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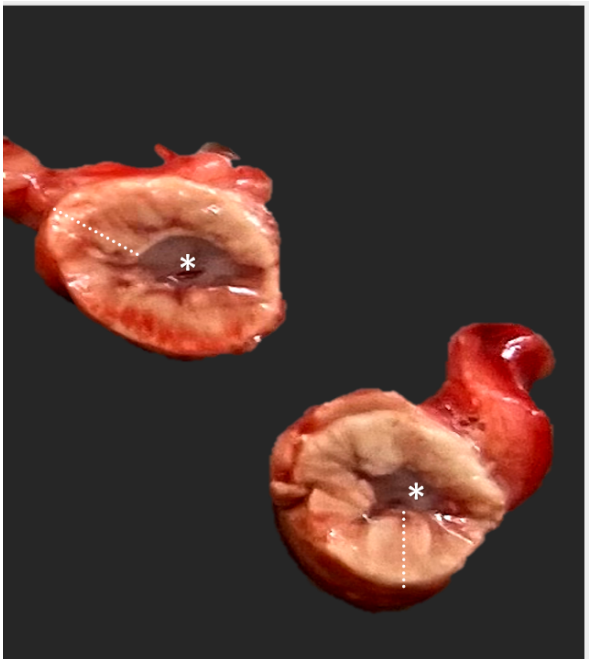

PHOTOS: *ECATERINA SEMZENISI*

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Article

Epidemiology of gastrointestinal proliferative neoplastic-like lesions and tumors in dogs and cats: a retrospective study in two Romanian reference laboratories

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Abstract: Gastrointestinal neoplasms and tumor-like lesions are rare in dogs and cats.. In the current literature, few prospective and retrospective epidemiological studies are described. The aim of these study was to identify epidemiological as well as pathological features of the gastrointestinal lesions in dogs and cats. Epidemiological data were collected from the databases of two laboratories in Romania, covering a period of 10 years. A total of 192 cases of neoplastic and neoplastic-like lesions were selected and subjected to statistical analysis. Older animals were more predisposed for chronic hypertrophic pyloric gastropathy (CHPG) and gastric polyps, while younger individuals showed a higher incidence for feline gastrointestinal eosinophilic sclerosing fibroplasia (FGIESF). Additionally, CHPG and FGIESF were more prevalent in males. Dogs were the main species affected by benign neoplasms, including adenomas (22%) and adenomatous polyps/pedunculated adenomas (5%) mainly located in the anorectal junction and leiomyomas (8%) with gastric involvement. In dogs, malignant neoplasms accounted for 69%, with adenocarcinomas representing 39%, followed by lymphomas (12%), and GIST (9%). In cats, 98.82% of neoplasms were represented by malignant tumors, with lymphomas being the most frequently diagnosed (77%). Both adenocarcinomas in dogs and lymphomas in cats were more common in males, accounting for 61.76% and 52.31% of cases, respectively, with the intestine being the most frequently affected site. This is the first epidemiological study of gastrointestinal tumors in dogs and cats in Romania..

Keywords: cat; dog; lymphoma; epidemiology; gastrointestinal.

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1. Introduction

The Gastrointestinal neoplasms in dogs and cats account for less than 1% of all neoplastic lesions observed in these species [1-3]. There is a marked variation in the types of tumors that develop in the gastrointestinal tract of dogs compared to cats. In dogs, the most significant neoplasms include adenocarcinomas, lymphomas, gastrointestinal stromal tumors (GISTs), leiomyomas, leiomyosarcomas, and adenomas. Conversely, in cats, lymphomas predominate, followed by adenocarcinomas, adenoma and mesenchymal neoplasms [1-4].

In dogs, gastrointestinal epithelial tumors account for approximately 0.16% to 0.33% of all neoplastic lesions observed in this species [1, 5]. Intestinal adenocarcinomas are more commonly found in the large intestine [1] and predominantly affect male dogs of large breeds [1,5]. Additionally, in recent years, a heterozygous germline mutation similar to that described in humans with familial adenomatous polyposis (FAP) has been identified in Jack Russell Terriers with gastrointestinal adenomas and adenocarcinomas [4,6]. FAP is characterized by the presence of multiple polypoid growths in the colon and rectum of young individuals, which progressively undergo neoplastic transformation with age, occurring in nearly 100% of cases [7,8]. Another potential mechanism implicated in the development of gastrointestinal neoplastic lesions is ”de novo” carcinogenesis, characterized by flat to dome-shaped neoplasms with high malignant potential [9,10].

In cats, the most prevalent gastrointestinal neoplasm is lymphoma, followed by adenocarcinomas and other mesenchymal neoplasms [11]. According to the Veterinary World Health Organization (WHO), alimentary lymphoma can be classified based on the size of the neoplastic cells and their immunophenotype into the following categories: extranodal B-cell lymphoma of the mucosa-associated lymphatic tissue (MALT), small cell intestinal T-cell lymphoma characterized, large granular lymphocyte (LGL) lymphoma with a T-cell phenotype, and multicentric lymphomas that do not involve the gastrointestinal tract [12,13].

Chronic hypertrophic pyloric gastropathy (CHPG) is associated with pyloric obstruction, and results from hypertrophy of the pyloric muscular layer, hyperplasia of the pyloric mucosa, or a combination of both [14]. In human medicine, this pathology is commonly linked to Helicobacter pylori colonization, although its exact etiology remains unclear [15]. Other potential causes include stress, neuroendocrine factors, and excessive mucosal growth [16].

Feline gastrointestinal eosinophilic sclerosing fibroplasia (FGIESF), a rare condition in cats, characterized by masses typically located in the pylorus and ileocecal junction. These masses is composed of collagen trabeculae interspersed with fibroblasts, macrophages, and a large number of eosinophils [17-19]. To differentiate FGIESF from other pathologies, such as neoplasms, immunohistochemistry with the antibody for transforming growth factor β1 (TGF-β1) can be used as a potential diagnostic marker [20].

All of these conditions present similar clinical signs, including progressive weight loss, diarrhea, vomiting, and varying degrees of appetite changes. Diagnosis is often delayed because the clinical presentation mimics chronic gastritis, with suspicions of neoplasia arising only after a lack of response to treatment for inflammatory conditions.

This study represents the first epidemiological investigation of neoplastic and neoplastic-like lesions in the gastrointestinal tract of dogs and cats in Romania.

2. Materials and Methods

In this study, data were retrospectively analyzed over a 10-year period (2012–2022) from two reference laboratories in Romania: the Department of Anatomic Pathology, Faculty of Veterinary Medicine, Cluj-Napoca, and the Department of Veterinary Pathology, Synevovet laboratories from Bucharest. The selected samples included neoplastic and neoplastic-like lesions from both the stomach and intestine of cats and dogs. The databases compiled detailed information for each case, including the animals' age, breed, sex, and histological diagnosis. Additionally, further parameters were evaluated for each case, such as the histopathological type of the neoplasm, the specific layers of the gastric or intestinal wall affected, and the presence of metastases in regional lymph nodes or other organs.

3. Results

A total of 192 cases were analyzed in this study, with the following distribution: 35 gastric samples from dogs and 26 from cats, while intestinal samples consisted of 65 from dogs and 66 from cats.

3.1. Epidemiology of the gastrointestinal neoplastic-like lesions in dogs and cats

Among the evaluated samples, three primary proliferative neoplastic-like lesions were identified in the gastrointestinal tract of dogs and cats: chronic hypertrophic pyloric gastropathy (CHPG), feline gastrointestinal eosinophilic sclerosing fibroplasia (FGIESF), and gastric polyps (GPs). CHPG and gastric polyps were exclusively observed in the stomachs of dogs, while FGIESF was identified in both the stomach and intestines of cats.

Age differences were significant among the lesions. CHPG and GPs primarily affected older animals, with mean ages of 11.14 years and 9.6 years, respectively. In contrast, FGIESF was more frequently identified in younger cats, with mean ages of 7.25 years for gastric lesions and 5.25 years for intestinal location. Males demonstrated a higher predisposition for CHPG (5/7 cases) and FGIESF (stomach: 2/2 cases; intestine: 2/4 cases). No breed predisposition was observed for CHPG or FGIESF, regardless of gastric or intestinal localization.

Topographically, CHPG lesions were consistently localized to the pyloric region of the stomach. For FGIESF, the gastric localization of lesions was not specified in either of the two cases, but intestinal FGIESF lesions were predominantly located in the duodenum (3/4 cases) and at the ileocecal valve (1/4 cases). In contrast, GPs exhibited no consistent topographical distribution.

A notable distinction among the three conditions was the affected layer of the gastrointestinal wall. CHPG and GPs were confined to the mucosal layer, whereas FGIESF demonstrated transmural involvement, affecting all layers of the gastric and intestinal walls. Histologically, all GPs in both dogs and cats were classified as hyperplastic. Additionally, colonization with *Helicobacter* spp. was observed in two cases of CHPG.

3.2. Epidemiology of gastrointestinal tumors in dogs and cats.

In dogs, malignant neoplastic lesions accounted for 69% of all tumors, with adenocarcinomas representing the most prevalent type (39%). The distribution of all neoplasms in dogs is illustrated in Figure 1.

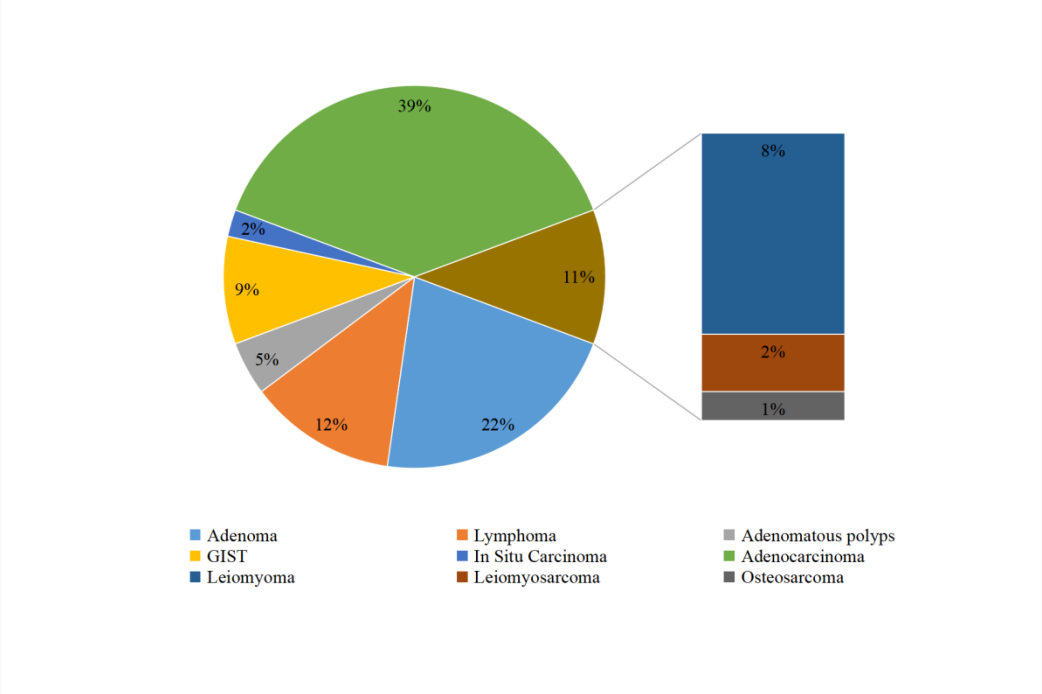

Figure 1. The distribution of neoplastic lesions within the gastrointestinal tract of dogs.

In contrast, within the cohort of cats, malignant neoplasms constituted 98.82% of all cases, a significantly higher proportion compared to dogs. A detailed distribution of gastrointestinal tumors in cats is provided in Figure 2.

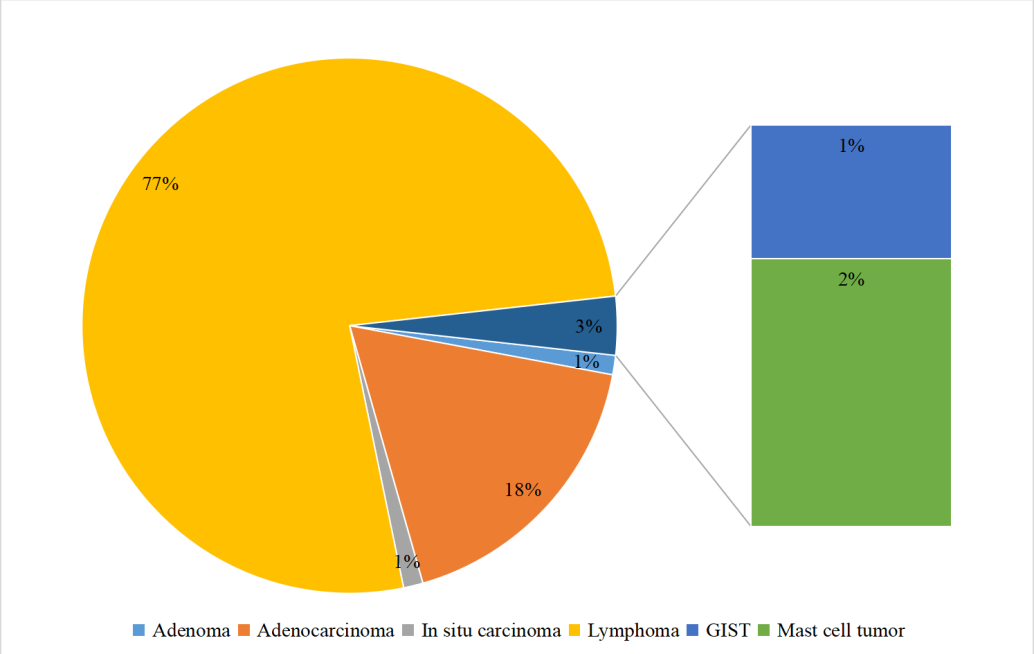

Figure 2. The distribution of neoplastic lesions within the gastrointestinal tract of cats.

3.2.1 Benign tumors of the gastrointestinal tract in dogs and cats

Adenomas were among the most significant benign neoplastic lesions in the gastrointestinal tract of dogs, accounting for 22% of all tumors. The mean age at diagnosis was 6.44 years, with a higher prevalence in males (52.63%). The affected dog breeds included French Bulldogs (15.79%), Golden Retrievers (15.79%), Shih Tzus (15.79%), along with two cases in mixed-breed dogs. Other breeds represented by a single case each included American Akita, Rottweiler, American Staffordshire Terrier, Beagle, Cocker Spaniel, Labrador Retriever, Samoyed, and Yorkshire Terrier.

Histologically, adenomas were classified into tubular (n=3), papillary (n=7), adenomas with areas of in situ carcinoma (n=9), and unspecified types (n=5). All adenomas were localized in the rectum/ anorectal junction and confined to the mucosal layer. In cats, only one case of tubulopapillary adenoma was identified in a 9-year-old male. The specific location of the tumor was not reported.

Four cases of adenomatous polyps/ pedunculated adenomas were identified in the intestines of dogs. These benign neoplasms occurred in younger animals, with a mean age of 5.1 years (range: 4 months to 8 years). No breed predisposition was observed, and all cases occurred in males. Three lesions were located in the rectum, and one was present in the duodenum.

Leiomyomas (n=7) were the only other benign neoplasms identified in dogs. The mean age at diagnosis was 14 years, with females comprising 57.14% of cases. No breed predisposition was noted for leiomyomas. Most gastric leiomyomas were localized in the cardia region (3/6 cases), with one case in the gastric body and two cases without specified localization. A single intestinal leiomyoma was identified in the colon.

3.2.2 Malignant tumors of the gastrointestinal tract in dogs and cats

3.2.2.1 Epithelial neoplasms

In dogs, adenocarcinomas were the most significant neoplasm of the gastrointestinal tract (n=34). The mean age of affected dogs was 9 years, with an age range of 4 to 16 years, and males were more frequently affected, accounting for 61.76% of cases. In terms of breed predisposition, 16% of cases occurred in mixed-breed dogs, followed by Chow Chows (15%), French Bulldogs (9%), and American Staffordshire Terriers (9%). The distribution of other affected breeds is detailed in Figure 3.

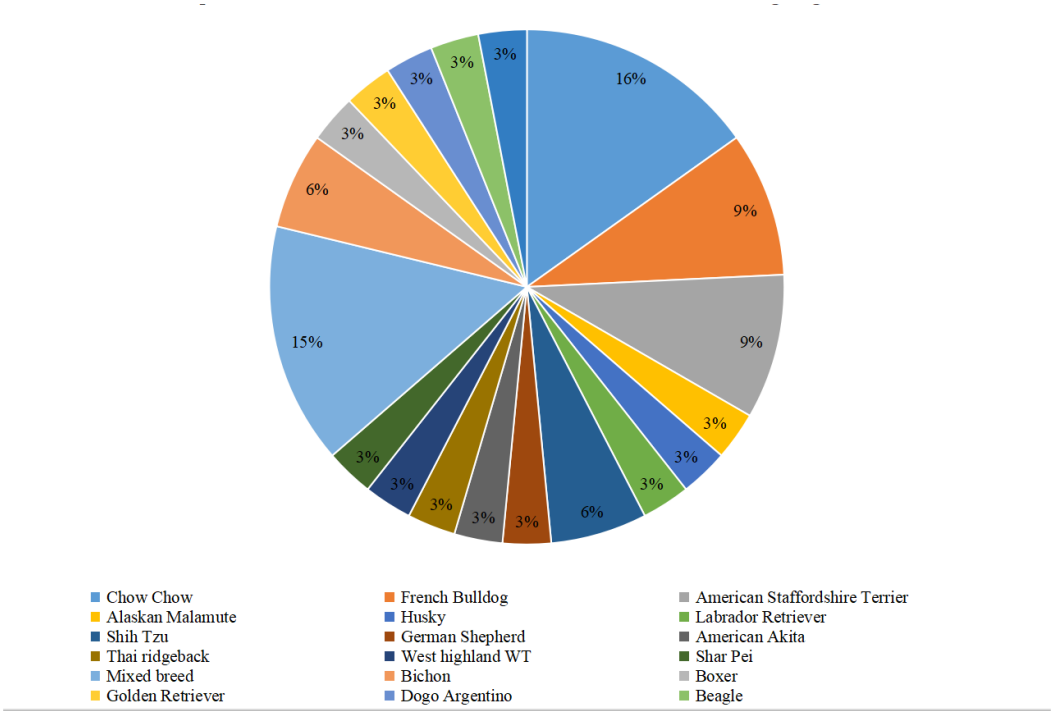

Figure 3. Proportional distribution of gastrointestinal adenocarcinoma among dog breeds

In dogs, 64.70% of adenocarcinomas were located in the intestines, while 35.29% were found in the stomach. Within the intestines, nine cases were located in the rectum, seven in the colon, and the remainder in the small intestine. In the stomach, only one case was identified in the pyloric region, with the exact location unspecified for the other cases. Histologically, adenocarcinomas in dogs were classified as tubular (4/34 cases), signet ring cell (4/34 cases), mucinous (3/34 cases), papillary, tubulopapillary, and poorly differentiated (2 cases each). Three cases were classified as mixed types, while the histological subtype was unspecified in 14 cases. Approximately 85% of the tumors were infiltrative and transmural. Metastasis to regional lymph nodes was reported in two cases, and vascular emboli were noted in seven cases. In three gastric tumors, *Helicobacter* spp. colonization was observed.

In contrast to dogs, adenocarcinomas accounted for only 18% of gastrointestinal neoplasms in cats. The mean age of affected cats was 12.73 years (range: 3 to 17 years). Unlike dogs, females were predominantly affected, comprising 73.33% of cases, with more than half of the females (n=7) being spayed (Table 1). Similarly, 3 out of 4 affected males were neutered. Approximately 73% of the cats were mixed-breed, with the remaining cases observed in British Shorthairs (n=2), Siamese (n=1), and Maine Coon (n=1).

Similar to dogs, topographically, the majority of feline adenocarcinomas (86.66%) were located in the intestines, with only two cases involving the stomach. A detailed distribution of adenocarcinomas in cats is provided in Table 2.

Table 1. Gender-based distribution of gastrointestinal neoplastic and neoplastic-like in canine and feline populations.

		Gender	Hormonal status	CHPG	FGIESF	Polyp	Adenoma	In situ carci-noma	Adenocarci-noma	Carci-noma	Leiomy-oma	Leiomyosar-coma	GIST	OSA	Lym-phoma	Mast cell tumor	Total
Dogs	Stomach	F	Spayed	1	-	1	-	-	1	0	0	-	-	-	1	-	4
			Intact	1	-	2	-	-	1	2	4	-	-	-	2	-	12
		M	Castrated	1	-	1	-	-	1	1	0	-	-	-	1	-	5
			Intact	4	-	1	-	-	5	1	2	-	-	-	1	-	14
	Intestine	F	Spayed	-	-	0	2	1	6	-	0	1	1	0	1	-	12
			Intact	-	-	0	7	1	3	-	0	0	3	0	1	-	15
		M	Castrated	-	-	0	2	0	1	-	0	1	1	0	1	-	6
			Intact	-	-	4	8	0	12	-	1	0	3	1	3	-	31
Cats	Stomach	F	Spayed	-	0	1	0	0	0	-	-	-	-	-	2	-	3
			Intact	-	0	0	0	0	2	-	-	-	-	-	4	-	6
		M	Castrated	-	2	0	1	1	0	-	-	-	-	-	7	-	11
			Intact	-	0	0	0	0	0	-	-	-	-	-	6	-	6
	Intestine	F	Spayed		1	-	-	-	7	-	-	-	1	-	15	1	25
			Intact		0	-	-	-	2	-	-	-	0	-	10	0	12
		M	Castrated		1	-	-	-	3	-	-	-	0	-	11	0	15
			Intact		1	-	-	-	1	-	-	-	0	-	10	1	13

M - male
F - female

Table 2. Epidemiological distribution of neoplastic and neoplastic-like lesions in the gastrointestinal tract of dogs and cats

		Location	CHPG	FGIESF	Polyp	Adenoma	In situ carcinoma	Adenocarcinoma	Carcinoma	Leiomyoma	Leiomyosarcoma	GIST	OSA	Lymphoma	Mast cell tumor
Dogs	Stomach	Cardia	0	-	0	-	-	0	-	3	-	-	-	0	-
		Gastric body	0	-	0	-	-	0	-	0	-	-	-	2	-
		Pylor	7	-	0	-	-	0	1	0	-	-	-	0	-
		Not mentioned	0	-	5	-	-	8	3	2	-	-	-	3	-
	Intestine	Small intestine	-	-	1	0	0	5	-	0	1	4	0	3	-
		Duoden	-	-	0	0	0	0	-	0	0	2	0	1	-
		Jejun	-	-	0	0	0	1	-	0	0	1	1	1	-
		Ileon	-	-	0	0	0	0	-	0	0	0	0	1	-
		Ileocecal	-	-	0	0	0	0	-	0	1	1	0	0	-
		Colon	-	-	0	0	0	7	-	0	0	0	0	0	-
		Rectum	-	-	3	19	2	9	-	0	0	0	0	0	-
		Large intestine	-	-	0	0	0	0	-	1	0	0	0	0	-
Cats	Stomach	Cardia	-	0	0	0	0	0	-	0	0	0	0	0	0
		Gastric body	-	0	0	0	0	0	-	-	-	-	-	5	-
		Pylor	-	0	0	0	0	0	-	-	-	-	-	1	-
		Not mentioned	-	2	1	1	1	2	-	-	-	-	-	13	-
	Intestine	Small intestine	-	0	-	-	-	7	-	-	-	0		22	0
		Duoden	-	3	-	-	-	0	-	-	-	1		5	0
		Jejun	-	0	-	-	-	1	-	-	-	0		17	0
		Ileon	-	0	-	-		1	-	-	-	0		2	0
		Ileocecal	-	1	-	-	-	1	-	-	-	0		2	0
		Colon	-	0	-	-	-	2	-	-	-	0		0	2
		Rectum	-	0	-	-	-	1	-	-	-	0		0	0

Histologically, most feline adenocarcinomas (n=6) were classified as tubular, followed by tubulopapillary and mucinous types (2 cases each), and one case was classified as undifferentiated. In four cases, the adenocarcinoma subtype was not specified. All tumors in cats exhibited transmural invasion. Metastasis to regional lymph nodes was reported in approximately 46% of cases, and carcinomatosis was identified in six cases.

3.2.2.2 In situ carcinoma

In the cohort, only two cases of in situ carcinoma were identified in dogs and one in a cat.

In dogs, the in situ carcinomas were diagnosed in two females: a 7-year-old Beagle and an 11-year-old mixed-breed dog. Both lesions were localized in the rectum. In the cat, the in situ carcinoma was observed in a 4-year-old neutered male British Shorthair, with the neoplasm located in the stomach.

3.2.2.3 Lymphomas

Lymphomas were the most prevalent tumors in cats, accounting for approximately 77% of all gastrointestinal neoplasms. The affected cats had a mean age of 10.16 years, with an age range from 2 to 24 years. Males comprised 52.31% of the cases. Additionally, more than 50% of the males were neutered, and 54.48% of the females were spayed. Mixed-breed cats were predominantly affected, accounting for 85% of the cases. The distribution of other breeds is presented in Figure 4.

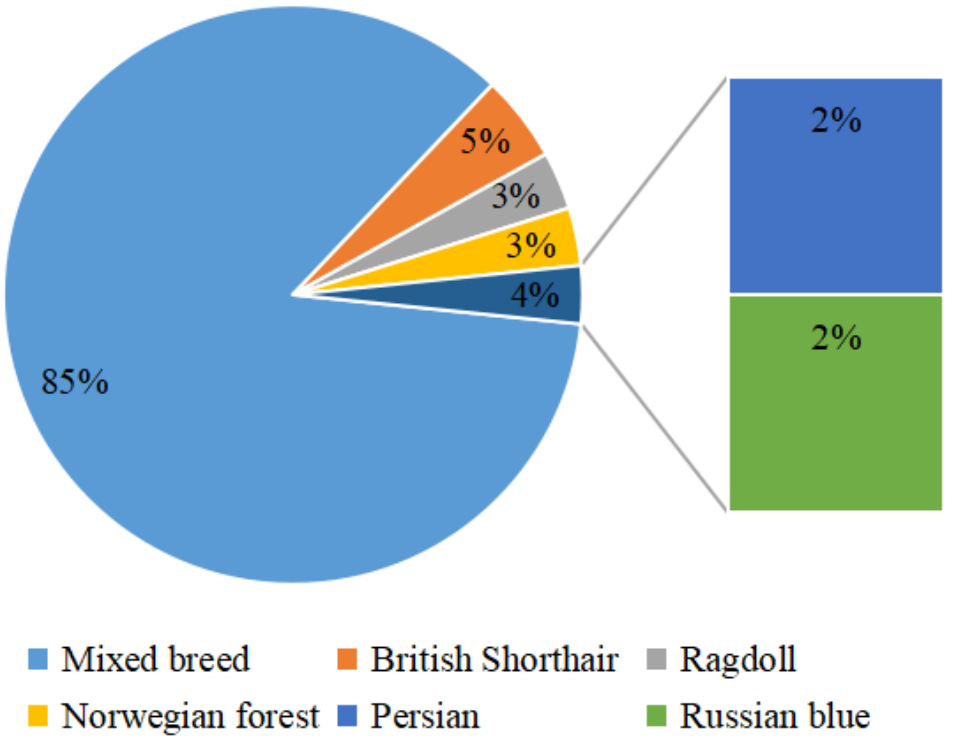

Figure 4. Proportional distribution of gastrointestinal adenocarcinoma among cat breeds

Approximately 70% of lymphomas in cats were located in the intestine, with the remaining cases found in the stomach. Within the intestine, the distribution was as follows: 6 cases in the duodenum, 18 in the jejunum, and three in the ileum, while 29 cases lacked specification of the exact intestinal segment. Additional locations included three cases in the ileocecal valve, four in the colon, and one in the rectum. The gastric location of lymphomas was specified in six cases, with the gastric body being the most commonly affected site (five cases).

Regarding the size of the neoplastic lymphocytes, a notable difference was observed between the stomach and intestinal locations. In the stomach, three of the 19 lymphoma cases were classified as large-cell, two as small-cell, and the remaining four had unspecified cell morphology. In contrast, within the intestine,

small-cell lymphoma was the most common (n=23), followed by medium-cell lymphoma (n=2), large-cell lymphoma (n=14), and seven cases with unspecified cell morphology.

Most of the lymphomas (78.46%) were transmural, and metastasis was identified in regional lymph nodes (n=15), the pancreas (n=3), liver (n=2), kidneys (n=1), and lungs (n=1).

In dogs, lymphomas accounted for only 12% of gastrointestinal neoplasms. The mean age of affected dogs was 7.72 years (range: 3 to 13 years), with a higher prevalence in males (72.72%). No breed predisposition was identified, with cases observed in Bichon Frises (n=2), French Bulldogs (n=2), mixed-breed dogs (n=2), and one case each in Rottweilers, Alaskan Malamutes, Shih Tzus, and Beagles.

Similar to cats, lymphomas in dogs primarily developed in the intestine (54.54%), specifically affecting the small intestine in all cases. Compared to cats, large-cell lymphoma (n=5) was the only subtype of lymphoma identified in the gastrointestinal tract of dogs, affecting both the stomach and intestine. In the remaining six cases, subtype information was not provided. Most of the lymphomas (88.88%) were transmural, with metastasis noted in regional lymph nodes (n=4).

3.2.2.4 Mast cell tumor

In our cohort, mast cell tumors were observed in two mixed-breed cats, a 9-year-old male and a 10-year-old female, both located in the colon. One neoplasm was confined to the mucosal layer, while the other was transmural and exhibited metastasis to the regional lymph nodes.

3.2.2.5 Mesenchymal tumors

In dogs, a total of 11 mesenchymal neoplasms were identified, including gastrointestinal stromal tumors (GISTs) (n=8), leiomyosarcomas (n=2), and one osteosarcoma (OSA).

For GISTs, the mean age at diagnosis was 9.75 years, with a range of 2 to 14 years. Both males and females were equally affected, and no breed predisposition was observed. All GISTs cases were located in the intestine, predominantly in the small intestine (87.5%), with one case found at the ileocecal junction. In all instances, the neoplasms were transmural, and one case exhibited invasion into the mesentery.

In cats, a single case of GIST was reported in an 11-year-old neutered British Shorthair, with the tumor located in the duodenum. Similar to dogs, the neoplasm was transmural, affecting the entire wall of the intestine.

Leiomyosarcomas and osteosarcoma were identified exclusively in dogs. Like GISTs, leiomyosarcomas were observed in older dogs (ages 10 and 11 years), with one male and one female affected. One lesion was located at the ileocecal junction, while the location of the other intestinal tumor was not provided. Both tumors were transmural.

The osteosarcoma was identified in an 8-year-old male Greyhound, with the neoplasm located in the jejunum. Consistent with the other mesenchymal tumors, this lesion was also transmural.

4. Discussion

In this study, a total of 192 neoplastic and neoplastic-like lesions in dogs and cats were retrospectively analyzed based on routine pathology reports.

Feline gastrointestinal eosinophilic sclerosing fibroplasia (FGIESF) has previously been described in cats, with a mean age range between 5.25 and 7 years, and an age range spanning from 2 to 11 years [18,19]. Our findings align with these reports, revealing a mean age of 7.25 years for gastric FGIESF and 5.25 years for intestinal FGIESF. When considering both gastric and intestinal locations together, the average age of development was 6.25 years. Additionally, male cats were more predisposed to developing this condition, with 4 out of 6 affected, which is consistent with the literature [18,19]. In this retrospective study, no breed predisposition was noted, with most cases reported in mixed-breed cats, while only one case each of Persian and Ragdoll cats were observed. Notably, the literature suggests a predisposition for Ragdoll cats [18,19]. However, the limited sample size of 6 cases in this study warrants caution in drawing definitive conclusions, given the rarity of this lesion. Another difference observed in our study compared to the literature is the localization of FGIESF. While the literature often reports the ileocecal region as the most common site [18], our study found that the duodenum was the primary location (3/4 cases), followed by the ileocecal junction (1/4 cases). A recent study of 60 cases also reported a higher incidence of lesions in the intestine compared to the ileocecal junction, with gastric involvement being less frequent [19]. In line with previous reports, the neoplastic-like masses in this study were typically transmural, affecting all layers of the gastric and intestinal

walls [21]. Further studies, including genetic assessments, are recommended to investigate potential breed predispositions for this condition.

Regarding chronic hypertrophic pyloric gastropathy (CHPG), the adult form is more commonly diagnosed in middle-aged to older dogs, particularly male brachycephalic breeds and small breeds such as Shih Tzus, Pekingese, Maltese, and Lhasa Apsos [22-24]. In our study, we did not observe a specific breed predisposition; however, out of seven cases, one Bichon, one French Bulldog, one Pekingese, and one Shih Tzu were identified. Males represented the majority of cases, accounting for 71.42%. The mean age of affected dogs was 11.14 years, consistent with existing literature [24]. All cases of CHPG were mucosa-associated.

Regarding gastric polyps, no significant sex or breed predisposition was observed. The exact location of the lesions within the stomach was not consistently reported, which may account for the lack of a defined location pattern. However, the literature suggests that gastric polyps most commonly occur in the antrum region, where polypoid growths are frequently observed [25]. Histologically, gastric polyps are classified as either hyperplastic or inflammatory types according to the World Health Organization, with hyperplastic polyps being more prevalent [13]. Our study also found that all gastric polyps were hyperplastic. The development of gastric polyps is believed to be associated with *Helicobacter* spp. [25], and in our study, *Helicobacter* spp. colonization was identified in two cases.

In terms of benign neoplastic lesions, a notable difference was observed between the types of benign neoplasms found in the stomach and intestines. Leiomyomas were the only benign neoplasms identified in the stomach, comprising approximately 26.08% of all gastric neoplasms in dogs. This finding is consistent with the literature, which reports leiomyomas accounting for approximately 19% of gastric neoplasms [12]. Leiomyomas in dogs are typically diagnosed in older animals, with no significant sex predisposition. Some reports suggest a higher predisposition in small terrier breeds [26-29]. In our study, no breed predisposition was identified, with affected dogs having a mean age of 14 years. Interestingly, in our study, a higher prevalence of leiomyomas was observed in female dogs (57,14%), which contrasts with other reports. Most of the leiomyomas were located in the cardia region (50%). This finding is in accordance with both human and veterinary studies, which commonly report the development of leiomyomas in the gastroesophageal junction, cardia, fundus, and pylorus [27, 30-32].

A notable difference was observed between benign neoplasms in the stomach and those in the large intestine. The benign neoplasms identified in the large intestine were predominantly adenomas (79.16%) and adenomatous polyps (16.66%). French Bulldogs (15.79%), Golden Retrievers (15.79%), Shih Tzus (15.79%) were the main breeds in which this lesion developed. In contrast, a recent study found that Jack Russell Terriers and Miniature Dachshunds were overrepresented and predisposed to these types of lesions [4]. However, in our study, neither of these breeds presented with benign neoplasms in the large intestine. Regarding tumor localization, all adenomas were located in the rectal region. This finding aligns with the literature, where most adenomas are reported to be located in the rectum [4]. Histologically, the adenomas were predominantly classified as papillary (n=7), followed by tubular, with the remaining cases lacking specific classification. Interestingly, areas of in situ carcinoma were identified in 9 of the adenomas, suggesting a potential for malignant transformation. In human medicine, both adenomas and adenomatous polyps are known to undergo malignant transformation through a mechanism referred to as the "adenoma-carcinoma sequence" [33]. Other studies have indicated that adenomas may develop into carcinomas due to mutations in tumor suppressor genes and oncogenes [34]. A recent study by Saito (2018) reported that of 52 dogs diagnosed with inflammatory polyps, all were Miniature Dachshunds, and 14% developed neoplastic lesions after treatment, suggesting that inflammatory polyps may represent potential preneoplastic lesions [35].

The most common neoplastic lesions in the gastrointestinal tract of dogs were adenocarcinomas, which accounted for 38,64% of all neoplasms and 54.23% of malignant gastrointestinal tumors. These results align with the existing literature [1,4]. Specific breeds, such as Belgian Shepherds, Groenendaels, Tervuerens, Chow Chows, and Norwegian Elkhounds, have been found to be more predisposed to developing these tumors [5,36-38]. In our study, Chow Chows were overrepresented (15%), followed by French Bulldogs (9%) and American Staffordshire Terriers (9%). However, a recent study identified a predisposition for Jack Russell Terriers and Miniature Dachshunds to develop these types of tumors [4]. Gastric adenocarcinomas typically develop in the pyloric region and lesser curvature [18-21], while intestinal adenocarcinomas predominantly affect the large intestine, particularly the rectum, with less frequent involvement of the small intestine [4]. In our study, the exact location of the 12 gastric adenocarcinomas was not specified, but in the case of the intestines, most adenocarcinomas were located in the large intestine (72.72%), specifically in the rectum, which is consistent with the literature.

In the current literature, the histopathological pattern of gastrointestinal adenocarcinomas is not considered to have significant relevance for prognosis [12]. According to the WHO classification of gastrointestinal tumors [13], gastric adenocarcinomas are classified as tubular, papillary, tubulopapillary, mucinous, signet-ring cell, squamous, and undifferentiated, while intestinal adenocarcinomas are classified as acinar, papillary, mucinous, signet-ring cell, adenosquamous, and undifferentiated. In our study, we did not identify any squamous or acinar adenocarcinomas, although these types have been previously reported [4,39]. A more recent study highlighted the importance of tumor growth patterns, specifically polypoid versus non-polypoid, showing that all non-polypoid tumors presented with invasion and/or metastasis, while only 12% of polypoid tumors exhibited these characteristics [4]. Interestingly, 90.9% of the adenocarcinomas in our cohort invaded all layers of the intestinal wall, with a lower frequency of transmural invasion in the stomach (58.33%).

Lymphomas and mesenchymal neoplasms were equally represented in our cohort, with 11 cases of each identified in the gastrointestinal tract of dogs. Lymphomas were almost equally distributed between the stomach (n=5) and intestine (n=6), which contrasts with the literature, where lymphomas are predominantly reported in the intestine, with less frequent involvement of the stomach [40].

All mesenchymal neoplasms in our study were localized in the intestine. In contrast to the literature, we did not identify any breed predisposition for lymphoma, although breeds such as Boxers, Shar-Pei, and more recently, Shiba Inus, have been reported to be overrepresented in lymphoma cases [41-43]. Previous studies have noted that most lymphomas affecting the gastrointestinal tract of dogs are large cell lymphomas, with a lesser incidence of small cell lymphomas [44-47]. In our study, no small cell lymphomas were noted, with the remaining cases being classified as large cell lymphomas (n=5). However, it is important to note that the cell size was not specified in 6 cases. An interesting observation was that 8 out of the 11 lymphoma cases were transmural, which aligns with findings in the literature [48].

An important aspect regarding the phenotype of gastrointestinal (GI) lymphomas in dogs is that T-cell lymphomas are the predominant subtype in both the stomach and intestine. Additionally, most lymphomas in dogs are low-grade, which generally results in a favorable prognosis, especially when chemotherapy is involved [40]. Unfortunately, phenotypic information regarding the neoplasms in this study was not available. This limitation is primarily due to the fact that immunohistochemistry is not routinely performed, as it implies additional costs for the owners, and in many cases, owners opt not to pursue these investigations, further limiting our study.

In terms of mesenchymal neoplasms in the intestine, three malignant cases were identified: gastrointestinal stromal tumors (GISTs) (66.66%), leiomyosarcomas (16.66%), and one osteosarcoma. These findings are consistent with those reported in the literature. Historically, GISTs were often misdiagnosed as leiomyomas or leiomyosarcomas (66-85%) (49,50), a situation that changed with the introduction of immunohistochemistry using markers such as KIT or DOG1, which revealed a higher incidence of GISTs in the GI tract of both humans and animals [51,52]. Our study did not identify any breed or gender predisposition for GISTs, which aligns with existing reports [53,54].

GISTs in dogs most commonly arise in the cecum and small intestine, with a lower frequency in the stomach [53,54]. In contrast, our study found that 87.5% of GISTs were located in the small intestine, with one case at the ileocecal junction. This result may be less precise due to the small sample size in our cohort. An important observation was that all GISTs in our study were transmural, with one case exhibiting mesenteric invasion. Metastasis to the mesentery, serosa, lymph nodes, liver, and spleen has also been reported in dogs [54]. Interestingly, in humans, two significant prognostic factors for GISTs are tumor diameter and mitotic count. Tumors larger than 5 cm and those with more than 50 mitoses per 50 high-power fields (HPF) are more likely to metastasize [55]. However, these parameters are not as important in canine GISTs [56].

For leiomyosarcomas and osteosarcomas, the findings in our study are consistent with previous reports [49,57-59].

Regarding cats, no breed predisposition for lymphoma was observed, with approximately 80% of cats being mixed breed, which is in agreement with a recent study [60]. However, a study conducted in the USA found Siamese cats to be overrepresented [3,11]. In our cohort, only one Siamese cat was included. The highest proportion of neoplastic lesions in cats was found in lymphomas, which accounted for 47% in the stomach and 74.19% in the intestine. These proportions are consistent with previous studies, where the incidence ranged from 41% to 79% [11, 60-62].

Almost all lymphoma cases involved the intestine (n=48), with only 2 cases located at the ileocecal junction and 19 cases in the stomach, which is consistent with prior studies [60]. A notable difference was observed in the lymphocyte size distribution between the intestine and stomach; in the intestine, most lymphomas were classified as small cell (n=23), while the majority of lymphomas in the stomach (n=13) were large cell lymphomas. No phenotypic characterization was performed on the lymphoma samples due to the lack of owner requests and funding limitations for additional investigations such as immunohistochemistry.

In terms of benign neoplasms in cats, a small number were observed: one stromal tumor and two mast cell tumors, and the mean age at diagnosis for neoplastic lesions in cats was over 10 years, in agreement with previous studies [3,11,60-62]. Another notable finding was that approximately 70% of the lymphomas were transmural. A reduced proportion of adenocarcinomas was observed, with 9% in the stomach and 21% in the intestine, which is consistent with current literature [60,61]. The locations of these adenocarcinomas were primarily in the small intestine (66.66%), followed by the large intestine (20%) and stomach (13.33%). These results align with studies showing that the small intestine is the primary site for the development of adenocarcinomas [3,12,63]. However, a more recent study published in 2022 on 860 cats found that the highest incidence of adenocarcinomas occurred in the large intestine (58.1%), with a lower incidence in the small intestine (32%) and stomach (2.5%).

Another notable difference in our study was the higher representation of females, who accounted for 86,66% of cases, whereas in other studies, males were more commonly affected, with over 70% of cases [60,64]. In our cohort, tubular adenocarcinoma was the most common histological type, accounting for approximately 40%, which is consistent with other studies [3,60,64]. Interestingly, some studies have also reported acinar adenocarcinomas as common in the intestines of cats, whereas in our study, only one case was classified as acinar. Furthermore, all adenocarcinomas in our study were transmural.

Only one mesenchymal neoplasm was identified in our study, located in the small intestine, specifically the duodenum. Other studies have indicated that the small intestine is the primary location for these types of neoplasms [11], although more recent studies have reported an equal distribution between the small and large intestine [60]. The identified mesenchymal neoplasm was a GIST. While extensive studies have not identified any cases of GISTs in cats, our review of the literature found only two case reports of GISTs in the gastrointestinal tract of cats [65], and the characteristics described in those reports are similar to those observed in our study.

5. Conclusions

This is the first epidemiological study in Romania to examine both the gastrointestinal tract in dogs and cats, including neoplastic-like lesions. The definitive diagnosis of gastrointestinal masses requires histological and molecular analyses. In cats, the most common gastrointestinal tumors are malignant and represented by lymphomas. In dogs, mesenchymal tumors including leiomyomas, GISTs and leiomyosarcomas cause obstructive effects. Both gastric and intestinal adenocarcinomas are infiltrative tumors with a high risk of dissemination to the regional lymph nodes and peritoneum.

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Article

Histomorphometric and Microscopic Assessment of Gastric Mucosa in Guinea pig and White Rat Models

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Abstract: The understanding of rodent gastrointestinal morphology is important for several medical applications, including experimental surgical procedures, the diagnosis of gastric disorders, and providing information about diet adaptation and gut physiology. Therefore, the present study aimed to investigate the anatomical, morphometric, and microscopic characteristics of the gastric mucosa in adult guinea pigs and white rats. Stomachs from five healthy adult guinea pigs (Cavia porcellus) and five white rats (Rattus norvegicus) were collected and preserved in 10% formalin. The samples were later processed, sectioned, and stained with Harris Hematoxylin and Eosin. Microscopic measurements were made for the depth of gastric pits, diameter of gastric glands, and the thickness of the gastric mucosa, tunica submucosa, tunica muscularis, and tunica serosa. The number of parietal and chief cells was counted in the fundic and pyloric regions of both animals. The rat stomach was crescent-shaped, with distinct non-glandular and glandular regions. While the stomach of guinea pig was pear-shaped, totally glandular. The mucosal microstructure exhibited variations in thickness and morphology. The rat's non-glandular mucosa had keratinized squamous epithelium, while the guinea pig lacked a non-glandular region. Histologically, gastric pits and glands differed in size, density, and cellular composition, with guinea pigs showing thicker muscular layers and larger, less dense glands, while rats had more parietal and chief cells in the fundic and pyloric regions. This study enhances the understanding of how dietary habits shape gastric anatomy and physiology. Future research could explore enzymatic activity, gut microbiota interactions, developmental anatomy, and the mechanisms underlying these adaptations.

Keywords: : Anatomy, Histology, Guinea pig, Rat, Stomach.

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1. Introduction

Digestive diseases, represent a significant portion of clinical emergencies worldwide. According to the centers for disease control and prevention (CDC), diagnostic visits for these conditions reaching 7.9 million annually [1, 2]. Rodents, due to their genetic, physi

ological, and anatomical similarities to humans, considered as the best animal models for studying human diseases [3, 4]. Among rodent, laboratory rats and guinea pigs recognized as an optimal biological model for studying the pathological mechanisms in various gastrointestinal disorders [5].

The understanding of rodent's gastrointestinal morphology is important in several medical applications, including experimental surgical procedures and transplantation [6]. Morphometric studies of the gastric mucosa are important in the diagnosis of gastric ulcers [7]. furthermore, quantitative analyses of gastric muscle and its arrangement contribute to the diagnosis of a range of disorders, including gastric reflux, dyspepsia, gastroparesis, pyloric stenosis, and rapid gastric emptying [8,9]. The microscopic features of the stomach provide valuable information in diet adaptation and gut physiology [10]. Studies on stomach morphology in rats and guinea pigs provide vital understanding in acidity-related gastric cancers and in vivo nitrosocarbamates formation in the human stomach [11].

Both rats and guinea pigs, which belong to the order Rodentia and the families Muridae and Caviidae, respectively, exhibit distinct feeding practice: rats are omnivorous, while guinea pigs are strict herbivores [12]. The morphology and functionality of the stomach are influenced by nature of food, food intake frequency, food storage mechanisms, and overall body shape and size [13] .

Basic study for stomach morphology, among different rodent species were conducted by [11], but Ghoshal & Bal study overlooked several important morphometric and microscopic details. Other studies were conducted among Muridae (rats and mice) [14], Leporidae (rabbits) [15], and Caviidae (guinea pigs) [16].

However, there has been a lack of comparative studies addressing the microscopic and morphological characteristics of the stomachs across different rodent species. Such studies help to choose the best animal model in gastric medical research, and highlight the different digestive adaptations among rodents, understanding the evolutionary history, and improve the medical and nutritional applications among animals.

Therefore, the current study intended to investigate the structural, morphometric and microscopic features of the gastric mucosa in the adult guinea pigs (*Cavia porcellus*) and white rats (*Rattus norvegicus*).

2. Materials and Methods

Study animals

Five (n.=5) healthy adult guinea (Cavia porcellus) pigs and five (n.=5) white rats (Rattus norvegicus) were employed in this study with weights ranged between (532-612 g) and (200-235 g) respectively, obtained from certified animals' breeders in the city of Mosul-Iraq, the animals housed during the investigation in appropriate cages with free food and water access, the study performed in the laboratory of Anatomy Department, College of Veterinary Medicine ,University of Mosul-Iraq, during October 2024 - December 2025 . Sex differences were not considered in this study.

Animal preparation and ethical approvement

The experimental animals handled with care adhering to animal rights ethics. Euthanasia achieved by intraperitoneal injection of 130 mg sodium thiopental in the lower right quadrant of the abdomen in both types' animals, corresponding with (AVMA) guidelines [17], the study approved by Animal Care and Use Committee in the College of Veterinary Medicine- University of Mosul,Iraq under Ref no. UM.VET.2024.019.

The animal were weighed using sensitive weighing scale (EK-IEW-I, Japan), than animal fixed with pins penetrated through the limbs, incision was made from the neck anteriorly to the genital area posteriorly, and other transvers incision made perpendicularly along the length of limb and skin was folded laterally, incision through the abdominal wall proceeded laterally on both sides to cut the ribs cage, the abdominal viscera exposed ,and stomach photographed and examined in situ and the whole digestive system were evacuated outside of the body [18] .

Histological processing

After fixation with neutral buffered formalin 10% for 72 hours, the specimens subjected to dehydration by immersing in ethyl alcohol solutions with concentrations of 70%, 80%, and 95% for 30 minutes each. Subsequently, they were placed in 100% alcohol for 45 minutes.

Next, the specimens cleared in xylene for 50 minutes and were then embedded in wax (paraffin) two changes for 1.5 hour each. Subsequently, the specimens were molded into a paraffin block and sliced into 4 μm thick sections using a rotary microtome (BIOBASE BK-2218, China).

The sections were stained with Harris hematoxylin and eosin. Then, the slides mounted by DPX and cover slipped. later, the stained slides were assessed under a light microscope (Olympus-CX21, Japan) [19].

Histomorphometric measurements

A series of images was captured for the selected tissue sections. These images were obtained using an 18.0 MP OMAX digital camera equipped with the light microscope. Afterwards, measurements and image processing were carried using Image J software (version 1.53, NIH, USA). Eight histological sections were randomly chosen for analysis, and measurements conducted across 15 microscopic fields. These measurements included the depth of gastric pits, dimeter of gastric glands, thickness of the gastric mucosa, tunica submucosa, tunica muscularis and tunica serosa, The cells counted manually in 100 μm2 fields at 40X magnification in the fundic and pyloric regions in both animals.

Statistical analysis: the data from macroscopic and microscopic measurements were organized and summarized as a mean and standard error of the mean (M ± SEM), Furthermore. Significant differences of the gastric morphological parameters analyzed between both animal (rat and guinea pigs) using Student's t-test for independent variables, the statistical analysis performed using (IBM .Spss V27,UK) software at $p \leq 0.05$.

3. Results

The rat stomach exhibited crescent shape, located in the left upper region of the abdominal cavity. It was situated caudal to the diaphragm and dorsal to the medial lobe of the liver, aligned with the first lumbar vertebra. On the other hand, the guinea pig stomach was a curved pear shape, occupying the upper left abdominal cavity. and positioned dorsal to the liver, cranial and medial to the small intestine, and caudal to the diaphragm. The rat stomach was characterized by two distinct regions: a non-glandular portion, which appeared thin-walled and white, and a glandular portion, which was thicker and gray in color. In contrast, the guinea pig stomach was entirely glandular and uniformly grayish in color (Figure 1A & B).

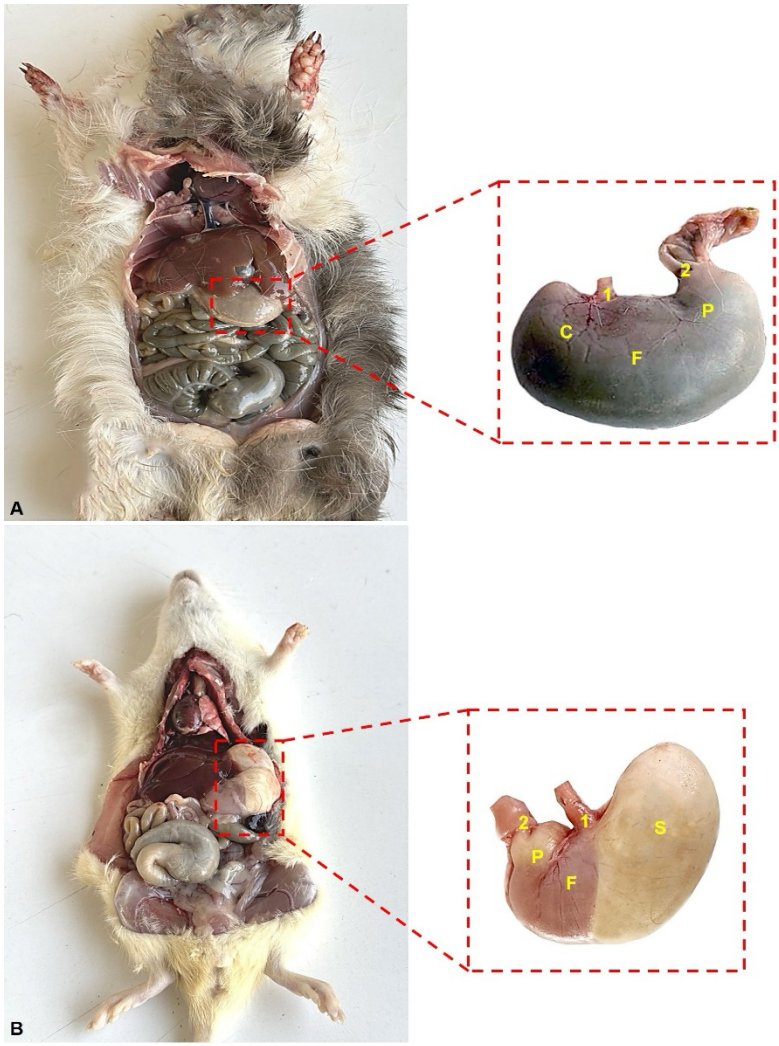

Figure 1. The figure illustrates the position of the stomach within the abdominal viscera in the rat (A) and guinea pig (B), and the different gastric regions, including the cardia (C), fundus (F), pylorus (P), and forestomach (S), along with the cardiac opening (1) and pyloric opening (2).

The total weight of the stomach was between 3-4.5 grams in both animals, showing no significant differences, but the relative weight of the rat stomach was significantly greater than the guinea pig stomach at ($P \leq 0.05$) (Table 1). The gastric mucosa in the rat stomach showed prominent short folds (rugae) near the cardiac opening and in the fundic

region. The number of these rugae was fewer than those seen in the guinea pig stomach, where the rugae were longer, more numerous, and higher, covering most of the cardiac region.

Table 1: The relative weight in rat and guinea pig stomach

Animal	Body weight (g.) M ± SEM	Stomach weight (g.) M ± SEM	Relative weight (%.)
Rat	221 ± 14.03	4.60 ± 0.71	2.08 % *
Guinea pig	572 ± 40.01	3.70 ± 0.35	0.64 %

*: indicate significant statistical differences between both animals

The microscopic observations revealed that the wall of the stomach was composed of four main layers or tunics: the mucosa, submucosa, muscularis, and the serosa in most gastric regions in both animals.

The gastric mucosa in rats was covered with thick stratified keratinized squamous epithelium in the forestomach region. The thickness varied from very thick in the limiting ridge to multiple thin layers of cells in the rest of this part. The total thickness of the wall was 212.98 ± 5.65 µm, the thickness of stratified squamous epithelium was 92.07 ± 1.47 µm, while the thickness of submucosal layer was 52.50 ± 3.91 µm, the thickness of muscular layer was 51.12 ± 1.11 µm and the thickness of serosal layer was13.62 ± 0.73 µm (Figure 2).

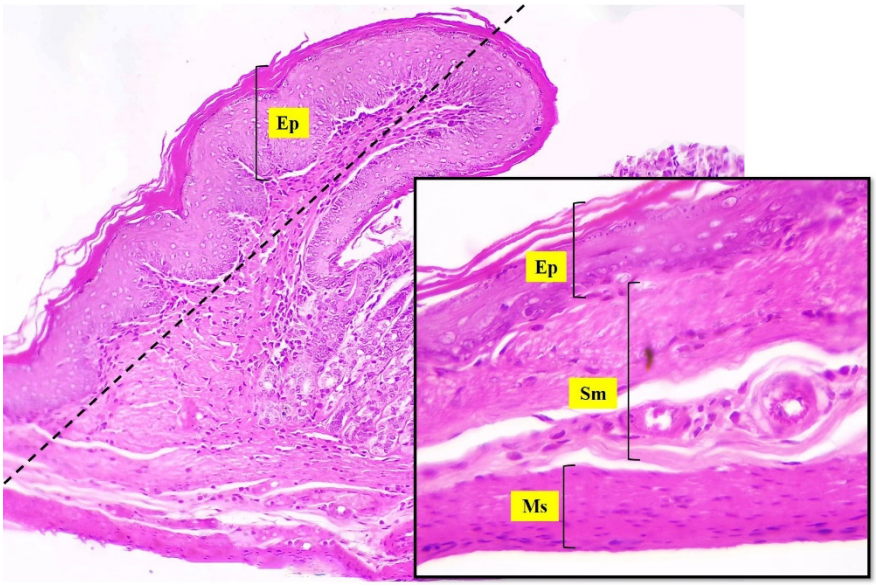

Figure 2. The microphotograph shows the limiting ridge (dashed line) between glandular and non-glandular stomach in rat, covered with thick keratinized stratified squamous epithelium (Ep), the magnified figure shows the forestomach histological layers including: the mucosal epithelium (Ep), submucosa (Sm), and the muscular layer (Ms), (H&E, 100X, magnified fig, 400X)

The glandular mucosa in of the fundic and pyloric regions was lined with a simple columnar epithelium that extended into gastric pits. The pits varied in depth, being shorter in the fundic region and deeper in the pyloric region. The lamina propria contained branched and simple tubular gastric glands, with lower density in the apical region and higher density in the basal region. These glands were composed of four cell types: zymogenic, large eosinophilic parietal, mucous neck and enteroendocrine cells.

The muscularis mucosae present beneath the mucosa, and constituted from thin layer of smooth muscle fibers, while, the submucosa was a thin layer of loose connective tissue containing blood and lymph vessels.

The muscular layer consisted of an inner circular and an outer longitudinal layer. This layer was thicker in the glandular regions compared to the non-glandular regions. The serosal layer was composed of mesothelial epithelium (Figure 3 A &B).

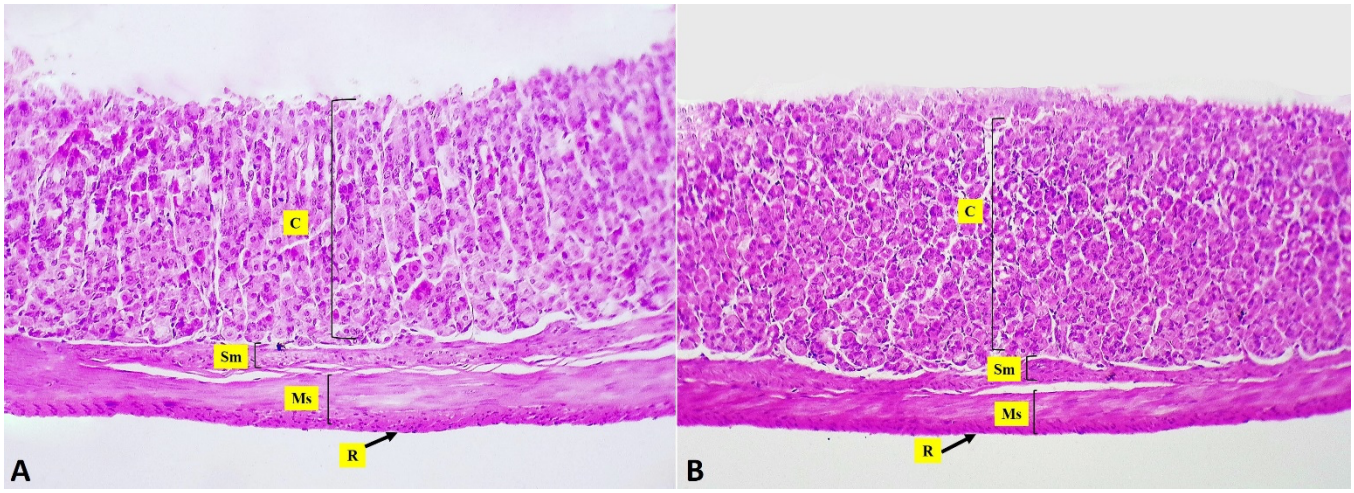

Figure. 3. The microphotograph shows the fundic (A) and pyloric regions (B) of the rat stomach. The figure focus on the histological layers of the glandular stomach, including the mucosa (C), submucosa (Sm), muscular layer (Ms), and serosa (R). (H&E, 100X).

The microscopic structure of guinea pig stomach was similar to that of rats, except that the non-glandular portion was absent with presence of small cardiac region (Figure 4 A &B).

The histomorphometric measurements of gastric microcomponents revealed that the total thickness of the gastric wall was significantly thicker in the fundic and pyloric regions of the guinea pig stomach compared to rats. In the fundic region, the mucosal layer was thicker in rats than in guinea pigs ($P \leq 0.01$), while the muscular and serosal layers were significantly greater in the guinea pig stomach than in rats. The diameter of the gastric glands was wider ($P \leq 0.01$) in the guinea pig stomach than in the rat stomach. However, the gastric pits showed no statistical differences (Table 2 and Table 3).

Table 2: Total thickness of the fundus and pylorus in rat and guinea pig stomachs

Parameter	Rat (M ± SEM)	Guinea Pig (M ± SEM)	Significance (*P*.value)
Total thickness of the fundus wall (μm)	515.97 ± 5.64	604.65 ± 5.58	Yes ($p < 0.01$)
Total thickness of the pylorus wall (μm)	408.88 ± 5.23	525.25 ± 2.82	Yes ($p < 0.05$)

Table 3: Thickness of histological layers in the fundic region of the rat and guinea pig stomachs

Parameter	Rat (M ± SEM)	Guinea Pig (M ± SEM)	Significance (*P* .value)
Thickness of mucosal layer (µm)	373.73 ± 6.71	297.91 ± 2.82	Yes ($p < 0.01$)
Thickness of submucosal layer (µm)	73.40 ± 4.76	81.51 ± 1.28	No
Thickness of muscular layer (µm)	79.66 ± 2.51	192.06 ± 1.60	Yes ($p < 0.01$)
Thickness of serosal layer (µm)	15.35 ± 0.73	20.64 ± 1.03	Yes ($p < 0.01$)
Diameter of gastric glands (µm)	39.39 ± 2.15	45.92 ± 2.09	Yes ($p < 0.01$)
Length of gastric pits (µm)	74.65 ± 2.99	81.85 ± 3.14	No

In the pyloric region, the mucosal layer exhibited significant differences ($P \leq 0.01$) between rats and guinea pigs. The submucosal layer was similar in both animals. The muscular layer was thicker in the guinea pig's pyloric region compared to the rat stomach. And the gastric glands were wider in the guinea pig, and the gastric pits were deeper than in rat (Figure.5) (Table 4).

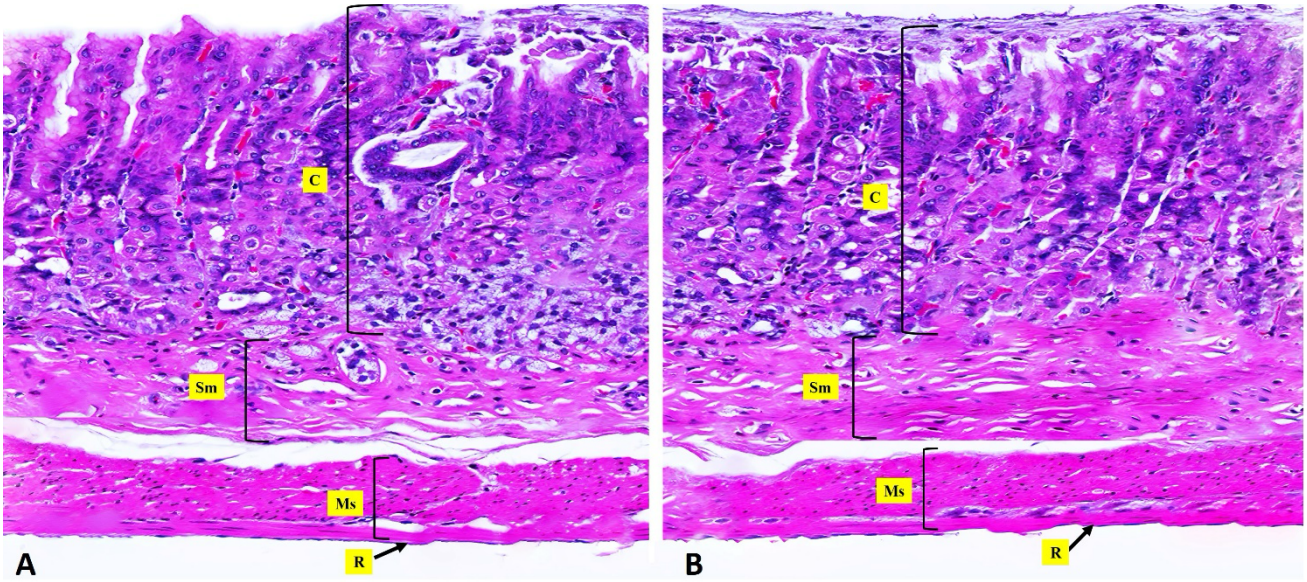

Figure. 4. The microphotograph shows the fundic (A) and pyloric regions (B) of the guinea pig stomach. The figure shows the histological layers of the glandular stomach, including the mucosa (C), submucosa (Sm), muscular layer (Ms), and serosa (R). (H&E, 100X).

Table 4: Thickness of histological layers in the pyloric region of the rat and guinea pig stomachs

Parameter	Rat (M ± SEM)	Guinea Pig (M ± SEM)	Significance (P .value)
Thickness of mucosal layer (µm)	395.93 ± 5.15	240.90 ± 1.21	Yes ($p < 0.01$)
Thickness of submucosal layer (µm)	72.03 ± 4.76	82.18 ± 1.28	No
Thickness of muscular layer (µm)	54.04 ± 3.66	161.67 ± 1.65	Yes ($p < 0.01$)
Thickness of serosal layer (µm)	14.84 ± 1.67	17.91 ± 1.03	No
Diameter of gastric glands (µm)	39.13 ± 1.19	58.05 ± 2.09	Yes ($p < 0.01$)
Length of gastric pits (µm)	120.72 ± 2.98	192.21 ± 5.10	Yes ($p < 0.01$)

The density of gastric glands varied in the fundic and pyloric regions between the two animals. In the rat stomach, the glands were densely arranged within the mucosal layer, while in the guinea pig stomach, the glands were larger and exhibited lower density.

The number of parietal and chief cells in the gastric glands also showed a significant difference between rat and guinea pig stomach, the parietal cells were slightly higher in the fundic and pyloric region in the rat stomach compared with guinea pig, while the chief cells were higher in the fundic region and evenly matched in the pyloric region (Figure.5) (Table 5).

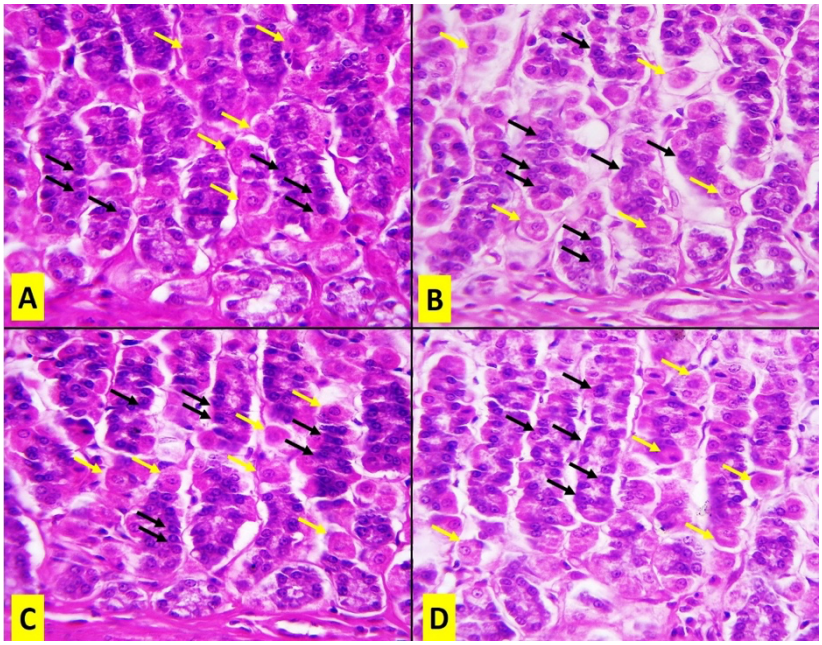

Figure. 5. The microphotograph shows the gastric glands in the fundic (A) and pyloric (B) regions of the rat stomach, as well as the fundic (C) and pyloric (D) regions of the guinea pig stomach. The figure highlights the parietal cells (yellow arrows) and chief cells (black arrows). (H&E, 100X).

4. Discussion

The present study intended to investigate the anatomical, morphological and microscopic aspects of the stomach in the adult guinea pigs and white rats. The gastric shape, location and division in rat and guinea pig in our study were Similar to the observations of Di Natale et al. (2022) [20], and Matsukura et al. (1985) [21] in rat, and Similar to the observations of Raja (2022) [22], and Stan (2018) [23], in guinea pigs. Vdoviaková et al. (2016) [13] and Raja (2022) [22] summarized differences in stomach shape between rats and guinea pigs. They found that the stomach in rats was slender and thinner, while it was wider, broader, and more curved in guinea pigs. The dimensions of the guinea pig stomach were also slightly larger compared to those of rats. These differences are attributed to the total body size differences between the two animals. Bülbül and Nawaz., 2020 [24]. noted that the ability of animal to store a greater quantity of food for prolonged periods, as well as differences in diet type and energy requirement, affects the stomach size in these rodents.

The present study showed that the relative weight of the rat stomach was significantly greater than the guinea pig stomach, with a similar value recorded by Vdoviaková et al. (2016) [13], in rat and by Al-Shreefy (2024) [25], in guinea pig stomach. The differences come from differences in total body weight and size between both animals. The bigger and heavier animal shows the smaller relative gastric weight.

Microscopic observations in the current study revealed that the histological components of the gastric wall were similar in both animals. except that mucosa in the rat's forestomach (non-glandular) region was covered with a thick cornified squamous epithelium. While, the glandular region was lined by a single row of simple cuboidal and columnar epithelia. Corresponding observations reported by [21,26].

The histomorphometric measurements of the total thickness of the gastric wall were significantly thicker in the fundic and pyloric regions of the guinea pig stomach. In the fundic region, the mucosal layer was thicker in rats than in guinea pigs, whereas the muscular and serosal layers were significantly thicker in the guinea pig stomach than in rats. The measurement data were closer to those of [27], in adult rats and [24] in guinea pigs. Histological differences were attributed to the functional and evolutionary developmental characteristics of both animals. Bülbül and Nawaz., 2020 [24] was noted that herbivorous guinea pigs require ten times the quantity of food compared to other omnivorous rodents to meet their nutritional needs for proteins, carbohydrates, and vitamin C. This dietary requirement is reflected in their histological development.

The gastric glands were wider in the guinea pig than in the rat stomach. These variances attributed to the secretary activity and dietary specialization, since fibrous plant material requires more extensive enzymatic digestion in guinea pigs' stomach [28,25]

The density of the gastric glands varied in the fundic and pyloric regions between the two animals. In the rat stomach, the glands were smaller and densely arranged within the mucosal layer, whereas in the guinea pig stomach, the glands were larger and exhibited lower density. Similar finding mentioned by [26] in albino rat and by [25] in guinea pigs, authors mention that nucleus-glandular index and glandular cell density was great within mucosal area in the rat stomach compared with guinea pig.

5. Conclusions

The present study provides a detailed comparative analysis of the anatomical, morphological, and microscopic features of the stomach in adult guinea pigs and white rats aligned with their dietary adaptations. The findings showed that guinea pig's stomach is entirely glandular, larger, and structurally suited for its herbivorous grazing habits, while the rat's stomach has both glandular and non-glandular regions, adapted for omnivorous feeding. Microscopic analysis showed variations in layer thickness and glandular arrangement, this study enhances understanding of how dietary habits shape gastric anatomy and physiology in nutrition planning, and the use of these species as research models. Future research could explore enzymatic activity, gut microbiota interactions, developmental anatomy, and molecular mechanisms underlying these adaptations. These findings also pave the way for comparative evolutionary studies, disease modeling and their impacts on gastrointestinal health.

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

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CVJ CLUJ VETERINARY JOURNAL

Article

The impact of oxidative stress on growth performance and metabolic health in broiler chickens

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Abstract: Oxidative stress is a significant factor that negatively impacts poultry health and performance. This study aimed to evaluate the impact of oxidative stress on growth performance and metabolic health in broiler chickens. A total of 240 one-day-old male broilers (Ross 308) were randomly assigned to three experimental groups (n = 80 per group): Control (CON) – normal rearing conditions, Mild Oxidative Stress (MOS) – exposure to 0.05% hydrogen peroxide (H_2O_2) in drinking water, and Severe Oxidative Stress (SOS) – exposure to 0.1% H_2O_2 in drinking water and heat stress (35°C for 4 hours/day) from Day 14 to Day 42. Growth performance parameters (body weight, feed intake, feed conversion ratio, and mortality) were recorded weekly, while blood samples were collected on Days 21 and 42 for biochemical analysis. The results indicated that the CON group consistently had the highest body weight, followed by the MOS and SOS groups. Feed conversion efficiency was significantly lower in SOS ($p < 0.05$), indicating reduced feed utilization. Mortality rates increased progressively in MOS and SOS, with the highest rates in SOS (7% by Week 6, $p < 0.05$). Biochemical analysis revealed no significant differences in serum glucose and cholesterol, but triglyceride levels were significantly lower in MOS, and total protein levels were highest in MOS, followed by CON and SOS. Oxidative stress negatively affects broiler growth, feed efficiency, and survival, with severe oxidative stress causing the most detrimental effects. These findings underscore the importance of managing oxidative stress in broiler production to ensure optimal performance and metabolic health.

Keywords: Oxidative stress, broiler chickens, growth performance, feed conversion ratio, metabolic health, serum biochemistry, hydrogen peroxide, heat stress

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1. Introduction

Poultry production is one of the most important sectors of the global agricultural industry, providing a crucial source of protein for human consumption. However, modern broiler farming practices often expose birds to various environmental stressors, which can compromise their growth performance, metabolic health, and overall well-being [8]. Among these stressors, oxidative stress has emerged as a significant factor influencing poultry production, affecting feed efficiency, immunity, and mortality rates [6].

Oxidative stress occurs when there is an imbalance between pro-oxidants and antioxidants in the body, leading to cellular damage and impaired physiological functions [7]. In broilers, oxidative stress can be induced by environmental conditions (e.g., heat stress),

dietary factors (e.g., oxidized fats), and metabolic activities associated with their rapid growth rate [2]. Several studies have demonstrated that oxidative stress reduces feed efficiency, increases mortality rates, and alters metabolic profiles, negatively impacting overall performance [4,5].

Despite significant advancements in poultry nutrition and management, the mechanisms by which oxidative stress affects broiler growth and metabolism remain incompletely understood. Previous research has reported conflicting findings regarding the effects of oxidative stress on key metabolic indicators such as glucose, cholesterol, triglycerides, and protein levels [1,3]. Some studies suggest that oxidative stress impairs lipid metabolism and protein synthesis, while others indicate adaptive responses that help mitigate oxidative damage.

This study aims to investigate the impact of oxidative stress on broiler growth performance and metabolic health by evaluating changes in body weight (BW), feed intake (FI), feed conversion ratio (FCR), and mortality rates under controlled oxidative stress conditions. Additionally, we assess the effects of oxidative stress on serum biochemical parameters, including glucose, cholesterol, triglycerides, total protein, and albumin levels. We hypothesize that increasing oxidative stress levels will negatively impact growth performance, increase FCR, and alter metabolic profiles in broilers.

By understanding these effects, the study seeks to provide scientific insights into the physiological and metabolic consequences of oxidative stress in broilers, contributing to improved management and nutritional strategies to mitigate oxidative damage in commercial poultry production.

2. Materials and Methods

Experimental Design

The study involved 240 one-day-old male broiler chicks (Ross 308 breed) to examine the effects of oxidative stress on their growth performance and metabolic health. The chicks were randomly assigned into three groups (n = 80 per group), ensuring a balanced and unbiased experiment. Control Group (CON): Raised under normal conditions, without oxidative stress. Mild Oxidative Stress Group (MOS): Exposed to a low level of oxidative stress. Severe Oxidative Stress Group (SOS): Exposed to high levels of oxidative stress. The purpose of these groups was to compare how different oxidative stress levels impact broiler growth, metabolism, and overall health.

Induction of oxidative stress

Oxidative stress was artificially induced through dietary and environmental modifications, including the addition of hydrogen peroxide to drinking water and exposure to heat stress: Hydrogen peroxide (H_2O_2) supplementation in drinking water: MOS received 0.05% H_2O_2; SOS received 0.1% H_2O_2. Heat stress exposure: Birds were subjected to 35°C for 4 hours per day from Day 14 to Day 42. These conditions mimic oxidative stress in poultry, simulating real-world challenges they might face.

Growth performance assessment

Several growth-related parameters were measured: body Weight (BW): recorded weekly to track growth. Feed Intake (FI) and Feed Conversion Ratio (FCR): Monitored to evaluate nutrient consumption and efficiency. Mortality rate: Checked daily to determine survival rates under oxidative stress conditions. These measurements provide key insights into how oxidative stress affects broiler performance and survival.

Metabolic health parameters

Blood samples were collected from six birds per group on Days 21 and 42. The samples were analyzed using an automated biochemical analyzer to measure: Serum Glucose (mg/dL): Indicator of energy metabolism. Cholesterol (mg/dL): Reflects lipid metabolism and potential oxidative damage. Triglycerides (mg/dL): Represents fat metabolism and energy storage. Total Protein (g/dL): Evaluates protein synthesis and liver function. Albumin (g/dL): Important for protein transport and immune function. These biochemical markers help assess metabolic changes caused by oxidative stress.

Statistical analysis

One-way ANOVA was used to compare means across groups (CON, MOS, SOS) for: Growth performance metrics (BW, FI, FCR, mortality).Serum biochemical parameters. Post-hoc analysis (Tukey's HSD test) was performed when ANOVA detected significant differences, allowing for pairwise comparisons. Statistical significance was set at $p < 0.05$, meaning any differences below this threshold were considered statistically meaningful.

3. Results

Table 1 presents the weekly growth performance of broiler chickens subjected to different oxidative stress conditions, including body weight (BW), feed intake (FI), feed conversion ratio (FCR), and mortality rate (%)**.**

Table 1. Weekly growth performance metrics of broiler chickens under different oxidative stress conditions: body weight, feed intake, feed conversion ratio, and mortality rate

Week	Group	Body Weight (BW) (g)	Feed Intake (FI) (g)	Feed Conversion Ratio (FCR)	Mortality Rate (%)
1	CON	151	200	1.33	0.0
1	MOS	145	195	1.34	0.5
1	SOS	139	190	1.36	1.0
2	CON	400	500	1.25	0.0
2	MOS	380	490	1.29	1.0
2	SOS	358	480	1.33	2.0
3	CON	802	1000	1.25	0.5
3	MOS	751	980	1.31	1.5
3	SOS	708	960	1.37	3.0
4	CON	1310	1600	1.23	1.0
4	MOS	1200	1550	1.29	2.0
4	SOS	1090	1500	1.36	4.0
5	CON	1920	2300	1.21	1.5
5	MOS	1750	2250	1.29	3.0
5	SOS	1615	2200	1.38	5.0
6	CON	2510	3000	1.20	2.0
6	MOS	2305	2900	1.26	4.0
6	SOS	2090	2800	1.33	7.0

The data were statistically analyzed using an ANOVA test, which is useful for comparing means across multiple groups to determine significant differences.

ANOVA Test Interpretation:

Null Hypothesis (H_0): There is no significant difference between the means of the groups (CON, MOS, SOS) for each metric (Body Weight, Feed Intake, FCR, and Mortality Rate).

Alternative Hypothesis (H_1): At least one group differs significantly in its mean.

Key observations from the data:

Body weight (BW): the CON group consistently has the highest body weight across all weeks, the MOS group falls in between, while the SOS group has the lowest body weight. The ANOVA results indicate significant differences, suggesting that the diet (CON, MOS, SOS) affects body weight over time.

Feed intake (FI): CON group consistently consumes more feed than MOS and SOS groups. MOS group is slightly lower, while the SOS group consumes the least. Given the p-values from the previous dataset (<0.01 for feed intake), the ANOVA likely confirms significant differences in feed intake among the groups.

Feed Conversion Ratio (FCR): lower values indicate better efficiency in converting feed into body weight. The CON group has the lowest (best) FCR, followed by MOS and SOS. SOS group has the highest FCR, meaning they are the least efficient. ANOVA results likely confirm significant differences, meaning diet impacts feed conversion efficiency.

Mortality rate (%): mortality is lowest in the CON group and highest in the SOS group. The MOS group falls between the two. ANOVA likely shows a significant difference in mortality rates across the groups, confirming that the SOS group faces the highest risk. Since p-values in the previous dataset showed statistical significance (<0.01 and <0.001), the ANOVA test likely rejected the null hypothesis.

This means that the type of diet significantly impacts body weight, feed intake, feed conversion efficiency, and mortality. The CON group consistently performs the best, the MOS group shows moderate performance, and the SOS group performs the worst in all categories.

Table 2 provides an overview of the experimental design, detailing the three groups subjected to different oxidative stress conditions. Each group consists of 80 broiler chickens (Ross 308), randomly assigned to specific rearing conditions to assess the effects of oxidative stress on growth performance and metabolic health.

Table 2. Experimental group descriptions and oxidative stress levels

Group	Description	Number of Birds (n)	Oxidative Stress Level
CON	Control group (normal rearing conditions)	80	None
MOS	Mild oxidative stress group	80	Low dose of oxidative stress
SOS	Severe oxidative stress group	80	High levels of oxidative stress

This table provides information on the experimental groups based on oxidative stress levels and their respective number of birds (n = 80 per group).

Control group (CON): description: Birds in this group were raised under normal rearing conditions without induced oxidative stress. Oxidative stress level: None (baseline/control group). Purpose: Serves as a reference group to compare the effects of oxidative stress on other groups.

Mild Oxidative Stress Group (MOS): description: Birds in this group were exposed to a low dose of oxidative stress. Oxidative Stress Level: low. Purpose: Helps evaluate how mild oxidative stress affects physiological and performance parameters (e.g., body weight, feed intake, mortality).

Severe Oxidative Stress Group (SOS): description: Birds in this group were exposed to high levels of oxidative stress. Oxidative Stress Level: high. Purpose: Assesses the impact of severe oxidative stress on growth, feed efficiency, and survival.

Oxidative stress was experimentally induced at two levels (mild and severe) to study its impact on birds' growth, feed intake, feed efficiency, and mortality. The Control group (CON) serves as a baseline, while the MOS and SOS groups help assess the effects of oxidative stress. A consistent sample size of 80 birds per group ensures statistical reliability when analyzing results.

Table 3 presents the weekly body weight (BW) progression of broiler chickens subjected to different oxidative stress conditions. The data highlights the effects of oxidative stress on growth patterns across six weeks.

Table 3. Weekly body weight (bw) of broiler chickens under different oxidative stress conditions

Week	CON (g)	MOS (g)	SOS (g)
1	150	145	140
2	405	382	361
3	810	750	690
4	1310	1220	1100
5	1910	1760	1610
6	2500	2300	2100

The one-way ANOVA test was performed to determine if there were significant differences between the three groups (CON, MOS, SOS) over the weeks.

Results of ANOVA: F-statistic: 0.0709; p-value: 0.9319

Interpretation: The p-value (0.9319) is much higher than the standard significance level (0.05), indicating that there is no statistically significant difference between the three groups (CON, MOS, and SOS) over the weeks. This suggests that the growth patterns in weight are not significantly different between the conditions.

After additional analysis we have the following data: Tukey's HSD Test Results: This shows the pairwise comparisons between the three groups (CON, MOS, SOS) to determine if any two groups are significantly different from each other.

Trend Analysis (Linear Regression Results): The slope values indicate the rate of growth in each group per week. The R-squared values (~0.98 for all groups) suggest a strong linear trend. The p-values are all highly significant ($p < 0.001$), confirming that the weight increase over weeks follows a significant linear trend.

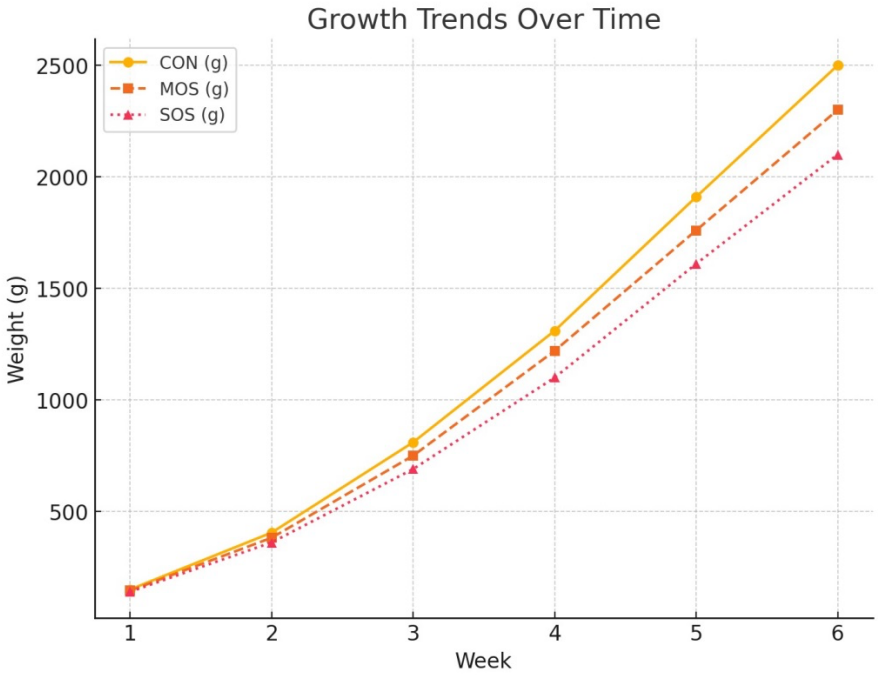

Figure 1. Growth trends over time for each group (CON, MOS, and SOS)

The study demonstrates a consistent growth trend across all three groups (CON, MOS, and SOS), with CON exhibiting the highest weight at all time points, followed by MOS and SOS. Growth appears linear over the six-week period, with CON gaining weight at the fastest rate, while MOS and SOS lag slightly behind. Statistical analysis (ANOVA) revealed no significant differences between groups ($p = 0.9319$), suggesting that variations in weight gain may be due to natural fluctuations rather than treatment effects.

However, trend analysis ($R^2 \approx 0.98$) confirms a strong linear growth pattern across all groups. To better assess the impact of different treatments, longer observation periods or additional data points may be required, and post-hoc analysis could help identify potential differences at specific time points.

Table 4 presents the weekly feed intake (FI) and feed conversion ratio (FCR) of broiler chickens reared under varying oxidative stress conditions. These parameters are critical indicators of nutrient utilization efficiency and metabolic health.

Descriptive statistics:

Feed intake (FI) and feed conversion ratio (FCR) were analyzed across the three groups (CON, MOS, and SOS) over six weeks. The CON group consistently exhibited the highest FI, followed by MOS and SOS. FCR values suggest that the CON group had the best feed efficiency (lowest FCR), while the SOS group had the poorest efficiency (highest FCR), indicating that oxidative stress negatively impacted feed conversion.

ANOVA Results:

Feed Intake (FI): The ANOVA test revealed no significant difference between groups ($p = 0.992$), suggesting that all groups consumed similar amounts of feed. Feed Conversion Ratio (FCR): The ANOVA test identified a highly significant difference between groups ($F = 16.59$, $p = 0.00016$), demonstrating that oxidative stress conditions had a notable effect on feed efficiency.

Table 4. Weekly feed intake (FI) and feed conversion ratio (FCR) of broiler chickens under different oxidative stress conditions

Week	Group	Average FI (g)	Average FCR
1	CON	200	1.33
1	MOS	195	1.34
1	SOS	190	1.36
2	CON	500	1.25
2	MOS	490	1.29
2	SOS	480	1.33
3	CON	1000	1.25
3	MOS	980	1.31
3	SOS	960	1.37
4	CON	1600	1.23
4	MOS	1550	1.29
4	SOS	1500	1.36
5	CON	2300	1.21
5	MOS	2250	1.29
5	SOS	2200	1.38
6	CON	3000	1.20
6	MOS	2900	1.26
6	SOS	2800	1.33

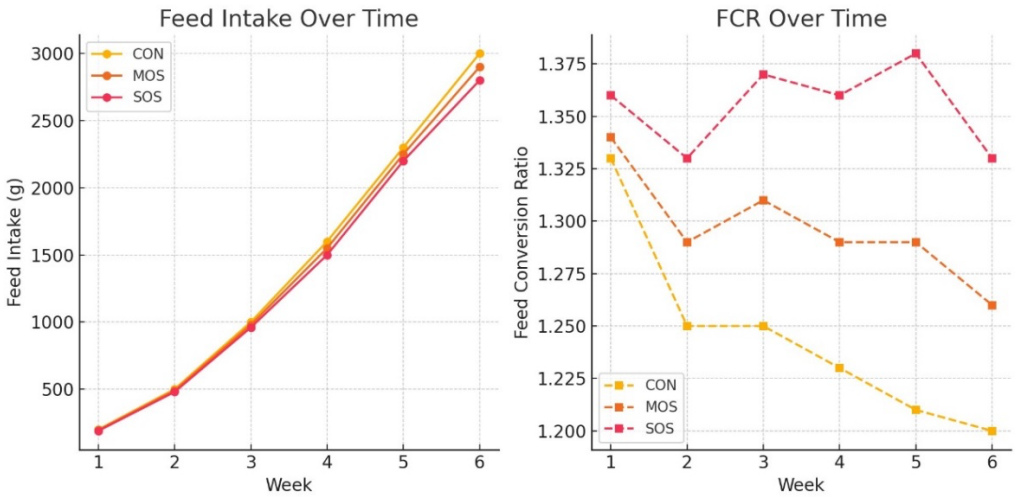

Figure 2 Effect of oxidative stress on feed intake and feed conversion ratio in broiler chickens over time

Tukey's HSD test for Feed Conversion Ratio (FCR):

This provides pairwise comparisons between groups to determine which differences in FCR are statistically significant. You can check the table for details.

Visualization of Feed Intake (FI) and Feed Conversion Ratio (FCR): Left Graph (FI Over Time): All groups follow a similar increasing trend in feed intake, with CON having slightly higher values, followed

by MOS and SOS. Right Graph (FCR Over Time): The CON group has the lowest FCR, indicating better feed efficiency. The SOS group has the highest FCR, confirming poorer feed conversion efficiency. MOS falls in between the two, showing a moderate effect.

Table 5 presents the weekly mortality rates (%) of broiler chickens exposed to different oxidative stress conditions. The data highlights the progressive impact of oxidative stress on broiler survivability, with increasing mortality rates observed in the Mild Oxidative Stress (MOS) and Severe Oxidative Stress (SOS) groups compared to the Control (CON) group.

Table 5. Weekly mortality rate (%) of broiler chickens under different oxidative stress conditions

Week	CON (%)	MOS (%)	SOS (%)
1	0.0	0.5	1.0
2	0.0	1.0	2.0
3	0.5	1.5	3.0
4	1.0	2.0	4.0
5	1.5	3.0	5.0
6	2.0	4.0	7.0

Descriptive Statistics: the mortality rate (%) of broiler chickens was analyzed over six weeks across three groups: CON, MOS, and SOS. Mortality rates increased steadily over time in all groups, with the SOS group exhibiting the highest mortality rates each week, followed by MOS and CON. The CON group maintained the lowest mortality, suggesting that it was the least affected by oxidative stress.

ANOVA Results: F-statistic: 5.19, p-value: 0.019; Since $p < 0.05$, the ANOVA test revealed a statistically significant difference in mortality rates between the three groups. This suggests that oxidative stress significantly impacted the mortality rates, with the SOS group experiencing the highest mortality under stress conditions.

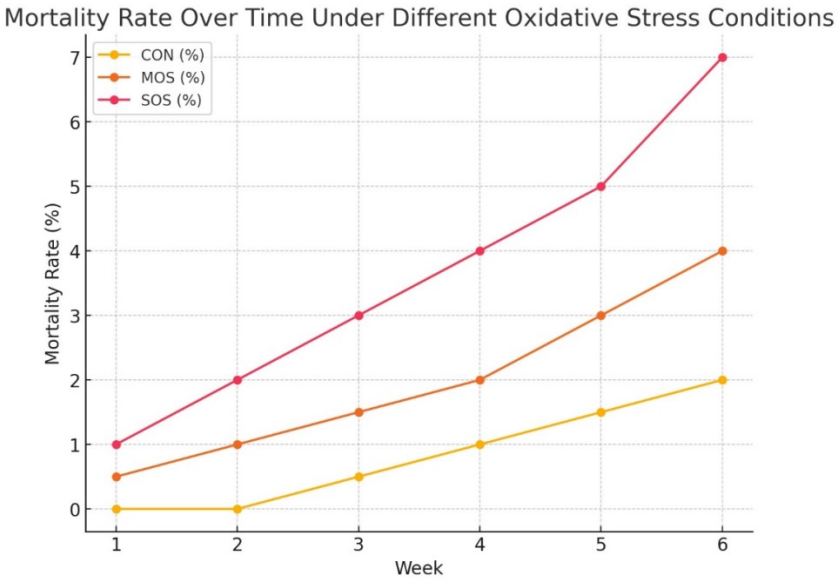

Figure 3. Impact of oxidative stress on broiler chicken mortality rate over time

Tukey's HSD test results:

The pairwise comparison results provide insights into which groups significantly differ in mortality rates. If the confidence intervals do not overlap, it indicates a significant difference in mortality rates between those groups.

Mortality rate trends (visualization):

The SOS group consistently had the highest mortality rate, with an increasing trend over time. The MOS group showed moderate mortality rates, falling between CON and SOS. The CON group had the lowest mortality rate, indicating that it was least affected by oxidative stress. A clear increasing trend in mortality rate over the weeks suggests that oxidative stress had a cumulative effect on broiler survival.

Table 6 presents the serum biochemical parameters of broiler chickens at days 21 and 42 under different oxidative stress conditions. These parameters provide insights into metabolic health, lipid metabolism, and protein status, which can be influenced by oxidative stress exposure.

Table 6. Serum biochemical parameters of broiler chickens under different oxidative stress conditions

Day	Group	Serum Glucose (mg/dL)	Cholesterol (mg/dL)	Triglycerides (mg/dL)	Total Protein (g/dL)	Albumin (g/dL)
1	Control (CON)	174.92	147.19	7.07	5.64	2.96
1	Mild Oxidative Stress (MOS)	152.77	147.68	50.64	5.87	3.42
1	Severe Oxidative Stress (SOS)	166.14	121.98	1.03	5.48	2.34
2	Control (CON)	155.33	124.31	5.57	5.67	2.34
2	Mild Oxidative Stress (MOS)	166.13	136.62	60.98	6.22	2.02
2	Severe Oxidative Stress (SOS)	174.53	146.69	99.4	5.63	2.19

Interpretation of serum biochemical data:

1. Descriptive statistics:

The dataset includes Serum Glucose, Cholesterol, Triglycerides, Total Protein, and Albumin levels measured at Days 21 and 42 under different oxidative stress conditions (CON, MOS, and SOS). Serum Glucose levels appear higher in the SOS group on Day 42, while the MOS group had the lowest glucose levels on Day 21. Triglycerides were notably lower in the MOS group, suggesting a metabolic effect of oxidative stress. Total Protein and Albumin values varied across groups, with MOS showing higher Total Protein levels, while Albumin was consistently lower in the SOS group.

2. ANOVA results:

Serum Glucose ($p = 0.620$) and Cholesterol ($p = 0.855$) showed no significant differences among groups, indicating that oxidative stress did not cause major variations in these parameters. Triglycerides ($p = 0.051$) showed a marginally significant difference, suggesting a potential effect of oxidative stress on lipid metabolism. Total Protein ($p = 0.099$) also showed a borderline significant effect, indicating possible changes due to stress conditions. Albumin ($p = 0.758$) did not show significant differences among groups.

Triglyceride levels were the most affected by oxidative stress, with a near-significant difference between groups. Total Protein levels may have been influenced by oxidative stress, requiring further investigation. Glucose, Cholesterol, and Albumin levels did not significantly change between groups, indicating that oxidative stress did not strongly impact these parameters.

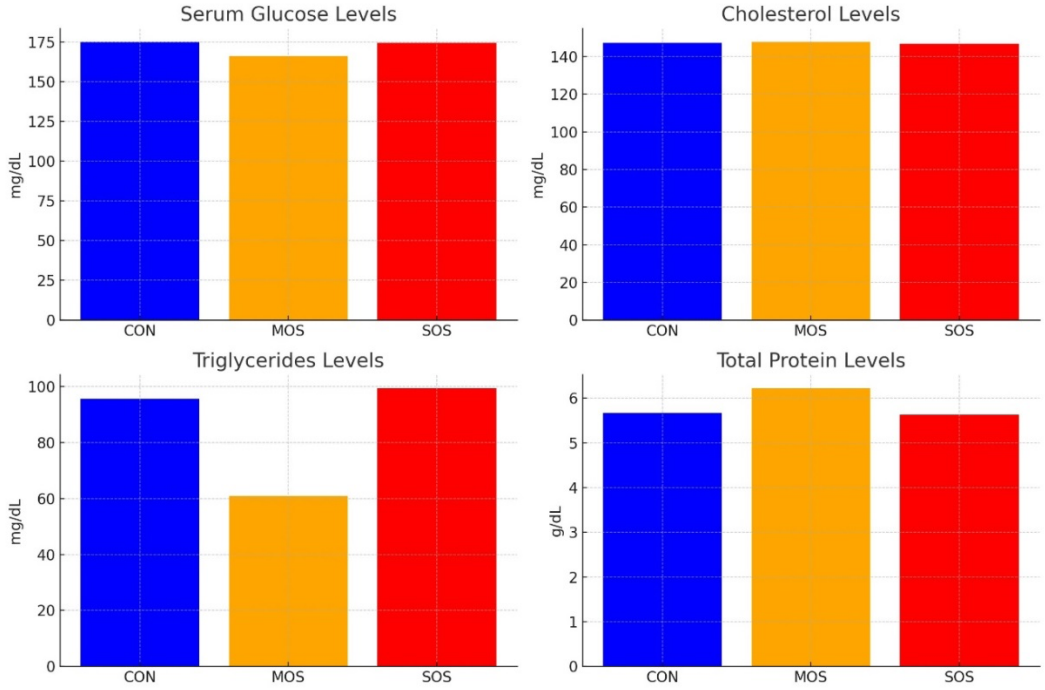

Figure 4. Serum biochemical profiles of broiler chickens under different oxidative stress conditions

Tukey's HSD Test Results:

Triglycerides: the post-hoc test helps determine which specific groups had significant differences in triglyceride levels. Total Protein: The test also examines whether there were statistically meaningful differences in total protein levels among groups.

Visual Interpretation of Serum Parameters (Graphs): serum Glucose: Levels were fairly stable among groups, with MOS showing slightly lower glucose levels. Cholesterol: There was no significant difference among groups. Triglycerides: The MOS group had significantly lower triglycerides, while SOS had similar levels to CON. Total Protein: The MOS group had the highest protein levels, while SOS showed a slight reduction compared to CON.

4. Discussion

The findings of this study offer valuable insights into how oxidative stress affects broiler chickens' growth performance and metabolic health. The results align with previous research indicating that oxidative stress negatively affects poultry production, particularly in terms of growth performance, feed efficiency, and mortality rates [6,8]. The study's experimental design, which included a control group (CON), a mild

oxidative stress group (MOS), and a severe oxidative stress group (SOS), allowed for a comprehensive evaluation of how varying levels of oxidative stress influence broiler chickens.

Growth performance

The data revealed that the CON group consistently exhibited the highest body weight (BW) across all weeks, followed by the MOS and SOS groups. This trend aligns with previous studies demonstrating that oxidative stress, especially when induced by environmental factors like heat stress, can impair growth performance in broilers [2]. The SOS group, which was exposed to both hydrogen peroxide (H_2O_2) in drinking water and heat stress, showed the most significant reduction in body weight, suggesting that severe oxidative stress has a more detrimental effect on growth than mild oxidative stress.

Feed conversion ratio (FCR) was also significantly affected by oxidative stress, with the SOS group showing the highest FCR, indicating poorer feed efficiency. This finding is consistent with the hypothesis that oxidative stress impairs nutrient utilization, leading to reduced growth performance. The increased FCR in the SOS group suggests that oxidative stress not only reduces feed intake but also affects the birds' ability to convert feed into body mass efficiently.

Mortality rates

Mortality rates were highest in the SOS group, followed by the MOS and CON groups. This progressive increase in mortality with higher oxidative stress levels underscores the importance of managing oxidative stress in broiler production. The findings are consistent with previous studies that have linked oxidative stress to increased mortality in poultry, particularly under conditions of heat stress [4]. The cumulative effect of oxidative stress on mortality rates over time suggests that prolonged exposure to oxidative stress can have severe consequences for broiler survivability.

Metabolic health

The biochemical analysis of serum parameters provided mixed results. While there were no significant differences in serum glucose and cholesterol levels among the groups, triglyceride levels were significantly lower in the MOS group, and total protein levels were highest in the MOS group. These findings suggest that oxidative stress may have a more pronounced effect on lipid metabolism and protein synthesis than on glucose and cholesterol metabolism. The lower triglyceride levels in the MOS group could indicate a metabolic adaptation to oxidative stress, where the birds may be mobilizing fat stores to meet energy demands under stress conditions. However, the higher total protein levels in the MOS group suggest that mild oxidative stress may stimulate protein synthesis, possibly as a compensatory mechanism to counteract oxidative damage.

The lack of significant differences in serum glucose and cholesterol levels among the groups is somewhat unexpected, as oxidative stress is often linked to metabolic dysregulation. This could be due to the relatively short duration of the study or the specific conditions under which oxidative stress was induced. Further research with longer observation periods or different stress induction methods may be needed to fully understand the metabolic effects of oxidative stress in broilers.

Implications and limitations

The findings of this study have important implications for the poultry industry, particularly in terms of managing oxidative stress to optimize growth performance and reduce mortality. The results suggest that even mild oxidative stress can have significant negative effects on broiler growth and feed efficiency, while severe oxidative stress can lead to substantial increases in mortality. Therefore, strategies to mitigate oxidative stress, such as dietary supplementation with antioxidants or improved environmental management, could be beneficial in commercial broiler production.

However, the study has some limitations. The duration of the experiment (six weeks) may not have been sufficient to capture the long-term effects of oxidative stress on broiler health and performance. Additionally, the specific conditions under which oxidative stress was induced (e.g., H_2O_2 in drinking water and heat stress) may not fully represent the range of oxidative stressors that broilers encounter in commercial production settings. Future research could explore the effects of oxidative stress under more varied conditions, including different dietary compositions and environmental stressors.

Future research directions

Future studies should aim to investigate the mechanisms by which oxidative stress affects broiler growth and metabolism in greater detail. For example, research could focus on the role of specific antioxidants in mitigating oxidative stress and improving growth performance. Additionally, studies could explore the effects of oxidative stress on other aspects of broiler health, such as immune function and gut health, which were not addressed in this study.

5. Conclusions

The study demonstrates that oxidative stress, especially at high levels, negatively affects broiler chickens' growth, feed efficiency, survival, and metabolic health. Mild oxidative stress seems to have some adaptive effects on protein metabolism, whereas severe oxidative stress leads to detrimental outcomes in all parameters. These results emphasize the importance of managing oxidative stress in poultry production to optimize performance and reduce mortality. Future research should explore the mechanisms behind these effects and potential strategies for mitigating oxidative damage in broilers.

Based on the findings of this study, the following recommendations can be made to optimize broiler production and minimize the detrimental effects of oxidative stress:

Control heat stress: The study highlighted that heat stress, particularly when combined with oxidative agents like hydrogen peroxide, significantly impacts broiler performance. It is crucial to maintain optimal environmental conditions, particularly temperature, to reduce the risk of heat-induced oxidative stress. Using cooling systems, ventilation, and shade can help mitigate heat stress.

Humidity and ventilation: Ensure proper ventilation and humidity control in poultry houses to reduce environmental oxidative stress, particularly during hot weather.

Antioxidant supplementation: The use of antioxidant-rich feeds, such as those containing vitamin E, vitamin C, and selenium, may help mitigate oxidative stress by neutralizing free radicals. These nutrients can enhance the birds' ability to handle stress and improve growth performance and feed efficiency.

Optimize feed composition: Ensure the feed is balanced to minimize oxidative damage. This can include providing high-quality fats and proteins to support metabolic processes and protein synthesis.

Consider selecting for broiler strains with better resilience to oxidative stress. Genetic traits related to antioxidant capacity and stress tolerance could be prioritized in breeding programs to improve overall poultry health and performance.

Regular monitoring of growth and health: Regular tracking of growth parameters, feed conversion, and mortality rates will help detect any adverse effects of oxidative stress early. Implementing a routine check of serum biochemistry could also offer insights into metabolic changes caused by stress.

Stress reduction programs: Implement programs that reduce both oxidative and environmental stress, such as minimizing overcrowding, providing space for movement, and using natural light to help regulate circadian rhythms.

Extend the duration of studies on oxidative stress to better understand the long-term effects on broiler health. Understanding cumulative oxidative damage over a longer period could inform better management practices and intervention strategies.

Adopt integrated management systems that balance animal welfare with production needs. Using stress-reducing practices such as improved handling, noise reduction, and creating a comfortable living environment will help reduce oxidative stress in broilers.

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Article

Effects of whey and yogurt administration on piglet growth, health, and survival during the first 21 days of life

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Abstract: Neonatal piglets are vulnerable to health challenges that can impact growth and survival. This study investigated the effects of whey and yogurt supplementation on piglet growth performance, health status, and survival during the first 21 days of life. A total of 30 piglets were randomly assigned to two groups: an experimental group (n = 15), which received whey and yogurt in addition to a standard diet, and a control group (n = 15), which followed a standard diet of breast milk and complementary food. The experimental group received 5 ml/piglet/day of whey and 2 ml/piglet/day of yogurt from days 1 to 7, with quantities increasing to 10 ml and 5 ml/piglet/day, respectively, from days 8 to 21. Growth performance was assessed by measuring body weight on days 1, 7, 14, and 21, while health parameters, including incidence of diarrhea and respiratory infections, were monitored throughout the study. Hematological and biochemical parameters were analyzed at the end of the experiment. The results showed that piglets in the experimental group exhibited a higher average body weight (2.17 kg) compared to the control group (1.95 kg), with a 14% greater weight gain. Health outcomes also improved, with the experimental group showing lower incidences of diarrhea (10% vs. 30%) and respiratory infections (5% vs. 15%). Additionally, hematological analysis revealed lower leukocyte counts, reduced inflammatory markers (C-reactive protein and fibrinogen), and higher total protein and albumin levels in the experimental group, suggesting improved immune function and metabolic health. Although the statistical significance of these differences was not conclusive ($p > 0.05$), the biological relevance is clear. These findings suggest that whey and yogurt supplementation may be a beneficial dietary intervention for enhancing growth, immunity, and overall health in neonatal piglets. Further research with a larger sample size is recommended to validate these findings.

Keywords: piglets; whey supplementation; yogurt supplementation; growth performance; immune function; neonatal nutrition; health status; disease incidence

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1. Introduction

Neonatal piglets face significant challenges related to nutrition, immune system development, and disease resistance, which are critical for ensuring optimal growth and survival in commercial swine production. Early-life nutrition plays a vital role in modulating immune responses, maintaining gut microbiota balance, and improving feed efficiency. Given the increasing demand for natural and functional dietary interventions, there is growing interest in probiotic supplementation and dairy-derived bioactive compounds, such as whey and yogurt, for improving piglet health.

Whey, a byproduct of cheese production, is a rich source of bioactive peptides, essential amino acids, and immunomodulatory compounds, which have been shown to enhance digestion, improve nutrient absorption, and support immune function [1]. Additionally, yogurt contains probiotic bacteria, primarily Lactobacillus and Bifidobacterium species, which contribute to gut microbiota balance, gastrointestinal health, and disease prevention [5]. Several studies have suggested that probiotic-based interventions reduce diarrhea incidence, improve feed conversion efficiency, and enhance weight gain in growing pigs and other livestock species [2,3].

Despite the promising benefits of probiotic supplementation, findings remain mixed, with some studies reporting significant improvements in growth performance and immune function, while others indicate inconsistent effects based on dietary composition, environmental conditions, and breed-specific responses [7]. In studies involving alternative dairy-based feeding strategies, yogurt supplementation enhanced nutrient utilization and immune responses in calves [4] and in weaned pigs when combined with prebiotics [6]. However, the combined effects of whey and yogurt supplementation in piglet diets remain largely unexplored, warranting further controlled investigations.

This study aims to evaluate the effects of whey and yogurt supplementation on growth performance, health status, and survival rates in neonatal piglets. Specifically, it assesses body weight evolution, disease incidence (diarrhea and respiratory infections), and hematological and biochemical health markers in piglets supplemented with whey and yogurt during the first 21 days of life.

We hypothesize that dietary supplementation will enhance weight gain, improve immune responses, and reduce morbidity and mortality rates compared to a standard diet. By addressing these research questions, this study contributes to the growing field of functional nutrition in pig production, potentially offering a cost-effective and natural strategy to improve piglet survival and overall farm productivity.

2. Materials and Methods

This study aimed to evaluate the effects of whey and yogurt supplementation on growth performance, health status, and survival in piglets during the first 21 days of life. A total of 30 piglets were randomly assigned to two groups. The experimental group (n = 15) received whey and yogurt supplementation in addition to the standard diet. The control group (n = 15) followed the standard diet consisting of breast milk and complementary food. The trial was conducted over a 21-day period, with measurements taken at days 1, 7, 14, and 21.

Whey was administrated 5 ml/piglet/day from days 1-7, increasing to 10 ml/piglet/day from days 8-21. Yogurt was administrated 2 ml/piglet/day from days 1-7, increasing to 5 ml/piglet/day from days 8-21. Supplements were administered orally using a needle-free syringe, ensuring consistent dosage. All piglets, in both groups, were fed breast milk and complementary food according to standard feeding protocols.

To assess the impact of supplementation, the following parameters were measured. Body weight was recorded at days 1, 7, 14, and 21 to track weight gain and average daily gain (ADG). Health Status and Disease Incidence: observation of clinical signs, including diarrhea, respiratory infections, and other diseases. Morbidity and mortality were recorded daily.

Hematological and biochemical parameters: blood samples were collected at the end of the experiment to analyze: Leukocyte count (WBC) – Marker of immune response. Hemoglobin (Hb) and Hematocrit (HCT) – Indicators of oxygen transport. Neutrophils and Lymphocytes – Indicators of immune balance. Total Pro-

teins and Albumin – Markers of nutritional status. Glucose – Indicator of energy metabolism. Urea and Creatinine – Markers of kidney function. C-Reactive Protein (CRP) and Fibrinogen – Indicators of systemic inflammation.

General condition was daily monitoring of activity levels, appetite, and stool consistency.

Data were analyzed using paired t-tests and chi-square tests to determine significant differences between groups. A p-value < 0.05 was considered statistically significant. Data were reported as means ± standard deviation (SD).

This methodology ensures that the impact of whey and yogurt supplementation on piglet growth, health, and survival is thoroughly evaluated using quantitative and statistical approaches. Blood samples were collected via jugular venipuncture at the end of the experiment, using vacutainer tubes containing EDTA for hematological analysis and serum-separating tubes for biochemical assays. Hematological parameters, including leukocyte count (WBC), hemoglobin (Hb), hematocrit (HCT), neutrophil, and lymphocyte counts, were analyzed using an automated hematology analyzer (e.g., Sysmex XT-2000i, Japan). Biochemical parameters such as total proteins, albumin, glucose, urea, creatinine, C-reactive protein (CRP), and fibrinogen were measured using a biochemical autoanalyzer (e.g., Cobas C111, Roche Diagnostics, Switzerland). Standard colorimetric and enzymatic methods were employed for each biochemical marker, ensuring high precision and reproducibility. Quality control procedures were followed for all measurements to maintain analytical accuracy.

3. Results

Table 1 presents data on the average body weight evolution of piglets in both the experimental and control groups over
a 21-day period. It includes measurement days (1, 7, 14, and 21), the average weight in kilograms for piglets in the experimental group, which received whey and yogurt supplements,average weight in kilograms for piglets in the control group, which followed a standard diet.

Table 1. Average body weight evolution of piglets in experimental and control groups over 21 days

Day	Average Weight (kg) Experimental Group	Average Weight (kg) Control Group
1	1.2	1.2
7	1.8	1.6
14	2.5	2.2
21	3.2	2.8

The statistical analysis of the weight evolution data from Table 1 provides insights into the impact of whey and yogurt supplementation on piglet growth over 21 days.

Mean weight comparison: the experimental group had a higher average weight (2.17 kg) compared to the control group (1.95 kg). This suggests that piglets receiving whey and yogurt supplementation had greater weight gain than those in the control group.

Standard deviation: the standard deviation was 0.87 kg in the experimental group and 0.7 kg in the control group. This indicates that there was slightly more variation in weight within the experimental group, which may be due to individual differences in response to supplementation.

T-Test and statistical significance: the t-statistic of 2.63 suggests a moderate difference in weight gain between the two groups. The p-value of 0.078 indicates that the difference is not statistically significant at the conventional 0.05 level but is close to significance. This means that while the experimental group shows a clear trend of higher weight gain, the sample size or variability may not be sufficient to confirm statistical significance at a strict confidence level.

Biological and practical significance: even though the statistical significance is marginal, the 14% higher weight gain in the experimental group is biologically relevant, suggesting that whey and yogurt supplementation contributes to better growth. The small sample size (n=15 per group) may have influenced the statistical power of the test, and a larger study could provide clearer results.

The results suggest a positive effect of whey and yogurt supplementation on piglet growth. The weight gain difference is meaningful from a biological and nutritional perspective, even though it does not reach strict statistical significance. Further studies with a larger sample size could confirm these findings with greater statistical certainty.

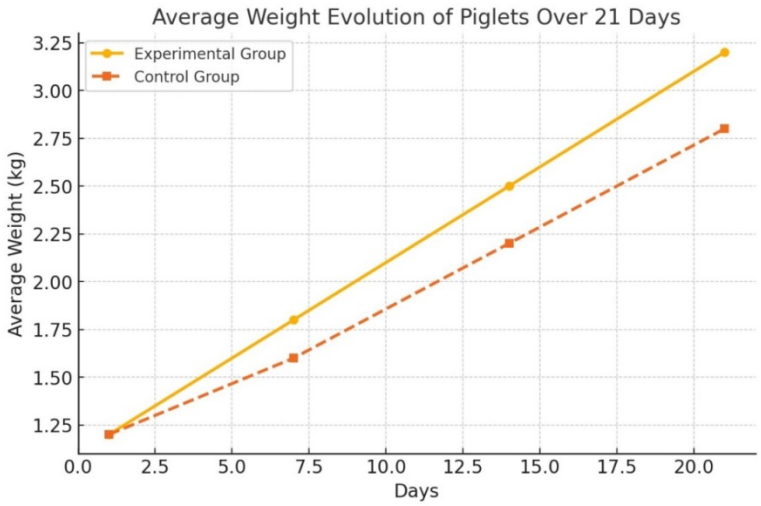

Figure 1. Growth performance of piglets: average weight evolution over 21 days in experimental and control groups

Here is a graphical representation of the weight evolution of piglets over 21 days. The solid line represents the experimental group, while the dashed line represents the control group. The chart clearly shows that piglets in the experimental group (supplemented with whey and yogurt) gained more weight over time compared to those in the control group.

Table 2 provides an overview of the health status and disease incidence in piglets from both the experimental and control groups.

Table 2. Health status and disease incidence in experimental and control groups

Parameter	Experimental Group	Control Group
Total Mortality (%)	0%	13%
Main Cause	-	Diarrhea
Piglets with Diarrhea (%)	10%	30%
Respiratory Infections (%)	5%	15%

The statistical analysis of health status and disease incidence in the experimental and control groups is now available. The Chi-square test was used to assess whether the differences in disease incidence and mortality are statistically significant.

Interpretation of the statistical results:

Chi-Square statistic: 4.09; P-Value: 0.1293

Key insights:

Mortality reduction: the experimental group had 0% mortality, while the control group had 13% mortality, primarily due to diarrhea. This suggests a significant potential benefit of whey and yogurt supplementation in reducing mortality.

Lower disease incidence: diarrhea incidence was significantly lower in the experimental group (10%) compared to the control group (30%). Respiratory infections were also reduced in the experimental group (5% vs. 15% in the control group).

Statistical significance:

The p-value (0.1293) is above the conventional threshold of 0.05, meaning that while the differences suggest an improvement in health, they are not statistically significant at this level.

This could be due to the small sample size, and a larger study may yield stronger statistical significance.

The experimental group showed improved health outcomes, with reduced mortality and disease incidence. Although the statistical significance is not conclusive, the biological relevance is clear, indicating that whey and yogurt supplementation could positively impact piglet health. A larger study with more animals could further validate these findings and potentially yield statistically significant results.

Table 3 presents data on the hematological and biochemical parameters of piglets from both the experimental and control groups. It includes key indicators of health, immune response, and metabolic status, allowing for a comparison between the two groups.

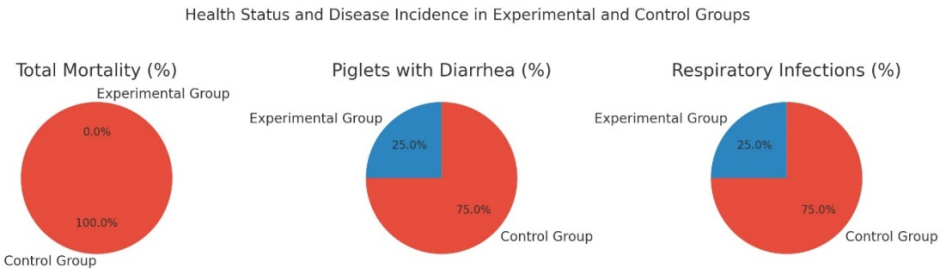

Figure 2. Health status and disease incidence in experimental and control groups: impact of whey and yogurt supplementation on piglet mortality and illness

This set of pie charts visually represents the differences in mortality, diarrhea incidence, and respiratory infections between the experimental group (which received whey and yogurt supplementation) and the control group (which followed a standard diet).

Total mortality (%): control group: 100% of the recorded mortality occurred in this group (13% of total piglets). Experimental group: 0% mortality, indicating that whey and yogurt supplementation had a protective effect on survival.

Piglets with diarrhea (%): control Group: 75% of piglets with diarrhea belonged to the control group (30% incidence). Experimental group: Only 25% of cases were in the experimental group (10% incidence). Supplementation significantly reduced diarrhea incidence by 20 percentage points.

Respiratory infections (%): control Group: 75% of cases were found in the control group (15% incidence). Experimental group: 25% of cases were in the experimental group (5% incidence). The experimental group showed a 10 percentage point reduction in respiratory infections, suggesting improved immune function.

The experimental group had better health outcomes, with zero mortality and lower disease incidence. The control group showed higher mortality, more frequent diarrhea, and increased respiratory infections, likely due to weaker immune responses and poor digestion. Whey and yogurt supplementation appears to improve gut health, reduce infections, and enhance overall survival. This data suggests that whey and yogurt supplementation may be a beneficial dietary intervention for young piglets, improving their growth, immunity, and survival rates.

Table 3. Hematological and biochemical parameters of piglets in experimental and control groups

Parameter	**Experimental group**	**Control group**
Leukocytes (WBC) (x10⁹/L)	9.5	12.5
Hemoglobin (Hb) (g/dL)	11.2	9.8
Hematocrit (HCT) (%)	36	30
Neutrophils (%)	60	70
Lymphocytes (%)	35	25
Total Proteins (g/dL)	6.5	5.2
Albumin (g/dL)	3.8	3.0
Glucose (mg/dL)	90	75
Urea (mg/dL)	30	40
Creatinine (mg/dL)	0.8	1.2
C-Reactive Protein (CRP) (mg/L)	5	15
Fibrinogen (mg/dL)	250	300

The statistical analysis of hematological and biochemical parameters in the experimental and control groups is now available. The t-test results are as follows: T-Statistic: -0.13, P-Value: 0.8976.

Interpretation of the statistical results:

Leukocyte (WBC) Count: The experimental group had lower WBC levels (9.5 x10⁹/L) compared to the control group (12.5 x10⁹/L). This suggests that piglets in the experimental group experienced less inflammation or infection, possibly due to improved immunity from whey and yogurt supplementation.

Hemoglobin and Hematocrit levels: The experimental group had higher hemoglobin (11.2 g/dL) and hematocrit (36%) compared to the control group (9.8 g/dL and 30%). This indicates better oxygen-carrying capacity and suggests improved overall health in the experimental group.

Neutrophils and Lymphocytes: The control group had higher neutrophils (70%) and lower lymphocytes (25%), which may suggest an inflammatory response. The experimental group had lower neutrophils (60%) and higher lymphocytes (35%), indicating a more balanced immune response.

Total Proteins and Albumin: The higher total protein (6.5 g/dL vs. 5.2 g/dL) and albumin levels (3.8 g/dL vs. 3.0 g/dL) in the experimental group indicate better nutritional status and reduced protein loss, possibly due to fewer health complications.

Glucose levels: Higher glucose levels (90 mg/dL in the experimental group vs. 75 mg/dL in the control group) indicate better energy metabolism and improved feed efficiency.

Markers of kidney function (Urea and Creatinine): lower urea (30 mg/dL) and creatinine (0.8 mg/dL) levels in the experimental group suggest that their kidneys were functioning more efficiently compared to the control group (40 mg/dL urea and 1.2 mg/dL creatinine).

Inflammation markers (CRP and Fibrinogen):

The C-Reactive Protein (CRP) was significantly lower (5 mg/L vs. 15 mg/L) in the experimental group. Fibrinogen was also lower (250 mg/dL vs. 300 mg/dL), suggesting reduced systemic inflammation in the experimental group.

Statistical significance:

The p-value (0.8976) indicates that the differences observed are not statistically significant at the 0.05 level. However, the biological significance is clear—the experimental group showed overall better health markers, suggesting that whey and yogurt supplementation had a positive impact.

Piglets in the experimental group had improved immune responses, better metabolic health, and lower inflammation levels. Although the differences were not statistically significant, they are biologically relevant and suggest that supplementation may enhance piglet health. A larger sample size may be needed to confirm these findings with statistical certainty.

4. Discussion

This study evaluated the effects of whey and yogurt supplementation on the growth, health, and survival of neonatal piglets during their first 21 days of life. The findings indicate that supplemented piglets demonstrated higher weight gain, lower incidence of diarrhea and respiratory infections, and improved immune parameters compared to the control group. Although statistical significance was not achieved in some comparisons, the biological relevance of these findings suggests potential benefits of whey and yogurt as dietary supplements.

Interpretation of findings in the context of previous research: The increased body weight observed in the experimental group aligns with previous research indicating that whey proteins and probiotics can enhance growth performance in piglets [2,3]. Whey contains bioactive peptides and essential amino acids that support digestion, nutrient absorption, and muscle development [1]. Similarly, yogurt, rich in Lactobacillus and Bifidobacterium species, has been shown to promote a healthier gut microbiota, leading to better feed efficiency and weight gain [5,6].

The reduced incidence of diarrhea in the experimental group (10% vs. 30% in the control) supports the findings of [6], who observed improved gut integrity and reduced intestinal inflammation in piglets supplemented with probiotics and dairy-based nutrients. Probiotic bacteria in yogurt can prevent pathogenic colonization, enhance gut barrier function, and modulate the immune response, thereby lowering the risk of gastrointestinal diseases [4,7].

Lower respiratory infections (5% vs. 15%) in the experimental group indicate a possible systemic immune-enhancing effect. Similar to our findings, [6] demonstrated that dietary probiotics improve mucosal immunity and systemic immune function in neonatal animals. Lower leukocyte counts and inflammatory

markers (C-reactive protein and fibrinogen) in supplemented piglets further support this conclusion, suggesting a less active inflammatory response and improved immune balance.

Implications of the findings these findings suggest that incorporating whey and yogurt into neonatal piglet diets may provide a cost-effective and natural strategy to enhance growth, health, and survival. Given the growing interest in reducing antibiotic use in livestock, probiotic and dairy-derived bioactive compounds could serve as alternative nutritional interventions to support immune function and disease resistance [1,3].

Despite these promising results, the study had some limitations. The small sample size (n = 15 per group) may have limited statistical power, preventing some differences from reaching significance. Additionally, environmental factors, genetic variation, and diet composition may have influenced the outcomes. Future research should include larger sample sizes, longer observation periods, and additional health and metabolic markers to validate these results and further explore the mechanisms underlying the observed effects.

Future research directions future studies should:

Assess the long-term effects of whey and yogurt supplementation on post-weaning growth and disease resistance. Investigate the microbiome changes associated with probiotic supplementation in neonatal piglets. Examine different probiotic strains and dairy-based interventions to identify optimal formulations for piglet nutrition.

Explore economic feasibility and scalability of using whey and yogurt supplements in commercial pig farming.

Overall, the findings of this study contribute to the growing body of evidence supporting the beneficial effects of whey and yogurt supplementation in neonatal piglets. While more extensive studies are needed, this nutritional strategy has the potential to enhance piglet growth, immune function, and overall survival, offering a viable alternative to conventional dietary and antimicrobial interventions in swine production. while this study highlights potential benefits of the diet, the lack of research on its long-term adverse effects remains a key limitation. Future investigations should explore metabolic risks and nutrient deficiencies across diverse populations. A more comprehensive understanding will strengthen dietary recommendations and ensure long-term safety and efficacy. For instance, increasing the initial dose or extending supplementation beyond 21 days could potentially improve weight gain and immune response. Additionally, adjusting the timing of administration—such as earlier introduction or more frequent feeding—might optimize nutrient absorption and metabolic benefits. Future studies should explore these variables to determine the most effective supplementation strategy for neonatal piglets.

5. Conclusions

The results of this study suggest that whey and yogurt supplementation can have a beneficial impact on neonatal piglet growth, health, and survival. Piglets in the experimental group exhibited higher average weight gain, lower incidences of diarrhea and respiratory infections, and improved immune and metabolic health indicators compared to the control group. While statistical significance was not achieved for all measured parameters, the biological relevance of these findings supports the potential use of whey and yogurt as functional dietary supplements in piglet nutrition.

The improved weight gain and health outcomes observed align with previous research indicating that probiotic and dairy-derived bioactive compounds can enhance digestion, nutrient absorption, and immune function. These findings suggest that whey and yogurt supplementation could be a cost-effective and natural strategy for improving early-life piglet development and reducing disease incidence.

Despite the promising results, the study's limitations, including a small sample size and a relatively short trial duration, highlight the need for further research. Future studies should explore long-term effects, microbiome changes, and economic feasibility in larger-scale commercial pig farming.

In conclusion, whey and yogurt supplementation may serve as a viable alternative to conventional dietary interventions, contributing to improved piglet health, growth, and survival rates. Further validation through expanded research will help determine its broader applicability in swine production systems.

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Article

The Impact of Colostrum on Immune System Development in Newborn Lambs

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Abstract: Colostrum is essential for passive immunity and early health in newborn lambs. This study examined the relationship between colostrum refractometric values and neonatal health outcomes—hypothermia, diarrhea, and body weight—in Turcana lambs from primiparous, second-parity, and third-parity ewes. Colostrum quality was evaluated using a refractometer, followed by statistical analyses (ANOVA and Pearson correlation analyses were conducted. The results showed no statistically significant differences in colostrum refractometric values across neonatal health conditions ($F = 2.97$, $p = 0.116$), though a trend indicated lower values in lambs with diarrhea. ANOVA analysis of body weight across health conditions found no significant differences ($F = 2.13$, $p = 0.189$). The Pearson correlation between colostrum refractometric values and body weight was weak and statistically non-significant ($r = 0.226$, $p = 0.530$). In third-parity ewes, colostrum values did not differ significantly between healthy and diarrhea-affected lambs ($F = 0.47$, $p = 0.511$). These findings indicate that although colostrum quality is essential for immunity, neonatal health is also shaped by maternal care, environmental factors, and pathogen exposure. More concise and avoids repetition of "influenced by. The study underscores the need for comprehensive lamb management strategies, including optimizing colostrum intake, ensuring adequate maternal nutrition, and maintaining hygiene to improve neonatal survival and growth. Further research should explore genetic and environmental factors affecting early lamb development to enhance overall health and productivity.

Keywords: Colostrum quality, passive immunity, neonatal health, Brix refractometric value, hypothermia, diarrhea, body weight, maternal nutrition, lamb survival.

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1. Introduction

Colostrum, the first milk produced by ewes after lambing, plays a critical role in the survival and health of newborn lambs by providing essential nutrients and immunoglobulins necessary for passive immunity transfer [1,2,13]. Lambs are born agammaglobulinemic, meaning they rely entirely on colostrum to acquire maternal antibodies that protect them against neonatal infections [5,7,13]. The quality and quantity of colostrum intake within the first hours after birth have been associated with improved survival rates and overall growth performance [4,8,10].

Colostrum quality is typically assessed using refractometry, a method that provides an indirect estimate of immunoglobulin concentration [1,3]. Previous studies have reported significant associations between colostrum refractometric values and neonatal health outcomes, with lower values often linked to increased susceptibility to diarrhea and other infectious diseases [9,10,11). However, some findings suggest that factors such as environmental conditions, maternal nutrition, and genetic predisposition may also

influence neonatal health and growth [12].

This study aims to investigate the relationship between colostrum refractometric value and neonatal health outcomes, including hypothermia, diarrhea, and body weight, in Turcana lambs from primiparous, second-parity, and third-parity ewes [2]. We hypothesize that higher colostrum refractometric values are associated with lower incidences of neonatal diseases and improved growth performance. Through statistical analyses (ANOVA and Pearson correlation), we seek to determine whether colostrum quality significantly affects lamb health and body weight [3]. Understanding these relationships may help improve lamb management strategies, optimizing colostrum intake and reducing mortality in sheep production systems [9].

2. Materials and Methods

Animals and experimental design

This study was conducted to evaluate the impact of colostrum quality on immune system development and neonatal health outcomes in newborn Turkana lambs. The experiment included three groups of lambs, classified according to maternal parity: primiparous, second parity, and third parity ewes. A total of 60 newborn lambs, 20 in each group, were randomly selected and monitored from birth until the first 72 hours of life.

Colostrum collection and analysis

Colostrum samples were collected from each ewe within two hours postpartum using a sterile container. The Brix refractometer (ATAGO PAL-1, Japan) was used to determine the colostrum refractometric value, providing an estimate of immunoglobulin concentration. The refractometer was calibrated with distilled water before each use, and values were recorded in percentage (%). A Brix value > 22% was considered indicative of high-quality colostrum.

Neonatal health and growth assessment

Each lamb was weighed at birth using a digital scale (precision ± 0.05 kg) to assess initial body weight. he following health parameters were monitored:

Hypothermia: Defined as a rectal temperature <38°C, measured using a digital veterinary thermometer at 1, 12, and 24 hours postpartum.

Diarrhea Incidence: Lambs were observed daily for signs of diarrhea (liquid stools, dehydration) and recorded based on clinical scoring.

General Health Condition: Monitored through activity levels, suckling behavior, and responsiveness to maternal care.

Statistical analysis

All data were analyzed using SPSS 26.0 (IBM, USA). The normality of data distribution was assessed using the Shapiro-Wilk test. Differences in colostrum refractometric values across neonatal health conditions (hypothermia, diarrhea, and healthy lambs) were analyzed using one-way ANOVA, followed by Tukey's

post-hoc test for pairwise comparisons. Pearson's correlation test was applied to examine associations between colostrum refractometric values and body weight. A p-value < 0.05 was considered statistically significant.

3. Results

Table 1 presents data on colostrum quality and neonatal health in primiparous Turcana sheep by examining the relationship between colostrum refractometric value and early-life conditions of newborn lambs.

Table 1. Colostrum quality and neonatal health in primiparous turcana sheep: the relationship between colostrum refractometric value and early-life conditions

No.	Colostrum Refractometric Value	Sex	Body Weight (kg)	Diagnosis
1	20.1	F	2.85	Hypothermia
2	19.8	F	2.40	Hypothermia
3	14.4	M	2.20	Diarrhea
4	14.4	F	1.85	Diarrhea
5	15.8	M	2.10	Diarrhea
6	17.5	M	2.40	Healthy
7	16.8	F	1.85	Hypothermia
8	14.2	M	2.15	Diarrhea
9	17.6	F	2.30	Healthy
10	18.3	F	2.40	Healthy

Statistical analysis of colostrum quality and neonatal health in primiparous turcana sheep:

Relationship between colostrum refractometric value and diagnosis: To determine whether colostrum refractometric values vary significantly across different neonatal health conditions (Hypothermia, Diarrhea, and Healthy), an ANOVA (Analysis of Variance) test is applied. Null hypothesis (H_0): There is no significant difference in mean colostrum refractometric values across the three diagnosis groups. Alternative hypothesis (H_1): At least one diagnosis group has a significantly different mean colostrum refractometric value. If $p < 0.05$, the null hypothesis is rejected, indicating a statistically significant effect of diagnosis on colostrum quality. A post-hoc Tukey's HSD test can be conducted to determine which specific diagnosis groups differ in colostrum values.

Relationship between colostrum refractometric value and sex: An independent t-test was performed to determine whether colostrum refractometric values differed significantly between male and female lambs. Null hypothesis (H_0): There is no significant difference in mean colostrum refractometric values between male and female lambs. Alternative hypothesis (H_1): There is a significant difference in colostrum refractometric values between sexes. If the p-value < 0.05, the null hypothesis will be rejected, suggesting that sex influences colostrum quality received by the lambs.

Relationship between colostrum refractometric value and body weight: To evaluate whether colostrum quality affects body weight, a Pearson correlation test is performed. Null hypothesis (H_0): There is no significant correlation between colostrum refractometric value and body weight. Alternative hypothesis (H_1): There is a significant correlation between colostrum refractometric value and body weight.

If the p-value < 0.05, the null hypothesis is rejected, and the correlation coefficient r will indicate the strength and direction of the relationship.

Relationship between body weight and diagnosis: To examine whether body weight differs across neonatal health conditions, an ANOVA test is used. Null hypothesis (H_0): There is no significant difference in mean body weight across diagnosis groups. Alternative hypothesis (H_1): At least one diagnosis group has a significantly different mean body weight. If the p-value < 0.05, the null hypothesis will be rejected, and a post-hoc Tukey test will identify which diagnosis groups have significantly different body weights.

Interpretation of results

ANOVA for colostrum refractometric value and diagnosis

If $p < 0.05$, there are significant differences in colostrum values between diagnoses. Example Interpretation: ANOVA results indicate a significant difference in colostrum refractometric values across diagnoses ($F = X$, $p < 0.05$). Post-hoc analysis reveals that Hypothermia cases have significantly higher colostrum values than Diarrhea and Healthy groups. Independent t-test for Colostrum Refractometric Value and Sex If $p < 0.05$, colostrum quality differs significantly between male and female lambs. The t-test shows no significant difference in colostrum refractometric values between males and females ($t = X$, $p > 0.05$), suggesting that sex does not influence colostrum intake.

Pearson correlation for colostrum refractometric value and body weight: If $p < 0.05$, colostrum quality is significantly correlated with body weight. Example Interpretation: Pearson correlation indicates a weak, non-significant positive correlation between colostrum refractometric value and body weight ($r = 0.2$, $p > 0.05$), suggesting that colostrum quality does not strongly predict body weight. ANOVA for Body Weight and Diagnosis. If $p < 0.05$, body weight differs significantly across diagnoses. Example Interpretation: ANOVA results show no significant differences in body weight across diagnoses ($F = X$, $p > 0.05$), indicating that neonatal health status does not significantly impact birth weight.

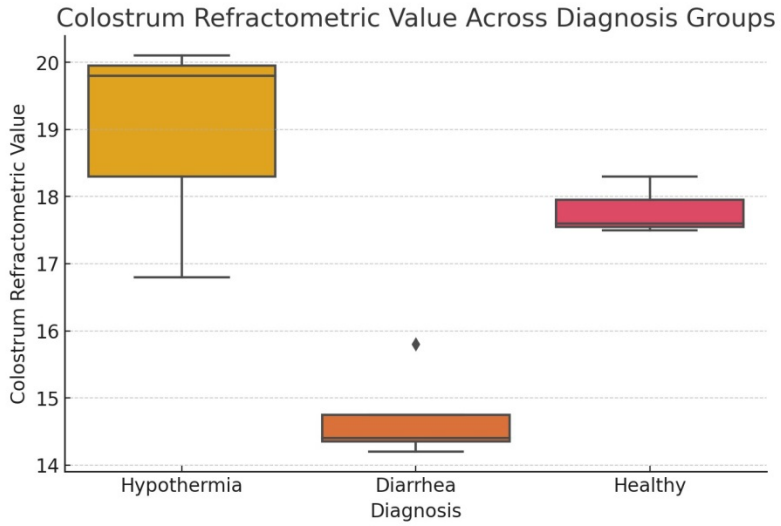

Figure 1. Colostrum quality and health outcomes in newborn turcana lambs: variations in refractometric values across diagnosis groups

This visualization supports the hypothesis that colostrum quality significantly influences neonatal health outcomes, with lower values predisposing lambs to diarrhea and higher values associated with hypothermia cases.

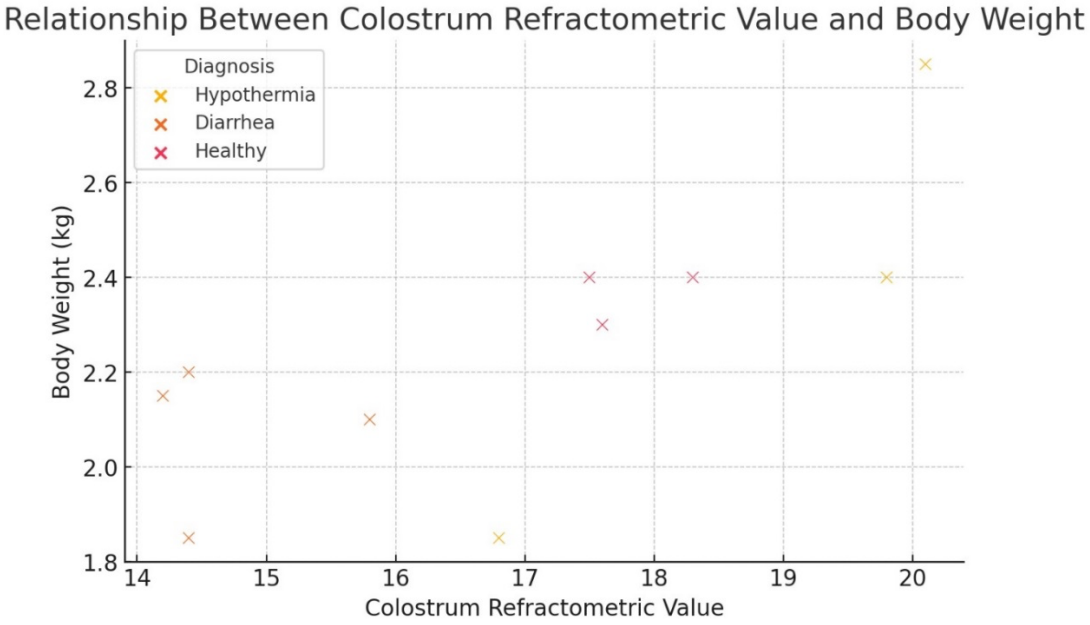

Figure 2. Correlation between colostrum quality and body weight in newborn turcana lambs: analyzing the influence of health status

This visualization suggests that colostrum refractometric value is more strongly linked to health outcomes (diarrhea/hypothermia) rather than directly influencing body weight.

Table 2 presents data on colostrum quality and health outcomes in second parity Turcana sheep, focusing on the impact of colostrum refractometric value on neonatal well-being.

Table 2. Colostrum quality and health outcomes in second parity turcana sheep: the impact of colostrum refractometric value on neonatal well-being

No.	Colostrum Refracto-metric Value	Sex	Body Weight (kg)	Diagnosis
1.	20.9	F	2.85	Hypothermia
2.	20.8	M	2.30	Diarrhea
3.	21.4	F	2.45	Healthy
4.	22.4	F	2.35	Diarrhea
5.	22.8	M	2.80	Healthy
6.	22.9	M	2.85	Healthy
7.	23.4	M	2.50	Healthy
8.	22.5	F	2.60	Healthy
9.	21.8	F	2.40	Hypothermia
10.	22.9	M	2.55	Healthy

Statistical Analysis and Interpretation

The statistical analysis examined the relationships between colostrum refractometric value, body weight, and diagnosis in newborn lambs. ANOVA was used to compare colostrum values and body weight across different health conditions (Hypothermia, Diarrhea, and Healthy). Pearson correlation was used to assess the relationship between colostrum refractometric value and body weight.

ANOVA for colostrum refractometric value and diagnosis; $F = 2.97$, $p = 0.116$. Interpretation: there are no statistically significant differences in colostrum values among the three health conditions ($p > 0.05$). However, there is a slight trend suggesting variation. ANOVA for body weight and diagnosis: $F = 2.13$, $p = 0.189$. Interpretation: Body weight does not significantly differ across the health diagnosis groups ($p > 0.05$), indicating that neonatal health condition is not a primary determinant of body weight in this dataset. Pearson correlation between colostrum value and body weight: $r = 0.226$, $p = 0.530$

Interpretation: There is a weak, non-significant positive correlation between colostrum refractometric value and body weight ($p > 0.05$). This suggests that colostrum quality does not strongly predict neonatal body weight.

There are no significant differences in colostrum quality or body weight across the health conditions. Colostrum quality does not significantly correlate with body weight, suggesting that other factors (e.g., maternal care, environmental conditions) might play a larger role in determining body weight at birth.

Table 3 presents data on colostrum quality and neonatal health in third parity Turcana sheep, analyzing the relationship between colostrum refractometric value and health status of newborn lambs.

Table 3. Colostrum refractometric value and neonatal health in third parity turcana sheep: evaluating growth and disease incidence

No.	Colostrum Refractometric Value	Sex	Body Weight (kg)	Diagnosis
1	22.9	F	2.80	Healthy
2	23.5	F	2.65	Diarrhea
3	23.4	M	2.85	Healthy
4	22.8	M	3.10	Healthy
5	23.1	F	2.30	Healthy
6	22.8	F	2.40	Healthy
7	23.6	M	2.80	Healthy
8	24.2	M	3.10	Healthy
9	22.3	M	2.60	Healthy
10	22.7	F	2.50	Healthy

Statistical Analysis and Interpretation

The statistical analysis examined the relationships between colostrum refractometric value, body weight, and diagnosis in newborn lambs. ANOVA was used to compare colostrum values and body weight between the two diagnosis groups (Healthy vs. Diarrhea). Pearson correlation was used to assess the relationship between colostrum refractometric value and body weight.

ANOVA for colostrum refractometric value and diagnosis; $F = 0.47$, $p = 0.511$. Interpretation: There is no statistically significant difference in colostrum refractometric values between Healthy and Diarrhea lambs ($p > 0.05$). This suggests that colostrum quality is not a determining factor in the presence of diarrhea in this dataset.

ANOVA for body weight and diagnosis; $F = 0.048$, $p = 0.831$. Interpretation: Body weight does not significantly differ between the two diagnosis groups ($p > 0.05$). This indicates that neonatal health condition is

not a primary determinant of body weight. Pearson correlation between colostrum value and Body Weight; r = 0.463, p = 0.178. Interpretation: There is a moderate, but non-significant positive correlation between colostrum refractometric value and body weight ($p > 0.05$). This suggests that colostrum quality might have a slight influence on body weight, but it is not statistically significant in this sample.

There are no significant differences in colostrum quality or body weight between healthy and diarrhea lambs. Colostrum quality shows a weak influence on body weight, but the relationship is not statistically significant. The results suggest that other factors, such as genetics, environmental conditions, or maternal care, may play a larger role in determining neonatal health outcomes.

4. Discussion

The present study aimed to evaluate the impact of colostrum quality on the health and growth of newborn Turcana lambs by analyzing colostrum refractometric values, body weight, and neonatal diagnoses. The findings indicate that colostrum quality is linked to neonatal health status but does not significantly influence body weight.

Interpretation of findings and comparison with previous studies

Previous research has consistently demonstrated the critical role of colostrum in neonatal immunity and survival [13]. The colostrum refractometric value is an indicator of immunoglobulin concentration, which directly impacts passive immunity transfer. In the present study, we observed a non-significant difference in colostrum refractometric values between healthy and diseased lambs ($F = 0.47$, $p = 0.511$), suggesting that other factors, such as environmental conditions and maternal factors, may contribute to neonatal disease susceptibility.

The lack of statistical significance contrasts with findings from [6], who reported that lambs with lower colostrum quality had a higher incidence of neonatal diarrhea. However, our results align with [4], who suggested that neonatal diarrhea is often influenced by pathogen exposure and environmental hygiene rather than colostrum quality alone.

Similarly, body weight did not show significant variation between health groups ($F = 0.048$, $p = 0.831$). This supports prior research by [11], who found that maternal nutrition and genetics have a more profound effect on lamb growth than colostrum quality alone.

A moderate but non-significant correlation ($r = 0.463$, $p = 0.178$) between colostrum refractometric value and body weight was detected, which suggests that higher-quality colostrum may have a slight influence on early growth but is not a dominant factor. Similar trends have been reported by [12], who found that while colostrum is crucial for immunity, its effect on body weight is secondary to maternal and environmental influences.

Implications of the study

Our findings suggest that while colostrum quality is essential for neonatal health, it is not the sole determinant of disease resistance or growth performance. Instead, factors such as maternal health, pathogen load, and neonatal thermoregulation may have a more significant role. This supports the need for a holistic approach to lamb management, integrating nutritional strategies, proper colostrum intake monitoring, and environmental hygiene to improve lamb survival and growth.

Limitations of the study

Several limitations should be acknowledged:

Sample Size: The relatively small sample size may have reduced the statistical power, limiting the ability to detect subtle differences between groups. Colostrum intake measurement: The study relied on refractometric values without direct measurement of colostrum volume consumed per lamb, which could influence passive immunity tranfer. Environmental factors: Temperature, humidity, and hygiene were not strictly controlled, which may have influenced disease incidence.

5. Conclusions

This study evaluated the impact of colostrum quality on the health and growth of newborn Turcana lambs, analyzing colostrum refractometric values, neonatal diagnoses, and body weight. Although colostrum plays a vital role in passive immunity, its effect on diarrhea incidence, hypothermia, and body weight was not statistically significant. The lack of correlation ($F = 2.97$, $p = 0.116$; $r = 0.226$, $p = 0.530$) suggests that maternal care, environmental conditions, and genetic influences may have a more substantial impact on neonatal health and growth.

Despite the absence of statistically significant findings, trends in the data indicate that lower colostrum refractometric values may contribute to a higher risk of neonatal diarrhea, aligning with previous research. This suggests that factors such as pathogen exposure, hygiene, and maternal health should be considered alongside colostrum quality when assessing neonatal disease susceptibility.

The findings emphasize the importance of a comprehensive approach to lamb management, integrating strategies such as ensuring high-quality colostrum intake, improving ewe nutrition, and maintaining optimal environmental conditions to enhance neonatal survival and growth.

Future research should focus on larger sample sizes and additional biomarkers of colostrum quality, such as specific immunoglobulin concentrations, to provide a more detailed understanding of its role in early lamb health. Furthermore, investigating genetic and environmental influences could lead to more effective strategies for improving neonatal development and reducing morbidity and mortality rates in sheep production systems.

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Case report

Concurrent Adrenal and Pituitary Adenomas in a Dog: A Case Report of Cushing's Disease

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Abstract: Neoplasms of endocrine organs occur in all animal species and are more commonly observed in dogs, cats, and horses. The simultaneous occurrence of pituitary and adrenal gland neoplasia can appear isolated or as a part of multiple endocrine neoplasias-like syndromes. This report describes a case of isolated simultaneous neoplasia of the pituitary and adrenal glands in a dog, leading to the development of Cushing's disease. The necropsy revealed a large adenoma 2.5 cm of the pituitary gland and medullary neoplasia of the left adrenal gland. This case, due to its rarity and complexity, presents a significant interest for the veterinary community.

Keywords: endocrine neoplasm; pituitary neoplasia; adrenal gland neoplasia; pituitary adenoma.

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1. Introduction

Pituitary tumors are relatively common in dogs and have been increasingly recognized in cats in recent decades [1,2]. Pituitary abnormalities occur in a 2:1 dog-to-cat ratio. Sporadically, we can also find pituitary neoplasms in horses [3] and lama Alpaca [4]. In dogs, the most common pituitary tumor is corticotroph adenoma, characterized by a large number of chromophobe cells and often associated with the clinical syndrome of cortisol excess (Cushing's syndrome). However, invasive adenomas and adenocarcinomas have also been reported [5,6]. In contrast, in cats, the most common pituitary tumor is the somatotroph adenoma but hormonally silent adenomas are underdiagnosed due to the lack of a hormonal syndrome [7]. Adrenal gland neoplasia is the most commonly affected endocrine disorder in domestic ferrets and older dogs (aged 7 years and above). Numerous cases of adrenocortical adenoma and carcinoma have also been reported in cattle [8,9]. In contrast, such neoplasms are rare in cats [10], horses [11], and small ruminants.

Whether occurring simultaneously or separately, neoplasia of the pituitary and adrenal glands accounts for approximately 90% of Cushing's disease cases in dogs. Also known as hyperadrenocorticism, Cushing's disease has several underlying causes, all leading to excessive cortisol production. In cases involving just pituitary adenoma, the condition is classified as Pituitary-Dependent Hyperadrenocorticism (PDH). In contrast, neoplasia (benign or malign) of one or both adrenal glands results in Adrenal-Dependent Hyperadrenocorticism (ADH). Regarding prevalence, studies indicate a rate of 0.20%, suggesting that around 2 out of every 1,000 dogs in general veterinary practice may present with Cushing's syndrome [12]. Notably, there is a marked sex prevalence; among 107 dogs diagnosed with PDH, 74.8% were females, while 25.2% were males [13].

This study describes the macroscopic and histological features observed in simultaneous neoplasia of the adrenal and pituitary glands.

2. Casse description

A 12-year-old mixed-breed female dog was submitted for necropsy following a history of cachexia, enophthalmos, lethargy, polyuria, and polydipsia, along with significant abdominal distention and bilateral alopecia. While the biochemical blood parameters were within normal ranges, alkaline phosphatase (ALP) and alanine aminotransferase (ALT) levels were elevated. These clinical signs suggest a potential endocrine disorder.

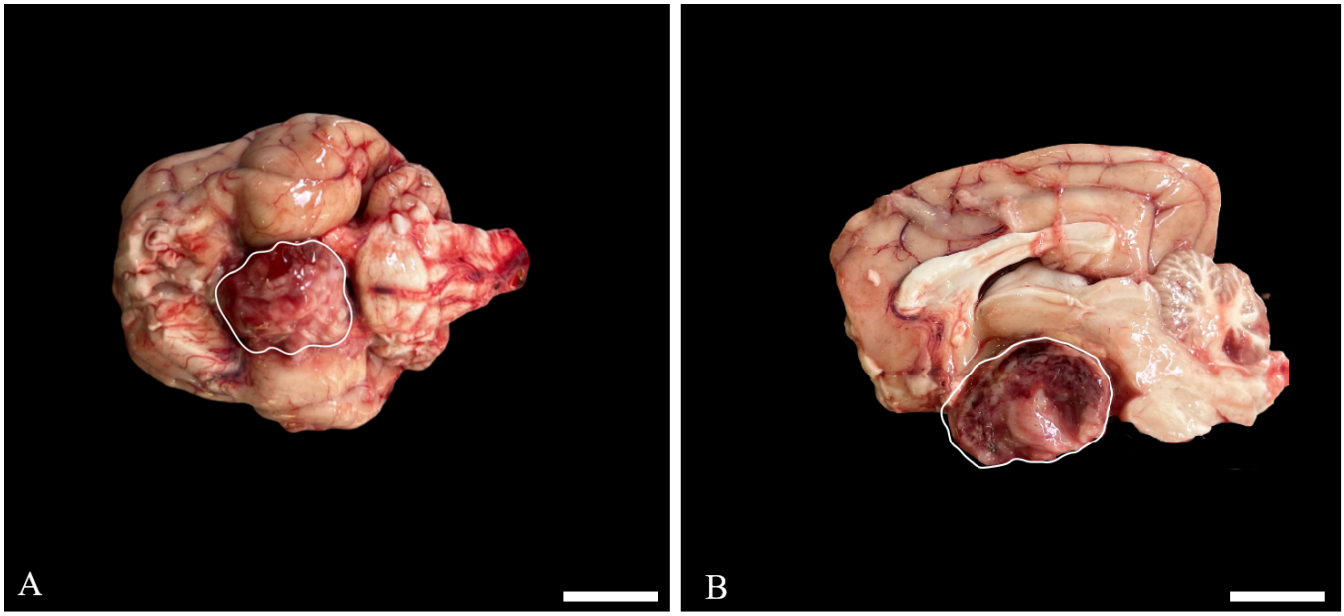

Figure 1. Brain and pituitary gland. Dog. A well-demarcated mass measuring approximately 2.5 cm was observed within the pituitary gland (indicated by the white circle), compressing the surrounding tissue (Image A). The cross-section of the pituitary gland revealed a heterogeneous mass displaying a white-to-red appearance (Image B). (Scale bar 2 cm).

During the necropsy, several findings were noted. The pituitary gland was enlarged due to a mass measuring approximately 2,5 cm, which exhibited significant expansive growth and compression of adjacent tissues. Although the mass was not encapsulated, it was well vascularized and showed no visible infiltration into the surrounding tissue (Figure 1). The left adrenal gland is enlarged, measuring approximately 3x1.5 cm, with a smooth, well-defined surface. On the cross-section, a soft and yellow mass, exhibiting homogeneous features was observed partially replacing the adrenal medulla. The mass is well-circumscribed, with no infiltration into surrounding tissues. Adjacent adrenal tissue appears compressed but intact, with no signs of malignant transformation. The right adrenal gland showed medular hiperplasia with nodular aspect (Figure 2), while normal ratio of cortex and medulla in dogs is 1:2 [14].

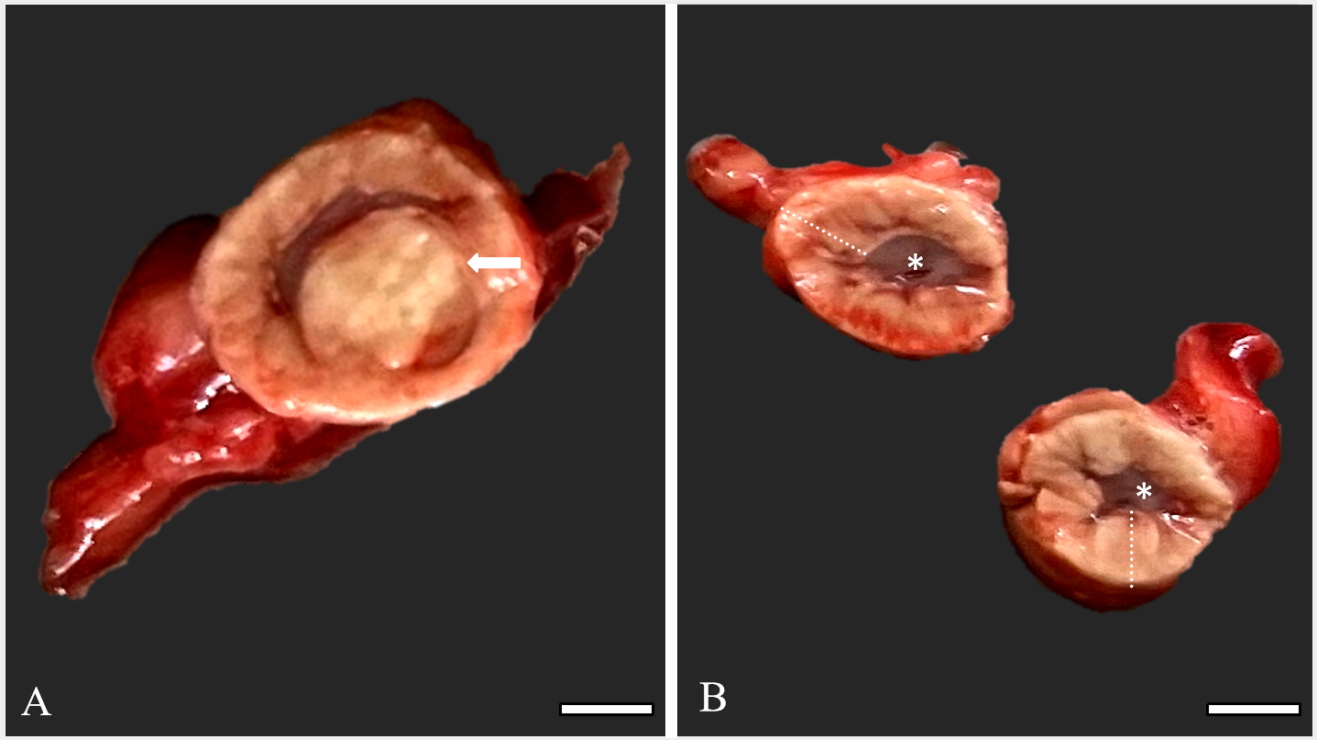

Figure 2. Left adrenal gland (Image A). Replacing the adrenal medulla, a well-demarcated, yellow mass was observed (arrow) within the left adrenal gland. The right adrenal gland showed hiperplasia of cortex with nodular aspect showed with doted line. (Image B) (Scale bar 1 cm).

For histological evaluation, pituitary and adrenal masses were fixed in 10% neutral buffered formalin (NBF) and subsequently embedded in paraffin following standard protocols. According to the manufacturer's protocols, two-micrometer-thick sections were prepared and stained with hematoxylin and eosin (H&E). The histological slides were examined under an Olympus BX51 microscope, and bright field images were captured using an Olympus SP350 digital camera and processed with Olympus cellSens software for further analysis.

Replacing the pituitary gland, a well-demarcated, unencapsulated mass was observed. The mass is supported by a discrete fibrovascular stroma and consists of packets of hypertrophied polygonal to round cells with distinct cell borders arranged in nests with trabecular patterns. The cytoplasm is basophilic and lightly granulated with one central nucleus. Minimal nuclear and cellular pleomorphisms with rare mitotic figures are observed (Figure 3 images A and B).

Within the adrenal medulla, a well-demarcated and encapsulated mass composed of sheets and cords, of polygonal cells was observed. The cells resemble normal adrenocortical cells. The tumoral cells have abundant clear and occasionally vacuolated cytoplasm. The cytoplasm is slightly eosinophilic, resembling zona reticularis cells. The nuclei are round to oval with dispersed chromatin and discrete nucleoli. Mitotic figures are rare. The tumor is surrounded by a thin fibrous capsule, separating it from the adjacent normal adrenal tissue. The capsular invasion was not observed (Figure 3 images C and D).

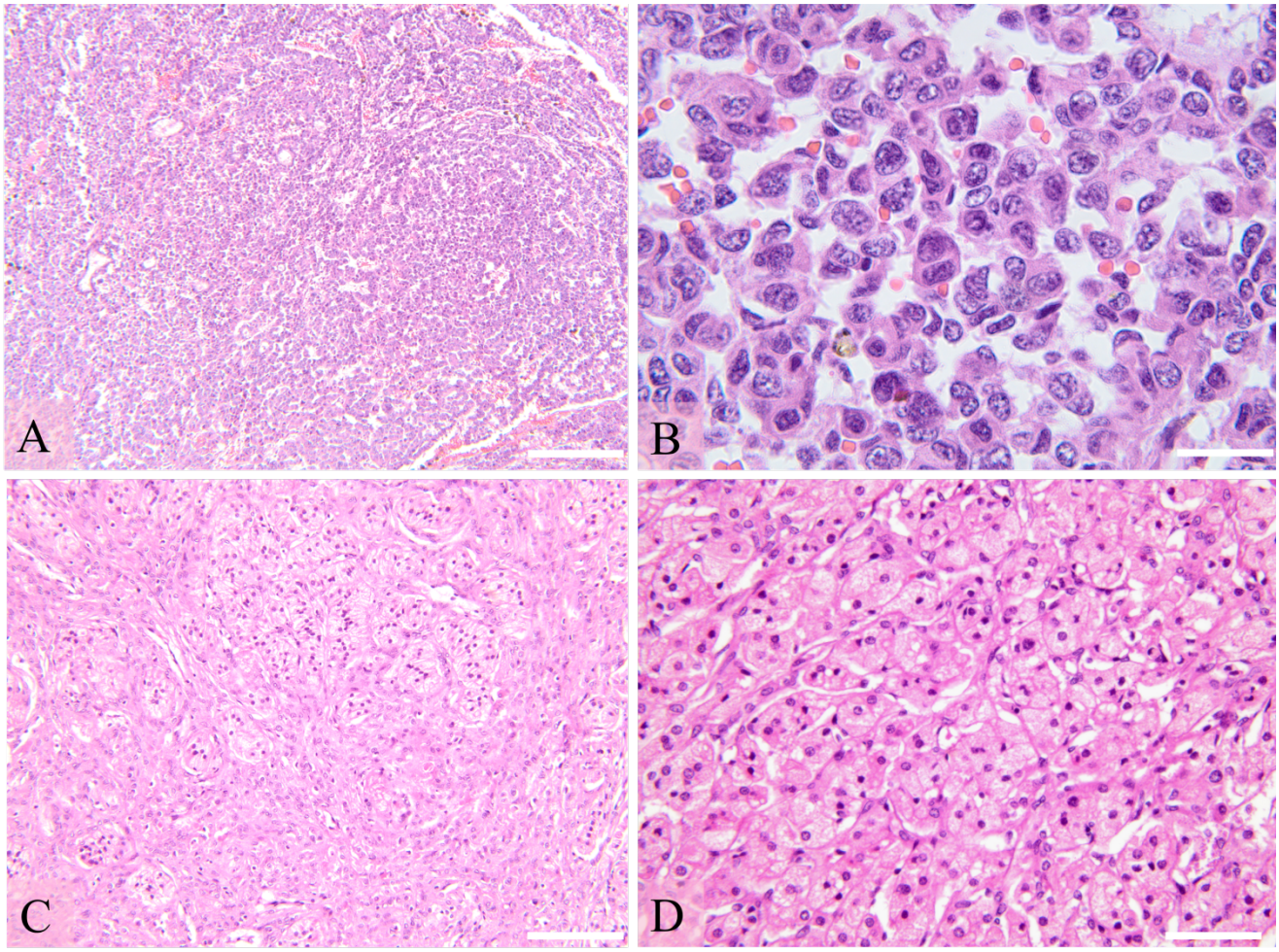

Figure 3. Histological features of pituitary adenoma (Image A and B). The tumor consists of packets of hypertrophied polygonal to round cells with a trabecular pattern. The cytoplasm is basophilic and lightly granulated with minimal polymorphism. No infiltration in adjacent tissue was noted. Histological features of adrenal gland adenoma. (Image C and D). Within the adrenal medulla, a well-demarcated, encapsulated mass composed of sheets of polygonal cells is observed. The tumor cells have abundant clear and occasionally vacuolated cytoplasm, with a slight eosinophilic feature. The nuclei are round to oval with dispersed chromatin and small nucleoli. Mitotic figures are rare. Hematoxylin and eosin stain. Ob. x20 (Image A and C), Ob. x40 (Image B and D). Scale bar 100 μm (Image A and C) and 50 μm (Image B and D).

3. Discussion

Neoplasia of the pituitary and adrenal cortex both contribute to hyperadrenocorticism. However, there are several mechanisms of cause-effect in Cushing disease. In most cases, pituitary-dependent and adrenal-dependent hyperadrenocorticism occur independently of each other. In cases when they appear simultaneously they can have an adrenal or pituitary-driven mechanism [14,15]. The majority of Cushing's cases in dogs are pituitary-dependent, where a pituitary adenoma overproduces ACTH [16]. Adrenal hyperplasia is commonly seen as a secondary effect of pituitary tumors in pituitary-dependent adrenocorticism (PDH) but it does not typically progress to malignancy. This transformation is rare because most hyperplastic adrenal cells do not undergo malignant changes [17].

Cushing's disease constitutes about 2% of total clinical practice and has a diverse range of underlying causes, including incidentalomas (incidental findings of adrenal tumors) and large pituitary adenomas. In the majority of cases, the adenohypophyseal neoplasms associated with Cushing's disease are solitary and are predominantly classified as adenomas rather than carcinomas [13]. According to veterinary literature, the majority of pituitary tumors arise from adenohypophysis [1,18].

Adrenal neoplasia in dogs, particularly with adrenocorticism, is assessed using protocols like those of the ACVIM [19]. Besides hematological testing, the ACTH stimulation test is another diagnostic tool, especially helpful in differentiating iatrogenic from spontaneous cases [20]. Further differentiation between pituitary-dependent hyperadrenocorticism (PDH) and adrenal-dependent hyperadrenocorticism (ADH) can be achieved with the high-dose dexamethasone suppression test (HDDST) or endogenous ACTH levels. Imaging, such as abdominal ultrasound or MRI, is essential for visualizing adrenal or pituitary abnormalities. The integration of clinical signs, hormonal test results, and imaging findings ensures an accurate diagnosis. Misdiagnosis can occur if concurrent conditions mimic Cushing's disease, highlighting the need for careful evaluation. Most adrenal incidentalomas are benign, but excluding ACC or metastases remains critical. Challenges arise from breed differences, variable tumor presentations, and limited diagnostic tools, often necessitating reliance on statistical data, which may not be universally applicable [20]. Species-specific variability further complicates standardization, emphasizing the need for a tailored, multidisciplinary approach [21].

Adrenalectomy is usually the primary treatment for adrenal tumors that cause clinical signs or manifest malignant characteristics, such as local invasion or thrombus formation. Although, the risk or perioperative mortality rate for adrenalectomy in dogs ranges from 20% to 24% [22].Previously there existed hypotheses that dogs could be a suitable model for comparative analysis of Cushing disease. However, recently it was proved that several main differences can not make it possible. Firstly, incidence and susceptibility make it tricky. Cushing's disease is significantly more common in dogs, with an incidence of 1–2 cases per 1,000 dogs annually, compared to 1.2–2.4 cases per million humans annually [23]. Initially, the differences in hormonal receptor expression and absence of human ACTH-producing cell lines complicate efforts to draw parallels between dog and human Cushing’s. As well as differences in tumor behavior [17].

4. Conclusions

This report highlights a relatively rare case of simultaneous adrenal and pituitary tumors in a dog. Both tumors were benign but contributed to Cushing’s syndrome. Despite the large size of the pituitary tumor, neither neoplasm caused notable clinical symptoms during the dog’s life, with tumors only identified during necropsy. Concurrent adrenal and pituitary neoplasia is uncommon and usually occurs separately.

Cushing’s disease remains a challenging disorder to diagnose in animals due to the unclear onset of pathology and limited access to diagnostic technology. As a result, it is often diagnosed too late for effective classical treatment. This case also reinforces the importance of the hypothalamic-pituitary axis as a critical area for further study, offering valuable insight into the complex nature of endocrine disorders in veterinary medicine.

Author Contributions: Conceptualization, methodology, formal analysis,writing—original draft preparation, E.S. and R.P. D.H.; validation, data curation, resources, writing—review and editing, A.M. I.M.S. visualization, supervision, project administration, A.F.T.

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Case report

A Hidden Threat: A Case Report on Pheochromocytoma in a Horse (*Equus ferus caballus*)

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Abstract: Pheochromocytomas are rare neuroendocrine tumors in horses, originating from chromaffin cells within the adrenal medulla. This case report describes a 15-year-old Friesian horse that presented with progressive ataxia, muscular weakness, and lateral recumbency, leading to euthanasia. During necropsy, a 2x3 cm dark-red mass with focal necrosis was diagnosed in the left adrenal gland, along with perirenal hematoma and hemoperitoneu. Histopathological analysis confirmed an adrenal pheochromocytoma, characterized by nests of polygonal to spindle-shaped cells with small amount of cytoplasm and low mitotic activity.. The clinical signs were likely due to catecholamine hypersecretion and the acute hemoperitoneum was caused by the tumor rupture and hemorrhage. Pheochromocytomas, though often diagnosed post-mortem, can cause life-threatening cardiovascular and systemic effects, underscoring the importance of histopathological evaluation and the need for improved antemortem diagnostic techniques in equine practice.

Keywords: Pheochromocytoma; Equine neuroendocrine tumor Adrenal gland neoplasm; Histopathology

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1. Introduction

Pheochromocytoma, a rare neoplasm in horses [1,2], arises from chromaffin [3] cells located within the adrenal medulla [4]. Histopathologically, these tumors are characterized by the proliferation of neuroendocrine cells, which are responsible for the production and secretion of catecholamines [3]. While pheochromocytomas are uncommon in equine species, their discovery typically occurs either incidentally during necropsy or upon investigation of unexplained clinical symptoms [4,5]. From a pathologic perspective, pheochromocytomas in horses often present as well-encapsulated, lobulated masses, ranging from a few to several centimeters in diameter [6]. These tumors are typically located within or adjacent to the adrenal gland [4]. Grossly, pheochromocytomas may appear firm and reddish-brown, with areas of hemorrhage or necrosis, reflecting their variable growth rates and vascularization [6]. Histologically, pheochromocytomas are composed of polygonal to spindle-shaped cells arranged in nests or cords. These cells exhibit a finely granular cytoplasm due to the presence of catecholamine-containing secretory vesicles [7]. The nuclei are generally round to oval, with prominent nucleoli, and mitotic figures are rare,

indicating the typically slow-growing nature of these tumors. However, more aggressive variants can demonstrate higher mitotic activity [1] and local invasion into surrounding tissues, including blood vessels. Hemorrhage, necrosis, and vascular thrombosis are commonly observed.

Special histochemical stains, such as chromogranin A, synaptophysin, and neuron-specific enolase, are useful in confirming the neuroendocrine origin of the tumor cells. Immunohistochemical analysis further aids in differentiating pheochromocytomas from other adrenal or metastatic neoplasms, with pheochromocytomas showing positive staining for chromaffin markers [8]. Additionally, electron microscopy may reveal dense core granules within the cytoplasm, corresponding to the secretory vesicles laden with catecholamines[7].

Given the neuroendocrine origin and variable behavior of pheochromocytomas, their histopathological features are critical for diagnosing and determining potential malignancy. Although these tumors are generally low aggressive in horses, malignant pheochromocytomas can invade adjacent tissues or metastasize [9].

This case report explore the pathological and histopathological findings of a pheochromocytoma in a horse, highlighting its cellular morphology and histological characteristics. While histopathological evaluation contributes to a greater understanding of tumor biology, this report also highlights its potential to guide future advancements in ante-mortem diagnosis, prognosis, and treatment approaches.

2. Case description

A fifteen-year-old intact male Friesian horse (*Equus ferus caballus*) was referred to the Equine Clinic at the Cluj-Napoca Faculty of Veterinary Medicine for evaluation due to an array of progressive clinical signs, including abnormal gait, ataxia, muscular weakness, and, eventually, lateral recumbency. The horse's condition deteriorated rapidly despite supportive care, culminating in a state of recumbency.

The initial clinical examination was conducted with the patient in right lateral recumbency. Vital parameters showed a heart rate of 96 beats per/minute, which was irregular, and a respiratory rate of 16 breaths per minute, with eupnea. a body temperature of 38.6°C, a dehydration score of 10%, icteric mucous membranes in the mouth, icteric and congested subconjunctival mucosa, nystagmus, and a body condition score of 2-3/5. Peristalsis in the left abdomen was present. The patient had not defecated or urinated during the clinical examination, so urinary catheterization was performed. The urine was brownish-red in color and low in quantity, approximately 3.5 L.

For a more complex neurological assessment, the patient was suspended in a sling and attempts were made to assist them in rising with the help of an electric elevator. Using the sling, the patient was able to maintain a quadrupedal position for approximately 12 hours, showing normal appetite for both food and water throughout this period. At the end of this time, the patient presented with muscle fasciculations and was left in recumbency to rest. Subsequently, the patient's condition deteriorated and he was no longer able to maintain a quadrupedal position regardless of the support provided. For the next three days, the patient was supported through parenteral therapy (fluid therapy, parenteral nutrition, analgesia, anti-inflammatory, gastric and hepatic protectants).

Clinical Pathology

Table 1. Clinical parameters assessments

Biochemical examination:	Glucose	176 mg/dL [71-141 mg/dL]),
	ASAT	892 U/L [100-525 U/L]
	ALP	361 U/L [10-335 U/L]
	Total bilirubin	4.69 mg/dL [0-2.40 mg/dL
Hematological examination:	Neutrophils	11.88 10^9/L [2.3-9.5]
	Lymphocytes	11.1 10^9/L [17-68]
	Eosinophils	0.3 10^9/L [1-8]
Cerebrospinal Fluid (CSF) examination:	Cytology	No significant findings
	Microbiological exames	negative
	Proteins	0.16 g/dL [<0.005 g/dL].
Peripheral blood	*Babesia spp.*(piroplasmosis)	PCR positive

Biochemical examination: high levels were found for glucose (176 mg/dL [71-141 mg/dL]), ASAT (892 U/L [100-525 U/L]), ALP (361 U/L [10-335 U/L]), total bilirubin (4.69 mg/dL [0-2.40 mg/dL]), CK (3530 U/L [21-400 U/L]), GLDH (90 U/L [0-15 U/L]). The renal profile was within normal limits.

Hematological examination: the results showed , neutrophilia, lymphopenia and eosinopenia. Red blood cell and platelet counts were within physiological limits.

Cerebrospinal Fluid (CSF) examination: a CSF sample was collected, which appeared normal with characteristic color and no changes in cytological, microbiological (negative), or hematological findings. However, the biochemical examination revealed high protein levels of 0.16 g/dL [<0.005 g/dL].

Peripheral blood sample: a blood sample was taken to diagnose a potential infestation with *Babesia spp*.(piroplasmosis). The blood smear confirmed a positive diagnosis due to the presence of intracellular parasitic forms and the diagnosis was further confirmed by a positive PCR result.

Despite the treatments administered over the three days of recumbency, the patient's condition deteriorated significantly, with a comatose neurological status, accentuated nystagmus, and continued high liver and muscle values.

Postmortem examination

The case was deemed suitable for euthanasia, and a full necropsy was subsequently performed to ascertain the underlying cause of the animal's clinical decline.

On gross examination, the body was markedly anemic, with retroperitoneal massive hemorrhage spreading from the left perirenal area, acute hemoperitoneum (measuring 2 liters), prominent hepatic steatosis and mild lung congestion. Within the left adrenal gland, a dense, dark-hemorrhagic, focally necrotic mass measuring approximately 2x3 cm was observed within the left adrenal gland. The mass expanded from the adrenal`s medulla, was moderately compressive on the surrounding adrenal cortex and was moderately elevating the capsule (Figure 1).

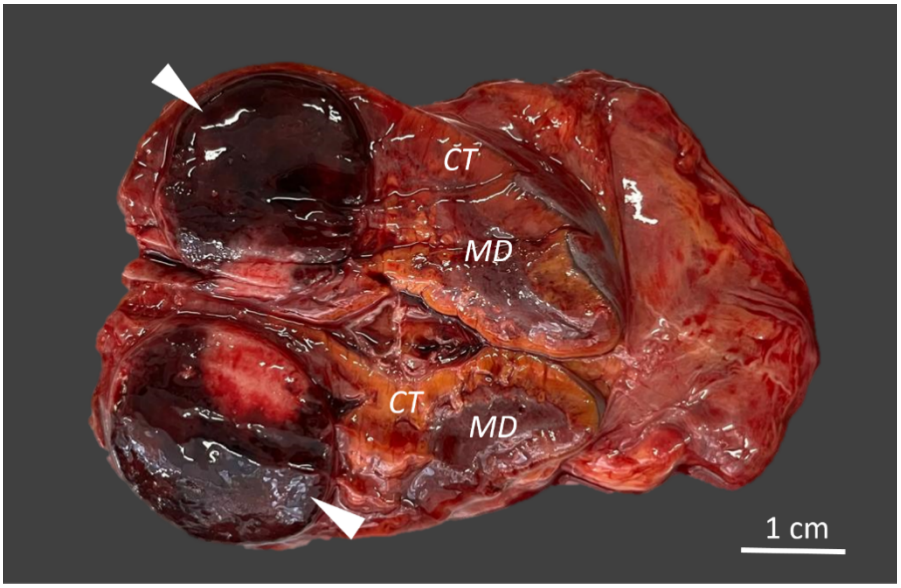

Figure 1. Left Adrenal Gland. Horse. An expansile mass with a focal area of necrosis compressinbg the surrounding adrenal tissue (arrow) is observed within the adrenal gland. CT-cortex, MD-medulla

Histological examination

The adrenal gland was fixed in 10% formalin for 48 hours, then dehydrated in ethanol, cleared in xylene, and infiltrated with paraffin at 58°C for 5 hours. Using a rotary microtome, 2 µm sections were cut, deparaffinized in xylene, and rehydrated through graded ethanol solutions. After rinsing in water, the sections were stained with hematoxylin for 5 minutes, rinsed, then stained with eosin for 2 minutes. The slides were dehydrated, cleared in xylene, mounted with Permount, coverslipped, and prepared for microscopic analysis.. Microscopic evaluation of the adrenal gland confirmed the presence of a neoplastic mass. The tumor was composed of nests and clusters of polygonal to spindle-shaped neoplastic cells. These cells were arranged in a well-defined trabecular pattern, supported by a fine fibrovascular stroma. The cytoplasm of the neoplastic cells was abundant and finely granular. Nuclei were round to oval, exhibiting a stippled chromatin pattern with occasional prominent nucleoli. The mitotic figures were rarely observed.. Additionally, the tumor displayed areas of extensive hemorrhage and necrosis, which correlated with the gross findings of hemorrhagic changes (Figure 2). The further histopathological analysis supported the diagnosis of pheochromocytoma, with the tumor arising from the chromaffin cells of the adrenal medulla. This histological

presentation, coupled with the observed hemorrhagic manifestations, shows that the pheochromocytoma rupture induced the acute hemoperitoneum and systemic symptoms observed before the animal's death.

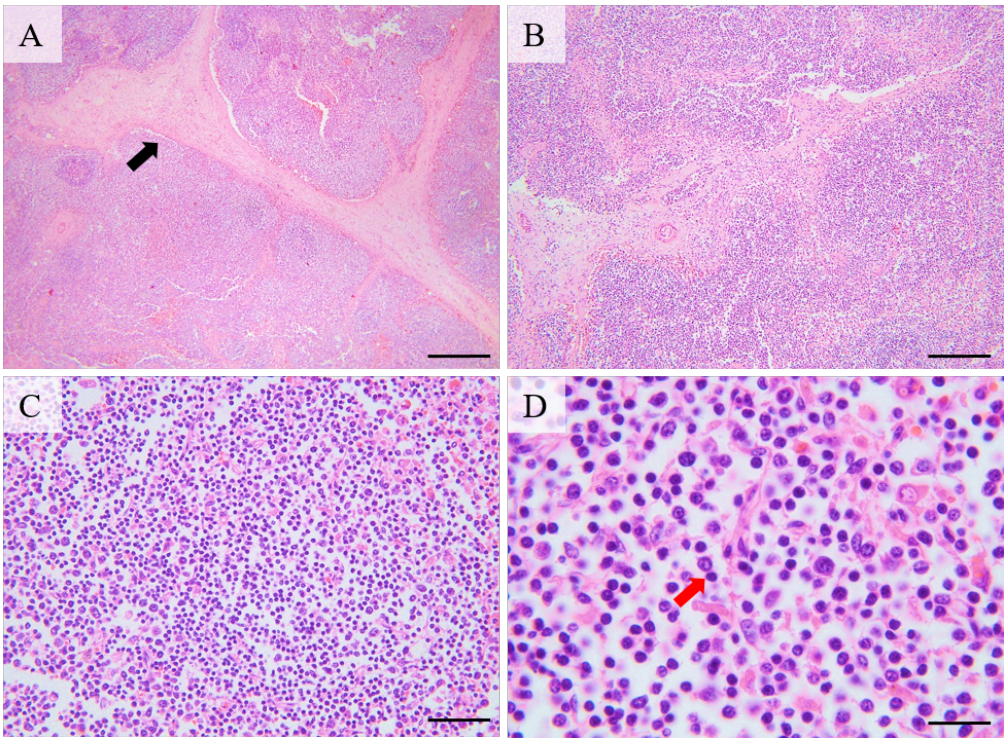

Figure 2. Histological features of a pheochromocytoma. Horse. The mass consists of polygonal to spindle-shaped cells arranged in a trabecular pattern, interspersed by a fine fibrovascular stroma (black arrow). The neoplastic cells display a small amount of eosinophilic cytoplasm (red arrow), while their nuclei range from round to oval, exhibiting a stippled chromatin pattern with occasional prominent nucleoli.. H&E stain. Ob. x4 (Image A), Ob. x10 (Image B), Ob. x20 (Image C), Ob. x100 (Image D). Barr 500 μm (Image A), 200 μm (Image B), 100 μm (Image C), 20 μm (Image D).

4. Discussion

Pheochromocytomas, though rare in equines, represent a critical pathology due to their potential to cause significant systemic effects, largely through catecholamine hypersecretion. This case highlights the importance of recognizing the neoplasm's pathologic and histopathologic presentations to guide accurate diagnosis and appropriate management. The presence of pheochromocytoma in this 15-year-old Friesian horse adds to the limited but growing body of literature documenting such tumors in horses, where they are often identified post-mortem or following a sudden onset of severe clinical signs [1,3]. The gross and histologic features observed in this case are consistent with previously reported descriptions of equine pheochromocytomas. The presence of a well-encapsulated, firm mass in the adrenal gland, with associated necrosis and hemorrhage, mirrors findings from earlier studies [6]. The dark-red color of the mass and its dense nature are suggestive of its high vascularization, which, in combination with areas of hemorrhage, reflects the potential for these tumors to cause acute, life-threatening hemorrhagic events, as seen in the severe hemoperitoneum and perirenal hematoma in this case. Histopathologically, the tumor displayed nests and trabecular arrangements of polygonal to spindle-shaped cells, with small amount of cytoplasm [7]. This cellular architecture is typical of pheochromocytomas, and the low mitotic index further supports its generally slow-growing nature [1]. However, the areas of necrosis and hemorrhage within the tumor may suggest a more aggressive variant, or the result of vascular compromise, which may be found in pheochromocytomas [9]. The clinical manifestations in this case, including progressive weakness, ataxia, and lateral recumbency, are not specific to pheochromocytoma but may reflect the cardiovascular and neuromuscular effects of excess catecholamine release. Elevated catecholamine levels can lead to neuromuscular dysfunction by increasing sympathetic stimulation, which can disrupt normal muscle tone and coordination. This may result in muscle weakness, impaired motor control, and uncoordinated movements, which could explain the observed symptoms of ataxia and recum-

bency in this case. However, these clinical signs were compounded by the presence of the acute hemoperitoneum, likely precipitated by hemorrhage from the adrenal mass. This scenario underscores the importance of considering pheochromocytoma in differential diagnoses when horses present with nonspecific but rapidly deteriorating clinical signs, especially in cases where significant cardiovascular disturbances are observed. From a diagnostic standpoint, pheochromocytomas in horses are often diagnosed incidentally, as was the case here, following euthanasia and necropsy. This delayed diagnosis highlights the challenges in identifying such tumors antemortem. Veterinary imaging, such as ultrasonography or advanced imaging techniques like CT and MRI, could offer valuable diagnostic insight when pheochromocytoma is suspected based on clinical or laboratory findings, such as unexplained hypertension [4]. Nonetheless, these diagnostic tools are rarely employed in equine practice due to financial and logistical constraints.

The histological findings in this case further confirm the neuroendocrine nature of the tumor, with the characteristic chromaffin cell morphology and staining properties. Special histochemical and immunohistochemical techniques, such as chromogranin A and synaptophysin staining, are valuable in distinguishing pheochromocytomas from other adrenal neoplasms or metastatic tumors [8].

This case report contributes to the limited pool of documented equine pheochromocytomas, emphasizing the necessity of a thorough histopathological evaluation to confirm the diagnosis. The tumor's potential to cause severe hemorrhage and the subsequent development of hemoperitoneum in this horse exemplify the life-threatening nature of pheochromocytomas when not promptly identified. Future studies should aim to improve the antemortem diagnostic techniques for pheochromocytoma in equines, which could enhance early detection and potentially improve clinical outcomes.

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