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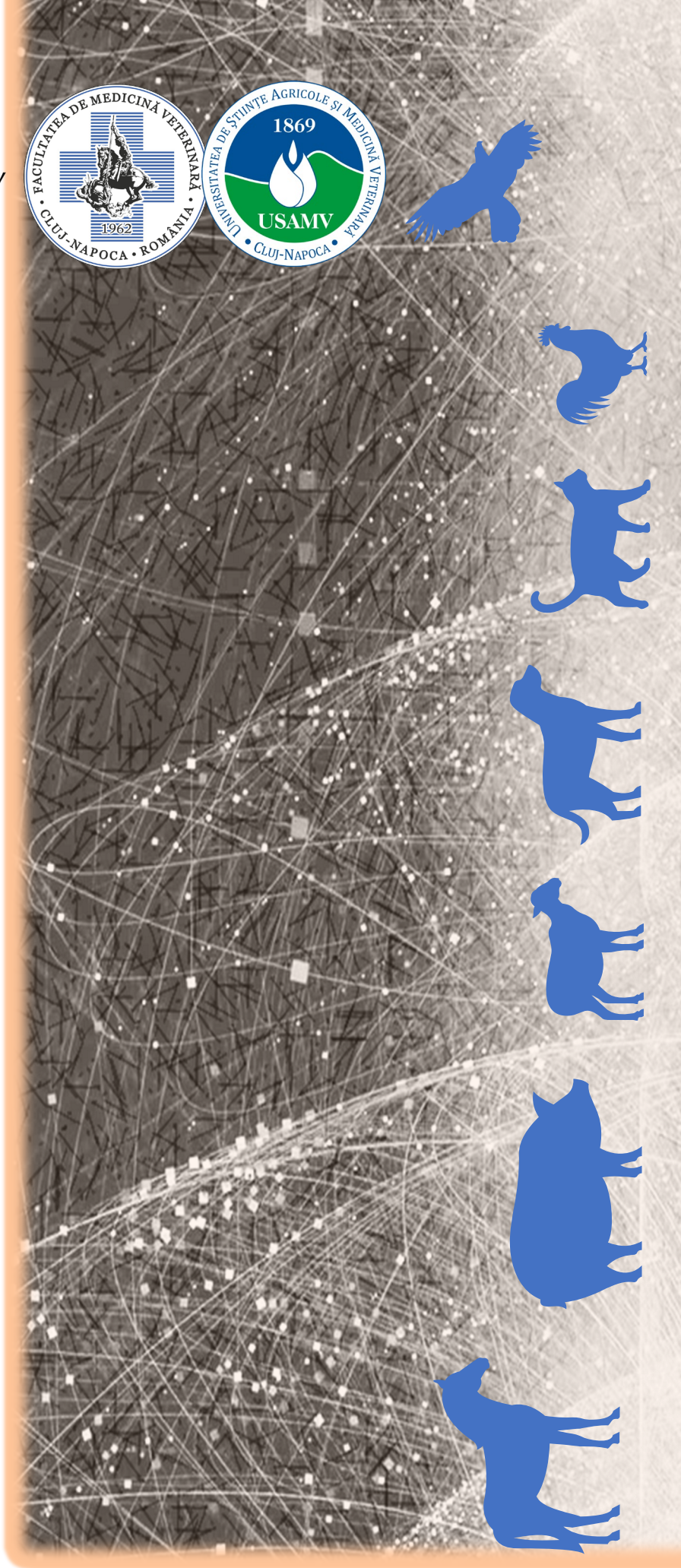

Societatea Romana Veterinara de Neurologie, Neurochirurgie si Medicina comportamentala
NEUROVET

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Research article

Macroscopic comparative aspects among two species of birds of prey: Falco tunninculus (Common kestrel) and Tyto alba (Barn owl)

Alexandra-Iulia Preja[1], Mircea Florin Cipou[1]*, Alexandru N. Stermin[2] and Aurel Damian[1]

[1] University of Agricultural Sciences and Veterinary Medicine, Department of Comparative Anatomy of domestic animals, Cluj-Napoca, Romania; e-mail: alexandraiuliap@gmail.com; mircea.cipou@usamvcluj.ro; damian56aurel@yahoo.com;
[2] Babeș-Bolyai University, Department of Taxonomy and Ecology, Romania; e-mail: alexandru.stermin@ubbcluj.ro;
* Correspondence: M.F.C. mircea.cipou@usamvcluj.ro;

Abstract: Birds of prey are at the top of the food chain and play an essential role in controlling populations of birds and rodents that are harmful to habitat. This study was conducted on 11 bird carcasses, 5 Falco tunniculus carcasses and 6 Tyto alba carcasses, donated by the UBB Zoological Museum of Academic Cultural Heritage, in order to examine the gross anatomical structures of the digestive system and to highlight the anatomical differences in both carnivorous species. Dissections were conducted at the Department of Comparative Anatomy at the Faculty of Veterinary Medicine in Cluj-Napoca, according to an established protocol. The beak is short and slightly curved in both species studied, the tomial tooth being highlighted in Falco tunniculus. Conical papillae and salivary duct openings are more numerous in both species. The oropharyngeal cavity has lateral longitudinal folds of the tongue and glottis, with a distensible esophagus along its whole length in Tyto alba. Falco tunniculus, however, has ingluvium and a less distensible oesophagus. The stomach is undeveloped in both species, with the appearance of an elongated pear, and the small intestine varies in length, shorter in Falco tunniculus than in Tyto alba, but in both species it is arranged in several loops with the help of the mesenterium. The cecum is different, poorly developed, vestigial type in Falco tunniculus, and well developed, with two elongated caecal protrusions in Tyto alba. The digestive system is characteristic of carnivorous species and is a reflection of how it has adapted to feeding behavior.

Keywords: oesophagus, ingluvium, cecum, nocturnal raptor, diurnal raptor

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1. Introduction

Birds of prey play a significant part in the ecosystem because they are at the top of the food pyramid, and their digestive system is adapted to a strictly carnivorous diet (Ford, 2010). The term "raptor" refers to a wide variety of bird species with different natural history, anatomical and dietary characteristics. In general, when we talk about raptors, we most often talk about eagles, falcons and owls. Although these birds share similar characteristics, they are framed into separate taxonomic groups [11].

The morphology of the gastrointestinal tract, metabolic performance and the physiology of digestion have evolved over time according to the principle of integrality, to satisfy nutritional requirements based on the food available in the natural habitat [9]. During evolution, the avian digestive system underwent changes to make the flight easier: the teeth disappeared, the digestive tract is shortened, and he avier organs (liver, muscle stomach) crowded around the centre of gravity [4]. The digestive system may be represented as a continuous tube, with an opening to two ends: the beak, at the oral end, and the anocecal orifice, at the posterior end [9]. Compared to mammals, birds do not have teeth and jawbone muscles are much less developed [4].

The purpose of this study is to examine the anatomical structures of the digestive tract for two species selected from the Falconiform and Strigiform orders, Falco tunniculus (Common kestrel), of the order Falconiformes, family Falconidae, and Tyto alba (Barn owl), of the order Strigiformes, family Tytonidae, and to bring to light the ana-

tomical differences of the digestive system in both carnivorous species.

Falco tunniculus or the Common kestrel is a common bird and one of the most common diurnal raptors in Romania, adapted to various habitats: it is found in mountain regions, in the plains, in the urban environment [7]; typically consumes small mammals, particularly mice, birds; in warmer areas, the diet is composed of insects and lizards [3].

Tyto alba or the Barn owl is a nocturnal bird, found in the western and northern parts of the country, nesting in agricultural areas with sparsely planted groves, gardens [7]. It feeds on small mammals, birds (including other Strigiformes, but smaller in size), frogs, moles, bats [3].

2. Materials and Methods

The birds we had examined came from the Zoological Museum of the University Cultural Heritage of BBU (Babeș-Bolyai University). Birds died from accidents (electrocution, road accident) or were euthanized due to injuries that no longer allowed them to be rehabilitated in nature. Each bird is accompanied by a note on which is written the year of death, the provenance and the name in Romanian/Hungarian or Latin.

The opening of the body was carried out in several stages. The dissection was performed after the protocol and instructions used in the Faculty of Veterinary Medicine of Cluj-Napoca [2].

The plucking of the corpses was carried out strictly in the area of the incisions. At the level of the head, the skin from the lateral commissure of the beak is incised. The skin incision continues on the ventral side of the neck, lateral to the trachea and up to the level of the cloacal orifice. With a pair of scissors, we made transversely section of the abdominal muscles from the posterior part of the sternum to the posterior of xiphoid appendix. On each side of the sternum, the initial abdominal incision is continued up to the level of the chondrocostal junctions. The abdominal wall is sectioned longitudinally up to the cloaca and is turned laterally. The sectioning of the chondrocostal joints is continued with scissors, bilaterally, up to the level of the scapulohumeral joints, the coracoid bones and the clavicle are sectioned, the sternum is removed after disengaging the pericardial sac. The organs located in the cavity are detached, both commissures of the beak are sectioned, the lower mandible is detached, along with the oesophagus and a portion of the trachea. The skin and muscles from the cloacal orifice were sectioned and we detach it together with the digestive tube and the accessory digestive organs. The digestive system was examined only macroscopically, *in situ*, and separated from the carcass. The oesophagus, proventriculus, ventriculus (gizzard), small intestine, large intestine, cloacal orifice were opened with scissors. A digital camera, Nikon COOLPIX P900, was used to make the images.

3. Results and discussions

In Falco tinnunculus, the beak is strong and curved; the upper jaw is more developed than the lower jaw. The tomial tooth, located in the upper jaw, is well developed. At the base of the beak, the ceroma is identified, which surrounds the nostrils (Fig 1). These peculiarities have been pointed out by Murray [11], Ford [6], Lacasse [10]. During examination of the oropharyngeal cavity, the partially hard, conical papillae may be identified, which surround the edge of the hard palate. Caudal to choana, the openings of the salivary glands can be highlighted in large numbers, particularly on the sides of the infundibular cleft. The tongue is short, with the rostral portion firm and rough. The conical, partially hard papillae can be identified at the base of the tongue, arranged in the form of the letter V, with an aboral opening. In addition, caudal to the glottis, the same hard, conical papillae can be seen. The oropharyngeal cavity has numerous openings of the salivary glands close to the base of the tongue and in the aboral part of the hard palate (Fig.2). These features have not been documented in the literature on this species.

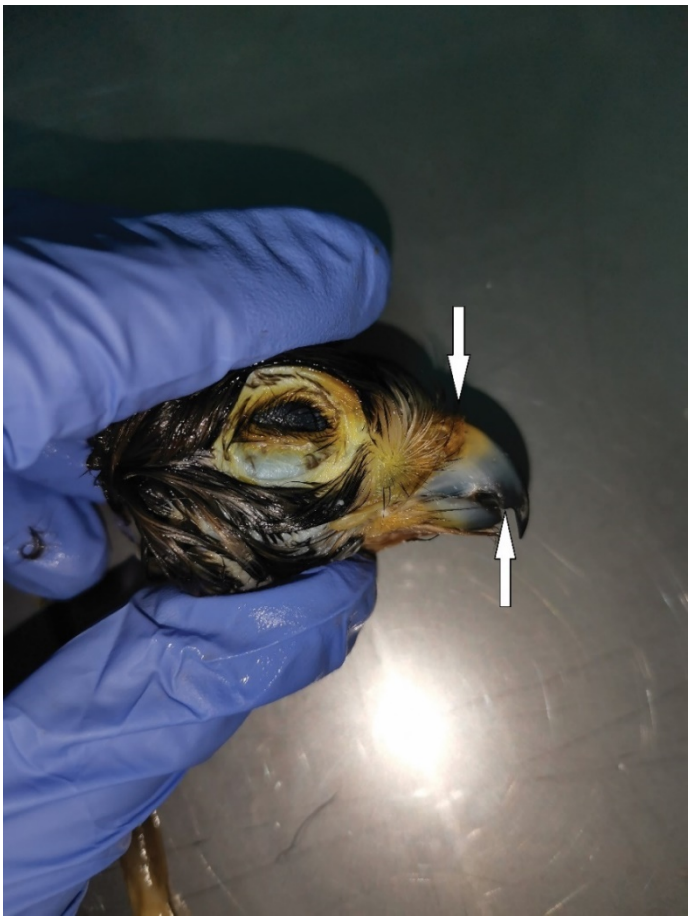

Figure 1. Head *Falco tunninculus*. Arrows-ceroma that encompasses the narinas and the tomial tooth

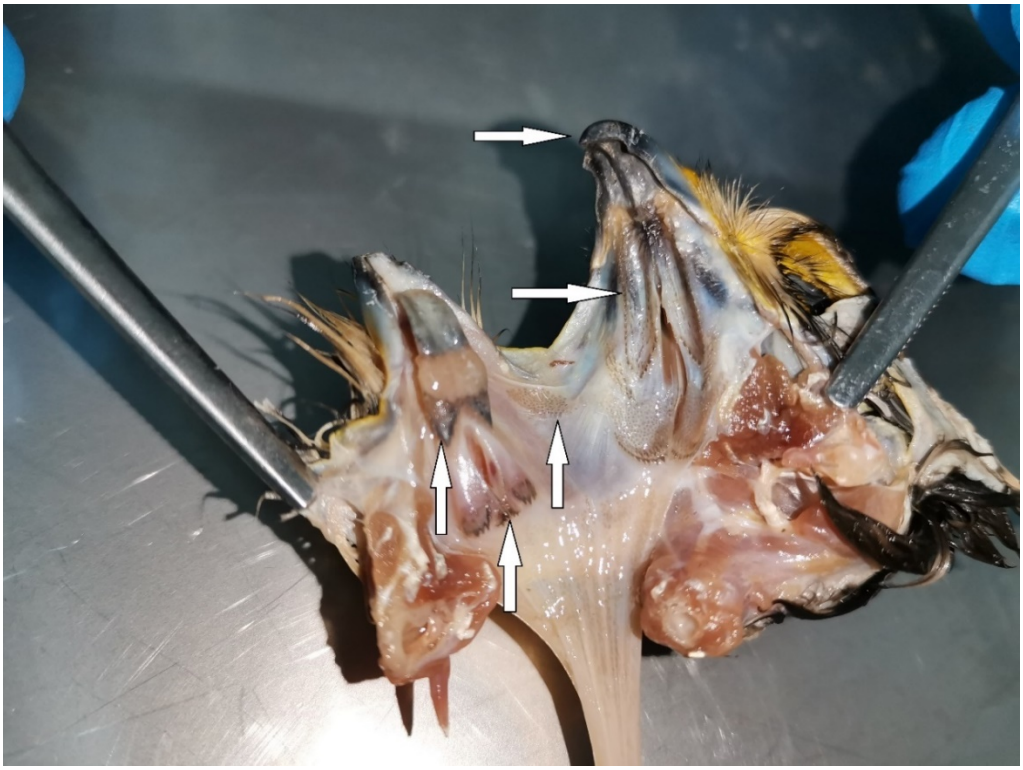

Figure 2. Oro-pharyngeal cavity of *Falco tunninculus*. The first arrow points at the tip of the beak. All the other arrows indicate the presence of hard papillae on the floor, tongue and glottis, respectively the openings of the salivary gland

Table 1. Measurements taken during dissections on the bodies of *Falco tunninculus*

Nr. ord.	Body weight	Length of body	TD weight without skull	Liver weight	Liver length	Liver width	TD weight without liver	Long. Esopha-gus+proven tric+ventric le	Length of small+lar ge in-testine
1	102 g	20 cm	4 g (without skull)	1 g	2 cm	4 cm	4 g	11,5 cm	40 cm
2	130 cm	29 cm	16 g (without skull)	4 g	3,5 cm	5 cm	10 g	10, 5 cm	54 cm
3	120 g	32 cm	11 g (without skull) 27 g (with skull)	1 g	2 cm	3 cm	8 g (with-out skull) 33 g (with skull)	11 cm	43 cm
4	107 g	30 cm	10 g (without skull) 30 g (with skull)	1 g	3 cm	3 cm	8 g (with-out skull) 28 g (with skull)	11 cm	45 cm
5	108 g	30 cm	8 g (without skull) 27 g (with skull)	2 g	2,5 cm	3,5 cm	8 g (with-out skull) 26 g (with skull)	12 cm	15 cm

Compared to the previously described species, Tyto alba has a short beak with a pointed ventral tip. The cerome was identified at the base of the upper jaw, but not around the nostrils. The oropharyngeal cavity doesn't have a soft palate and a pharyngeal isthmus. Conical and hard papillae are present on the surface of the hard palate, most highlighted caudal in the choana and lateral in the infundibular cleft (Fig.3), on the lingual surface, in the caudal part, at the lingual base, on the surface of the glottis (Fig.4). Salivary gland channel openings are evident on the floor of the oropharyngeal cavity and its lateral surfaces (Fig.4). The oropharyngeal cavity has longitudinal folds, most visible near the lingual base and on the glottis side, at the entrance of the esophagus. These characteristics are not reported in the literature for this species.

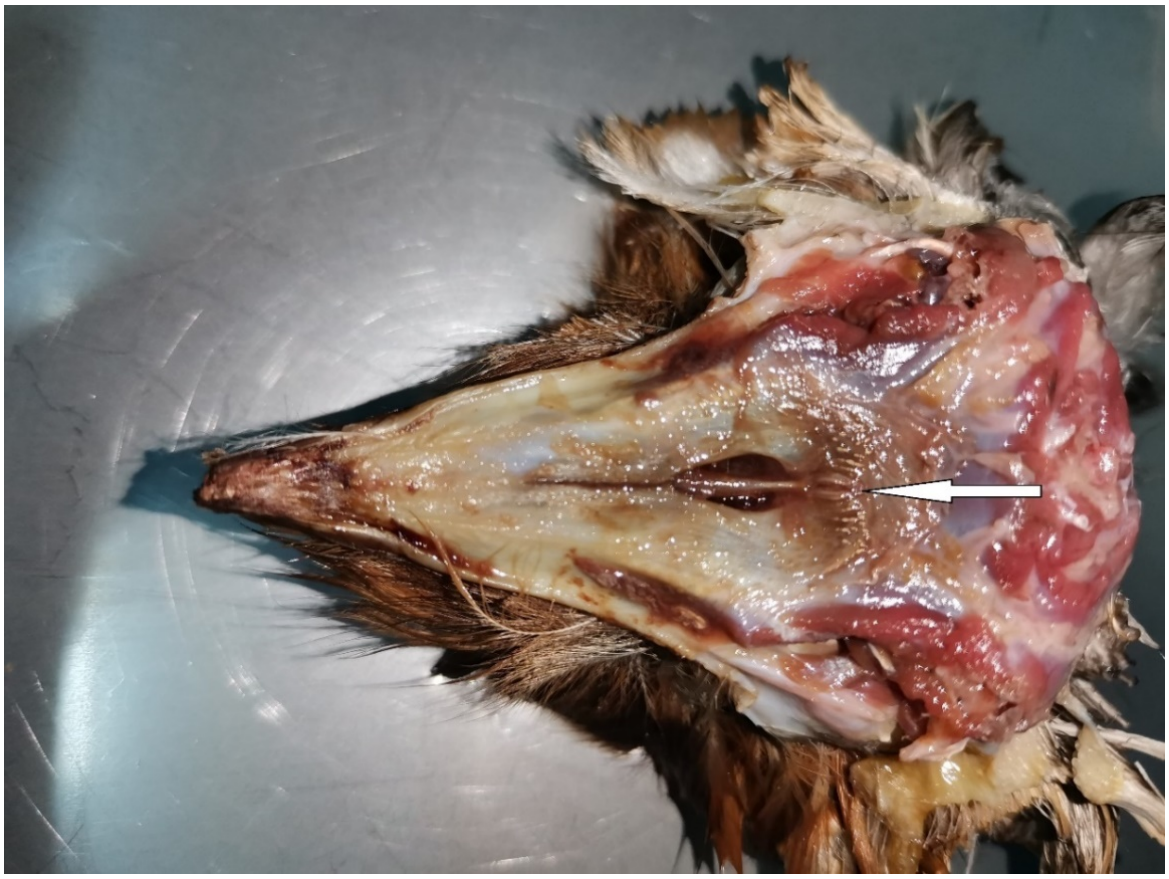

Figure 3. The top of the Oro-pharyngeal cavity at *Tyto alba*. Arrow-presence of conical papillae

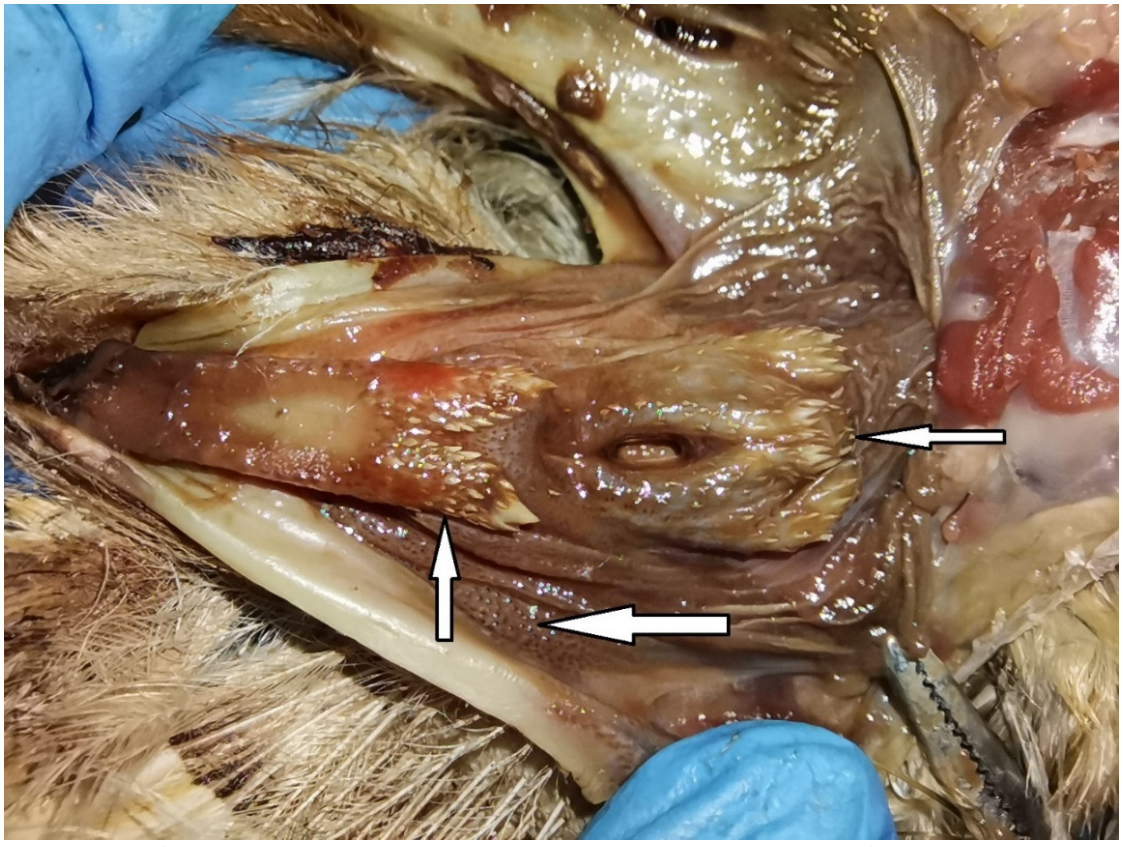

Figure 4. The top of the Oro-pharyngeal cavity at *Tyto Alba*. Arrows-presence of hard papillae and salivary glands openings

Table 2. Measurements taken during dissections on bodies of *Tyto alba*

Nr. ord.	Body weight	Length of body	TD weight without skull	Liver weight	Liver length	Liver width	TD weight without liver	Long. Oe-sophagus+ proventric+ ventricle	Length of small+ large intestine
1	287 g	30 cm	x	X	x	x	x	x	x
2	291 g	33 cm	x	X	x	x	x	x	x
3	295 g	31 cm	x	X	x	x	x	x	x
4	309 g	30 cm	26 g (without skull)	6 g	5 cm	4 cm	20 g	13 cm	41 cm
5	203 g	32 cm	14 g (without skull) 50 g (with skull)	3 g	3,5 cm	4,5 cm	13 g (without skull) 58 g (with skull)	14 cm	35 cm
6	274 g	31 cm	26 g (without skull)	X	x	x	20 g	15 cm	44,5 cm

In the case of the Common kestrel, the esophagus is short and it presents a crop or ingluvies. The crop is represented by an enlargement of the cervical part of the esophagus, with the function of food storage, with a fusiform aspect. This aspect was highlighted by Duke et al. [5], who note that the crop, the glandular and the muscular stomach are similar in appearance and have the same dimensions as other birds from the family Falconidae. The stomach, the next part of the digestive tract, is considered a dilated continuation of the oesophagus, has a pear-shaped form and is situated to the left of the median and dorsal line to the liver; it is divided into proventriculus and gizzard (the proventriculus is placed before the gizzard). Cranially, the proventriculus is separated from the esophagus by a constrictive region. Caudal, the proventriculus is separated from the gizzard by a reduced constriction zone, and no obvious boundaries can be observed inside. The longitudinal folds observed on the inner surface of the oesophagus do not occur at the proventricular level. Proventriculus is underdeveloped, and many openings in the secretory gland can be seen on its surface. The gizzard is similar to a biconvex lens with thick sides (Fig. 5). Due to the advanced state of decomposition of the cadavers, the internal particularities of this intestinal segment cannot be highlighted.

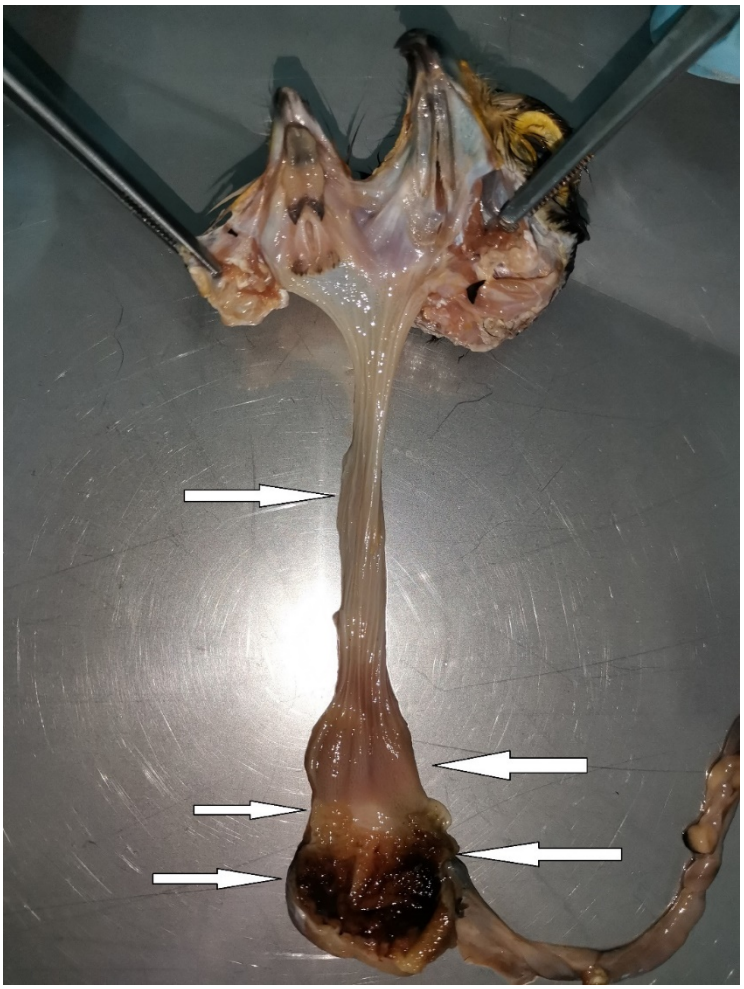

Figure 5. Cranial portion of the digestive tract in Falco tunninculus. The arrows (in order) the ingluvies or the crop, the proventriculus, the passage area between the glandular and muscular stomach, the gizzard and the pyloric orifice, near the passage area between the proventriculus and the gizzard

Tyto alba has a long, narrow, straight esophagus which stretches from the level of the oropharyngeal cavity to the level of the glandular stomach. This aspect was highlighted by Umar and Atabo, [12]. The longitudinal folds are visible throughout the surface of the esophagus; they suddenly disappear with the transition between the esophagus and the glandular stomach. The crop is not observed. The transition between the glandular stomach and the muscular stomach is abrupt, without a isthmus or zona intermedia gastric. The glandular stomach is small in size, with many openings of the secretory gland on its surface. The ventricle is well developed, with thick walls (Fig. 6), but because of the advanced state of decomposition of the corpses, no other morphological peculiarities can be distinguished.

Figure 6. Gastrointestinal tract in Tyto alba. The arrows indicate, in order, the oesophagus, the proventricle, the opening of the pyloric hole, near the passage between the glandular and muscular stomach, the gizzard.

For both species studied, the pyloric orifice opens on the right side of the gizzard, close to the transition zone between the proventriculus and gizzard (Figs 5, 6). Falco tunniculus has a short small intestine arranged in multiple loops, it occupies mainly the caudal part of the coelomic cavity, situated on the right side of the proventriculus and the gizzard. This aspect was underlined by Al-Aaraji and Al-Kafagy, [1]. Because of the advanced state of decomposition of the bodies, a clear dividing line between duodenum, jejunum and ileum cannot be established. Tyto alba has a short small intestine; the duodenum comes from the proximal part of the gizzard, is long and folded in several loops using the mesentery fold. This aspect was highlighted by Umar and Atabo, [12]. Because of the advanced state of decomposition of the corpses, a clear line between duodenum, jejunum and ileum cannot be drawn.

The ceca is located at the ileo-cecal junction. Falco tunniculus presents a poorly developed, vestigial cecum (Fig. 7). Hongxing et al., [8], argues that the ceca of this species is underdeveloped compared to herbivorous species. The ceca in Tyto alba is a well-developed organ, with two long tubular projections, which attach to the small intestine with the help of the mesentery (Fig. 8), a feature brought up by Umar and Atabo, [12]. For both species, the large intestine is short and straight, it opens outward through the cloaca opening.

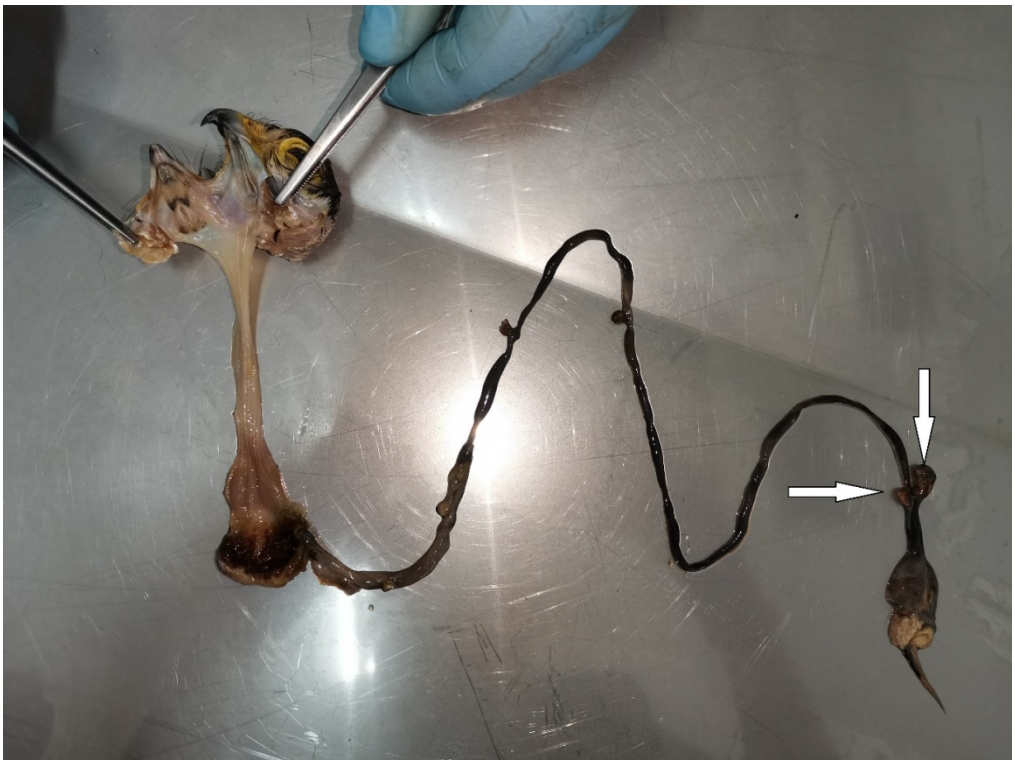

Figure 7. Falco tunninculous digestive tract. The arrows indicate the ceca.

Figure 8. Digestive system in Tyto alba. The arrow indicates the ceca.

The pancreas has not been identified in any examined bodies of the Falco tunninculus species. In Tyto alba, the pancreas may be identified at the first duodenal loop, with an elongated appearance (Fig. 9). It has not been reported in the literature.

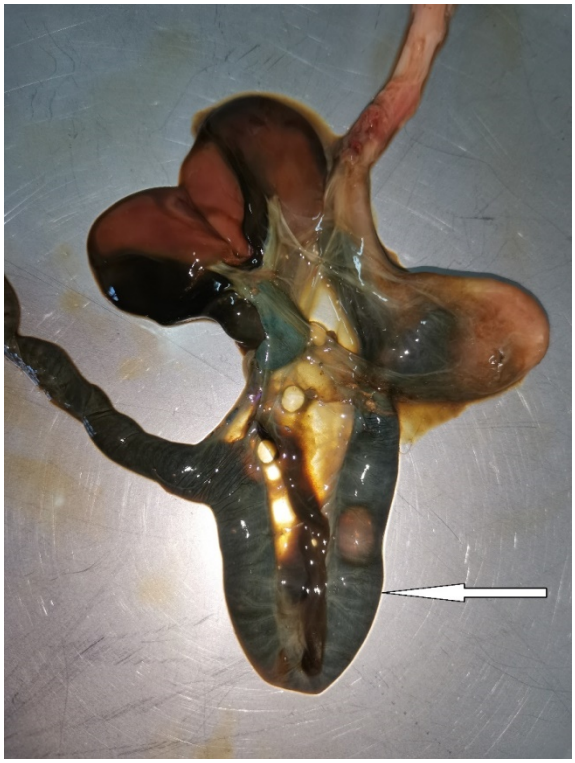

Figure 9 First duodenal loop in Tyto alba. In the duodenum loop, the pancreas is indicated by an arrow.

In both species examined, the liver consists of two lobes, the right liver lobe is more developed than the left liver lobe, which cranially surrounds the apex of the heart and joins on the midline. The Falco tunninculus gallbladder is well developed, located on the visceral surface of the right lobe of the liver, as reported in the literature by Murray [11]. (Fig 10, 11). In the bodies examined by Tyto alba, the gall bladder is not well developed (Fig 12, 13), located on the visceral side of the right lobe of the liver. This aspect was not reported in the literature.

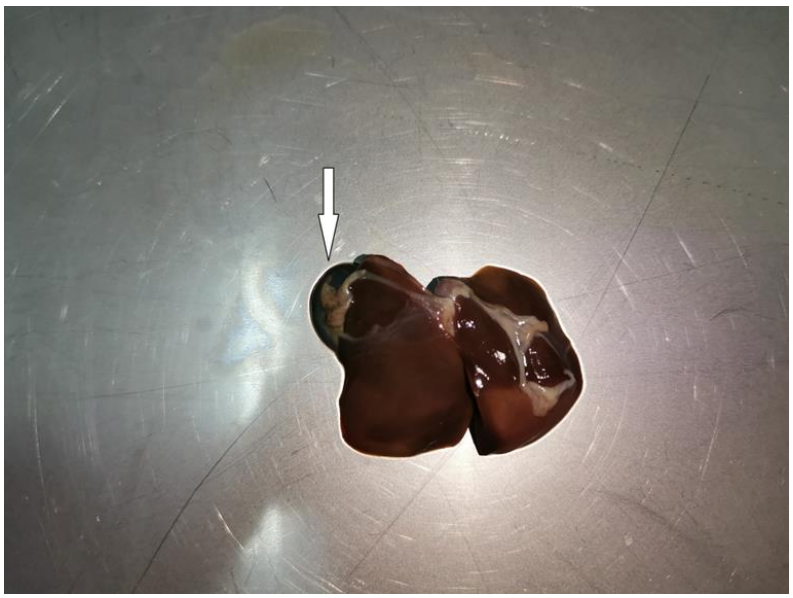

Figure 10. The appearance of the parietal surface of the liver in Falco tinnunculus. The arrow indicates the gall bladder.

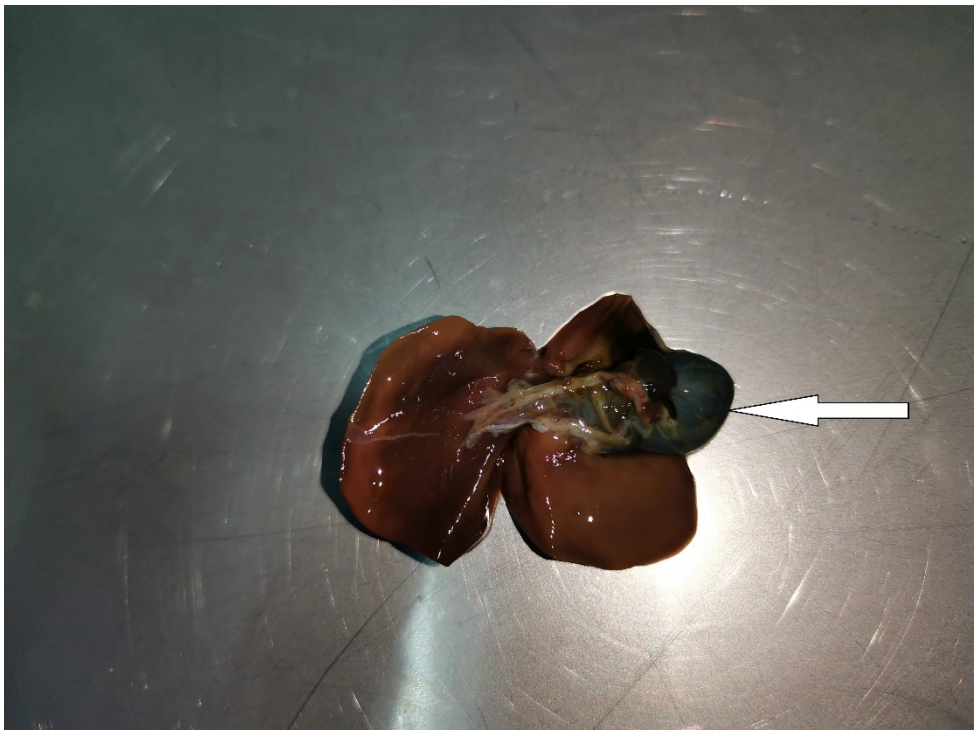

Figure 11. The visceral surface of the liver in Falco tunninculus. The arrow indicates the gallbladder

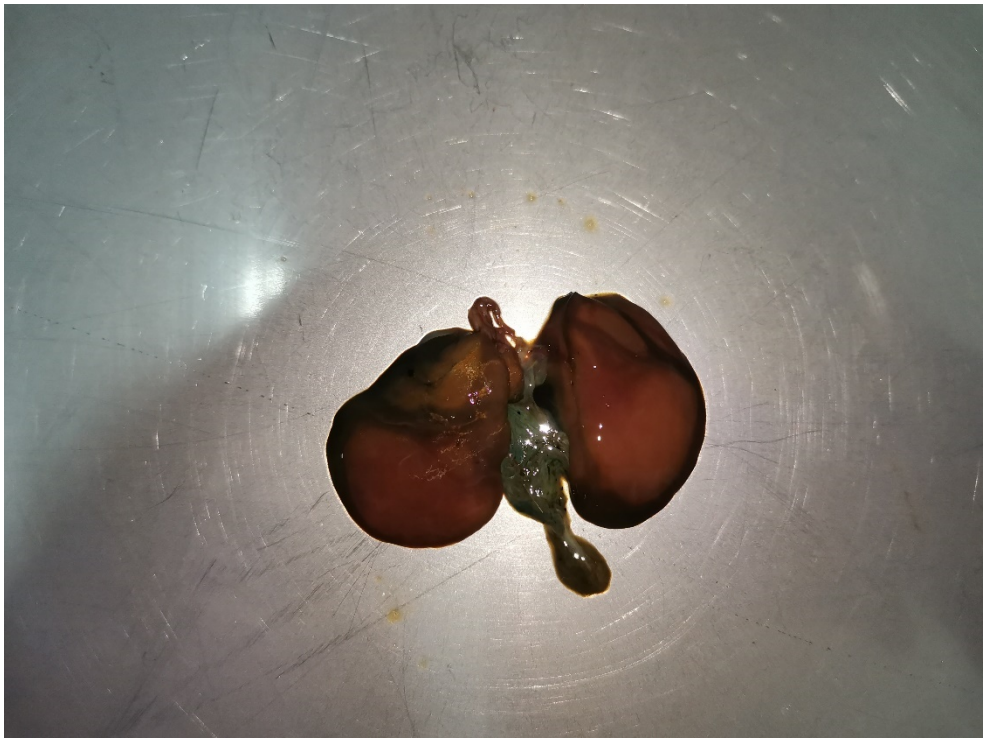

Figure 12. Parietal liver surface in Tyto alba

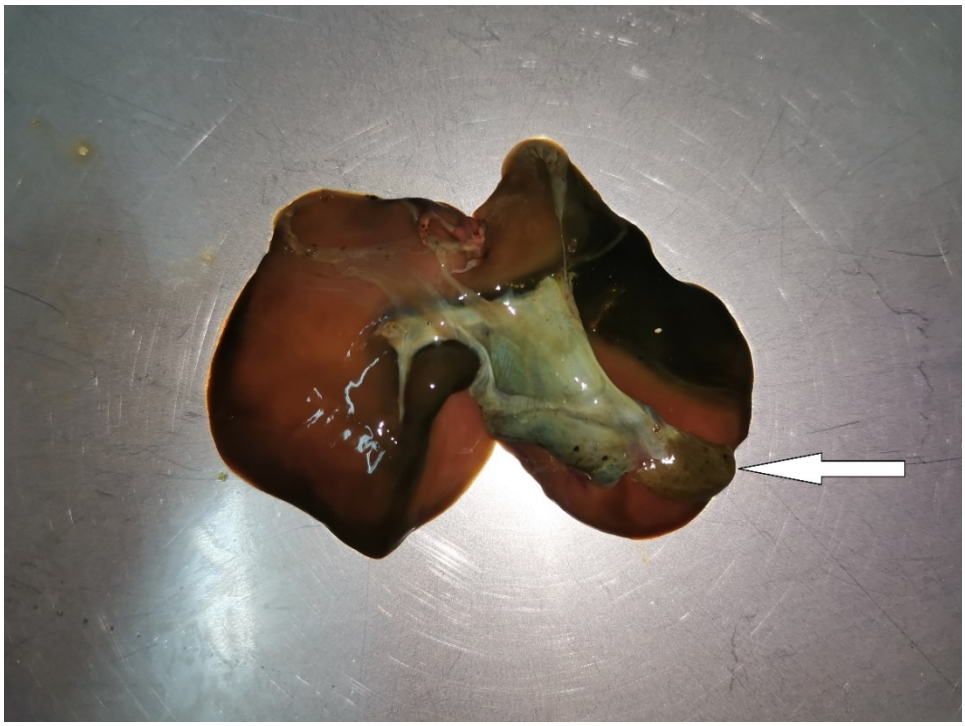

Figure 13. The visceral liver surface in Tyto alba. The arrow indicates the gallbladder.

4. Conclusions

The digestive system of the two species studied is adapted to a strict carnivore diet. The beak varies depending on the reference species, but both species have short, light beaks. *Falco tunninculus* has a tomial tooth, a characteristic formation of the upper jaw and found in many bird species, in particular those of the family *Accipitridae, Falconidae* and *Laniidae*.

Both species have hard, conical papillae in the oropharyngeal cavity, with multiple openings of the secretory gland. In *Tyto alba*, the oro-pharyngeal cavity can increase its volume with the help of longitudinal folds, an appearance observed near the lingual and lateral base of the glottis at the entrance to the oesophagus. In the case of Falco tunninculus species, the esophagus shows a crop in its cranial segment, with fusiform-like aspect. The crop is absent in *Tyto alba*. *Tyto alba* has a distensible esophagus throughout its surface, with well-highlighted longitudinal folds, which suddenly disappear at the boundary between the esophagus and the glandular stomach. Both species have weak, pear-like stomachs, divided into two chambers, the proventriculus or glandular stomach and the gizzard or muscular stomach, separated, on the internal surface, by a weak isthmus. The small intestine is short in both species in relation to the length of the body, but is arranged in multiple loops with the help of the mesentery fold. The cecum, located at the ileoceal junction, is little developed at *Falco tunninculus*, with a vestigial aspect, and well developed at Tyto alba, with elongated cecal extensions. The liver has two liver lobes, the right liver lobe is more developed than the left liver lobe. *Falco tunninculus* has a well-developed gallbladder in comparison with Tyto alba, located on the visceral side of the right liver lobe.

Supplementary Materials: Figure S1: title, Table S1: title, Video S1: title.

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Data Availability Statement: In this section, please provide details regarding where data supporting reported results can be found, including links to publicly archived datasets analyzed or generated during the study. You might choose to exclude this statement if the study did not report any data.

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Conflicts of Interest: “The authors declare no conflict of interest.” “The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results”.

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Research Article

Comparative evaluation of florfenicol and polymeric nanoparticles loaded with florfenicol against bacterial strains isolated from chickens

Emilia TRIF, Constantin CERBU, Marina SPÎNU, Diana Ioana OLAH, Adrian Valentin POTÂRNICHE, Sergiu Dan ZĂBLĂU, Florina MARIAN, George HERȚANU, Emoke PALL, Gheorghe Florinel BRUDAȘCĂ

University of Agricultural Sciences and Veterinary Medicine Cluj Napoca, Faculty of Veterinary Medicine, 3-5 Calea Mănăștur, 400372, Cluj-Napoca, Romania
* Correspondence: constantin.cerbu@usamvcluj.ro

Abstract: Antimicrobial resistance (AMR) poses a significant threat to both human and animal health, necessitating the search for alternative antimicrobial agents and strategies. In this study, we aimed to identify and isolate clinical bacterial strains from chickens and evaluate their sensitivity to florfenicol, a common antimicrobial agent that is used exclusively in veterinary medicine, along with polymeric nanoparticles loaded with florfenicol at various concentrations. Three clinical bacterial strains (*Escherichia coli*, *Enterococcus faecalis* and *Enterobacter cloacae*) were successfully isolated and identified from chicken presenting clinical signs. In order to assess their susceptibility, the isolated strains were subjected to a standard disc diffusion assay using florfenicol. Subsequently, polymeric nanoparticles loaded with florfenicol were tested at six different concentrations and compared their efficacy against the bacterial strains. Our results demonstrated that all three clinical bacterial strains exhibited varying degrees of resistance to florfenicol. Interestingly, the use of polymeric nanoparticles loaded with florfenicol did not displayed enhanced antimicrobial activity compared to the free drug. Notably, the efficacy of the loaded nanoparticles did not significantly vary with different concentrations of active substance. This study highlights the importance of exploring novel therapeutic approaches to combat antimicrobial resistance. The use of polymeric nanoparticles loaded with florfenicol presents a promising avenue for overcoming resistance mechanisms and improving the efficacy of antimicrobial treatments both in human and veterinary medicine. Further investigations are needed to elucidate the underlying mechanisms and optimize the formulation of polymer nanoparticles for enhanced therapeutic outcomes in combating AMR.

Keywords: florfenicol; antibiotic-loaded nanoparticles; antimicrobial resistance;

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1. Introduction

The discovery and integration of antimicrobial substances in the twentieth century stands as a remarkable achievement in modern medicine. This category of active substances has revolutionized the treatment of infectious diseases, ranging from minor to life-threatening complex surgical procedures, feasible organ transplantation to more effective chemotherapy treatment protocols [1]. However, the alarming rise of antimicrobial resistance (AMR) on a global scale poses a significant threat, potentially reversing the progress made and returning us to a time similar to the pre-antibiotic era. Furthermore, the economic impact of AMR is staggering, with a significant loss of $3 trillion in gross domestic product [2]. AMR is an inevitable consequence of the evolutionary process, as organisms develop genetic mutations to evade the lethal selective pressures imposed by antibiotics [3]. As long as antimicrobial substances continue to be utilized against human, veterinary or agriculture pathogens, bacteria will persistently develop and employ resistance mechanisms. Currently, more that 70% of pathogenic bacteria display resistance to at least one antibiotic [4]. Being ubiquitous, microorganisms serve as a reservoir of AMR in various ecological niches. The intrinsic network of interactions among microbial communities in diverse environments facilitates the transfer of genetic material, thereby expanding the spread of AMR, leading to a global concern [5].

Many classes of antibiotics used in human infections are shared with the veterinary sectors and vice versa, exerting cumulative selective pressure on microorganisms and leading to reduced efficacy on antimicrobial based treatments [7]. Traditionally, antibiotics have been used in animal husbandry for the treatment of infectious diseases, as well as for preventive measures and as growth-promoting factors. The latter application is based on observations linking the administration of subtherapeutic doses of antibiotics to significant weight gain in treated animals [4]. Although the precise mechanism behind this phenomenon is not yet fully understood, is has been observed that prolonged administration of antibiotics at subtherapeutic doses affects multiple organs and physiological processes [4]. The later mentioned processes represent a reduced diversity of the intestinal microbiota and diminished competition for nutrients, a decrease in harmful bacteria, reduced immune stimulation or increased vitamin biosynthesis in the intestines. Collectively, these effects improve the net energy balance and enhance animal performance from a zootechnical standpoint [8]. Furthermore, sublethal doses of antibiotics act as selective pressure, stimulating bacterial evolutionary mechanisms to adapt to environmental stressors and allowing the survival and propagation of more resistant strains carrying AMR traits. Similar effects can be observed with the use of antibiotics for prophylactic purposes. In this context, antimicrobial compounds are commonly administered via drinking water or feed, ensuring prolonged exposure of animals to low antibiotic doses over an extended period. However, the protective effects are reversed once antibiotic administration is suspended, leaving the animals susceptible to infections [9], [10].

In the context of poultry farming, the use of antibiotics has been a common practice with far-reaching consequences, particularly concerning AMR. Antibiotics have been employed in poultry production for therapeutic purposes, and they are typically administered through drinking water [11]. Penicillins, aminoglycosides, tetracyclines, macrolides, and a combination of sulfonamide/trimethoprim are among the commonly used classes of antibiotics in this sector [12]. However, the extensive use of antibiotics in poultry farming raises concerns about the development and spread of antimicrobial resistance. The repetitive and widespread use of antibiotics in poultry production contributes to the selection and proliferation of resistant bacteria [13]. As a result, various resistance genes emerge, compromising the effectiveness of antibiotics not only in poultry sector but also in human medicine [14]. Florfenicol, a broad-spectrum antibiotic, is frequently employed in poultry to combat respiratory, enteric or septicemic infectios. In addition, it has beed utilized for prophylaxis and growth promotion, due to its lower risk of promoting resistance development when compared to other amphenicols. [15]. However, studies have identified resistance genes associated with florfenicol in poultry populations, that poses a significant challenge for public health. These genes, when transferred to human pathogens, can diminish the effectiveness of antibiotics used for treating human infections [16]. Therefore, the emergence and dissemination of resistance genes in poultry population warrant careful monitoring and intervention strategies to mitigate the spread of antimicrobial resistance. Understanding the impact of antibiotic usage in poultry and the prevalence of resistance genes, such as those linked to florfenicol, is crucial for implementing effective control measures [17]. Is is essential to develop alternative strategies that promote responsible antibiotic use in poultry farming, prioritize animal welfare, and minimize the risk of antimicrobial resistance transmission between animals and humans. By addressing these issues, we can safeguard the efficacy of antibiotics and ensure the continued protection of both animal and human health [18].

In order to determine the antimicrobial resistance of clinical bacterial strains, we employed a technique involving a previous isolation and identification of three strains using chemical identification. Our focus was on evaluating the sensitivity of these bacterial strains for florfenicol, as well as for six different concentrations of florfenicol-loaded nanoparticles. The antibiotic was chosen based on the interest in poultry farming, since florfenicol is an antibiotic commonly employed to combat bacterial infections in this species [19]. The methodology consisted in testing the susceptibility of the bacterial strains by using a diffusimetric method, hence the inhibitory effect of the antimicrobial agents was assessed by measuring the diameter of the inhibition zones around the wells. The obtained results were then interpreted by comparing the inhibiting diameters of the different concentrations of florfenicol and florfenicol-loaded nanoparticles. This analysis provided insights into the effectiveness of these agents against the tested bacterial strains and allowed for the determination of the minimum inhibitory concentration (MIC) required to inhibit bacterial growth. By employing the diffusimetric method and measuring the inhibiting diameter, we could evaluate the antimicrobial resistance of the clinical bacterial strains to florfenicol and assess the potential enhancement of its efficacy through the use of florfenicol-loaded nanoparticles. These findings contribute to our

understanding of the susceptibility patterns of bacterial strains and aid in the development of more effective antimicrobial strategies to combat antimicrobial resistance.

2. Materials and Methods

2.1. Sample Collection: Swab sampleswere collected from 10-day-old chickens exhibiting non-specific clinical signs such as weight loss and decreased appetite. A total of 10 chickens were selected for this study. The birds were carefully examined, and samples were collected using aseptic techniques to avoid contamination.

2.2 Isolation and identification of bacterial strains: upon sample collection, the specimens were inoculated onto nutrient agar plates using the streaking method. The plates were then incubated at 37°C for 24 hours to allow bacterial growth. Following incubation, individual bacterial colonies were isolated based on their morphological characteristics. The isolated bacterial strains were subjected to identification using the API 20 E biochemical rapid test (Biomérieux SA). This test utilizes a panel of biochemical reactions to identify the bacterial species. Each bacterial strain was inoculated into the API 20 E strip and incubated according to the manufacturer's instructions. The results obtained from the test were recorded and used for further analysis.

2.3. Preparation of Bacterial Cultures: to prepare 24-hour cultures of the identified bacterial strains, a loopful of each isolate was streaked onto nutrient agar plates. The plates were then incubated at 37°C for 24 hours. After incubation, a single colony from each plate was selected and inoculated into Mueller-Hinton broth at a reference scale of 0.5 McFarland. The broth cultures were incubated under optimal conditions for the respective bacterial strains.

2.4. Sensitivity testing: a plate containing 12 ml of Mueller-Hinton agar was used to perform the sensitivity testing for the three isolated bacterial strains. The tests aimed to evaluate the ability of florfenicol to inhibit bacterial growth using the diffusion method recommended by the Clinical and Laboratory Standards Institute (CLSI). Commercial susceptibility disks loaded with 30 μg of florfenicol were employed in the testing. Additionally, polymeric nanoparticles loaded with florfenicol were used as an alternative formulation. The nanoparticles were reconstituted at a concentration of 30 μg/ml and subjected to successive dilutions to obtain concentrations of 15 μg/ml, 7.5 μg/ml, 3.75 μg/ml, 1.875 μg/ml, and 0.937 μg/ml. The reconstituted nanoparticles were prepared in 38 ml of sterile saline solution in Wheaton scintillation vials made of borosilicate glass.

Table 1. Inhibition zone diameters of florfenicol and nanoparticle-loaded formulations against isolated bacterial strains

BACTERIAL STRAIN	FLORFENICOL DISK (30 μG)	FLORFENICOL-LOADED NANOPARTICLES (μG/ML)
E.COLI	15 mm	30 μg/ml: 8 mm (± 1) 15 μg/ml:7.5 mm (±0.5) 7.5 μg/ml: 8 mm (±1) 3.75 μg/ml: 6.8 mm (±0.2) 1.875 μg/ml: 7 mm (±0.5) 0.937 μg/ml: 8 mm (±1.5)
ENTEROCOCCUS FAECALIS	18 mm	30 μg/ml: 6 mm 15 μg/ml: 6 mm 7.5 μg/ml: 6 mm 3.75 μg/ml: 6 mm 1.875 μg/ml: 6 mm 0.937 μg/ml: 6 mm
ENTEROBACTER CLOACAE	17 mm	30 μg/ml: 7 mm (± 1.5) 15 μg/ml: 6.5 mm (± 1) 7.5 μg/ml: 6 mm (±1.8) 3.75 μg/ml: 7.5 mm (±1.8) 1.875 μg/ml: 6.8 mm (±1.2) 0.937 μg/ml: 6 mm (±1.5)

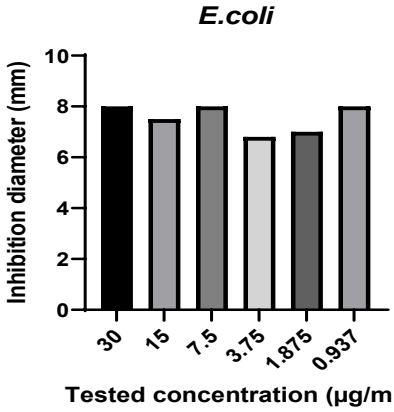
2.5 Interpretation of results: The interpretation of the sensitivity testing results was performed by measuring the diameter of inhibition zones formed around the susceptibility disks and nanoparticle-loaded wells. The diameter measurements were recorded for each concentration of florfenicol tested. Statistical analysis was carried out using GraphPad Prism 9.3.0 to determine the significance of the differences observed between the susceptibility of the bacterial strains to florfenicol and the nanoparticle-loaded formulation. The results were analyzed, and relevant statistical parameters such as mean, standard deviation, and p-values were calculated.

3. Results

3.1. Isolation and identification of bacterial strains: from the examined chickens, three bacterial strains were isolated and identified as follows: *Escherichia coli*, *Enterococcus faecalis*, and *Enterobacter cloacae*. It is important to note that these bacteria can also be part of the normal gut bacterial flora. However, further investigation is required to determine whether these isolated strains have any pathogenic effects.

3.7. Sensitivity testing results: the susceptibility testing was performed to evaluate the effectiveness of florfenicol and nanoparticle-loaded formulations against the isolated bacterial strains. The inhibition zone diameters were measured and are summarized in Table 1.

3.2 Data analysis: statistical analysis (one-way ANOVA test) was performed to assess the significance of the differences observed between the susceptibility of the bacterial strains tested at six different concentrations of florfenicol-loaded nanostructures. The analysis revealed no statistical significance between them ($p>0.05$), as shown also in figures 1, 2 and 3. It is worth noting that the bacterial strains exhibited a significantly higher susceptibility to the florfenicol disk compared to the nanoparticles loaded with florfenicol.



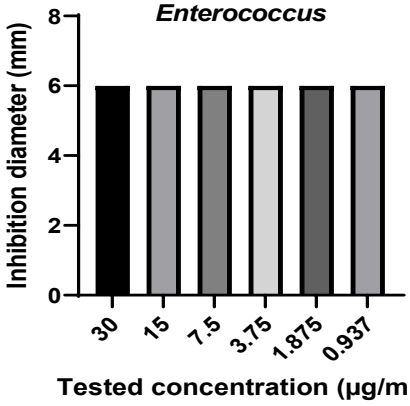


Figure 1 and 2: Graphical representation of the *E.coli* (1) and *Enterococcus* (2) susceptibility to different concentrations of florfenicol-loaded nanoparticles

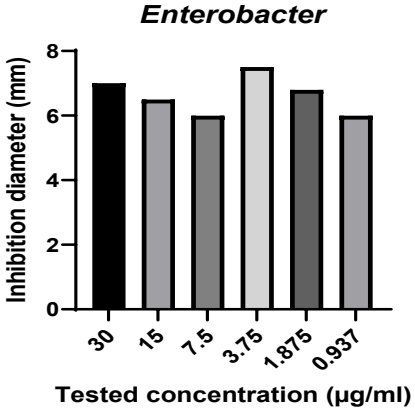


Figure 3: Graphical representation of the *Enterobacter* susceptibility to different concentrations of florfenicol-loaded nanoparticles

4. Conclusions

The main objective of this study was to investigate the susceptibility of bacterial strains (*Escherichia coli*, *Enterococcus faecalis*, and *Enterobacter cloacae)* isolated from chickens exhibiting non-specific clinical signs to florfenicol and nanoparticle-loaded formulations. The identified bacterial strains represent bacteria commonly found in poultry and fresh chicken meat and their presence suggest that they may have a significant effect on human colonization and the dissemination of antibiotic resistance in the environment. The results of the susceptibility testing revealed a higher susceptibility to he florfenicol disk compared to the nanoparticle-loaded formulation. However, it is important to consider that the aqueous solution used for the nanoparticles preparation and the potential influence of variables like the release rate of florfenicol from the nanostructures, temperature variations, and other nanostructure-related properties might have influenced these results . Statistical analysis indicated no significant differences between the six different concentrations tested, suggesting that the susceptibility of the bacterial strains to the nanoparticle-loaded formulations remained consistent across the concentration range. The findings from this study highlight the potential limitations of the nanostructures in terms of antimicrobial efficacy compared to the conventional florfenicol disk. Further investigations are warranted in order to characterize the nanostructures, including their release kinetics, as well as the loading with the active substance, and the impact of various other parameters on their antimicrobial activity. This additional research will aid in optimizing the nanoparticle formulation and overcoming the observed limitations. Moreover, considering that the isolated bacterial strains (*E. coli, Enterococcus faecalis*, and *Enterobacter cloacae*) can be part of the normal gut bacterial flora, it is crucial to conduct further studies to determine whether these strains possess pathogenic properties or are associated with the observed non-specific clinical signs in the examined chickens.

In conclusion, this study provides valuable insights into the susceptibility of bacterial strains isolated from chickens to florfenicol and nanoparticle-loaded formulations. The results suggest a higher susceptibility to the conventional florfenicol disk compared to the nanoparticle formulation, highlighting the need for further investigation and optimization of the nanoparticle system. The findings also emphasize the importance of assessing the pathogenic potential of these isolated bacterial strains to elucidate their role in the observed clinical signs. Overall, this study sets the foundation for future research aiming to enhance antimicrobial strategies and promote animal health and welfare.

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CVJ CLUJ VETERINARY JOURNAL

Review

Unravelling the Antioxidant Potential of Resveratrol and Quercetin in Animal Models: A Comprehensive Review

Ioana Craciun[1,*] and Florinel Gheorghe Brudașcă[1,]

[1] Department of Infectious Diseases, Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Calea Mănăştur nr. 3-5, 400372 Cluj-Napoca, Romania. ioana.craciun@usamvcluj.ro, florinbrudasca@yahoo.com

Abstract: Resveratrol and quercetin are naturally occurring polyphenolic compounds widely studied for their potential health benefits, particularly their antioxidant properties. This abstract provides an overview of the extensive research conducted on resveratrol and quercetin as antioxidants in animal models, highlighting their mechanisms of action and therapeutic potential. Animal models, such as rodents, have been instrumental in elucidating the oxidative stress pathway and evaluating the efficacy of various antioxidants. Resveratrol and quercetin have demonstrated significant antioxidant effects in animal models through multiple mechanisms. These include direct scavenging of reactive oxygen species (ROS), upregulation of endogenous antioxidant enzymes, inhibition of lipid peroxidation, and modulation of oxidative stress-related signaling pathways.

Keywords: polyphenols; therapeutic implications; animal models

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1. Introduction

Resveratrol and quercetin supplementation has been found to reduce oxidative stress in a wide range of systems, including the liver [1], heart [2], brain [3], and kidneys [4] in animal models. It has been discovered that these antioxidants can reduce oxidative damage caused by disorders like ageing, inflammation, diabetes, neurological diseases, and cardiovascular conditions [1]. The adaptation of resveratrol and quercetin research to human applications faces several difficulties despite the encouraging results in animal studies [5]. To achieve efficient and secure therapeutic interventions, factors like bioavailability, metabolism, and dosage optimization need to be meticulously taken into consideration. Oxidative stress, resulting from an imbalance between reactive oxygen species (ROS) production and antioxidant defense mechanisms, has been implicated in the pathogenesis of numerous diseases, including ageing, cardiovascular disorders, neurodegenerative diseases, and cancer [1]. Consequently, there is a growing interest in identifying natural compounds that possess potent antioxidant properties to counteract oxidative damage and promote overall health. Resveratrol and quercetin, two polyphenolic compounds abundantly found in various fruits, vegetables, and plant extracts, have garnered considerable attention for their potential as antioxidants in animal models [6].

The authors reported that quercetin treatment suppressed oxidative stress markers, including malondialdehyde (MDA) and protein carbonyl levels, while upregulating the expression of antioxidant enzymes, such as heme oxygenase-1 (HO-1).

Moreover, several mechanistic studies have shed light on the underlying antioxidant mechanisms of resveratrol and quercetin in animal models. For instance, in their comprehensive review, Han et al. (2007) summarized the multiple pathways through which resveratrol exerts its antioxidative effects, including ROS scavenging, activation of nuclear factor erythroid 2-related factor 2 (Nrf2)-mediated antioxidant response, and modulation of signalling pathways like mitogen-activated protein kinases (MAPKs) and nuclear factor-kappa B (NF-κB)[11]. In a similar vein, Xu et al. (2019) elucidated the antioxidant mechanisms of quercetin, highlighting its ability to inhibit ROS generation, restore cellular redox balance, and activate antioxidant enzymes through Nrf2 signalling pathways in animal models. The evidence that resveratrol and quercetin have antioxidant functions in animal models is carefully increasing, and it includes the outcomes of this study in addition to a large number of additional studies. Understanding the processes by which these chemical compounds exert their effects is crucial for the development of effective medical therapies for oxidative disorders in individuals linked to stress. Additional research is required to bridge the gap between animal models and clinical trials. This will facilitate the development of safe and effective therapies for human health based on these findings.

2. Mechanism of Action of Resveratrol

Due to its extraordinary pharmacological potential, resveratrol, also known as 3,4′,5-trihydroxystilbene, is a nutraceutical that has attracted a lot of academic interest. It is a naturally occurring phytoalexin that is frequently found on numerous plants, notably berries, grapes, and peanuts. Resveratrol was initially extracted from the *Veratrum grandiflorum* plant, frequently referred to as white hellebore, in the 1940s, which is when it was first discovered [12, 13]. Red wine represents a processed plant product that is known to have a high resveratrol content. Due to its potential involvement in the "French paradox," which refers to the unusually low rate of heart disease among Southern French people despite their consumption of diets high in saturated fat, this substance has garnered interest. Red wine contains resveratrol, which has been proposed as a potential explanation for this phenomenon. Resveratrol concentrations in red wine can range from 0.1 to 14.3 mg/L [12, 14-16]. Numerous studies have elucidated the mechanisms through which resveratrol exerts its antioxidative effects [17, 18].

2.1. Activation of Nrf2 Pathway

Resveratrol has been shown to activate the nuclear factor erythroid 2-related factor 2 (Nrf2) pathway, a crucial regulator of antioxidant defence systems. Nrf2 translocate into the nucleus upon activation, leading to the upregulation of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). For instance, Jia et al. (2019) demonstrated that resveratrol treatment increased the expression of Nrf2 and its target genes, resulting in reduced oxidative stress and liver injury in a rat model [9].

2.2. ROS Scavenging

Resveratrol exhibits direct scavenging properties against reactive oxygen species (ROS). By neutralizing ROS through electron transfer, resveratrol mitigates their damaging effects on cellular components. Aminjan et al. (2019) reported that resveratrol administration led to a decrease in ROS levels, contributing to the preservation of cellular redox balance and protection against oxidative stress-induced liver injury [19].

2.3.Modulation of Intracellular Signalling Pathways

Resveratrol modulates several intracellular signalling pathways associated with oxidative stress, such as mitogen-activated protein kinases (MAPKs) and nuclear factor-kappa B (NF-κB). Du et al. (2021) highlighted that resveratrol inhibits the activation of MAPKs, including ERK, JNK, and p38, thereby reducing oxidative stress-mediated cellular damage. By interfering with NF-κB signalling, resveratrol can suppress inflammation and oxidative stress-induced damage [20].

2.4. Activation of Sirtuins

Resveratrol activates sirtuins, a family of NAD+-dependent deacetylases, particularly the SIRT1 enzyme. SIRT1 activation has been associated with enhanced antioxidant defences and improved mitochondrial function [21]. Kaeberlein et al. (2021) emphasized that resveratrol-mediated SIRT1 activation contributes to cellular resilience against oxidative stress and overall cellular homeostasis [22].

3. Therapeutic Potential of Resveratrol

The mechanisms of action of resveratrol in animal models transfer into its potential medicinal applications for a variety of conditions linked to oxidative stress. Resveratrol, for instance, has been demonstrated to reduce renal oxidative stress, inflammation, and fibrosis in a mouse model of diabetic nephropathy[23]. Resveratrol appears to protect against myocardial ischemia/reperfusion injury in a rat model by reducing oxidative stress while improving cardiac function, according to Yu et al. (2021) [24]. Furthermore, Ibrahim et al. (2020) demonstrated that resveratrol reduced renal oxidative stress and inflammation, which attenuated cisplatin-induced nephrotoxicity[25]. Reservatrol was given to rats with chronic cerebral hypoperfusion in a study by Wang et al. (2014) [26]. According to the study's findings, resveratrol administration enhanced mental performance while lowering oxidative stress markers in the brain. In a study conducted by Wang et al. (2019), resveratrol was administered to rats with experimentally induced colitis [26]. Resveratrol treatment reduced oxidative stress preserved colonic tissue integrity, and ameliorated inflammation in the colon[27]. Wang et al. (2018) investigated the neuroprotective effects of resveratrol in a mouse model of Alzheimer's disease. They found that resveratrol supplementation improved cognitive function, reduced amyloid-beta plaque deposition, and alleviated oxidative stress in the brain [28]. In a study by Hu et al. (2020), resveratrol was administered to rats with acute lung injury induced by lipopolysaccharide (LPS)[29]. Resveratrol treatment attenuated lung injury, reduced oxidative stress markers, and inhibited inflammatory responses in the lung tissues. Aged mouse treatment with resveratrol was studied by Barger et al. (2008). According to their findings, resveratrol increased lifespan, lowered oxidative stress, and enhanced metabolic health in the treated mice compared to the control group [30]. Resveratrol's preventive properties were examined by Lan et al. (2022) in a rat model of renal ischemia-reperfusion injury. Administration of resveratrol reduced oxidative stress and inflammation in the renal tissues, improved renal function, and minimized kidney damage [31]. Resveratrol has been studied in a rat model of liver fibrosis induced on by carbon tetrachloride in a study by Abdu et al. (2017). By lowering oxidative stress, inflammation, and hepatic collagen deposition, resveratrol therapy improved liver fibrosis [32, 33]. These investigations collaboratively provide a spotlight on resveratrol's various medicinal benefits. Collectively, these studies have provide insight on the numerous therapeutic effects of resveratrol in animal models. They back up the idea that resveratrol has the potential to be an effective natural substance for both the prevention and treatment of many different oxidative illnesses associated with stress.

4. Mechanism of Action of Quercetin

The chemical compound 3,5,7-trihydroxy-2-(3,4-dihydroxy phenyl)-4Hchromen-4-one, typically known as quercetin, is a dietary flavonoid that exists in a variety of plant sources, including capers, black chokeberries, onions, tomatoes, and lettuce [34]. Quercetin can be identified in plants in a conjugated state, paired with phenolic acids, sugars, ethers, and other substances. The precise forms of quercetin derivatives can affect how quickly they are absorbed in the stomach and small intestine [35]. The mechanism of action of quercetin involves its interactions with multiple cellular targets, leading to its diverse pharmacological effects. Quercetin is an excellent free radical scavenging antioxidant [36]. Consuming foods that contain flavonoids lowers the chance of developing long-term illnesses including diabetes, coronary heart disease, and stroke that are brought on by oxidative stress [36–38]. The flavonoid quercetin, which can be discovered in fruits and vegetables, has unique biological properties that could enhance cognition and physical performance while reducing the risk of illness [8]. The foundation for possible benefits to general health and disease resistance is established by these characteristics, which include the ability to suppress lipid peroxidation, platelet aggregation, and capillary permeability as well as the capacity to induce mitochondrial biogenesis [39]. It additionally possesses anti-inflammatory, antiviral, anti-inflammatory, antioxidant, and stimulant effects.

4.1 Antioxidant Activity

One of the primary mechanisms through which quercetin exerts its effects is its potent antioxidant activity. Quercetin acts as a free radical scavenger, effectively neutralizing reactive oxygen species (ROS) and inhibiting lipid peroxidation. It also enhances the activities of endogenous antioxidant enzymes, such as superoxide dismutase (SOD) and catalase (CAT), thereby augmenting the cellular defence against oxidative stress [40, 41].

4.2 Anti-Inflammatory Effects

Quercetin demonstrates notable anti-inflammatory properties by modulating various inflammatory signalling pathways. It inhibits the production and release of pro-inflammatory mediators, including cytokines (such as tumour necrosis factor-alpha and interleukins) and inflammatory enzymes (such as cyclooxygenase-2 and inducible nitric oxide synthase). Quercetin achieves this by suppressing the activation of nuclear factor-kappa B (NF-κB), a key transcription factor involved in the inflammation [41, 42].

4.3 Modulation of Cellular Signalling Pathways

Quercetin influences several cellular signalling pathways involved in cellular homeostasis and disease processes. It activates the adenosine monophosphate-activated protein kinase (AMPK) pathway, which plays a critical role in cellular energy metabolism and oxidative stress response. Quercetin-mediated activation of AMPK promotes cellular antioxidant defences and inhibits oxidative stress-induced damage [43, 44].

4.4 Epigenetic Modifications

Quercetin has been shown to exert epigenetic modifications, particularly through its influence on DNA methylation and histone acetylation. It can alter the expression of genes involved in various physiological processes, including antioxidant defence, inflammation, and cell cycle regulation [45]. By modulating epigenetic mechanisms, quercetin may exert long-term effects on cellular function and disease development [46]. These mechanisms collectively contribute to the pharmacological effects of quercetin, including its antioxidant, anti-inflammatory, and cytoprotective properties.

5. Therapeutic Potential of Quercetin

The diverse mechanisms of action of quercetin in animal models translate into its potential therapeutic applications for various conditions. Neuroprotection: quercetin has shown neuroprotective effects in animal models of diabetic neuropathy, Alzheimer's disease, and ischemic brain injury. Its antioxidant and anti-inflammatory properties contribute to the preservation of neuronal function and protection against neurodegeneration [47, 48]. Hepatoprotection, animal studies have demonstrated that quercetin can attenuate liver injury, fibrosis, and inflammation in models of liver fibrosis and hepatotoxicity. Its antioxidant and anti-inflammatory actions contribute to the preservation of liver function and the reduction of liver damage [49-51]. Cardiovascular Health: quercetin supplementation has shown cardioprotective effects in animal models of myocardial ischemia-reperfusion injury. It reduces oxidative stress, suppresses inflammation, and improves cardiac function, suggesting its potential in preventing cardiovascular diseases [52-54]. Anti-Inflammatory Effects: Quercetin exhibits anti-inflammatory effects in animal models of colitis and other inflammatory conditions. It mitigates inflammation, preserves tissue integrity, and reduces oxidative stress, highlighting its potential as an adjunct therapy for inflammatory bowel diseases [54, 55].

6. Discussions

In the field of both human and veterinary medicine, various studies using animal models have provided important light on the mechanisms of action and therapeutic potential of quercetin and resveratrol. The results of these investigations are all featured in this review. The review of quercetin and resveratrol's mechanisms of action and therapeutic potential in both veterinary and human medicine delivers important insights into the potential applications of these organic compounds as therapeutic agents. Although preliminary evidence is provided through animal research, it is crucial to take into account the parallels and discrepancies between animal models and human patients to ensure the reliability and practicality of the results. The variation in species and their physiological peculiarities are an important factor that must be taken into consideration. The pharmacokinetics and therapeutic response to quercetin and resveratrol can vary amongst individuals and animals based on individual metabolism, organ structure, and genetics. In light of this, extreme caution should be taken when extrapolating findings from animal studies to human patients, and their findings should be confirmed more thoroughly in meticulously planned clinical trials. The effectiveness and safety of quercetin and resveratrol in both veterinary and human medicine are significantly affected by factors other than the biodiversity of species, particularly dosage, formulation, and method of administration. Understanding the optimal dosages and delivery methods for each species is essential to achieve desired therapeutic outcomes. Furthermore, the bioavailability and metabolism of these compounds may differ between animals and humans, emphasizing the need for further research to establish appropriate dosing regimens in clinical practice. Long-term safety and potential drug interactions are important considerations in both veterinary and human medicine. While animal studies provide insights into short-term effects, long-term studies are necessary to evaluate any potential adverse effects or interactions with other medications commonly used in clinical practice. Rigorous monitoring and assessment of safety profiles are required to ensure the well-being of patients. In both veterinary and human medicine, quercetin and resveratrol have the potential for alleviating an extensive spectrum of diseases. These chemicals have demonstrated promise in animal models for the treatment of neurological conditions, liver diseases, cardiovascular problems, and metabolic disorders. Similar to animal research, there is growing evidence that indicates

antioxidants could have a role in the management and prevention of chronic illnesses like cancer, cardiovascular disease, and neurodegenerative disorders. However, more clinical studies are required to confirm these results and determine accurate characteristics of patients, optimal dosages, and lengths of treatment. Research on the application of quercetin and resveratrol for veterinary and human medicine should be expanded by cooperation between scientists, doctors, and pharmaceutical companies. For researchers to provide accurate information regarding safety, efficacy, and optimal methods of treatment, well-designed clinical trials taking into consideration species-specific features and involving a variety of patients must be conducted. These investigations may additionally look at potential interactions with currently administered pharmaceuticals, establish early warning signs or contraindications, and contribute to developing clinical practice guidelines.

7. Conclusion

The research conducted with animal models provides significant insights into the therapeutic potential of resveratrol and quercetin in both human and veterinary medicine. While the findings from animal studies are promising, caution should be exercised when translating these results to human applications, considering species differences and variations in pharmacokinetics. Further research, including well-designed clinical trials, is necessary to establish the optimal dosages, safety profiles, and efficacy of these compounds in both veterinary and human patients. Collaboration among researchers, clinicians, and pharmaceutical companies will play a crucial role in advancing the research and application of quercetin and resveratrol, ultimately improving the health outcomes of both animals and humans.

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