

Comparative study of the liver Histological and histochemical in barn owl (*Tyto alba*) and black francolin (*francolinus*)

Hawraa faisal Mishal¹ and Eyhab R. Al-samawy²

AL- Muthanna University / College of Vet.Medicine / Anatomy Department, Iraq¹

AL- Muthanna University / College of Medicine / Anatomy Department, Iraq.²

Corresponding author: fgbkjhg25@gmail.com.

ABSTRACT

The aim of study is provide basic data about the histological features of the liver of two species of birds, which differ in their classification, habitat, nutrition and activity. The birds were omnivorous Black francolin(*francolinus*) and carnivorous Barn Owl (*Tyto alba*). To make this purpose a 10 mature healthy males birds were bought from the local suppliers at common markets at Al-Samawa city .(5 birds from each species) , all birds were anesthetized and dissected directly. The results of histological study, our results explained that the liver in the two species of birds were surrounded by a thin layer of dense connective tissue called Glissons capsule, this capsule different in size in owl and francolin thick layer in francolin than of owl . The hepatic parenchyma in studied birds were not clearly identified divided into distinct lobules due to absence of indiscernible hepatic connective tissue septa. The basic unit of the parenchyma of liver is hepatocyte which arranged in radially around the central vein as a hepatocords consists from two – several cell in thickness in barn owl, but in francolin bird, hepatic cells are composed of chains of hepatic cell. The lobules surrounded by portal area peripherally which made from dense connective tissues , it constitutes of portal vein, hepatic artery, and bile duct, in addition to lymphatic vessels which lining by endothelium.The histochemical study showed formed differ size of glycogen granules in cytoplasm of hepatocytes and The glycogen appears as pink-color deposits as identified throughout the cellular parenchyma of the liver especially in francolin,while glycogen appears less frequent in owl.

Key words: Histology, Owl, Francolin and Histochemical.

Introduction:

Birds are consider as one of bigger classes in kingdom of Animalia including (9990) species which distributed in twenty orders, many countries legislated some laws to protect birds from hunting in Breeding season to prevent it from extinction (Abu- al Hubb ,1994) and(Grimmett , Inskipp, 2011).

The digestive system is a long hollow tube or tract that has many functions such as digestion and absorption of nutrients that are necessary for organisms, the system begins at the beak and finishes at the vent in sequential order it is composed of a mouth, oesophagus, crop, proventriculus, ventriculus (gizzard), intestine, caeca, rectum and cloaca. However, associated with the digestive tract, the accessory digestive organs which including the liver, pancreas and gall bladder that deliver

their secretory products to the small intestine by excretory ducts, which are the common bile duct from the liver and the main pancreatic duct from the pancreas that join in the duodenal loop to form a single duct common to both organs (Eroschenko, 2008), (Denbow, 2015) and (Ali and Hussein, 2020).

The liver is one of the accessory organs of the alimentary canal that deliver their secretory products to the small intestine by excretory ducts (Eroschenko, 2008) and (Faraj and Al-Bairuty, 2016) is the largest gland of the bird's body which have dark brown or red brown colour. The right side of the liver has both endocrine and exocrine gland, which are releasing several substances directly into the blood stream and secreting bile into the duct system (Krause; Cuts, 1986, Dyce *et al.*, 2010). The current study aimed to comparative study of the liver histological and histochemical finding of the above birds.

Method:

Ten birds were used to conduct the current study. They were distributed into two groups each comprises five birds of the selected male species. The two different species were used are Barn Owl (*Tyto alba*) and Black francolin (*francolinus*) as a carnivores and omnivores birds, respectively. These birds were collected from common markets at Al-Samawa city. The birds were bought from the local suppliers at these markets.

Birds were weighed before euthanasia. The birds were euthanized prior to its dissection with an intramuscular injection of sodium pentobarbitone (80 mg/kg) (Mitchell and Smith, 1991). After that, it was dissected by fixing them on a dissecting board. A mid-line incision was made in the abdominal wall of each bird to view the abdominal viscera. These organ was identified and photographed in situ using digital camera (Canon X91). The liver of the dissected birds was well described. The organ then after were washed by normal saline to remove blood or any other adhering debris. The contents of the liver was emptied by gentle pressure on each of them, and then washed by normal saline again.

For histological study, half of the specimens (liver) from each dissected birds were collected and fixed in Bouin's solution and the other half were fixed in 10% neutral buffered formalin. After well fixation the specimens were dehydrated by passing them through a series of ascending ethanol alcohol each for two hours (70%, 80%, 90%, 95% and 100%) and then specimens were cleared in xylene for one hour after that embedded in paraffin wax and then the blocks were sectioned at

6µm thickness and stained with either one of the following stains: Mayer's Hematoxylin and Eosin routine stain for general features identification and Masson trichrome stain for the staining of the collagenous and smooth muscle fibers (Bancroft and Stevens, 2010).

To conduct the histochemical study for used Periodic acid Schiff (PAS) for the neutral mucin of the liver.

Results and Discussion:

The histological results in this finding of two species of birds show the liver gland is surrounding by capsule this capsule different in size in owl and francolin thick layer in francolin than of owl this results depending on the nutrition of birds, were surrounded by dense connective tissues is mostly consist of collagen fibers with little cells most of it fibroblast. Above these connective tissues there is a layer of mesothelium of peritonitis, these collagen fibers and mesothelium forms together the capsule which called Glasson's capsule. These collagen fibers appear with blue color while mesothelium cells appear with dark blue color when coloring with Masson trichrome stain (fig.1) as shown previously (**Bacha and Wood, 1990; Subhan, 2009 and Khaleel *et al.*, 2017**)

The mean thickness of capsule in owl and francolin were (20) µm, (22.5) µm, respectively. The statistical analysis revealed significant differences at the level ($P < 0.05$) in capsule of liver between the studies birds.

The hepatic parenchyma in studied birds were not clearly identified divided into distinct lobules due to absence of indiscernible hepatic connective tissue septa while we find abundance of the connective tissue in the portal area surrounding with hepatic lobules by using the method of coloring (H&E), the visceral parenchyma of liver that formaed from parenchymal cells and non parenchymal cells (fig.2) this results agreed with reports of **El-Zoghby, (2005)** in quail, **Bacha and Wood (1990)** in chicken, and **Al-AAaraji (2015)** in turkey but not compatible with observations previously made by **Selman, (2013)** in coot bird.

The cells of gland in this study show the two types hepatic cells (hepatocytes and macrophage cells), hepatocytes occupy almost 80% of the total liver volume and perform numerous liver functions. The shape of these cells varied in the twspecies;

they were polyhedral in owl, but irregular to oval in francolin. This variation in the shape of these cells could be related to the species differences.

The hepatocytes composed of plates of hepatocytes that are arranged radially around the central vein as a hepatocords consists from two – several cell in thickness in barn owl, it is more compacted and mostly polyhedral in shape and contains large nuclei with prominent nucleoli . But in francolin bird, hepatic cells are composed of chains of hepatic cell and hepatic cord which separated from each other by a small opening representing the blood sinusoids, the hepatocytes contains a round, large and centrally placed nucleus with a prominent dark nucleolus, this results resembled description in the common moorhen (**Hussein and Hussein, 2016**), and differs with report of Hickey and Elias (**1954** who reported that the hepatocytes arranged in cords mostly with one cell thick in birds, in contrast to just two thick cells formed hepatocyte plates in turkey and chicken (**Bacha and Wood, 1990; Bhatanagar and Singh, 1982**).

The portal zone is built up of four structures: as mentioned above, in addition to lymphatic vessels. The hepatic vein of both species was wide and lined by simple squamous epithelium in the tunica intima and supported by the tunica media that consist from smooth muscle fiber. The hepatic artery was also lined by simple squamous epithelium; the smooth muscles of the tunica media were separated by the tunica intima by internal elastic lamina (fig.3). as shown by **Hussein and Hussein (2016)** in the liver of moorhen and domestic fowl.

Histochemical, the stains showed that the main stored substances in hepatocytes are glycogen but the amount of these materials varied among the two species of birds. The hepatocytes were moderate reactive to PAS reaction in the owl bird but the interaction was stronger in francolin than the owl. The glycogen appears as pink-color deposits as identified throughout the cellular parenchyma of the liver especially in francolin(fig.5)., while glycogen appears less frequent in owl (fig.4).

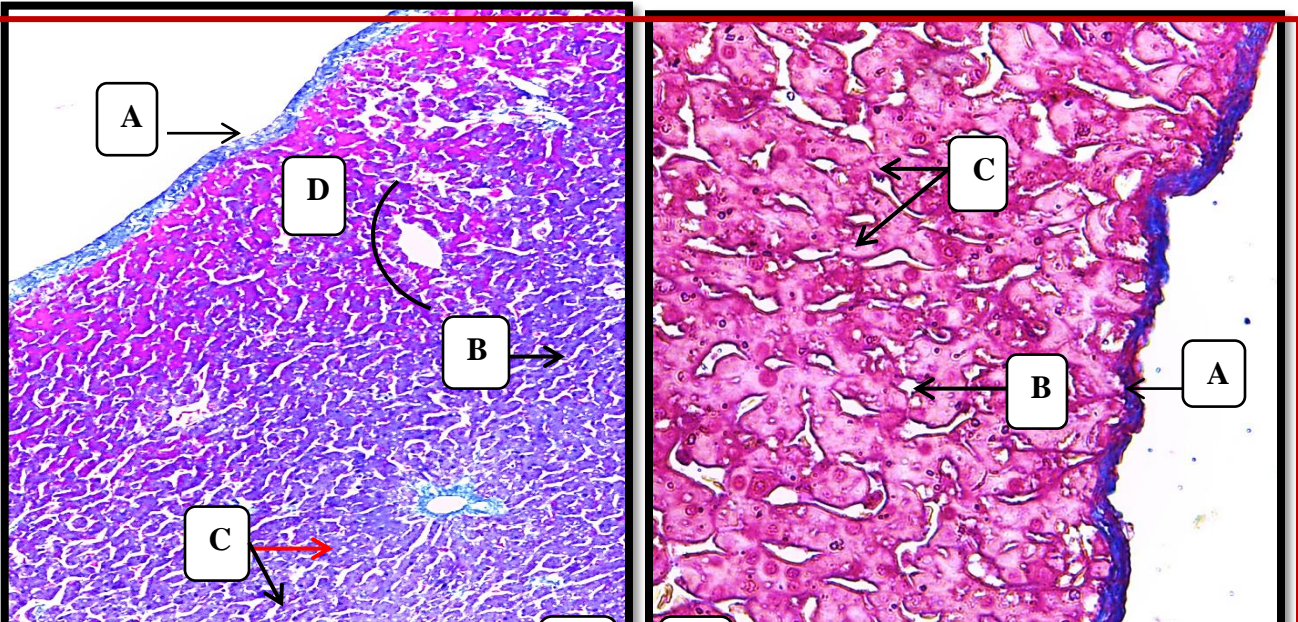


Fig.1. photography of the liver show the : A. Capsule, B. Hepatocytes, C. Sinusoid, D. Central vein.

(a) owl (b) francolin X400 Masson Trichrome stain

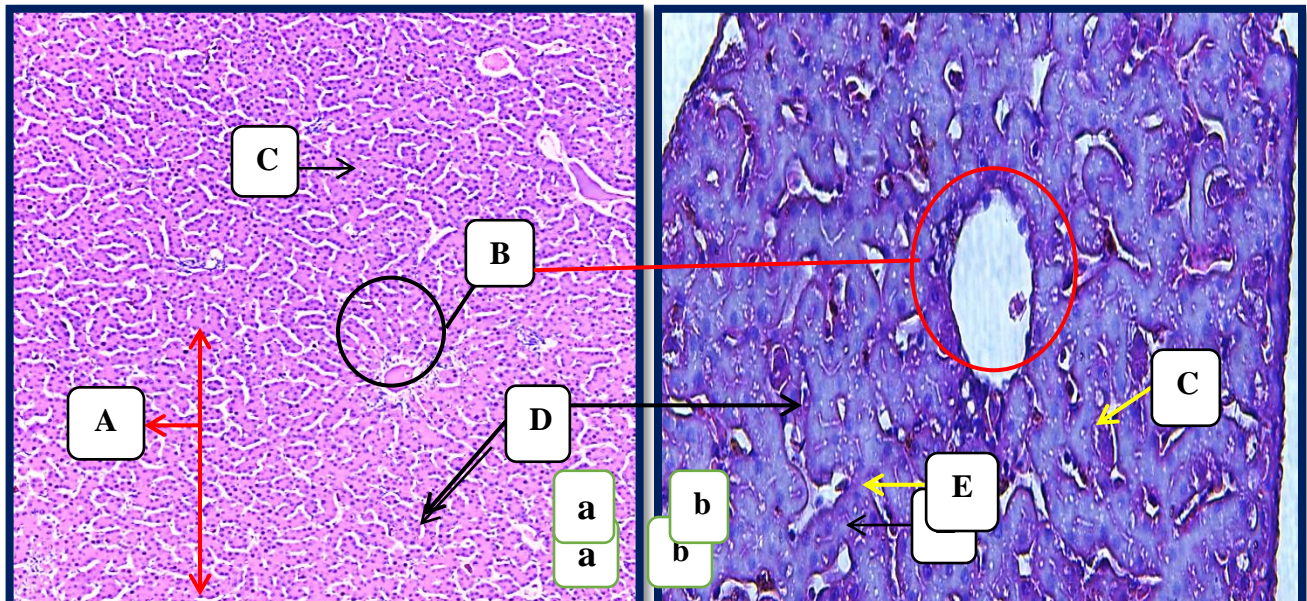


Fig.2. photography of the liver show : A. Stroma of liver, B. Central vein. C. Hepatocyte, D. Sinusoid, E. Kuppfer cell.

Fig.3. photography of the liver show the : **A.** Stroma of liver, **B.** Central vein, **C.** Hepatocyte, **D.** Sinusoid, **E.** Kupffer cell.

(a) X40 owl and (b) X400 francolin H & E

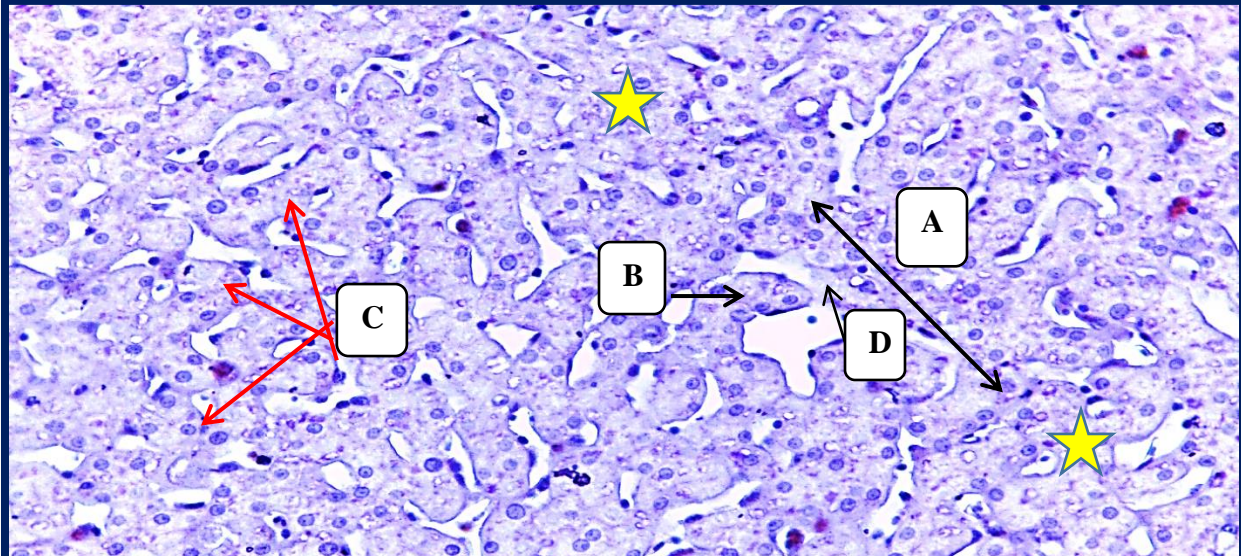
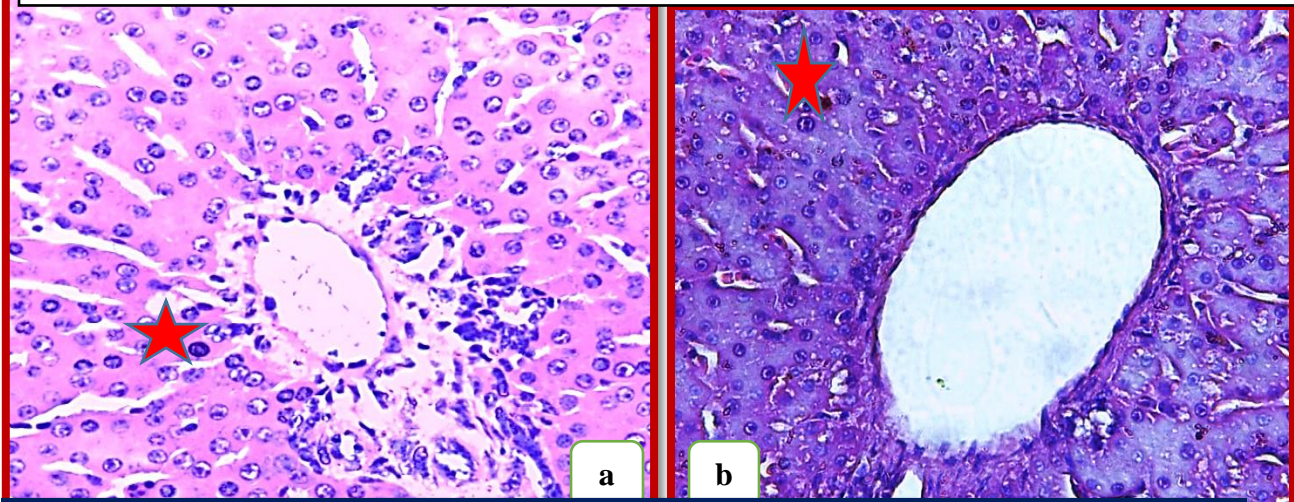


Fig.4. photography of the liver in owl show the : **A.** Hepatocyte, **B.** Central vein, **C.** Sinusoid, **D.** Endothelium of central vein and star yellow show the mucin with cells.

X400 PAS.

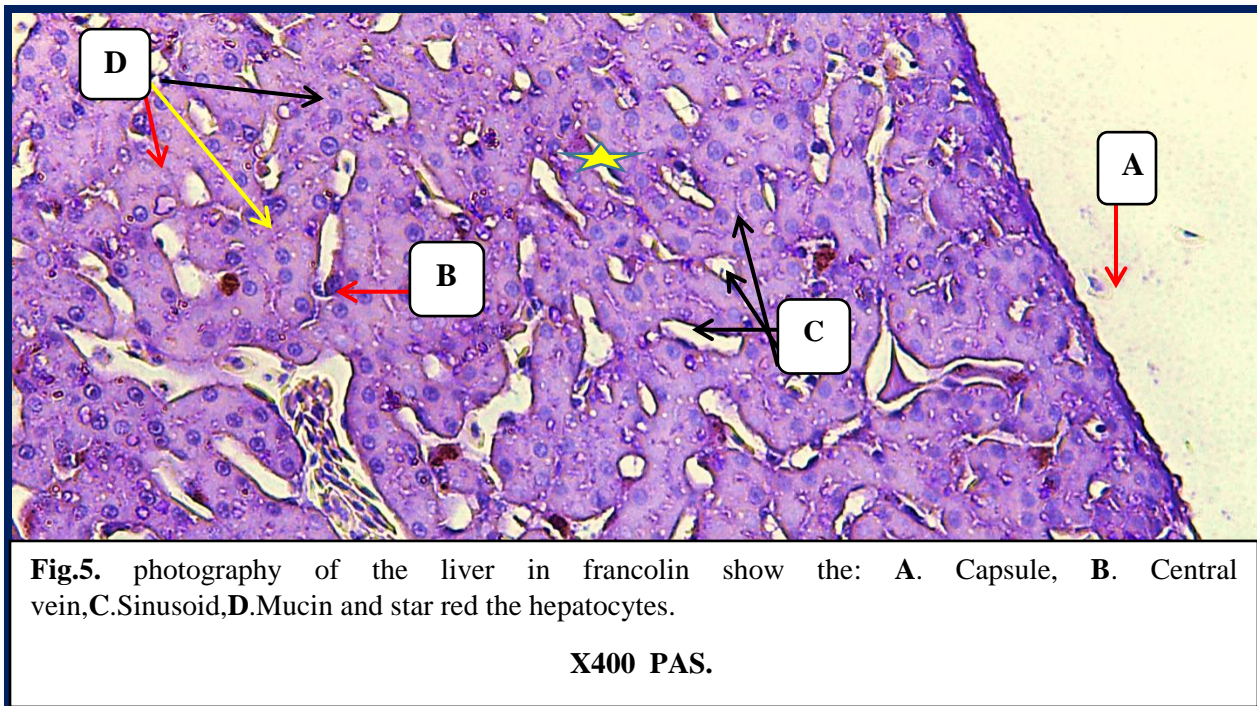


Fig.5. photography of the liver in francolin show the: **A.** Capsule, **B.** Central vein,**C.**Sinusoid,**D.**Mucin and star red the hepatocytes.

X400 PAS.

growth rate on mucosal and muscle weights in the different regions of the small intestine of the Domestic fowl (*Gallus Domesticus*). *Comp. Biochem. Physiol.* 99A: 251-258.

Bancroft, J. D. and Stevens, A. (2010): *In Theory and Practice of Histological Techniques*. 2nd (Ed), Churchill Livingstone. New York.

Al-A'araji, A. S. (2015). Study of some anatomical and histological characteristics in liver of male indigenous turkey (*Meleagris gallopava*) *Bas. J. Vet. Res.*, 14(2), 150-157.

Bacha, W.J. and Wood, G.L.M. (1990). *Avian Digestive System .Color Atlas of Veterinary Histology .William and Wikins . Waverly . Company .Hong Kong* Pp:113-150. Selman, H. A. (2013). Morphological and histological study for liver local coot birds (*Fulica Atra*). *Bas. J. Vet. Res.* 12(1), 152-158.

Dyce K M; Sack W O and Wensing C J G (2010). *Text book of veterinary anatomy fourth edition*. Sanders Elsevier. pp:135-138.

Bacha, W. J. and Wood, L. M. (1990). *Color Atlas of Veterinary Histology*. Philadelphia, Lea and Febiger. William and Wilkins a Waverly Company. PP: 113-114.

Denbow, D.M. (2015). *Gastrointestinal Anatomy and Physiology*. In, Scanes CG (Ed): *Academic Press, London. Sturkie's Avian Physiology*. 337-366. El-Zoghby, I.

- M. A.(2005). Pre and Post Hatching Developmental Studies of the Quail's Liver. Zag. Vet. J., 33(1): 185-193.
- Eroschenko, V.P. (2008). Difior's Atlas of Histology with Functional Correlations. 3rd, Lippincott Williams & Wilkins/Wolters Kluwer health, Philadelphia., USA, Pp: 235.
- Faraj, S.S. and Al-Bairuty, G.A. (2016). Morphological and Histological Study of Liver in Migratory Starling Bird (*Sturnus vulgaris*). J. Al-Mustansiriyah Journal of Science. VOL.27: 1-6.
- Grimmett, R.C. and Inskipp, T. (2011). Birds of the India, Pakistan, Nepal, Bangladesh, Bhutan, Sri Lanka and the Maldives. Princeton University Press, New Jersey 528.
- Hussein, A.J. and Hussein, D.M. (2016). Morphological and Histomorphological Comparative Study of the Liver in Adult Female Domestic Fowl (*Gallus gallus*), common moorhen (*Gallinula chloropus*) in south Iraq. Kufa J. Vet. Med. Sci. 7(1): 36-45.
- Khaleel, I.M., Al-Khazraji, K.I. and Al-Aameli, M.H. (2017). A Comparative Study in Some Morphological and Histological Features of the Liver in Gull (*laruscanus*) and Mallard Duck (*anas platyrhynchos*). Adv. Anim. Vet. Sci. 5(7): 307-311.
- Subhan , S. (2009). Anatomical and Histological and Radiological Study of the Liver, Gall bladder and Biliary Duct System of Male Local Breed Geese, (*Anser anser*) (Grelag Goose). M. Sc.Thesis. University of Sulaimani. College of veterinary medicine.Krause W. J. and Cuts J. H. Concise text of Histology, Second edition Pp: 330-334, 1986.
- Ali N A and Hussein AJ. (2020): Histo-Morphological and Histochemical Study on (Liver, Pancreas, Gall bladder) with Ultra-Structural Aspects of Pancreas between White – Eared Bulbul (*Pycnonotus leucotis*) and European Starling (*Sturnus vulgaris*). M.Sc.Tesis.University of Basra. Basra. Iraq.
- Abu- al Hubb J (1994) Prevention from harmful birds. General House of Cultural Affairs. Baghdad .No. deposit in the Library and Archives 222.