeprapto et al., 2022; Mariappan et al., 2023). Collaboration with local communities and indigenous knowledge holders presents valuable opportunities for ethnoveterinary research to assess the effectiveness of conventional herbal medicines in improving fish welfare.

Development of standardized plant products: Fish parasites are a major concern for fish health and fish farm performance (Castro et al., 2023; Ghorbani and Garedaghi, 2023). Chemical drugs can kill these parasites, but can also harm the environment. Instead, the use of natural plant-based substances is safer for controlling fish parasites (Dezfuli and Scholz, 2022; Buchmann, 2022). Ahmad et al. (2023) and Castro et al. (2023) reported that plant compounds, such as tannins, alkaloids, phenols, and saponins can fight many fish parasites. Proteases from fruits, such as figs, pineapples, papayas and kiwi can also help control animal parasites, bugs, and worms that damage plants (Özil, 2023). Although these plant substances have great potential, only a few have been extensively studied for their ability to fight parasites (Liu et al., 2023). Few studies have examined the effectiveness of

herbal preparations against fish parasites. Further studies are needed to maximize the benefits of plant-based parasite control in fish as alternatives to synthetic drugs and pesticides that provide safety and convenience.

Concerns are growing about the problem of antibiotics' poor performance and their impact on the environment. This has led to increased interest in using plants to produce natural medicines to control fish parasites (Özil, 2023; Ribeiro et al., 2023). Finding the right parts of these natural products to make good antiparasitic drugs is important, but it can take a long time and costs (Geisshirt et al., 2023). Scientists need to find new methods to use plants as medicines for fish parasites by studying plant parts that can fight these parasites (Ranasinghe et al., 2023). Fish farms can reduce the use of antibiotics and vaccines by using active plant parts, such as essential oils and other natural substances as a safe and effective way to control parasites. Medicinal plants, which are full of active substances, have been proven to keep fish healthy, help them grow, allow them to better cope with stress, and prevent diseases (Singh et al., 2022). These plants provide fish with immune-boosting and antioxidant benefits for less money and with less harm than usual treatments, which helps fish remain healthier overall (Nunez et al., 2022). Because more people are buying herbal medicines, there is not much information about plants that can fight parasites in fish, which is stopping them from being used more in fish farming. More research is needed on the use of medicinal plants to fish healthy because this is still a new idea.

Collaborative research and sharing the knowledge:

Understanding the importance of traditional methods in controlling animal parasites, the World Association for the Advancement of Veterinary Parasitology is working together to bridge the gap between veterinarians and traditional animal care practitioners (Riyaz and Ignacimuthu, 2023). These traditional practices, which are part of local customs and have been taught from one generation to the next, provide useful information on the use of medicinal plants for animal health (Güneş et al., 2022). Herbal treatments help manage different health issues associated with these practices (Wani et al., 2022). It's important to maintain and mix traditional animal healing knowledge with current veterinary methods to improve animal care in rural areas.

Working with traditional animal health workers and veterinarians is important for confirming the value of traditional knowledge and developing treatments for animals that are based on evidence. Through clinical trials, this partnership can evaluate the effectiveness of traditional herbal treatments (Nwafor and Nwafor, 2022). Knowledge passed down through generations from indigenous communities plays a significant role in livestock disease treatment (Gandasari et al., 2023). Documenting and verifying traditional practices such as the use of turmeric and cinnamon to enhance poultry care could improve farming methods (Sujeetha and Ashokan, 2022). It is crucial to preserve and share indigenous knowledge on the diagnosis and treatment of cattle diseases (Asefa et al., 2022).

Encouraging cooperation between those who hold traditional knowledge and veterinarians can help manage animal parasites, which is beneficial for both developing and developed countries. There is an increasing worldwide interest in traditional plant-based therapies (Casagrande et al., 2023). In many cultural groups, traditional medicine is very important (Musa et al., 2023). To combine traditional plant treatments with scientifically proven therapies, we need to work together and share information (Scherrer et al., 2023). Teaching about the environment is important for keeping knowledge about traditional medicinal plants alive for future generations, according to research (Yusransyah et al., 2023). The importance of connecting traditional knowledge with scientific understanding to create effective plant-based treatments is becoming increasingly evident as research in this area grows (Singh, 2022). Bringing together and sharing traditional knowledge can help improve scientifically supported plant-based therapies.

Conclusion

Using plants to treat fish diseases has been successful in traditional farming methods. This plant extracts, which have been used for generations to fish healthy in fish farms, represent a valuable resource. It is important to keep these traditional health practices alive so that future generations can benefit from these natural health care systems. Farmers can determine the best plant-based treatment for specific fish parasites by trying different methods, even though the effectiveness of these plants can change due to different factors like the type of active substance when they are available, how they are prepared, and the amount used. More research is needed to understand how these plant treatments work and the effects they have on the environment. More studies need to be conducted to decide whether medicinal plants should be used as purified substances or as live extracts and to find the best way to give them to animals. It is also important to acknowledge the traditional knowledge of animal care that has been passed down through generations. It is suggested that using medicinal plants will benefit fish health and reduce the need for chemical treatments.

Author Contribution

A single author completed all the studies (literature searches and edited and reviewed the manuscript).

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Conflicts of Interest

The author declares no conflict of interest.

Acronym/Abriviations

CPs: Cysteine proteases,; HPLC: high-performance liquid chromatography; MAE: Microwave-assisted extraction; NTA: Natural Tropical Area; TEK: Traditional ecological knowledge ; TLC: Thin-layer chromatography; UAE: ultrasound-assisted extraction.

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Hematological and blood biochemical differences in dogs with mammary dysplasia/ hyperplasia, bening or malignant mammary tumors

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ABSTRACT

Mammary tumors are the most common neoplasms in female dogs. There are many factors involved in the development of mammary tumors. Recently, there is an increasing need for cost-effective prognostic markers derived from hematological parameters in dogs and cats with cancer. Our study was designed as a retrospective study to determine the significance of hematologic parameters in female dogs bearing with mammary tumors. For this, hematocrit (HCT), blood biochemistry, and some clinical and histopathological findings of female dogs with mammary gland masses that had undergone mastectomy (n = 100) were included in our study. Mammary masses in female dogs are divided into five histopathological groups: Group 1 (malignant epithelial tumors), Group 2 (malignant mesenchymal tumors), Group 3 (malignant mixed tumors), Group 4 (benign tumors) and Group 5 (hyperplasia/dysplasia). The following hematologic parameters were evaluated: leukocytes (WBC), neutrophils (NEU), lymphocytes (LYM), monocytes (MON), eosinophils (EOS), basophils (BAS), erythrocytes (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cell distribution width (RDW), platelets (PLT),mean platelet volume (MPV), and neutrophil to lymphocyte ratio (NLR). Biochemical analysis included blood urea nitrogen (BUN), creatinine (CRE), alanine aminotransferase (ALT), glucose (GLU), total protein (TP), alkaline phosphatase (ALP), and albumin/globulin ratio (AGR). Comparisons were made between the groups according to histopathological tumor types. The most common histopathological type after mastectomy is malignant epithelial tumors (72%). For TP, a difference was found between G1 and G5 ($p<0.05$). For MON, a difference was found between G1 and G5, between G2 and G5, between G3 and G5, between G1 and G4, between G3 and G4 ($p<0.05$). For PLT, a difference was found between G2-G3 and G3-G4 ($p<0.05$). Although NLR was different between the groups and gets higher values with malignancy no statistical significance was found between the groups ($p>0.05$). While our study revealed some potential associations between hematologic parameters and histopathological tumor types, further studies with a larger and more diverse canine population are needed before these parameters can be reliably used as prognostic markers in female dogs with mammary masses.

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Introduction

Mammary tumors are the most common neoplasms (%50 of all tumors) in female dogs (Ariyarathna et al., 2020). While 80-90% of mammary tumors in cats are malignant, the malignancy in dogs is 50% (Kırşan and Canooğlu, 2016). Most of the mammary tumors in female dogs are of epithelial origin (Namagerdi et al., 2020). Female dogs with spontaneous mammary tumors have a variety of epidemiological, clinical, biological and genetic characteristics similar to those of women. For this reason, some researchers

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recommend using these canine mammary tumors as models for comparative studies with humans (Estrela-Lima et al., 2010). The development of mammary tumors in female dogs is influenced by a complex interplay of factors, including breed predisposition, dietary habits, advanced age, environmental influences, neutering status, pseudo pregnancies and radiation exposure (Marquardt, 2003). The incidence of mammary tumors in female dogs increases significantly with age, while benign tumors can also occur at a younger age. The incidence of mammary tumors in female dogs is significantly influenced by the time of spaying. In prepubertal spayed bitches, the risk is remarkably low at 0.5%. However, the risk increases substantially to 8% for dogs spayed after their first estrus cycle and further rises to 26% for those spayed after their second estrus cycle (Egenvall et al., 2005). Although rare, mammary tumors can also occur in male dogs (Kırşan and Canooğlu, 2016). While the incidence of mammary tumor is generally higher in small breeds than in large breeds, certain large dog breeds also have a significant risk (Sorenmo et al., 2013). Several purebred dog breeds have a significantly higher predisposition to mammary tumors compared to other breeds. These breeds include the English Springer Spaniel, the Cocker Spaniel, English Setter, Pointer, German Shepherd, Maltese, Yorkshire Terrier, Dachshund, Puli, Toy Poodle and Miniature Poodle (Moe L, 2001).

There is a growing need for the identification of cost-effective prognostic markers derived from hematologic parameters in dogs and cats with neoplastic or inflammatory diseases. Cancer-related anemia, a common complication observed in patients with various types of tumors, can adversely affect prognosis and response to treatment. In addition to cancer-related anemia, a range of biochemical abnormalities have been found in oncology patients, including hypercalcemia, hypoglycemia, hypoproteinemia, and elevated levels of total alkaline phosphatase and the corresponding isoenzymes. A study by Oliveira et al. (2022), which examined female dogs with mammary tumors, found that the most common hematological and biochemical changes were normocytic normochromic anemia, neutrophilic leukocytosis, monocytosis, elevated ALT and AST and hypoalbuminemia. A comparative analysis of hematological parameters in dogs with benign and malignant mammary tumors revealed that anemia was the most significant abnormality in the benign group, while the malignant tumors were characterized by leukopenia, predominantly neutropenia, thrombocytosis and hyperproteinemia (Lallo M.A et al., 2016). Red blood cell distribution width (RDW), neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR), determined as part of routine blood count analysis, have been shown to be significant biomarkers related to systemic inflammation and cancer biology, providing valuable insights for cancer detection, progression and survival prognosis (Divsalar et al., 2021). Another study conducted in dogs with lymphoma, osteosarcoma, mast cell tumors and soft tissue sarcoma has shown that WBC and NLR provide prognostic information (Petrucci et al., 2021). In dogs with mast cell tumors and soft tissue sarcomas, higher NLR was associated with more aggressive tumors. In cats with injection-site sarcomas, WBC and NLR have been shown to be prognostic factors for local recurrence (Petrucci et al., 2021). In a study on dogs, it was found that a high pre-treatment NLR value (NLR > 5) was associated with a lower survival rate. However, AGR (Albumin/ Globulin Rate) was not shown to have a significant impact on tumor malignancy and it is hypothesized that NLR could be used as a prognostic marker for disease severity (Uribe Querol et al., 2023). Breast tumors that are Estrogen receptor (ER), Progesterone receptor (PR) and Human epidermal growth factor receptor 2 (Her-2) negative are referred to as Triple Negative Breast Cancers (TNBC), and a high rate of NLR has been shown to be associated with recurrence and poor prognosis (Petrucci et al. al., 2021). The prognostic utility of routinely determined hematologic and biochemical parameters in oncologic patients in veterinary medicine remains poorly understood. The aim of our study is investigate the presence of significant differences in clinically assessed hematologic and biochemical serum parameters in female dogs with malignant mammary tumors, benign mammary tumors and hyperplasia/dysplasia.

Materials and Method

Patients

Female dogs with mammary masses (n = 100) aged between 3-17 years, who applied to Istanbul University -Cerrahpaşa Faculty of Veterinary Medicine, Obstetrics and Gynecology Clinic between 2018 and 2022, were included in our study. Our retrospective case study includes the comparison of blood tests performed before mastectomy in female dogs with the histopathological results of mammary tissue taken after mastectomy. The female dogs were divided into five groups based on their mammary gland pathology (Goldschmidt et al., 2011): female dogs with malignant epithelial tumors (Group 1, n = 72), female dogs with malignant mesenchymal tumors (Group 2, n = 9), female dogs with malignant mixed tumors (Group 3, n =

5), female dogs with benign tumors (Group 4, n = 7) and female dogs with hyperplasia/dysplasia (Group 5, n = 7). The breed distribution of the female dogs included in the study was as follows: mixed breeds (n=20), Cocker Spaniel (n=18), Terrier (n=18), Golden Retriever (n=14), Yorkshire Terrier (n=5), Pinscher (n=3), Cavalier King Charles Spaniel (n=3), Beagle (n=3), Russian Fino (n=2), Pekingese (n=2), Boxer (n=2), German Shepherd Dog (n=2), Setter (n=1), Rottweiler (n=1), Labrador Retriever (n=1), Sivas Kangal (n=1), Jack Russell Terrier (n=1), French Bulldog (n=1), Chow Chow (n=1) and Akita Inu (n=1). The mean age and neuter status for each group are shown in Table 1. Written or verbal informed consent was obtained from dog owners for the use of their dogs' blood and tissue results. As our study is a retrospective study, ethics committee approval was not required.

Blood Samples

Blood samples are routinely taken from female dogs that are brought to our hospital with the complaint of a mass in the mammary gland and are subsequently scheduled for mastectomy. For this purpose, blood is taken from the V. cephalica antebrachii into EDTA-containing tubes for the hemogram and into heparin-containing tubes for the biochemical analysis of the serum. Blood tests were performed in the central laboratory of the university. The data of the female dogs included in our retrospective study were obtained from the laboratory's information system. For complete blood count, the results obtained from the hemogram device (Procyte Dx Hematology Analyzer, Idexx, USA) and biochemistry device (DRI-CHEM NX600, Fujifilm, Japan) in the laboratory were taken from the laboratory information system and used. The following parameters in the hemogram results were included in the study: leukocyte (WBC), neutrophil (NEU), lymphocyte (LYM), monocyte (MON), eosinophil (EOS), basophil (BAS), red blood cells (RBC), hemoglobin (HGB), hematocrit (HCT), mean erythrocyte volume (MCV), mean erythrocyte hemoglobin (MCH), mean erythrocyte hemoglobin concentration (MCHC), red blood cell distribution width (RDW), platelets (PLT) and mean platelet volume (MPV). In the biochemistry results, the following parameters were included in the study: Blood urea nitrogen (BUN), creatinine (CRE), alanine aminotransferase (ALT), glucose (Glu), total protein (TP), albumin (ALB) and alkaline phosphatase (ALP). To high GLU levels were excluded from the groups for the correct statistical results of ANOVA. The neutrophil-to-lymphocyte ratio (NLR) was calculated by dividing the total number of neutrophil by the total number of lymphocyte. The albumin-to-globulin ratio (AGR) was calculated by dividing the albumin concentration by the globulin concentration (Uribe Querol et al., 2023).

Histopathology

The histopathology results of the female dogs included in our retrospective study were obtained from the hospital's automation system and used. Routinely, mammary tissue examinations of dogs that have undergone mastectomy in our faculty are carried out in the laboratory of our university's Department of Pathology. Briefly, for histopathology, mammary tissue samples taken from mastectomies are fixed in 10% buffered formalin solution for 24 hours and embedded in paraffin blocks. Embedded tissue and cell block samples were processed using routine tissue processing procedures, and 5-µm-thick serial sections were prepared from all samples using a rotary microtome. Section samples from each mammary tissue and lymph node sample are stained with hematoxylin-eosin (H&E) and evaluated under a light microscope. As a result of examining the tissues stained with H&E under a light microscope, the mammary gland tissues of the bitch were histopathologically classified into five groups (Figure 1). In female dogs with multiple mammary tumors, all comparisons were based on histopathological result of primary tumor.

Statistical Analysis

All data analyses were performed using IBM SPSS version 27.0. The categorical and continuous variables were expressed using descriptive statistics (mean±standard deviation). In addition, the Levene's test was used to check the homogeneity of variance, a prerequisite for parametric tests. The normality assumption was verified using the Kolmogorov-Smirnov and Shapiro-Wilk test. The groups (group 1-5) were added to the statistical model as between-

Table 1. Mean age and sterilization situations of the groups.

Variables	G1	G2	G3	G4	G5	Total
N	72	9	5	7	7	100
Age (Mean ± SD)	10.35 ±2.87	10.55 ±3.43	10.6 ±0.48	8.92 ±2.17	7.21 ± 2.74	10.06 ±2.93
OVH +	16 (22.22%)	2 (22.22 %)	0 (0 %)	3 (42.85 %)	1 (14.28 %)	22 (22 %)
OVH -	56 (77.78%)	7 (77.78 %)	5 (100 %)	4 (57.15 %)	6 (85.72 %)	78 (78 %)

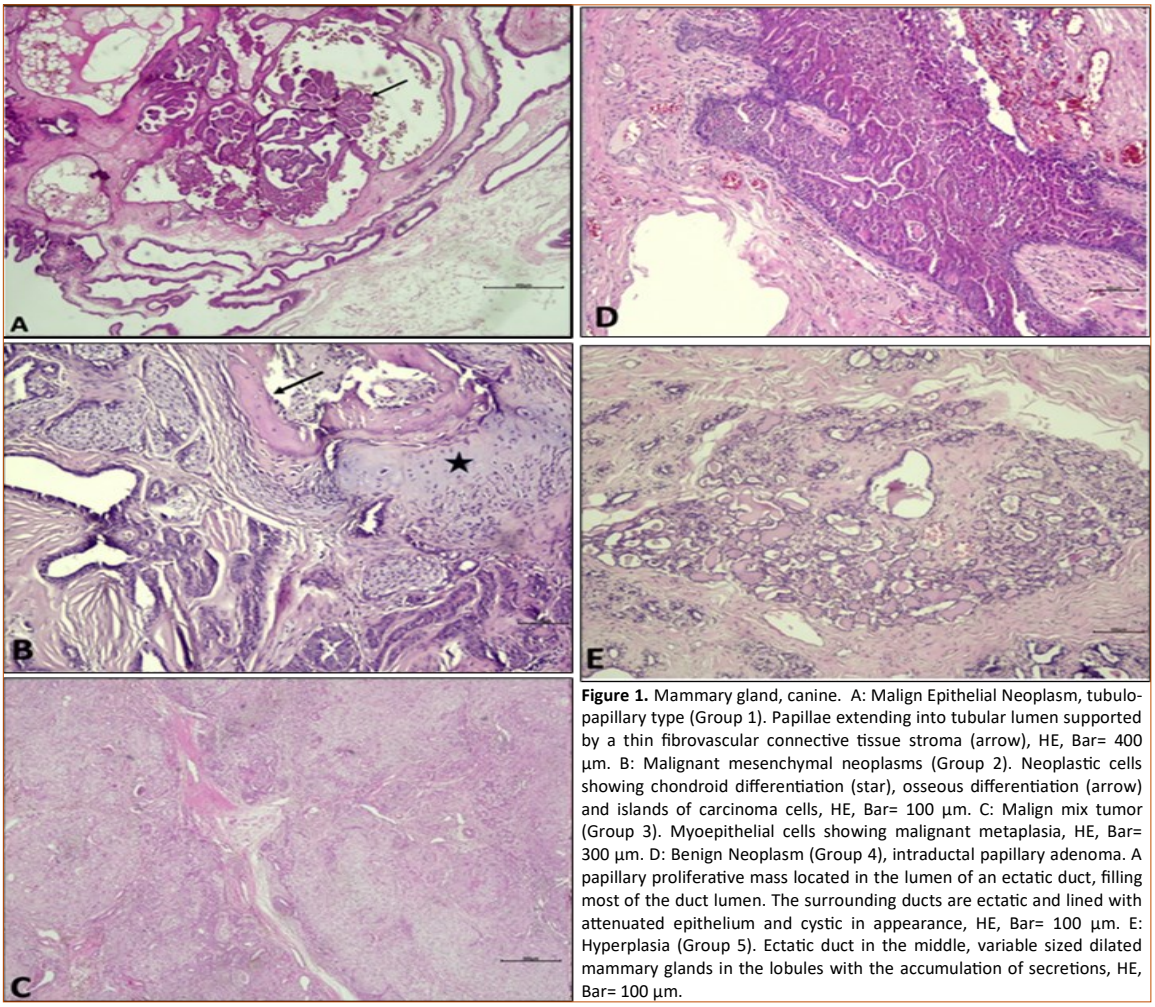

Figure 1. Mammary gland, canine. A: Malign Epithelial Neoplasm, tubulo-papillary type (Group 1). Papillae extending into tubular lumen supported by a thin fibrovascular connective tissue stroma (arrow), HE, Bar= 400 µm. B: Malignant mesenchymal neoplasms (Group 2). Neoplastic cells showing chondroid differentiation (star), osseous differentiation (arrow) and islands of carcinoma cells, HE, Bar= 100 µm. C: Malign mix tumor (Group 3). Myoepithelial cells showing malignant metaplasia, HE, Bar= 300 µm. D: Benign Neoplasm (Group 4), intraductal papillary adenoma. A papillary proliferative mass located in the lumen of an ectatic duct, filling most of the duct lumen. The surrounding ducts are ectatic and lined with attenuated epithelium and cystic in appearance, HE, Bar= 100 µm. E: Hyperplasia (Group 5). Ectatic duct in the middle, variable sized dilated mammary glands in the lobules with the accumulation of secretions, HE, Bar= 100 µm.

-subjects effects, and a one-way ANOVA and Tukey's HSD (as a post hoc test) tests was performed to compare mammary tumor disease on each hematological parameter between the groups. Additionally, the Kruskal-Wallis test was used in cases where the assumptions were not valid. A value of $p < 0.05$ was used to indicate statistical significance.

Results

Data on hematologic and blood biochemistry results for each group are shown in Table 2 and Table 3, respectively. An increased mean MON count was observed in Group 2 (Table 2). MCV value was below the normal reference range in Group 2 (Table 2). PLT was above the normal reference range in Group 3 (Table 2). In addition to the average hematology values, the WBC count was found to be above the reference range in ten female dogs in Group 1 and in three female dogs in Group 2. Despite an increased mean MON value in Group 2, MON values above the reference range were found in 21 female dogs in Group 1. LYM levels were above the normal reference range in only two female dogs in Group 1. While the mean NEU value was within the reference range in all groups, NEU values above the reference range were found in 19 female dogs in Group 1 and in two female dogs each in Group 2 and Group 3. Although the mean RBC value was within the reference range in all groups, nine female dogs in Group 1 and two female dogs in Group 2 had RBC values below the normal reference range. While mean HGB and HCT values were within the reference range in all groups, HGB values were found to be below the normal reference range in nine female dogs in Group 1 and four female dogs in Group 2. Similarly, HCT levels were below the normal reference range in 12 female dogs in Group 1 and five female dogs in Group 2. Despite an decreased mean MCV value in Group 2, 15 female dogs in Group 1, three female dogs in Group 3, a female dog in Group 4 and 2 female dogs in Group 5 had MCV values below the normal reference range. While the mean PLT value was elevated in Group 3, 12 female dogs in Group 1, two female dogs in Group 2 and a female dog each in Group 4 and Group 5 had PLT values above the normal reference range. While the mean values for all biochemical variables in the blood were within the normal reference range in all groups, the BUN and CRE levels were below the normal reference range in ten female dogs in Group 1. ALT levels were above the normal reference range in six female dogs in Group 1 and a female dog in Group 5. ALP levels were above the normal reference range in six female dogs in Group 1, two female dogs in Group 2 and a female dog in Group 3. The TP levels were above the normal reference range in eight female dogs in Group 1. GLU levels were below the normal reference range in a female dog in Group 1 and above the normal reference range in a female dog in Group 2.

Table 2. Data of the hemogram variables and NLR (Neu/ Lym) values of the groups.

Parameters	G1	G2	G3	G4	G5	Reference Ranges	P
WBC (K/µL)	13.37 ±11.52	15.64 ±8.36	12.62 ±2.95	8.79 ±3.46	9.18 ±1.87	5.05-16.76	0.126^{K}
Lym (K/µL)	2.22 ±1.53	2.18 ±0.99 ^{b}	2.22 ±0.25	1.56 ±0.48	1.90 ±0.66	1.05-5.10	0.296^{K}
Neu (K/µL)	9.61 ±9.59	11.48 ±6.45	8.32 ±2.11	6.22 ±2.76	6.00 ±1.48	2.95 - 11.64	0.191^{K}
Mon (K/µL)	1.07 ±0.74	1.40 ±1.46	1.05 ±0.28	0.59 ±0.29	0.59 ±0.15	0.16 - 1.12	**0.025**^{K}
Bas (K/µL)	0.02 ± 0.02^{a}	0.02 ± 0.02^{a}	0.00 ± 0.00^{a}	0.02 ± 0.01^{a}	0.07 ±0.11	0.00 - 0.10	0.368^{K}
Eos (K/µL)	0.42 ±0.28	0.54 ±0.52	0.84 ±0.60	0.39 ±0.22	0.60 ±0.33	0.06 - 1.23	0.299^{K}
RBC (K/µL)	6.73 ±0.96	6.44 ±1.24	7.45 ±0.84	7.01 ±0.35	6.75 ±0.98	5.65 - 8.87	0.427^{A}
HGB (g/dL)	15.61 ±2.16	14.21 ±2.91	16.7 ± 2.03	16.24 ±1.04	15.57 ±1.60	13.1 - 20.5	0.256^{A}
HCT (%)	43.26 ±6.68	39.22 ±8.54	46.94 ±6.19	45.07 ±3.62	40.91 ±6.73	37.3 - 61.7	0.227^{A}
MCV (fL)	64.14 ±3.49	60.73 ±3.46	62.88 ±3.08	64.25 ±3.19	63.00 ±2.73	61.6 - 73.5	0.086^{A}
MCH (pg)	23.21 ±1.39	22.03 ±1.61	22.40 ±0.65	23.15 ±1.25	23.17 ±1.32	21.2 - 25.9	0.156^{A}
MCHC (g/dL)	36.18 ±1.56	36.30 ±2.12	35.64 ±0.86	36.08 ±1.26	36.78 ±0.76	32.0 - 37.9	0.796^{A}
RDW (%)	16.64 ±2.46	16.48 ±3.14	17.80 ±1.51	16.25 ±1.69	15.21 ±1.20	13.6 - 21.7	0.466^{A}
PLT (K/µL)	364 ±120.74 ^{a,b}	271 ±150.29 ^{a}	494 ±155.65 ^{b,A}	236 ±117.27 ^{a,b,B}	312.8 ±43.7 ^{a,b,A,B}	148 – 484	0.005^{A}
MPV (fL)	10.05 ±2.32	11.43 ±3.54	10.58 ±1.35	10.74 ±1.37	9.41 ±1.50	8.7 - 13.2	0.446^{A}
NLR (Neu /Lym)	4.48 ± 2.84	5.80 ±3.38	3.74 ±0.86	4.05 ±1.15	3.43 ±1.24		0.500^{K}

BAS: Basophil; EOS: Eosinophil; HCT: Hematocrit; HGB: Hemoglobin; LYM: Lymphocyte; MCH: Average Amount of Erythrocyte Hemoglobin; MCHC: Mean Erythrocyte Hemoglobin Concentration; MCV: Mean Erythrocyte Volume; MON: Monocyte; MPV: Average Platelet Volume; NEU: Neutrophil; NLR: Neutrophil-to-Lymphocyte Ratio; PLT: Platelet; RBC: Erythrocyte; RDW: Erythrocyte Distribution Width; WBC: White Blood Cells; a,b,A,B: Different superscript letters on the same row indicate statistically significant difference (P<0.05). ANOVA, K: KRUSKAL-WALLIS

The one-way ANOVA revealed that there was a statistically significant difference in the mean value of TP levels between G1 and G5 (p<0.05). For PLT, a difference was found between G2-G3 and G3-G4 (p<0.05). Kruskal-Wallis revealed that there was a statistically significant difference in the test mean values of MON levels between G1-G5, G2-G5, G3-G5, G1-G4 and G3-G4 (p<0.05). Although NLR was different between the groups and gets higher values with malignancy no statistical significance was found between the groups (p>0.05).

The mastectomy revealed that malignant epithelial tumours were the most common histopathological type (72%) among the histopathological findings obtained. The most common histopathological subtype was complex carcinoma (13%), followed by tubular carcinoma (8%) and tubulopapillary carcinoma (7%). Table 4 shows the types of mammary tumors found in the dog breeds included in the study.

Table 3. Data of the biochemical variables of the blood belonging to the groups.

Parameters	G1	G2	G3	G4	G5	Reference	P
ALB (g/dL)	3.29 ±1.24	2.811 ±0.37	3.26 ±0.38	3.14 ±0.25	3.27 ±0.27	2.2 – 4.0	0.160^{K}
ALP (U/L)	83.63 ±53.61	72.57 ± 30.59	97.75 ±35.78	70.42 ±29.87	44.28 ±12.88	23 – 212	0.121^{K}
ALT (U/L)	60.77 ±40.77	42.44 ±20.65	68 ±8.62	53.14 ±28.28	62.71 ±30.11	10–125	0.130^{K}
BUN (mg/dL)	13.82 ±5.62	17.84 ±10.44	13.80 ±6.24	15.01 ±2.77	15.15 ±5.62	7–27	0.548^{A}
CRE (mg/dL)	0.98 ±1.56	1 ±0.32	0.9 ±0.16	0.84 ±0.21	0.91 ±0.31	0.5-1.8	0.400^{K}
BUN/CRE	19.05 ±9.86	17.72 ±6.29	14.6 ±4.49	18.66 ±4.41	20.53 ±10.63		0.819^{K}
GLU (mg/dL)	103.76 ±11.76	104 ±11.19	100.6 ±11.25	101.42 ± 6.13	115.14 ±9.56	74–143	0.131^{A}
TP (mg/dL)	7.39 ±0.70 ^{a}	7.05 ±0.35 ^{a,b}	7.56 ± 0.34 ^{a,b}	6.92 ± 0.46 ^{a,b}	6.61 ±0.46 ^{b}	5.2-8.2	0.013^{A}
AGR	0.77 ± 0.20	0.70 ±0.13	0.78 ±0.20	0.85 ±0.15	1.07 ±0.23	≤ 1.0	0.056^{K}

AGR: Albumin-globulin ratio; ALB: Albumin; ALP: Alkaline Phosphatase; ALT: Alanine Aminotransferase; BUN: Blood Urea Nitrogen; CRE: Creatinine; GLU: Glucose; TP: Total Protein; a,b: Different or combinations of superscript letters on the same row indicate statistically significant difference (P<0.05). ANOVA, K: KRUSKAL-WALLIS

Table 4. Dog breeds and histopathological subtypes distribution of the groups.

Dog Breeds	Histopathologic subtypes and number of patients (n)
German Shepherd Dog	Carcinosarcoma (n=1), Malignant Mixed Tumor (n=1)
Akita Inu	Carcinosarcoma (n=1)
Beagle	Tubular Carcinoma (n=1), Carcinoma (n=1), Malignant Mixed Tumor (n=1)
Boxer	Adenosquamos Carcinoma (n=1), Carcinoma Mixed Type (n=1)
Chow Chow	Carcinoma (n=1)
Cocker Spaniel	Adenocarcinoma (n=3), Adenosis (n=2), Basic Papillary Adenocarcinoma (n=1), Duct Ectasia and Adenozis (n=1), Fibroadenomatous Change (n=1), Carcinoma Complex Type (n=2), Malignant Mixed Tumor (n=1), Sarcomas (n=1), Solid Adenocarcinoma (n=2), Solid Carcinoma (n=1), Tubular Carcinoma (n=2), Tubulopapillary Carcinoma (n=1)
French Bulldog	Tubular Adenocarcinoma (n=1)
Golden Retriever	Tubulopapillary Carcinoma (n=3), Tubulopapillary Adenocarcinoma (n=2), Malignant Myoepithelium (n=2), Malignant Mixed Tumor (n=1), Complex Carcinoma (n=2), Carcinosarcoma (n=1), Carcinoma Mixed Type (n=1), Intraductal Xanthomatous Fibrous Breeding (n=1), Ductal Carcinoma (n=1)
Jack Russel Terrier	Benign Mixed Tumor (n=1)
King Charles	Solid Adenocarcinoma (n=1), Carcinosarcoma (n=1), Adenoma (n=1),
Sivas Kangal	Complex Carcinoma (n=1)
Labrador Retriever	Tubular Carcinoma (n=1)
Mixed Breed	Tubular Carcinoma (n=1), Tubulopapillary Carcinoma (n=1), Solid Adenocarcinoma (n=1), Tubular Adenocarcinoma (n=3), Complex Carcinoma (n=2), Tubular Carcinoma- Mixed Type (n=1), Carcinoma (n=2), Adenocarcinoma (n=1), Complex Adenocarcinoma (n=1), Lipid-Rich Carcinoma (n=1), Squamous Cell Carcinoma (n=1), Carcinosarcoma (n=1), Osteosarcoma (n=1), Fibroadenoma (n=1), Chondroma (n=1), Fibroadenomatous Change (n=1)
Pekingese	Adenoma (n=1), Rhabolomyosarcoma (n=1)
Pinscher	İntraductal Papillary Adenoma (n=1), Tubulopapillary Carcinoma (n=1), Endocrinopathy (n=1)
Rottweiler	Tubular Carcinoma (n=1)
Russian Fino	Comedocarsinoma (n=1), Papillar Carcinoma (n=1)
Setter	Tubulopapillary Carcinoma (n=1)
Terrier	Benign Mixed Tumor (n=1), Ductal Adenocarcinoma (n=1), Hyperplasia (n=1), Carcinoma (n=1), Carcinoma Mixed Type (n=1), Carcinosarcoma (n=1), Complex Adenocarcinoma (n=1), Complex Carcinoma (n=1), Malignant Myoepithelioma (n=1), Tubular Adenocarcinoma (n=1), Tubular Carcinoma (n=1),
Yorkshire Terrier	Tubular Adenocarcinoma (n=1), Malignant Mixed Tumor (n=1), Complex Carcinoma (n=1), Intraductal Papillary Carcinoma (n=1), In Situ Carcinoma (n=1)

Discussion and Conclusion

In a study hematological examination of women with breast tumors, the mean values of WBC, RDW and MPV were higher but the mean values of RBC, HGB, HCT, MCV and MCH were lower than those of healthy women (Divsalar et al., 2021). A study of 30 dogs with advanced-stage tumor masses in the mammary glands showed a significant decrease in RBC, HGB and PCV values compared to healthy dogs. In addition, slight reductions in MCV and MCH were observed (Hasan S et al., 2015). A study comparing healthy female dogs and female dogs with mammary tumors showed a decrease in RBC, HGB and HCT levels in the mammary tumor group (Satilmis F et al., 2022). In our study, the MCV value is also below the mean group MCV value in Group 2. The study conducted by Divsalar et al. showed that the parameters RDW and MCV represent the best differential diagnostic hematological potential for mammary tumor in female dogs. In our study, although no statistically significant difference in WBC values was found between the groups, ten female dogs in Group 1 and three female dogs in Group 2 were found to have an increased WBC count. Consistent with previous studies, the mean WBC values were within the normal range in all groups. Divsalar et al. reported an increase in WBC count in the patients compared to the control group, but the average WBC remained within the normal reference range (Divsalar et al., 2021). A similar situation was found by Satilmis et al.in which there was no statistical difference in the WBC value between dogs with mammary tumors and healthy dogs, but it was found that the WBC value increased significantly when the size of the tumor increased and was seen in many mammary lobes (Satilmis F et al., 2022). In another study, a 1.5 to 3 fold increase in the WBC count was found in the examined group compared to healthy animals (Hasan et al., 2015). In a study conducted on 246 female dogs with mammary tumors, it was found that hematological abnormal changes were less pronounced in dogs with benign mammary tumors. In dogs with malignant mammary tumors, hematological abnormal changes were found in 55% of the samples examined, and it was found that there is a close

relationship between cancer progression and hematological changes. When the blood count of female dogs with malignant tumors is compared with that of female dogs with benign tumors and the healthy control group, thrombocytosis was found in 38%, hyperproteinemia in 34% and leukopenia with predominant neutropenia in 34% (Lallo M.A et al., 2016). In our study, neutropenia and leukopenia were not observed in any groups. But increased mean PLT count was observed in Group 3. Additionally, 12 dogs in Group 1, two dogs in Group 2 and a dog in Group 4 and Group 5 were found to have a value above the normal reference range. Our statistical analysis showed that the PLT value was significant differences between G2-G3 and G3-G4 ($p<0.05$). PLT values can be used as a predictor for different types of cancer in humans (Jurasz P. et al., 2004). Breast cancer patients with elevated PLT levels tend to have poorer survival (Taucher S. et al., 2003). A study of hematologic parameters in dogs with different types of cancer showed no significant differences in PLT counts among the different cancer groups (Andreasen E.B. et al., 2012). Another study found that PLT levels in female dogs with mammary carcinoma were 3.3% above the normal range (Stockhaus C. et al., 1999). In another study, it was observed that the increased TP was observed in female dogs with mammary tumors (Satilmis F et al., 2022). A study of dogs with mammary gland neoplasms showed a statistically significant decrease in the mean MON count compared to a group of healthy control dogs (Gangwar K. et al., 2024). In contrast to our results, in a study investigating tumor progression in 43 female dogs with mammary tumors, an increase in MON levels was observed in dogs with inflammatory carcinomas (Oliveira M.R. et al., 2022). In our study, a significant difference in mean monocyte counts was found between different groups. These results suggest that monocytes could serve as a potential biomarker to differentiate between malignant and benign/hyperplastic tumors, but further studies are needed to support this hypothesis.

A study in which the values of BUN, CRE, ALT and AST were compared in 30 dogs with mammary tumors and 10 healthy dogs showed no significant difference between these values in the two groups (Srikanth N., et al 2021). In a separate study of 30 dogs with advanced-stage mammary tumors, the values for BUN, CRE, ALT and AST were within the normal range (Hasan et al, 2015). In our study, 100 female dogs complaining of mammary tumors were grouped according to their histopathological subtypes. The mean BUN, CRE and ALT values were within the normal reference ranges in all groups, which is consistent with the results of this study. In another study based on previous research, ALT enzyme activity was found to decrease in dogs with malignant mammary tumors (Satilmis F et al., 2022), but in our study, we only found an increase in ALT enzyme in six female dogs in Group 1, and in all groups, the mean value was within the normal reference range. Therefore, further studies are needed to establish a relationship between mammary tumor and ALT enzyme level. In a study on dogs with mammary tumors, a non-significant increase in TP values was observed in a few patients (Nandhini S. et al., 2022). In our study, although the mean TP value was within the normal reference range in all groups, there was a statistically significant difference between G1 and G5 ($P<0.05$).

Recent studies have found consistent results between the diabetes and the risk of pancreatic, liver, endometrial and colon/rectal cancer in humans. Data on esophageal, stomach, prostate and breast cancer are more limited and conflicting. In a nearly 10-year study examining the association between fasting serum glucose levels and cancer in Korean women and men between the ages of 30 and 95, the mortality rate for women with breast cancer was 6.0 for those with fasting glucose levels below 90, 7.5 for those with fasting glucose levels of 90-109, and 5.2 for those with fasting glucose levels of 110-125, while the rate for those between 126-139 and 140 and above 140 was 6.1, the rate for those with diabetes was 9.8. The breast cancer incidence rate for women is 60.2 for fasting glucose levels below 90, 63.8 for fasting glucose levels 90-109, 68.7 for levels 110-125, 65.2 for fasting glucose levels 126-139, 55.4 for fasting glucose levels 140 and above 140, and 76.9 for those with diabetes. Hyperinsulinemia is considered a possible risk factor for breast cancer, which is confirmed by laboratory findings. In that study, fasting blood glucose levels were found to increase the risk of breast cancer in women (Jee, S et all., 2005). In our study, no statistically significant difference was found in mean GLU values between the groups. Further studies with more dogs are needed to determine the prognostic relevance of GLU level and mammary tumour development.

Inflammation is part of the tumor microenvironment and influences every step of tumorigenesis, cellular and biochemical blood markers of systemic inflammation, such as neutrophil-to-lymphocyte ratio (NLR) and albumin-to-globulin ratio (AGR), have been proposed as prognostic factors for cancer development in humans. The NLR was calculated by dividing the total number of neutrophils by the total number of lymphocytes. The AGR was calculated by dividing the albumin concentration to the globulin concentration. Although NLR and AGR levels

have not been adequately studied as prognostic factors for cancer development in veterinary medicine, 45 dogs with mammary tumors and 25 healthy dogs were used in a study investigating the relationship between NLR and AGR levels and mammary tumors in female dogs (Uribe Querol et al., 2023). A comparison between the healthy, benign and malignant mammary tumor groups, the NLR values were found to be statistically different ($p<0.05$). The AGR value was calculated preoperatively, and similar between the healthy and female dogs with mammary tumor. According to those results, the AGR value alone could not provide a prognostic value for mammary tumors in female dogs (Uribe Querol et al., 2023). Additionally, the NLR value is not a sufficient indicator for determining tumor type. The NLR value could become a simple and cost-effective tool to help make therapeutic decisions from the outset, as an accurate diagnosis often requires a more invasive and expensive procedure (Uribe Querol et al., 2023). In veterinary medicine, AGR varies in bacterial infections in cats and parasitic infections in dogs (Uribe Querol et al., 2023). The combination of NLR and AGR is reported to have better predictive value in patients with triple-negative breast cancer (Uribe Querol et al., 2023). In our study, the mean AGR value was lower in female dogs with mammary tumor than in female dogs with mammary hyperplasia/ dysplasia, but no statistically significant difference was found for the AGR value between the groups. It was observed that NLR was highest in Group 2 and lowest in Group 5. No statistical significance was found for the NLR value between the groups ($p>0.05$).

In the study by Lallo et al. the average age of female dogs with mammary tumors was 10.5 ± 3.7 years and the most common breed was mixed breed with 48%, Poodles with 29%, Rottweilers with 6% and the rest were various other breeds. In our study, the mean age was 10.06 ± 2.93 years and the most common breed was mixed breed at 20%, followed by Cocker Spaniel at 18%, Terrier at 18% and the rest were various other breeds. As can be seen in Table 1, the average age of Groups 1-2-3 is higher than that of Groups 4-5, and it was seen that the average age of dogs with malignant mammary tumors is higher than that of dogs with benign mammary tumors which is a similar result of the study by Uribe-Querol et al., 2023. In another study conducted on 12 dogs with mammary tumors, the average age was 8.42 ± 1.88 years (Satilmis F et al., 2022).

In another study conducted on 51 female dogs with mammary tumors, 31 dogs were found to have benign mixed tumors and 20 dogs were found to have carcinomas. When dogs with carcinomas are divided into subtypes, the most common are four Papillary Carcinomas, four Solid Carcinomas, three Tubulopapillary Carcinomas and three Tubular Carcinomas (Estrela Lima, 2010). In our study of 100 dogs, in contrast to this study, the most common histopathological type was a malignant epithelial tumor (72%). The most common histopathological subtype was complex carcinoma (13%), followed by tubular carcinoma (8%) and tubulopapillary carcinoma (7%), respectively.

In conclusion, in this study the hemogram and biochemical analysis of female dogs (n=100) complaining of a mass in the mammary glands were examined and compared according to their histopathological types. Further studies in more animals are needed to use the differences in hemogram and biochemical values as predictive markers in patients complaining of mammary tumors/masses. According to the results of our study, it will be possible to make a prognosis based on the blood test results during the examination of the patient in the clinical setting. Our study is a guide for future studies.

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Viral infections; affect genital system in female cats

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ABSTRACT

Infertility in cats is a common reproductive disorder caused by genetic, environmental, and infectious factors. This review focuses on infertility caused by viral infections in cats. Previous studies have demonstrated that viruses such as Feline Immunodeficiency Virus (FIV), Feline Leukemia Virus (FeLV), Feline Panleukopenia Virus (FPLV), Feline Calicivirus (FCV), and Feline Herpesvirus (FeHV) contribute to infertility in cats by directly damaging reproductive organs or by weakening the immune system. Furthermore, the immunosuppression caused by these viruses makes cats more susceptible to secondary infections, which severely impacts reproductive health. This article highlights the critical importance of understanding, preventing, and managing infertility associated with viral infections in cats.

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Introduction

Infertility not only leads to significant economic losses in farm animals such as cows, sheep, and goats, but also poses a serious threat to the lineage of cats and dogs. Although the causes of infertility in dogs are well-known, there is a lack of extensive research on this topic in female cats (Evecen & Demir, 2017). This condition significantly impacts animal welfare and may present risks to both public and environmental health (Risvanli et al., 2009). Infertility can be defined as the loss or reduction of an animal's reproductive ability, manifesting as the inability to produce offspring after mating. Infertility in cats is a common problem that can occur in both female and male individuals and can arise due to various causes. The causes of infertility in cats include genetic factors, environmental influences, nutritional deficiencies, hormonal imbalances, and infections (Fontbonne et al., 2020; Lamm & Njaa, 2012; Tek & Beceriklisoy, 2020).

Viral infections represent a major contributing factor to infertility in cats. Viruses including Feline Immunodeficiency Virus, Feline Leukemia Virus, Feline Panleukopenia Virus, Feline Calicivirus, and Feline Herpesvirus may contribute to infertility by directly damaging reproductive organs or by compromising overall health (Fontbonne et al., 2020). These viruses can weaken the cat's immune system, making them more susceptible to secondary infections and severely impacting reproductive health (Tek & Beceriklisoy, 2020).

Viral diseases causing infertility

1. Feline leukemia virus

Feline leukemia virus (FeLV) is a Gammaretrovirus observed in domestic and wild felids, categorized into four subtypes (A, B, C, and T) based on the spectrum of host cells. Since this virus belongs to the Retrovirus family and is species-specific, it does not cause disease in species other than cats (Tek & Beceriklisoy, 2020). FeLV-type A is acquired from the environment, while FeLV-type B arises from the recombination between FeLV-A and endogenous retroviral sequences. FeLV-C results from a mutation in the env gene, and FeLV-T is characterized by T lymphotropism (Decaro et al., 2012).

The primary transmission occurs through saliva. Therefore, animals living in multi-cat households, shelters, and breeding farms are at high risk of FeLV

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infection due to sharing food and water bowls, close contact, and shared litter areas (Decaro et al., 2012).

FeLV includes various clinical forms caused directly or indirectly by virus replication in lymphoid tissues and bone marrow. The primary consequence of FeLV infection is immune suppression. Additionally, secondary infections caused by mild pathogens such as Mycoplasma hemofelis and other feline hemoplasmas, Cryptococcus spp., Toxoplasma gondii, feline coronavirus, and calicivirus can exacerbate the clinical course (Hartmann, 2011).

From a gynecologic perspective, queens infected with FeLV may exhibit reproductive disorders. It can lead to intrauterine infection, fetal resorption, abortion, and neonatal death. Fetal resorption may be responsible for what appears to be prolonged infertility. Abortion often occurs in the later stages of pregnancy and may be accompanied by bacterial endometritis (Hartmann, 2011). In a case report published by Axnér et al. (2008), it was noted that uterine pathologies, which could cause infertility, were observed in 4 out of 7 cats (57%) in which FeLV antigen was detected (Axnér et al., 2008).

Reproductive problems in cats diagnosed with FeLV can be mitigated by taking certain precautions, with regular vaccination being the most important measure. Kittens should receive the first dose of the vaccine at 8–10 weeks of age, followed by a second dose at 12 weeks, and yearly vaccinations should continue thereafter (Lutz et al., 2009).

2. Feline panleukopenia virus

Feline Panleukopenia Virus, together with canine parvovirus type 2 (CPV-2) and other parvoviruses, belongs to the Parvoviridae family's Parvovirus genus, specifically within the feline parvovirus group. This deoxyribonucleic acid (DNA) virus is shed in the feces of infected cats and is highly transmissible via the fecal-oral route. Following the onset of viremia, the virus shows a particular affinity for lymphoid organs, bone marrow, intestinal crypts, and the fetus in pregnant cats (Kilham et al., 1971). In a study performed in Turkey, positivity rates of 27.7% (5/18) and 16.6% (1/6) were determined in samples collected from the districts of Bolvadin and İscehisar, respectively (Gür & Avdatek, 2016).

The clinical course of the disease can vary significantly depending on the time of infection. Infections occurring postnatally in kittens aged 2 to 6 months often result in the classic form of feline panleukopenia, characterized by fever, loss of appetite, depression, hemorrhagic diarrhea, vomiting, and dehydration. Severe leukopenia, affecting all white blood cell (WBC) populations, is consistently observed, with WBC counts ranging between 50 and 3000 cells/µL (Binn et al., 1970). Intrauterine infections can lead to various gynecological disorders, depending on the stage of pregnancy at which the infection occurs. Early-stage uterine infections typically result in infertility, early fetal death, and resorption, while mid-pregnancy infections are more likely to cause abortion or fetal mummification. For example Cave et al. (2002), in their study conducted on 274 cats, reported that a significant proportion (25%) of abortions and neonatal deaths were caused by FPLV (Cave et al., 2002). Similarly, Oliveira et al. (2018), in their study on reproductive failures in 42 female cats, reported that infertility problems such as stillbirths and premature births were largely caused by FPLV (Oliveira et al., 2018). In late pregnancy, the virus shows affinity for fetal neural tissues, including the cerebrum, cerebellum, optic nerve, and retina (Csiza et al., 1971). Consequently, virus-induced lesions in the offspring may manifest as hydrocephalus, hydranencephaly, cerebellar hypoplasia, optic nerve atrophy, and retinopathy. In cats, since this part of the central nervous system develops during late pregnancy and the early neonatal period, the cerebellum is the most affected tissue. These lesions can also be observed in kittens infected within the first 10 days after birth (Sharp et al., 1999).

Accurate and early diagnosis is crucial for preventing FPLV, which causes all these reproductive issues. Vaccination against FPLV, confirmed via Polymerase Chain Reaction (PCR) testing, is one of the most effective prevention methods, particularly when administered to kittens in two doses starting at 8–9 weeks of age. Additionally, preventing direct contact between domestic cats and wild cats, environmental management, and regular vaccination schedules help reduce the incidence of FPLV (Truyen et al., 2009).

3. Feline immunodeficiency virus

Feline immunodeficiency virus (FIV) is a retrovirus belonging to the Lentivirus genus. It shares pathogenic features with human immunodeficiency virus (HIV). FIV is now recognized as an endemic pathogen in domestic cat populations worldwide, with prevalence rates reaching up to 28% in some countries. To date, at least five genetically distinct subtypes have been identified based on the sequence diversity of the env gene (Dunham & Graham, 2008; Hosie et al., 2009).

FIV transmission typically occurs through the transfer of free virus or virus-infected leukocytes via bite wounds. While transmission from infected female cats to their kittens is not very common, it can be experimentally achieved. Important routes of FIV transmission include vertical transmission, transplacental passage within the uterus, direct contact

with genital secretions during birth, or ingestion of infected colostrum or milk postpartum. Studies have detected high titres of the virus in the milk of infected cats, indicating an affinity of the virus for mammary epithelial tissues. Vertical transmission is more effective when pregnant female cats are infected during gestation. An increased rate of FIV infection with advancing pregnancy has been demonstrated. Fetuses from cats infected with FIV at 3 weeks of gestation were found not to be infected, but up to 60% of fetuses were virus-positive when the queens were infected later in pregnancy. This indicates that FIV infection in late pregnancy poses a significant risk for cats (Rogers & Hoover, 1998). For example, in a study by Weaver et al. (2005) on experimental FIV infection in cats, 15 out of 25 concepti (60%) were not viable, and FIV was isolated in 21 out of 22 fetuses (95%) (Weaver et al., 2005).

In hosts that acquire the pathogen through bites, clinical symptoms may appear months or even years later. The most commonly observed gynecological signs include: inhibition of fetal development, fetal mummification, abortion, stillbirths, shortened intervals between births, and congenital anomalies in the offspring (Hosie et al., 2009; Tek & Beceriklisoy, 2020).

Currently, there is no vaccine registered in Europe to protect against the effects of FIV. Therefore, rather than vaccination, it is recommended to prevent FIV transmission by adhering to general preventive measures such as avoiding the use of contaminated materials like needles and surgical instruments on multiple cats, and eliminating potential vectors like flies and insects that could transmit the virus to cats (Hosie et al., 2009).

4.Feline herpes virus

Feline herpesvirus 1 (FeHV-1) is a DNA virus belonging to the Alphaherpesvirinae subfamily. It primarily causes an upper respiratory tract infection, termed feline viral rhinotracheitis, in domestic cats. Both domestic and wild felids are at risk for herpesvirus infection. Infected cats may harbor the virus in a latent state, which can reactivate intermittently in response to stress, immunosuppression, or parturition (Gaskell et al., 2007; Karapinar et al., 2014).

The virus is transmitted through direct contact with acutely or latently infected cats, and indirectly in environments with high cat populations such as shelters and breeding catteries. The primary carriers of the virus are oronasal and conjunctival secretions. Newborn kittens typically become infected through contact with their mother's oronasal secretions. Intrauterine infections have been reported only under experimental conditions (Westermeyer et al., 2009). Herpesvirus-induced abortions in cats are much rarer compared to herpesvirus-induced abortions observed in dogs. When FeHV-1 DNA is isolated from the fetuses of cats that have aborted as a result of FeHV-1 infection, no definitive findings are observed. This suggests that the abortion may be indirectly caused by the immunosuppressive effects of the virus. Studies have shown that intravenous inoculations administered to pregnant cats in the late stages of gestation result in abortion, stillbirth, or generalized neonatal infections, whereas intranasal inoculation does not have a negative impact on pregnancy (Tek & Beceriklisoy, 2020). Additionally, following intravenous inoculation, herpesvirus has been isolated from the genital tract of the queens and the tissues of their aborted fetuses. This unnatural route of infection is the only one that causes necrotic lesions in the uterus, placenta, and vagina of the queens (Johnson, 1964).

The most important way to protect against FeHV-1, which causes serious problems, is through vaccination. Initial vaccinations at 9 and 12 weeks of age, followed by annual boosters, significantly reduce the risk of infection. In addition, infected animals can be treated with various antiviral drugs (Thiry et al., 2009).

5.Feline calicivirus

Feline Calicivirus (FCV) is an ribonucleic acid (RNA) virus belonging to the Vesivirus genus of the Caliciviridae family. It is a highly contagious pathogen commonly found in domestic cats, known for its resilience to environmental conditions and significant genetic variability. FCV infections pose serious problems, particularly in environments with large numbers of cats, such as shelters and breeding facilities. The most common clinical signs observed in cats infected with FCV are related to upper respiratory tract diseases, and it can lead to fatal lung infections, especially in young kittens (Hofmann-Lehmann et al., 2022).

While Feline Calicivirus (FCV) is primarily transmitted via oral and nasal secretions from infected cats, the virus can also be detected in their blood, urine, and feces. Transmission can occur through contact with these materials. Even after clinical recovery, cats may continue to shed the virus for durations ranging from 30 days to several years (Coyne et al., 2006). Studies reporting the rates related to FCV affecting the genital system of female cats are not available in the literature.

Similar to Herpesvirus, Calicivirus is believed to cause abortions due to immunosuppression as it adversely affects the overall health of the cat. Additionally, in contrast to Herpesvirus, experimental intranasal Calicivirus infections in pregnant cats have

shown that the virus can be transmitted to the fetuses, leading to severe abortions. For this reason, FCV vaccination is not recommended for pregnant cats, regardless of the route of administration (Hofmann-Lehmann et al., 2022; Tek & Beceriklisoy, 2020).

The most effective way to protect against FCV is through vaccination. All healthy cats should be vaccinated against FCV. Early vaccination should be considered for kittens from queens that have previously given birth to infected litters or for cats at risk of infection. Two vaccinations are recommended at 9 and 12 weeks of age, followed by a first booster one year later. In high-risk situations, a third kitten vaccination at 16 weeks is recommended. Booster vaccinations should be administered every 3 years; however, in high-risk situations, annual revaccination is advised (Radford et al., 2009).

Conclusion

This review addresses the various mechanisms by which viral infections can lead to infertility in cats. Viruses such as Feline Immunodeficiency Virus (FIV), Feline Leukemia Virus (FeLV), Feline Panleukopenia Virus (FPLV), Feline Calicivirus (FCV), and Feline Herpesvirus (FeHV) have been observed to have significant impacts on feline reproductive health. These viruses can cause direct damage to the reproductive organs, as well as indirectly lead to infertility by negatively affecting the overall health of the cats.

In cats infected with these viruses, serious reproductive issues such as immunosuppression, susceptibility to secondary infections, pregnancy losses, congenital anomalies, and neonatal mortality are observed. The effects of infection become more pronounced during pregnancy, negatively impacting fetal development and leading to outcomes such as abortion, mummification, and stillbirth.

In this context, the prevention and management of viral infections in cats are of paramount importance for preserving reproductive health. To prevent the spread of these viruses, appropriate vaccination protocols must be implemented, the risk of infection minimized, and infected cats excluded from breeding programs. Furthermore, the early diagnosis and treatment of viral infections play a critical role in safeguarding feline reproductive health. Future studies should focus on more thoroughly investigating the effects of these viruses on reproductive health and developing protective strategies.

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The relationship between ketosis and transitional nutritional diseases

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ABSTRACT

Ketosis is a highly prevalent nutritional condition that affects fresh dairy cows during the transition period. Ketosis occurs when there is a negative energy balance. Clinical findings of ketosis include excessive loss of body condition, decreased feed consumption (especially of concentrated feed), reduced milk yield, and nervous signs. Subclinical ketosis is a serious nutritional disease that may result in displaced abomasum, decreased milk yield, poor reproductive performances, early culling of herds, and economic losses, among other adverse effects. Ketosis is linked to nutritional disorders that are commonly observed after calving including, metritis, mastitis, milk fever, lameness, retained placenta, and displaced abomasum. The two most crucial strategies for preventing ketosis are reducing negative energy balance and increasing dry matter consumption. The density of triacyl-glycerides and non-esterified fatty acids in plasma is higher after calving. Non-esterified fatty acids are oxidized to ketone bodies. A cow starts to mobilize its body fat for energy when it reaches a negative energy balance. Live weight and body condition score fall under such circumstances.

Keywords: dairy cattle, ketosis, negative energy balance

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Introduction

Due to low feed consumption and high milk output, energy needs cannot be physiologically met at the start of lactation, leading to an energy deficit during this period. The body uses its fat reserves to make up for this deficit. Ketosis usually occurs in high milk producing cows at the start of lactation, when the energy balance is negative. However, it can also occur when dry matter intake is reduced and the diet lacks sufficient energy density in conditions such as displaced abomasum, mastitis, metritis, fatty liver syndrome, and retained placenta, which may reduce appetite and feed intake, increasing the risk of secondary ketosis (Atalay and Eseceli, 2015).

Ketosis is a nutritional disease characterized by liver degeneration, decrease in blood glucose concentration, and increase in ketone bodies in the blood and other body tissues particularly in the postpartum period. In a negative energy balance, the mobilization of body fat results in an increase in the concentration of free fatty acids in various tissues. Free fatty acids in the liver are limited by the oxidation capacity of the liver. When the capacity is surpassed, free fatty acids are converted to triglycerides, which accumulate in the liver. The incidence of ketosis increases when plasma free fatty acid concentrations exceed 1000 mEq/L (Arslan and Tufan, 2010b).

Dry matter consumption

Dry matter intake, milk output, and blood glucose levels have all been shown to be reduced in ketosis cows, although NEFA, BHBA, and serum total lipid levels are higher (Dann et al., 2005). Cows with subclinical ketosis had higher amounts of BHBA and NEFA, but lower levels of glucose than healthy cows (Zhang et al., 2010).

The following processes supply the glucose and metabolic energy required during the transition period due to low dry matter consumption: hepatic

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gluconeogenesis; lactic acid accumulated from anaerobic glycolysis in muscle tissue and transported to the liver; glucogenic amino acids released from protein catabolism; and glycerol released from lipolysis in adipose tissue (Arslan and Tufan, 2010a; Reynolds et al., 2003).

Obesity in cows during the parturition period leads to a significant decrease in dry matter consumption and an increased release of NEFA (Stockdale, 2001; Roche et al., 2009).

Subclinical acidosis is caused by the addition of easily digestible non-structural carbohydrates to the diet to balance energy and boost dry matter consumption. Subclinical acidosis quickly progresses to laminitis. The cow with laminitis prefers to rest rather than feed, thus dry matter consumption drops, and the negative energy balance further exacerbates (Kelley, 2014).

Dry matter consumption is one of the most key factors that influence milk output. Hormonal and metabolic changes during the transition phase may affect dry matter consumption (Ingvartsen and Andersen, 2000). Dry matter consumption decreases by more than thirty percent in the last three weeks of gestation (Hayırlı et al., 2002).

Numerous clinical indicators of ketosis are nonspecific, including decreased milk production and dry matter consumption as well as environmental indifference. Abnormal behaviours like aggression, lack of coordination, and chewing on nonfeed items are displayed by some cows. Ketone levels in milk, urine, or blood must be measured for a conclusive diagnosis. The value and accuracy of different tests have previously been reviewed (Tatone et al., 2016).

Nutritional diseases

High milk yielding cows experience decreased blood glucose levels, depleted liver glycogen stores, formation of ketone bodies from unesterified fatty acids in the body, and ketosis because of their high energy requirements during the transition period and their inability to meet these needs. Hypocalcemia, metritis, retained placenta, ketosis, and displaced abomasum may occur simultaneously. Abomasal atony is the biggest contributor to displaced abomasum. Atony is caused by RPT, ulcers, metritis, mastitis, retentio secundinarum, acidosis, elevated volatile fatty acids, and low blood calcium levels (Rukkwamsuk et al., 1999).

BHBA > 1.2 mmol/ L is linked to abomasum displacement, mastitis, metritis, and increased milk loss in cows experiencing ketosis in the first one or two weeks postpartum (LeBlanc, 2010). The cut-off value for subclinical ketosis in blood BHBA is 1-1.4 mmol/L (Ospina et al., 2010). The likelihood of displaced abomasum, clinical ketosis, and metritis is 2.6–8 times, 3–6 times, and 1–5.8 times higher, respectively, if BHBA levels are elevated in cows during the first two weeks following calving (Dubuc et al., 2010). Metabolic diseases are frequently associated with low blood glucose levels as well as high blood NEFA and BHBA levels, both of which suggest negative energy balance. Increased ketone bodies in the blood raise the possibility of left-displaced abomasum. Ketone bodies can disrupt the activities of milk and blood immune system cells (Graber et al., 2010). Negative energy balance is linked to diseases including mastitis, metritis, and retained placenta, which are not metabolic diseases (Grummer et al., 2004).

It is critical to assess the prevalence of ketosis in the herds in the enterprise. The occurrence of ketosis is influenced by a variety of factors, including geographical location, temperature, barn structure, roughage structure, pasture structure, season, and lactation number. There is a negative correlation between ketosis prevalence and herd size during lactation. It has been suggested that feeding with Total Mix Ration minimizes the risk of ketosis. The highest prevalence of ketosis was reported in cows calving during the second quarter of the year (April-June). Low consumption of concentrated feed has been linked to an increased risk of ketosis due to reduced energy consumption. Cows that had only one calving showed the lowest levels of ketosis. Cows in the second lactation are reported to have a higher prevalence of ketosis, whereas cows in the third to seventh lactation have the highest prevalence of ketosis. It has been stated that ketosis in the first week after calving is linked to increased abomasum displacement, metritis, and mastitis. Another study indicates a significant link among ketosis, mastitis, and clinical laminitis (Berge and Vertenten, 2014).

Fatty liver is a major risk factor for abomasal displacement, ketosis, and immune dysfunction. In contrast, if these disorders reduce dry matter intake and induce a more severe negative energy balance, they may increase the likelihood of developing fatty liver. Obesity is a significant risk factor for developing fatty liver (Bobe et al., 2004). In the early postpartum period, fatty cows consume less dry matter than cows in proper body condition, which results in higher mobilization of body fat (Stockdale, 2001).

Hormonal change

Insulin levels in cows have been reported to be low after calving (Holtenius et al., 1993). Lactating cows produce only half the insulin that non-lactating cows do (Lomax et al., 1979). Insulin resistance develops in

peripheral tissues during early lactation (Holtenius and Holtenius, 1996). Insulin levels vary during the transition period. Low insulin levels after parturition decrease glucose uptake in insulin-sensitive organs, such as muscle and adipose tissue. This condition favors glucose uptake in the mammary gland, which is not influenced by insulin action. Thus, the concentration of triglycerides in the liver rises in response to adipose tissue mobilization in the body (Aschenbach et al., 2010).

The mammary gland does not require insulin for glucose metabolism (Komatsu et al., 2005). NEFA circulating freely during negative energy balance contributes to milk fat formation (Bauman and Griinari, 2001). In a lactating cow experiencing negative energy balance, milk fat concentration tends to increase, while milk protein concentration tends to decrease. The fat to protein ratio expresses negative energy intensity and the risk of metabolic disease. Milk samples should be evaluated within 9 to 30 days to estimate the risk factors for metabolic diseases (Duffield et al., 1997; Toni et al., 2011).

Insulin inhibits the mobilization of fatty acids from adipose tissue and reduces blood glucose levels by increasing glucose absorption into tissues. Insulin resistance refers to a decrease in the tissues' response to insulin actions. As insulin resistance in adipose tissues increases, so does fatty acid mobilization and the concentration of non-esterified fatty acids in the blood. The body condition score used in determining energy balance provides information regarding the change in live weight. It has been stated that one unit change in body condition score corresponds to an 80 kg change in live weight. The fatty tissues that are mobilized or the live weights that are regained are located in the abdominal, intramuscular, and subcutaneous regions. Energy balance is expressed by the blood molecule β-Hydroxybutyric acid (BHBA). If the amount of non-esterified fatty acids entering the liver exceeds the capacity for fatty acid oxidation, ketone bodies increase. Blood analysis should be performed in the second week of lactation and 5 hours after feeding to assess BHBA levels in the blood. The BHBA blood threshold is 1.2 to 1.4 mmol/L. There is a strong link between milk yield and energy status. When there is an energy deficiency, microbial protein synthesis and the number of amino acids in mammary tissue decrease, lowering the level of milk protein. A milk protein content of less than 2.7% relates to a negative energy value. Blood analysis should be used to determine milk protein concentration between weeks 1 and 12 of lactation, and 5 hours after feeding. Milk fat levels increase but milk protein levels decrease during lactation due to a negative energy balance. A high fat/protein ratio at the start of lactation suggests a negative energy balance. If the fat/protein ratio exceeds 1.4, it indicates that fat is being mobilized in the adipose tissue and that the animal is in a negative energy state. Milk acetone levels are commonly used to diagnose subclinical ketosis. If the milk acetone concentration exceeds 0.7 mmol/L, the animal may be in ketosis. It is important to focus on the number of samples that surpass the threshold values rather than the average value when interpreting metabolic indicator data. If more than 10% of the cows in the herd surpass the BHBA threshold, this is a strong indication of a negative energy balance in the herd (Serbester et al., 2012).

Holtenius and Holtenius (1996) classified ketosis as type I and type II. Type I ketosis usually develops a few weeks after parturition, when milk supply and mammary gland glucose demands are high. However, it is not generally associated with high hepatic fat concentrations. Type II occurs at or around parturition and is typically linked with fatty liver. Type II ketosis is often more difficult to treat than type I (Herdt, 2000; NASEM, 2021). This classification method indicates two major causes of ketosis. The risk factors and causes of type II ketosis are similar to those of fatty liver. Type I cows had lower blood glucose and insulin concentrations, as well as higher ketone concentrations compared to healthy cows. Low insulin increases fatty acid oxidation, likely by reducing hepatocyte malonyl-CoA levels and decreasing the sensitivity of carnitine palmitoyl-transferase 1 to malonyl-CoA. (Emery et al., 1992; NASEM, 2021). Carnitine palmitoyltransferase 1 is responsible for the transport of fatty acids from the cytosol to the mitochondria for oxidation, and it is highly active in type I ketosis. Thus, this suggests that type I ketosis is caused by an insufficient supply of gluconeogenic precursors. Low dry matter intake could be the cause of limited substrate supply. Studies reveal that increasing dietary starch postpartum lowers blood BHBA while increasing glucose (Rabelo et al., 2005; McCarthy et al., 2015; NASEM, 2021). Propylene glycol administered as a drip or bolus can lower blood BHBA levels (Nielsen and Ingvartsen, 2004).

Fatty liver

Ketone bodies are produced when excess NEFA enters the liver. Subclinical ketosis may occur because not all NEFA can be completely oxidized. Negative energy balance leads to ketosis. When the body's energy reserves are mobilized, the concentrations of NEFA and ketone bodies (acetoacetate, BHBA, and acetone) increase. Increased NEFA concentration contributes to ketosis (Weber et al., 2013).

Fat accumulates in the liver when fatty acid intake exceeds the liver's ability to oxidize or eliminate fatty acids, which occurs when blood concentrations of stored fatty acids increase (Bobe et al., 2004; Grummer, 2008). Ketosis almost always develops when cows have moderate (5 to 10% of liver wet weight raised as triacylglycerides) to severe (more than 10% increased as triacylglycerides) fatty liver. Plasma NEFA concentrations in healthy cows are low (less than 0.2 mEq/L) until a few days before parturition, increases to as high as 0.8 mEq/L during parturition, remain high for several days, and then gradually fall (Bertics et al., 1992; NASEM, 2021). Cows with a higher risk of postpartum metabolic disorders often have plasma NEFA concentrations higher than 0.5 mEq/L prepartum and higher than 1.0 mEq/L postpartum (LeBlanc et al., 2005; NASEM, 2021).

The uptake of NEFA by the liver is proportional to NEFA concentrations in the blood (Emery et al., 1992) and NEFA taken up by the liver can be esterified or oxidized (Drackley, 1999). Triglycerides, the primary result of esterification, can be absorbed or retained as part of very low density lipoprotein. Compared to other species, ruminants absorb triglycerides at a very slow pace (Kleppe et al., 1988; Pullen et al., 1990). As a result, fatty acid esterification and triglyceride buildup occur in the liver when it takes up excessive levels of hepatic NEFA.

Negative energy balance

When milk production begins, the cow's energy need increases by around threefold compared to the period before parturition (Van Dorland et al., 2009). As milk production begins, glucose and metabolic energy needs increase two to threefold by the 21st day postpartum, compared to the 21st day prepartum (Drackley et al., 2001).

More than fifty percent of cows are thought to have at least one subclinical illness throughout the transition phase (Overton and Waldron, 2004). The negative energy picture is shaped by the imbalance between stagnant dry matter consumption during the transition period and increased energy demand during lactation.

The relationship between energy expended through milk production during lactation, the mobilization of body fat, and energy intake from feed defines the condition known as ketosis (Collard et al., 2000; Mulligan et al., 2006). The transition period for high-yielding dairy cattle is extremely challenging. During this time, the animal frequently enters a negative energy state. A cow with a negative energy balance requires more energy than it can receive from feed (De vries et al., 1999). Dry matter consumption during this time is most likely determined by NEFA oxidation in the liver (Allen et al., 2009).

Microorganisms in the rumen produce the volatile fatty acids butyric acid, propionic acid, and acetic acid. The liver uses acetic acid as a source of acetyl coenzyme A to make ATP. The rumen absorbs butyric acid, which is then transformed into ketone bodies (BHBA). The liver uses propionic acid in the process of gluconeogenesis (Reynolds et al., 1988).

During the far off dry period, 4 to 6 weeks before parturition, insulin level was found to be consistent with liver lipid concentrations in cows fed a high energy diet compared to those on a restricted diet. However, because it was higher, this level was found to be inconsistent with the NEFA and BHBA levels (Dann et al., 2006). Compared to cows fed a high energy intensive diet, more significant and relevant results were found in dry matter consumption and energy balance in cows fed a restricted diet throughout the far off dry period. It was reported that during the far-off dry period, cows fed a restricted energy diet eliminated negative energy balance and achieved a balanced energy state more quickly than those fed an intensive diet. Additionally, it was noted that cows overfed during the far-off dry period following parturition showed signs of health issues (Dann et al., 2006, Kelley, 2014). Cows tend to consume more feed during the dry period to gain fat, which provides energy after parturition.

Cows that are in negative energy balance which can happen when their dry matter intake decreases during the prepartum phase and almost always happens during the first few weeks of lactation have higher plasma NEFA levels because their energy needs are high and their intake is low, which causes their body reserves to be mobilized. Most cows in the early stages of lactation have some degree of hepatic fat accumulation since NEFA levels are elevated in most of them during the peripartum period (Jorritsma et al., 2001; Bobe et al., 2004; NASEM, 2021).

Ketosis, also known as hyperketonaemia, occurs when an excess of long chain fatty acids is oxidized by B -oxidation. Cows with a severe negative energy balance after calving release an excessive amount of long-chain fatty acids. Ketone bodies (B-hydroxybutyric acid and acetoacetate) are the byproducts of B-oxidation, and when they accumulate in the blood, clinical symptoms can appear. When plasma insulin levels are low and glucagon levels are high, fatty acid release from adipose tissue and subsequent B-oxidation are triggered (Holtenius and Holtenius, 1996; NASEM, 2021).

Conclusion

The transition period includes the three weeks preceding parturition and the three weeks following parturition. Almost all high-milk-yielding cows

experience a negative energy balance throughout the transition period. This negative energy balance results from an increased need for energy following parturition, which is caused by a decrease in dry matter intake and the onset of milk secretion. In a negative energy balance, the cow mobilizes body fat to meet its NEFA (Non-Esterified Fatty Acids) requirements. The cow with a negative energy balance attempts to correct the imbalance by boosting NEFA and BHBA (Beta Hydroxy Butyric Acid) levels. Because the cow is in negative energy balance during ketosis, ration energy cannot be used to make up the difference. Thus, conducting a ration study during ketosis might not yield useful results.

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Comparison of hemogram, amylase and lipase values in cats with feline panleukopenia disease and healthy cats

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ABSTRACT

Feline panleukopenia (FPV), commonly known as the feline distemper virus, is a type of DNA virus. Known for its high transmissibility, FPV primarily affects young cats, particularly targeting the bone marrow, intestines, and lymphatic system. Without treatment, it can be fatal. The aim of this study is to compare the hemogram parameters of an infected cat with those of a healthy cat, and to facilitate diagnosis and prognosis by also comparing amylase and lipase levels. In the study, 30 cats were evaluated and divided into two groups: the control group and the patient group. The patient group consisted of 15 cats that tested positive for FPV using a rapid antigen test, while the control group consisted of 15 cats that tested negative for the antigen and showed no clinical symptoms. In the study, white blood cell, lymphocyte, neutrophil, neutrophil, monocyte, eosinophil, red blood cell and platelet counts, haematocrit and haemoglobin values were found lower in sick cats ($p<0.05$). Amylase and lipase values of sick cats were found to be higher than healthy cats ($p<0.05$). According to the study's results, white blood cell count, neutrophil, monocyte, lymphocyte counts, and platelet count were found to be highly reliable parameters for diagnosing FVP infection.

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Introduction

Feline Panleukopenia Virus (FPV) is a disease called Feline Distemper or Feline Parvovirus infections. The causative agent of this disease is a single-stranded non -enveloped DNA virus of the Parvoviridae family. This virus is highly contagious in cats and causes many physiological changes. FPV, also known as feline infectious enteritis, pseudomembranous enteritis, feline agranulocytosis due to its pathogenesis, is a disease agent with symptoms such as high fever, vomiting and diarrhoea. It is seen in cats of all ages, but young cats are more affected. The disease is most characterised by a decrease in white blood cells and an affected bowel structure. Mortality and morbidity rates are high (Bet aler ve et al., 1983, Scott, 1987, Steinel et al., 2001). It affects other members of the felidae family as well as cats. It also infects mink, raccoons and foxes.

Many methods have been developed to diagnose and follow the prognosis in FPV to date. Detection of faecal FPV antigen is one of the most valuable diagnostic methods. The faecal antigen test has excellent specificity. However, due to its limited sensitivity, false positive results are very rare, so false negative results are more likely. Therefore, a negative faecal ELISA does not rule out FPV infection. Additional tests may be valuable in these cases (Tuzio, 2021).

Antibodies against FPV can be detected by haemagglutination inhibition (HI), ELISA or indirect immunofluorescence. As these tests do not distinguish between vaccine-derived and infection-derived

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antibodies, their use for the diagnosis of FPV infection in sick animals is limited. However, they are useful for identifying animals protected against panleukopenia (Tuzio, 2021).

Haematological parameters may show pancytopenia and mild anaemia due to decreased erythrocyte production.

Serum biochemistry panel results are not specific for FPV. Hypoalbuminemia is the most common abnormality, probably due to decreased protein intake and leakage from mucosal lesions into the gastrointestinal tract (Kruse ve et al, 2010).

Since parvovirus causes pathognomonic microscopic changes of intestinal crypt necrosis with villus blunting in the jejunum and ileum, histopathology is the confirmatory test. Cellular depletion of bone marrow and lymphoid tissues may also occur. In any suspicious case, samples from these four tissues should be sent for histopathology (Tuzio, 2021).

One of the common problems in virus detection is the rapid decline of virus particles in faeces when an enteric infection occurs and false negatives caused by a decrease in the number of virus particles with dilution (diarrhoea) (Tuzio, 2021).

Although many laboratory and pathological tests are used in the diagnosis of FPV, it is known that tests can yield false negative results in the early stages of the disease, and existing leukopenia may not be reflected in laboratory results. Therefore, there is a need for additional parameters that can support diagnosis in cats carrying the disease and in co-infection situations, provide early findings of organ damage, and be used to monitor prognosis during treatment.

In our study, the differences in hemogram, serum amylase, and lipase values of FPV-infected cats were compared to healthy cats. It is known that leukopenia, neutropenia, lymphopenia, thrombocytopenia, and anemia are observed in the hemogram. However, since studies have shown that hemogram parameters may not always provide disease-specific and sensitive results, serum amylase and lipase levels were also examined in our study, as they are thought to indicate gastrointestinal system damage and facilitate diagnosis when supported by hemogram parameters. The elevated serum amylase value, which indicates gastrointestinal system involvement, showed an increase compared to normal cats. For the lipase enzyme, it was concluded that it can be elevated in secondary infections as well as in gastrointestinal damage. Since both enzymes are released from the gallbladder, other gallbladder-related pathologies were also examined.

Some factors limiting this study include the disease presenting with nonspecific symptoms in the early stages and progressing in the late stages, delaying diagnosis and treatment; difficulties in performing diagnostic tests due to the patient's clinical findings and general condition deterioration; challenges in controlling patients while collecting blood samples; and the inability of pet owners to cooperate with relevant veterinarians during the diagnosis and treatment process.

The aim of the study is to compare the hemogram parameters, amylase, and lipase values of an FPV-infected cat with those of a healthy cat.

Materıals and Methods

Ethical statement

The study was conducted within the scope of the Ethics Committee Report obtained from Sivas Cumhuriyet University Animal Experiments Local Ethics Committee (decision dated 08.03.2023 and numbered 65202830-050.04.04-711).

Animal material

As part of the study, the animal material consisted of 15 cats diagnosed with Feline Panleukopenia and 15 healthy cats without any signs of illness, all of which were owned and brought to the Mascote Veterinary Clinic in the Bornova district of Izmir province.. The animal owners were provided with necessary information about the blood and stool sample collection process and the interventions to be applied to the animals during these procedures within the scope of the study and a consent form was signed. No anesthetic or analgesic drugs were administered to the animals during the collection of samples.

Rapid antigen test method

After positioning the cat appropriately for the rapid antigen test, a swab was gently inserted about 1 cm into the rectum and moved around the rectal pouch lumen to collect a fecal sample. The fecal sample on the swab was mixed in the sample diluent fluid for about 10 seconds. The sample was left to stand for about 20 seconds to allow large particles to settle. The test kit (Vet Expert FPV Ag, South Korea) was placed on a flat surface. Using the dropper provided with the test kit, 4 drops of the sample were added vertically to the sample well. The test was left for 10 minutes before reading. Cats whose tests were read and determined to be positive were included in the patient group.

Collection of blood samples and processing in the laboratory

Blood samples were collected by veterinarians from the cephalic veins of cats, with 0.5 ml drawn into a 2 ml

tube containing Ethylenediamine Tetraacetic Acid (K2EDTA) and 1 ml into a 5 ml tube with Clot Activator. After collecting blood in the EDTA tube for complete blood count, it was gently mixed by inverting to prevent coagulation and sent to the laboratory. The blood collected in the Clot Activator tube for biochemistry results, was left to rest for 10 minutes in the laboratory before centrifugation. A Mindray BC-30 Vet Hemogram device was used to determine the hemogram data. After entering the device settings, the EDTA tube containing the blood sample was placed in the device's sample intake apparatus to deliver the sample. Results were digitally transferred by the device and a physical copy was obtained.

After the biochemistry tube arrived at the laboratory, it was centrifuged in a Medwelt 800d centrifuge at 3000 rpm for 10 minutes. The centrifuged samples were then placed in a Fujifilm Dri-Chem NX600 biochemistry analyzer. Results were digitally transferred by the device and a physical copy was obtained.

Statistical analyses

It was determined that the data (haemogram parameters, amylase and lipase) did not show normal distribution by performing normality test. The comparison of health status (patient and healthy), gender (male and female) parameters were compared by Mann-Whitney U test and age group (3-5 months, 6-11 months, 12-17 months and 18 months and over) were compared by Kruskall-Wallis test and 5% significance level was accepted.

Results

The study evaluated 30 cats. The age range of the patient group was between 2 months and 22 months, while the control group's age range was between 5 months and 11 years. In both groups, eight male and seven female cats were assessed.

Examining the hemogram parameters, leukopenia and neutropenia were observed in all 15 cats in the patient group. Lymphopenia was detected in 9 patients, anemia in 2 patients, and thrombocytopenia in 9 patients. The hemogram parameters in the control group were within normal ranges (Yarsan, 2023).

The values of white blood cell (WBC) count, lymphocyte count, lymphocyte percentage, neutrophil count, neutrophil percentage, eosinophil count, eosinophil percentage, erythrocyte count, and monocyte count in the patient cats were found to be lower and statistically significantly different compared to healthy cats ($p<0.05$).

Hemoglobin, hematocrit, and platelet values were lower in patient cats compared to healthy cats, with statistically significant differences ($p<0.05$). The patient cats were anemic and thrombocytopenic.

When examining amylase and lipase values, although these values were within the reference range for both groups, they were found to be higher in patient cats compared to healthy cats, with statistically significant differences ($p<0.05$) (Yarsan, 2023). The blood parameter values, amylase, and lipase values for patient and healthy cats are given in Table 1.

When comparing the blood test parameters of male and female patient cats, no statistically significant differences were found between male and female patient cats for hemogram and biochemistry parameters ($p>0.05$).

In the comparison of blood test parameters according to the age groups of cats, no statistically significant differences were found between the groups ($p>0.05$).

Discussion

FPV is the causative agent of a highly contagious disease characterized by high fever that can result in death (Tuzio, 2021). The study examined the effects of FPV disease on hemogram parameters, amylase, and lipase enzymes in cats.

In a study conducted by Gülersoy et al. (2023), hemogram analysis revealed that the panleukopenia group had lower levels of WBC, lymphocytes, monocytes, granulocytes, erythrocytes, hematocrit, erythrocyte distribution volume, and hemoglobin compared to the control group.

Kadam et al. (2022) observed anemia, neutropenia, and lymphopenia in cats affected by FPL in their study. Modi et al. (2024) detected leukopenia, anemia, thrombocytopenia, neutropenia, and lymphopenia in the hematological analysis of cats affected by FPV.

In the present study, similar to previous studies, WBC, lymphocyte count, lymphocyte percentage, neutrophil count, neutrophil percentage, eosinophil count, eosinophil percentage, erythrocyte count, and monocyte count values were found to be lower in infected cats compared to healthy cats.

The study also found lower hemoglobin, hematocrit, and platelet values in infected cats compared to healthy cats, consistent with previous studies.

After entering the body, the virus multiplies in the oropharyngeal lymphoid tissue. It then spreads to all tissues via the bloodstream. Lymphoid tissue infection causes lymphoid tissue necrosis. Bone marrow infection and suppression lead to a decrease in blood parameters. Clinical signs related to leukopenia and thrombocytopenia appear approximately 2 weeks after bone marrow necrosis.

Table 1. Blood values of healthy and patient cats

Parameters	Group	N	Mean Rank	P (sig.)
WBC	Patient	15	8.00	0.000*
	Healthy	15	23.00	
NEU	Patient	15	8.00	0.000*
	Healthy	15	22.50	
LYM	Patient	15	8.60	0.000*
	Healthy	15	22.40	
MON	Patient	15	8.00	0.000*
	Healthy	15	23.00	
EOS	Patient	15	11.23	0.008*
	Healthy	15	19.77	
NEU%	Patient	15	8.67	0.000*
	Healthy	15	22.33	
LYM%	Patient	15	22.20	0.000*
	Healthy	15	8.80	
MON%	Patient	15	15,13	0.819
	Healthy	15	15.87	
EOS%	Patient	15	19.50	0.013*
	Healthy	15	11.50	
RBC	Patient	15	11.67	0.017*
	Healthy	15	19.33	
HGB	Patient	15	12.50	0.062
	Healthy	15	18.50	
HCT	Patient	15	11.87	0.024*
	Healthy	15	19.13	
MCV	Patient	15	13.63	0.245
	Healthy	15	17,37	
MCH	Patient	15	17.23	0.280
	Healthy	15	13.77	
MCHC	Patient	15	20.60	0.001*
	Healthy	15	10.40	
RDW_CV	Patient	15	17.50	0.213
	Healthy	15	13.50	
RDW_SD	Patient	15	15.97	0.771
	Healthy	15	15.03	
PLT	Patient	15	9.80	0.000*
	Healthy	15	21.20	
MPV	Patient	15	14.70	0.618
	Healthy	15	16.30	
PDW	Patient	15	19,50	0.013*
	Healthy	15	11.50	
PCT	Patient	15	9.53	0.000*
	Healthy	15	21.47	
Lipase	Patient	15	22.70	0.000*
	Healthy	15	8.30	
Amylase	Patient	15	18.77	0.042*
	Healthy	15	12.23	

Irgashev et al. (2023) detected alpha-amylase hyperenzymemia associated with pancreatic damage, a significant increase in bilirubin levels, a 20% increase in serum protein levels, and elevated creatinine and urea levels in their study. This study concluded that cats with panleukopenia have a complex pathogenesis with characteristic changes in hematological and biochemical parameters, along with the development of immunosuppression and multiple organ failure.

Parent et al. (1995) and Armstrong et al. (2012) observed increases in serum amylase and lipase activities in their feline pancreatitis studies.

In the present study, similar to other studies, when examining the biochemical parameters of the infected group and the control group, amylase and lipase values were found to be significantly higher in the infected group compared to the control group. Pancreatic enzyme levels in infected cats are higher than in healthy cats. This increase is thought to be associated with pancreatitis.

Conclusion

In conclusion, FPV is a highly contagious disease agent with high mortality that affects cats of all ages and manifests itself with numerous symptoms. Hemogram parameters will guide clinicians in the diagnosis and prognosis of the disease. The elevated amylase and lipase values in patients compared to the control group are important for diagnosis. However, the fact that amylase and lipase values are within normal reference ranges in all samples makes it difficult to use these enzymes in the diagnosis of FPV.

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JIVS JOURNAL OF ISTANBUL VETERINARY SCIENCES

Prevalence of gastrointestinal parasites in domestic pigeons in Van province

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ABSTRACT

This study aimed to determine the prevalence of intestinal parasites in pigeons raised for hobby or different purposes in Van province and to help develop protective measures for their healthy lives. The fecal material of the study was collected from 240 domestic pigeons randomly selected from 26 different cages raised for hobby purposes in Van province. Native, flotation, and sedimentation techniques were used for the examination of fecal samples. Eimeria spp. oocysts were sporulated in 2.5% potassium dichromate to identify the species. In the study, 219 of the 240 (91.25%) - samples were found to be infected with one or more parasite species. There were five helminth species among the identified parasite species: *Capillaria* spp., *Ascaridia columbae*, *Heterakis gallinarum*, *Raillietina* spp. and *Syngamus* spp. In addition, two protozoan species, Eimeria *columbarum* and *Eimeria labbeana*, were detected as enteric protozoa. The most common parasite species in the examined fecal samples were as follows: *Eimeria columbarum* (63 samples, 26.25%), *Eimeria labbeana* (58 samples, 24.17%), *Capillaria* spp. (34 samples, 14.17%), *Ascaridia columbae* (22 samples, 9.17%), *Heterakis gallinarum* (11 samples, 4.58%), *Raillietina* spp. (18 samples, 7.5%) and *Syngamus* spp. (13 samples, 5.42%). In conclusion, it was observed in this study that the rate of detection of intestinal parasites in pigeons raised for hobby or different purposes in Van province was high. Therefore, it was concluded that in order to reduce the prevalence of parasitic infections in pigeons in the region, animal owners should be informed, attention should be paid to the cleanliness and hygiene of pigeon cages, and more importance should be given to the diagnosis and treatment of intestinal parasites in these animals.

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Introduction

Pigeons are birds belonging to the Columbidae family of the Columbiformes order, used for meat production, hobby, competition, show, and experimentation purposes (Sales and Janssens, 2003; Yılmaz and Boz, 2012; Attia et al., 2022). These birds can transmit important diseases to other bird species and mammals and contribute to the spread of zoonotic diseases in humans. They are also important because they are reservoirs of many parasitic diseases (Sari et al., 2008; El-Dakhly et al., 2018). Pigeons pollute the environment through various pathogenic agents and cause the spread of parasites among different animal and bird species with their feces (Attia and Salem, 2021). Many parasites detected in pigeons reduce the performance of these animals, can lead to developmental delays, cessation of egg production, and death (Dranzoa et al., 1999; Şenlik, 2005; Gül et al., 2009). Helminths, in particular, are of great importance due to endoparasite infections in pigeons. In studies conducted worldwide, the presence of

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Capillaria spp., *Ascaridia columbae*, *Heterakis* spp., *Dispharynx* spp., *Tetrameres* spp., *Syngamus* spp., *Raillietina* spp., *Cotugnia digonophora* and *Strongyloides avium* have been reported in pigeons (Şenlik, 2005; Gül et al., 2009; Elmajdoub and Mshiheet, 2016; Mehmood et al., 2019; Ali et al., 2020; Walteros-Casas et al., 2021; Das et al., 2022; Thankachan et al., 2022). Helminths are the main responsible for important clinical and subclinical infections in domestic pigeons (Ali et al., 2020). Coccidiosis is one of the important protozoan parasites in pigeons as in other poultry animals. The presence of *Eimeria labbeana, E. columbae, E. columbarum, E. pfeifferi, E. tropicalis, E. janovyi, E. waiganiensis, E. curvata, E. gourai* and *E. duculai* species have been reported in studies conducted in the environment. In Turkey, the presence of *E. labbeana, E. columbarum* and *E. columbae* has been reported in domestic pigeons (Sarı et al., 2008; Gül et al., 2009). There are a limited number of studies on the detection of parasites in domestic and wild pigeons in Turkey. This study aimed to determine the prevalence of intestinal parasites in pigeons raised for hobby or different purposes in Van province and to help develop preventive measures for their healthy lives.

Materials and methods

The study material consists of the feces of 240 domestic pigeons randomly selected from 26 different cages in different cages raised for hobby purposes in Van province between February and September 2022. The necessary permission for the study was obtained from Van Yuzuncu Yil University Animal Experiments Local Ethics Committee (decision dated 24/02/2022 and numbered 2022/02-04). The fecal samples collected in the pigeon cages were placed in feces containers and brought to Van Yuzuncu Yil University, Faculty of Veterinary Medicine, Department of Parasitology laboratory. Samples were examined on the same day as much as possible, and those that could not be examined on the same day were stored in the refrigerator at +4°C. Native, flotation, and sedimentation techniques were used to examine helminths in fecal samples. For enteric protozoa, stool samples were mixed with 2.5% potassium dichromate (K2Cr2O7) solution and placed in separate petri dishes to form a thin layer and left to sporulate in the laboratory (Kaya, 2003). After sporulation, the preparations were examined microscopically for the morphological structures of Eimeria oocysts and species were distinguished (Levine, 1985; Mimioğlu et al., 1969; Soulsby, 1968).

Statistical analysis

Statistical analyses were performed using SPSS (Version. 26) package program. As statistical analysis, the relationship between the species was determined using the Chi-square test. Descriptive statistics are given as numbers and The significance level was accepted as $p<0.05$.

Results

In the study, 219 out of 240 (91.25%) pigeon feces samples examined were found to be infected with one or more parasite species. Among the parasite species identified, there were five helminth species: Capillaria spp. Ascaridia columbae, *Heterakis gallinarum*, Raillietina spp. and *Syngamus* spp. In addition, two protozoan species, *Eimeria columbarum* and *Eimeria labbeana*, were found as enteric protozoa.

The most common parasite species in the examined feces samples were as follows: *Eimeria columbarum* (63, 26.25%), *Eimeria labbeana* (58, 24.17%), *Capillaria* spp. (34, 14.17%), *Ascaridia columbae* (22, 9.17%), *Heterakis gallinarum* (11, 4.58%), *Raillietina* spp. (18, 7.5%) and *Syngamus* spp. (13 5.42%). In the study, the number of pigeons infected with helminths was found to be 98 (%40.83), and the number of those infected with enteric protozoa was found to be 121 (%50.41). The number of pigeons infected with both helminths and enteric protozoa was found to be 19 (%7.92). Among pigeons infected with one or more parasite species, the number of those infected with a single species was determined to be 68 (%28.33), the number of those infected with two species was determined to be 56 (%23.33) and the number of those infected with three species was determined to be 5 (%2.08) (Table 1).

Discussion

Infections caused by endoparasites in pigeons can cause developmental delays, loss of condition and sometimes death. Various studies have been conducted to determine endoparasites in domestic pigeons. In these studies conducted in different regions of our country; *Ascaridia* spp., *Heterakis* spp., *Capillaria* spp., *Raillietina* spp. and *Syngamus* spp. helminths and *Eimeria* spp., *Isospora* spp. and *Cryptosporidium* spp. protozoa were detected (Sarı et al., 2008; Gül et al., 2009; Gökpınar et al., 2023). In this study, five helminth species (*Capillaria* spp*, Ascaridia columbae, Heterakis gallinarum, Raillietina* spp. and *Syngamus* spp.) and two enteric protozoan species (*Eimeria colum*barum and *Eimeria labbeana*) were detected. As a result of this study, it was determined that 219 (91.25%) of the 240 pigeon feces samples examined were infected with one or more parasite species. In studies conducted in different parts of the world, these rates were reported as between 69.16% and 87.1% in Nepal (Gurung and Subedi, 2016; Adhikari et al., 2022), 81% in Indonesia

Table 1. Coccidia and helminth species and their infection rates in pigeons subjected to fecal examination.

Parasites	Types of Parasites	Total number of positive cases in 240 pigeons	Infection rate (%)	Chi-Square	p
Helminths	*Capillaria spp*	34 (16)[a]	14.17 (6.67)[a]	50.062	0.000*
	Ascaridia columbae	22 (12)[a]	9.17 (5)[a]		
	Heterakis gallinarum	11	4.58		
	Raillietina spp	18 (13)[a]	7.5 (5.42)[a]		
	Syngamus spp	13	5.42		
	Capillaria spp+ Ascaridia columbae	4	1.67		
	Capillaria spp+ Raillietina spp	3	1.25		
Total		98	40.83		
Enteric protozoa	*Eimeria columbarum*	63 (15)[a]	26.25 (6.25)[a]	10.949	0.004*
	Eimeria labbeana	58 (12)[a]	24.17 (5)[a]		
	E.columbarum+E. labbeana	35	14.58		
Total		121	50.42		
Intestinal protozoa+ Helminth	*Eimeria columbarum+ Capillaria spp*	8	3.33	4.886	0.180**
	E. labbeana+ Ascaridia columbae	6	2.5		
	Eimeria columbarum+ E. labbeana + Capillaria spp	3	1.25		
	Eimeria columbarum+ E. labbeana + Raillietina spp	2	0.83		
Grand total		219	91.25		

a = Infection rates with a single species, * According to the Chi-square test, there is a significant difference between helminths and enteric protozoa infestation rates ($p<0.001$), * * According to the Chi-square test, the relationship between multiple infestations of helminths and enteric protozoa was not found to be statistically significant ($p>0.05$).

(Ashfiyah et al., 2022), 84.56% in Poland (Bartosik et al., 2020), and 44.10%-86.8% in India (Sivajothi and Sudhakara, 2015; Das et al., 2022; Thankachan et al., 2022). In Turkey, these rates were determined as between 59.6% and 71.72% (Sarı et al., 2008; Gül et al., 2009). In general, the results obtained from endoparasite studies conducted in pigeons around the world are similar to the results obtained in this study. In this study, 40.83% of the positive samples had helminth infection, 50.41% had protozoan infection, and 7.91% had mixed helminth and protozoan infection. In a study conducted in Niğde, it was reported that 58% of domestic pigeons were coccidia positive and 42% were coccidia+helminth positive (Sarı et al., 2008). While other studies found the mixed infection rate in domestic pigeons as 31.8% (Sivajothi and Sudhakara, 2015) and 35.1% (Thankachan et al., 2022), this rate was found to be 60% in pigeons kept at home and 85.6% in pigeons kept around temples (Adhikari et al., 2022). When the fecal samples analyzed in our study were examined in terms of infection with one or more parasite species; were determined as 68 (28.33%) infected with a single species, 56 (23.33%) infected with two species and 5 (2.08%) infected with three species. In studies conducted worldwide on *Ascaridia* spp. in domestic pigeons based on fecal examination; 13.58% in Poland (Bartosik et al., 2020), between 21.66% and 22.6% in Nepal (Gurung and Subedi, 2016; Adhikari et al., 2022), 22% in Libya (Alkharigy et al., 2018), 42% in Indonesia (Ashfiyah et al., 2022) and between 18.60% and 33.3% in India (Sivajothi and Sudhakara, 2015; Das et al., 2022). In studies conducted in Turkey, *Ascaridia* spp. rates were determined as 5.1% in Niğde (Sarı et al., 2008), 11.03% in Van (Gül et al., 2009) and 41.9% in Kırşehir (Gökpınar et al., 2023). In this study, 9.17% Ascaridia spp. was detected. The rate in our study was lower than studies worldwide; it was similar to Niğde and Van, but lower than the study in Kırşehir. It is thought that the differences in the results are due to the number of samples examined, the diagnostic methods used and environmental factors. In our study, *Capillaria* spp. eggs were found in 14.17% of the pigeon droppings examined. In other studies conducted in

et al., 2009), 19.9% in Niğde (Sarı et al., 2008) and 33.3% in Kırşehir (Gökpınar et al., 2023). In studies conducted worldwide, it was determined as 32.71% in Poland (Bartosik et al., 2020), 41% in Indonesia (Ashfiyah et al., 2022), 31.67% in Nepal (Gurung and Subedi, 2016) and 9.30% in India (Das et al., 2022). The results of this research are similar to studies conducted in the world and Turkey.

Heterakis spp. detected in different provinces of Turkey. egg rates were reported as 3.7% in Niğde (Sarı et al., 2008), 6.2% in Van (Gül et al., 2009) and 12.4% in Kırşehir (Gökpınar et al., 2023). It was determined as 45% in Tuban, Indonesia (Ashfiyah et al., 2022) and 2.5% in Nepal (Gurung and Subedi, 2016). In this study, the rate of *Heterakis* spp. was found as 4.6%, which is similar to the studies conducted in Niğde, Van, and Nepal. However, lower rates were found in the studies in Kırşehir and Indonesia. It is estimated that the differences in the results regarding *Heterakis* spp. may be due to the number of samples examined, rearing conditions and environmental factors.

Coccidiosis is one of the most important protozoal infections of poultry. In previous studies conducted in Turkey, *Eimeria* spp. rates in domestic pigeons were determined as 59.6% in Niğde, 67.58% in Van, and 68.6% in Kırşehir (Sarı et al., 2008; Gül et al., 2009; Gökpınar et al., 2023). In studies conducted worldwide, rates between 8.13% and 39.5% in India (Sivajothi and Sudhakara, 2015; Das et al., 2022; Thankachan et al., 2022), 8.1% in Iraq (Ul-Jabbar et al., 2019), 36% in Colombia (Walteros-Casas et al., 2021), 40.9% in Iran (Radfar et al., 2012) and 80.86% in Poland (Bartosik et al., 2020) were reported. In this study, the *Eimeria* spp. rate was found to be 50.41%. While this rate is similar to the rates detected in other studies conducted in Turkey, it differs from studies conducted worldwide.

Conclusion

As a result, it is observed that the rate of intestinal parasites detected in pigeons raised for hobby or different purposes in Van province is high in this study. This situation shows that parasitic control is not carried out at an adequate level. It was concluded that in order to reduce and control the rate of parasitic infection in pigeons in Van province, animal owners should be informed about this issue, attention should be paid to the hygiene of pigeon cages and coops, and importance should be given to the diagnosis and treatment of parasitic diseases in animals.

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A case of secondary ectopic pregnancy and *schistosoma reflexum* in a cat

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ABSTRACT

This report describes a case of ectopic fetus and *Schistosoma reflexum* encountered in a 3-year-old, 4 kg tabby cat brought to our clinic for routine neutering. Gingivitis and multiple intraabdominal masses were detected in the clinical examination of the cat. Leukocytosis and anemia were determined in the haemogram. Ovariohysterectomy was performed to remove two ectopic fetuses covered with fibrous capsules beyond the ovaries. *Schistosoma reflexum* was detected in one of the ectopic fetuses. The cat received postoperative care for one week and recovered without any problems. In conclusion, it was observed that ectopic fetus cases in cats can be associated with *Schistosoma reflexum*. These cases can be diagnosed using imaging methods, and surgical intervention can provide treatment.

Keywords: cat, ectopic pregnancy, *Schistosoma reflexum*

The summary of the case report will be shown in a poster presentation at the 24th International Veterinary Students Scientific Research Congress to be held on 11-13 December. Istanbul, Türkiye.

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Introduction

The gestation period in cats is 61-72 days from the first day of mating, with an average of 65 days (Kustritz et al., 1995). Ultrasonography and hormone measurements are the most commonly used and highly accurate methods for early pregnancy diagnosis in domestic animals (Alaçam, 2010). Ultrasonography is preferred in pregnancy diagnosis and detection of ovarian and uterine pathologies because it provides accurate and rapid results and has no harmful effects on the operator or the patient (Rendano, 1983; Atmaca, 1985; Biller and Haibel, 1987; Barr, 1988). Diagnostic imaging methods such as radiography, ultrasonography, diagnostic laparoscopy, computed tomography and magnetic resonance imaging are used in veterinary gynecology (Thrall, 1994; Kao et al., 2014). Pathological conditions such as subinvolution of placental regions, postpartum metritis, pyometra, cystic endometrial hyperplasia, uterine rupture or tumours can also be determined by ultrasonography (Barr, 1992; Kahn, 1994; Alaçam, 1998; England, 1998a and 1998b; Wright and Watt, 1998; Luvoni and Grioni, 2000; Son et al. , 2001; Kutzler et al., 2003; Eker and Salmanoğlu, 2005b). Radiography should not be used during pregnancy unless necessary to avoid the side effects of X-rays on developing fetuses (Burke, 1986). Mineralization can be observed from the 35th day of pregnancy (Dennis et al., 2010). Lateral radiographs can be taken 5-10 days before parturition to determine the number and location of offspring (Simpson et al., 2004; Peterson and Kutzler, 2011). Extrauterine pregnancy refers to a condition in which pregnancy develops outside the uterus (Corpa, 2006). Primary abdominal extrauterine pregnancy occurs when the fertilized oocyte cannot be transferred to the uterus due to oviductal obstructions or oviductal contraction waves, causing it to fall into the abdominal cavity (Corpa, 2006; Curtseit et al., 2016; Hughes, 2019; Bhatta et al., 2020). Abdominal ectopic

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pregnancy develops as a result of the implantation of the fetus in the abdominal cavity and is classified as primary or secondary (Corpa, 2006). Primary ectopic pregnancy can be encountered in rodents and lagomorphs because they possess a discoid, hemomonochorial placenta, similar to humans (Dzięcioł et al., 2008). Secondary ectopic pregnancy occurs when the oocyte develops into a fetus within the uterus but falls into the abdominal cavity as a result of a rupture in the uterine wall. Secondary ectopic pregnancy can occur due to trauma, uterine rupture, uterine anomalies, increased uterine pressure, or the administration of high doses of oxytocin (Jerome and Hendrickx, 1982; Corpa, 2006; Sagar et al., 2017). In veterinary literature, long-standing mineralized abdominal ectopic fetuses have been described in monkeys, rabbits, dogs, and cats (Carrig et al., 1972; Segura et al., 2004; Corpa, 2006). The primary treatment principle is often the surgical removal of the mummified ectopic fetus (Johnson, 1986).

Schistosoma reflexum is a congenital anomaly which have been rarely described in cats (Kawata and Tiba, 1961). The etiology of various congenital anomalies, including *Schistosoma reflexum*, remains unknown. However, genetic mutations, chromosomal anomalies, environmental factors, infectious agents, or combinations of these factors are thought to contribute to the formation of ventral body wall defects and associated internal organ anomalies (Timurkan and Mert, 1987; Özsoy et al., 2009).

This case report aims to describe the diagnosis and treatment approaches of an extrauterine pregnancy in a cat, which may be accompanied by a pregnancy pathology such as *Schistosoma reflexum*.

Case History

A three-year-old domestic cat weighing 4 kg was brought to our clinic for routine neutering. The mucous membranes were rosy pink, respiration was 20/min, heart rate was 120/min, body temperature was 38.7 °C, and infection was found in the gums and right upper molar tooth. Haematological examination revealed anaemia and leukocytosis (Figure 1). Abdominal palpation revealed two hard, movable masses approximately 6-7 cm in diameter. Palpation of the mass did not cause pain. Abdominal radiography was taken in the laterolateral position (BMI, Vet System + Plus Digital X-Ray Device, Italy) and mineralised structures measuring 5.5x5 cm and 2.5x3 cm were visualized (Figure 2). The head and spine of the fetus, which was curled up on the radiograph, were identified. B-mode abdominal ultrasonography (SIUI, Apogee 2100V, People's Republic of China) was performed to determine fetal viability and no fetal heartbeat was detected. According to the transversal body diameter, the fetus was 44 days old. Laparotomy was applied to remove the dead fetuses and a standard ovariohysterectomy under general anaesthesia was performed (Rosset et al., 2011).

Fetuses encapsulated in a fibrous capsule surrounded by the mesentery were removed from the right ovary, approximately 3 cm ahead, and from the left abdomen (Figure 3). Following the incision of the capsule of the larger fetus, schistosoma reflexum was diagnosed in the cat (Figure 4). Absorbable suture material was used for all sutures. To prevent postoperative pain and infections, meloxicam hydrochloride (0.2 mg/kg, subcutaneous, SID, Meloxicam, Bavet®, Turkey) and amoxicillin-clavulanic acid (20 mg/kg, subcutaneous, SID, Synulox, Zoetis, USA) were administered for seven days. On the 10th day postoperatively, the skin sutures were removed, and the cats general condition was observed to be satisfactory.

TEST	RESULT	REFERENCE VALUE	
RBC	3.67	6.54 - 12.20 M/µL	L
Haematocrit	12.6	30.3 - 52.3 %	L
Haemoglobin	5.0	9.8 - 16.2 g/dL	L
MCV	34.3	35.9 - 53.1 fL	L
MCH	13.6	11.8 - 17.3 pg	
MCHC	39.7	28.1 - 35.8 g/dL	H
RDW	23.6	15.0 - 27.0 %	
% Reticulocyte	1.0	%	
Reticulocytes	38.2	3.0 - 50.0 K/µL	
Reticulocyte Haemoglobin	15.3	13.2 - 20.8 pg	
WBC	48.51	2.87 - 17.02 K/µL	H
% Neutrophils	*47.8	%	
% Lymphocytes	*49.5	%	
% Monocytes	*2.1	%	
% Eosinophils	0.4	%	
% Basophils	0.2	%	
Neutrophils	*23.22	2.30 - 10.29 K/µL	H
Bands	*Suspected		
Lymphocytes	*24.00	0.92 - 6.88 K/µL	H
Monocytes	*1.01	0.05 - 0.67 K/µL	H
Eosinophils	0.20	0.17 - 1.57 K/µL	
Basophils	0.08	0.01 - 0.26 K/µL	
Platelets	380	151 - 600 K/µL	
MPV	17.0	11.4 - 21.6 fL	
Pleteletcrit	0.65	0.17 - 0.86 %	

Figure 1. Hematological parameters of the cat.

Discussion and Conclusion

Although ectopic pregnancy is a well-known pathology in humans due to regular gynecological examinations, it is rarely diagnosed in animals because routine examinations are not conducted. Detailed epidemiological studies on ectopic pregnancy in animals have not been carried out (Hong and Armstrong, 1978; Van Den Eeden at al., 2005). According to the literature, ectopic pregnancy is rare in

animals and is mostly reported in cats (Bodle, 1979; Corpa, 2006; Çetin et al., 2014; Rosset et al., 2011). Since the placenta of cats cannot support the growth and development of a fetus outside the uterus, there have been no reports of an ectopic fetus reaching maturity in the abdominal cavity of felines (Bodle, 1979). In our case, the exact time when the fetus fell into the abdominal cavity is unknown, but the fact that the fetuses were dead indicates that the ectopic fetus could not survive in the abdominal cavity.

Figure 2. Abdominal radiograph of the cat taken in a ventrodorsal position (Red arrows indicate the fetuses).

Most cats with ectopic pregnancy do not show clinical signs, and some may even remain fertile. An ectopic fetus may remain in the abdominal cavity for several months or even years. A systemic inflammatory response can result from necrotic ectopic tissues or mechanical stimulation of the ectopic fetus, leading to clinical symptoms. Some cases may result in pyometra (De Nooy, 1979; Botcherby, 1980; Nack, 2000; Corpa, 2006; Tu et al., 2016). Some cats may exhibit symptoms such as fever, anorexia, vomiting, lethargy, depression, hematuria, pollakiuria, or urinating outside the litter box (Johnston et al., 2013). In the presented case, no clinical signs or pyometra were observed in the cat. The lack of clinical signs may be due to the fibrous capsule surrounding the ectopic fetus not adhering to vital organs.

Since ectopic fetuses usually do not cause noticeable symptoms, they are often diagnosed

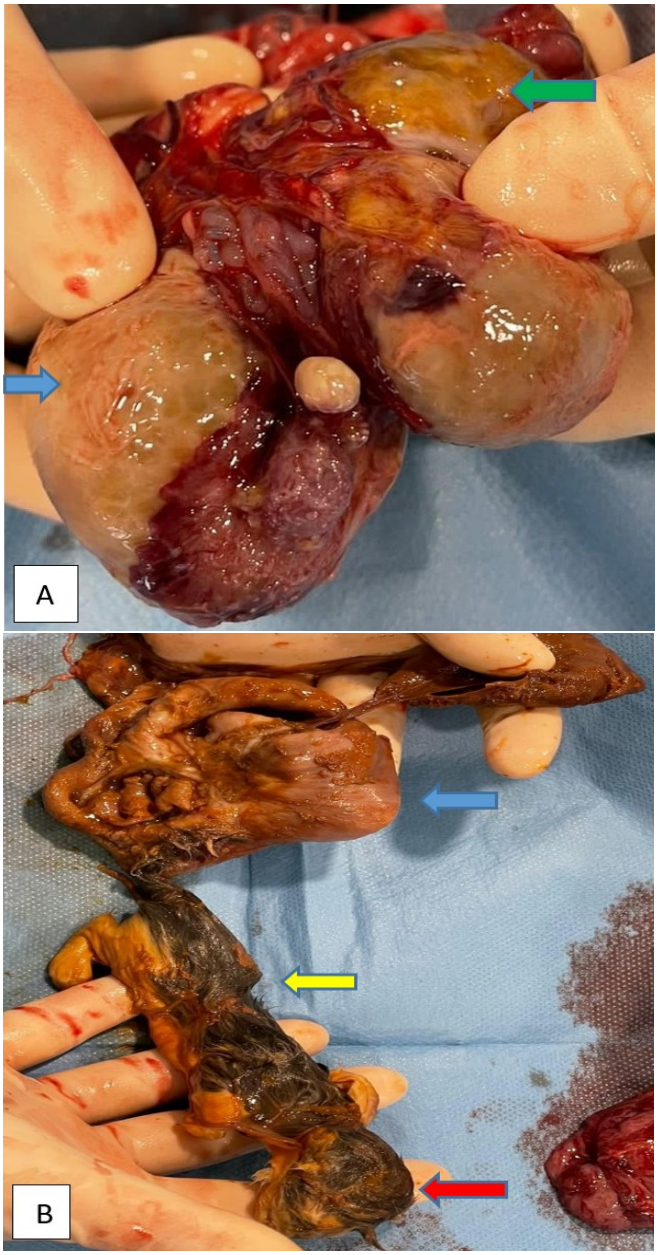

Figure 3. Ectopik fetuses. A: Extrauterine fetus within a fibrous capsule (body of the ectopic fetus; blue arrow, head of the ectopic fetus; green arrow). B: Fetus removed from fibrous capsule (head of the ectopic fetus; red arrow, body of the ectopic fetus; yellow arrow, the fibrous capsule; blue arrow).

incidentally (Rosset et al., 2011). The diagnosis of ectopic pregnancy can be made using imaging methods such as radiography, ultrasonography, diagnostic laparoscopy, or computed tomography. Although ultrasonography is the most preferred imaging method for pregnancy examinations, computed tomography and magnetic resonance imaging techniques provide more useful information for determining the exact location of an extrauterine fetus (Kao et al., 2014). In this case, the ectopic fetuses were diagnosed through abdominal radiography. Radiographic findings of an

Figure 4. Fetus with *Schistosoma reflexum* anomaly removed from the fibrous capsule (the fibrous capsule; blue arrow, head of the ectopic fetus; green arrow, exposure of the abdominal organs of the fetus; yellow arrow).

abdominal ectopic fetus include a round, curled fetal outline, bones that are more radio-opaque than usual, a more contrasted image due to the lack of fluid in the fetal sac, and the fetus being located in a site unrelated to the uterus (Thrall, 1994). In agreement with other researchers, the lack of fluid in the fetal sacs and the fetus being located outside the uterus were detected on a lateral abdominal radiograph.

Mateo and Camon (2008) reported clinical approaches to a cat carrying a fetus with multiple congenital malformations that met the criteria for true *Schistosoma reflexum*. Similarly, abdominoschisis and exposure of the abdominal organs were identified in the fetus with *Schistosoma reflexum* in this case.

In conclusion, this study highlights the association between ectopic fetus cases and schistosoma reflexum in cats. Advanced imaging techniques play a crurial role in diagnosis, and surgical intervention remains the primary treatment option.

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Effects of non-steroidal anti-inflammatory drug administration following parturition on milk yield, postpartum disorders and reproductive parameters in lactating dairy cows

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Research Article

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ABSTRACT

In this study, effects of carprofen, as a long-acting non-steroidal anti-inflammatory drug (NSAID), administration to decrease pain following calving on changes in body weight (kg) between days 1-15, 15-30 and 1-30 postpartum, the first 150 days milk yield, postpartum diseases, milk electric conductivity scores, estrous activities based on pedometer and reproductive parameters were investigated in lactating cows. Physiologic saline (Control; n=50) or Carprofen (1.4 mg/kg; Rimadyl®, n=50) was administered subcutaneously within 12 hours following parturition. All cows were normally calved (eutocia). Vaginal discharge was visually examined on 25-32 days postpartum. Cows were artificially inseminated (AI) following Ovsynch protocol or estrus detected with pedometer. Incidences of retained foetal membranes, metritis, mastitis were not differed between groups. Occurrence of pathological vaginal discharge was significantly ($P<0.05$) higher in Carprofen (25.6% [10/39]) group than that in Control (7.3% [3/41]) group. There were no differences in intervals from calving to the first detected estrus and AI, pregnancy per AI, changes in body weight nor 150 days milk yield between groups. Rate of at least one detected estrus until 70 days postpartum was significantly ($P<0.05$) higher in Control (97.7% [42/43]) group than that in Carprofen (84.6% [33/39]) group. In conclusion, Carprofen administration following calving did not increase milk yield or fertility in eutotic Holstein cows in this study. Furthermore, no increase in the incidence of retained foetal membranes or metritis following carprofen administration in this study could allow to use of carprofen immediately after calving for therapeutic purposes in eutotic cows.

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Introduction

Parturition is a crucial moment for the health and welfare of the cow (Laven et al., 2012). The welfare of an animal is defined as its state with regard to its efforts to deal with its environment (Broom, 1986). In this matter, inflammation and pain are anticipated to be more prevalent and critical in primiparous cows due to narrower pelvic region, slower parturition and deficient vulva dilatation (Mee, 2004). Pain care following parturition could be useful for a cow by causing a better appetite leading to more milk production (Stilwell et al., 2014). Although non-steroidal anti-inflammatory drugs (NSAIDs) contain analgesic, anti-inflammatory, anti-endotoxic and anti-pyretic impacts in cattle, NSAID's are less likely to be used pain management following calving (Laven et al., 2012). Pregnancies in cattle may be subject to dystocia

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ectopic at a rate of 3-25%, which causes a significant economic loss in breeding. Nutrition and environmental factors are among the causes of dystocia. Body condition score, oxidative stress, mineral substance balance in the diet and dry period nutrition, housing conditions, climate and geographical location are factors that predispose to dystocia. The incidence of dystocia can be reduced with a balanced diet, appropriate reproductive management and good livestock practices (Günay Uçmak and Kurban, 2023).

NSAIDs have light organic acid constitution with a broad range of structures suppress cyclooxygenase (COX) enzyme in the arachidonic acid cascade leading to lessen the release of prostaglandins (Espinasse et al., 1994; Vane, 1971). Xie et al. (1991) reported that COX enzymes are in two isoforms including COX1, plainly fundamental, and COX2, an inducible. Because COX2 enzyme primarily leads to production of prostaglandins associated with inflammation, fever and pain, therapeutic impacts of NSAIDs, including peripheral and central analgesia, anti-inflammatory, anti-pyretic and anti-endotoxic effects are attributed to suppression of COX2 enzyme (Fitzpatrick et al., 2004). Among NSAIDs, carprofen non-selectively supresses COX-1 and COX-2 enzymes in cattle (Brentnall et al., 2012). That is why carprofen was reported as a treatment of choice, as an analgesic, for painful cases in cattle (Stilwell et al., 2008).

Antanaitis et al. (2018) reported decrease in concentration of blood cortisol reflecting stress for 48 hours following parturition in primiparous dairy cows administered carprofen within one hour after from calving. Moreover, higher pregnancy rate, shorter calving interval, lower somatic cell count in milk and lower incidences of retained fetal membranes (RFM), metritis and clinical mastitis have been reported in primiparous dairy cows administered carprofen within one hour after from calving (Antanaitis et al., 2018). Stilwell et al. (2014) observed 2.593 L more milk in primiparous but not in multiparous dairy cows administered carprofen (1.4 mg/kg) within six hours after from calving. Stilwell et al. (2014) attributed this beneficial effect of carprofen administration on milk yield to its analgesic effect resulting in sooner feed consumption following parturition in primiparous cows.

Higher reproductive performance based on pregnancy rates were reported in response to NSAID administration following calving (Antanaitis et al., 2018; Giammarco et al., 2018; Stilwell et al., 2014). In literature, there was no study to investigate effect of NSAID administration following calving on estrous expression rate and subclinical mastitis based on milk electrical conductivity during postpartum period. Therefore, it was aimed to investigate effect of carprofen administration following calving on milk yield, reproductive performance and incidences of postpartum diseases/disorders along with mastitis in lactating cows in this study.

Materials and Methods

Animals and experimental design

This study was approved by Local Ethical Committee for Animal Experiments at Balıkesir University (File Number: 2020/4-11, 25.06.2020). This study was conducted in commercial dairy farm located in Balıkesir, Türkiye. Holstein dairy cows were milked freely for 24 hours in Robotic milking system. Lactating Holstein cows were fed (26 kg dry matter/head/day) with total mixed ration (%3.6 wheat hay, %12 alfalfa, % 51.6 corn silage, %3 soy bean meal, %9 corn flake, %3.6 crushed barley grain, %9.6 sugar beet pulp, %0.6 molas, %4.8 concentrated feed, %0.96 by-pass fat, %0.96 buffer, %0.3 yeast) including 2900 kcal metabolic energy and %14.6 crude protein. Primiparous (n=27) and multiparous (n=73) cows with single offspring were randomly divided into two groups, and then physiologic saline (Control; n=50) or Carprofen (1.4 mg/ kg; Rimadyl®, Zoetis Animal Health, Türkiye; n=50) were administered subcutaneously within 12 hours following calving.

Clinical examinations and records

Calving scores in 1-4 scale (1: normal; 2: slight calving difficulty, assistance with one person without use of mechanical equipment; 3: severe dystocia, assistance with more than one person or use of mechanical equipment; 4. severe dystocia resulted in C-section) were recorded as reported by Meyer et. al., (2001).

Cows were visually examined for vaginal discharges at 25-32 days postpartum, and vaginal discharges were classified in 0-3 scale (0: bright or transparent; 1: discharge containing particles of white or off-white purulent content; 2, including ≤50% white or off-white mucopurulent content; and 3, including ≥50% purulent content, generally white or yellow, but rarely bloody discharge) as reported by (Williams et al., (2005). In order to perform discrete analyses, vaginal discharge scores below two were accepted as normal, and vaginal discharge scores equal to or above two were accepted as pathologic.

Cows did not expel the placenta 48 hours after from calving was accepted as RFM case. Cows having malodorous, purulent or bloody vaginal discharge, along with at least one systemic symptoms including fever, anorexia, fatigue were accepted as a metritis case. Presence of oedema, redness, sensibility, swelling, colour changes, fleck of pus, odor and positive results of CMT test in milk in at least one mammary gland was accepted as clinical mastitis. Subclinical

mastitis cases were determined based on milk electrical conductivity by milking system for the first 150 days during lactation.

Data for total milk yield (kg) for 150 days, live body weight (kg) on the day 0 (calving), 15 and 30 postpartum, clinical and subclinical mastitis cases, postpartum health records, estrous records based on pedometer system, the first service pregnancy rates was obtained from computerized herd management system (Lely™, Lely T4C-Time for cows, Netherlands).

In this study, routine postpartum reproductive protocol of farm was followed. In this matter, cows were mainly inseminated following Ovsynch protocol, or detection of estrus based on pedometer. In all artificial inseminations (AI), conventional sperma with proven fertility were used. Pregnancies were diagnosed with transrectal ultrasonography 30-35 and 60-70 days after from AI.

Statistical Analysis

All statistical analysis were performed with MedCalc (MedCalc® Statistical Software version 20.007 [MedCalc Software Ltd, Ostend, Belgium; https://www.medcalc.org; 2021]) program. Differences for milk yield, body weight, period time lengths from calving to the first estrous and from calving to the first AI between two experimental groups were analysed with two way ANOVA test. Mathematical model for all two way ANOVA analysis includes treatment, parity and interaction of treatment, by parity. Results were expressed as mean and standard error of mean.

All categorical analysis including rates of dystocia, RFM, pathological vaginal discharge, metritis, clinical and subclinical mastitis, estrous detection, the first service pregnancy between two experimental groups were analysed with Chi-squared test.

Results

There was no dystocia, and all cows calved normally (calving score: 1). There were no differences for the incidence of RFM, metritis, clinical mastitis and subclinical mastitis between experimental groups (Table 1). Rate of pathological vaginal discharge on days 25-32 postpartum was statistically higher (P<0.05) in Carprofen treated group (%25.6 [10/39]) compared to that in Control group (%7.3 [3/41], Table 1).

There were no differences for days in milk at the first detected estrus based on pedometer and the first AI between experimental groups (Table 2). However, rate of detected estrus until 70 days postpartum was significantly (P<0.05) higher in Control group (%97.7 [42/43]) compared to that in Carprofen treated group (%84.6 [33/39]) (Table 2). Whereas, P/AI following the first AI postpartum did not differ between experimental groups (Table 2).

Table 1. Distribution of postpartum diseases and disorders between experimental groups.

Disease/Disorder	Control	Carprofen
Dystocia (%; n/n)	% 0 (0/50) [a]	% 0 (0/50) [a]
Retained fetal membranes (%; n/n)	% 6 (3/50) [a]	% 10 (5/50) [a]
Pathological vaginal discharge (%; n/n)	% 7.3 (3/41) [a]	% 25.6 (10/39) [b]
Metritis (%; n/n)	% 2 (1/50) [a]	% 4 (2/50) [a]
Clinical mastitis (%; n/n)	% 0 (0/50) [a]	% 2 (1/50) [a]
Subclinical mastitis (%; n/n)	% 26.1 (12/46) [a]	% 43.2 (19/44) [a]

a,b Different superscripts within row indicates statistical significance (P<0.05).

Table 2. Days in milk (mean and standard error of mean) at the first detected estrus and the first AI along with rate of the detected estrus and pregnancy per AI after the first service

Reproductive parameters	Control	Carprofen
Days in milk at the first detected estrus until 70 days post-partum.	37.95 ± 2.58 [a] (n=42)	35.22 ± 3.95 [a] (n=33)
Rate of detected estrus until 70 days postpartum (%; n/n).	%97.7 (42/43) [a]	%84.6 (33/39) [b]
Days in milk at the first AI	84.07 ± 3.69 [a] (n=49)	81.82 ± 4.84 [a] (n=46)
Pregnancy per AI after the first service (%; n/n)	%20.5 (9/44) [a]	%15.4 (6/39) [a]

a,b Different superscripts within row indicates statistical significance (P<0.05).

Table 3. Differences and changes in body weight (kg; mean and standard error of mean) between experimental groups.

Time of body weight (kg) measurement	Control (n= 37)	Carprofen (n= 42)
On the day (0) of calving	648.95 ± 11.69 [a]	646.56 ± 15.86 [a]
15 days after from calving	615.63 ± 11.49 [a]	609.38 ± 15.59 [a]
30 days after from calving	600.65 ± 11.66 [a]	596.26 ± 15.81 [a]
Between 0-15 days after from calving	-33.32 ± 5.20 [a]	-37.18 ± 7.06 [a]
Between 15-30 days after from calving	-14.98 ± 4.41 [a]	-13.12 ± 5.98 [a]
Between 1-30 days after from calving	-48.30 ± 7.23 [a]	-50.30 ± 9.81 [a]

a,b Different superscripts within row indicates statistical significance (P<0.05).

Table 4. Distribution of 150 days milk yield (kg; mean and standard error of mean) among treatment groups and parity.

Parity	**Control (n=44)**	**Carprofen (n=39)**
Primiparous (n=21)	5256.38 ± 281.35 [a] (n=16)	4926.28 ± 503.29 [a] (n=5)
Multiparous (n=62)	6517.45 ± 212.68 [a] (n=28)	6166.59 ± 193.00 [a] (n=34)
All cows (n=83)	5886.91 ± 176.34 [a]	5546.43 ± 269.51 [a]

a, b Different superscripts within row indicates statistical significance (P<0.05).

No differences were detected for body weight of cows on days 1, 15 and 30 following calving between experimental groups (Table 3). Similarly, there were no differences in body weight changes during the first fifteen days, the second fifteen days or the first 30 days following parturition between experimental groups (Table 3).

There was no difference for 150 days milk yield between experimental groups (Table 4). Likewise, there was no interaction effect of treatment by parity for 150 days milk yield, and similar trends were observed for 150 days milk yield between experimental groups in both primiparous and multiparous cows (Table 4).

Discussion

It has been known that uterine muscles do not contract in response to excessive amounts of PGF2α secretion from uterine and non-uterine sources due to uterine infections, and uterine involution is delayed (Kindahl et al., 1992). Tended to be lower incidence of RFM following ketoprofen administrations immediately after from parturition and 24 hours later in Holstein dairy cows was reported (Richards et al., 2009). In this matter, Richards et al. (2009) postulated that ketoprofen with anti-inflammatory and anti-endotoxic effects decrease inflammatory response could cause normal involution leading to faster expulsion of fetal membranes. Newby et al. (2014) reported that meloxicam administration within one hour following calving did not increase the incidence of RFM and periparturient diseases in Holstein dairy cows. No differences for the incidence of RFM were reported meloxicam administration prior to or after parturition compared to control group in cows (Swartz et al., 2018). Similarly, Stilwell et al. (2014) reported no difference for the time of the expulsion of the fetal membranes and incidence of clinical diseases during three days postpartum following carprofen administration with in six hours after parturition compared to control cows in Holstein dairy cows. Moreover, Antanaitis et al. (2018) reported lower incidence of RFM in response to carprofen administration within one hour following calving in first -lactation cows. Furthermore, no differences were reported for incidence of RFM in cows administered carprofen or flunixin meglumine within 12 hours after calving compared to control group; however, lower incidence of RFM was reported in multiparous cows administered flunixin meglumine (Giammarco et al., 2018). In contrast, Newby et al. (2017) reported that administration of flunixin meglumine within one hour after calving increased the risk of RFM in cows. Swartz et al. (2018) postulated that different results from different NSAID administrations could be due to

different effects of different NSAID's administered at different times following calving. In this regard, Newby et al. (2017) and Swartz et al. (2018) concluded that higher risk of RFM following flunixin meglumine administration in cows could be owing to stronger suppression of COX-1 enzyme in response to flunixin meglumine administration as reported by Beretta et al. (2005). Likewise, no detrimental effect of carprofen administration following calving on the incidence of RFM in current study is in agreement with previous studies.

Similar to Giammarco et al. (2016) no effects of carprofen administration following calving on the incidences of metritis, clinical and subclinical mastitis were observed in current study. Likewise, no effect of administration of meloxicam prior to or following calving on the incidences of metritis and clinical mastitis (Swartz et al., 2018). Similarly, Richards et al. (2009) reported no effect of ketoprofen administrations immediately after from parturition and 24 hours later on endometritis score in Holstein dairy cows. Contraversely, Antanaitis et al. (2018) reported lower incidences of metritis and clinical mastitis and lower somatic cell count in milk with regards to carprofen administration following calving. No effect of carprofen administration on incidences of metritis, clinical and subclinical mastitis could be attributed to no dystocia cases and robotic milking system with better milking hygiene in present study.

In spite of increase in the rate of pathological vaginal discharge, carprofen administration following calving did not increase incidence of metritis in this study. In this matter, higher incidence of pathological vaginal discharge could be attributed to suppression of PGF2α from endometrium, in response to NSAID administration following calving, leading to impaired involution in present study. In this matter, Thun et al., (1989) reported that a single dose of carprofen (0.7 mg/kg) administration effectively suppressed PGF2α release in the postpartum cow. However, it has been thought that normal release of PGF2α after completion of half-life of carprofen, for almost three days, could stimulate uterine involution; thereby, it could prevent the presence of metritis in current study. Similar to this assumption, it has been reported that administration of carprofen either on days 1, 3, 5 or 19, 21, 23 following calving did not have any effect on vaginal mucus score based on Metri-check device and proportion of polymorph-nucleated cells following cytobrush technique on 13-24 and 30-49 days postpartum (Meier et al., 2014).

No differences for the time intervals from calving to the first detected estrus and from calving to the first AI could indicate the absence of any detrimental effect of carprofen administration following calving on fertility in current study. Likewise, Giammarco et al. (2016) reported no differences for the time intervals from calving to the first detected estrus and from calving to the first AI in response to carprofen or flunixin meglumine administrations within 12 hours following calving compared to those in untreated cows. Similarly, no effects of ketoprofen administrations immediately after from parturition and 24 hours later on incidence of corpus luteum detected with transrectal ultrasonography at a pre-breeding examination on days 20 to 25 postpartum and interval from calving to the first AI in Holstein dairy cows were reported (Richards et al., 2009). Furthermore, Priest et al. (2013) reported no differences for the time of the first ovulation (based on plasma progesterone ≥1 ng/mL) during postpartum period in grazing dairy cows with three subsequent carprofen administrations once in three days between days 21 – 31 following calving. Lower rate of detected estrus until day 70 postpartum in carprofen treated group could indicate the negative effect of the delay of the uterine involution in current study. However, no relationship between rates of pathological vaginal discharge and estrous detection could reveal any other mechanism to suppress estrous expression in response to carprofen administration following calving in current study.

While, there was no effect of carprofen administration following calving on the first service pregnancy rate in this study, Giammarco et al. (2016) reported higher pregnancy rates at the first service in cows administered either carprofen or flunixin meglumine within twelve hours following calving. Similarly, Antanaitis et al. (2018) indicated 8.5% increase in pregnancy rate in primiparous Holstein cows administered carprofen within one hour following calving. In contrast, Stilwell et al. (2014) reported lower pregnancy rate in cows administered carprofen within six hours following calving, and they assumpted that higher milk production following carprofen administration could result in delay of resumption of ovarian cyclicity leading to lower pregnancy rate. These discrepancies for fertility among these studies could be due to differences in sample sizes, parity distributions and reproductive management systems. That is why more controlled studies with adequate sample sizes under standard reproductive management to investigate effect of NSAID administration on fertility following calving are required. Numerically lower pregnancy rate in carprofen treated group could be attributed to lower ovarian cyclicity rate based on estrous detection during postpartum period in

carprofen treated group in this study. In this matter, lower rate of detected estrus until day 70 postpartum in carprofen treated group could indicate the negative effect of delayed involution on ovarian cyclicity; however, this significantly lower estrous detection rate until day 70 postpartum did not reflect on pregnancy rates in carprofen treated group in this study. Since most of the cows were inseminated at fixed time insemination (FTAI) following synchronization of ovulation (Ovsynch) protocol, acyclic cows could recover following GnRH injections until FTAI in this study.

No changes for body weight during monitored period could indicate no increase in feed consumption following a single carprofen administration with its analgesic and antipyretic effects post calving in this study. Similarly, Giammarco et al. (2016) reported no effect of carprofen administration within 12 hours after calving on body weight during postpartum period. No beneficial effect of carprofen administration following calving on body weight changes could be due to absence of dystocia cases in present study, and more controlled studies including both eutotic and dystosic cows would be required for better comparison.

Effect of NSAID administration on milk yield was more effectively observed during peak of lactation in dairy cows (Carpenter et al., 2016; Trevisi & Bertoni, 2008). Since 150 days total milk yield obtained from automated daily milk records was provided in current study, it has been thought that the effect of NSAID administration on milk yield could be strictly revealed. Similar to no main effect of treatment nor interaction effect of treatment by parity on 150 days total milk yield following carprofen administration in this study, Giammarco et al. (2016) reported no main effect of treatment nor interaction effect of treatment by parity on 60 days total milk yield following carprofen or flunixin meglumine administration within 12 hours after calving. Likewise, Meier et al. (2014) reported no effect of carprofen administration on days 1, 3, 5 or 19, 21, 23 following calving on six weeks total milk yield. Similarly, no effect of ketoprofen administrations immediately after from parturition and 24 hours later on milk production at the first recording was reported (Richards et al., 2009). In contrast, Antanaitis et al. (2018) noted 252 kg more milk for 305 days milk yield in primiparous Holstein cows following carprofen administration within one hour after calving. Stilwell et al. (2014) reported an increase in 305 days milk yield in response to carprofen administration within six hours following calving in primiparous but not in multiparous cows. Swartz et al. (2018) indicated a tendency ($P<0.07$) for increase in milk yield following meloxicam administration after parturition in eutotic but not in dystosic cows. Swartz et al. (2018) speculated that increase in milk yield following NSAID administration could be due to proliferation of mammary epithelial cells or decrease in involution along with apoptosis in mammary gland. In this regard, Bertoni et al. (2008) reported that cows with higher degree of inflammation produced 20% lower milk compared to those with lower degree of inflammation. No difference for milk yield following carprofen administration could be attributed to higher proportion of multiparous cows and no dystocia cases in current study. Better animal welfare could overlap any beneficial effect of NSAID administration following calving on milk yield since cows voluntarily were milked for 24 hours in robotic milking system in this current study.

Conclusion

In conclusion, NSAID administration following calving as a farm routine did not increase milk yield or fertility in eutotic Holstein cows in this study. However, no increase in the incidence of RFM or metritis in response to NSAID administration following calving in eutotic cows could indicate that carprofen could be used immediately after calving upon needed for therapeutic purposes. Because no dystocia cases were observed in this study, further research is warranted to investigate any beneficial effect of NSAID administration following calving on milk yield and fertility in eutotic versus dystosic cows with different parities.

Conflicts of Interest: The authors declare no conflict of interest.

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Closure of a chronic inguinal wound in a cat using a flank fold flap: A case report

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Case Report

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ABSTRACT

Open wounds are common cases diagnosed in small animals without distinction regarding species, breed, gender, and age. They could be aseptic, contaminated, or infected. The condition can caused by traumas such as high-rise syndrome, car accidents, etc. Depending on the condition of the wound and patient, it could be treated primarily or secondarily with surgical and medical treatment. In veterinary medicine despite many of reconstructive surgical techniques are useful and viable for open wounds their understanding among veterinary surgeons is limited.Reconstructive surgical techniques require relatively advanced knowledge and hand skills to apply. Also, soft tissue reconstruction is useful for open wounds and allow more quick tissue healing in comparison with medical treatment. In this case report we tried to share the applicability of the flank fold flap, which is a relatively less preferred treatment method, for more widespread use in inguinal area injuries in cats.

Keywords: skin, chronic, reconstruction, flap, flank fold

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Introduction

Wounds can be defined as tissue destruction caused by trauma or a cytotoxic agent in the skin and/or subcutaneous tissue (Iskefli and Bayrakal, 2021; Samsar and Akin, 2000; Kirpensteijn and Haar, 2013). Since it frequently appears in veterinary practice, veterinarians need to have knowledge of wound healing processes and wound management options (Kirpensteijn and Haar, 2013). Although wounds can occur due to different reasons such as sharp body, abrasion, burn, laceration, penetration, tear, bite and gunshot injuries, the main principles of wound healing are the same for all types of wounds. The cellular phases of wound healing in veterinary medicine have been significantly understood over the last 25 years, and in this context, it has an important role in determining appropriate treatment based on the type of wound and the condition of the patient (Balsa and Culp, 2015).

Wounds can be classified in many different ways. The most fundamental classification during this process is whether they are open or closed. An open wound refers to one where the integrity of the skin and mucosa has been compromised, while a closed wound is used for those where the outer layer has not been damaged (Kirpensteijn and Haar, 2013; Fossum, 2019; Balsa and Culp, 2015). Additionally, wounds can be classified according to their level of contamination (categories 1,2,3,4) as clean, clean-contaminated, contaminated, or infected wounds, or based on the time of occurrence as acute/chronic wounds (Iskefli and Bayrakal, 2021; Kirpensteijn and Haar, 2013). While acute and clean wounds are primarily closed, chronic or contaminated/infected wounds require debridement. Wound debridement can be performed surgically, but it can also be carried out using many different methods such as mechanical, autolytic, enzymatic, chemical, and bio-surgical techniques (Kirpensteijn and Haar, 2013). In addition to these,

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methods like maggot therapy can also be used specifically for the debridement of chronic wounds (Choudhary and Choudhary, 2016).

Wound healing can occur as primary, secondary, or mixed. For primary healing to take place, the wound must be new or renewed, the wound edges must be brought together without tension, there should be no foreign body or necrotic tissue at the suture line, hemostasis of the area must be ensured, and good vascularization of the area must be provided (Iskefli and Bayrakal, 2021; Kirpensteijn and Haar, 2013; Fossum, 2019; Balsa and Culp, 2015; Tobias and Johnston, 2012). The primary goal of the veterinarian should be to ensure these conditions are met and to achieve primary closure of the wound (Iskefli and Bayrakal, 2021). In primary wound healing, a wound paste called lymphoplastic seeps from the surrounding lymphatic vessels into the area between the wound edges, and the wound is closed in a way that will leave a scar tissue (Iskefli and Bayrakal, 2021; Fossum, 2019).

If these conditions cannot be provided or if the wound is too large to be primarily closed, secondary wound healing or healing by contraction may be preferred. This type of healing occurs in 4 phases: the inflammatory phase, the debridement phase, the proliferation phase, and the maturation phase. First, inflammatory cells come to the wound area through leakage from damaged blood and lymph vessels after the injury, and the inflammatory phase begins. Subsequently, necrotic tissues are debrided from the area through drainage after macrophage activity. The proliferation phase begins 3-5 days after the injury. In the wound cleaned of dead tissues and microorganisms, granulation occurs in sequence, and after the granulation tissue covers the area, contraction occurs between the healthy skin and granulation, and finally, the proliferation phase ends with epithelialization, followed by a long maturation phase. In the maturation phase, the scar tissue strengthens, and the newly formed skin gains resistance. This phase is completed in a duration that varies from a few weeks to 1 year (Kirpensteijn and Haar, 2013; Balsa and Culp, 2015).

Primary wound healing for the inguinal region poses many challenges due to the high mobility of the area and its complex vascular anatomy. Advancement flaps and transposition flaps are useless because they cannot provide sufficient suture tension relief for inguinal region (Kirpensteijn and Haar, 2013). These methods could cause complications such as necrosis or wound dehiscence could occur. Also general reconstructive techniques like Z-plasty or V-Y plasty are useless due to insufficient tension relief and blood supply(Fossum, 2019; Kirpensteijn and Haar, 2013). Several advanced techniques can be utilized to close the defects in this region effectively. Flank fold flap is one of these techniques, which includes using the loose skin between femur and flank.It is an effective axial pattern flap for inguinal defects (Fossum, 2019). The flap can be transposed bilaterally if needed for large defects. Proper dissection and preservation of vascular integrity are critical for ensuring flap survival. Another axial pattern flap could utilized for inguinal region injuries is caudal superficial epigastric axial pattern flap which includes multiple caudal mammary glands, adjacent skin tissue and the caudal superficial epigastric artery (Tobias and. Johnston, 2012). This versatile flap also could be used for caudal abdominal and flank region defects. Careful dissection along the aponeurosis of external abdominal oblique muscle ensures the inclusion of vascular structures and minimizes necrosis risk (Kirpensteijn and Haar, 2013; Tobias and Johnston, 2012).

The main purpose in wound treatment is to heal the wound as quickly as possible. At this point, the veterinarian's mastery of reconstruction techniques will prevent tissue losses, shorten the treatment duration, and reduce complications that may arise during the treatment process. Stages of treating an inguinal region wound that was attempted to be closed primarily twice but was unsuccessful, using a flank fold flap will shared in this case report.

Case description

Our case involved a 5-year-old, neutered male, 6.5 kg weighted domestic shorthair cat presented to the Small Animal Clinic of the Department of Surgery, Faculty of Veterinary Medicine, Istanbul University-Cerrahpaşa Veterinary Hospital, with a history of a non-healing wound cranial to the inguinal region. The patient had previously undergone surgery for a cyst near the left inguinal mammary lobule, which resulted in seroma formation and subsequent development of an open wound post-excision. The owner reported that after repeated attempts at primary closure of this wound had failed, prompted them to seek consultation at our clinic. Clinical examination revealed an infected open wound in the inguinal region, showing no signs of contraction or epithelialization. Due to the constant tension on the suture line caused by skin movement in the affected area, which was thought to preclude wound closure, a flank fold flap procedure was planned.

Discussion

Preoperatively the patient was prescribed topical ointments containing nitrofurantoin (0.2% Furacin, Sanofi), *Centella asiatica* extract (1% Madecassol

cream, Bayer), and zinc oxide (40% Oksizinc) to be applied regularly for 10 days. Three days prior to surgery, systemic broad-spectrum antibiotic treatment was initiated with ceftriaxone sodium (Novosef 1g vial, Sanofi) at a dose of 30 mg/kg. Complete blood count (CBC) revealed no significant abnormalities other than mild monocytosis, and no clinically significant changes were detected in serum biochemistry analyses.

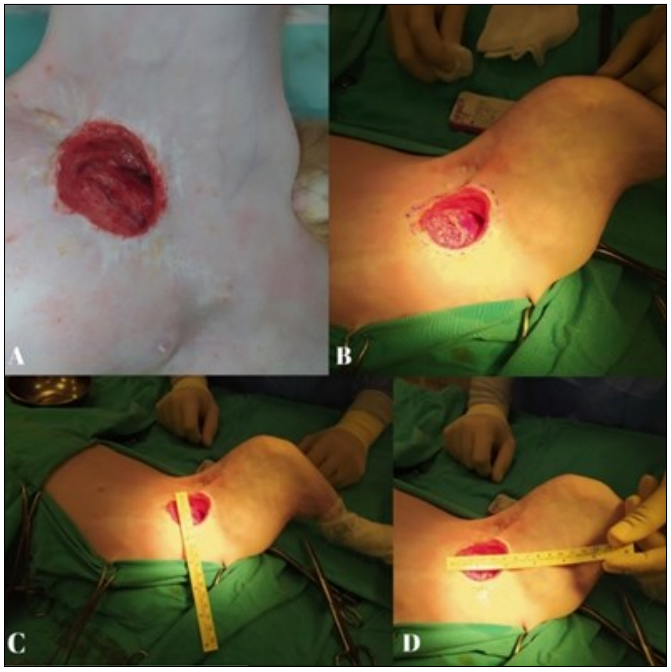

Figure 1. A, the shaved appearance of the wound. B, the sterilely draped appearance of the wound. C, measurement of the short axis diameter of the wound. D, measurement of the long axis diameter of the wound.

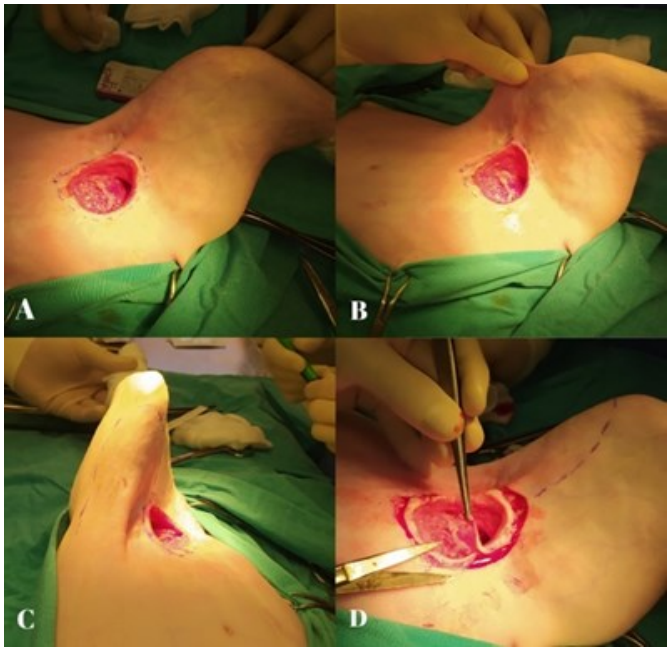

Figure 2. A, Marking of the wound with a sterile marker. B, Determination of the flap. C, marking of the flap with a sterile marker. D, Revision of the wound.

The patient was initially premedicated with intravenous xylazine (Vetaxyl, VetAgro) at a dose of 0.5 mg/kg, followed by intravenous ketamine (Ketax, VEM) at a dose of 5 mg/kg for general anesthesia induction. Five minutes after induction, the patient was orotracheally intubated via 3.5 mm endotracheal tube, and general anesthesia was maintained with 100% oxygen and 2% isoflurane (Isoflurane USP, ADEKA). After the anesthetic initiation, the patient was placed in dorsal recumbency, and the area from the xiphoid process of the sternum to the pubis, as well as the lateral and medial surfaces of the femur adjacent to the wound, was shaved and disinfected. The patient was draped with sterile surgical drapes, including the left hind limb. The borders necessary for excision of the circular wound, measuring 5.5 x 4 cm, were marked on the surrounding skin using a sterile marker (Figure 1). To determine the free skin area from which the flank fold flap would be rotated, the loose skin on the anterior aspect of the femur, from the stifle joint to the body, was lifted between the thumb and index finger, and the area of sufficient width to easily close the wound and donor site was marked with a sterile marker. Subsequently, the wound edges were removed with a circular incision using a no. 22 scalpel blade, thus the revision of the wound edges completed. (Figure 2).

In the second step, the flap was incised along the marked borders. The flap was dissected from the subcutaneous connective tissue using blunt-tipped Metzenbaum scissors and transferred to the revised recipient site using towel clamps (Figure 3). First, the proper positioning of the flap was determined, and the donor site skin was attached to the recipient site skin at several points using simple interrupted sutures. The subcutaneous tissue transferred to the recipient site was then sutured circumferentially using a simple continuous technique. After closure of the subcutaneous tissue, the skin was also closed using a simple interrupted suture technique. 3/0 monofilament absorbable suture material (polydioxanone) was used for the subcutaneous tissue, and 3/0 monofilament non -absorbable suture material (polypropylene) was used for the skin (3). A Penrose drain was placed as a passive -open drain to prevent fluid accumulation under the skin (Figures 4, 5, and 6).

Due to both the unsuitability of the surgical site for bandaging and the risk of compromising local blood supply, no bandage was applied. Post-operatively, ceftriaxone sodium at a dose of 50 mg/kg intramuscularly once daily for 7 days was prescribed as a broad-spectrum antibiotic; meloxicam at a dose of 0.1 mg/kg subcutaneously once daily for 5 days was prescribed as a non-steroidal anti-inflammatory drug (NSAID); sucralfate at a total dose of 250 mg orally

twice daily for 5 days was prescribed as a gastroprotective agent to prevent gastrointestinal side effects of the NSAID; and ascorbic acid at a total dose of 75 mg intramuscularly once daily for 7 days was prescribed to enhance collagen synthesis and accelerate wound healing. The drains were removed on the fourth day. On the 12th post-operative day, healing was observed, and the skin sutures were removed under sedation.

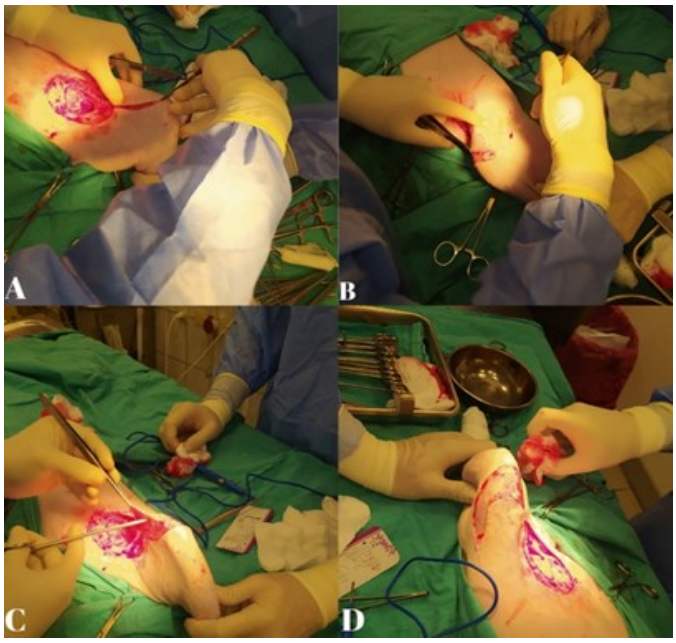

Figure 3. Flap incision and seperation of subcutaneous tissue.

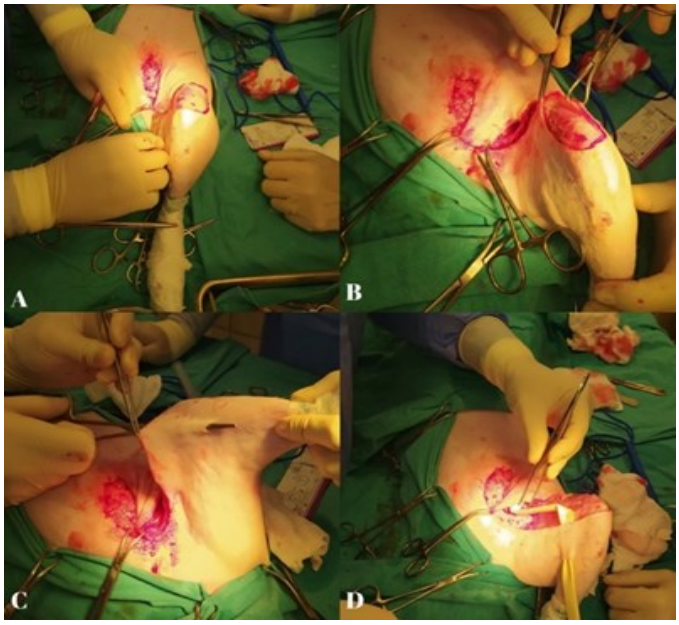

Figure 4. A, B Positioning of the flap via towel clamps. C, D drain placement.

Open wounds are frequently encountered cases in small animal clinics. Although many cases can be treated without resorting to secondarily wound healing by using transposition and advancement techniques of adjacent skin, as well as V-Y plasty and axial pattern flap techniques, but the lack of knowledge of these techniques by many veterinarians leads to prolongation of wound healing time and significant financial losses (Kirpensteijn and Haar, 2013).

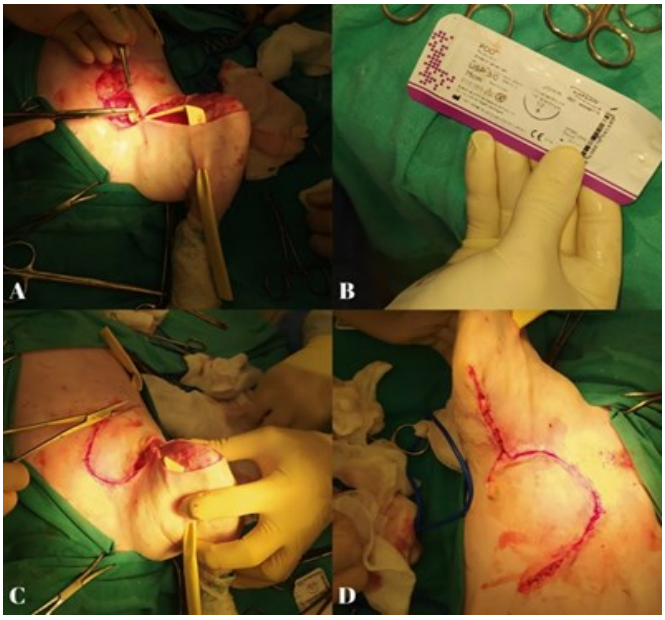

Figure 5. A, C, D closure of subcutaneous tissue with simple interrupted sutures. B the suture material.

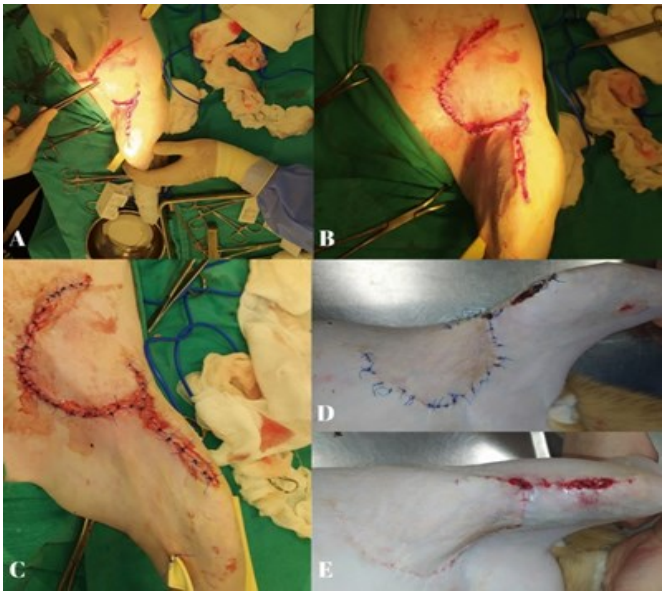

Figure 6. A, B, C skin closure with simple interrupted sutures. D appearance of the wound on postoperative 12th day. E appearance of the wound after suture removal.

Especially as wounds become chronic, closure becomes much more difficult due to both bacterial resistance and cellular mortification, and it is necessary to consider treatment methods beyond traditional ones. For this purpose, methods such as maggot debridement therapy (Choudhary and Choudhary, 2016), topical use of Momordica charantia (bitter melon) extract due to its antiseptic, astringent, anti-inflammatory, antimicrobial, and biostimulatory properties (Kisacik and Gunes, 2017) and low-dose ultrasound therapy to accelerate granulation

Gokulakrishnan et al 2018) are currently used/ recommended. It is expected that treatment success in chronic wounds will also increase with new approaches. Nevertheless, closing the wound as soon as possible is important in wound treatment, and after sufficient granulation tissue has formed in chronic wounds, it is important to close the skin defect with reconstructive surgery and apply a combined treatment with granulation-stimulating techniques(Kirpensteijn and Haar, 2013).

However, considering both the long treatment duration, cost, and potential complications (infection, seroma, and wound dehiscence being the most common) (Cantatore et al, 2014) of all these secondary wound healing methods, it is anticipated that more widespread use of primary closure and reconstructive techniques will provide a solution in this regard.

Reconstructive surgery is more practical to perform as it does not require special equipment such as maggot debridement therapy (Choudhary and Choudhary, 2016) or negative pressure therapy (Nolff, 2021), but it requires deeper anatomical and surgical knowledge (Kirpensteijn and Haar, 2013; Fossum, 2019, Tobias and Johnston, 2012). In addition, following current studies on this subject and applying the developed techniques or inferences made as a result of the studies will increase post-reconstruction success in wound healing. Reconstructive surgery is a surgical procedure that requires special care, especially due to reasons such as asepsis-antisepsis, contamination, and tissue viability. Therefore, to achieve a good post-operative outcome, unlike a routine surgical suture application, modifications such as PRP or omentum graft should be performed in the operation area (Gokulakrishnan et al, 2016; Gray, 2005), and/or attention should be paid to such nuances because the local circulation will be affected if the frequency of sutures to be placed and the distance of the needle entry point to the wound are too great (Millbourn et al., 2009).

It is also thought that techniques such as subcutaneous tissue release to reduce tension in areas where the skin is mobile or thin, walking sutures, advancement flaps, and greater utilization of these techniques that use the loose skin in these areas will contribute to success.

Furthermore, veterinarians should not forget that before deciding on primary wound closure, they must ensure that they have met all the necessary conditions for primary wound healing. For primary healing to occur, the wound must be new or revised, the wound edges must be brought together without tension, there should be no foreign bodies or necrotic tissue in the suture line, hemostasis of the area must be provided, and good regional vascularization must be ensured (Iskefli and Bayrakal, 2021; Kirpenstejin and Haar, 2013; Fossum, 2019; Balsa and Culp, 2015; Tobias and Johnston, 2012).

An important point in the primary closure of wounds is the suture material used. The preference of monofilament suture material, especially in cases with suspected contamination, will reduce infection and suture complications due to its low capillarity. Therefore, the use of absorbable monofilament materials made of substances such as polyglecaprone 25 and polydioxanone in the body and subcutaneously; and the use of non-absorbable monofilament materials containing polypropylene or stainless steel skin staples in the skin tissue should be preferred. The use of multifilament threads in contaminated wounds is not appropriate because bacteria settling between the braids will cause persistent infection.

Again, during the preparation phase of operative procedures performed for the treatment of open wounds; in order to avoid iatrogenic damage to healthy skin tissue, it is recommended that shaving be done with a 0.40mm clipper instead of a razor, and that antiseptic solutions not be applied roughly.

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A cross-sectional study on animal nutrition and management evaluation of selected small ruminant enterprises in Sakarya and Balıkesir.

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Research article

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ABSTRACT

This study was conducted to determine the deficiencies or improvable aspects in this area in order to carry out sustainable and more efficient small ruminant farming in Balıkesir and Sakarya provinces, to reveal the demographic and structural status of small ruminant farming, animal health conditions, production techniques applied by enterprises with existing opportunities, biosecurity, health-protection, care-feeding and other elements. For this purpose, the results obtained from a face-to-face survey conducted on a total of 200 enterprises, 150 in Balıkesir and 50 in Sakarya, were evaluated. It has been observed that while most small ruminant enterprises in Balıkesir do not have cattle, cattle farming is more prominent in Sakarya. In both provinces, a significant proportion of owners were over 46 years old, and only a small percentage were university graduates. When the enterprises in the two provinces are compared, the rates obtained regarding the types of small ruminant enterprises in Balıkesir such as fattening (84%), the dominance of extensive and semi-extensive enterprises in terms of enterprise structure (33%), the presence of poultry in the enterprise (43%), environmental spraying (45%), the use of factory feed in feeding the animals (98.7%), vitamin-mineral supplements (41.3%), the use of pasture (93.3%), the use of vetch hay (16%), the use of automatic water dispensers (15.6%), buckets (70.9%), water tanks (25.2%) in water supply to the animals, the presence of a maternity area in the enterprise (75.3%) and umbilical cord care and hygiene in neonates (67.1%) were found to be higher than the enterprises in Sakarya ($p<0.05$). On the other hand, the proportions obtained regarding the enterprise type of small ruminant enterprises in Sakarya being combined (meat-milk) (94%), the dominance of closed farms in terms of farm structure (94%), quarantine application for new animals entering the farm (26%), the use of barley (98%), oats (24%), alfalfa (66%), oat straw (76%), the use of licking stones (98%), the use of troughs for water supply (100%), keeping rams separate from the herd (46%), the ability of the farm owner to help during difficult births (100%) and postpartum cleaning (87%) were found to be significantly higher than the rates obtained from the farms in Balıkesir. Therefore, in order enhance the efficiency of small livestock enterprises in both provinces and to eradicate the shortcomings, negative aspects, and faults found in these businesses, educational activities and practices are required.

Keywords: sheep, goat, survey, small ruminant, farm, nutrition

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Introduction

As in every country, the livestock sector has a great importance in meeting the need for animal protein in Türkiye. The contributions of animal husbandry on a national basis include national nutrition and development, increase in exports, provision of raw materials, employment and consequently prevention of hidden unemployment in rural areas, balanced development between sectors and ensuring stability (Aydemir and Pıçak, 2007). While pork and beef have a large share in red meat production in the world, in

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Türkiye this demand is mainly met by beef and sheep meat. Considering the geographical, climatic and agricultural structure, sheep breeding, which provides significant advantages in terms of cost and quality, has continued in our country until recently as both an economic animal production activity and a part of cultural and family life (Çiçek et al., 2022).

It is noteworthy that the sheep population in Türkiye fluctuates from year to year. As a matter of fact, the number of sheep, which was approximately 40 million heads in 1975, increased to 45-50 million between 1980-1985, decreased to approximately 33.7, 28.5, 25.2 and 21.7 million in 1995, 2000, 2004 and 2009, respectively, and increased again in 2019 and 2021 to 37.3 and 45.2 million, respectively (Aydemir and Pıçak, 2007; Çiçek et al., 2022). According to a recent report by the Turkish Statistical Institute, the number of sheep was recorded as 42,060,470 in 2023, showing a 5.9% decrease compared to the previous year, and this number increased by 3.2% in 2024, totaling 43,393,709 heads in June (TÜİK, 2024). In the same report, it was also reported that the number of goats, which was 10,302,940 in 2023, increased by 2.6% to 10,571,297 in 2024.

Today, although animal production and the amount of consumption of the products obtained from this production are used as an indicator of development, the consumption of animal products in Türkiye, which is a country with a significant potential in terms of animal population and suitable climatic conditions for animal breeding, has remained at a low level compared to other countries, and the reason behind this is the effort of producers to meet the need for animal products within a closed system production model that adopts self-sufficiency due to their long-standing habits (Aydemir and Pıçak, 2007).

In Türkiye, small ruminant breeding is generally carried out extensively based on pasture with domestic breeds with low combined productivity, and the number of enterprises engaged in intensive breeding with improved breeds imported from abroad is quite small. Especially since sheep breeding is carried out in the form of extensive production, flock management practices differ in the same or different regions. For example, while some livestock breeders do not separate the ram from the flock at all, some livestock breeders add rams in different seasons according to their own preferences, while some farms do milking, some farms do not milk and make the lambs suckle during lactation, and the suckling period of the lambs also varies from farm to farm (Ülsü and Çilek, 2024).

As in all other sectors, increasing productivity, maintaining stability, ensuring progress and minimizing or preventing the adversities encountered in the livestock industry depend on scientific studies in this field. Considering that the increase in capacity and efficiency especially in the field of small ruminant breeding depends on factors such as pasture characteristics, pasture grazing capacity, climate, roughage source, market security and stability, breeding animal supply, disease and pest risk, labor force and similar factors, it is obvious how important scientific researches on this sector are. In this regard, survey studies that provide data revealing the current situation of enterprises on a general or regional basis are as essential as experimental researches carried out on animals in order to search for alternative ways to increase yield performance. As a matter of fact, surveys are the best known and most frequently used method for obtaining original data and primary data in agricultural researches are mostly obtained through surveys as a time-saving and less costly technique (Oruç and Gürler, 1994).

In this context, in this study, it was aimed to carry out a comprehensive cross-sectional study to reveal the deficiencies of the livestock farmers in Balıkesir and Sakarya provinces, which provide a large part of the demand for small ruminant products of the surrounding provinces, especially Istanbul, in order to carry out sustainable and more efficient animal husbandry, to determine the deficiencies of the breeders or to determine the aspects that can be improved, to obtain data on issues such as the structural status of small ruminant farming, animal health conditions, production techniques applied with the existing facilities, basic problems such as health-protection, care-feeding and socio-economic status.

Materials and Methods

This study was designed in cross-sectional descriptive study. The main material of the study consisted of original data obtained from small ruminant farming enterprises through face-to-face questionnaires. The prepared questionnaire was planned to take approximately 10 minutes and this time was proved to be sufficient in the preliminary test on people who were not related to the subject. The data collection process involved the period between 01 October and 30 December 2023. Balıkesir and Sakarya provinces, which are considered to be representative of the region, were preferred as the study area since they provide a large part of the small ruminant demand of the surrounding provinces, especially Istanbul, in the Marmara Region. In this context, data on the number of enterprises and the number of small ruminants in the enterprises were obtained from the Provincial and District Directorates of Agriculture and Forestry and Animal Information System, and a face-to-face survey

was conducted in a total of 200 enterprises, 126 and 24 in Bigadiç and Sındırgı districts of Balıkesir province, respectively, and 50 in Taraklı district of Sakarya province, and the structure of the relevant enterprises, protection control methods against diseases, feeding methods of animals, habits of the enterprises on issues such as hygiene, vaccination and disinfection were discussed. The number of enterprises to be surveyed was calculated by simple random sampling method.

It was estimated that there were approximately 1,000 small ruminant enterprises in Balıkesir and Sakarya. The sample size was calculated using the following formula and OpenEpi, Version 3, Open Source Calculator-SSPropor software. A sample size of 186 was needed for a population size of 1000, a prevalence of 50% ± 5%, 95% confidence level, 6.5% margin of error (precision) and 1 design effect.

$$n = deff \times \frac{N\hat{p}\hat{q}}{\frac{d^2}{1.96^2}(N-1) + \hat{p}\hat{q}}$$

n = number of samples, $deff$ = design effect, N = population size,

$\hat{p}$ = estimated proportion, $\hat{q} = 1 - \hat{p}$, d = desired absolute precision

The data obtained from the standard and study-specific questionnaires were analysed using SPSS v.20 package programme. Number and percentage distributions of categorical variables were used in the analyses. Chi-square and Fisher's exact Chi-square tests were used in the comparisons to reveal the differences between the two provinces.

Results

The number of animals of the enterprises included in the study is presented in Figure 1. When the percentages of the groups of animal numbers were evaluated, it was observed that the herds in Sakarya were smaller and Balıkesir had more small ruminant population. While most of the small ruminant farms in Balıkesir do not have cattle, it was determined that cattle are more prominent in Sakarya.

The evaluation of the demographic and structural status of small ruminant farming enterprises was presented in Table 1. There was no difference in the age distribution of enterprise owners in both provinces, and it was determined that a significant portion of enterprise owners in both provinces were over 46 years of age. According to the data obtained regarding the educational status of the enterprise owners, it was found that 8% of the owners of the enterprises included in the study from Balıkesir province were university graduates, but none of the owners of the enterprises in Sakarya were university graduates. It was observed that 61% and 80% of the enterprise owners in Balıkesir and Sakarya provinces, respectively, were at secondary school and before, and 31% and 20% were at high school level. While 84% of the enterprises in Balıkesir were of fattening type, only 6% in Sakarya were of fattening type; 33% of the enterprises in Balıkesir were of extensive and semi-extensive type, but only 6% in Sakarya were of the same type (p=0.001).

The evaluation of the enterprises in terms of precautions taken to minimize possible disease risks and general biosecurity practices was presented in Table 2. According to the data on the presence of different animal species in the related enterprises, it was found that 43% of the enterprises in Balıkesir had poultry while this rate was 18% in Sakarya. In addition, 90% of the enterprises in Balıkesir and 43% of the enterprises in Sakarya contained cats and dogs, but there was no significant difference between the two

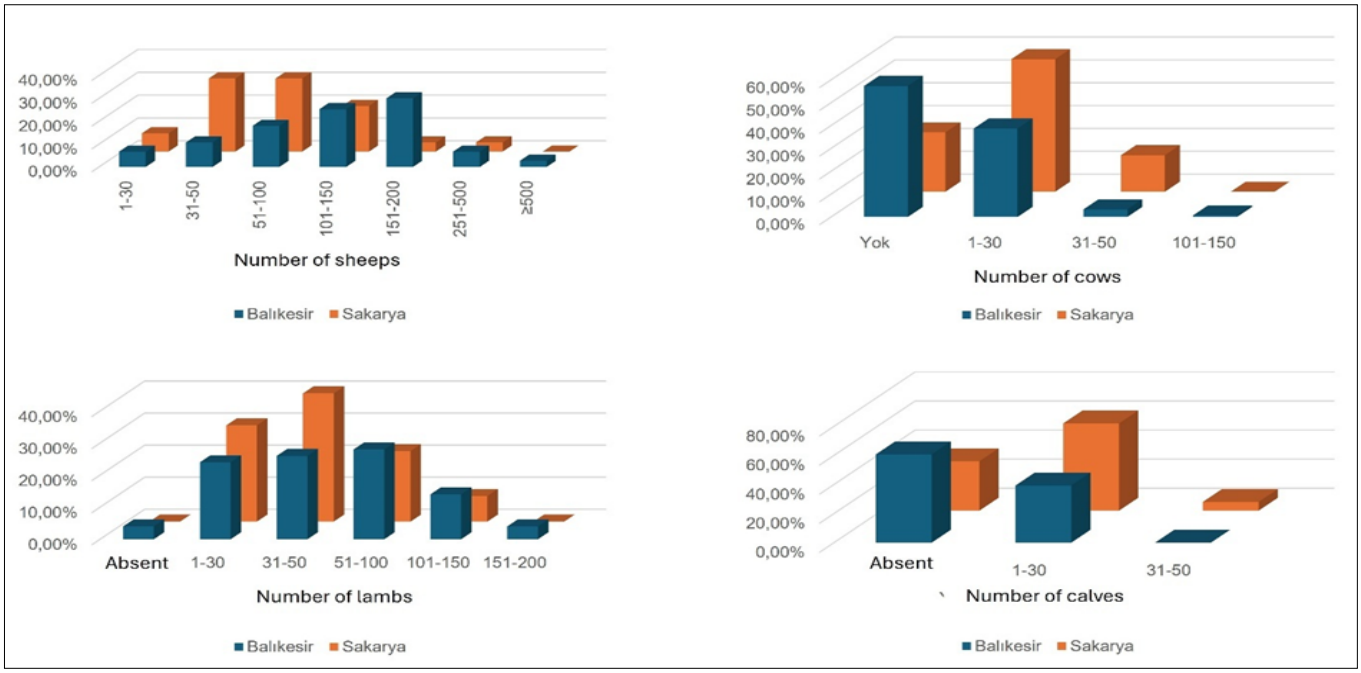

Figure 1. Distribution of the number of animals in the enterprises

Table 1. Demographic and structural characteristics of the enterprises.

	Balıkesir	Sakarya	Overall	*p-value*
Age distribution of enterprise owners				
≤24	4 (2.7 %)	2 (4 %)	6 (3 %)	
25-35	18 (12 %)	8 (16 %)	26 (13 %)	
36-45	38 (25 %)	8 (16 %)	46 (23 %)	0.70
46-55	43 (28 %)	16 (32 %)	59 (29 %)	
>=56	47 (31 %)	16 (32 %)	63 (31 %)	
Education status of the enterprise owner				
Secondary school and before	92 (61 %)	40 (80 %)	132 (66 %)	
High school	46 (31 %)	10 (20 %)	56 (28 %)	0.06
University	12 (8 %)	0 (0 %)	12 (6 %)	
Type of enterprise				
Fattening	126 (84 %)	3 (6 %)	129 (64 %)	0.001
Dual-purpose	24 (16 %)	47 (94 %)	71 (36 %)	
Structure of enterprise				
Intensive system	100 (66 %)	47 (94 %)	147 (73 %)	0.001
Extensive and semi-extensive system	50 (33 %)	3 (6 %)	53 (26 %)	

provinces (p=0.44). The use of parasite medicines in cats and dogs in Balıkesir and Sakarya provinces was 57% and no significant difference was found (p=0.55). It was determined that 45% of the enterprises in Balıkesir applied environmental disinfestation while this rate was approximately 14% in Sakarya (p=0.003). Depending on environmental spraying, the rates of fly presence were 59% and 94% for Balıkesir and Sakarya, respectively. It was also observed that 62% of the enterprises in Sakarya and 71% in Balıkesir did not perform fly control. In both provinces, the proportion of enterprises with new animal entries in the last one year was determined as 26% and 14% in Balıkesir and Sakarya, respectively, and no significant difference was found (p=0.14). It was determined that the participants were quite insufficient in terms of quarantine application for new animals and disinfection of vehicles entering the enterprise from outside.

The data on the feed and feed ingredients used in the enterprises, excluding the flushing period, was presented in Table 3. In the small ruminant farming enterprises participated from Balıkesir and Sakarya provinces, the proportions of enterprises using concentrate feed were determined as 64% and 78% for wheat, 28% and 98% for barley, 10% and 24% for oat and 99% and 62% for factory feed (pellet), respectively. Although there was a significant difference between the two provinces in terms of the proportions of enterprises using oat (p=0.02), pellet feed (p=0.001) and barley (p=0.001), no significant difference was found for wheat. Vitamin and mineral supplements were not used in any of the enterprises from Sakarya,

Table 2. Data on selected biosecurity practices in the enterprises.

	Balıkesir		Sakarya		Overall		*p-value*
	Yes	No	Yes	No	Yes	No	
Presence of different animal species							
Poultry	65 (43 %)	85 (57 %)	9 (18 %)	41 (82 %)	74 (37 %)	126 (63 %)	0.001
Cat, dog	135 (90 %)	15 (10 %)	43 (86 %)	7 (14 %)	178 (89 %)	22 (11 %)	0.44
Administration of antiparasitic to cats and dogs							
	85 (57 %)	63 (42 %)	28 (57.1 %)	21 (42.9 %)	113 (57 %)	84 (42 %)	0.55
Application of environmental disinfestation							
	39 (45 %)	48 (55 %)	4 (13.8 %)	25 (86.2 %)	43 (37 %)	73 (63 %)	0.003
Presence of flies							
	88 (59 %)	62 (41 %)	47 (94 %)	3 (6 %)	135 (67 %)	65 (32 %)	0.99
Applications to rid flies							
	43 (28 %)	107 (71 %)	19 (38 %)	31 (62 %)	62 (31 %)	138 (69 %)	0.22
New animal entry (last year)							
	111 (74 %)	39 (26 %)	43 (86 %)	7 (14 %)	154 (77 %)	46 (23 %)	0.14
Quarantine for new animals							
	17 (11 %)	133 (88 %)	13 (26 %)	37 (74 %)	30 (15 %)	170 (85 %)	0.02
Application of vehicle disinfection							
	12 (8 %)	138 (92 %)	8 (16 %)	42 (84 %)	20 (10 %)	180 (90 %)	0.11

whereas 41% of the enterprises from Balıkesir used them (p=0.001). In order to meet the mineral requirements of animals, 86% of the enterprises in Balıkesir and 98% of the enterprises in Sakarya preferred to use licking stones. In Balıkesir and Sakarya, it was determined that 93% and 76% of the enterprises participating in the survey preferred to graze their animals on pasture, respectively, and a significant difference was found in pasture preference between the two provinces (p=0.03). For Balıkesir and Sakarya provinces, the proportions of enterprises using roughage were 44% and 66% for alfalfa, 8% and 76% for oat straw, 97% and 90% for wheat straw, 12% and 10% for grass hay, 16% and 2% for vetch hay, 3% and 8% for beet pulp, 1% and 2% for forage pea, respectively. However, preference for sainfoin was found only in Sakarya with 4% of enterprises. There was a significant difference between the two provinces only in terms of preference for pasture (p=0.03), alfalfa (p=0.01), oat straw (p=0.001) and vetch hay (p=0.006), and it was also noteworthy that the use of valuable and alternative forages such as beet pulp, forage pea and sainfoin was very rare.

The data on the methods of water provision to animals in the enterprises were presented in Table 4. It was determined that the rate of enterprises using automatic water dispensers was 16% in Balıkesir and 2% in Sakarya (p=0.03). The proportions of the enterprises using buckets, water troughs and water tanks to supply water to animals were determined as 71% and 37%, 70% and 100%, 25% and 0% for Balıkesir and Sakarya, respectively, and a significant difference was found between the two provinces in terms of these water supply methods (p=0.001).

The data on parturition management and practices in small ruminant enterprises were presented in Table 5. It was observed that the proportion of enterprises separating rams from the flock was 18% in Balıkesir province and 46% in Sakarya province, and the proportion of enterprises not preferring to separate rams from the flock in Balıkesir province was significantly higher than those of in Sakarya province

Table 3. Data on feeds and feedstuffs used outside the flushing period in the enterprises.

	Balıkesir		**Sakarya**		**Overall**		***p-value***
	Yes	No	Yes	No	Yes	No	
Concentrate feeds and feedstuffs							
Wheat	96 (64 %)	54 (36 %)	39 (78 %)	11 (22 %)	135 (67.5%)	65 (32.5 %)	0.07
Barley	42 (28 %)	108 (72 %)	49 (98 %)	1 (2 %)	91 (45.5%)	109 (54.5 %)	0.001
Oat	15 (10 %)	135 (90 %)	12 (24 %)	38 (76 %)	27 (13.5 %)	173 (86.5 %)	0.02
Factory feed	148 (98.7 %)	2 (1.3 %)	31 (62 %)	19 (38 %)	179 (89.5 %)	21 (10.5 %)	0.001
Vitamin-mineral supplements							
	62 (41.3 %)	88 (58.7 %)	0 (0 %)	50 (100 %)	62 (31 %)	138 (69 %)	0.001
Licking stone							
	129 (86 %)	21 (14 %)	49 (98 %)	1 (2 %)	178 (89 %)	22 (11 %)	0.04
Forages							
Pasture	140 (93.3 %)	10 (6.7 %)	38 (76 %)	12 (24 %)	178 (89 %)	22 (11 %)	0.03
Alfalfa	66 (44 %)	84 (56 %)	33 (66 %)	17 (34 %)	99 (49.5 %)	101 (50.5 %)	0.01
Oat straw	12 (8 %)	138 (92 %)	38 (76 %)	12 (24 %)	50 (25 %)	150 (75 %)	0.001
Wheat straw	145 (96.7 %)	5 (3.3 %)	45 (90 %)	5 (10 %)	190 (95 %)	10 (5 %)	0.12
Grass hay	18 (12 %)	132 (88 %)	5 (10 %)	45 (90 %)	23 (11.5 %)	177 (88.5 %)	0.08
Vetch hay	24 (16 %)	126 (84 %)	1 (2 %)	49 (98 %)	25 (12.5 %)	175 (87.5 %)	0.006
Beet pulp	4 (2.7 %)	146 (97.3 %)	4 (8 %)	46 (92 %)	8 (4 %)	192 (96 %)	0.1
Forage pea	2 (1.3 %)	148 (98.7 %)	1 (2 %)	49 (98 %)	3 (1.5 %)	197 (98.5 %)	0.99
Sainfoin hay	0 (0 %)	150 (100 %)	2 (4 %)	48 (96 %)	2 (1 %)	198 (99 %)	0.06

Table 4. Data on the methods of water provision to animals in enterprises.

	Balıkesir		**Sakarya**		**Overall**		***p-value***
	Yes	No	Yes	No	Yes	No	
Automatic	23 (15.6 %)	124 (84.4 %)	1 (2.4 %)	49 (98 %)	24 (12.8 %)	164 (87.2 %)	0.03
Bucket	105 (70.9 %)	43 (29.1 %)	16 (37.2 %)	27 (62.8 %)	121 (63.4 %)	70 (36.6 %)	0.001
Water trough	103 (70.1 %)	44 (29.9 %)	50 (100 %)	0 (0 %)	153 (77.7 %)	44 (22.3 %)	0.001
Water tank	37 (25.2 %)	110 (74.8 %)	0 (0 %)	40 (100 %)	37 (19.8 %)	150 (80.2 %)	0.001
From pasture	129 (87.8 %)	18 (12.2 %)	44 (91.7 %)	4 (8.3 %)	173 (88.7 %)	22 (11.3 %)	0.6

Table 5. The data on parturition management and practices in the enterprises.

Balıkesir		Sakarya		Overall		*p-value*
Yes	No	Yes	No	Yes	No	
Separation of rams from the flock						
28 (18.7%)	122 (81.3%)	23 (46%)	27 (54%)	51 (25.5%)	149 (14.5%)	0.001
Vaccination of pregnant animals						
127 (84.7%)	23 (15.3%)	39 (78%)	11 (22%)	166 (83%)	34 (17%)	0.28
The presence of a maternity area						
113 (75.3%)	37 (24.7%)	18 (36%)	32 (64%)	131 (65.5%)	69 (34.5%)	0.001
The presence of birthing pen						
5 (3.3%)	145 (96.7%)	3 (6%)	47 (94%)	8 (4%)	192 (96%)	0.41
Enterprise owner knows the signs of parturition						
147 (98%)	3 (2%)	50 (100%)	0 (0%)	197 (98.5%)	3 (1.5%)	0.57
Enterprise owner can assist during case of dystocia						
132 (88%)	18 (12%)	48 (100%)	0 (0%)	180 (90.9%)	18 (9.1%)	0.008
Postpartum cleaning of the parturition area						
49 (32.7%)	101 (67.3%)	42 (87.5%)	6 (12.5%)	91 (46%)	107 (54%)	0.001
Administration of the colostrum to neonates within first two hours						
140 (93.3%)	10 (6.7%)	44 (88%)	6 (12%)	184 (92%)	16 (8%)	0.23
Forced administration of colostrum when necessary						
113 (75.3%)	37 (24.7%)	40 (80%)	10 (20%)	153 (76.5%)	47 (23.5%)	0.56
Umbilical cord care and disinfection						
100 (67.1%)	49 (32.9%)	17 (34%)	33 (66%)	117 (58.8%)	82 (41.2%)	0.001

(p=0.001). The rate of enterprises not vaccinating pregnant animals was determined as 15% and 22% for Balıkesir and Sakarya provinces, respectively, and no significant difference was found between the two provinces. In Balıkesir and Sakarya provinces, the proportion of enterprises with a maternity area was determined as 75% and 36%, respectively, and this difference between the two provinces was statistically significant (p=0.001). Although there was no statistically significant difference, it was found that there was no owner who did not know the signs of parturition in Sakarya, whereas 2% of the owners in Balıkesir did not know the signs of parturition (p=0.57). While 88% of the owners of small ruminant enterprises in Balıkesir provided assistance in the case of dystocia, it was determined that all of the owners in Sakarya provided assistance (p=0.008). While 33% of the enterprise owners perform postpartum cleaning in Balıkesir, this rate was 87% in Sakarya, and in Balıkesir, 67% of enterprises did not perform postpartum cleaning, compared to 13% in Sakarya (p<0.001). Data on colostrum management and umbilical cord care and disinfection in neonates were also presented in Table 5. While the proportion of enterprises administered colostrum to neonates within 2 hours after birth was 93% in Balıkesir, this proportion was determined as 88% in Sakarya. Although there was no statistically significant difference, it was understood that there were a considerable number of enterprises that did not administer colostrum to neonates in both provinces (p<0.23). It was also determined that 75% and 80% of enterprises participated from Balıkesir and Sakarya provinces, respectively, performed forced administration of colostrum when necessary (p=0.56). The proportion of the enterprises applied tincture of iodine and antibiotic spray etc. for umbilical cord of neonates after parturition was 67% in Balıkesir and 34% in Sakarya. In Balıkesir, 33% did not implement umbilical cord care and disinfection initiatives, whereas in Sakarya, the number was 66%, which was significant. (p=0.001).

Discussion

In this survey study, it was observed that Balıkesir province has a higher number of small ruminants than Sakarya province. According to the 2021 report published by Balıkesir Provincial Directorate Animal Health and Breeding Department in 2023, the total number of cattle and small ruminants in Balıkesir was 550,054 and 1,685,029, respectively, and the total number of cattle and small ruminants in Bigadiç and Sındırgı districts were 104,419 and 225,089, respectively (Ercan, 2023). According to this report, it is understood that these two districts have a share of

approximately 32% and 68% in terms of cattle and small ruminants, respectively. According to the Sakarya Agricultural Investment Guide published by the Ministry of Agriculture and Forestry, Strategy Development Directorate, Agricultural Investor Advisory Office in 2022, the total number of cattle and small ruminants in Sakarya province in 2021 was 195,829 and 94,493, respectively. It is clear that small ruminant farming is more prominent in Balıkesir than in Sakarya, and this situation is in accordance with the comparison of small ruminants and cattle in the present study. In addition, in the 2023 Situation and Forecast Report on red meat by the Institute of Agricultural Economics and Policy Development, it is observed that the number of small ruminants in Balıkesir in 2021 (1,685,029) is considerably higher than the number of small ruminants in Sakarya (94,493), and when compared to other provinces, small ruminant population in Balıkesir is higher than other provinces except Ankara, Diyarbakır, Konya, Van and Şanlıurfa.

Although there was no significant difference in the age distribution of the enterprise owners in both provinces, it was observed that the majority of them were between the ages of 46-55 and 56 or older, and there were very few young owners. In general, it can be said that middle and older age is dominant. In a survey conducted on sheep farming enterprises in the Selçuklu district of Konya province, it was determined that 45.2% of the enterprise owners were between the ages of 26-44 and 6.5% were between the ages of 18-25 (Mohamud et al., 2023), and the low proportion of young enterprise owners is similar to the situation in the current study. Also, in a recent survey study conducted on small ruminant enterprises, it was determined that the owners between the ages of 41-60 constituted the most important age range with a rate of 52.1% (Demir and Tuncer, 2023) and it was noteworthy that the owners were mostly in the middle age group as in the present study. This situation shows that the interest of the young population in this sector is low and that small ruminant farming should be made more attractive for the young generation. When the educational status of the enterprise owners participating in the study was considered, it was understood that all of them were literate. It was determined that 66% of the total participants had primary and secondary school education, 56% had high school education and 6% had university education. On a provincial basis, although the rate of secondary school and high school graduates of the small ruminant enterprise owners in Balıkesir was partially lower than that of the enterprise owners in Sakarya, it was determined that Balıkesir had a high rate of high school and university graduates, whereas no university graduate was found among the small ruminant enterprise owners from Sakarya. The fact that the proportion of those with secondary and primary education is considerably higher than the proportion of those with high school and university education is expected as a result of the fact that primary education is compulsory. When the educational status of the enterprise owners who participated in the survey in this study was compared with the recent studies, it was found that the rate was lower than the rate determined by Mohamud et al. (2023) and Tavalı and Çak (2023), higher than the rate determined by Demir and Tuncer (2023), Göncü (2023), Yıldız (2023) in terms of primary and secondary school educational attainment rate. In terms of high school educational attainment, the rate determined in this study was lower than the rate determined by Çetinkaya et al. (2023) and Göncü (2023), higher than the rate determined by Demir and Tuncer (2023), Mohamud et al. (2023), Tavalı and Çak (2023), and almost similar to the rate determined by Yıldız (2023). In terms of university education, the rate obtained in this study was lower than the rate reported by Çetinkaya et al. (2023) and Yıldız (2023), higher than the rate reported by Demir and Tuncer (2023), Tavalı and Çak (2023) and Göncü (2023), and close to the rate determined by Mohamud et al. (2023). When both age and education status were taken into consideration, the data obtained showed that young and educated people stayed away from the related sector. Since the livestock sector requires large areas of land, enterprises are generally concentrated in towns and villages, so the limited infrastructure and social facilities in rural areas cause young and highly educated people to prefer urban life with a wider choice in terms of relevant opportunities (Güven and Yavuz, 2020). As a matter of fact, the possible reflections of this situation were also observed in the data on age and education level obtained in the present study.

When the results of this survey were evaluated in terms of enterprise type, it was observed that small ruminant fattening enterprise type was more prominent in Balıkesir province compared to Sakarya province, whereas dual-purpose small ruminant enterprise type was more preferred in Sakarya. Balıkesir Provincial Directorate of Agriculture and Forestry reported that Balıkesir ranks third in Türkiye in terms of red meat production with 4%, 10th in terms of sheep milk production with 2.5%, and 17th in terms of goat milk production with 1.9% (Republic of Türkiye Ministry of Agriculture and Forestry Balıkesir Provincial Directorate of Agriculture and Forestry, 2024). This situation explains why fattening enterprise type is

preferred more in small ruminant farming in Balıkesir. In addition, it is also possible that Balıkesir has a climate where fodder crops required for fattening can be grown more easily and small ruminant enterprises aiming to obtain faster results in terms of production in this province may find the fattening enterprise type more attractive. As a matter of fact, in the same report prepared by Balıkesir Provincial Directorate of Agriculture and Forestry, it was stated that this province ranked 1st for wheat, rye, broad bean, 2nd for maize, oat, 3rd for forage pea, maize for silage, fodder turnip, 4th for Italian ryegrass, 6th for vetch, green grass and sorghum in Türkiye in terms of fodder crops and green grass production. In terms of enterprise structure, it was determined that the majority of the enterprises (73%) participating in the survey were in closed enterprise structure, whereas 26% were in extensive and semi-extensive enterprise structure. On a provincial basis, it was determined that the enterprises in Sakarya had a significantly higher proportion of closed enterprise structure compared to the enterprises in Balıkesir, whereas extensive and semi -extensive enterprise structure was dominant with a significantly higher proportion in Balıkesir. It can be said that regional climate structure is an effective factor in the preference of the enterprise structure. Balıkesir province has a transitional climate type between the Mediterranean and the Black Sea (Aliağaoğlu and Miroğlu, 2020), while Sakarya has humid weather conditions due to the influence of the Black Sea in the north and the Marmara Sea in the west, and continental climate conditions due to the land masses originating from the Saman Mountains in the south and east (Ustaoğlu, 2018). Şişman et al. (2009) reported that 66.6% of small ruminant enterprises in Bolu region had closed housing type and 33.4% had open housing type, Elmaz et al. (2014) reported that 84.4% of the enterprises in Burdur province had semi-open, 6.3% had open and 9.3% had closed housing type, Bakır et al. (2017) reported that 95.8% of enterprises had closed housing type, 4.2% had open housing type in Siirt province due to the harsh climate conditions, and Aydın and Keskin (2018) reported that the majority of small ruminant enterprises in Muğla province had semi-open (54% for sheep and 36% for goats, respectively) or open housing type (36% for sheep and 56% for goats, respectively).

Biosecurity, which is a set of proactive routine precautions to protect the health of the herd by limiting the risk of transmission of agents that can cause herd disease in an animal enterprise, is also an important issue in terms of minimizing the risk of exposure of farmers, their families and workers to zoonotic diseases and reducing food safety risks (Erzurum et al., 2021). In order to carry out livestock farming activities in a sustainable and efficient manner, it is of great importance to act in a herd-oriented manner beyond individual animal treatments and to create a enterprise structure away from infectious diseases (Kristiensen and Jakobsen, 2011). In the current study, when the biosecurity data obtained from the enterprises were examined, it was determined that some of the small ruminant enterprises participating in the survey harboured different types of animals in addition to the relevant animal species. In terms of different animal species, it was observed that the proportion of enterprises keeping poultry was significantly higher in Balıkesir province compared to Sakarya province (43% vs. 18%). Although there is no significant difference between these two provinces in terms of cat-dog harbouring rates, it was observed that 89% of all participant enterprises harbour cats-dogs, in addition to this, 42% of all participant enterprises do did not carry out routine antiparasitic treatment of cats -dogs. Considering that parasites such as neosporosis, sarcocytosis and tapeworms can be transmitted from dogs to livestock (Atton, 2021), this can be considered a biosecurity weakness. In a study conducted in Malatya by Şeker et al. (2017), who reported that keeping animals of different species together in farms caused significant disadvantages, the rate of farms kept animals of different species together was 37.2% and this was a high rate. Another issue addressed under the biosecurity framework is combat against flies. Since manure waste in livestock farms is a suitable spawning environment for flies, the fly problem related to livestock farming is an important problem that both negatively affects animal productivity and interrupts livestock activities in areas close to settlements (Kaya and Uzmay, 1995). In the present study, although there was no significant difference between the two provinces in terms of fly problem and fly control, it was determined that there was a high rate (67%) of fly problem, whereas the rate of enterprises that did not fight against flies was high (69%). In this study, the low proportion of enterprises that combated flies, which can cause serious risks such as sheep-goat pox, Rift Valley fever, Wasselbron disease, Cache Valley virus (Sevinç and İder, 2021), myiasis (Uslu, 2021), bluetongue infection and Schmallenberg disease (Bulut, 2021), demonstrated that the fly control was not at the desired level.

The quarantine programme to be applied to new animals joining the herd in an enterprise is an important biosecurity issue for herd health. Rams and bucks selected as strong in terms of genotype and

phenotype should also be free from diseases, all vaccinations and controls should be carried out at least 8 weeks before the breeding season, and rams or bucks newly purchased during the breeding season should not join the herd before completing the quarantine process as much as possible (Güler and Satılmış, 2021). It is also essential to adopt an effective quarantine program of at least 15 days for new additions to the flock against foot diseases of sheep and goats (Alkan et al., 2021). In the current study, it was determined that the quarantine process was not applied to new animals entered the enterprises at a high rate of 85%, and the disinfection of new vehicles entered the enterprise was not carried out at a rate of 90%, which revealed the inadequacy in this regard.

Balanced and adequate feeding is of great importance for a successful herd performance and sustainability in small ruminant farming. In the present study, although there were differences in the preference rates of cereal grain feeds between the two provinces, the preference rates of barley and oat, except wheat, were statistically higher in the enterprises in Sakarya than in Balıkesir; however, it was determined that wheat preference was the first with a rate of 68%, barley preference was the second with a rate of 46% and oat preference was the third with a rate of 14% in terms of all participated enterprises. It was thought that these differences in cereal grain feed preferences for both the provinces and all participants were probably related to factors such as local agricultural production, feedstuff prices, feeding habits of the owners, regional conditions, feedstuff supply chain and economic reasons. The proportion of enterprises preferring to use factory feed was found to be as high as 90% for all participants and it was observed that this proportion was significantly higher in Balıkesir province than Sakarya province (99% vs. 62%). Considering the fact that factory feed, which is expensive, has a large share in feed costs, this high proportion of enterprises preferring to use factory feed both in the enterprises in Balıkesir on a provincial basis and among all participating enterprises can be interpreted as the fact that most of the enterprise owners are able to tolerate this cost. However, it was determined that there were also enterprises that did not use factory feed at all, this rate was 38% especially in enterprises participated from Sakarya province and 10% for all participated enterprises. This situation may probably be due to economic reasons. As a matter of fact, in the studies conducted by Demir et al. (2015), Karadaş (2018) and Ünal and Dellal (2023), it was reported that feed prices ranked first among the problems experienced in enterprises.

In this survey study, differences were found between the enterprises participating from Balıkesir and Sakarya provinces in terms of forage preferences. According to the results obtained from all participants, it was observed that wheat straw was preferred the most, followed by alfalfa. According to the results obtained on provincial basis, it was understood that the enterprises in Sakarya preferred alfalfa and oat straw at a significantly higher proportion than the enterprises in Balıkesir, whereas the enterprises in Balıkesir preferred grass hay and vetch hay at a significantly higher proportion than the enterprises in Sakarya. The fact that the enterprises in Balıkesir have lower preference rates for oat and oat straw and higher preference rates for wheat and wheat straw is probably related to the production potential of Balıkesir. As a matter of fact, according to TÜİK (2022), Balıkesir ranks 16th in oat grain production in Türkiye, while it ranks 2nd in wheat hay production. However, alternative roughages such as beet pulp, forage peas and sainfoin were preferred at very low rates in both provinces and in all participated enterprises. It is highly probable that factors such as local vegetation, feeding habits and availability of forage supply play a role in the formation of these differences. In a study conducted by Gökmener (2023) on sheep farms in Uzundere district of Erzurum province, the usage rates of straw, dry clover, sainfoin and hay were found to be 39.9%, 24.4%, 11.1% and 24.4%, respectively.

In this study, it was determined that pasture was utilized at high rates on the basis of provinces and all participating enterprises. In particular, this proportion was 93% for the enterprises in Balıkesir, 72% for Sakarya and 89% for all enterprises participated in the survey. This is an expected situation since small ruminant farming is based on pasture in our country. However, pasture grazing preference proportion of the enterprises in Balıkesir was significantly higher than the enterprises in Sakarya. It was thought that this situation was related to the geographical structure and the area of meadow-pasture. According to the Balıkesir Provincial Directorate of Agriculture and Forestry of the Ministry of Agriculture and Forestry, Balıkesir meadow-pasture area in 2022 is 82,715 hectares and constitutes 5.67% of its surface area, and in the Provincial Briefing made by the Governorship of Sakarya in 2024, Sakarya meadow-pasture area is 7080.5 hectares and constitutes 1.47% of its surface area.

One of the important issues in terms of nutrition in animal farming is to meet the vitamin and mineral requirements of animals. In this study, it was determined that 41% of the small ruminant enterprises from Balıkesir supplemented their animals with

vitamins and minerals, whereas 59% of the enterprises from Balıkesir and none of the enterprises from Sakarya did not do this supplementation. It was noteworthy that the majority of the enterprises (89%) used licking stones, and this rate was higher in Sakarya than in Balıkesir. Considering the finding that none of the participating enterprises from Sakarya used vitamin-mineral mix, it is estimated that they tried to cover this gap by using licking stones. The use of licking stone alone without mineral supplementation to the ration is not sufficient especially for meeting mineral requirements (Pump et al., 1976; Burghardi et al., 1982; Zervas et al., 2001).

Provision of water, which is one of the most basic needs for growth, reproduction and milk yield in livestock, is an important issue. In this study, most of the enterprises (89%) provided water from pasture water sources, followed by the use of troughs (78%) and buckets (63%). On the other hand, the use of water tank and automatic drinker remained at a low level with 20% and 16% respectively. When compared on the basis of provinces, no significant difference was found between Balıkesir and Sakarya provinces in terms of the use of pasture water sources, however, it was found that the use of automatic drinker, bucket and water tank was significantly higher in enterprises from Balıkesir than enterprises from Sakarya province, except the use of trough. It is recommended to use automatic water dispensers in animal farms so that the herd can constantly access clean water and not get sick by drinking contaminated water (Şeker et al., 2017). The finding that automatic water dispensers, which provide advantages in terms of labor, water saving and hygiene, were preferred less than equipment such as buckets and troughs in this study demonstrated that those enterprises had not sufficiently adopted mechanization and modernization in the provision of water to animals. Nevertheless, in this study, the trough and automatic drinker preference proportion determined for water supply of total enterprises were lower and higher, respectively, than the trough preference proportion (95.5%) and automatic drinker preference proportion (5.5%) determined in the recent study conducted by Şeker et al. (2022) on sheep farms.

In the present study, variables such as whether rams are separated from the herd, whether pregnant animals are vaccinated, whether there is a maternity area or birthing pen for parturition, whether the owners know the signs of birth, whether they help in case of dystocia and whether disinfection and hygiene procedures are carried out after birth were also evaluated in the context of herd management related to birth. It was found that 74.5% of the enterprises in the survey did not separate the rams from the flock and this rate was significantly higher in the enterprises from Balıkesir compared to the enterprises from Sakarya (81% vs. 54%). This situation can be considered as a problem related to flock management. Because keeping rams together with ewes in the flock throughout the year may cause negative effects on the ram effect, which is known to have a direct effect on the mating season, synchronization of estrus and fertility, delaying the seasonal estrus of ewes by about 6 weeks, decreasing the mating desire of rams, and keeping rams among lactating ewes may cause resistance to rams in ewes (Ungerfeld et al., 2004; Sunderland et al., 1990; Yılmaz et al., 2009). In sheep farming with different flock management in the same region or in different regions, some farms do not separate the ram from the flock at all, while some farms introduce rams in different seasons (Ülsü and Çilek, 2024). In this study, the proportion of the enterprises separating rams from the flock among all enterprises was close to the proportions determined by Dönmez (2008) for Bursa and Gökmener (2023) for Edirne Uzundere (29.8% and 24. 4%), higher than the proportion determined by Bilginturan and Ayhan (2009) for enterprises in Burdur (3%), lower than the proportions determined by Ceyhan et al. (2015) for enterprises in Niğde and Mohamud et al. (2023) for enterprises in Konya-Selçuklu (69.8% and 69.4%, respectively). When compared on the basis of provinces, the proportions of enterprises keeping rams separate from the flock in the participating enterprises from Balıkesir and Sakarya were lower and higher respectively, than the proportions reported by Gökmener (2023), higher than the proportions reported by Bilginturan and Ayhan (2009), and lower than the proportions reported by Ceyhan et al. (2015) and Mohamud et al. (2023).

In small ruminant farming, both the sheep and the offspring can be immunized against possible diseases by vaccinating pregnant animals, and in this context, it is recommended to vaccinate pregnant animals against clostridial infections, contagious ecthyma caused by Orf virus, contagious agalactia caused by mycoplasma, and agents such as B. melitensis, C. abortus, T. gondii that cause abortions (Fthenakis et al., 2012). Especially during pregnancy, Clostridial vaccines are important in preventing lamb and kid losses due to enterotoxaemia, lamb dysentery and tetanus, and the application of Mannheimia haemolytica and Bibersteinia trehalosi vaccines can prevent lamb losses due to pneumonia (İder and Ertürk, 2023). In the present study, although 83% of the enterprises applied mixed vaccination to pregnant animals, 17% did not vaccinate, which was

considered as a risk in terms of herd health and this situation necessitated the requirement to inform the owners about possible risks. In the evaluation on whether there was a maternity area in the enterprises, it was found that there was a statistically significant difference on the basis of provinces and it was observed that the enterprises from Balıkesir had a maternity area at a significantly higher proportion than the enterprises from Sakarya (75.3% vs. 36%). Among all participant enterprises, this rate was 65.5% and it was noteworthy that 34.5% of enterprises used common barns for parturition. It has been reported that, in general, individual maternity areas should account for approximately 10% of the total sheep and goat population on the farm, and if parturition is synchronized, the number of maternity area may need to be increased up to three times that amount (Ünal et al., 2018). The reason why maternity areas were rarely found in enterprises in the current study was probably due to insufficient space. It was determined that most of the enterprises participating in the study both on the basis of province and in general did not have a birthing pen. This situation indicated that the enterprises in the region preferred to use the existing barns and other areas rather than investing in a separate structure for parturition. Postpartum cleaning in the place of birth within the framework of hygiene rules is an important safety precaution against the risk of disease transmission of surfaces contaminated with birth fluids and residues. In the present study, significant differences were found between the enterprises handled on a provincial basis regarding postpartum cleaning, and this proportion was 46% for all enterprises. This situation drew attention as a weak point in postnatal biosecurity in terms of hygiene.

In the present study, it was determined that the owners knew the signs of birth at a high proportion (98.5%) both on the basis of the province and on the basis of all participant enterprises, and it was understood that almost all owners were aware of the signs of birth in the herd. Considering that the experience and knowledge of the owners to recognise signs such as loss of appetite, restlessness, swelling and discharge in the vulva, swelling of the milk glands, vocal changes in the form of silence or shouting in the animal is critical for the protection of both maternal and offspring health and rapid intervention in possible complications that may occur during the birth process, the finding that the owners in the current study had this knowledge and competence can be considered as a positive development. One of the most common difficulties encountered in ruminant farming is to ensure the continuity of the survival of newborn offspring and the main objective is to increase the number of offspring born and to reduce offspring losses during the period in these enterprises (Koyuncu and Duymaz, 2017). In small ruminant farming, umbilical cord care and colostrum are of vital importance in increasing the survival chances of neonatal lambs and kids (Fesseha et al., 2023). In order to cut and disinfect the umbilical cord properly following birth in lambs and kids, the cord is cut 3-4 cm below the abdominal region and immersed in 7% iodine solution or alcohol-based 2.5% iodine solution to dry the umbilicus and to reduce the risk of disease transmission through the umbilical cord (Tepeli, 2021; Menzies and Bailey 1997). In this study, significant differences were found between the participant enterprises from Balıkesir and Sakarya in terms of the proportions of disinfection and initial care of the umbilical cords in neonates immediately after birth (67% and 34%, respectively). This rate was 58.8% in terms of all enterprises evaluated in the study and it was noteworthy that the proportion of enterprises that did not perform umbilical cord disinfection and care both on a provincial basis and on a general basis was at a considerable level. This situation revealed that there were still small ruminant farms in these provinces that need to be raised awareness in terms of umbilical cord care and hygiene, which is of vital importance. In a study conducted by Kandemir et al. (2015) in Izmir region, it was reported that umbilical cord disinfection of neonates was not performed in 91.3% and 76.5% of enterprises in mountainous and plain regions, respectively, and 83.3% of all enterprises. When these proportions were compared with the results of the present study, the proportions of enterprises performing umbilical cord disinfection both on the basis of provinces (Balıkesir and Sakarya) and on the basis of all enterprises were higher than the proportions in the study conducted by Kandemir et al. (2015). Similarly, the umbilical cord disinfection proportion (40.4%) obtained in the study conducted by Şahin (2019) in Tokat province was lower than the proportion determined for Balıkesir and all enterprises in the present study. Since newborn lambs and kids are susceptible to hypothermia due to their low energy reserves and large body surface areas compared to their body weights, they should receive sufficient colostrum in the first 30-60 minutes after birth, and the survival of these animals can be increased by consuming sufficient colostrum in the first 2-3 hours (Tepeli, 2021). Since small ruminants have epitheliochorial placenta structure, immunoglobulins cannot pass the placental barrier and therefore, neonates should receive the maternal immunoglobulins through the colostrum (Koyuncu and Duymaz, 2017). In

the present study, it was determined that the majority of the enterprises in the survey both on provincial and general basis paid attention to the colostrum intake of neonates within the first two hours and these proportions were 93.3% and 88% in the enterprises participated from Balıkesir and Sakarya provinces, respectively, and 92% on the basis of all enterprises. However, the presence of 6.7% and 12% of the enterprises that did not use colostrum in the related provinces clearly showed that there were still enterprises that did not comprehend the importance of colostrum, which is vital for newborn animals, and that there were still enterprises with poor colostrum management. In the present study, the proportion of colostrum usage in the enterprises participated from Balıkesir province and on the basis of all enterprises was similar to the proportion (93.4%) reported in the study conducted by Kandemir et al. (2015) in Izmir. In cases when newborn lambs and kids could not benefit from colostrum due to reasons such as weakness, hypothermia, lack of sucking reflex or rejection by their mothers, colostrum should be given by bottle or gastric catheter (Tepeli, 2021; Ermetin, 2021). In the present study, it was determined that 76.5% of the enterprises performed forced administration of colostrum to neonates, when necessary, whereas 23.5% did not do so. It was thought that this situation may probably be due to the inadequacy of the enterprises in terms of technical knowledge, equipment and experience related to colostrum administration.

Conclusion

The fact that the owners of most of the small ruminant farms included in this survey study from Balıkesir and Sakarya provinces are older than 46 years of age and the fact that there are very few young owners shows that the interest of young generation in this sector should be increased, and small ruminant farming should be made more attractive. In terms of the educational status of the owners of small ruminant enterprises, the majority of them have primary and secondary school education, but the number of those with university education is generally low, especially in the enterprises included in the study from Sakarya province, no university graduates were found, which shows that this sector should be informed and encouraged for higher education graduates that this sector can be an attractive option. It is noteworthy that there are considerable deficiencies and mistakes in biosecurity in small ruminant enterprises from both provinces. Informing the owners and employees about the correction of this situation and strengthening the weak points will contribute to minimizing both the economic losses of the enterprise and the factors that will adversely affect human and animal health. The feed and feedstuff preferences for feeding animals in the enterprises in the two provinces differed, and although it is considered normal that these preferences include differences due to regional differences, soil structure, budget, and product supply chain variability, deficiencies and errors in feeding were noted, and especially the failure to use vitamin-mineral mixture and the attempt to meet this need from licking stones were evaluated as a wrong and negative situation. Owners and personnel should be informed about appropriate, balanced and rational feeding strategies for animals and deficiencies in this regard should be eliminated. In the enterprises examined in the study, different equipment is used to meet the water requirements of the herd. It has been observed that the use of automatic water dispensers in water supply is preferred at low rates, and it is thought that this is probably due to the inability to provide the necessary mechanization for economic reasons. There is a need for initiatives to encourage the use of automatic water dispensers in these enterprises, and to inform those who use equipment such as buckets, troughs and barrels about the importance of frequent cleaning and disinfection of these equipment. In the small ruminant sector, deficiencies and errors have also been identified at some critical points regarding the management of pregnant animals, animals approaching birth, animals giving birth, and the postpartum period, which are of particular importance in maintaining the herd and increasing the number of animals. In the majority of the enterprises examined in the study, it was evaluated as a positive finding that the enterprise owners knew the signs of the beginning of parturition in animals and had the ability to help in cases of dystocia. However, the existence of enterprises that made incorrect and incomplete applications in vital points such as vaccination of pregnant animals, postpartum cleaning, colostrum management and umbilical cord care, disinfection and hygiene in neonates has led to the necessity of teaching informative practical applications to the relevant responsible persons in these matters.

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Authors' contributions

All authors have read and agreed to the published version of the manuscript. E.D., T.A., T.T.G., and T.B.

were responsible for the creation of the questionnaire. T.A., T.T.G. shared the questionnaire and collected the responses. E.D. and T.A. performed the statistical analysis and interpreted the data O.K. and S.B.Ö. drafted the manuscript. T.B. revised the first versions of the manuscript.

Disclaimer

No potential conflict of interest was reported by the authors.

Ethical statement

This study was conducted with the approval of the ethics committee for feasibility in accordance with the decision of Istanbul University Cerrahpaşa Social and Human Sciences Research Ethics Committee dated 09.10.2023 and numbered 2023/344.

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Evaluation of the efficiency of TENS therapy to the regeneration in N. ischiadicus injured rats

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ABSTRACT

Transcutaneous electrical nerve stimulation (TENS) is one of the electrotherapy methods, used for physical therapy, to relieve neuropathic pain in the nervous system lesion. However, the effect on peripheral nerve regeneration has been unknown. This work aims to examine peripheral nervous system diseases in which fullness is preserved, the efficiency of TENS to hasten healing, and the suitability of magnetic resonance imaging to diagnose peripheral nervous system diseases. Also, electrophysiologic findings of functional nerve recuperation will be considered after comparing with histopathologic and magnetic resonance imaging. 72 Sprague Dawley rats were randomly assigned to four groups. Group 1; normal without crush lesion, Group 2; control group with crush lesion, Group 3; stimulated group on lesion area, Group 4; stimulated group on gastrocnemius muscle. The animals were sacrificed post-operatively 21. day and 45. day after the electrophysiological, assessment and walking trace analysis, magnetic resonance imaging and nerve samples were obtained for histologic analysis. According to this study, low-frequency TENS leads to delayed regeneration after a crush lesion of the sciatic nerve in rats.

Keywords: rat, TENS, EMG, nerve degeneration, electrophysiology

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Introduction

Traumatic peripheral nerve and root injuries are common in companion animals. These injuries frequently occur as a result of motor vehicle accidents, leading to fractures of the humerus, pelvis, and proximal femur, as well as iliosacral and sacrocaudal fractures and dislocations. Additionally, bite wounds and gunshot injuries are also significant causes of peripheral nerve damage. Iatrogenic nerve injury, arising from incorrectly administered intramuscular injections or surgical procedures, is another common cause. Peripheral nerve damage can manifest as compression, contusion, stretching, avulsion, or complete transaction (Dewey, 2003; Forterre et al., 2007; Rodkey & Sharp, 2003).

In recent studies focused on nerve healing have shed light on the pathophysiological mechanisms and molecular changes associated with peripheral nerve injuries. Despite all the research on nerve healing, adequate nerve recovery has not been achieved to the desired extent following severe injuries. The primary goal in the treatment of peripheral nerve injuries is to restore nerve integrity, thereby re-establishing the transmission of signals and the full functional recovery of the target organs innervated by the nerve. For successful nerve regeneration, processes such as axonal sprouting, axonal growth, target organ reinnervation, and reintegration of the regenerated fibers with the central nervous system need to be completed (Wolthers et al., 2005).

Electrical stimulation has positive effects on regeneration in nerve compression injuries has been proven. Transcutaneous electrical nerve stimulation (TENS) is a widely used electrotherapy modality in physical rehabilitation, primarily aimed at alleviating neuropathic pain associated with nerve lesions. There is currently no consensus regarding the effectiveness of TENS in improving nerve regeneration, as its efficacy is influenced by various factors such as the type, frequency, intensity, and method of application (Alarcon et al., 2022). While some studies suggest that TENS may accelerate reinnervation, other research indicates that it could potentially delay regeneration

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(Baptista et al., 2008). The effects of TENS on peripheral nerve regeneration have yet to reach a consensus in the literature.

The purpose of our study is to evaluate the efficacy of TENS in promoting the healing of peripheral nerve injuries where the integrity of the nerve has not been compromised.

Materials and Methods

In this experimental study, 72 male Sprague Dawley rats, weighing between 200 and 300 grams, were used. The rats were obtained from the TÜBİTAK Marmara Research Center. During the study, the rats were housed in a room with a 12-hour light/dark cycle, controlled ventilation, and maintained at room temperature. They were provided unlimited standard food (dry pellets) and drinking water. All experimental procedures were performed by the ethical guidelines set by the Istanbul University Animal Ethics Committee.

The rats were divided into four groups, each containing 18 animals (n=18). Group 1 (n=18): Sham group, where a surgical incision was made without inducing nerve injury. Group 2 (n=18): Control group, where crush injury was induced to the sciatic nerve without any further intervention. Group 3 (n=18): Experimental group I, where a crush injury was induced to the sciatic nerve, followed by transcutaneous electrical nerve stimulation (TENS) applied to the injured area. Group 4 (n=18): Experimental group II, where a crush injury was induced to the sciatic nerve, followed by TENS applied to the gastrocnemius muscle.

Surgical procedure

General anesthesia was induced by intramuscular injection of a mixture of 8 mg/0,1 kg Ketamine HCl (Ketalar®, Pfizer) and 1 mg/0,1 kg Xylazine HCl (Rompun®, Bayer). Following anesthesia, the left femur areas of the rats were shaved. The rats were positioned in the right lateral recumbent position, and the surgical area was disinfected with povidone-iodine. The surgical field was covered with sterile drapes, leaving the incision site exposed. An oblique incision was performed on the left femur to open the skin. The biceps femoris muscle was bluntly dissected, and the edges were retracted to expose the sciatic nerve. The sciatic nerve was carefully freed from surrounding tissues, from the sciatic notch to the branching area of the nerve. A surgical clamp was used to induce crush injury to the sciatic nerve. The sciatic nerve compression injury was induced by applying pressure through the single tooth of this clamp for 30 seconds, 10 mm above the branching region (figure 1). This procedure aimed to standardize the sciatic nerve injury . After completing the surgical procedures, the muscle tissue was sutured with continuous 5/0 Vicryl, and the skin was closed using continuous 5/0 silk sutures.

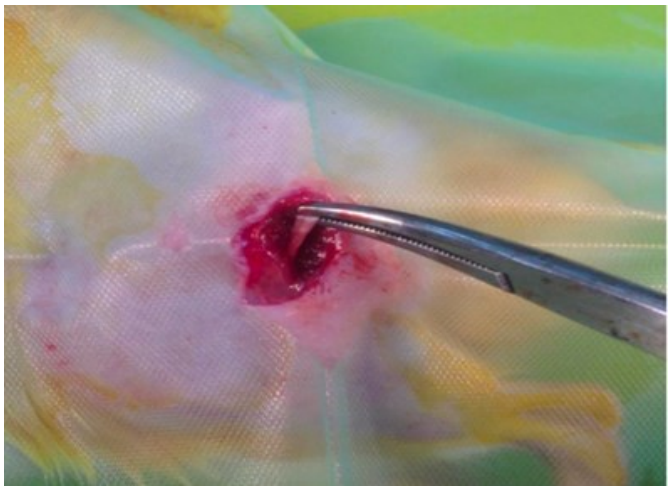

Figure 1. Creation of crush injury in the sciatic nerve

TENS application in experimental groups

In the 3rd experimental group, consisting of 18 rats in Group 3, TENS was applied to the region of the nerve injury. In the 4th experimental group, comprising 18 rats in Group 4, TENS was applied to the gastrocnemius muscle, which is innervated by the injured sciatic nerve. TENS was started 4 days post-surgery in all rats. The electrical stimulation was administered daily for 20 minutes for 15 days, using a 4 Hz frequency and 200 µs pulse duration, via the Physiomed Vetri Combi device.

Over the 45-day follow-up period, general changes were observed in the rats. During the experiment, partial autophagy was observed in the feet of 2 rats in the control group, 2 rats in experimental group 1, and 1 rat in experimental group 2. Since these conditions interfered with the SFI measurements, these rats were excluded from the study. To balance the number of animals across the groups, 1 rat was also excluded from experimental group 2, and the findings were evaluated based on 16 animals in each group. Functional recovery of the sciatic nerve was assessed by gait analysis , and the Sciatic Functional Index (SFI) was calculated for all animals before surgery (2 days prior) and on the 21st and 45th days post-surgery. For the gait analysis, a walking pathway apparatus was prepared with a length of 50 cm, a width of 10 cm, and a side height of 12 cm, ending in a dark room. White sheets of paper, cut to the same size as the corridor, were placed inside the walking path. The rats' left hind paws were pressed onto an ink stamp soaked in black India ink, and the rats were made to walk through the prepared corridor to leave footprints (Figure 2). In general, several sampling attempts were required to

Figure 2. The walking corridor ending in a dark room

obtain clear and distinct footprints. Using the most suitable footprints on the paper strips, the following measurements were taken with the help of a millimeter ruler: the distance between the heel and the toe (print length, PL), the distance between the first and fifth toes (step width, SW), and the distance between the second and fourth toes (mid-step width, MSW). The values obtained from the measurements were placed into the formula developed by Medinacelli and later modified by Bain-Mackinnon-Hunter to calculate the SFI. In values ranging from 0 to -100, an index of 0 indicates normal function, while an index of -100 signifies complete loss of function. The differences in the SFI values between the groups were statistically analyzed.
Magnetic resonance imaging (MRI) of the left leg was performed before the TENS application on the 4th day, and again on the 21st and 45th days, prior to nerve sample collection, for three randomly selected rats from each group.

Electrophysiological measurements were taken under deep anesthesia from the left leg of the rats before surgery and on the 21st and 45th days after surgery. Three electrodes were used: one active, and the others as reference and ground electrodes. For measurement, while the rats were under anesthesia, the area to be measured was shaved and cleaned with alcohol. To prevent direct contact with the surface and maintain a constant temperature, a hot water bottle was placed beneath the rats. The active electrode was placed on the mid-region of the gastrocnemius muscle, the reference electrode on the tendon region, and the ground electrode on the tail. The stimulus electrode was positioned between the L3-L4 vertebrae. After delivering a stimulus with a frequency of 1 Hz and a duration of 0.1 ms, the responses were analyzed using Neurosoft software. In the second stage, electrophysiological measurements were taken by exposing the sciatic nerve through an incision of approximately 1 cm at the mid-thigh level. The stimulation unit, with an inter-electrode distance of 1.1 cm, was placed on the sciatic nerve, and the first stimulus was delivered from the first electrode. Recordings from the gastrocnemius muscle were obtained using a needle electrode and transferred to a computer with the help of an amplifier. Similarly, a stimulus was delivered through the second stimulation electrode, and recordings were made. In both cases, three stimuli were delivered, and the conduction velocities were calculated and averaged. The within group and between group analysis included the evaluation of peak-to-peak amplitude values, latencies, and nerve conduction velocities.

After the electrophysiological assessments, the rats were euthanized under general anesthesia, and pathological samples were collected. Sciatic nerve samples, including the gastrocnemius muscle attached to the nerve, were harvested with the damage site medial to the specimen. The collected tissues were processed through routine histological procedures, and 5-7 µm thick sections were obtained from paraffin blocks. These sections were stained using the hematoxylin-eosin method and examined under a light microscope at 400x magnification. Axons in the observed field were counted, and the presence of edema cells was evaluated.

Results

During the study, partial autophagy (self-cannibalization) in the feet was observed in 2 rats from the control group, 2 rats from experimental group 1, and 1 rat from experimental group 2. Since this condition interfered with the Sciatic Functional Index (SFI) measurements, these rats were excluded from the study. To equalize the group sizes, 1 rat was also excluded from experimental group 2, and the findings were evaluated based on 16 rats per group.

All rats in each group were walked on the gait path before the experiment, and the Sciatic Functional Index (SFI) values were recorded on Day 0. The average pre-experiment SFI value (Day 0) for all groups was found to be -5.50 (± 3.80). On Day 21 and Day 45 post-surgery, no significant differences were observed between the control and TENS groups when comparing the average SFI values. However, when each group was assessed individually, significant differences were found

in the mean SFI values between the pre-experiment (Day 0), post-experiment Day 21 (SFI 21st Day), and Day 45 (SFI 45th Day), indicating nerve regeneration was supported.

Magnetic resonance imaging (MRI) scans taken before the TENS application on Day 4 revealed hyperintense areas in T2 sequences, consistent with inflammation and edema in the surgical site. No significant differences in appearance were observed between Day 21 and Day 45 in either inter-group or intra-group comparisons. Compared to Group 1, there was significant denervation atrophy in the gastrocnemius muscle, consistent with nerve degeneration.

When examining the latency values of all rats, a significant increase was observed post-injury compared to the pre-surgical baseline. However, no significant differences were found in latency values between the control and experimental groups. Regarding peak-to-peak amplitude values, a significant decrease was observed in all groups that underwent surgical procedures. No significant difference in amplitude values was detected between the control and experimental groups. Nerve conduction velocity (NCV) measurements were also obtained., the pre-injury average sciatic nerve conduction velocity in all groups was measured as 46.22 ± 3.25 m/s. In this study, where sciatic nerve injury was performed in the left leg, the average left sciatic nerve conduction velocity in the sham group was 51.3 ± 2.65 m/s. In the control group, where injury was induced, the nerve conduction velocity decreased to 18.6 ± 1.64 m/s. In the TENS-treated Groups 3 and 4, the conduction velocities decreased to 19.4 ± 1.6 m/s and 18.8 ± 1.7 m/s, respectively.

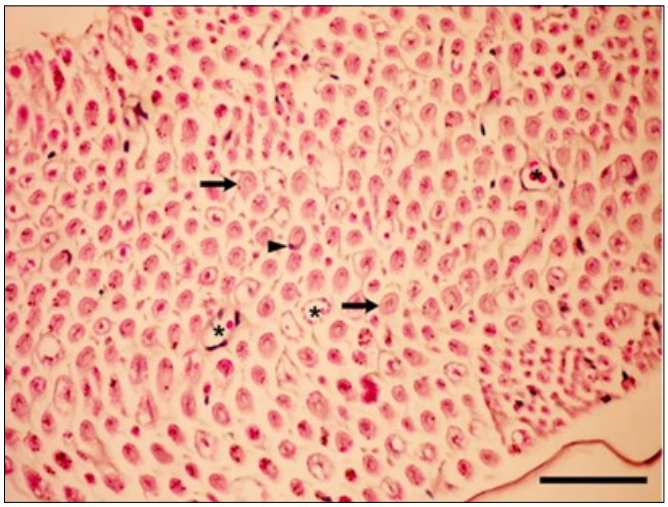

Figure 3. Normal nerve section (sham-operated), myelinated axons (arrows), Schwann cells (arrowheads), and capillary vessels (stars) H&E, Bar = 100 µm.

In Group 1 biopsy samples obtained from the sciatic nerve (n.ischiadicus), the nerve fibers were found to be wrapped in myelin sheaths and arranged in a normal pattern, with the presence of a small number of Schwann cells (Figure 3). No pathological changes were observed, apart from slight artifacts due to routine tissue processing. No pathological changes were observed in tissue samples taken from the gastrocnemius muscle (m. gastrocnemius) either.

In Group 2 biopsy samples taken from the distal portion of the sciatic nerve (n.ischiadicus) injury site on Day 21, the epineurium exhibited an edematous appearance, with a small number of mononuclear inflammatory cell infiltrates. The perineurium was separated from the nerve fibers, and the endoneurium appeared irregularly scattered. Myelinated axons showed largely dissolved myelin sheaths, with evidence of vacuolar degeneration. Additionally, an increase in the number of Schwann cells and a small number of mononuclear inflammatory cell infiltrates were observed (Figure 4). Histopathological examination of gastrocnemius muscle (m.gastrocnemius) samples from the control group on Day 21 revealed no changes except for mild myositis.

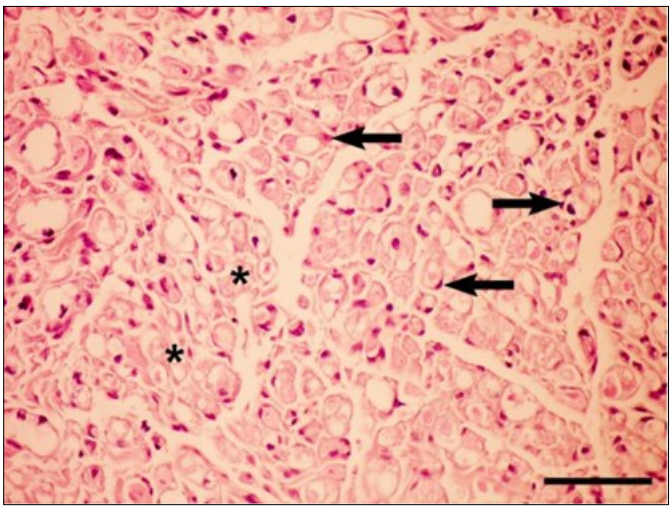

Figure 4. Control Group, Day 21, increase in the number of Schwann cells (arrows) and degeneration in the myelin sheath and axons (stars), H&E, Bar = 50 µm.

In Group 3 and Group 4, similar to the control group, in animals that underwent TENS application to both the sciatic nerve (n.ischiadicus) and gastrocnemius muscle (m.gastrocnemicus), histological sections taken from the distal part of the injury site of the sciatic nerve on day 21 showed edema in the epineurium and infiltration of a few mononuclear inflammatory cells. The endoneurium exhibited an irregular, scattered structure, with myelinated axons having mostly lost their myelin sheaths, and vacuolar degeneration was observed. Additionally, an increase in the number of

Schwann cells and infiltration by a small number of mononuclear inflammatory cells was detected (Figure 5). On day 21, no histopathological differences were observed between the control group and the TENS-treated groups.

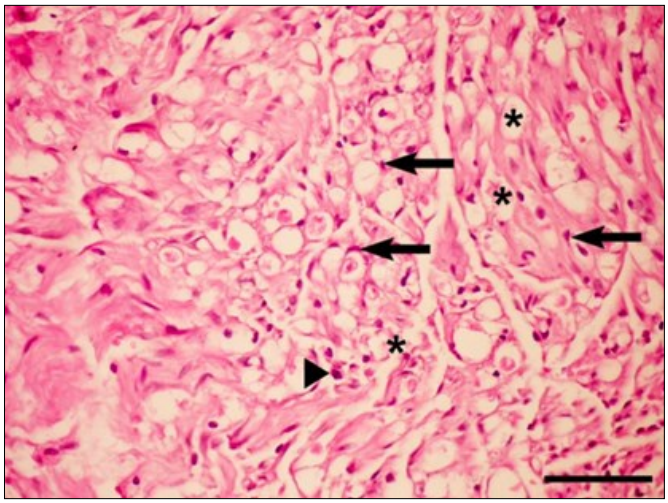

Figure 5. In the experimental group on day 21, an increase in the number of Schwann cells (arrow), mononuclear cells between nerve cells (head of arrow), and degeneration in the myelin sheath and axons (star).

In biopsy samples taken from the distal portion of the sciatic nerve (n.ischiadicus) injury site of control group rats on day 45, there was significant Schwann cell proliferation and the presence of mononuclear inflammatory cell infiltrates. It was observed that the degenerative axonal structures were progressively disappearing. Histopathological examination of gastrocnemius muscle (m.gastrocnemius) samples from the control group on day 45 revealed mild degenerative changes (Figure 6).

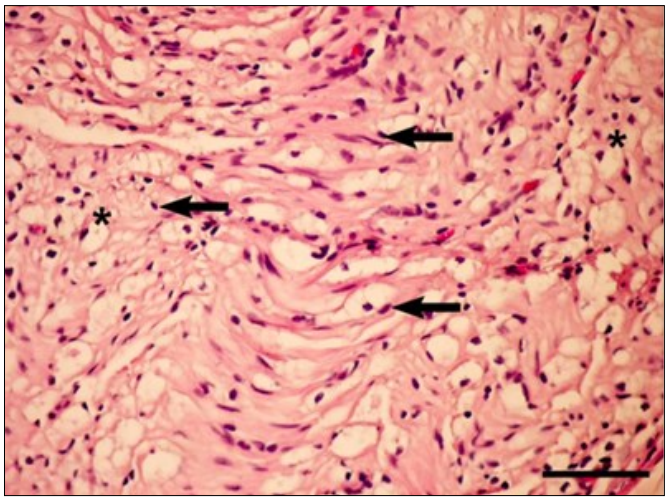

Figure 6. Control group, Day 45, significant increase in the number of Schwann cells (arrows), minimal degeneration in myelin sheath and axons (stars), H&E, Bar = 50 µm.

Similar to the control group, in animals treated with TENS on both the sciatic nerve (n.ischiadicus) and gastrocnemius muscle (m.gastrocnemius), sections taken from the distal portion of the sciatic nerve injury site on Day 45 showed significant axonal and myelin degeneration. However, no Schwann cell proliferation was observed compared to the control group on Day 45 (Figure 7). When histopathological comparisons were made between the control group and the TENS-treated groups on Day 45, it was found that regeneration in the control group was much more pronounced than in the TENS groups. However, no differences were observed between the n.ischiadicus and m.gastrocnemius groups. In the examination of gastrocnemius muscle (m.gastrocnemius) samples from animals treated with TENS on both the sciatic nerve and gastrocnemius muscle on Day 45, atrophic changes in muscle cells were observed, along with hyalinization and activation of connective tissue between muscle fiber.

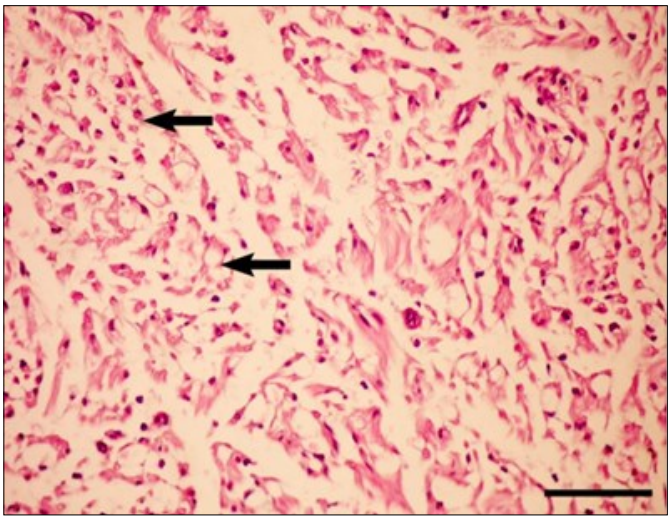

Figure 7. In the experimental group, Day 45, severe degeneration in myelin sheath and axons (arrows), H&E, Bar = 50 µm.

Discussion

The rat sciatic nerve is the most widely used model for examining functional, histological, and electrophysiological changes after peripheral nerve injury, and for evaluating the effectiveness of different surgical and medical treatment approaches (Varejao et al., 2004). The long course of the rat sciatic nerve, its location in the middle femur area that allows easy dissection, makes it indispensable in nerve research. Another reason for the preference of this nerve in experimental models is that it is a mixed, polyfascicular type nerve, containing axons of various sizes and types, providing a comprehensive research opportunity. This allows for the simultaneous evaluation of both sensory and motor functions (Martin et al., 2006).

In peripheral nerve research, the crush injury model is commonly used for axonotmesis damage, particularly with the rat sciatic nerve. The crush injury model is an

ideal model for studying the cellular and molecular mechanisms of peripheral nerve regeneration and for investigating the effects of various factors on the regeneration process. There are several defined techniques for inducing crush injuries in peripheral nerves in experimental models. The primary challenge in these types of studies is the lack of standardization of the injury extent. Even when a fixed pressure is applied, some nerve fibers may remain unaffected and retain their continuity, which can lead to errors, particularly in electrophysiological measurements. To minimize this issue, Luis and colleagues designed a clamp that applies a pressure of 54 newtons in their experimental models (Luis et al., 2007). Varejao et al. demonstrated in their studies that crush injury with this pressure results in complete functional deficit, with normal values returning no earlier than the 7th week (Varejao et al., 2004). In this study, the use of a clamp was employed to induce similar and consistent nerve damage in the majority of the sciatic nerves. The injury was standardized by applying a single-tooth compression with the clamp. Following the compression injury, a complete loss of function was observed in all subjects. During the subsequent follow-up period, the degree of regeneration was found to be consistent within the respective groups.

In sciatic nerve regeneration, gait analysis is one of the most commonly used methods to assess functional recovery. The Sciatic Functional Index (SFI) was defined by De Medinacelli in 1982 and has since been modified by various researchers. The index is based on measurements taken from the paw prints of the animals, which provides insight into the functional recovery of the nerve. This simple, non-invasive technique is the most frequently used evaluation method because it can be repeated at different time points on the same animal, and it assesses coordinated movement, which results from both sensory and motor recovery (Shen & Zhu, 1995). Kanaya et al. proposed that the SFI is the best method for evaluating nerve regeneration, as the final stage of nerve regeneration is stepping. In this study, the modified SFI formula by Bain et al. was used to make measurements (Kanaya et al., 1996).

One of the main disadvantages of SFI measurements is that the results can vary depending on the technique used and the researcher's precision, increasing the risk of error or inaccurate measurements. Before collecting paw prints, animals need to undergo a training period. Another potential source of error is the unwanted development of contractures in the affected limb. Even if reinnervation occurs in the muscles, joint movement loss and contractures can prevent proper stepping. Hare et al. reported in their study that, even after a year following autologous nerve graft repair, there was no significant improvement in the SFI, likely due to contractures (Hare et al., 1992). In the current study, no joint contractures that would interfere with paw print measurements were encountered. However, varying degrees of autophagia were observed in 10 animals, and four animals with severe autophagia were excluded from the study. The incidence of autophagia is closely related to the type of nerve injury, with more frequent occurrences in complete nerve transections and less so in crush injuries (Martins, 2006). Although different degrees of autophagia were observed in the animals, no behavioral changes suggesting hyperalgesia were noted.

When assessing the sequential measurements of each group, no significant differences were found in the sham group, as expected. In contrast, in the other three groups with crush injuries, significant differences were observed between their sequential measurements, supporting nerve regeneration. When comparing groups by weeks, the sham group was significantly different from the other three groups, with values closer to normal. The control group (Group 2) and the TENS-treated groups (Groups 3 and 4) did not show significant differences. Furthermore, no significant difference was observed between the TENS groups, which applied TENS to different areas. These results suggest that TENS treatment, both at the injury site and in the muscles innervated by the damaged nerve, does not significantly influence functional recovery.

Electrophysiological tests are frequently used to evaluate peripheral nerve regeneration. These tests are based on the measurement of action potentials generated by nerve fibers stimulating muscle fibers, which are then amplified and analyzed. The action potentials obtained are compound muscle action potentials (CMAPs), and various parameters can be measured from these potentials for evaluation. Although electrophysiological tests provide information about the axons passing through the nerve repair site, they do not indicate whether these axons are able to make sufficient distal connections. In this study, electrophysiological measurements were taken at baseline, as well as on days 21 and 45 post-surgery, using electromyography (EMG) on all groups of rats. The electrophysiological measurements included the latency, amplitude, and peak-to-peak amplitude of the CMAPs. The latency refers to the time between the stimulus and the beginning of the muscle contraction, and it is considered an important indicator of myelination (Baykal et al., 2002). In a study by Chen et al. in 2007, an increase in latency values was reported following nerve damage. Similarly, this study showed a significant increase in latency values post-injury.

However, no significant difference in latency was observed between the control and experimental groups.

The amplitude and peak-to-peak amplitude of the CMAPs reflect the total depolarization waves of active muscle fibers that can reach the electrode. Therefore, amplitude is directly related to the number of active neurons. Studies by Baykal et al. in 2002 and Wolthers et al. in 2005 demonstrated a significant decrease in amplitude after injury. In accordance with the literature, results of this showed a significant decrease in amplitude following injury in the control group and TENS groups. The peak-to-peak amplitude results also showed a similar change. These EMG measurements demonstrated that there was no change in electrophysiological parameters between rats treated with or without TENS.

Nerve conduction velocity (NCV) is another important parameter that provides information about the condition of nerve fibers. It measures the speed at which a stimulus travels between two electrodes and is widely used to assess the structural integrity of nerve fibers. In 2004, Varejao et al. reported an NCV of approximately 45.6±3.2 m/s for the intact limbs of rats. Similarly, studies by Arnaoutoglou et al. (2006) and Sayyed et al. (2006) reported average NCVs of 48.02±1.92 m/s and 50.39±2.17 m/s for the intact limbs of rats. In our study, the pre-injury average sciatic nerve conduction velocity was measured at 46.22±3.25 m/s for all groups, which is consistent with the literature. In the sham group, the average conduction velocity for the left sciatic nerve was 51.3±2.65 m/s. In the control group, where injury was inflicted on the left sciatic nerve, the NCV dropped to 18.6±1.64 m/s. In the TENS-treated groups (Groups 3 and 4), the NCV decreased to 19.4±1.6 m/s and 18.8±1.7 m/s, respectively. According to Varejao et al., Cragg and Thomas (1964) reported in their long-term follow-up studies after crush injuries that regenerated fibers never fully returned to normal in electrophysiological measurements.

In the light microscopic examination of the sciatic nerve sections obtained from the experimental control group on the 21st and 45th days, which were stained with hematoxylin-eosin, it was observed that although a significant portion of the myelinated nerve fibers maintained their normal structure, there was a notable decrease in the axon diameters and myelin sheath thickness compared to the intact group. In the light microscopic examination of the sciatic nerve sections with TENS application after nerve crush injury, it was striking that the cell debris related to degeneration could not be cleared from the region. A reduction in the number of myelinated axons was present. This situation was interpreted as an indication that nerve healing was delayed.

Transcutaneous Electrical Nerve Stimulation (TENS) is an electrotherapy method that involves placing superficial electrodes on the skin for pain relief (Akyüz, 2001; Bockstahler, Levine, Millis, 2004). The exact effect of peripheral nerves is not fully understood. In this study, we observed that low-frequency TENS application had a negative impact on the histological healing of the nerve. However, this result was not consistent with the functional and electrophysiological recovery of the nerve. In the light microscopic examination of the sciatic nerve sections obtained from the experimental control group on the 21st and 45th days, which were stained with hematoxylin-eosin, it was observed that although a significant portion of the myelinated nerve fibers maintained their normal structure, there was a notable decrease in the axon diameters and myelin sheath thickness compared to the intact group. In the light microscopic examination of the sciatic nerve sections with TENS application after nerve crush injury, it was striking that the cell debris related to degeneration could not be cleared from the region. A reduction in the number of myelinated axons was present. This situation was interpreted as an indication that nerve healing was delayed.

In conclusion, as the use of TENS becomes more widespread in veterinary medicine, we recommend that it be applied only after the type and localization of the existing neurological damage have been accurately determined to avoid potentially adverse effects on nerve healing.

Acknowledgments

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