Brazilian Journal of Poultry Science
Revista Brasileira de Ciência Avícola

ISSN 1516-635X Oct - Dec 2019 / v.21 / n.4 / 001-006

http://dx.doi.org/10.1590/1806-9061-2018-0917

Original Article

Hematology and Histopathology of Broiler Chickens Supplemented with Tuna Black Flour

■Author(s)

Rebolledo OFP[I] https://orcid.org/0000-0001-8802-0177
Barrera JEM[II] https://orcid.org/0000-0001-9344-1873
Márquez JG[III] https://orcid.org/0000-0001-7066-4660
Vázquez AR[I] https://orcid.org/0000-0002-0742-4864
Casillas ACG[I] https://orcid.org/0000-0002-7716-210X

[I] Faculty of Veterinary Medicine and Zootechnics, University of Colima.
[II] Department of Animal Production and Agriculture, University Autonomous Metropolitan. Mexico City.
[III] University Center for Agricultural Research and Development, University of Colima.

■Mail Address

Corresponding author e-mail address
Arturo César García Casillas
Doctor of Philosophy (PhD) in Agricultural Sciences - Universidad de Colima Ringgold standard institution - Faculty of Veterinary Medicine, Tecomán, Colima, Colima 28040, Mexico.
Phone: 313 3229407
Email: cesargarciacasillas@hotmail.com

■Keywords

Broiler, Hematology, Histopathology, ω-3 polyunsaturated fatty acids, Tuna black flour.

Submitted: 14/July/2019
Approved: 08/September/2019

ABSTRACT

The present study evaluated the effect of the sex and dietary intake of tuna black flour (TBF)on the hematology and histopathology of broiler chickens. A total of 200 sexed broilers (Ross 380) were distributed according to a completely randomized design into four treatments (25 males and 25 females/experimental group). Different levels of inclusion of TBF (0, 1, 2, and 3%) were offered as a source of ω-3 polyunsaturated fatty acids (ω-3 PUFAs), for 42 d. At the end of the experiment, blood samples and tissues were collected. The hematocrit value (Hemat), total protein (PROT-T), hemoglobin concentration (Hemog), erythrocytes concentration (Erythro) and total count and differential of leucocytes (Leuco): lymphocytes, heterophils, monocytes, eosinophils, and basophils were determined. Statistical analysis of the data was performed using the Analysis of Variance test, Duncan's multiple range test, and descriptive analyses ($p<0.05$). Samples of heart, liver, and bursa of Fabricius were processed with the routine histological technique. The sex had no significant effect on the hematology. The feeding additional with 3% TBF had lower level of Hemat value and PROT-T. The Leuco had lower value with 1 and 2% of TBF. The Monocytes showed low value with 1 and 2% of TBF, and the eosinophils had high level with 1% of TBF. No cardiac lesions were detected in the broiler chickens in all treatments, normal deposition of fat in the epicardium, endocardium and pericardium were seen in all treatments.

INTRODUCTION

Cardiovascular disease (CVD) is one of the leading causes of death in men and women of all ethnic groups. Consuming saturated dietary fats and ω-6 polyunsaturated fatty acids (ω-6 PUFAs) increases the risk of CVD, whereas consumption of ω-3 polyunsaturated fatty acids (ω-3 PUFAs) may reduce CVD incidence (González & Leeson, 2000; Zuidhof *et al.*, 2009). In recent years, ω-3 PUFAs have received considerable attention in both human and animal nutrition. Dietary supplementation with fish oil (FO), which is rich in ω-3 PUFAs, is reported to be nutritional (Pilevar *et al.*, 2011; Al-Khalifa *et al.*, 2012; Ganesan *et al.*, 2014). Diet manipulation by incorporating FO or oilseeds in the hen diet is the usual way of producing ω-3 PUFAs modified chicken eggs (González & Leeson, 2000; Castro, 2002; Cherian & Hayat, 2009; Khatibjoo *et al.*, 2011; Koppenol *et al.*, 2014), but in many countries the consumption of marine products is very low; hence the benefit that could be derived from a diet rich in ω-3 PUFAs does not reach the majority of a population. Fatty acid (FA) content of the meat chicken can

be modified through nutrition. Currently, linseed oil and marine products are used commercially to achieve this effect (Zuidhof *et al.*, 2009; Morales *et al.*, 2013b; Morales *et al.*, 2013a; Ribeiro *et al.*, 2013; Martínez *et al.*, 2016). In Mexico, black tuna meat is not used for human consumption but is considered a by-product destined for animal consumption in the form of flour. The aim of the present study was to evaluate the effect of the sex and dietary intake of tuna black flour (TBF) on the hematology and histopathology of broiler chickens.

MATERIALS AND METHODS

Animals and experimental design

Institutional and national guidelines for the care and use of animals were followed and all experimental procedures were approved by the Committee of Colima University ethical review.Two hundred sexed broilers: (100) 1 d old male (Ross 308) and (100) 1 d old females (Ross 308), were obtained from a local commercial hatchery. The birds were housed in floor pens of identical size (1×2 m) using wood shaving as litter. The room was thermostatically controlled to produce an initial temperature of 32 °C on day 1 and reduced in by 0-5 °C per d, reaching 21 °C. The birds were distributed according to a completely randomized design into four treatments (25 males and 25 females/experimental group). All data were analyzed as a 2 x 4 factorial arrangement, taking into account the incorporation of sex and TBF levels as the main factors.

Determination of fatty acids in tuna black flour (TBF)

The FA and fatty acids to methyl esters (FAME) analyses were carried out on a Varian CP3800 gas chromatograph (JVA; Analytical Ltd., Dublin, Ireland) equipped with a flame ionization detector (FID). The column was a CP FFAP CB capillary column (30 m × 250 µm i.d., 0.32-µm phase thickness; Agilent Technologies Ireland Ltd., Little Island, Cork, Ireland). The concentration of the main fatty acids in TBF are shown in table 1.

Dietary treatments

Different levels of inclusion of TBF (0, 1, 2, and 3%) were offered as a source of ω-3 PUFAs in commercial sorghum-soybean diets, for 42 d. All diets were isonitrogenous and isocaloric. Diets were formulated in three phases: i) starter (1-14 d), ii) grower (15-35 d), and iii) finisher (36-42 d), the diets formulations are shown in table 2.

Table 1 – Concentration of fatty acids in tuna black flour.

Total lipids, %	8.32 ± 0.01
Total saturated fatty acids (SFA), mg/100 g	1814.68
Main SFA	
Myristic ($C_{14:0}$)	137.25 ± 0.72
Palmitic ($C_{16:0}$)	1120.78 ± 35.12
Stearic ($C_{18:0}$)	356.40 ± 4.58
Total monounsaturated fatty acids (MUFAs), mg/100 g	1063.41
Main MUFAs	
Palmitoleic (C16:1)	213.85 ± 8.09
Oleic acid (C18:1, ω-9)	708.40 ± 15.85
Total polyunsaturated fatty acids (PUFAs), mg/100 g	1579.80
Main PUFAs	
Linoleic ($C_{18:2, ω-6}$)	118.83 ± 3.50
α Linolenic ($C_{18:3, ω-3}$)	33.72 ± 1.86
γ Linolenic ($C_{18:3, ω-6}$)	11.44 ± 3.28
Arachidonic ($C_{20:4}$)	86.26 ± 3.28
Eicosapentaenoic ($C_{20:5, ω-3}$)	258.88 ± 14.15
Docosahexaenoic ($C_{22:6, ω-3}$)	1039.07 ± 47.79

Data expressed as mean ± standard deviation.

Analytical testing

The diets were fed in mash form, mixed in house, and were analyzed for gross energy by bomb calorimetry (Robbins & Firman, 2006). The crude protein (CP) was measured to the method 968.06 of Association of Official Analytical Chemists (AOAC, 2002) using the Nitrogen/Protein Determinator (FP-428; LECO Instruments Ltd., Mississauga, Ontario, Canada). Phosphorus and calcium content were measured to the method 985.01 (AOAC, 2002) using the Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES). Methionine, cysteine, and lysine were measured by High-Performance Liquid Chromatography (HPLC).

Blood collection and determination of hematology parameters

All birds were sacrificed by exsanguination and necropsied immediately. Blood samples were collected from the jugular vein using 6 mL vacuum tubes with Ethylene Diamine Tetra Acetic Acid (EDTA) as anti-coagulant (BD Vacutainer 367863; Becton-Dickinson Co., Franklin Lakes, United States). The serum was separated by centrifuging at 1 500 x *g* for 15 min using a portable centrifuge (Porta-Spin C828; UNICO, Dayton, United States). The Hemat value was counted using standard Wintrobe method and haemocytometer. The PROT-T was measured by Biuret using UV/Vis spectrophotometer (Biochemistry Analyzer ES-218; KONTRO Lab.,

Table 2 – Composition and nutrient content of starter, grower and finisher experimental diets (% inclusion of raw materials)..

Ingredient	Starter diets (1-4 d)				Grower diets (15-35 d)				Finisher diets (36-42 d)			
	Tuna black flour (TBF, %)											
	0	1	2	3	0	1	2	3	0	1	2	3
Sorghum	65.16	66.56	67.96	69.36	71.80	73.21	74.45	76.01	74.49	75.44	76.32	77.04
Soybean meal	28.03	26.26	24.48	22.70	21.54	19.77	18.06	16.21	18.55	16.94	15.41	14.03
Calcium phosphate	2.60	2.31	2.01	1.72	2.33	2.03	1.74	1.45	2.19	1.90	1.60	1.31
Calcium carbonate	0.76	0.71	0.66	0.62	0.71	0.66	0.62	0.57	0.73	0.68	0.63	0.58
Oil	1.97	1.57	1.18	0.78	1.70	1.31	1.00	0.52	2.23	2.23	2.23	2.23
Lysine	0.43	1.47	0.48	0.49	0.43	0.44	0.45	0.46	0.36	0.36	0.37	0.37
Methionine	0.41	0.39	0.39	0.39	0.41	0.40	0.40	0.40	0.32	0.32	0.32	0.32
Salt	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Vitamin premix[1]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Mineral premix[2]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Coccidiostate	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Pigment	0.00	0.00	0.00	0.00	0.45	0.45	0.45	0.45	0.50	0.50	0.50	0.50
Calculated Analysis												
CP %	20	20	20	20	18	18	18	18	17	17	18	18
ME kcal/kg	3000	3000	3000	3000	3050	3050	3050	3050	3070	3084	3121	3127
Calcium %	1.00	1.00	1.00	1.00	0.90	0.90	0.90	0.90	0.85	0.85	0.85	0.85
Phosphorus%	0.50	0.50	0.50	0.50	0.45	0.45	0.45	0.45	0.42	0.42	0.42	0.42
Methionine + cysteine %	1.00	1.00	1.00	1.00	0.90	0.90	0.90	0.90	0.83	0.83	0.83	0.83
Lysine %	1.40	1.40	1.40	1.40	1.20	1.20	1.20	1.20	1.06	1.06	1.06	1.06

[1]Vitamin premix supplied the following per kilogram of complete feed: vitamin A 12 000 IU, vitamin D3 2 500 IU, vitamin E 30 IU, vitamin k3 2 mg, thiamine 2.25 mg, riboflavin 7.5 mg, pyridoxine 3.5 mg, cobalamin 0.02 mg, niacin 45 mg, D-pantothenic acid, 12.5 mg, biotin 0.125 mg and folic acid 1.5 mg; [2]Mineral premix supplied the following per kilogram of complete feed: zinc 50 mg, copper 12 mg, iodine 0.3 mg, cobalt, 0.2 mg, iron 100 mg, selenium 0.1 mg, and manganese 110 mg.

Guidonia, Italy). The Hemog concentration was analyzed by the HemoTest. The Erythro value was counted using Improved Neubauer, and total count and differential of Leuco were analyzed by blood smear and Giemsa dye.

Tissue processing and histological analysis

The portion of the heart, liver and bursa tissues were fixed in 10% neutral buffered formalin, they were trimmed, processed, (Pathology Laboratory of Colima University, Mexico), sectioned at approximately 5 µm, mounted on a glass slide, stained with hematoxylin and eosin. The evaluation of hematoxylin and eosin stained sections was conducted by a pathologist blinded to the treatments. The histological structures of the heart, liver, and bursa of Fabricius were observed using a light microscope under low (×10) and high (×40) magnification. Photographs were taken for better illustration of the results.

Statistical analysis

The comparison between groups was assessed by Analysis of Variance using (PROC ANOVA, SAS, System, v. 8.2, Cary, NC). Differences among treatments were examined by Duncan's multiple range test. Significance (*p*-value) was evaluated at 0.05.

RESULTS

The effects of the sex and dietary intake of TBF on the hematology of broiler, are shown in table 3 and 4. The sex had no significant effect ($p>0.05$) on the Hemat value, PROT-T, Hemog, Erythro and total count and differential of Leuco. Dietary intake of TBF had no significant effect ($p>0.05$) on the Hemog, Erythro, lymphocytes, heterophils, and basophils.

The feeding with thea dditional3% TBF had lower level of Hemat value and PROT-T ($p<0.05$). The Leuco had lower concentration with 1 and 2% of TBF. The Monocytes showed low value with 1 and 2% of TBF, and the eosinophils had high level with 1% of TBF. The interaction between sex and TBF affected Hemat with the increased dietary intake of TBF level (table 3). The Leuco concentration increased in female with 1 and 2% of TBF and male with 0, 1, 2 and 3% of TBF. Thus, the sex with the increased dietary intake of TBF had lower value of monocytes. No significant gross abnormalities were noted in any of the birds examined. No cardiac lesions were detected in the broiler chickens in all treatments, normal deposition of fat in the epicardium, endocardium and pericardium were seen in all treatments. Liver with multifocal hepatitis in all broiler chickens and the bursa of Fabricius was not affected by the dietary intake of TBF (figure 1).

Table 3 – Effects of the sex and dietary intake of tuna black flour (TBF) on the hematology of broiler chickens, *n*= 200 sexed broilers (Ross 380).

Factor	Hemat[1]	PROT-T[2]	Hemog[3]	Erythro[4]	Leuco[5]
Sex					
Female	32.27±3.56a	3.95±0.51a	11.07±3.25a	1.98±0.41a	1.78±0.61a
Male	32.22±2.64[a]	3.90±0.51[a]	11.34±3.03[a]	2.11±0.46[a]	1.81±0.32[a]
p[6]	0.95	0.67	0.77	0.40	0.59
EEM[7]	1.77	0.71	1.77	0.66	0.38
Tuna black flour (TBF)					
0%	33.10±1.96[a,b]	4.12± 0.04[a]	11.33±3.04[a]	2.06±0.60[a]	2.14±0.54[a]
1%	33.80±3.32[a]	4.04±0.63[a,b]	11.65±3.63[a]	2.21±0.50[a]	1.64±0.39[b]
2%	32.05±2.72[a,b]	3.90±0.04[a,b]	10.85±3.04[a]	1.93±0.30[a]	1.47±0.29[b]
3%	30.04±2.86[b]	3.64± 0.11[b]	10.98±3.11[a]	2.00±0.11[a]	2.00±0.29[a]
p[6]	0.01	0.03	0.93	0.55	0.00
EEM[7]	1.69	0.62	1.79	0.66	0.63
Interaction					
Female *0%TBF	32.30±1.78[a,b]	4.22±0.25[a]	12.72±3.95[a]	2.00±0.22[a]	2.27±0.71[a]
Male *0%TBF	34.00±1.87[a]	4.02±0.08[a]	9.95±0.70[a]	2.26±0.82[a]	2.01±0.32[a,b]
Female *1%TBF	35.40±3.57[a]	4.04±0.50[a]	12.69±4.31[a]	2.29±0.54[a]	1.45±0.35[a,b]
Male *1%TBF	32.20±2.38[a,b]	4.04±0.49[a]	10.61±2.90[a]	2.14±0.50[a]	1.82±0.37[a,b]
Female * 2%TBF	33.30±2.13[a,b]	4.04±0.25[a]	9.72±0.69[a]	1.82±0.37[a]	1.36±0.86[b]
Male *2%TBF	30.80±2.86[a,b]	3.76±0.46[a]	11.97±4.15[a]	2.05±0.17[a]	1.57±0.39[a,b]
Female * 3%TBF	28.18±2.33[a,b]	3.50±0.33[a]	9.13±1.47[a]	1.98±0.19[a]	2.43±0.77[a]
Male *3%TBF	31.90±3.04[b]	3.78±0.52[a]	12.83±3.33[a]	2.01±0.80[a]	1.83±0.47[a,b]
p[6]	0.01	0.35	0.05	0.55	0.06
EEM[7]	1.60	0.62	1.74	0.67	0.61

[1]Hematocrit value (%); [2]total protein (g/dL); [3]hemoglobin concentration (g/dL); [4]erythrocytes concentration (x10^{12}/L); [5]leucocytes value (x10^{9}/L); [6]significance; [7]standard error of measurements; data expressed as mean ± standard deviation; significant differences were obtained between groups indicated with different letters.

Table 4 – Effects of the sex and dietary intake of tuna black flour (TBF) on the leucocytes of broiler chickens, *n* = 200 sexed broilers (Ross 380).

Factor	Lymphocytes	Heterophils	Monocytes	Eosinophils	Basophils
Sex					
Female	64.25 ± 16.75[a]	26.80 ± 14.60[a]	2.70 ± 2.02[a]	3.05 ± 2.06[a]	3.25 ± 2.29[a]
Male	54.75 ± 17.66[a]	35.70 ± 14.58[a]	3.60 ± 2.56[a]	2.60 ± 2.21[a]	2.85 ± 2.43[a]
p[1]	0.07	0.05	0.08	0.86	0.58
EEM[2]	4.14	3.81	1.51	1.45	0.52
Tuna black flour (TBF)					
0%	52.10 ± 9.88[a]	36.80 ± 8.41[a]	4.30 ± 2.26[a]	2.90 ± 0.73[b]	3.90 ± 1.10[a]
1%	53.30 ± 15.41[a]	33.00 ± 14.46[a]	5.10 ± 1.96[a]	4.80 ± 2.14[a]	3.80 ± 1.87[a]
2%	69.90 ± 10.08[a]	24.60 ± 10.04[a]	1.30 ± 1.05[b]	2.30 ± 0.61[b]	2.80 ± 3.35[a]
3%	62.70 ± 26.06[a]	30.60 ± 22.79[a]	1.90 ± 1.44[b]	2.00 ± 0.52[b]	1.70 ± 2.05[a]
p[1]	0.06	0.30	0.00	0.00	0.13
EEM[2]	4.08	3.87	1.31	0.53	0.71
Interaction					
Female *0%TBF	53.40 ± 2.40[a]	35.40 ± 3.84[a]	4.60 ± 2.40[a,b]	2.80 ± 0.83[a]	3.80 ± 1.09[a]
Male *0%TBF	50.80 ± 14.48[a]	38.20 ± 11.81[a]	4.00 ± 2.34[a,b,c]	3.00 ± 0.70[a]	4.00 ± 1.22[a]
Female *1%TBF	65.20 ± 6.72[a]	21.80 ± 5.31[a]	3.60 ± 0.89[a,b,c]	4.80 ± 1.92[a]	4.60 ± 2.07[a]
Male *1%TBF	41.40 ± 11.63[a]	44.20 ± 11.34[a]	6.60 ± 1.51[a]	4.80 ± 2.58[a]	3.00 ± 1.41[a]
Female * 2%TBF	72.80 ± 9.73[a]	20.00 ± 6.36[a]	1.20 ± 0.44[c]	2.60 ± 1.34[a]	3.40 ± 3.13[a]
Male *2%TBF	67.00 ± 10.63[a]	29.20 ± 11.56[a]	1.40 ± 1.51[b,c]	2.00 ± 1.50[a]	2.20 ± 3.83[a]
Female * 3%TBF	65.60 ± 30.76[a]	30.00 ± 27.12[a]	1.40 ± 1.51[b,c]	2.00 ± 2.91[a]	1.20 ± 1.30[a]
Male *3%TBF	59.80 ± 23.70[a]	31.20 ± 20.80[a]	2.40 ± 1.34[b,c]	2.00 ± 1.87[a]	2.20 ± 2.68[a]
p[1]	0.46	0.35	0.06	0.96	0.56
EEM[2]	4.03	3.79	1.27	0.79	1.02

[1]Significance; [2]standard error of measurements; data expressed as mean ± standard deviation; significant differences were obtained between groups indicated with different letters.

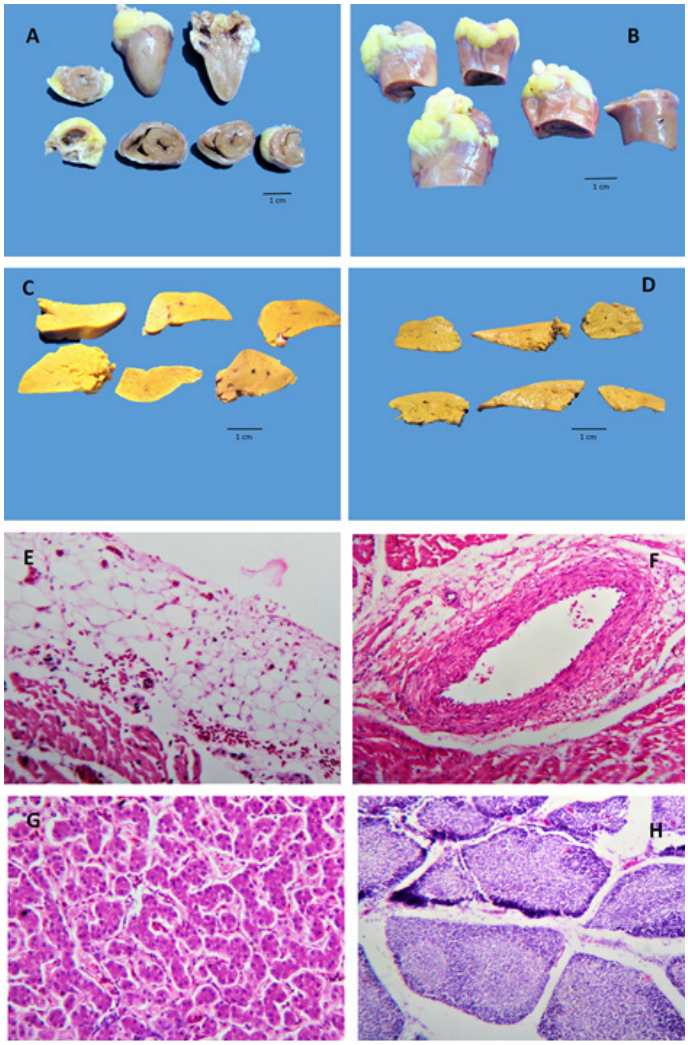

Figure 1 – Effect of the dietary intake of tuna black flour (TBF) on the histopathology of heart, liver and bursa of Fabricius of broiler chickens.

A/B. Normal deposition of fat in epicardium;

C/D. Liver fixed in neutral buffered formalin, without evidence deposition of fat;

E. Normal deposition of fat in epicardium;

F. Myocardium and blood vessel, without evidence deposition of fat;

G. Liver without morphological changes; and

H. Bursa of Fabricius without morphological changes.

DISCUSSION

The diets that contain ω-6 PUFAs, can promote many diseases, including CVD (Simopoulos, 2016). Conjugated linoleic acid (CLA) has been reported to reduce CVD(Shen *et al.*, 2018).To enhance human consumption of CLA, several enriched foods, mostly animal products, are being produced either by adding ω-3 PUFAs directly during the finisher phase of rearing or by modifying animal diets. The ω-3 PUFAs contribute to increase the-number of calories for broiler chickens. However, it has been shown that the inclusion of over 6% FO in poultry diets, can exert some negative effects, such as a compromised oxidative balance in live animals and a higher oxidative susceptibility of the derived meat (González *et al.*, 2013; Leskovec *et al.*, 2018).In the present study up to 3% of the dietary intake of TBF was supplied, as a source of ω-3 PUFAs. Blood biochemical parameters may provide useful information for the evaluation of the health status of broiler chickens and reflect many metabolic alterations of organs and tissues for example heart, liver and bursa of Fabricius (Kudair & Al-Hussary, 2010). Serum proteins are mainly synthesized in the liver, and among other functions, participate in cell coagulation, and the body defense against foreign agents (Melillo, 2013). According to Harr (2002), PROT-T values of broiler chickens tend to be lower than those of mammals, ranging from 2.5 to 4.5g/dL. The values found for PROT-T (table 2), are consistent with the reference values. The 3% of the dietary intake of TBF had lower Hemat value and PROT-T, this situation assumes a low level in globulin (GLOB) rejecting a process of hepatic inflammation. High levels of ω-3 PUFAs affect the immune function in broiler chickens by reducing lymphocyte proliferation, mainly monocytes (Wang *et al.*, 2011). The monocytes transform into macrophages and are actively involved in phagocytosis. Therefore, a reduction of monocytes (table 3) also reduces phagocytosis (Al-Khalifa *et al.*, 2012).

The tight link between hepatic metabolic, dietary intake of methionine in all isonitrogenous and isocaloric diets of the experiment, regulation of adiponectin receptor2 (AdipoR2), fattyacyl-coenzyme Aoxidase1(Acox1), and carnitine palmitoyltransferase 1 (Cpt1) seems to explain the absence of cardiac lesions in broiler chickens. Considering the above, we maintain that it is reasonable to add 3% of the TBF dietary intake to commercial chicken feed, making it a significant source of ω-3 PUFAs for consumers of chicken.

REFERENCES

Al-Khalifa H, Givens DI, Rymer C, Yaqoob P. Effect of n-3 fatty acids on immune function in broiler chickens. Poultry Science 2012;91:74-88.

AOAC - Association of Official Analytical Chemists. Official methods of analysis. 17th ed. Arlington; 2002. p.152-169.

Castro GMI. Ácidos grasos omega 3: beneficios y fuente. Interciencia 2002;27:128-136.

Cherian G, Hayat Z. Long-term effects of feeding flaxseeds on hepatic lipid characteristics and histopathology of laying hens. Poultry Science 2009;88:2555-2561.

Ganesan B, Brothersen C, McMahon DJ. Fortification of foods with omega-3 polyunsaturated fatty acids. Critical Reviews in Food Science and Nutrition 2014;54:98-114.

González ER , Leeson S. Effects of menhaden oil and flaxseed in broiler diets on sensory quality and lipid composition of poultry meat. British Poultry Science 2000;41:481-488.

González OG, Sala R, Cánovas E, Abed N, Barroeta AC. Consumption of dietary n-3 fatty acids decreases fat deposition and adipocyte size, but increases oxidative susceptibility in broiler chickens. Lipids 2013;48:705-717.

Harr KE. Clinical chemistry of companion avian species: a review. Veterinary Clinical Pathology 2002;31:140-151.

Khatibjoo A, Kermanshahi H, Golian A, Zaghari M. The effect of dietary n-6:n-3 ratio and sex on broiler breeder immunity. Poultry Science 2011;90:2209-2216.

Koppenol A, Delezie E, Aerts J, Willems E, Wang Y, Franssens L, et al. Effect of the ratio of dietary n-3 fatty acids eicosapentaenoic acid and docosahexaenoic acid on broiler breeder performance, egg quality, and yolk fatty acid composition at different breeder ages. Poultry Science 2014;93:564-573.

Kudair IM , Al-Hussary NAJ. Effect of vaccination on some biochemical parameters in broiler chickens. Iraqi Journal of Veterinary Sciences 2010;24:59-64.

Leskovec J, Levart A, Nemec Svete A, Peric L, Ethukic Stojcic M, Zikic D, *et al.* Effects of supplementation with alpha-tocopherol, ascorbic acid, selenium, or their combination in linseed oil-enriched diets on the oxidative status in broilers. Poultry Science 2018;97:1641-1650.

Martínez AJA, González AMJ, Miranda RLA, Carrillo DS, Castillo DRM. Sustitución de aceite de soya por aceite de atún en la dieta de pollos como alternativa para enriquecer la carne con ácidos grasos omega-3. Interciencia 2016;41:851-856.

Melillo A. Applications of serum protein electrophoresis in exotic pet medicine. Veterinary Clinics of North America. Exotic Animal Practice 2013;16:211-225.

Morales BJE, González AMJ, Castillo DRM, Prado ROF, Vázquez JL, Hernández VX, *et al.* Effect of time and fatty acid composition in eggs of white Leghorn hens supplemented with tuna oil. Food and Nutrition Sciences 2013a;4:39-44.

Morales BJE, González AMJ, Castillo DRM, Prado ROF, Hernández VX, Menconi A, *et al.* Fatty acid deposition on broiler meat in chickens supplemented with tuna oil. Food and Nutrition Sciences 2013b;4:16-20.

Pilevar M, Arshami J, Golian A, Basami MR. Effects of dietary n-6:n-3 ratio on immune and reproductive systems of pullet chicks. Poultry Science 2011;90:1758-1766.

Ribeiro T, Lordelo MM, Alves SP, Bessa RJ, Costa P, Lemos JP, *et al.* Direct supplementation of diet is the most efficient way of enriching broiler meat with n-3 long-chain polyunsaturated fatty acids. British Poultry Science 2013;54:753-765.

Robbins DH , Firman JD. Evaluation of the metabolizable energy of poultry by-product meal for chickens and turkeys by various methods. International Journal of Poultry Science 2006;5:753-758.

SAS. SAS/STAT user's guide. Version 8.2. Care: SAS Institute; 2001.

Shen P, Kershaw JC, Yue Y, Wang O, Kim KH, McClements DJ, *et al.* Effects of conjugated linoleic acid (CLA) on fat accumulation, activity, and proteomics analysis in Caenorhabditis elegans. Food Chemistry 2018;249:193-201.

Simopoulos AP. An increase in the omega-6/omega-3 fatty acid ratio increases the risk for obesity. Nutrients 2016;8:128.

Wang MZ, Ding LY, Wang JF, Wang HR, Yu LH. Effects of n-6:n-3 polyunsaturated fatty acid ratio on heterophil: lymphocyte ratio and T lymphocyte subsets in the peripheral blood of the Yangzhou gosling. Poultry Science 2011;90:824-829.

Zuidhof MJ, Betti M, Korver DR, Hernandez FI, Schneider BL, Carney VL, *et al.* Omega-3-enriched broiler meat: 1. Optimization of a production system. Poultry Science 2009;88:1108-1120.