Análise histomorfométrica do fígado e pâncreas de três espécies de aves de rapina: *Caracara plancus, Rupornis magnirostris* e *Coragyps atratus*

Histomorphometric analysis of the liver and pancreas of three species of birds of prey: *Caracara plancus, Rupornis magnirostris* and *Coragyps atratus*

DOI:10.34117/bjdv7n4-019

Recebimento dos originais: 07/03/2021
Aceitação para publicação: 01/04/2021

Isabelly Fernanda Santos Barbosa
Mestre Em Morfotecnologia - Universidade Federal De Pernambuco
Av. Prof. Moraes Rego, 1235
Cidade Universitária, Recife- PE, Brasil
E-mail: isabelly_barbosa@hotmail.com

Carolina Peixoto Magalhães
Professora Associado I Da Universidade Federal De Pernambuco-Centro Acadêmico De Vitória
R. Alto do Reservatório - Alto José Leal, Vitória de Santo Antão - PE, Brasil,
E-mail: carolina.magalhaes@*ufpe*.br

Francisco Carlos Amanajás de Aguiar Júnior
Professor Associado Ii Da Universidade Federal De Pernambuco-
Centro Acadêmico De Vitória
R. Alto do Reservatório - Alto José Leal, Vitória de Santo Antão - PE, Brasil,
E-mail: famanajas@yahoo.com.br

Kleber Botelho Fraga
Mestre Em Saúde Humana E Meio Ambiente - Universidade Federal De Pernambuco -
Centro Acadêmico De Vitória
R. Alto do Reservatório - Alto José Leal, Vitória de Santo Antão - PE, Brasil,
CEP 55608-250
E-mail: kbfraga@hotmail.com

Fernanda Alda da Silva
Mestre Em Saúde Humana E Meio Ambiente - Universidade Federal De Pernambuco -
Centro Acadêmico De Vitória
R. Alto do Reservatório - Alto José Leal, Vitória de Santo Antão - PE, Brasil,
CEP 55608-250
E-mail: fernandaalda.s@hotmail.com

Marcos Aurélio Santos da Costa
Mestrando Em Morfotecnologia - Universidade Federal De Pernambuco
Av. Prof. Moraes Rego, 1235
Cidade Universitária, Recife- PE, Brasil, CEP 50670-901
E-mail: marcosxp17@gmail.com

Juliana Pinto de Medeiros
Professora Associada Ii Da Universidade Federal De Pernambuco
Av. Prof. Moraes Rego, 1235
Cidade Universitária, Recife- PE, Brasil, CEP 50670-901
E-mail: jupinto2@gmail.com

RESUMO

As aves de rapina Caracara plancus; Rupornis magnirostris e Coragyps atratus têm hábitos alimentares baseados em animais vivos ou mortos. Este estudo tem como objetivo realizar uma análise histomorfométrica descritiva do fígado e pâncreas de aves de rapina diunais encontradas em áreas urbanas e rurais. Esses órgãos foram coletados de sete animais, um de cada espécie, fixados em formol a 10%, processados e corados com hematoxilina e eosina (HE) para fígado e pâncreas e histoquímica em ácido de Schiff periódico (PAS) e prata de reticulina para fígado. As lâminas foram fotomicrografadas em microscópio óptico, a morfometria foi feita por meio do software ImageJ. Os dados foram analisados por meio dos testes estatísticos Kolmogorov Smirnov, Kruskal Wallis e Mann Whitney para comparar os resultados entre as espécies. As três espécies não variaram em relação à composição dos tecidos do pâncreas e do fígado, semelhante às descrições na literatura para outras espécies de aves. Na coloração PAS, os fígados das três espécies eram semelhantes, com baixa concentração de glicogênio. Na morfometria do fígado, foram observadas diferenças entre a área dos núcleos, o número total de núcleos, o número de células mononucleadas e binucleadas entre as três espécies. Quanto às fibras reticulares, quando comparadas entre si, as espécies C. plancus e R. magnisrostris não apresentaram diferenças, enquanto a espécie C. atratus diferiu das demais. As ilhotas pancreáticas também diferiram entre as espécies. Essas variações podem ser indicativas de adaptações do organismo aos diferentes hábitos alimentares.

Palavras-chave: Aves de Rapina. Hábitos alimentares. Histologia. Pâncreas. Fígado. Histomorfometria.

ABSTRACT

The birds of prey *Caracara plancus*; *Rupornis magnirostris* and *Coragyps atratus* have feed habit based in alive or dead animals. This study has objective of realize a descriptive histomorfometric analyse of liver and pancreas of diunal birds of prey found in urban and rural areas. These organs were collected from seven animals, one of each species, fixed in 10% formaldehyde, processed and stained with hematoxylin and eosin (HE) for liver and pancreas and histochemistry in Periodic Schiff Acid (PAS) and Reticulin Silver for liver. The slides were photomicrographed in an optical microscope, the morphometric were done through the software ImageJ. The data were analyzed using the statistical tests Kolmogorov Smirnov, Kruskal Wallis and Mann Whitney to compare the results between species. The three species did not vary in relation to the composition of the tissues of the pancreas and liver, similar to the descriptions in the literature for other species of birds. In PAS staining, the livers of the three species were similar, with low glycogen concentration. In liver morphometry, differences were observed between the area of the nuclei, the total number of nuclei, the number of mononucleated and binucleated cells between the three species. As for the reticular fibers, when compared to each other, the species C. plancus and R. magnisrostris did not present differences, whereas the species C. atratus differed from the others. The pancreatic islets also differed between species. These variations may be indicative of the body's adaptations to different eating habits.

Keywords: Birds of prey. Feeding habits. Histology. Pancreas. Liver. Histomorphometry

1 INTRODUCTION

Birds in general have a high diversity on dietary, mainly, birds of prey feed on vertebrate and invertebrate animals and depending on the species, may include decomposing animals in the diet. Moreover, according to Granzinolli (2003), the morphological adaptations of the digestive system reflect the dietary diversity that these birds have. These animals show differences when compared to other vertebrates, which is the absence of teeth and thus the inability to chew, this function is then transmitted to the other organs of this system (Pough et al., 2008).

In the organs of the digestive system, the liver and the pancreas, classified as attached organs, are responsible for controlling energy metabolism through the digestive process, acting as an interface between the digestive system and the blood (Benez 2004; Cormack 2008; Junqueira and Carneiro 2013, 2017; Denbow 2014).

According to the descriptions made by Saviani et al. (2012) in bird livers, these organs usually have a low glycogen deposit, which may be associated with fasting period, stress, physiological conditions of the species and the genetics. Turner et al. (1999) in studies carried out in Galliformes, affirm that diets rich in carbohydrates raise the level of glycogen in the liver, while diets rich in proteins and low in carbohydrates do not have this same capacity, in this case the demand for glucose by the body is supplied by the process of gluconeogenesis. Ferrer et al. (1987) and Mighorini et al. (1973) demonstrated in their studies that birds of prey have a high gluconeogenic process, important for these them due to poor habits of eating carbohydrate. Also, the liver of birds also has the capacity to produce vitamin C from glucose stored in the liver (Benez 2001). The pancreas, on the other hand, acts to regulate the concentration of glucose in the bloodstream, in birds of prey there is a hormonal release different from granivorous birds, similar to mammals. Carnivorous birds are still able to maintain a constant glucose concentration even after long periods of fasting, which shows that even in these situations the pancreas works by maintaining the metabolism homeostasis (Harr 2002).

Among these birds of prey, *Coragyps atratus* (Bechstein 1793), *Rupornis magnirostris* (Gmelin 1788) and *Caracara plancus* (Miller 1777) are abundant day birds in urban and rural areas and consequently have greater contact with humans. They belong to the orders Cathartiformes, Accipitriformes and Falconiformes, respectively (Sick 2001). *C. atratus*, the American black culture, has a diet based on decomposing animals;

R. magnirostris, the Roadside hawk feeds on fruits, arthropods, small mammals, reptiles and birds; *C. plancus*, southern crested carcara , has a general diet, covering all the diets mentioned above (Panasci and Whitacre 2001; Vargas 2007; Montalvo 2011; Sick 2001).

2 MATERIAL AND METHODS

2.1 ANIMALS

21 animals were used, 7 individuals of *C. plancus*, 7 individuals of *R. magnirostris* and 7 individuals of *C. atratus*. The first two species came from previous collections by our research group at UFPE - Vitória Academic Center. The vulture individuals were collected in the wild at the Natuba landfill in the municipality of Vitória de Santo Antão in the South Forest of Pernambuco, this collection and research was also authorized by the Ethics Committee on the Use of Animals (Comitê de Ética no Uso de Animais) - CEUA through authorization No. 23076.023966 / 2016 -19 and by the Biodiversity Authorization and Information System (Sistema de Autorização e Informação em Biodiversidade) - SISBIO nº 57230-1.

2.2 SAMPLING OF *C. ATRATUS* (AMERICAN BLACK VULTURE)

To sample these specimens, we used the Tomahawk trap according to the methodology of Mangini & Nicola (2003). These traps are made of metal and are widely used in the capture of birds, reptiles and mammals. They have a trigger system in which the animal enters the trap, activates it, closing the door, this trigger consists of a hook where we fix pieces of chicken from butcher shop. The traps were placed at a distance of approximately 10 meters between each one, at strategic points of high incidence of vultures, until the capture occurred. After being captured by the traps, the animals were transported to the Anatomy Laboratory of the Federal University of Pernambuco - Vitória Academic Center for immediate euthanasia.

2.3 EUTHANASIA AND SAMPLING OF BIOLOGICAL MATERIAL

The birds were weighed, measured and then anesthetized through the administration of xylazine and ketamine 1.6 mg/kg and 30 mg/kg intramuscularly injection. Then 100 mg/kg of sodium pentobarbital was administered for euthanasia. After the death was confirmed, the whole digestive tract was removed immediately, from the oropharynx to the cloaca.

All organs were preserved in 10% formaldehyde for 48 hours. After fixation, fragments of the liver and pancreas of each animal were collected and subjected to dehydration in ethyl alcohol at increasing concentrations, diaphanization by xylol, impregnation and inclusion in liquid paraffin. The blocks obtained were cut to a thickness of 5 µm in a digital microtome (Leica RM2245), the cut tissues were then transferred to the histological slide previously prepared with albumin to be stained. The liver was stained with Hematoxylin-Eosin (HE), Schiff's Periodic Acid (PAS) and Silver Reticulin, while the pancreas was stained with HE.

3 HISTOLOGICAL ANALYSIS

The histological slides were photomicrographed and analyzed with a digital camera (Moticam 2300) coupled to the optical microscope (Olympus CX22), using the Multimage image analysis system.

To counting the nucleus, binucleated, uninucleated cells, sinusoid capillary area and nucleus area, we performed 20 photomicrographs in HE for each liver histological section (7 histological slides for each of the 3 species), in total 420 photos were analyzed in this stain. The liver was also stained with PAS and followed by a qualitative analysis of the intensity of cellular glycogen deposits. As for livers stained with silver reticulin, we did new photomicrographs of the 21 histological slides of the 3 species to estimate the volume of reticular fibers. The photographed areas corresponded to the periphery of the portal spaces, this was necessary to standardize the same area in all analyzes.

The photomicrographs were analyzed using the Image J 1.44 software (Research Services Branch, U.S. National Institute of Health, Bethesda, MD, USA), the statistical data analyzes were throught the SPSS software version 20.0.

4 STATISTICAL ANALYSIS

4.1 INTRASPECIFIC STATISTICAL ANALYSIS

For the comparison between individuals of the same species in terms of the number of nuclei, the number of mononucleated cells, the number of binucleated cells, the area of the nuclei, the area of the capillaries and the area of the pancreatic islets by tissue area, the Kruskal-Wallis. To compare these same variables between males and females of the same species, the Mann-Whitney test was used. We decided to use these tests after verifying that the data were non-parametric, as can be seen through the

Kolmogorov-Smirnov test. For the analyzes, a significance value of p <0.05 was considered.

4.2 STATISTICAL ANALYSIS AT INTERSPECIFIC LEVEL

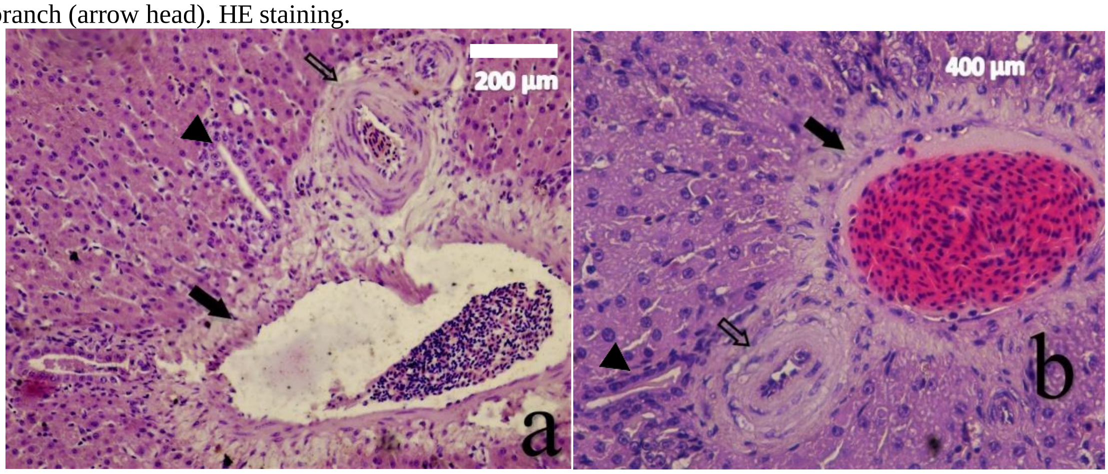
To test for differences among individuals of different species in terms of the number of nuclei, the number of mononucleated cells, the number of binucleated cells, the area of the nuclei, the area of the capillaries and the area of the pancreatic islets by tissue area. Kruskal-Wallis test. After finding differences with the previous test, Mann-Whitney tests were performed to identify where these differences are and the values of each specific group. For example: binucleated cells of species A x (versus) binucleated cells of species B; binucleate cells of the A species x binucleate cells of the C species; binucleate cells of species B x binucleate cells of species C. For all analyzes, a significance value of p <0.05 was considered.

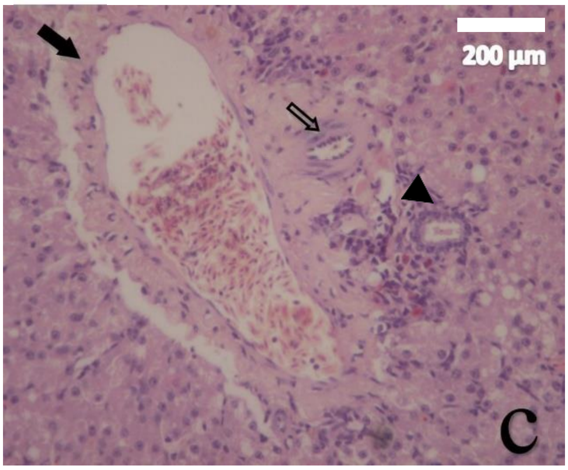
5 RESULTS

5.1 HISTOLOGICAL DESCRIPTION OF THE LIVER OF *C. PLANCUS*, *R. MAGNIROSTRIS* AND *C. ATRATUS*

The liver of the three species of birds of prey showed the same pattern of tissue organization, being composed of a capsule of layer of mesothelial cells covering the connective tissue. The hepatic lobes of all species did not present defined limits, making it possible to identify the portal spaces formed.by branches of the portal vein, branches of the bile duct and the hepatic artery (Fig.1 a, b, c). These portal spaces were randomly distributed by histological section.

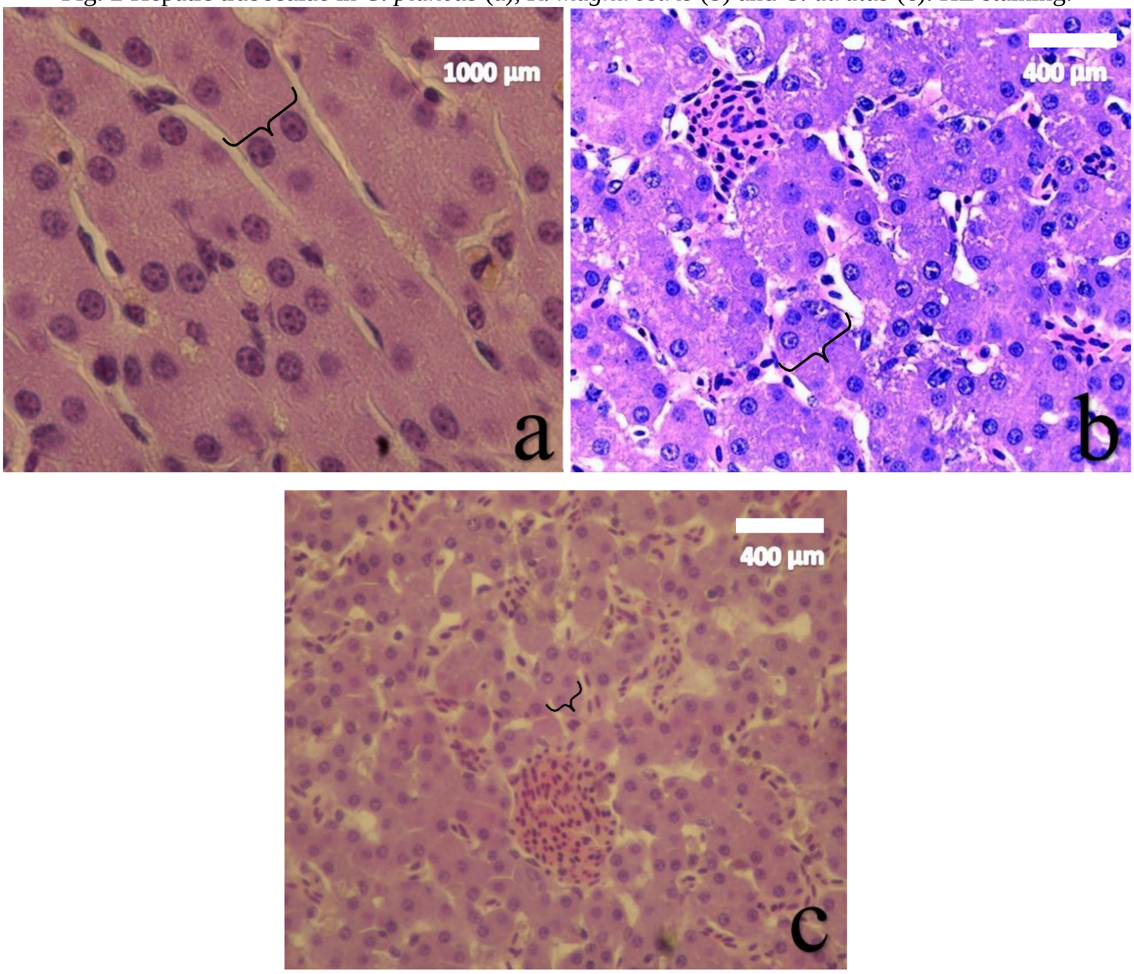
Fig.1 Porta-hepatic spaces of *Caracara plancus* (a), *Rupornis magnirostris* (b) and *Coragyps atratus* (c). We highlight the portal vein branch (black arrow), hepatic artery branch (hollow arrow) and bile duct branch (arrow head). HE staining.





The hepatocytes were organized in lines formed by a double layer of liver cells, separated by sinusoid capillaries (Fig. 2 a, b, c).

Fig. 2 Hepatic trabeculae in *C. plancus* (a), *R. magnirostris* (b) and *C. atratus* (c). HE staining.



Intraspecific results (individuals compared to each other within the same species) showed that in relation to hepatocytes there is a similarity between the total amount of nuclei among individuals of the species *C. atratus* (p> 0.05), the same was observed among individuals of the species *C. plancus* (p> 0.05) and *R. magnirostris* (p> 0.05).

In the total amount of mononucleated cells, they are similar among individuals of the species *C. atratus* (p> 0.05), while between the birds of *R. magnirostris* (p <0.05) and *C. plancus* (p <0.05), males had a higher average of mononucleated cells than females. About the number of binucleated cells, differences were observed in the three species (p <0.05), in these males had a greater amount when compared to females. The results for area of the sinusoid capillary showed that the individuals of the 3 species differ from each other (p <0.05), the area was larger in males than in females. For the area of the nuclei, there were also differences between the individuals of the three species (p <0.05), of these, only *R. magnirostris* presented a larger area in males (table 1).

Table 1 Average area and standard deviation of morphometric analyzes in μm² of the area of the nuclei of the hepatocytes, the area of the sinusoid capillaries, the total number of nuclei, mononucleated and binucleated cells of males and females of *C. plancus*, *R. magnirostris* e *C. atratus*.

Species	**Area of nucleus**	**Area of sinusoid capillaries**	**Total of nucleus**	**Mononucleated cells**	**Binucleated cells**
C. atratus Male	-	-	-	-	-
C. atratus Female	27,1±4,4	11,5±2,8	374,6±27,1	352,6±24,8	11,7±3,5
R. magnirostris Male	31,1±7,3	14,4±6,9	258,5±42,3	249,2±41,8	3,6±2,3
R. magnirostris Female	33,7±5,9	11,3±3,1	237,4±39,1	232,3±37,9	2,5±1,7
C. plancus Male	30,1±6,1	21,9±4,4	219,2±44,4	216,7±39,8	5,2±3,5
C. plancus Female	26,2±4,6	21,7±5,4	217,6±54,8	180,1±33,2	2,5±1,7

Statistical analyses showed that there are differences between the total nuclei of the three species (p <0.001). Between *C. atratus* x *C. plancus* (p <0.001); between *C. atratus* and *R. magnirsotris* (p <0.001) and between *R. magnirostris* and *C. plancus* (p <0.001). The highest average was found in *C. atratus* and the lowest in *C. plancus*. About the mononucleated cells, differences between the three species also occurred (p <0.001). Between *C. atratus* x *C. plancus* (p <0.001); between *C. atratus* and *R. magnirsotris* (p <0.001) and between *R. magnirostris* and *C. plancus* p <0.001). The highest average was found in *C. atratus* and the lowest in *C. plancus*. Among binucleated cells, differences between the three species occurred (p <0.001). Between *C. atratus* x *C. plancus* (p <0.001); between *C. atratus* and *R. magnirotris* (p <0.001) and between *R. magnirostris* and *C. plancus* (p = 0.001). The highest average was found in *C. atratus* and the lowest in *R. magnirostris*. For the capillary area, the highest average was found in *C. plancus* and the lowest in *C. atratus*. Among the area of the nuclei, there were differences between the three species (p <0.001). Between *C. atratus* x *C. plancus* (p <0.001);

between *C. atratus* and *R. magnirotris* (p <0.001) and between *R. magnirostris* and *C. plancus* (p = 0.001). The highest average was found in *R. magnirostris* and the lowest in *C. atratus* (table 2).

Table 2 Average area and standard deviation of morphometric analyzes in µm² of the area of the nuclei of the hepatocytes, of the total number of nuclei, mononucleated and binucleated cells of *C. plancus* (a), *R. magnirostris* (b) and *C. atratus* (c) by area. For statistical significance, the P-value was p<0,05.

Species	Area of nucleus	Area of sinusoid capillaries	Total nucleus of	Mononucleated cells	Binucleated cells
Caracara plancus (a)	28,9 ± 6,2	21,8 ± 4,7	218,7 ± 47,5	216,7 ± 39,8	5,2 ± 3,5
Rupornis magnirostris(b)	32,3 ± 6,9	13,1 ± 5,8	249,5 ± 42,1	241,9 ± 40,9	3,2 ± 2,2
Coragyps atratus (c)	27,1 ± 4,4	11,5 ± 2,8	374,6 ± 27,1	352,6 ± 27,1	11,7 ± 3,5
Statitical significance	<0,001/<0,001/	0,001/<0,001/	<0,001/<0,001/	<0,001/<0,001/	0,001/<0,001/

The slides stained with the Silver Reticulin technique (Fig 3. a, b, c), the highest average of the amount of reticular fibers was observed in *C. plancus* and the lowest in *C. atratus*. Statistical analysis showed that the three species are not similar when compared to each other (p <0.001). When compared individually, the species *C. atratus* x *C. plancus* (p <0.001) and *C. atratus* and *R. magnirotris* (p <0.001) differed, however, *R. magnirostris* and *C. plancus* (p> 0.05) showed similar (Table 3).

Fig. 3 Reticular fibers stained black in *C. plancus* (a), *R. magnirostris* (b) and *C. atratus* (c). Cell nuclei reacted to staining, however, for quantitative analysis, they were erased. Silver Reticulin

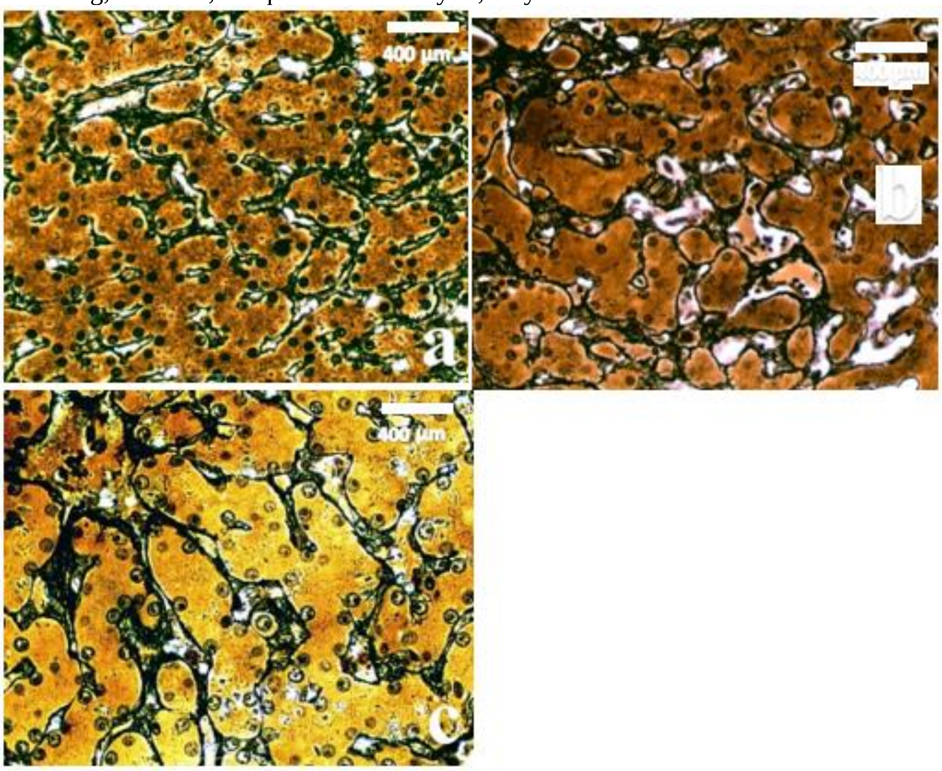
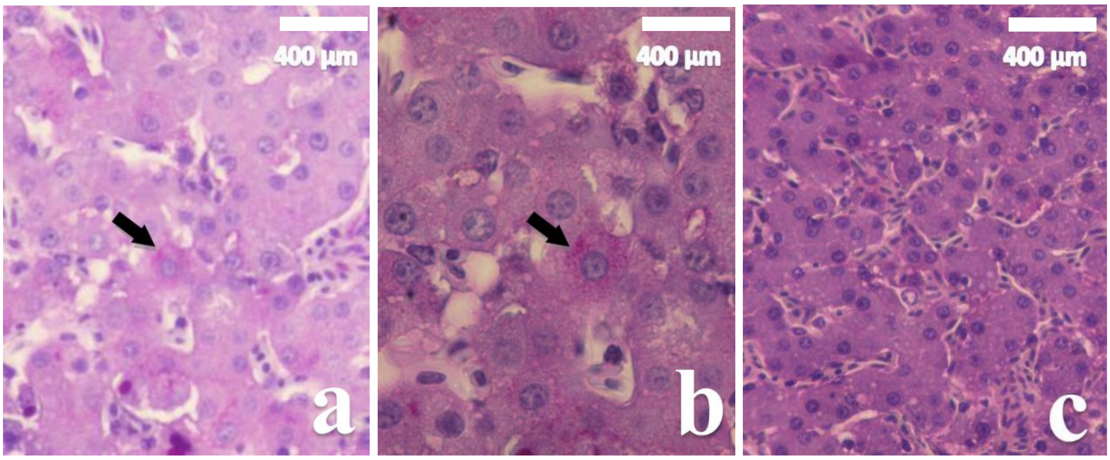


Table 3 Mean and standard deviation of the volume of reticular fibers in *C. atratus* (a), *C. plancus* (b) and *R. magnirostris*.

1. Species	Reticular fibers
C. atratus (a)	18,5 ± 7,2
C. plancus (b)	25,9 ± 30,6
R. magnirostris (c)	23,1 ± 6
(a+b)/(a+c)/(b+c)	**<0,001/<0,001/0,497**

With PAS histochemical staining, tissues showed low glycogen accumulation in hepatocytes (Fig. 4).

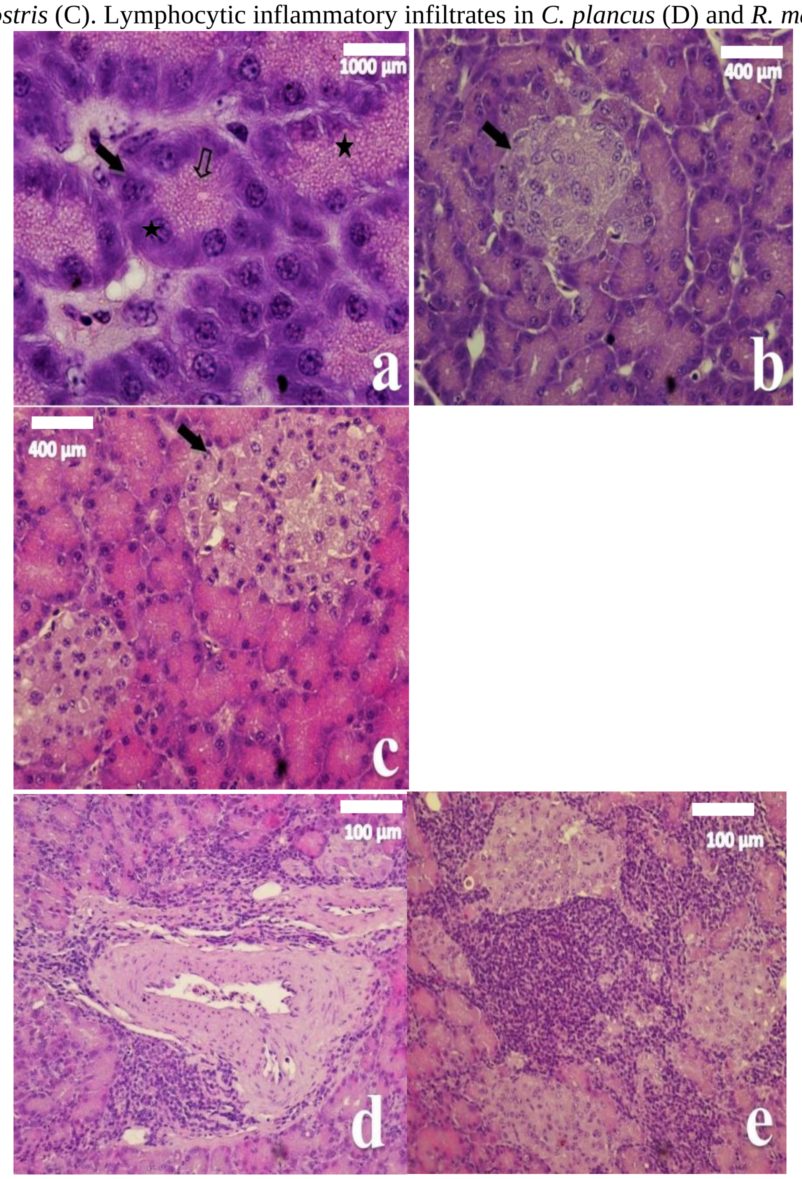
Fig. 4 Qualitative analysis of hepatic glycogen deposit. Black arrows point to hepatocyte containing glycogen. *C. plancus* (a), *R. magnirostris* (b) and *C. atratus* (c). Schiff's Periodic Acid.



5.2 HISTOLOGICAL DESCRIPTION OF THE PANCREAS OF *C. PLANCUS, R. MAGNIROSTRIS* AND *C. ATRATUS*

We saw the connective tissue layer that covers the organ. The exocrine region of the pancreas in all species showed similarities, they are composed of acinous cells forming the serous acini, where it was possible to identify the lumen of the acini that flow into the intercalated ducts. The pancreatic lobes are separated by septa. The cells have an acidophilic cytoplasm where you can see the secretion granules (zymogen) in their apical part and the spherical nuclei located in the cell base (Fig.5 a, c). The endocrine region is also similar among these birds, where it is made up of pancreatic islets. The islets come in various shapes and sizes and are randomly distributed throughout the organ in the three species (Fig. 5 b, c). Caracara and Roadside hawks also showed inflammatory lymphocytic infiltrates (fig. 5 d, e). As for the morphometry of the area of the islets of Langherans in the pancreas, among the three species the highest average was found in *C. plancus*, while the lowest was found in *C. atratus*. According to statistical analysis, these species differ from each other (p <0.001). When compared to each other, the species *C. atratus* X *C. plancus* (p <0.001); between *C. atratus* and *R. magnirotris* (p <0.001) and between *R. magnirostris* and *C. plancus* (p <0.001) (Table 4).

Fig. 5 Photomicrograph of pancreas showing the exocrine pancreas in *C. plancus* (a) and *R. magnirostris* (c): we highlight the acinous cells forming the pancreatic acini (black arrow), in the center there is the acino lumen (hollow arrow). The black star indicates the acidophilic zymogen granules in the apical cell region. The endocrine pancreas is represented by the pancreatic islets pointed by a white arrow in *C. plancus* (B) and *R. magnirostris* (C). Lymphocytic inflammatory infiltrates in *C. plancus* (D) and *R. magnirostris* (E).



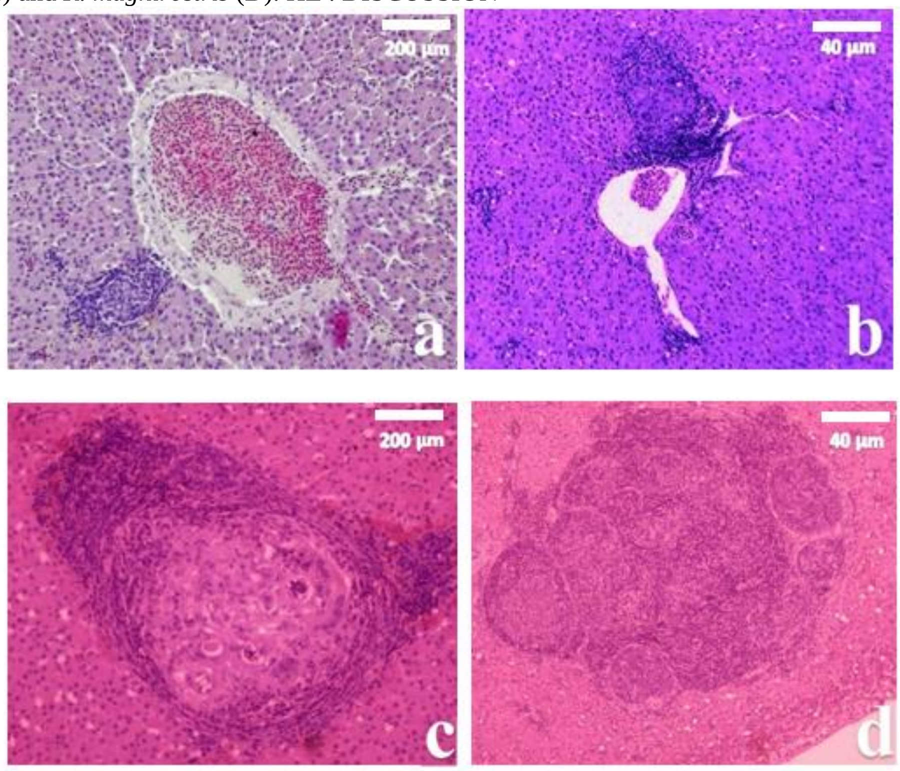
ISSN: 2525-8761

Table 4 Mean and standard deviation of the pancreatic islet area in *C. atratus* (a), *C. plancus* (b) and *R. magnirostris* (c).

2. **Species**	**Islets of Langerhans**
C. atratus (a)	853,5 ± 443,8
C. plancus (b)	2547,9 ± 1408,5
R. magnirostris (c)	1713,7 ± 1085,2
(a+b)/(a+c)/(b+c)	**<0,001/<0,001/<0,001**

In some individuals of Caracara and Roadside hawk, the presence of a large amount of inflammatory lymphocytic infiltrates was observed throughout the histological section, mostly concentrated between the portal spaces (Fig. 6A and 4B), in some individuals of Caracara also had the presence of chronic granulomatous inflammations (Fig. 6C and 4D).

Fig. 6 Cross-sectional photomicrograph of liver from *C. plancus* (A) and *R. magnirostris* (B). A full arrow points to an inflammatory infiltrate next to a blood vessel. Chronic granulomatous inflammation in*C. plancus* (C) and *R. magnirostris* (D). HE . DISCUSSION



5.3 HISTOLOGICAL ANALYSIS OF THE LIVER AND PANCREAS OF *C. PLANCUS*, *R. MAGNIROSTRIS* AND *C. ATRATUS*

The livers of these species showed a similar morphology to those described for orders of non- prey birds, such Rheiformes by Rodrigues et al. (2012), Psittaciformes by Matsumoto et al. (2009) and Struthioniformes by Saviani (2012). In these analyses and the birds of prey in this study, the absence of defined hepatic lobes was evidenced, usually found in mammals. Also, these authors observed the presence of radial hepatic trabeculae organized in lines composed of two cells, each cell having one or two nuclei. The presence of centrilobular veins randomly distributed by the liver was a common feature among birds, in addition to portal spaces composed of branches of the portal vein, branches of the hepatic artery and branches of the bile duct.

After the PAS histochemical staining, we performed a qualitative analysis of the presence and intensity of intracellular glycogen deposits. We observed that in the three analysed species there is a low amount of cellular glycogen deposit. This characteristic was mentioned in the studies by Saviani et al. (2012) who observed that birds have a low glycogen content in the liver, which can generally be associated with several factors such as the fasting time, stress, the individual's physiological conditions and the genetics of the species. The result was expected because birds of prey basically feed on animals, vertebrates and invertebrates, with low consumption of foods rich in carbohydrates. Moreover, Turner et al. (1999) through studies in Galliformes, observed that diets with higher amounts of carbohydrates elevated hepatic glycogen, while diets low in carbohydrates, as in the case of raptors, essentially rich in protein, observed a decrease in hepatic glycogen. In this case, according to the same author, the body's need for glucose is met by the process of gluconeogenesis. This process is known from the studies of Ferrer (1987) and Mighorini (1973) that demonstrated that birds of prey have a high gluconeogenic process, important for these due to their feeding habits. Explaining the fact that the birds in this study also have low glycogen reserves in hepatocytes.

The pancreas of the three species has morphological similarities with Orders of non-predatory birds such Orders Rheiformes, Psittaciformes and Galliformes (Matsumoto et al. 2009; Kadhim 2010; Rodrigues et al. 2012), they are composed of serous acinos divided by septa in the exocrine pancreas and pancreatic islets randomly distributed by the organ in the endocrine pancreas. Structures such as excretory ducts, blood vessels and lymphatic vessels were also visualized distributed throughout the organ.

Harr (2002) says that carnivorous birds have the ability to maintain balanced concentrations of glucose over prolonged periods of fasting, differently from what occurs in granivorous birds that mostly presented hypoglycemia. In cases of pancreatectomy, it was observed that in carnivorous birds there was an induction of diabetes mellitus, differently from what occurred in granivorous birds, which after the procedure, the birds presented cases of hypoglycemia.

During our analysis, some individuals of caracara and roadside hawk, had inflammatory infiltrates in both the liver and pancreas, also, to the presence of chronic granulomatous inflammation in individuals of caracara. Free-living and captive birds are subject to diseases resulting from infectious agents. Studies by Cepeda (2016) and Freitas (2008) showed the presence of mononuclear inflammatory infiltrates in the liver of *Gallus gallus* infected by *Borellia arsenia* and *Eimeria acervulina*, respectively. Santos et al. (2015) detected individuals of Accipitriformes and Falconiformes in captivity infected by *Eimeria sp.* and *Capillaria sp.* These results suggest the possible disease of some individuals in this study by parasitic agents.

In the individuals analysed of *C. atratus* (Black vulture), only one had an inflammatory infiltrate in the liver, this species that showed less inflammation in both the liver and the pancreas, suggesting that despite life in highly contaminated environments, these birds have a high adaptability of the organism to the presence of microorganisms.

Studies by Carvalho et al., (2003) showed that in *C. atratus* there is an antagonism between predominant bacteria of the intestinal flora, such as enterobacteria and gram-positive bacteria inhibiting the action of pathogenic microorganisms. Lima et al. (2011) showed that heteroantagonism occurred between strains of the bacterium *Enterobacter agglomerans* isolated from *C. atratus* against the bacterium *Pseudomonas aeruginosa*, in this study the authors showed the formation of inhibition halos in 60.2% of the samples.

This study did not show significant differences in histological analyses between the organs of the three species used, so we assumed that its high adaptability to infectious agents from the food occurs through acquired resistance due to the presence of a varied microbiota capable of inhibit the pathological action of microorganisms.

The high presence of inflammations in organs of the individuals of caracara and roadside hawk obtained in captivity suggests that healthy animals came into contact with infected animals during the captivity period, causing contamination of the environment in which they were housed. According to Daszak (2000), the management of possibly infected animals and their reintroduction into the wild can be considered a serious problem in conservation programs. This refers to human exposure to these pathogens and animal populations never before exposed. Finally, Santos et al. (2015) still state about the need for biosafety measures for the management of these species in wildlife conservation centers.

Related to the use of histomorphometry for this study, Davydova (2017) cite that its use in quantitative analysis is more objective than qualitative analysis, however this type of quantitative analysis is little used in scientific studies. Considering that all individuals of *C. atratus* studied are females, the fact that there was intraspecific variation in the concentration of binucleated cells suggests that the animals probably presented different responses of the organism to environmental conditions.

Veiga (1982) said binucleated cells are generally related to the process of liver regeneration, which in the case of *C. atratus*, is due to the diet essentially based on putrefying foods. The histological variation between male and female hepatocytes from *C. plancus* suggests that, in fact, this is a variation pattern found in nature. The same has also been seen in other species of the same order (Madeira et al. 2006; FRANZO et al. 2010). However, it is important that future studies carry out more depth analyses of the level of histological variation. Similar to *C. plancus*, the variations between males and females of *R. magnirostris* found in our analyses may represent a pattern observed in nature. The same cannot be said about the differences related to the area of the nuclei. When we observe a greater volume of the nucleus of hepatocytes in some individuals, we can infer that this characteristic is due to the transcription activity of nucleic acids (Silva and Veiga 2013). In this way, it is possible this result is random variation, which can be seen in later studies with a larger sample.

Our results about the comparison between the liver of these three different orders of birds of prey, it is natural to analyse these variations in the light of evolution. Basically, the literature considers that birds belonging to the order Accipitriformes, as is the case of *R. magnirostris* and Cathartiformes here represented by *C. atratus*, are phylogenetically closer to each other than they are to birds of the order Falconiformes, here represented by *C. plancus* (Hackett 2008). Given that, *R. magnirostris* and *C. atratus* were expected to share more histological similarities with each other than they share with *C. plancus*. Regarding the number of mononucleated cells, *C. atratus* and *C. plancus*, which are distant from a phylogenetic point of view, were also the most distinct from a histological point of view.

As previously mentioned, a higher occurrence of binucleated cells may be related to liver regeneration processes (Veiga 1982; Davydova 2017). Provided that, it is expected that species exposed to environmental conditions and diets that contribute to contamination in the liver have a higher index of binucleated cells per tissue area. Following this tendency, the species *C. atratus*, considered a scavenger bird, was one of the three that presented the highest amount of binucleated cells. On the other hand, the *R. magnirostris* species, which has a good part of the diet based on live animals, presented the lowest indexes of binucleated cells by tissue area, which is understandable in view of a lower risk of infections.

In the literature, the sinusoid capillaries of the liver, in birds and mammals, play the role of controlling bidirectional exchanges between liver cells and blood, and thus the area of liver capillaries for a species is probably related to a greater or lesser demand for transport (Collardeau-Frachon & Scoazec 2008). However, individuals of species *C. atratus* which had the highest number of cells per tissue area were the ones with the lowest sinusoid area indexes, whereas *C. plancus*, which had the lowest number of cells per tissue area, had the least sinusoid area than the other species.

It is important to discuss that the knowledge about the liver and other appendage glands of birds of prey is still incipient and future studies may contribute to elucidate the reasons for such differences in the area of sinusoids. The fact that no species was similar in terms of the nucleus area and a greater average of the nucleus area observed in *R. magnirostris*, may be related to the different rates of metabolic activity of these birds. According to Popescu et al. (2012) and Davydova (2017), the cellular metabolic rate and responses to tissue repair processes can be estimated by analysing the relationship between the size of the nucleus and the cytoplasm. Also, Popescu et al. (2012) observed that after a hepatectomy procedure in rats, there was an increase in liver nuclei. This process can be explained by the fact that hepatocytes have shapes and sizes related to metabolic activity (Rašković et al. 2011).

5.4 MORPHOMETRIC ANALYSIS OF THE RETICULAR FIBERS OF *C. PLANCUS*, *R. MAGNIROSTRIS* AND *C. ATRATUS*

A lower volume of reticular fibers observed for *C. atratus* and a higher average of these fibers for *C. plancus* characterizes different demands for protection of the hepatic framework for different species. Junqueira & Carneiro (2013) said reticular fibers are formed by type III collagen, associated with glycoproteins and proteoglycans. They form a framework that supports and protects cells in organs that normally undergo modifications. In the liver it is common to find the presence of reticular fibers, mainly forming networks in the walls of blood vessels as described by Ribeiro et al. (1997) in Galliformes.

5.5 MORPHOMETRIC ANALYSIS OF THE ISLET AREA OF LANGERHANS IN THE PANCREAS OF *C. PLANCUS*, *R. MAGNIROSTRIS* AND *C. ATRATUS*

Palmieri (2014) affirm that birds of prey have different islet configurations, these adaptations being directly involved in feeding habits, mainly in granivorous and carnivorous birds. Among carnivorous birds, which is the case of birds of prey, some studies have observed the occurrence of more insulin-producing β cells. In contrast, granivorous species tend to have more α cells, producing glucagon (STEVENS 2004). Our data on the morphometry of the area of the islets of Langherans, demonstrated that among the three species compared, none was equal to each other, which indicates differences regarding the production and release of pancreatic hormones of these species. The fact that there is a higher average of the islet area in *C. plancus* and a lower average in *C. atratus*, possibly due to a greater amount of α, β and δ cells in the first.

ACKNOWLEDGMENT

We thank the Federal University of Pernambuco, Academic Center of Vitória, especially the Anatomy and Biotechnology laboratory for the necessary support to all procedures of this research. This work was carried out with the support of the Coordination for the Improvement of Higher Education Personnel - Brazil (CAPES) - Financing Code 001.

REFERENCES

Benez SM (2001) Aves – Criação – Clínica – Teoria – Prática. Robe Editorial. São Paulo
Benez SM (2004) Aves (4ed). Tecmed. São Paulo

Carvalho CEA, Marini MA (2007) Distribution patterns of diurnal raptors in open and forested habitats in south-eastern Brazil and the effects of urbanization. Bird Conserv. Int. 17(4): 367-380. https://doi.org/10.1017/S0959270907000822

Cepeda MB, Cepeda PB, Baêta BA, Gaudêncio FN, Cordeiro MD, Magalhães-Matos PC, Brito MF, Fonseca AH (1891) Alterações bioquímicas, anatômicas e histopatológicas em fígado de *Gallus gallus* Linnaeus, 1758 experimentalmente infectados por *Borrelia anserina* Sakharoff. Pesq. Vet. Bras. 36(8): 687-693. https://doi.org/10.1590/S0100-736X2016000800002.

Cormack DH (2003) Fundamentos de Histologia. 2ª edição. Guanabara Koogan, Rio de Janeiro

Daszak P, Cunningham AA, Hyatt AD (2000) Emerging infectious diseases of wildlife: threats to biodiversity and human health. Science. 87: 443-449. DOI: 10.1126/science.287.5452.443

Davydova YA, Nesterkova DV, Mukhacheva SV, Chibiryak MV, Sineva NV (2017) Distinctive features of hepatocytes in five small mammal species (insectivores and rodents): taxonomic versus ecological specificity. Zoomorphology 136:539–548. https://doi.org/10.1007/s00435-017-0368-5

Denbow DM (2014) Gastrointestinal anatomy and physiology. In: Scanes CG (edit) Sturkie's avian physiology 337-366

Ferrer M, Garcia-Rodriguez T, Carrillo JC, Castroviejo J (1987) Hematocrit and blood chemistry values in captive raptors (*Gyps fulvus*, *Buteo buteo*, *Milvus migrans*, *Aquila heliaca*). Comp. Biochem. Phys. 87(4): 1123-1127. Doi: 10.1016/0300-9629(87)90049-1

Freitas MFL, Oliveira JB, Cavalcanti MDB, Leite AD, Magalhães VS, Oliveira RA, Sobrinho AE (2008) Parasitos gastrointestinales de aves silvestres em cautiveiro em el estado de Pernambuco, Brasil. Parasitologia Latinoamericana. 57: 50-54

Granzinolli MAM (2003) Ecologia Alimentar do gavião-do-rabo-branco *Buteo aubicaudatus* (Falconiformes:Accipitridae) no município de Juiz de Fora, sudeste do estado de Minas Gerais. São Paulo: Instituto de Biociências da Universidade de São Paulo; Universidade de São Paulo.

Junqueira LC, Junqueira, LMMS (1983) Técnicas básicas de citologia e histologia. Livraria e editora Santos. São Paulo

Junqueria LC, Carneiro J (2013) Histologia Básica, 12ª edição. Guanabara Koogan, Rio de Janeiro

Kadhim KK et al (2010) Morphological study of pancreatic duct in red jungle fowl. Afr. j. biotechnol. 9(42): 7209-7215

Lima LA, Lima CDLC, Carvalho LR, Margutti-Pinto MEB (2011) Heteroantagonismo entre estirpes de Enterobacter agglomerans isoladas de urubu (Coragyps atratus) como produtoras e Pseudomonas aeruginosa como reveladoras. Arq. Bras. Med. Vet. Zootec. 63(3): 595-599. https://doi.org/10.1590/S0102-09352011000300010.

Madeira LA et al. (2006) Morfologia das fibras musculares esqueléticas de frangos de corte de diferentes linhagens criados em sistemas de confinamento e semiconfinamento. R. Bras. Zootec. 35(6): 2322-2332. https://doi.org/10.1590/S1516-35982006000800018.
Matsumoto FS et al (2009) Topografia e morfologia das vísceras do Periquito-Australiano (*Melopsittacus undulatus*, Shaw 1805) Ciênc. anim. bras. 10(4): 1263-1270

Mighorini RH, Linder C, Moura JL, Veiga JAS (1973) Gluconeogenesis in a carnivorous Bird (black vulture). Am. J. Physiol. 225: 1389-1392. Doi:10.1152/ajplegacy.1973.225.6.1389

Montalvo CI, Talladea PO, Fernández FJ, Moreira GJ, Rafusee DJ, De Santis LJM (2011) Bone damage patterns found in the avian prey remains of crested caracara Caracara plancus (Aves, Falconiformes**)**. J. Archaeol. Sci. Elsevier. 38(12):3541-3548. Doi:10.1016/j.jas.2011.08.021

Panasci T, Whitacre D (2000) Diet and Foraging Behavior of Nesting Roadside Hawks in Pete´n, Guatemala. The Wilson J. of Ornithology, 112(4):555-558. https://doi.org/10.1676/0043- 5643(2000)112[0555:DAFBON]2.0.CO;2

Rodrigues MN et al. *(*2012) Morfologia e topografia do fígado e pâncreas de emas Rhea americana. Ciência Rural, Santa Maria. 42(3): 474-479

Santos PMS, Silva SGN, Fonseca CF, Oliveira JB (2015) Parasitos de aves e mamíferos silvestres em cativeiro no estado de Pernambuco**.** Pesq. Vet. Bras. 35(9): 788-794. https://doi.org/10.1590/S0100- 736X2015000900004

Saviani G et al. (2012) Histologia do fígado de avestruz (*Struthio camelus*, Linnaeus 1758) Braz. J. Vet. Res. Anim. Sci. 49(2): 122-129

Sick H (2001) Ornitologia Brasileira*.* 4º Ed. Nova Fronteira. Rio de Janeiro

Turner K, Applegate TJ, Lilburn MS (1999) Effects on feeding high carbohydrate and fat fiets. 1. Growth and metabólica status of the posthatch poult following immediate or delayed access tu feed. Poultry Science.78: 1573-1580

Vargas RJ et al. (2007) Diet of the southern caracara (*Caracara plancus*) in mar Chiquita reserve, southern Argentina. J. Raptor Res. 2(41): 113-121. DOI: 10.3356/0892-1016(2007)41[113:DOTSCC]2.0.CO;2

Veiga JAS et al. (1982) Effects of fasting and adrenalectomy on the kinetics of glucose metabolism in gramnivorous and carnivorous bird. Brazilian J. Med. Biol. Res. 15(2-3): 175-180