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RESEARCH ARTICLE

Analysis of Fatty Acids of Liver in the Embryo and Adult of Domesticated Chicken Gallus Gallus Domesticus

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Abstract

The fatty acids in the embryo's liver at ages (7, 11, 14 and 19) days incubation, small chicken aged (14) days after hatching and adult were analyzed, and found (5) fatty acids, the highest concentration of fatty acid in the adult of domesticated chicken and lowest concentration in small chicken age (14) days after hatching. Statistically, there were high significant differences at the probability level ($P \leq 0.001$) between all ages together, and the highest concentrations of Oleic acid (C18:1) and Linoleic acid (C18:2) were in embryo age (7) days incubation, while in embryo age (11) days incubation Stearic acid (C18:0) and α-Linolenic acid (C18:3) were higher concentration and Palmitic acid (C16:0) was the highest concentration in the adult. Stearic, Palmitic, Linoleic and α-Linolenic acids were recorded as the lowest concentration as well as in a small chicken age (14) days after hatching. Oleic acid had the lowest concentration in the embryo (19) days incubation, as well as α-Linolenic acid in the embryos age (7, 19) days incubation and the adult chicken did not record any concentration.

Key words: *Fatty acid, Chicken, Embryo, Liver.*

Introduction

The fatty acids are the aliphatic acids long-chain and they are considered the basic building block for the synthesis of several varieties of fats that given by the fatty or oily nature. Additionally, they are not free in cells and tissues but can be obtained by hydrolipic analysis of lipids, this acids characterized by a monocarboxylic group (COOH) with a hydrocarbonic chain, and different in the number of carbon atoms may be even or odd.

The fatty acids are found either saturated or unsaturated. The saturated fatty acids include Lauric, Myristic, Palmitic, Stearic, Arachedic and Lignoceric acids, the most prevalent being Palmitic and Stearic acids. While the unsaturated fatty acids include Palmitoleic, Oleic, Linoleic, α-Linolenic and Arachiodonic, the most prevalent of which are Palmitoleic and Oleic acids.

Unsaturated fatty acids are more prevalent than saturated types. Fatty acids are synthesized in the cytoplasm, while the catabolism of acids is take place in the mitochondria. Because of mitochondrial membrane is not permeable to the fatty acid therefor it turns into the derivative of the coenzyme fatty acyl CoA which reacts in turn with the carnitine which is the material that it synthesized by the body from Lysine amino acid, then formation fatty acid carnitine that enters into mitochondria to oxidize the fatty acids [1]. There are many hormones that control on the lipogenesis in the hepatic cells such as the insulin hormone that stimulates lipid synthesis, and there are hormones that have inhibiting effectiveness to the lipogenesis as thyroxin, glycogen and epinephrine [2, 3].

Lipid metabolism occurs in the liver in this process lipids are oxidized for the purpose of producing energy within the liver and synthesis of lipoproteins, cholesterol and phospholipids, and the liver converts excess carbohydrates and proteins into fatty acids and triglycerides then exports to be stored in the adipose tissue [4].

Samanez *et al.* stated that under conditions where the concentration of glucose is high, the hepatocytes increase the secretion of glycoproteins for lipogenesis [5].

The embryo of the bird is formed in a very fatty concentration environment, but the lipogenesis process is low and rapidly changing due to the efficacy of fat-forming enzymes in the liver that are increasing rapidly. The birds depend on the liver in the synthesis of fatty acids, which is the main site for lipogenesis and represents about 90-95% of the fatty acids is a new formation as opposed to the lipogenesis process that occur in the liver and adipose tissue [6, 8].

During the early stages of incubation, the embryo absorbs a small amount of lipid in the yolk. In fact, embryonic liver does the fat absorption, and the absorption of fat from the yolk during the second half of the incubation [15, 18] days was increased, resulting an increase in the weight of the liver about 15%. For this reason the color of the liver is yellowish at hatching directly.

The fetus consumes the fat that is visible during the second half of the incubation. As well as the appearance of pools under the skin of the embryo and estimated to store about 25% of the yolk lipids that used as a source of energy at hatching [9, 10, 13]. Speake *et al* [11].Stated that the embryo uses most of the lipids, as triglycerides are used as a source of energy, while phospholipids act as essential precursors in the formation of the bilipids layer in cell membranes. There are many local studies at different field about the birds such as [14] Al-Kenany Abed and Al-Bakri [15]; Abid and Al-Bakri [16] and Al-Bakri *et al.* [17], but no local studies about fatty acid in the liver of birds in Iraq. Therefore, the aim of the current study is to identify the fatty acids in the liver of the domesticated chicken embryo for ages [7, 11, 14, 19] days incubation and small chicken aged [14] days after hatching as well as adult.

Materials and Methods

Fatty acids were separated using standard samples for the diagnosis of fatty acids and the use of high performance liquid chromatography (H.P.L.C) device. The analysis was conducted according to [18] Chouinard *et al.* (1999); Cortinas *et al.* [19], Feng *et al.* [20], as follows:

- 100 mg of domesticated chicken liver tissue for ages (7, 11, 14, 19) days incubation and (14) days after hatching, and adult chicken liver were homogenised using manual homogenizer with (100) ml of ionized water. After that the fat was separated according to ISOIDF (2001) method using a mixture (20) ml N-pentane and diethyl ether by rate (V/V 50:50) after the first addition of (100) ml of ammonium hydroxide solution (0.1) M to the extract.
- The fat layer was transported to micro tubes and left at approximately 30 minutes before the micro centrifuged for (20) g at room temperature, and after the centrifuged, the top layer of the analysis was removed where the separated fatty acids was stored in amber vials then has been shown to stream of N_2 and froze to -20°C.
- An acid is measured using a high performance liquid chromatographic (H.P.L.C) device.
- According to the concentration of fatty acids using the following equation:
- Concentration of amino acid=$\frac{package\ area\ in\ sample}{Standard\ package\ area}$ ×standard concentration× times the number of dilution

Results

Table (1) and Figure (1) showed that the highest concentration of the fatty acid was at the adult and reached (73.79±15.20). The lowest concentration was when the small chicken was 14 days old after hatching (15.41±2.17). Statistically, there were high significant differences (P≤0.001) among all age together, while non significant differences (p>0.05) was showed between the adult whose concentration as mentioned above (73.79±15.20) and embryos aged (7, 11, 14) days incubation with concentrations of fatty acids (71.65±17.74, 47.71±7.83, 59.49±12.69) in respectively, and embryo aged (7) days incubation where the concentration of fatty acids (71.65±17.74) with embryos aged (11, 14) days incubation, which were their concentrations of fatty acids (47.71±7.83, 59.49±12.69) respectively. Also, embryo aged (11) days incubation which were their concentrations of fatty acids (47.71±7.83) with embryos aged (14, 19) days incubation, which reached their concentrations of fatty acids (59.49±12.69, 17.15±0.71) respectively. Finally the embryo aged 19 days incubation that had the concentration of fatty acids (17.15±0.71) with a small chicken aged (14) days after hatching

which was their concentration (15.41±2.17), but other than that it showed a significant difference (P≤0.05) between each day and the other separately.

Table 1: Fatty acids in the liver of domesticated chicken *Gallus Gallus domesticus* during incubation days, after hatching and adult

Group	Concentration (mean± S.E)					
	7 days incubation	11 days incubation	14 days incubation	19 days incubation	14 days after hatching	Adult
Fatty acids	71.65±17.74	47.71±7.83	59.49±12.69	17.15±0.71	15.41±2.17	73.79±15.20

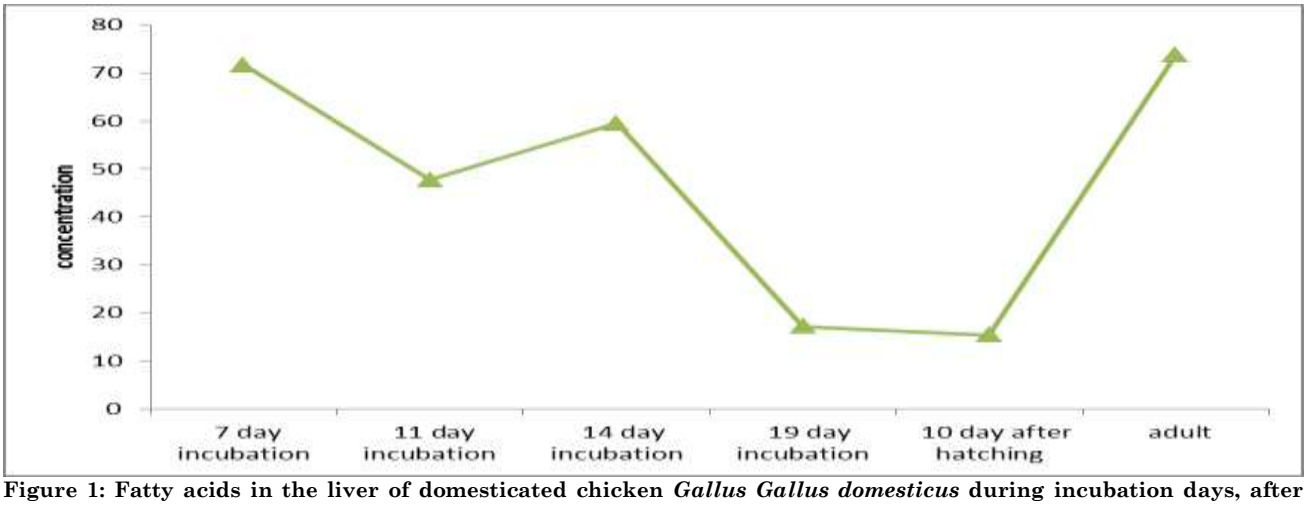

Figure 1: Fatty acids in the liver of domesticated chicken *Gallus Gallus domesticus* during incubation days, after hatching and adult

As for the fatty acid found in the liver of the chicken embryo aged (7, 11, 14, 19) days incubation and small chicken age (14) days after hatching and adult .It was found that there are five fatty acids and that the highest concentration of the Oleic and Linoleic acids at (7) days incubation as follows: 138.37±9.29 and 90.38±10.20 respectively. The Stearic acid has had the highest concentration 41.36±24.95 at the age of (11) days incubation as well as α-Linolenic acid which showed the highest concentration 8.41±1.46 in the embryo age (11) days incubation. The highest concentration of Palmitic acid was 67.35±9.06 in adults. The lowest concentration of fatty acids Palmitic, Stearic, Linoleic and α-linolenic acids in small chicken aged (14) days after hatching and as follows: 13.45±1.51, 14.65±7.41, 18.29±7.27 and 5.33±0.56 respectively. While embryo showed (19) day's incubation lowest concentration of Oleic acid is 16.61 ± 5.40. Whereas each of Linoleic acid did not show any concentration in the embryo aged (19) days incubation, as well as α-Linolenic acid did not show any concentrations in the embryos of the age (7, 19) days incubation and adult. The statistical findings indicated that there were high significant differences between ages and fatty acids, except Stearic acid, which did not show the existence of significant differences (P>0.05) in their concentrations within the aforementioned stages (Table 2). In general, Oleic acid was the highest concentration of fatty acids during the ages studied.

Table 2: Fatty acids concentrations in the liver of domesticated chicken *Gallus Gallus domesticus* during incubation days and after hatching and adult

Groups	Concentration (mean± S.E)					
	7 days incubation	11 days incubation	14 days incubation	19 days incubation	14 days after hatching	Adult
Palmitic C16: 0	42.26±1.88	46.62±3.86	58.98±4.20	15.51±0.67	13.45±1.5	67.35±9.06
Stearic C18: 0	15.59±2.46	41.36±24.95	30.58±15.32	19.31±7.43	14.65±7.41	18.70±1.54
Oleic C18: 1	138.37±9.29	77.57±14.04	113.48±20.98	16.61±5.40	25.31±1.38	131.93±4.51
Linoleic C18: 2	90.38±10.20	64.57±1.67	87.18±23.38	0.00±0.00	18.29±7.27	77.18±10.48
α-Linolenic C18: 3	0.00±0.00	8.41±2.11	7.24±1.46	0.00±0.00	5.33±0.56	0.00±0.00

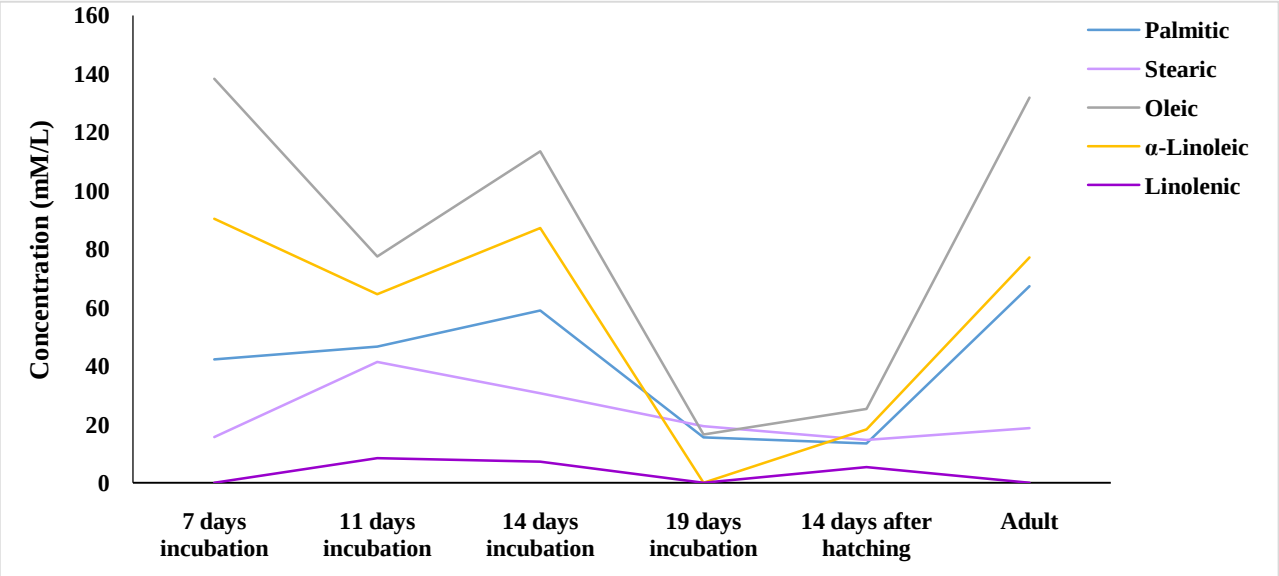

Figure 2: Fatty acids concentrations in the liver of domesticated chicken *Gallus Gallus domesticus* during incubation days and after hatching and adult

Discussion

Fatty acids are the main component of the biological cell membranes that play an important role in intra-cellular and precursor. They act as vital energy store that the body needs for natural growth and in the organogenesis [21, 22]. The results of the acids analysis for the embryos liver of aged (7, 11, 14, 19) days incubation and a small chicken age (14) days after hatching and adult showed presence of (5) fatty acids include Palmatic (C16:0) acid, Stearic acid (C18:0), Oleic acid (C18:1), Linoleic acid (C18:2) and α-Linolenic acid (C18:3).This agree with Cieślik *et al* [23].

Who study of the content the liver lipids and fatty acids of adult chicken as their results indicate the presence of Palmatic, Stearic, Oleic, Linoleic and α-Linolenic acids, but their analysis showed that there were other fatty acids were not recorded in current study, which are Palmitoleic (C16:0), Archedonic acids (C20:4) and conjugated linoleic CLA acid, which was found in the adult chicken [24].

Ding and Lilburn (1996) study showed that the content of turkey liver of the fatty acids and its concentrations during the final stages of incubation and hatching and after hatching stages found (6) fatty acids; Palmatic, Palmitoleic, Stearic, Linoleic, Archedonic and Oleic acids and found little or no appear of α-Linolenic acid. This result was demonstrated the current study except for the appearance of Palmitoleic and Archedonic during embryonic stages, hatching, after hatching and adult. The results of the presence or absence of α-Linolenic acid were also consistent with the results of the current study as no concentration was recorded in embryo ages (7, 19) days incubation and adult chicken in addition to its recorded few concentration compared to the rest of the fatty acids in the current study. As well as the results of the current study agree with Noble and Moore [25] study of lipid metabolism in the liver of the chicken embryo (13, 15, 17, 19) days incubation. Additionally, it was found that the fatty acids was the same acids in the results of the fatty acids analysis of the embryos liver and adult chicken, except the presence of Archedonic acid, which was not registered in current study.

The egg yolk is the most important source of the fatty acids required for embryogenesis. The embryo derives about 90% of the energy required from yolk lipids oxidation consisting of 72% triglycerides and 22% phospholipids [13, 26].

The absorption of lipids from the yolk was occurring during the second half of the incubation (12-21) days. The percentage of lipids at (13) day incubation was 65% and then transferred into 44% at (21) day incubation [10]. Peebles *et al* [12]. Stated that lipids were absorbed by embryo liver between 15 and 18 days incubation, therefore the increasing weight of the liver about 15% and the clear evidence is the color of the yellowish liver at hatching and one day after hatching. In Cherian *et al* [27].Study for egg content of fatty acids was found the following acids:

Palmatic, Stearic, Palmitolic, Oleic, Linoleic, Archedonic and Docosahexaenoic acids. Oleic acid represents the highest concentrations compared to the remaining fatty acids in above study, followed by Palmatic acid. The study of Orensanz *et al* [28].That included fatty acid that present in triglycerides of domestic chicken eggs yolk was found that more than 45% of Oleic acid and more than 30% of Palmatic acid from total triglycerides. So the embryo and embryonic liver derived it needs of the fatty acids from the egg that is share in its composition and growth. Noy *et al* [29].Stated that the residual yolk represents about 50% lipids and that it accounts for 20-30% of the body weight at hatching and is a source of energy for the newly hatching chicken.

However, Ding and Lilburn [24] stated that lipids and fatty acids are reduced rapidly during the last stages of the incubation and that residual yolk is not an important source of energy for a newly hatching chicken, as it relies on external nutrition to get the energy it needs. Wu *et al* [30] .Mentioned in them studies the processes of hepatic metabolism in hatching and after hatching at (42) days and stage adult at (35) weeks in the roster.

It was found that metabolism of lipids, including Palmatic and Oleic acids, which increases their concentrations at hatching stage to reach the highest value at the stage (42) days after hatching and then reduced concentrations at puberty (35) weeks. This partial contrasts with the results of current study which recorded the highest concentration of Palmatic acid in the adult domesticated chicken and the lowest concentration was in the small chicken aged (14) days after hatching.

While the Oleic acid was the highest concentration of the incubation stage aged (7) days and the lowest concentration was in the chicken embryo aged (19) day incubation. Thus, the role of the Palmatic acid is exposed, as it is saturated fatty acids (SFAS) that enters into the synthesis of triglycerides and it is an interactive material in the synthesis of triglycerides and it is considered as a reactive material substrate important in the formation and secretion of lipoproteins in the liver [31]. Ricchi *et al* [32].And Zhang *et al* [33].Have a role in inducing cellular death apoptosis and the concentration of lipids in the hepatocytes in rats and mice.

The results of the current study agree with Ding and Lilburn (24) due to the fact that the concentration of Oleic acid in the liver of the turkey embryo increases during the incubation period, hatching and after hatching. Also Oleic acid is a highest concentration compared to the rest of the fatty acids and also stated that this increased concentration of Oleic acid may be a reflection of different species in the activity of Δ-9desaturase.

Noble and Shand [34] stated that the efficacy of this enzyme was recorded during the second period of incubation (12-20) day in the chicken embryo. The results of the analysis fatty acids in the liver of a domesticated chicken embryo (7) days incubation, the highest concentration recorded for Linoleic acid which considered as essential fatty acids that the body cannot synthesis so it must be obtained from external nutrition.

This essential fatty acid represents the potential power in lipids and included in addition to the linoleic acid other acids such as α-Linolenic and Archedonic, which represent polyunsaturated fatty acids (PUFAs). These acids have a role in regulating the formation and natural growth of human and animal, contributes to the formation of cell membranes and composition prostaglandins which are substances similar in composition largely fatty acids and have a role in many physiological functions such as blood pressure, blood clotting and cell regeneration [32, 35]. This is demonstrated by the results of the current study in the high concentration of Linoleic acid in the embryo age (7) day's incubation because of it plays a significant role in the health, composition and safety of the liver.

Since Linoleic acid is one of the acids that cannot be synthesized by the body, therefore it gets from the fatty acids in the egg yolk. May be the changes in fatty acid concentrations and the different in their types during the embryogenesis period, after hatching and the adult of domesticated chicken bird in current study due to the structural and functional maturation of liver .We concluded from this that found (5) fatty acids in the analysis of the embryo liver of the domesticated chicken for ages (7, 11, 14, 19) day incubation, small chicken age (14) day after hatching and adult.

The highest concentration of fatty acid in the adult chicken and the lowest concentration in small chicken age (14) day after hatching.

There were high significant differences among all ages together and the Oleic acid and Linoleic acid scored the highest concentration in the embryo age (7) day incubation. Stearic and α-Linolenic acids were the highest concentrations in embryo age (11) day incubation and Palmatic acid was in the highest concentration chicken, and found in a small chicken age (14) day after hatching. The acids of the Palmatic, Stearic, Linoleic and α-Linolenic acids recorded their lowest concentration and Oleic acids had the lowest concentration in embryo age (19) day incubation, which considered the highest concertation among the fatty acids during the studied ages.

Conclusion

The statistical results showed the existence of high significant differences between ages and all fatty acids except Stearic acid did not show any significant differences in their concentrations within the aforementioned stages at the probability level ($P>0.05$). The aim of the current study is to identify the fatty acids found in the liver of embryos age (7, 11, 14, 19) days incubation and small chicken age (14) days after hatching and adult.

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