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# ABSTRACT

The objective of this study was to investigate the postnatal development of liver in “Cob-500” broilers from day 1 to day 32. Four groups of Cob-500 broilers were taken at day 1, day 14, day 28 and day 32. Five apparently healthy broilers were taken in each group. They were killed in halal method, dissected in systemic way and liver were collected. Then their shape, size, weight of liver and diameter of hepatic lobule, number of hepatic lobule were recorded. Samples from different segments were prepared and stained with haematoxylin and eosin staining technique to study the histology under light microscope. The average length (mm), weight (gm) of liver and diameter (µm) of hepatic lobule, number of hepatic lobule were significantly higher in Cobb 500 at day 35than that at day28, day 14, and day 1.

**Key words:** Liver, hepatic lobule, gross morphology, histology.

TABLE OF CONTENTS

[ACKNOWLEDGEMENT i](#_Toc409304199)

[ABSTRACT ii](#_Toc409304200)

[LIST OF TABLE v](#_Toc409304201)

[LIST OF PHOTOGRAPHS vi](#_Toc409304202)

[LIST OF ABBREVIATIONS vii](#_Toc409304203)

[CHAPTER I INTRODUCTION 1](#_Toc409304204)

[CHAPTER II REVIEW OF LITERATURE 3](#_Toc409304206)

[CHAPTER III MATERIALS AND METHODS 6](#_Toc409304208)

[3.1 Selection of study population: 6](#_Toc409304210)

[3.2 Source of the samples: 6](#_Toc409304211)

[3.3 Experimental design: 6](#_Toc409304212)

[3.4 Management: 6](#_Toc409304213)

[3.5 Determination of live weight of bird 7](#_Toc409304214)

[3.6 Sacrifice of broilers 7](#_Toc409304215)

[3.7 Collection of sample 7](#_Toc409304216)

[3.8 Gross Anatomical investigation 7](#_Toc409304217)

[3.9 Preservation of the sample 7](#_Toc409304218)

[3.10 Histometrical investigation 7](#_Toc409304219)

[3.10.1 Dehydration 8](#_Toc409304220)

[3.10.2 Cleaning 8](#_Toc409304221)

[3.10.3 Paraffin infiltration 8](#_Toc409304222)

[3.10.4 Embedding of tissue 8](#_Toc409304223)

[3.10.5 Sectioning of tissues 9](#_Toc409304224)

[3.10.6 Placing the tissues in the water bath 9](#_Toc409304225)

[3.10.7 Staining of the tissue: 9](#_Toc409304226)

[3.10.8 Mounting of slide 10](#_Toc409304227)

[3.10.9 Visualization under microscope 10](#_Toc409304228)

[CHAPTER IV RESULTS AND DISCUSSION 11](#_Toc409304229)

[4.1 Anatomical changes of liver 11](#_Toc409304231)

[4.2 Hepatic lobule shape 13](#_Toc409304232)

[4.3 Hepatic lobule diameter 13](#_Toc409304233)

[4.4 Hepatic lobule number 14](#_Toc409304234)

[CHAPTER V CONCLUSION 16](#_Toc409304235)

[REFERENCES 17](#_Toc409304237)

# LIST OF TABLE

|  |  |  |
| --- | --- | --- |
| **Serial No.** | **Title** | **Page** |
| TABLE 1 | Length, Width, and Weight change occur in relation to age | ……..11 |
| TABLE 2 | Hexagonal shape hepatic lobule found in different slides | ……..12 |
| TABLE 3 | Liver lobule diameter change in relation to age and weight | ……..13 |
| TABLE 4 | Hepatic lobule number changes with the age | ……..14 |

# LIST OF PHOTOGRAPHS

|  |  |  |
| --- | --- | --- |
| **Serial No.** | **Title** | **Page** |
| Photograph 4.1 | Anatomical changes occur in relation to age | 12-12 |
| Photograph 4.2 | Lobule diameter in relation to age and weight | 14-14 |
| Photograph 4.3 | Liver lobule at day 1 | 15-15 |
| Photograph 4.4 | Liver lobule at day 14 | 15-15 |
| Photograph 4.5 | Liver lobule at day 28 | 15-15 |
| Photograph 4.6 | Liver lobule at day 35 | 15-15 |

# LIST OF ABBREVIATIONS

|  |  |
| --- | --- |
| **Abbreviations** | **Elaborations** |
| % | Percent |
| ° | Degree |
| °C | Degree Celcius |
| μm | Micrometre |
| cm | Centimetre |
| Gm  KOH  Kg | Gram  Potassium hydroxide  Kilogram |
| M | Metre |
| Mm | Milimetre |
| Ml | Mililitre |

# CHAPTER I

# INTRODUCTION

The liver is a vital organ of the digestive system present in vertebrates and some other animals. It is necessary for survival; there is currently no way to compensate for the absence of liver function in the long term, although new liver dialysis techniques can be used in the short term (Beresford et al, 1984). Liver is a bilobbed organ that lies in the mid-coelomic cavity of avian species. It is divided into right and left lobes which are joined cranially at the midline. The right lobe is larger than the left lobe in the domestic fowl and the left lobe is subdivided into the dorsal and ventral parts. Each lobe is drained by separate bile ducts into the distal ascending loop of duodenum. It is positioned ventral and caudal to the heart (as there is no diaphragm) and surrounds the cranioventral portion of heart. It is closely associated to the proventriculus and spleen (King et al 1984).

It is dark brown colored (except just after hatching where it is yellow).The avian liver changes in color and consistency during the life of the bird. The liver of the newly hatched chick is very pale and contains a large amount of fat. It changes to a more normal brownish red color at 5-7 days of age at which time the yolk sac has been completely reabsorbed (Khenenou et al, 2013). Fat again accumulates in the liver of the female chicken when it is starting egg production. This change is physiological and under the control of estrogen. With fat infiltration the liver of a laying hen tends to be larger, paler and more friable than that of a male bird of the same age (Craig et al, 1999).

This gland plays a major role in metabolism and has a number of functions in the body, including glycogen storage, decomposition of red blood cells, plasma protein synthesis, hormone production, and detoxification. It produces bile, an alkaline compound which aids in digestion via the emulsification of lipids contains amylase and lipase. The liver's highly specialized tissues regulate a wide variety of high-volume biochemical reactions, including the synthesis and breakdown of small and complex molecules, many of which are necessary for normal vital functions.

Liver has a thin capsule. Its parenchyma resemble the liver of mammalian but there is some difference in histological features such as hepatic lobules are indistinct (except near hilus) due to a lack of perilobular connective tissue. Two bile ducts enter the distal duodenum from each lobe of the liver. The duct from the right lobe is connected to the gall bladder. Hepatocytes are the principal cell type in the liver accounting for ∼70% of the mass of the adult organ. Hepatocytes, along with biliary epithelial cells (BECs; also known as cholangyocytes) are derived from the embryonic endoderm, while the stromal cells, stellate cells, kuppfer cells and blood vessels, are of mesodermal origin.

Liver lobules of the birds are commonly described as five- or six-sided polyhedra of a fairly definite size, the variation in the same liver is very great, and the largest lobules may be five or six times the volume of the smallest ones. Those on the surface are usually fairly regular in shape, being pyramidal with a hexagonal base which is slightly convex and situated immediately beneath Glisson's capsule, and having an apex from which emerges the central vein. The lobule diameter increased from 370μm at 5 days after birth to 970μm at 35days (E.G.White et al, 1996).Mall (1906) gave an approximate estimate of the number of lobules in the dog's liver based on the area of surface lobules and the volume of the liver and, as a result of numerous calculations; he considered 480,000 to be the probable number.

There are sufficient literatures available on broiler’s different organ. But limited study was conducted on anatomy and histology of liver of broiler. Therefore the present study was conducted with an aim at explore the anatomical and histological development occur during different stage of life of broiler chicks.

The objectives of my study were:

* To know the postnatal anatomical development of liver.
* To know the postnatal histological development of liver.

# CHAPTER II

# REVIEW OF LITERATURE

The main purpose of my study was to determine the anatomical and histological development of liver happened in different ages of broiler. Several literatures (e.g*.* Craig et al, 1999; Gridley, M.F et al, 1960; J. Mclelland et al, 1984; Luna et al, 1968; Michael et l, 2007; Carmen et al, 2008;etc.) were reviewed on liver morphology of broiler. They had observed that the length and width of liver and liver lobule was significantly influenced by the ages of birds.

Liver is the largest gland in the body and it can be regarded as the central organ in the maintenance of energy supply moreover, the liver catalyzes biosynthetic and biodegrative processes and excretes final metabolic products (Katz et al, 1992). It has secretary capabilities, this organ also is able to excrete, provide, storage, detoxify, metabolize, esterifies, and phagocytize, in short, it play roles as the control center for digestive system it also functions as both endocrine and exocrine gland (Caceci et al, 2006).

Liver lies in the ventral part of the body cavity and seems red-brown in color and divided into two lobes, right and left and left one was subdivided into two parts. Right lobes were larger than left one (Henan Ali Selman et al, 2013). Liver of domestic fowl consist of two lobes the left one is small and subdivided into the dorsal and ventral parts while there were no further lobular subdivisions in the liver (Dellman et al,1979);

The liverplays important roles in digestion via the production of bile. It is also the major organ for metabolism and detoxification. It has also endocrine roles, secreting compounds into the bloodstream. The hepatocytes produce albumin, fibrinogen, and thrombin, for example.The liver, gallbladder, and pancreas receive blood supply from the celiac trunk. One main branch is the common hepatic artery, leading to the hepatic artery properthat branches into band right hepatic arteries to supply the liver. The right hepatic artery gives off the cystic artery to supply the gallbladder (Whitlo et al, 2000).

For the histology of liver of broiler we had stained the liver tissue with the recommended procedure. The birds were slaughtered by the name of Allah (Khenenou, 2013). Livers was collected carefully, set in a 10% formalin (Gridley, 1960).The achievement of blades for histological examination was made according to the technique described below; a successive passage through the different compartments of the automaton, whose goal is dehydration (passages in alcohols of different degrees), the clarification (xylene) and impregnation (infiltration) in paraffin (Bennoune, 2008). The residence time of the fragments in the automaton is 24h. Blocks were then cut to a thickness of 5μm using the microtome (MIC 509), (2009). The sections were floated on a warm water bath at 37°C, fix it on the slide and dried it. The sections were stained with the hematoxylin and eosin (H and E), Mayer's (2009).

For the assessment of histology of liver of broiler we have reviewed (Hodges et al,1974; Bacha and Wood et al,1990;Beresford and Henninger et al,1986; Randall et al,1996; Bhatnagar and Singh et al,1982).It was found from their research that liver bird is large lobed gland enclosed by serosal lining that contain a thin capsule of connective tissue which continue to subdivided the liver into lobes and to a lesser extend into lobules that provided physical support with this indicates that liver of chicken covered by mesothelium called Glisson`s capsule (Hodges et al,1974)

The Parenchymal cells of liver in broiler consist of hepatocytes which arranged radially around the central vein as hepatocords in two cells thickness, this hepatocytes it is polygonal in shape, and have rounded nucleus and there is present of sinusoids between hepatocords (Bacha and Wood et al,1990).Scientist revealed that the radiating plates of hepatocytes are two cells thick in the chicken but this result was unliked with (Randall et al,1996; Bhatnagar and Singh et al,1982) who observed that hepatocytic plates composed of (1-2) cells in thickness.

Inflow to the liver involves hepatic arteries, which bring oxygenated blood to hepatic tissue, and portal veins, which bring nutrients and other compounds absorbed by the GI tract to be processed and/or stored in the liver. Outflow also involves two routes – hepatic veins which drain into the inferior vena cava and the common hepatic duct which joins the cystic duct and empties bile into the duodenum (Bailey,T.A. et al, 1997).Major characteristics of the liver are portal triads and central veins. The portal triad contains the portal vein, the hepatic artery, and the bile duct. Each has its typical appearance. The central vein is lined with endothelial cells, with perforations into which the sinusoids empty (Seiferk et al,1977)

Liver acinus of Rappaport is the most functionally important classification. The acinus is roughly oval in shape with 2 central veins and 2 portal triads on opposite ends. Based on the blood flow within hepatic tissue, the acinus is divided into 3 zones. Cells in different zones are specialized for different activity. Zone 1 cells, being closest to the portal triads and hence most oxygenated blood, have the most drug-metabolizing enzymatic activity. Following that same reasoning, zone 3 hepatocytes near the central veins are most susceptible to ischemia (Michel et al,2007).

Liver lobules of the birds are commonly described as five- or six-sided polyhedra of a fairly definite size, the variation in the same liver is very great, and the largest lobules may be five or six times the volume of the smallest ones. Those on the surface are usually fairly regular in shape, being pyramidal with a hexagonal base which is slightly convex and situated immediately beneath Glisson's capsule, and having an apex from which emerges the central vein. The lobule diameter increased from 370μm at 5 days after birth to 970 μm at 35 days. Two livers show even higher values than the latter and, had the animals been allowed to survive longer, liver weights of 3000 g. might well have been obtained.(E.G. White et al,1997)

The hepatic lobules in birds increase in size during growth has long been known, but the question as to whether this increase is sufficient to account for the growth of the liver has not been decided. Killing (1905) suggested that the increase in size of the liver might result from the formation of new lobules, enlargement of pre-existing ones, increase of interstitial tissue, or a combination of these. He did not find any evidence of the new formation of lobules. The age and liver weight of the animals were not given. Mall (1906) gave an approximate estimate of the number of lobules in the dog's liver based on the area of surface lobules and the volume of the liver and, as a result of numerous calculations; he considered 480,000 to be the probable number. This rough estimate, unsupported by maceration experiments, proved to be remarkably close to the values subsequently obtained by Johnson (1918 b).

# CHAPTER III

# MATERIALSANDMETHODS

The present work was carried out to demonstrate the morphometrical and histometrical details of liver of COBB 500 broiler chickens. The study was carried out in the Dept. of Anatomy & Histology, Faculty of veterinary medicine, Chittagong Veterinary and Animal Sciences University.

## 3.1 Selection of study population:

The study was carried out in broiler chickens COBB 500 of different ages, ranging from day 1 to day 35 days. The samples were collected at weekly intervals each group consists of 3 birds of either sex.

## 3.2 Source of the samples:

The broilers were collected from broiler farms situated in vary remote place in the Chittagong district. The samples were collected at weekly intervals from “Rezaul Poultry Farm”. The birds were apparently healthy without any detectable signs of disease and abnormalities that could influence the result of the study.

## 3.3 Experimental design:

Prior to the start of the experiment an experimental schedule was fixed specifying different aspects of the total work and it was maintained during the whole study. Samples were divided into 5 groups according to their ages and regardless of sex. Each group consists of three broiler chickens of equal ages- group I (1 day), group II (14days), and group III (28 days), group IV (35 days) .The experiment was carried out on each group at different days for efficiency of work and to minimize the result errors.

## 3.4 Management:

After collection, all the chickens were preserved in a cage, in the department of Anatomy and Histology, Faculty of Veterinary Science, Chittagong Veterinary and Animal Sciences University (CVASU). Optimum temperature, lighting and ventilation were maintained. Water and feed were provided ad libitum. All procedures were approved by the Animal Care and Welfare Committee of our institute.

## 3.5 Determination of live weight of bird

Live weight was measured using sensitive electronic balance (Mettler Toledo B154, ± 0.001g, China) prior to sacrifice of the broilers. In the laboratory of Department of Anatomy and Histology, Faculty of Veterinary Science, CVASU

## 3.6 Sacrifice of broilers

The birds were sacrificed by Halal method.

## 3.7 Collection of sample

After slaughtering of chicken and ceasation of the heartbeat and respiratiom the blood samples were collected by thymectomy and bursectomy**.** The collected samples were washed properly using normal saline and dried properly.

## 3.8 Gross Anatomical investigation

The shape, color, consistency and position of the liver were observed. After that weight of the samples were taken and recorded. By using digital caliper the longitudinal diameter, transverse diameter are measured and tabulated. The percentage of liver weight in relation to the body weight was tabulated.

## 3.9 Preservation of the sample

After the gross anatomical investigation the samples are cut to a suitable size and tagged with a piece of paper to identify the sample name and number and then fixed by chemical fixation using 10% formalin solution.

## 3.10 Histometrical investigation

The lobule shape, increase diameter, and the total number change at different stage of broiler life was calculated and recorded and shows them graphically with Microsoft Excel. The mean diameter of the lobules was then calculated from the mean area of cross-section (Area) assuming them to be regularly hexagonal. The formula is:

**Diameter of regular hexagon=1.24 √ Area.**

The total number of lobules was calculated from the liver weight-actual or estimated-after allowing for the shrinkage occurring during fixation, dehydration and embedding.

For this purpose at first histological slide was prepared first. Procedure of histological slide preparation is given bellow:

### 3.10.1 Dehydration

The tissues are washed in water overnight prior to the dehydration process. The tissues are dehydrated in the dehydration process using different

Concentration of alcohol (ethyl alcohol ETOH) and xylene.

50% alcohol (ethyl alcohol) (starting from water) - 2 hours

70% alcohol- 2 hours.

90%alcohol- 2 hours.

95% alcohol- 2hours.

100% alcohol (first time) - 1 hour

100% alcohol (second time) - 1 hour

### 3.10.2 Cleaning

Alcohol+ xylene(50%) – 2 hours or overnight.

Toluene or xylene (first time)- 1/2–1 hour

Toluene or xylene (second time) - 1/2–1 hour

### 3.10.3 Paraffin infiltration

From the second change of xylene the tissues are moved through several changes of xylene+paraffin and kept in oven for maintaining specific temperature (58-600 c).

### 3.10.4 Embedding of tissue

The tissues are then embedded in 100% paraffin and paraffin blocks were mounted using suitable pieces of metal (two L shaped metal join together to make a block).

### 3.10.5 Sectioning of tissues

Prior to sectioning of the tissues using rotary microtome machine the face of each paraffin blocks were trimmed properly so that it is in the shape of a trapezoid with the top and bottom sides being parallel. The block was then cut at a diameter of 6 µm.

**Fig: Shape of the paraffin block**

### 3.10.6 Placing the tissues in the water bath

Bundles of cut sections (paraffin ribbon) were then placed in water bath at a temperature of 58ºc in order to melt the paraffin and also for spread the tissues properly. Necessary amount of slides are taken and cleaned properly using blotting paper. Then with a diamond marker one end of the each slide were scratch marked to identify the sample name and number. Egg albumin was applied as an adhesive to hold up the tissues so that they won’t fall down or detach during further processing. The tissues from the water bath are then wiped into the slides very gently. The slides were then dried overnight and placed in slide tray for further processing.



**Fig: ribbons of paraffin sections floating on water on a slide.**

### 3.10.7 Staining of the tissue:

For the staining procedure Hematoxylin and Eosin stain was used. Prior to staining the paraffin was completely removed from the tissues using xylene. The slides are then washed in water and rehydrated again using graded series of alcohol (ethyl alcohol ETOH) after staining with hematoxylin and eosin the slides are then washed in xylene.

**Staining schedule:**

* Toluene (or xylene) I — ↓ 20 min.
* Toluene (or xylene) II — ↓ 20 min.
* 100% alcohol— ↓ 5 min.
* 95% alcohol — ↓ 5 min.
* 70% alcohol — ↓ 5 min.
* Distilled water — (I) 5 min.
* Alcian blue - 10 min.
* Distilled water — (II) 5 min.
* Hematoxylin -2 min.
* Tap water — several changes 1 min. each
* 70% alcohol — ↑ 5 min.
* Alcoholic eosin 5 min 95% alcohol — ↑ 1 min. dipping
* 100% alcohol I — ↑ 1 min. dipping
* 100% alcohol II — ↑ 1 min. dipping
* Xylene — ↑ 2 min.

### 3.10.8 Mounting of slide

After completing the staining procedure the slides were air dried properly. Then cover slips were mounted in the slides very carefully using a suitable mounting medium (Canada balsam) without producing any bubbles. The excess mountant was removed very carefully with tissue paper.

### 3.10.9 Visualization under microscope

When the slides were properly fixed and dried the slides were taken under microscope. Slides are then visualized at 4x objective and still images were taken by using a computer operated (top view) software. After capturing still photo image these images were opened in a different computer operated software named canvas 14 for hystometrical measurement of different parameters like cortical diameter, medullary diameter, height and width of the lobe and folds. And the values of no. of lobe were tabulated chronologically. Along with these operations different cellular structures like hepatocytes, portal triad, bile duct, and gall bladder were observed and marked.

# CHAPTER IV

# RESULTS AND DISCUSSION

## 4.1 Anatomical changes of liver

The table1 and the graph1 bellow shows that the length and width of liver was increased in relation to ages, this data was supported by (king et al, 1974) he was reported that liver length and diameter was increased during postnatal development due maintain body metabolism according to age. The graph1 shows that the weight of liver was increased in relation to age which was supported by (George et al, 2013) who were reported that the fresh weight of liver was 41.33 ± 10.11gm.

**Table 1: Length, Width, and Weight change occur in relation to age**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Age  (days) | **Length(mm)** | **Width(mm)** | **Weight(gm)** |  |
| Mean ± Std. error | Mean ± Std. error | Mean ± Std. error | Picture |
| Day 1 | 25.03 ± 1.1 | 28.43 ± 0.09 | 14.56 ± 5.66 | **H:\TAREQ\Report\IMG_2172.JPG** |
| Day 14 | 38.69 ± 1.37 | 39.09 ± 1.1 | 23.16 ± 6.11 | **H:\TAREQ\Report\IMG_2164.JPG** |
| Day 28 | 45.78 ± 2.16 | 48.96 ± 1.87 | 34.21 ± 8.27 | H:\TAREQ\Report\IMG_2167.JPG |
| Day 35 | 55.30 ± 2.52 | 58.15 ± 2.36 | 41.33 ± 10.11 | **H:\TAREQ\Report\IMG_2148.JPG** |

**Histological changes of liver**

## 4.2 Hepatic lobule shape

The result shows in table 2 in cross-section, almost all the lobules have five or six sides, the pentagonal cross-section being rather more common. No evidence was obtained of any change in shape of the lobule during growth; in histological sections and in macerated specimens the shape was similar in animals of all ages, the length and breadth being approximately in the ratio 3:2 this result is supported by (E.G. White et al, 1997)

**Table 2: Hexagonal shape hepatic lobule found in different slides**

|  |  |
| --- | --- |
| No. of sides | No. of lobules |
| 3 | 1 |
| 4 | 66 |
| 5 | 308 |
| 6 | 246 |
| 7 | 28 |

## 4.3 Hepatic lobule diameter

The table 3 bellows shows that the liver lobule diameter changes in relation to age and weight this data was supported by (E.B. White et al, 1997) he was reported that liver lobule diameter was increased during postnatal development when the size of liver was increased. He reported that when the liver weights 30gm the diameter was 0.37mm.

**Table 3: Liver lobule diameter change in relation to age and weight**

|  |  |  |
| --- | --- | --- |
| Age (days) | Weight(gm) | Lobule Diameter(μm) |
| Day 1 | 14.56 | 370 |
| Day 14 | 23.16 | 560 |
| Day 28 | 34.21 | 790 |
| Day 35 | 41.33 | 970 |

## 4.4 Hepatic lobule number

The result shows from the table 4 thatif the increase in size of the liver occurs with age, the number of lobules will increase. We have shown that the liver lobule, between one and 35 days after birth, increases in number from 0.35 x 106to 0.79 x 106 i.e. 1.5-2 times. This result is supported by (E.G. White et al, 1997; Johnson et al, 2006), they reported that the increase in size of the liver were due solely to increase in the number of lobules, an increase of about eighty times would be needed. But this does not occur due to different reason.

**Table 4: Hepatic lobule number changes with the age**

|  |  |  |
| --- | --- | --- |
| Age (days) | Weight(gm) | No of lobules |
| Day 1 | 14.56 | 0.35 x 106 |
| Day 14 | 23.16 | 0.51 x 106 |
| Day 28 | 34.21 | 0.71 x 106 |
| Day 35 | 41.33 | 0.79 x 106 |

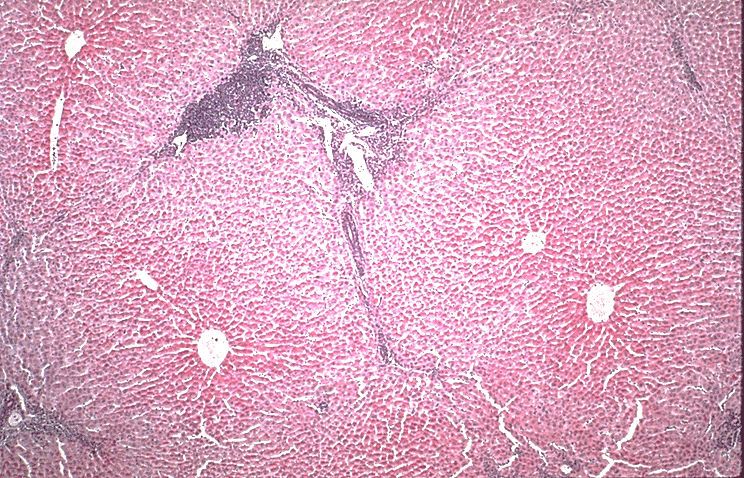
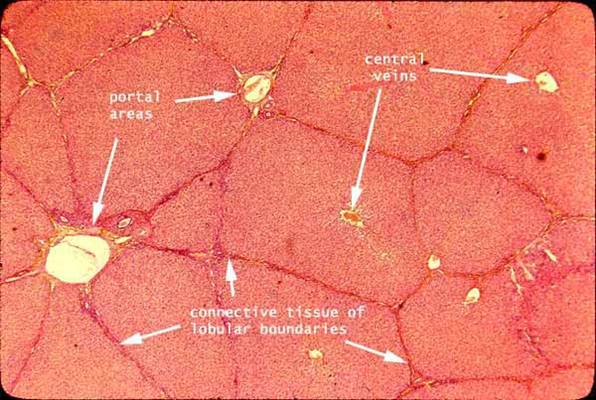
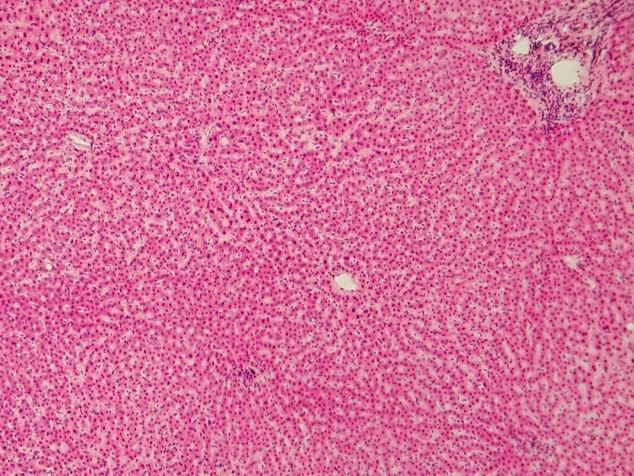
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Fig 4.6Liver lobule at day 35

Fig 4.5Liver lobule at day 28

Fig 4.4Liver lobule at day 14

Fig 4.3Liver lobule at day 1

# CHAPTER V

# CONCLUSION

The objective of this study was to investigate the postnatal development of liver in “Cob-500” broilers from day 1 to day 32. Four groups of Cob-500 broilers were taken at day 1, day 14, day 28 and day 32. Five apparently healthy broilers were taken in each group. They were killed in halal method, dissected in systemic way and liver were collected. Then their shape, size, weight and diameter of hepatic lobule, number of hepatic lobule were recorded. Samples from different segments were prepared and stained with haematoxylin and eosin staining technique to study the histology under light microscope. The liver length, width and weight were 25.03mm, 28.43mm, and 14.56gm respectively. It was increased to 55.30mm, 58.15mm, and 41.33gm respectively at day 35. The lobule became hexagonal and their diameter and number of hepatic lobule at day one were 370μm and 0.35 x 106 which was increased to 970 and 0.79 x 106 day 35 respectively. The average length (mm), weight (gm) of liver and diameter (µm) of hepatic lobule, number of hepatic lobule were significantly higher in Cobb 500 at day 35 than that at day28, day 14, and day 1.

# 

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