

Comparison of Fat and Carbohydrate Metabolisms in Chicken and Rat Liver

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ABSTRACT

The aim of this study is to reveal histological differences between prenatal and adult liver carbohydrate and fat metabolism in chickens and rats. In this study, 30 Wistar albino rats and 30 Ross breed broiler chickens were used. The rats and chickens were divided into groups of 10. These groups were categorized as adult, 14- and 18-day-old fetus groups. In this study, the fat and carbohydrate metabolisms in rat and chicken liver were studied by histological methods, and changes in adult and fetal periods were evaluated comparatively. In the light microscopic examinations of liver carbohydrate accumulation, hepatic glycogen accumulation was similar in the adult rats and chickens. When the hepatic glycogen amount was compared in the 14-day-old rat and chicken fetuses, while glycogen granules were found in most of the chicken fetal hepatocytes, almost no glycogen granules were found in the rat fetuses. This difference between the animals was found to be statistically significant ($p<0.001$). It was observed that glycogen accumulation in the 18-day-old rat fetuses was less than that in chicken fetuses. This difference between the animals was found to be statistically significant ($p=0.013$). Light microscopic examinations of liver lipid accumulation showed that similar ratios of lipid droplets were observed in the hepatocytes of adult rats and chickens. Significantly higher amounts of lipid droplets were detected in the 14- and 18-day-old chicken fetus hepatocytes compared with 14- and 18-day-old rat fetus hepatocytes, which was statistically significant ($p<0.001$).

Keywords: chicken, fetus, hepatocyte, rat.

Tavuk ve Rat Karaciğerinde Yağ ve Karbonhidrat Metabolizmalarının Karşılaştırılması

ÖZ

Bu çalışmanın amacı, tavuklarda ve ratlarda doğum öncesi ve erişkin dönem karaciğer karbonhidrat ve yağ metabolizması arasındaki histolojik farklılıkları ortaya koymaktır. Çalışmada, Wistar albino türü 30 adet rat ile 30 adet Ross ırkı broiler tavuk kullanıldı. Ratların ve tavukların her biri 10' arlı gruplara ayrıldı. Bu gruplar erişkin, 14 günlük ve 18 günlük fötüsler halinde düzenlendiler. Bu çalışmada, rat ve tavuk karaciğerindeki yağ ve karbonhidrat metabolizmaları histolojik yöntemlerle incelenmiş, erişkin ve fetal dönemlerdeki değişimler karşılaştırmalı olarak değerlendirilmiştir. Karaciğer karbonhidrat birikiminin ışık mikroskopik incelemelerinde, Erişkin dönem ratlar ile tavuklarda hepatik glikojen birikiminin benzer yoğunlukta olduğu görüldü. 14 günlük rat ve tavuk fötüslerinde hepatik glikojen miktarı karşılaştırıldığında, tavuk fötüs hepatositlerinin çoğunda glikojen granüllerine rastlanırken rat fötüslerinde yok denecek kadar az miktarda glikojen granülüne rastlandı. Hayvanlar arasındaki bu fark istatistiksel olarak da anlamlı bulundu ($p<0.001$). 18 günlük rat fötüslerindeki glikojen birikiminin tavuk fötüslerine göre daha az miktarda olduğu gözlemlendi. Hayvanlar arasındaki bu fark istatistiksel olarak da anlamlı bulundu ($p=0.013$). Karaciğer lipid birikiminin ışık mikroskopik incelemelerinde, erişkin rat ve tavuk hepatositlerinde benzer oranlarda lipid damlacığı görüldü. 14 ve 18 günlük tavuk fötüsü hepatositlerinde 14 ve 18 günlük rat fötüslerine oranla belirgin derecede fazla miktarda lipid damlacığı tespit edildi. Hayvanlar arasındaki bu farklar istatistiksel olarak da anlamlı bulundu ($p<0.001$).

Anahtar Kelimeler: fötüs, hepatosit, rat, tavuk.

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INTRODUCTION

The liver, the body's factory, has important functions such as bile secretion, storage of substances such as glycogen, lipid, vitamins, and iron, purification of blood from metabolism residues and toxic substances, and hematopoiesis in the fetal and neonatal periods (Junqueira and Charneiro 2009, Petorak 1986). These functions of the liver occur in tandem with the morphological development of the organ in the prenatal and postnatal period (Aydin et al. 2000). In order for the fetus to grow and develop normally, resources such as carbon, nitrogen, ions and water are required. These resources are used in important biochemical events such as the formation of new tissues, the storage of molecules such as glycogen and fat, and the production of energy for growing tissues. Therefore, although the total nutritional requirement of the fetus varies depending on its growth rate, it also differs by species (Fowden 2001). The fetal growth in chickens occurs through physiological phenomena that take place in a closed system independent of the mother. In rats, on the other hand, fetal growth occurs when physiological requirements are met as a result of the mutual interaction of the maternal and placental environment with the fetus (King and Loke 1994).

The liver has many roles in carbohydrate and lipid metabolisms. A significant part of these tasks is performed by hepatocytes, which constitute approximately 80% of the organs (Mitra and Metcalf 2012). Glucose, the most important fuel for living things, is stored in hepatocytes in the form of glycogen. Glycogen deposits can be visualized with Periodic acid-Schiff (PAS) and Best's carmine stains. The lipid droplets stored in the form of triglycerides in hepatocytes can be visualized using dyes such as Sudan Black B and Oil Red O after appropriate detection (Junqueira and Charneiro 2009, Altınışık 2010, Kierszenbaum and Tres 2016).

The liver is a key organ involved in various metabolic processes that regulate the growth and productivity of living things. Considering the yield characteristics of animals, the importance of liver carbohydrate and fat metabolism is understood. When the fetal and adult periods are considered comparatively, it is thought that the findings obtained from the research can be a guide in the development of yield characteristics, and thanks to this information, new clues can be obtained at the level of basic information on fat and carbohydrate metabolism. In this study, to compare the fat and carbohydrate metabolisms in chicken and rat liver, liver sections of 14- and 18-day-old rat and chicken fetuses and adult rats and chickens were investigated by light microscopy after staining with relevant histological techniques.

MATERIAL AND METHODS

This study was approved by the Ankara University Animal Experiments Local Ethics Committee (dated: 14.03.2018, approval number: 2018-6-55). In the study, rats supplied from Ankara University Experimental Animal Production and Research Laboratory and broiler chickens obtained from Sökmenler Poultry Chicken Production and Marketing were used.

In the study, 30 Wistar albino rats and 30 Ross broiler chickens were used as study material. The rats were divided into 3 equal groups including 10 rats in each group. These groups were categorized as adult (6–8 weeks) rats, 14- and 18-day-old fetuses. The broiler were divided into 3 equal groups including 10 chickens in each group. These groups were categorized as adult (39–43 days) chickens, 14- and 18-day-old chicken fetuses. The rats were cared for and fed in Ankara University Experimental Animal Production and Research Laboratory. Animals were fed with standard pellet feed and tap water in 12 hours of light and 12 hours of darkness without restriction of feed and water. In the care and nutrition of the chickens, they were fed in a 24-hour light environment by giving the basic ration without any feed and water restriction in the Sökmenler Poultry Chick Production Center. Eggs were incubated in an incubator under optimal conditions (Incubation temperature 36.5-37.5C, Humidity: 85%). Considering the basic embryological information available on the development of rat and chicken fetal liver and literature reviews on the carbohydrate and fat metabolism of these animals (Elias 1955, Luzzatto 1981, Hamburger and Hamilton 1951, Suksaweang et al. 2004), liver tissue samples were obtained from 14- and 18-day-old fetuses.

On the 14th and 18th days of pregnancy the abdomen of the rats was opened under ketamine-xylazine anesthesia and their fetuses were removed from the dissected uterus. After their macroscopic examination, the abdomen of the fetuses was opened and their livers were removed. Tissue samples were also obtained from various parts of the liver from the adult rats under anesthesia.

The developmental stages of the embryos in the chicken fetuses were determined according to the Hamburger and Hamilton (1951) stages, the formation of organs was evaluated according to this scale, and embryos that were out of the scale or showing developmental disorders were not included in the experiment. The chicken eggs were carefully broken and the liver tissues of the fetuses were dissected. Tissue samples were taken from various parts of the liver after cervical dislocation of the adult chickens.

Light Microscopic Examinations

The liver samples obtained from the animals were placed in tissue plates in 10% buffered formol, formol–alcohol at + 4°C and Baker’s 10% formol–calcium solutions at + 4°C for histological preparation. Both fetal and adult liver tissue samples, which were fixed in formol–alcohol for 24 h at + 4°C, were passed through 96% alcohol, absolute alcohol, methyl benzoate, and benzol series and blocked in paraplast. Best’s carmine staining and PAS staining were performed on 5 µm sections taken from the blocks to visualize glycogen in hepatocytes. In Best’s carmine and PAS staining, the specificity of the reaction was determined with control preparations. For this purpose, α -amylase that hydrolyzes glycogen (diastase digestion) was applied to control sections and the non-stained structures were evaluated as glycogen (Bancroft and Gamble 2002). The liver tissue samples fixed in 10% buffered formol for 24 h were washed with tap water for 24 h and then passed through graded alcohols, methyl benzoate, and benzol series and blocked in paraplast. Mallory’s trichrome staining technique, modified by Crossmon for general histological examinations were applied on 5 µm sections taken from the blocks (Bancroft and Gamble 2002). The preparations obtained were examined under the Leica DM 2500 model research microscope and were photographed.

In contrast, 8–10 µm thick cryostat sections were taken from the tissue samples that were fixed in 10% formol–calcium solution for 24 h at + 4°C. The Sudan Black B and Oil Red O stainings were performed on these sections to visualize the oils (Bancroft and Gamble 2002). The preparations obtained were examined under the Leica DM 2500 model research microscope and were photographed.

Evaluation of Histological Data

Statistically, the level of carbohydrate accumulation was graded using a Likert scale ranging from 1 to 5. The grading was conducted by two different histologists. Glycogen granules seen in pink-red color in the hepatocyte cytoplasm of the liver sections stained by PAS were evaluated and graded as follows. Descriptive statistics for the obtained data were calculated. The statistical control of the difference between the species and the developmental periods of the same species in terms of the evaluation of carbohydrate accumulation was done with the Chi-Square Test. SPSS 14.01 package program was used. In order to evaluate the fat accumulation in the liver statistically, with the Image J program, the fat droplets in a 40× (enlarged 400 times) area were counted manually. In the hepatocyte cytoplasm of the liver sections stained with Oil Red O Red, lipid droplets were counted. Descriptive statistics for the

obtained data were calculated. Differences between species in terms of oil droplet count statistical control was done with Independent Samples t-Test. The statistical control of the difference between the developmental stages of the same species was done by One-Way Analysis of Variance. In case of any statistical difference between the groups with One-Way Analysis of Variance, the Tukey Test was applied as a post hoc analysis to determine which two groups caused the difference. SPSS 14.01 package program was used.

RESULTS

Light Microscopic Results

In the triple-stained liver sections of the adult rats and chickens, it was observed that the parenchyma of the organ consisted of polygon-shaped epithelial cell cords that anastomosed with each other. Between the cords were sinusoids opening into the vena centralis. The portal triad consisting of artery, vena, and bile duct in the connective tissue mass between the lobes, which were not very pronounced, showed a similar appearance in adult rats and chickens. In the triple-stained liver sections of the fetuses taken on the 14th and 18th days of the development of rats and chickens, vena centralis and sinusoids were seen in the center of the hepatic lobules whose borders could not be clearly identified. Small hepatocytes were found around the vena centralis that did not show regular localization as in adult hepatocytes (Figure 3.1).

In the adult rat and chicken PAS-stained liver sections, dense glycogen accumulation was observed in a large number of hepatocytes around the vena centralis and portal area. Best’s carmine method used for visualization of glycogen in the liver showed the same results as PAS staining. In PAS and Best’s carmine staining methods, the specificity of the reaction was determined by the application of α -amylase, which hydrolyzes glycogen (Figure 3.2). When the fetal livers obtained on the 14th day of development of rats and chickens were compared, it was observed that there were distinct glycogen granules in most of the chicken fetal hepatocytes, whereas they were almost absent in the rat fetuses. Best’s carmine method showed the same results as PAS staining (Figure 3.3). When microscopic liver images of the rat fetuses (18 days old) and chicken fetuses (18 days old) were compared, glycogen accumulation in the rat fetuses was less than that in the chicken fetuses (Figure 3.4). The specificity of the reaction was determined by the application of α -amylase, which hydrolyzes glycogen.

In the Oil Red O and Sudan Black B stainings made to visualize fats, positive lipid droplets were found in the adult rat and chicken hepatocytes. When the liver

cryostat sections of the rats and chickens were compared, similar proportions of lipid droplets were found in hepatocytes (Figure 3.5). In the fetal livers obtained on the 14th day of the development of rats and chickens, the amount of lipid droplets in the chicken fetus hepatocytes was observed to be significantly higher than that in the rat fetus hepatocytes (Figure 3.6). When the liver cryostat sections of 18-day-old rat and chicken fetuses were compared, it was observed that lipid droplets in the fetal chicken hepatocytes were more than those in the fetal rat hepatocytes (Figure 3.7).

As a result of light microscopic examinations, while both glycogen granules and lipid droplets were seen in the adult rat hepatocytes, glycogen granules were almost never observed on the 14th day of fetal development. However, glycogen granules were prominent in the 18-day-old rat fetuses, although not as much as in the adult rats. The amount of hepatic lipid droplets in the 18-day-old rat fetuses was higher than that in the 14-day-old rats. While the number of lipid droplets in the adult rats was higher than that in the 14-day-old rat fetuses, it was similar to that in the 18-day-old rat fetuses (Figure 3.8).

As a result of light microscopic examinations, both lipid droplets and glycogen granules were found in the liver of all groups of chickens. The group of chickens ranked from highest to lowest based on the density of hepatic glycogen concentration was adult chickens, 18-day-old chicken fetuses, and 14-day-old chicken fetuses. The group of chickens ranked from highest to lowest based on the amount of hepatic lipid droplet accumulation was 18-day-old fetuses, 14-day-old fetuses, and adult chickens, respectively (Figure 3.9).

Statistical Results

The average number of lipid droplets was counted in the liver sections of the adult rats and chickens, 14- and 18-day-old rat and chicken fetuses using the ImageJ program. As a result of this, no statistically significant difference was found in terms of average fat droplet counts in the adult rat and chicken livers (Table 3.1). The average fat droplet count of 14- and

18-day-old chicken fetuses was found to be higher compared with that of 14- and 18-day-old rat fetuses ($p < 0.001$; Table 3.2, $p < 0.001$; Table 3.3).

The average fat droplet count of the adult rats was found to be statistically higher than that in rats on the 14th day of fetal development. However, no statistically significant difference was found between the adult rats and 18-day-old rat fetuses in terms of average fat droplet count. There is a statistically significant difference between the 14- and 18-day-old rat fetuses in terms of average fat droplet count ($p = 0.009$; Table 3.4).

The average fat droplet count of the adult chickens was statistically lower than that of the 14- and 18-day-old chicken fetuses. The average fat droplet count of the chickens on the 18th day of fetal development was statistically higher than that of the 14-day-old rat fetuses ($p < 0.001$; Table 3.5).

The liver tissue samples of the adult rats and chickens, 14- and 18-day-old rat and chicken fetuses were rated by two different histologists using a Likert scale ranging from 1 to 5. As a result of the evaluation, no statistically significant difference was found between the adult rats and chickens in terms of carbohydrate accumulation in the liver (Table 3.6). However, there was a significant difference in terms of carbohydrate accumulation between the 14- and 18-day-old rat fetuses and chicken fetuses, and it was found that hepatic glycogen density in the chicken fetuses was higher than that of the rat fetuses ($p < 0.001$; (Table 3.7, $p = 0.013$; Table 3.8).

The liver carbohydrate accumulation in adult rats was found to be higher compared with that in the 14- and 18-day-old rat fetuses. In addition, a statistically significant difference was observed between the 14-day-old rat fetuses and the 18-day-old rat fetuses in terms of carbohydrate accumulation, and more glycogen accumulation was observed in the 18-day-old rat fetus livers ($p < 0.001$; Table 3.9).

A statistically significant difference was found in terms of carbohydrate accumulation between the adult chickens and the 14- and 18-day-old chickens ($p = 0.044$; Table 3.10).

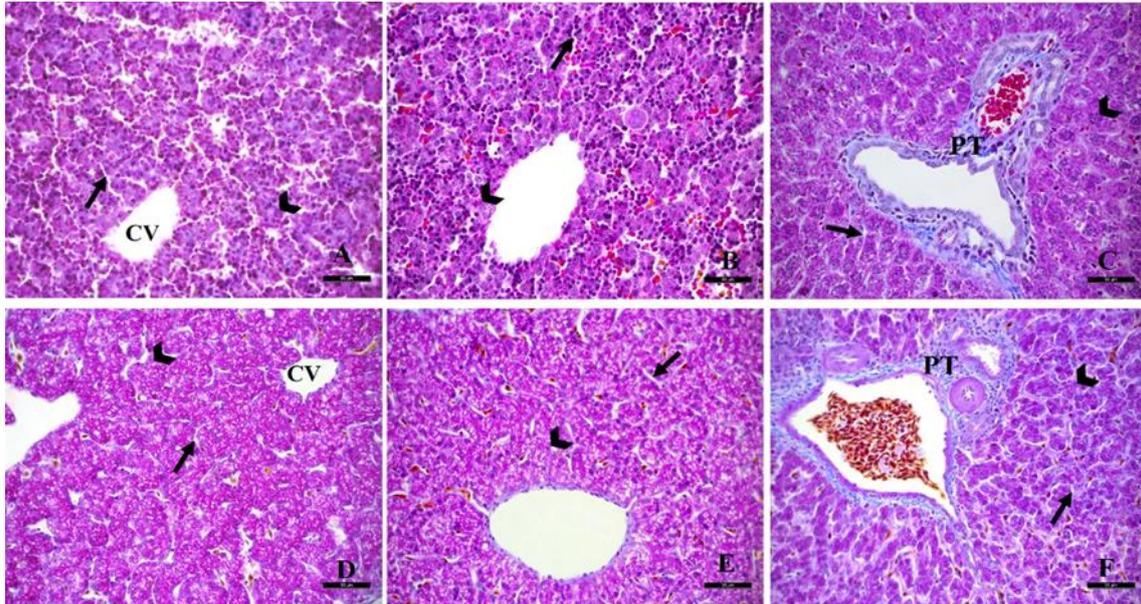


Figure 3.1: Liver sections of a 14-day-old rat (A), 18-day-old rat (B), adult rat (C), 14-day-old chicken (D) 18-day-old chicken (E) and adult chicken (F). PT: portal triad, Arrowheads: hepatocytes, arrows: sinusoids, cv: central vein. Mallory's trichrome. Bar: 50 μ m.

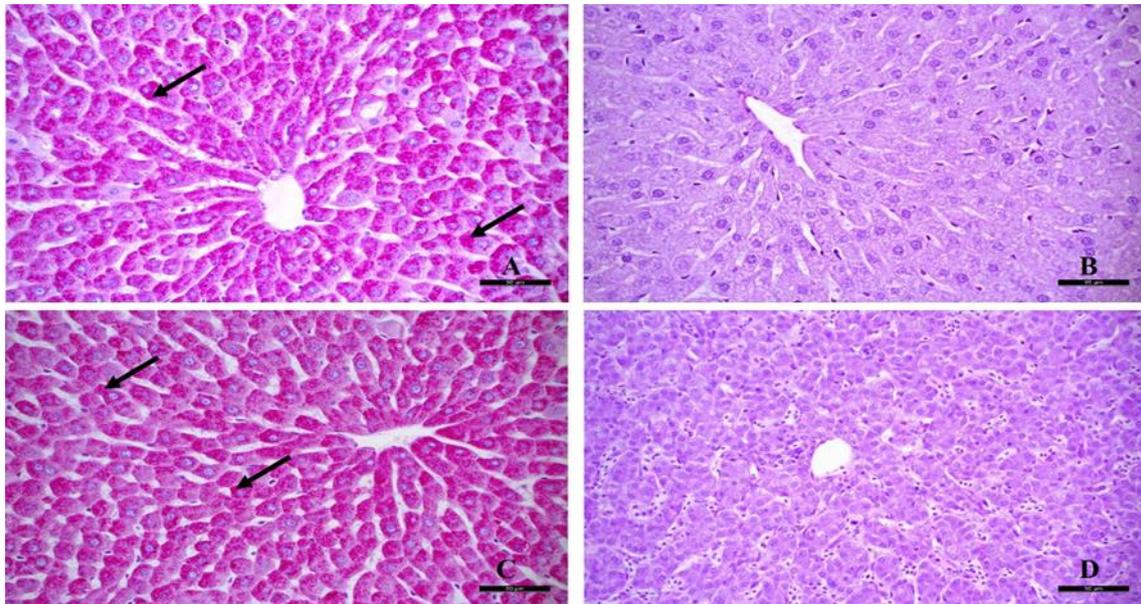


Figure 3.2: Strong PAS positive reaction (arrows) in adult rat (A) and adult chicken (C) hepatocytes. PAS. Bar: 50 μ m. PAS negative reaction after diastase ingestion in adult rat (B) and adult chicken (D) hepatocytes. Diastase / PAS. Bar: 50 μ m.

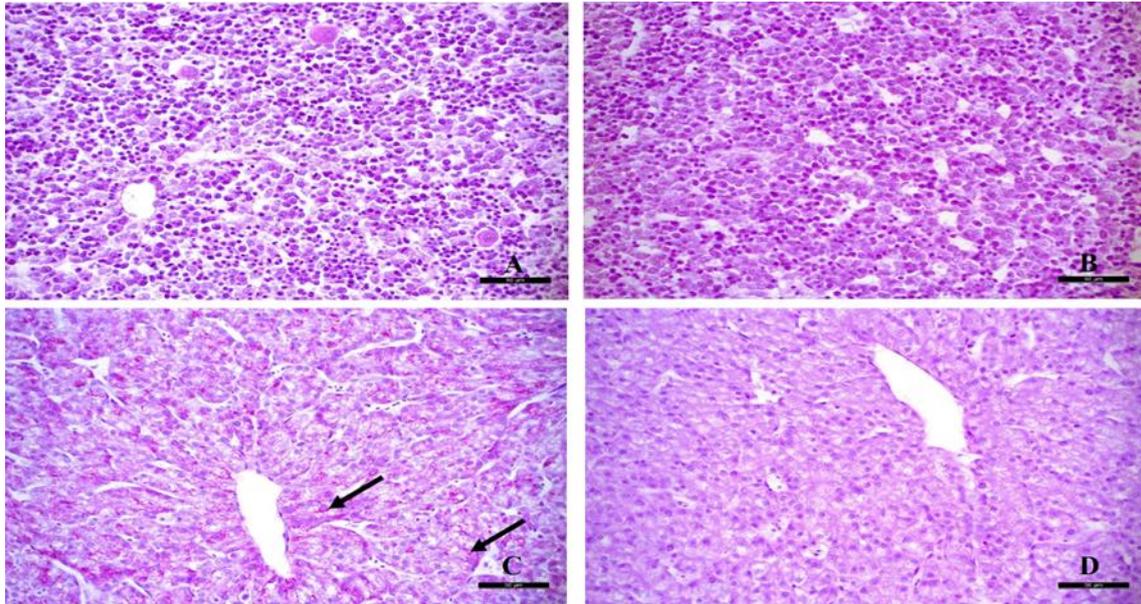


Figure 3.3: PAS negative reaction in 14-day-old rat hepatocytes (A), PAS-positive reaction (arrows) in 14-day-old chicken hepatocytes (C). PAS. Bar: 50 μ m. PAS negative reaction in hepatocytes after diastase ingestion of 14-day rat (B) and 14-day chicken (D). Diastase / PAS. Bar: 50 μ m.

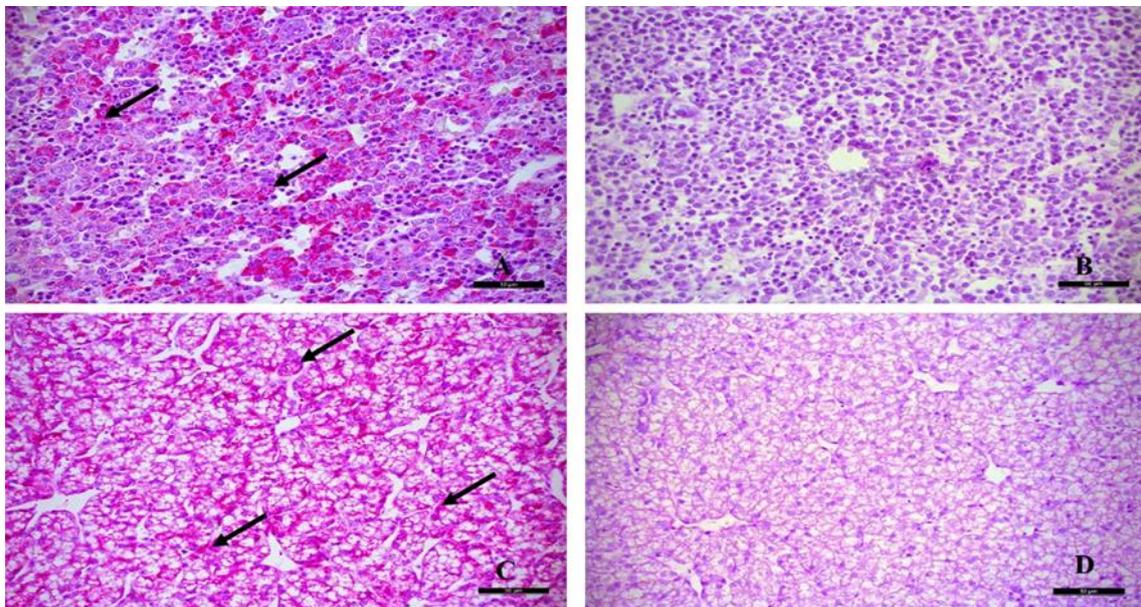


Figure 3.4: Glycogen accumulation (arrows) in 18-day-old rat (A) hepatocytes, dense glycogen accumulation (arrows) in 18-day-old chicken hepatocytes (C). Best Carmine. Bar: 50 μ m. Best Carmine negative reaction after diastase digestion in 18 day-old rat (B) and 18 day-old chicken hepatocytes (D). Diastase / Best Carmine. Bar: 50 μ m.

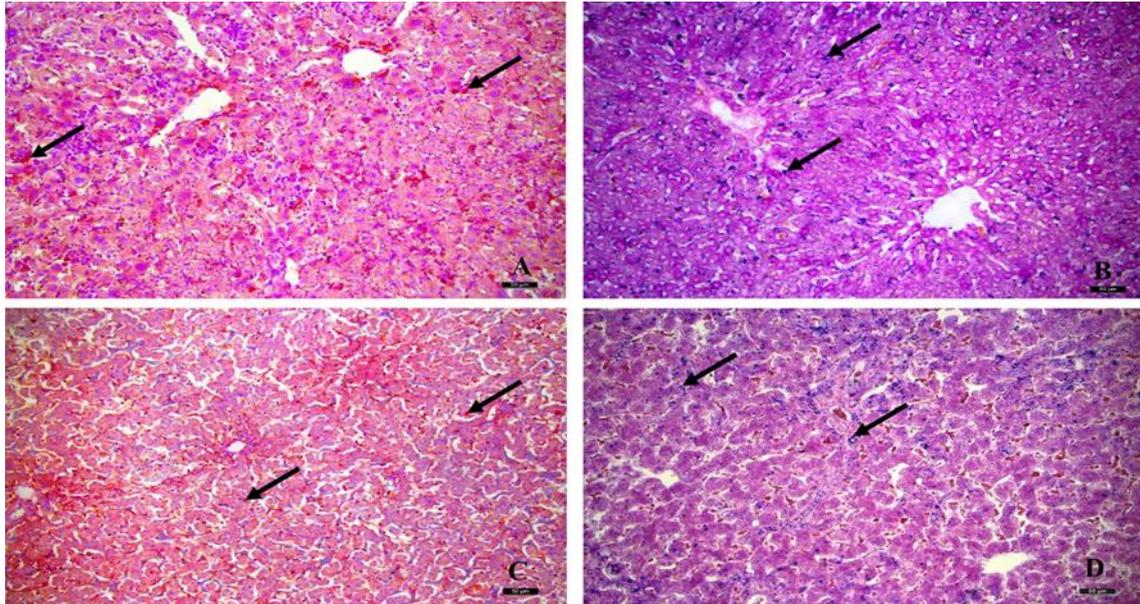


Figure 3.5: Positive lipid droplets (arrows) in adult rat (A, B) and adult chicken (C, D) hepatocytes. A, C: Oil Red O. Bar: 50 μ m. B, D: Sudan Black B. Bar: 50 μ m

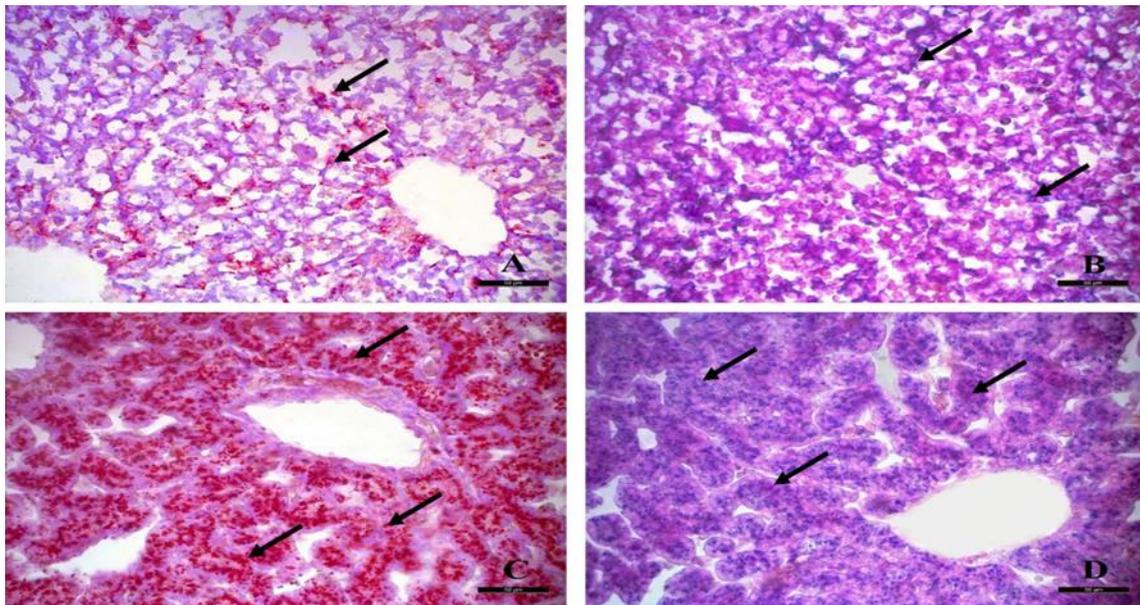


Figure 3.6: Cryostat sections of 14-day-old rat (A, B) and 14-day-old chicken (C, D). Oil Red O positive lipid droplets (arrows) in hepatocytes (A, C). Oil Red O. Bar: 50 μ m. Sudan Black B positive lipid droplets (arrows) in hepatocytes (B, D). Sudan Black B. Bar: 50 μ m.

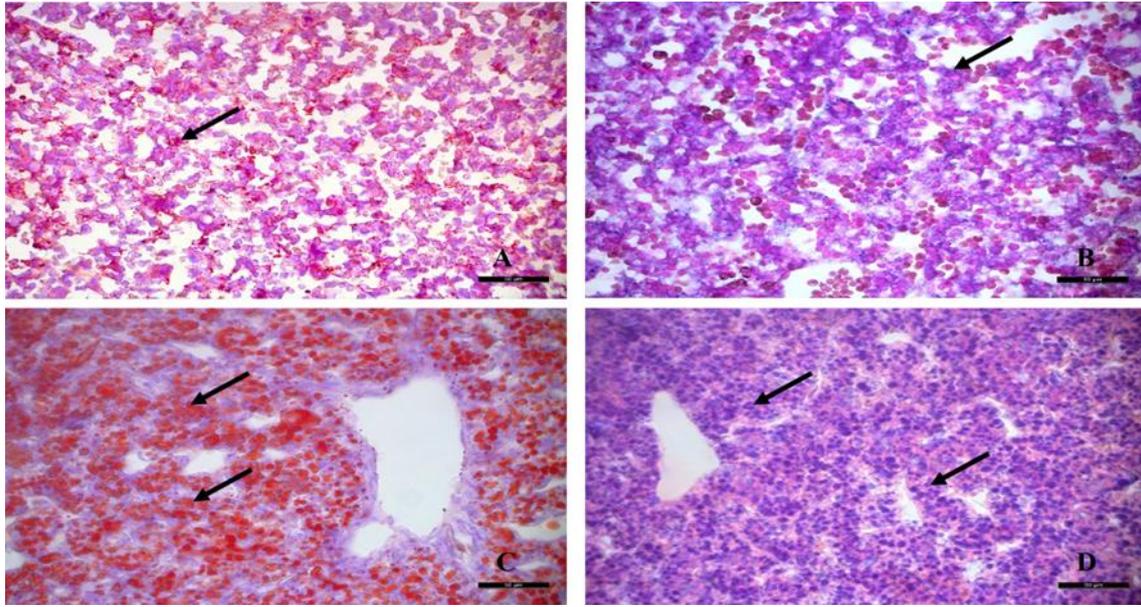


Figure 3.7: Positive lipid droplets (arrows) in of 18-day-old rat (A, B) and 18-day-old chicken (C, D) hepatocytes. A, C: Oil Red O. Bar: 50 µm. B, D: Sudan Black B. Bar: 50 µm.

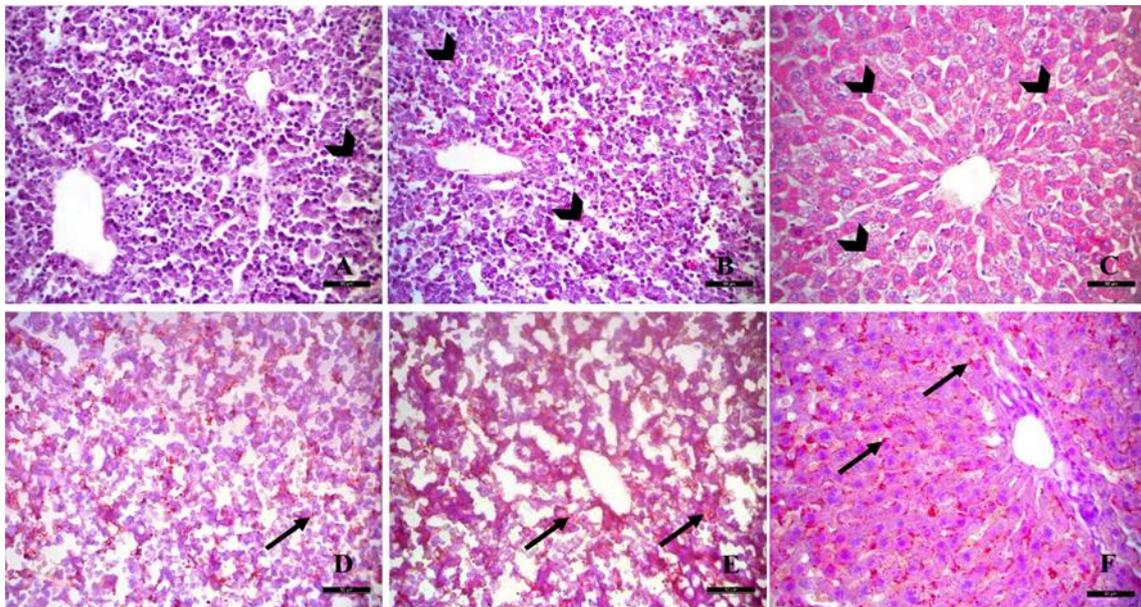


Figure 3.8: Liver sections of a 14-day-old rat (A, D), 18-day-old rat (B, E), and adult rat (C, F). B, C: dense glycogen accumulation (arrowheads) in hepatocytes. A: very little glycogen accumulation in hepatocytes (arrowhead). Best's carmine. Bar: 50 µm. D, E, F: positive lipid droplets (arrows) in hepatocytes. Oil Red O. Bar: 50 µm.

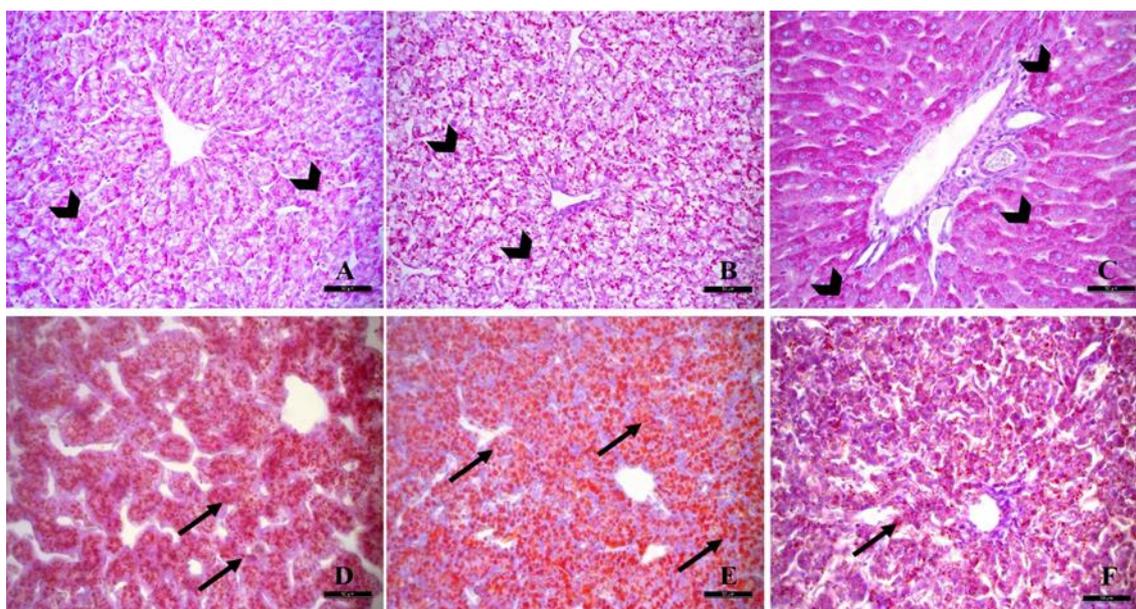


Figure 3.9: Liver sections of an adult chicken (C, F), 14-day-old chicken (A, D), and 18-day-old chicken (B, E). A, B, C: dense PAS positive reaction in hepatocytes (arrowheads). PAS. Bar: 50 μ m. D, E, F: positive lipid droplets (arrows) in hepatocytes. Oil Red O. Bar: 50 μ m.

Table 3.1. Hepatic lipid droplet count results in adult rat and adult chicken.

Group	N	Arithmetic Mean	Standard deviation	Standard error	Median	Maximum	Minimum	p
AR	10	297,5	84,1	26,59	314,05	395,70	141,80	0,130
AC	10	357,41	84,89	26,84	340,95	503,10	256,10	

AR, adult rat; AC, adult chicken; N, sample size.

Table 3.2. Hepatic lipid droplet count results in 14-day-old rat fetus and 14-day-old chicken fetus.

Group	N	Arithmetic Mean	Standard deviation	Standard error	Median	Maximum	Minimum	p
FR14	10	222,75	33,09	10,47	226,55	273,40	176,20	<0,001
FC14	10	803,7	79,48	25,13	793,15	944,40	664,60	

FR14, 14 day-old rat fetus; FC14, 14 day-old chicken fetus; N, sample size.

Table 3.3. Hepatic lipid droplet count results in 18-day-old rat fetus and 18-day-old chicken fetus.

Group	N	Arithmetic Mean	Standard deviation	Standard error	Median	Maximum	Minimum	p
FR18	10	282,59	15,05	4,76	283,90	297,60	244,00	<0,001
FC18	10	1147,72	75,42	23,85	1150,95	1237,60	1017,10	

FR18, 18 day-old rat fetus; FT18, 18 day-old chicken fetus; N, sample size.

Table 3.4. Hepatic lipid droplet count results in adult rat, 14-day-old rat fetus and 18-day-old rat fetus.

Group	N	Arithmetic Mean	Standard deviation	Standard error	Median	Maximum	Minimum	p
AR ^a	10	297,5	84,1	26,59	314,05	395,70	141,80	
FR14 ^b	10	222,75	33,09	10,47	226,55	273,40	176,20	0,009
FR18 ^a	10	282,59	15,05	4,76	283,90	297,60	244,00	

AR, adult rat; FR14, 14day-old rat; FR18, 18day-old rat; N, sample size.

Table 3.5. Hepatic lipid droplet count results in adult chicken, 14-day-old chicken fetus and 18-day-old chicken fetus.

Group	N	Arithmetic Mean	Standard deviation	Standard error	Median	Maximum	Minimum	p
AC ^c	10	357,41	84,89	26,84	340,95	503,10	256,10	
FC14 ^b	10	803,7	79,48	25,13	793,15	944,40	664,60	<0,001
FC18 ^a	10	1147,72	75,42	23,85	1150,95	1237,6	1017,1	

AC, adult chicken; FC14, 14day-old chicken; FC18, 18day-old chicken; N, sample size.

Table 3.6. Carbohydrate accumulation grading results in adult rat and adult chicken.

	Group		Total	p
	AR	AC		
1	1 (%10,0)	0 (%0,0)	1 (%5,0)	
2	2 (%20,0)	1 (%10,0)	3 (%15,0)	
3	1 (%10,0)	1 (%10,0)	2 (%10,0)	
4	3 (%30,0)	3 (%30,0)	6 (%30,0)	0,766
5	3 (%30,0)	5 (%50,0)	8 (%40,0)	
Total	10 (%100)	10 (%100)	20 (%100)	

AR, adult rat; AC, adult chicken.

Table 3.7. Carbohydrate accumulation grading results in 14-day-old rat fetus and 14-day-old chicken fetus.

	Grup		Total	p
	FR14	FC14		
1	10 (%100,0) ^a	0 (%0,0) ^b	10 (%100,0)	
2	0 (%0,0) ^a	5 (%50,0) ^b	5 (%25,0)	
3	0 (%0,0) ^a	3 (%30,0) ^a	3 (%15,0)	
4	0 (%0,0) ^a	1 (%10,0) ^a	1 (%5,0)	<0,001
5	0 (%0,0) ^a	1 (%100,0) ^a	1 (%5,0)	
Total	10 (%100)	10 (%100)	20 (%100)	

FR14, 14 day-old rat fetus; FC14, 14 day-old chicken fetus.

Table 3.8. Carbohydrate accumulation grading results in 18-day-old rat fetus and 18-day-old chicken fetus.

	Grup		Total	p
	FR18	FC18		
2	8 (%80,0) ^a	1 (%10,0) ^b	9 (%45,0)	0,013
3	2 (%20,0) ^a	5 (%50,0) ^a	7 (%35,0)	
4	0 (%0,0) ^a	3 (%30,0) ^a	3 (%15,0)	
5	0 (%0,0) ^a	1 (%10,0) ^a	1 (%5,0)	
Total	10 (%100)	10 (%100)	20 (%100)	

FR18, 18 day-old rat fetus; FT18, 18 day-old chicken fetüs.

Table 3.9. Carbohydrate accumulation grading results in adult rat, 14-day-old rat fetus and 18-day-old rat fetus.

	Grup			Total	p
	AR	FR14	FR18		
1	1 (%10,0) ^a	10 (%100,0) ^b	0 (%0,0) ^a	11 (%36,7)	<0,001
2	2 (%20,0) ^a	0 (%0,0) ^a	8 (%80,0) ^b	10 (%33,3)	
3	1 (%10,0) ^a	0 (%0,0) ^a	2 (%20,0) ^a	3 (%10,0)	
4	3 (%10,0) ^a	0 (%0,0) ^a	0 (%0,0) ^a	3 (%10,0)	
5	3 (%10,0) ^a	0 (%0,0) ^a	0 (%0,0) ^a	3 (%10,0)	
Total	10 (%100)	10 (%100)	10 (%100)	30 (%100)	

AR, adult rat; FR14, 14day-old rat; FR18, 18day-old rat.

Table 3.10. Carbohydrate accumulation grading results in adult chicken, 14-day-old chicken fetus and 18-day-old chicken fetus.

	Grup			Total	p
	EC	FC14	FC18		
2	1 (%10,0) ^b	5 (%50,0) ^a	1 (%10,0) ^b	7 (%23,3)	0,044
3	1 (%10,0) ^b	3 (%30,0) ^b	5 (%50,0) ^a	8 (%30,0)	
4	3 (%30,0) ^b	1 (%10,0) ^b	3 (%30,0) ^b	7 (%23,3)	
5	5 (%50,0) ^a	1 (%10,0) ^b	1 (%10,0) ^b	7 (%23,3)	
Total	10 (%100)	10 (%100)	10 (%100)	30 (%100)	

AC, adult chicken; FC14, 14day-old chicken; FC18, 18day-old chicken.

DISCUSSION

The liver is metabolically the most active organ in the fetus. It is the only organ in which all metabolic pathways and metabolic enzymes are active (Krebs 1972). Glycogen and lipids are large forms of energy storage, which are tightly controlled by enzymes, hormones, and metabolic signaling pathways. For this reason, various studies have been conducted on enzymes that play an active role in liver carbohydrate and lipid metabolisms in both fetal period and adulthood using various animal species (Yeung et al. 1967, Leskes et al. 1971, Rideau et al. 2008). In the study conducted by Leskes et al. (1971), liver glucose-6-phosphatase activity of rats was determined in adult and fetal periods. The glucose-6-phosphatase activity in 18-day-old rat fetuses was found to be as frequent as 5% of the activity in adults. Therefore, glycogen granules were not found in all hepatocytes in the liver. In another study conducted by Dvorak (1971) with rats, it was reported that glycogen granules were found in the liver on the 18th day of fetal life. In the study we conducted, while large amounts of glycogen granules were found in most adult hepatocytes, less glycogen granules were found in the fetal rats. When these results were compared with chickens, it was noticed that there was a higher amount of glycogen accumulation in the 18-day-old chicken fetuses compared with that in the rat fetuses. In most of the adult rat and chicken hepatocytes, similar proportions and dense amounts of glycogen granules were observed. On the 18th day of fetal rat development, more glycogen granules were found in many hepatocyte cytoplasm, albeit not very dense, compared with the 14th day. This difference was found to be statistically significant. This situation can be explained by the ability of the animal to meet its increasing energy needs during the fetal rat development period, to complete its development in a healthy manner, and to guarantee the glucose reserves of the liver for postnatal metabolic transition. Thus, an increase in glycogenesis and gluconeogenesis metabolisms is observed in the fetal liver (Trefts et al. 2017). In the study conducted by Luzzatto (1981) with rat fetuses, he reported that glycogen granules in hepatocytes could be identified on the 18th day and lipid droplets on the 12th day of the fetal development. In this study, hepatic glycogen granules were observed in the light microscopic examinations of the 18-day-old rat fetuses. In addition, it was observed that glycogen granules in the 18-day-old rat fetuses were much less compared to those in the chicken fetuses. Machida et al. (1990) evaluated lipoprotein lipase (LPL) activity in the liver as an indicator of hepatic lipid metabolism and measured lipoprotein lipase activity in the fetal liver (15th, 17th, 19th, and 20th days). They found that LPL activity in the liver increased in the fetal period.

The biochemical findings of Machida et al. (1990) are consistent with the light microscopic examinations of our liver tissue samples because the amount of hepatic lipid droplets in the 14- and 18-day-old rat fetuses also increased in our cross-sections. The increased lipolytic activity in the mother during the fetal period in order to meet the increasing energy need and the effect of maternal energy metabolism on the fetal energy metabolism may be the reason for pronounced hepatic fat accumulation in both periods (Hendrickse et al. 1985, Herrera et al. 2006, Rao et al. 2013). In rats, the fetus constantly receives substrate support from the placenta. With birth, this support suddenly stops and the baby begins to use endogenous substrates for glucose homeostasis. In order to prepare for a switch from exogenous support to endogenous substrate support, the fetus begins to store more glycogen and lipids at the end of pregnancy (Kimura 1991). This information clarifies the increase in the hepatic lipid and glycogen content in the fetal period in our study. In a study by Borrebaek et al. (2007) who measured the fetal liver enzyme activities of 14- and 19-day-old chickens, hexokinase enzyme activity and glycogen content was reported to have increased in the liver. In the study conducted by Willemsen et al. (2010) with chicken fetuses, it was reported that the amount of glycogen in the liver was higher on the 18th day compared with that on the 16th day in the control groups. In the light microscopic examinations of this study, it was seen that the amount of glycogen stored in the liver was higher on the 18th day compared with that on the 14th day. The reason for the difference in liver glycogen accumulation in rats and chickens is that fetal rats have maternal support during the embryonal development process, whereas fetal chickens do not have any external support.

Metabolic pathways related to lipids are very active in chicken embryos from mid-incubation until 2–3 days before hatching. At this stage, the embryo uses egg yolk fatty acids as its main source of energy. Therefore, fatty acid synthesis and beta-oxidation must be highly active at this stage of embryonic development (De Oliveira et al. 2008). In the study conducted by Zhao et al. (2007) with broiler chickens, tissue samples were taken from the liver and the hepatic triglyceride amount was measured. It was observed that the amount of liver triglyceride was higher on the 19th day of incubation compared with that on the 14th day. Wong and Cavey (1992) found that on the 14th day of incubation, all hepatocytes contained both lipid and glycogen. When these results were compared with the amount of hepatic lipid in the rats in our study, fewer lipid droplets were observed compared with the 14- and 18-day-old chicken fetuses and it was determined that there was a statistically significant difference between the 14- and 18-day-old rat and chicken fetuses in terms of

average fat droplet count. In this study, it was noticed that the amount of hepatic lipid increased significantly on the 18th day of chicken fetal development compared with that on the 14th day. Lipid droplets are observed more intensely with the development of fetal chicken liver and it can be explained by the fact that lipid metabolism is very active until a few days before hatching. Chickens need a greater amount of energy to survive and grow, both during and after the process of hatching. However, because the development of embryos takes place within the egg surrounded by certain limits, there is no external nutritional support. For this reason, the embryo stores a greater amount of glycogen and fat in order to meet the energy it will need in the last period of its development. This may be the reason why, in light microscopic examinations of our study, we observed that more glycogen and fat were stored in hepatocytes on the 18th day of incubation compared with that observed on the 14th day Yang et al. (2010) measured the amount of hepatic triglyceride and cholesterol in their study with broiler chickens. In the control group of the study, it was found that the amount of hepatic triglyceride and cholesterol in 14-day-old chickens was higher than that in adult chickens (63 days old). It was also reported that total cholesterol and triglycerides in the blood decreased in adulthood. The results of our study also support this situation histologically because it was determined that the amount of fat droplets in the adult chicken liver was lower compared with that in the fetal periods.

In chickens, the only source of lipid for the embryo during the incubation period is the egg yolk, and increased absorption of lipids in the egg yolk in the last week of the incubation period causes lipid concentration in the fetal liver to increase up to eight times (Uni et al. 2012). Providing substrate support to rats during the fetal period, the mother undergoes two metabolically differentiated periods in order to meet the increasing energy needs of the fetus. First, because of the limited fetal development, the excess food taken by the mother is stored as fat, the second is that the transfer of dietary lipids to the fetus increases with the mother's storage fat (lipolytic activity increases) during the period when fetal growth is much faster (López-Luna et al. 1986, Lopez et al. 1991, Chaves and Herrera 1978, Knopp et al. 1970, Martin et al. 1994). These reasons suggest that the amount of hepatic lipid in the 18-day-old rat and chicken fetuses is higher than that of the 14-day-old rat and chicken fetuses.

When histological and statistical results were evaluated, significant differences were found in carbohydrate and fat metabolisms of the liver in rats and chickens because in addition to an endogenous source, rats also receive exogenous support during fetal development, whereas chickens only have an endogenous source. When it comes to adult rats and

chickens, there was no difference between fat and carbohydrate storage. When the yield characteristics of animals are taken into consideration, it is understood how important liver carbohydrate and fat metabolisms are. In order to improve information about these metabolisms, the fetal and adult periods were compared and it is believed that the findings obtained may provide new clues at basic knowledge level on liver metabolism and may be a guide to the improvement of yield characteristics.

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