Effects of feeding different lipid sources on hepatic histopathology features and growth traits of broiler chickens

Vincenzo Tufarelli [a,*], Giancarlo Bozzo [b], Antonella Perillo [b], Vito Laudadio [a]

[a] *Department of Emergency and Organ Transplantation (DETO), Section of Veterinary Science and Animal Production, University of Bari 'Aldo Moro', Valenzano 70010, Bari, Italy*

[b] *Department of Veterinary Medicine, University of Bari 'Aldo Moro', Valenzano 70010, Bari, Italy*

Running title: Dietary lipid sources on chickens liver histopathology

* Corresponding author. Tel.: +39 080 544 39 16; Fax: +39 080 544 38 11.

E-mail address: vincenzo.tufarelli@uniba.it (V. Tufarelli)

Complete correspondence address: Dr. Vincenzo Tufarelli, Department of Emergency and Organ Transplantation (DETO), Section of Veterinary Science and Animal Production, University of Bari 'Aldo Moro', Valenzano 70010, Bari, Italy, Strada Prov. le per Casamassima, km 3, 70010 Valenzano (Ba), Italy; vincenzo.tufarelli@uniba.it

Abstract

The effects of different dietary lipid sources on growth traits and hepatic histopathology of broiler chickens were investigated. Hubbard strain one-day old chickens (n = 120) were kept in pens and were fed one of the three corn-soybean meal-based diets until 49 days of age. The dietary treatments consisted of 2.5% added oil or fat from three sources as follows: SFO diet containing sunflower oil; LRD diet containing lard, and EVOO diet containing extra-virgin olive oil. Dietary oil or fat type improved significantly body weight and gain as well as feed efficiency in birds fed EVOO compared to those fed the other treatments. Based on our findings, after the whole experimental feeding period it was possible to observe relevant injuries to the liver of the chicks fed with lard, whereas the hepatic histopathological changes appeared less marked or absent in the chicks fed vegetable oils from sunflower or olive. Thus, we can conclude that dietary lipid source affected chicks performance and hepatic histopathology especially when chicks fed diet containing animal fats; whereas feeding extra-virgin olive oil supported positively growth traits and did not result in hepatic histopathological effects.

Keywords: Lipids; Histopathology; Liver; Growth; Chickens

Introduction

In modern poultry production, fat is the natural component of the feed mixtures, an additive increasing the energy value and a factor improving the consistency and tastiness of the feed. Vegetable fats, such as soybean and sunflower oil, as well as animal fats such as beef tallow, bone and poultry fat are commonly used (Burlikowska et al., 2010). Previous investigations have demonstrated that broilers have the ability to use considerable levels of dietary fat as energy source; on the other hand, the efficiency of its utilization mostly depends on the fatty acids composition (Zduńczyk et al., 2001). It was reported that fats of animal origin high in saturated fatty acids are not easy to digest in poultry digestive system when compared to unsaturated oil from vegetables

(Poorghasemi et al., 2013). The significance of fats from different sources in poultry nutrition is studied not only from a productive point of view, but also in relation to human nutrition and wellbeing. The fat type in diet affects not only the blood biochemical traits but also the organs metabolic processes particularly in the liver (Krasnodębska-Depta and Koncicki, 2000). Thus, studies have been focused to discover possible clarifications for broiler feeding supporting production traits as well as wellbeing. Different high-fat rations utilized in laboratory animal research include more saturated fats such as beef tallow, lard, or vegetable oils and these diets are rather able to induce obesity indifferent strains (Buettner et al., 2006). Sunflower oil is the main source of vegetable fat in poultry diet due to the high metabolizable energy amount (Smulikowska and Rutkowski, 2005), however its cost is relatively high, whereas lard is a cheaper energy source. Extra-virgin olive oil (EVOO), the major dietary fat component in the Mediterranean diet, has shown to possess health-protective effects ascribed to its high polyunsaturated fatty acids content (Laudadio et al., 2015). Moreover, EVOO is rich in phenolic compounds which have been shown to delay *in vitro* metal-induced and radical-dependent low density lipoprotein oxidation (Owen et al., 2000). However, information on the effect of these dietary fat sources on the liver histopathology in broiler chickens is quite scan. The influences of different lipid sources in diet on hepatic features have been evaluated in previous investigations conducted using mice and rats; nevertheless, to the best of our knowledge no trials have been performed in broiler chickens. Therefore, the present study aimed to evaluate the effects of different dietary fats supplementation on growth traits and liver histopathological features of broiler chickens.

Materials and methods

Experimental design and diets

Experimental procedures followed in this study were strictly adhered to the protocols approved by the University of Bari “Aldo Moro”, Italy. A total of 120 day-old male chicks (Hubbard strain), from a commercial hatchery, were raised in a conventional environment. The trial

lasted for 49 days, and pens were randomly assigned to three dietary treatments with each having four replicates of 10 birds (each subject occupied 0.095 m^2 of floor space). In preparation to the study, the facility was deeply cleaned and rinsed using pressurized water to disinfect the environment. The trial was performed in a completely randomized design with three different dietary treatments. Each diet was replicated four times and each replicate including one pen of ten broilers. Birds were vaccinated following a standard vaccination schedule, and in order to reduce the vaccination stress, 24 h before and after vaccination, a solution of multi-electrolytes was supplemented in the drinking water. Broilers were raised under controlled environmental conditions as indicated by Laudadio et al. (2012). Each pen was equipped with feeders, drinkers and wood shaving was used as bedding material.

A feeding program including single-phase was applied in the present study. Up to the slaughtering age, broilers were fed one of the three diets supplemented with different oil or fat sources formulated to meet or exceed birds nutrient requirements according to NRC (1994). The dietary treatments consisted of 2.5% added oil or fat from three sources as follows: LRD, diet containing 2.5% lard; SFO, diet containing 2.5% sunflower oil; and EVOO, diet containing 2.5% extra-virgin olive oil.

Lard was heated to a liquid state and then added to the feed and mixed. The oils were kept in cold room at 4°C prior mixing and the each diet was weekly prepared and kept in cold room in air-tight containers. The extra-virgin olive oil (from *Coratina* variety) used for experimental diet had a high total polyphenols concentration (Laudadio et al., 2015). The sunflower oil used for control diet had very low levels of total polyphenols (De Leonardis et al., 2005). The detailed ingredients and chemical composition of the basal diet are reported in Table 1.

Sampling procedure, histological and cytological analysis

Diet samples were ground in a hammer mill with a 1 mm screen and analysed in triplicate for dry matter (DM, method 945.15), crude protein (Kjeldahl N×6.25, method 990.03), ether extract

(method 945.16) and ash (method 967.05) according to AOAC (2000). Feed and water were provided *ad libitum* throughout the experimental period. Body weight and feed consumption by replicate were weekly assessed for all birds. Average daily gain, feed intake and feed conversion ratio were then calculated. Mortality was daily recorded as it occurred.

At the end of the trial (49 days of age), after a 12 h feed withdrawal, broilers (n = 12/ treatment) were selected according to the mean body weight and euthanized by cervical dislocation, and the left lateral lobe of the liver was dissected and fixed in 10% neutral buffered formalin. The fixed tissues were trimmed and embedded in paraffin. Thin sections (4 µm) were sliced and mounted on a slide, and stained with haematoxylin-eosin for histopathological examination by a pathologist that was blinded to treatment when evaluating slides. Moreover, for the detection of lipids, the slides for liver cytological analysis, on frozen sections, were stained with Oil red O (Cat. No. O9755, Sigma-Aldrich, St. Louis, MO, USA) (Lillie, 1965) and Sudan black B (Cat. No. 199664, Sigma-Aldrich, St. Louis, MO,USA) (Humason, 1972), respectively.

Statistical analysis

Data were statistically analyzed using a statistical software (SAS, 2006). Means were compared using one-way analysis of variance in completely randomized experimental design. Means having significant difference were analyzed with Duncan's Multiple Range Test (Duncan, 1955). Post-hoc pairwise comparisons between diets were made when effect of diet was significant. Moreover, differences between treatment means for significant effects were also detected using LSD procedure. The P-values less than 0.05 were considered as statistically significant.

Results and discussion

The effect of dietary treatments on growth traits of broiler chickens are reported in Table 2. The average final body weight tended to increase when birds fed dietary EVOO and it was significantly higher than those in the LRD and SFO groups, respectively. Chicks from EVOO and

LRD groups were characterized by a higher daily body weight gain compared to SFO treatment. Conversely, chickens fed dietary SFO exhibited significantly higher feed consumption compared to the other groups. After 49 days of feeding period, including EVOO in diet led to a positive effect on chickens' feed efficiency resulting significantly improved compared to LRD and SFO treatments. There were significant differences between dietary treatments regarding the mortality rate that resulted higher in broilers fed lard over the entire experiment. The significant finding of EVOO diet on birds' growth traits could be explained by the positive effect of olive oil on the digesta passage rate the through the gastrointestinal trait that resulted in a decrease, leading for an improved absorption and utilization of the nutrients in diet (Latshaw, 2008). In a previous study by Golian and Polin (1984), it was found that supplementing diet with plant or animal fats did not influence the food passage time through the chickens intestine. Nevertheless, the dissimilarity of these results with our findings may be due to the difference the dietary fat source supplemented in diet and also to the age of broilers. According to our findings, Gallardo et al. (2012) reported that poultry fed diet containing canola oil resulted in enhanced growth performance compared to birds fed rations supplemented with soybean oil or tallow. The present findings demonstrates the favourable effect in supplementing EVOO compared to lipids of animal origin as dietary energy source in poultry. Moreover, variations in responses of random-bred and modern-type broiler strains to supplemented lard in diet could be a result derived by the genetic selection (Poorghasemi et al., 2013).

The main aim of the present experimental study was to investigate the influence of different dietary lipid sources in diet of chickens slaughtered at 49 days of age, the most common commercial slaughter age of modern broiler strains. On the basis of our findings, after the entire experimental feeding period it was possible to observe relevant injuries to the liver of the animals fed with LRD diet. By cytological observation, the gross lesions were exclusively evidenced in the animals fed diet containing lard as dietary lipid source (Fig. 1). In particular, the liver appeared enlarged, firm and showed a diffuse yellowish colour of the surface that is indicative of significant fatty infiltration of hepatocytes.

By histological analysis of liver (Fig. 2), the fatty infiltration was confirmed showing: (i) the presence of groups of vacuolated and heavily stained hepatocytes scattered among the pale stained hepatic cells; (ii) numerous hepatocytes with fatty infiltration; (iii) large vacuoles containing fat distend many hepatocytes, and moreover several cells showed histological features of necrosis scattered throughout the liver parenchyma. The histopathological changes appeared less marked or absent in the livers of chicks fed vegetable oils from sunflower (Fig. 3) or olive (Fig. 4) compared with birds fed diet including animal fat. This is indicative of lipid storage and thus liver malfunction (Plaa and Charbonneau, 2008). Lipidosis is reversible and there were no histological indicators of permanent damage of the liver (Blevins et al., 2010). Thus, it can be hypothesized that the health of the liver would be enhanced in poultry fed diet supplemented with vegetable-origin lipids compared to animal-origin fats. As a result, the dynamics of three different type of poultry feed supplied showed that a diet containing lard resulted injurious and detrimental in broiler production.

Bioactive molecules (mainly polyphenols) in EVOO support positively the reduction of pathogens that might increase in the digestive system of poultry, while reducing the toxins formation in the feedstuff and improving the activity of the digestive enzymes (Cayan and Erener, 2015). According to our findings, there was no occurrence regarding poultry health disorders, in all dietary treatments because of our rearing environment was thoroughly monitored. Nevertheless, the enhancement in broilers' live body weight in our study might be due to the polyphenols in EVOO. In fact, the hydroxytyrosol and other phenolic compounds have multiple biological actions related to activity as scavenging of free radicals (Rice-Evans, 1995), but the current evidence strongly supports that natural biophenols may also provide indirect protection by increasing endogenous defence systems (Pereira-Caro et al., 2012).

In our opinion, the results of the present study could be due to the high amount of monounsaturated fatty acids in EVOO, compared to LRD and SFO which are less susceptible to lipid peroxidation than polyunsaturated fatty acids. Moreover, the impairments of hepatic functions and metabolism induced by dietary lard could mirror the reduced performance as well as the liver

functions in broilers. The influences of both animal fat and plant oils need to be further investigated not only for productive performances, but also for meat quality and blood profile relative to the human health (Ozdogan and Aksit, 2003; Dhama et al., 2015; Laudadio et al., 2015).

Based on our findings, we can conclude that dietary lipid source affected negatively chicks growth performance and hepatic histopathology especially when chicks fed diet containing animal fats; whereas feeding extra-virgin olive oil supported positively growth traits and did not resulted in hepatic histopathological effects.

References

AOAC. International. Official Methods of Analysis of AOAC International. 17th ed. Gaithersburg, MD, USA; 2000.

Blevins S, Siegel PB, Blodgett DJ, Ehrich M, Saunders GK, Lewis RM. Effects of silymarin on gossypol toxicosis in divergent lines of chickens. Poult Sci 2010; 89:1878–86.

Buettner R, Parhofer KG, Woenckhaus M, Wrede CE, Kunz-Schughart LA, Schölmerich J, Bollheimer LC. Defining high-fat-diet rat models: metabolic and molecular effects of different fat types. J Mol Endocrinol 2006; 36:485-501.

Burlikowska K, Piotrowska A, Szymeczko R. Effect of dietary fat type on performance, biochemical indices and fatty acids profile in the blood serum of broiler chickens. J Anim Feed Sci 2010; 19: 440-51.

Cayan H, Erener G. Effect of olive leaf (*Olea europaea*) powder on laying hens performance, egg quality and egg yolk cholesterol levels. Asian-Austral J Anim Sci 2015; 28: 538-543.

De Leonardis A, Macciola V, Di Domenico N. A first pilot study to produce food antioxidant from sunflower seed shells (*Helianthus annus*). Eur J Lipid Sci Technol 2005; 107:220–227.

205 Dhama K, Latheef SK, Mani S, Samad HA, Karthik K, Tiwari R, Khan RU, Alagawany M, Farag
206 MR, Alam GM, Laudadio V, Tufarelli V. Multiple Beneficial Applications and Modes of Action
207 of Herbs in Poultry Health and Production - A Review. Int J Pharmacol 2015; 11: 152-76.
208 Duncan DB. Multiple range and multiple F-test. Biometrics 1955; 11: 1–42.
209 Golian A, Polin D. Passage rate of feed in very young chicks. Poult Sci 1984; 63: 1013-9.
210 Humason GL. Animal tissue techniques. W. H. Freeman and Company, San Francisco; 1972.
211 Latshaw JD. Daily energy intake of broiler chickens is altered by proximate nutrient content and
212 form of the diet. Poult Sci 2008; 87: 89-95.
213 Krasnodębska-Depta A, Koncicki A. Physiological values of selected serum biochemical indices in
214 broiler chickens. Med Wet 2000; 56: 456-60.
215 Laudadio V, Ceci E, Lastella NMB, Tufarelli V. Dietary high-polyphenols extra-virgin olive oil is
216 effective in reducing cholesterol content in eggs. Lipids in Health Dis 2015; 14: 5.
217 Laudadio V, Dambrosio A, Normanno G, Khan RU, Naz S, Rowghani E, Tufarelli V. Effect of
218 reducing dietary protein level on performance responses and some microbiological aspects of
219 broiler chickens under summer environmental conditions. Avian Biol Res 2012; 5: 88-92.
220 Leeson S, Summers JD. Scott's nutrition of the chicken. University Books, Guelph, Ontario; 2001.
221 Lillie RD. Histopathologic technic and practical histochemistry. McGraw-Hill Book Co., Inc., New
222 York; 1954.
223 NRC. Nutrient Requirements for Poultry. 9th rev. ed. Washington, DC: National Academy Press;
224 1994.
225 Owen RW, Giacosa A, Hull WE, Haubner R, Würtele G, Spiegelhalder B, Bartsch H. Olive-oil
226 consumption and health: the possible role of antioxidants. Lancet Oncol 2001; 1: 107–12.
227 Ozdogan M, Aksit M. Effects of feeds containing different fats on carcass and blood parameters of
228 broilers. J Appl Poult Res 2003; 12: 251-6.
229 Pereira-Caro G, Mateos R, Sarria B, Cert R, Goya L, Bravo LHydroxytyrosyl acetate contributes to
230 the protective effects against oxidative stress of virgin olive oil. Food Chem 2012; 131: 869-878.

231 Plaa GL, Charbonneau M. Detection and evaluation of chemically induced liver injury. pp. 1456–95,
232 In: Principles and Methods of Toxicology. A. W. Hayes, ed. CRC Press, Boca Raton, FL; 2008.
233 Poorghasemi M, Seidavi A, Qotbi AAA, Laudadio V, Tufarelli V. Influence of dietary fat source on
234 growth performance responses and carcass traits of broiler chicks. Asian-Austral J Anim Sci
235 2013; 26: 705-710.
236 Rice-Evans, C. Plant polyphenols: free radicals scavengers or chain breaking antioxidants?
237 Biochem Soc Symp 1995; 61: 103–16.
238 SAS. SAS/STAT User's Guide. Release 9.1. SAS Inst. Inc., Cary, NC; 2006.
239 Smulikowska S, Rutkowski A. Nutrient Recommendations and Nutritive Value of Feedstuffs.
240 Nutritional Standards for Poultry The Kielanowski Institute of Animal Physiology and Nutrition
241 Ed. Jabłonna, Poland; 2005.
242 Zduńczyk Z, Jankowski J, Koncicki A. Fats in Poultry Feeding. Selected Problems. University of
243 Warmia and Mazury Ed., Olsztyn, Poland; 2001.
244

245 **Table 1**. Ingredients and chemical analysis of the basal diet fed to broiler chickens.

Ingredients	Diet
	g/kg as-fed basis
Corn	541.0
Soybean meal (48% CP)	175.0
Corn gluten meal (60% CP)	45.0
Dicalcium phosphate	17.0
Oil or Fat[1]	25.0
Calcium carbonate	90.0
L-Lys HCl	2.0
DL-Met	2.0
Vitamin-mineral premix[2]	2.5
Sodium chloride	2.5
L-Thr	1.0
Yeast	1.5
Sodium bicarbonate	2.5
Chemical analysis, %	
Dry matter	88.13
Crude protein	19.00
Crude fibre	2.80
Crude fat	5.35
Starch	42.87
Ash	5.59
Calculated analysis	
ME (kcal/kg of diet)	3,050
Lys, %	0.98
Ca, %	1.01
Met, %	0.44
Na, %	0.17
Met + Cys, %	0.65
Thr, %	0.64
Available P, %	0.42

246 [1]Each diet contained one of the following oil or fat sources at 2.5% of inclusion level: lard (LRD),
247 sunflower oil (SFO) and extra-virgin olive oil (EVOO), respectively.

248 [2]Supplied per kilogram of diet: vitamin A 12,000 IU; vitamin E, 10 mg; vitamin D 2,200 IU; niacin
249 35.0 mg; D-pantothenic acid 12 mg; riboflavin 3.63 mg; pyridoxine 3.5 mg; thiamine 2.4 mg; folic
250 acid 1.4 mg; biotin 0.15 mg; vitamin B 0.03 mg; Mn 60 mg; Zn 40 mg; Fe 1,280 mg; Cu 8 mg; I 0.3
251 mg; Se 0.2 mg.

252

253 **Table 2.** Effect of the experimental diets on growth performance of broiler chickens.

Item	Diet[1]					
	LRD	SFO	EVOO	SEM	*P*-value	$LSD_{0.05}$
Body weight, g/bird[2]	2,570[b]	2,424[c]	2,643[a]	21.07	0.027	0.032
Body weight gain, g/d	52.6[ab]	49.7[b]	53.9[a]	0.34	0.032	0.037
Feed intake, g/bird/d	139[b]	143[a]	138[b]	0.72	0.037	0.029
Feed conversion ratio, g/g	2.65b	2.88a	2.56c	0.08	0.019	0.021
Mortality, %	1.7[a]	1.1[b]	1.0[b]	-	0.044	0.047

254 [1]Each diet contained one of the following oil or fat sources at 2.5% of inclusion level: lard (LRD), sunflower oil (SFO) and extra-virgin olive oil

255 (EVOO), respectively.

256 [2]Body weight at 49 days of age.

257 SEM, standard error of the means.

258 Means within a row with no common letter (a-c) differ significantly (P\0.05)

259

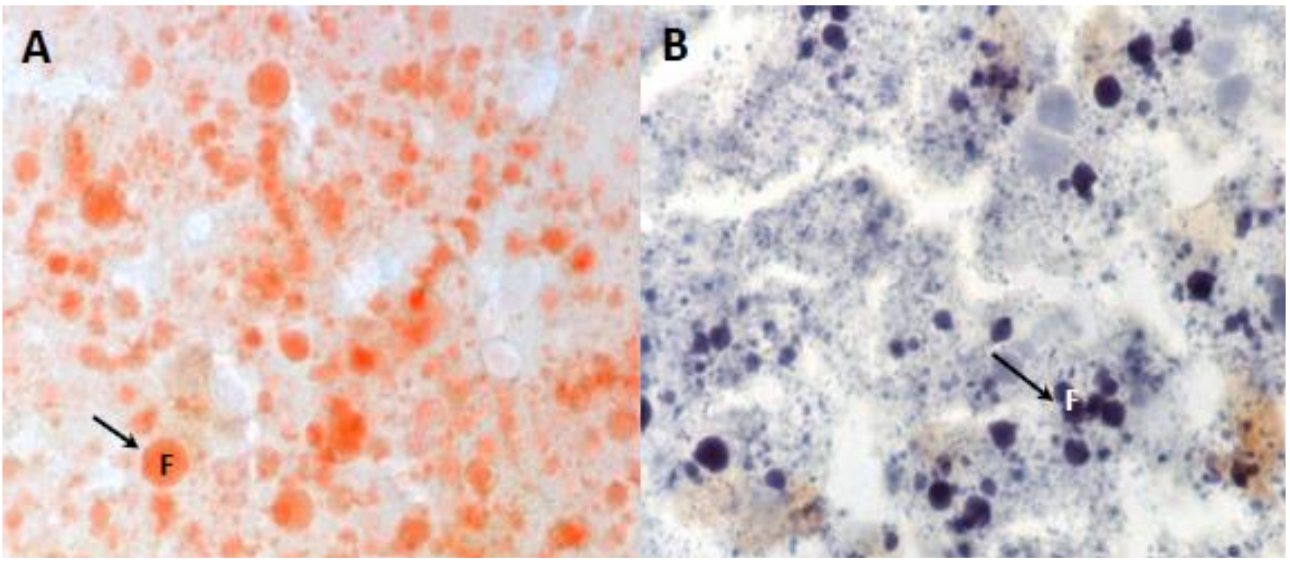

Figure 1. Liver of chickens fed dietary lard (LRD). Numerous hepatocytes with fatty (**F**) infiltration and vacuolated hepatocytes. Oil red O (**A**) and Sudan black B (**B**) × 100, respectively.

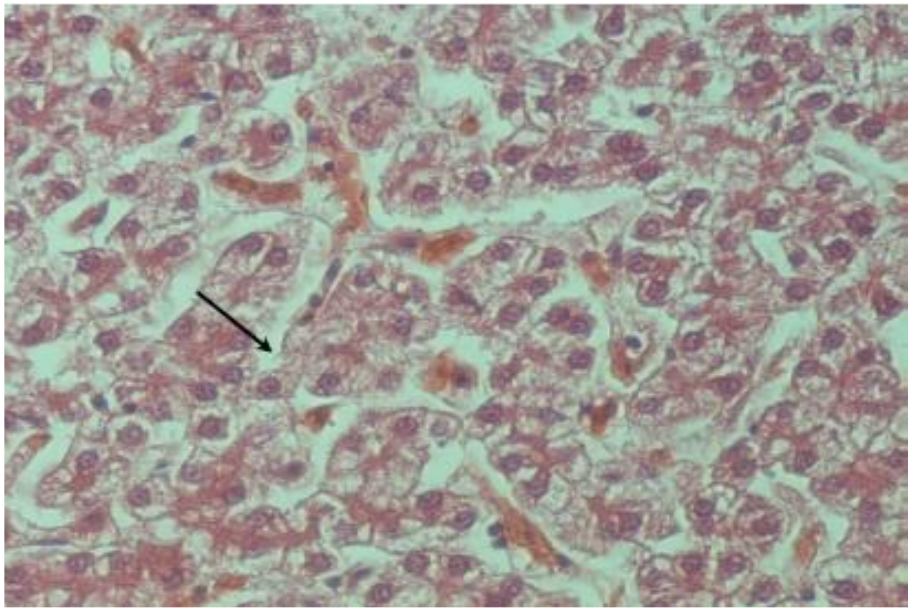

Figure 2. Liver of chickens fed dietary lard (LRD). Group of vacuolated hepatocytes with fatty infiltration. Haematoxylin-Eosin 40×.

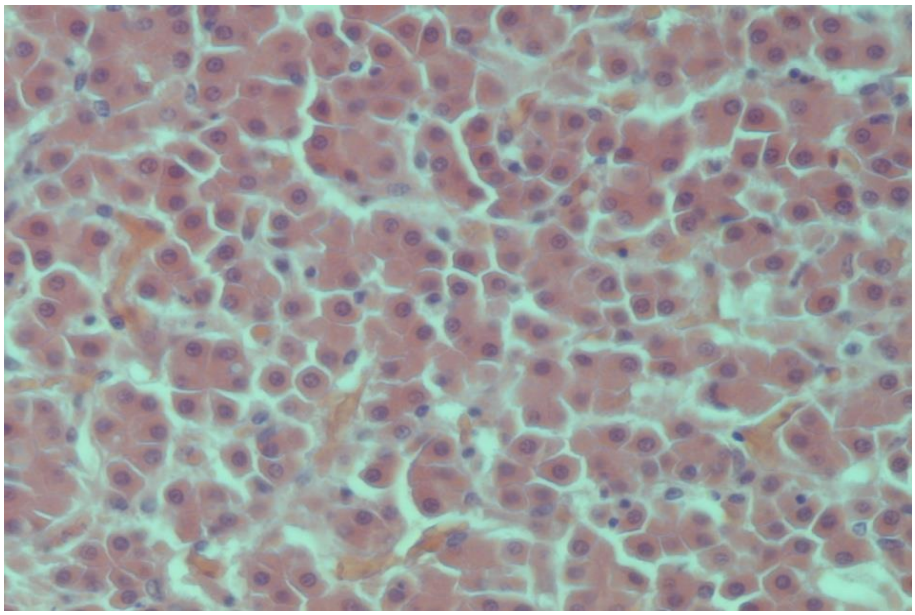

Figure 3. Normal liver chickens fed dietary sunflower oil (SFO). Haematoxylin-Eosin 40×.

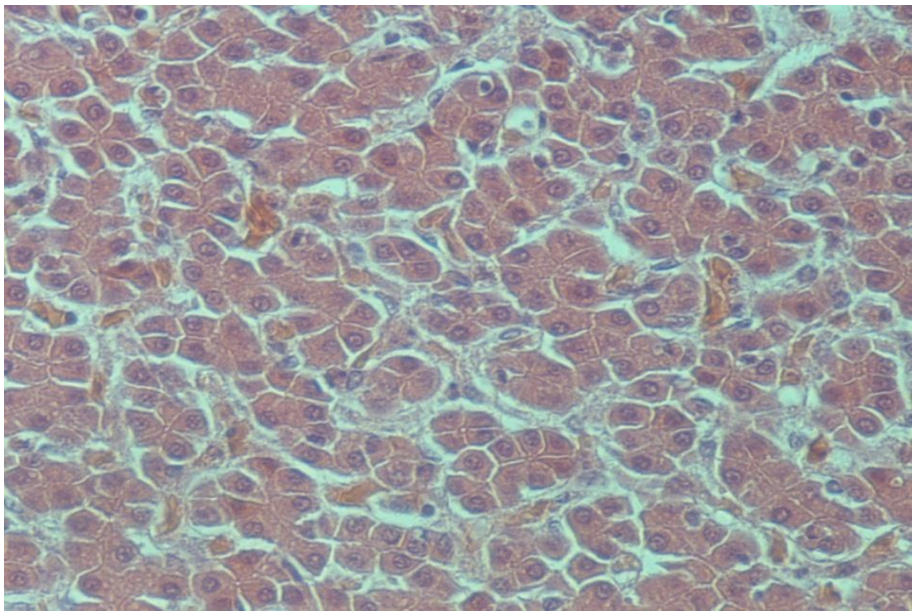

Figure 4. Normal liver chickens fed dietary extra-virgin olive oil (EVOO). Haematoxylin-Eosin 40×.