

Effect of flaxseed oil supplemented diets on growth performance and meat quality traits in broilers

Petra Ivanova¹, Petar B. Petrov¹, Vasko Gerzilov^{1*}, Neli Keranova², Zhana Petkova³, Olga Teneva³, Ginka Antova³, Pavlina Hristakieva⁴, Ivan G. Penchev⁵

¹Agricultural University, Faculty of Agronomy, 4000 Plovdiv, Bulgaria

²Agricultural University, Faculty of Economics, 4000 Plovdiv, Bulgaria

³University of Plovdiv "Paisii Hilendarski", Faculty of Chemistry, 4000 Plovdiv, Bulgaria.

⁴Agricultural Academy, Agricultural Institute, 6000 Stara Zagora, Bulgaria.

⁵Trakia University, Faculty of Agriculture, 6000 Stara Zagora, Bulgaria.

*Corresponding author: v_gerzilov@abv.bg

Abstract

Ivanova, P., Petrov, P. B., Gerzilov, V., Keranova, N., Petkova, Zh., Teneva, O., Antova, G., Hristakieva, P. & Penchev, I.G. (2022). Effect of flaxseed oil supplemented diets on growth performance and meat quality traits in broilers. *Bulg. J. Agri. Sci.*, 28 (Supplement 1), 101–110

The aim of the present study was to determine the effect of two flaxseed oil levels in the diet of male Ross 308 broilers on the growth performance, feed consumption, slaughter analysis, meat quality, chemical and fatty acid composition of breast and thigh muscles. A total of 180 one-day-old broiler chicks were weighed individually, randomly distributed in 3 groups (n=60 birds in each group; 4 replicates × 15 birds per replicate) and fed to 42 days of age with following diets containing: first group (G₁) – 0%; second group (G₂) – 1.5% and third group (G₃) – 3.0% flaxseed oil. At the end of the experiment, chickens attained average body weight of 2485 ± 49 g (G₁), 2500 ± 49 g (G₂) and 2551 ± 47 g (G₃) without statistically significant differences – P > 0.05. Feed conversion ratio (FCR) for all periods was 1.74 kg/kg (G₁), 1.58 kg/kg (G₂) and 1.57 kg/kg (G₃). Higher water-holding capacity (WHC) was found for breast fillet in G₁ and G₂ compared to G₃ – P < 0.05, and for thigh muscle in G₁ and G₃ compared to G₂ – P < 0.05. No differences between the groups were found with regard to the tenderness of the meat – P > 0.05. With regard to fatty acids, with increasing the amount of flaxseed oil in the diet, the content of oleic, palmitic and palmitoleic acids in the breast fillet decreased while the quantity of linoleic and linolenic acids increased drastically – from 4.60 ± 0.81 (G₁) to 5.80 ± 0.87 (G₂) and to 13.27 ± 0.15 (G₃), and from 0.07 ± 0.03 (G₁) to 0.80 ± 0.23 (G₂) and to 6.77 ± 0.55 (G₃). The tendency of changes in fatty acids in thigh meat was similar – palmitic and palmitoleic acids decreased, and linoleic and linolenic acids increased dramatically – from 6.47 ± 0.03 (G₁) to 10.20 ± 0.12 (G₂) and to 11.23 ± 1.59 (G₃), from 0.10 ± 0.06 (G₁) to 2.60 ± 0.23 (G₂) and to 5.67 ± 1.13 (G₃). The addition of flaxseed oil in the diet decreased the amount of SFA, increased UFA, and PUFA in particular, which once again was due to the increased content of linoleic and linolenic acid in the lipids. The diet supplemented with 3.0% flaxseed oil showed the highest influence on the lipid composition of the broiler's meat.

Keywords: broiler, flaxseed oil, growth performance, meat quality, fatty acid

Introduction

The majority of functional foods, important part of healthy human nutrition, are rich in unsaturated omega-3 and omega-6 fatty acids (Grashorn, 2005; Singh et al. 2011; Calder, 2014). Some of them, e.g. the alpha-linolenic acid (ALA) and linoleic acid are essential for the human body and have to be provided by the food (de Lorgeril et al. 2001). Meat quality and proximate composition are largely dependent on the content of poultry feeds (Haug et al., 2007). It has been demonstrated that fatty acid profile of poultry meat may be altered through the poultry feed fatty acid profile (Salamatdoustnobar et al., 2008; Qi et al., 2010; Rahimi et al., 2011). The addition of flaxseed or flaxseed oil, rich in polyunsaturated fatty acids (PUFA), increases the content of long – chain omega-3 PUFA (LC n-3 PUFA) of poultry meat (Zduńczyk & Jankowski, 2013). The consumption of the latter improves human health (Grashorn, 2007) and helps preventing cardiac diseases (Harris et al., 2007). The α -linolenic acid ($C_{18,3}$) is the primary polyunsaturated fatty acid in flaxseed oil, whose content varies from 51.80 to 60.42% followed by oleic, linoleic, palmitic and stearic acids (Choo et al., 2007; Aguillón-Páez et al., 2020). That is why, it is commonly used for enrichment of poultry meat with n-3 PUFA (Betti et al., 2009 a,b; Zuidhof et al., 2009; Konieczka, et al., 2017; Kanakri et al., 2017; Abbasi et al., 2019; Lalev et al., 2021).

Therefore, the aim of this study was to determine the effect of two different dietary flaxseed oil levels on the growth performance, feed consumption, slaughter traits, meat quality, chemical and fatty acid composition of breast and thigh muscles in broiler chickens.

Material and methods

Experimental design

The experiment was conducted in the poultry farm of the Agricultural University – Plovdiv, with day-old male Ross 308 broiler chickens, reared until 42 days of age. The chickens were divided into 3 groups – one control and 2 experimental. Each group consisted of 4 subgroups with 15 birds each, with uniform body weight. Depending on the amount of dietary flaxseed oil in feed, groups were labelled as followed:

- First group (G_1) – control ($n = 60$); without flaxseed oil supplementation to compound feed.
- Second group (G_2) – ($n = 60$); with supplementation of 1.5% flaxseed oil to compound feed.
- Third group (G_3) – ($n = 60$); with supplementation of 3.0% flaxseed oil to compound feed.

The broilers were housed on deep permanent litter in compliance of all hygiene parameters recommended by the hybrid manufacturer. The birds were fed according to directions of Aviagen group (<http://eu.aviagen.com>) – three-stage mode including starter (1 – 10 day), grower (11 – 24 day) and finisher (25 – 42 day) period. The nutritional value and components of the compound feed are presented in Table 1.

Growth performance and feed consumption

The following growth performance parameters were monitored throughout the trial:

Live body weight, g – by individual weighing of birds with precision of 0.1 g on days 10, 24 and 42 of life;

Feed intake, g – by periods and total for the trial period;

Feed conversion ratio (FCR) – cumulative feed intake (kg)/total weight gain (kg)

Slaughter analysis

At 42 days of age, after 6-hour fasting, 8 broilers from each group (2 from each of subgroups) with live body weight as close as possible to the group average, were slaughtered. The ethical requirements for humane slaughtering of birds were met. The carcass weight, broiler meat cuts, and edible offal (neck, heart, liver and gizzard) were analysed after 24-hour stay at 3°C. The weight of the different cuts and edible offal was determined with precision of 0.01 g.

Meat quality analysis

Meat pH determination

Meat pH was measured on the 24th *post mortem* hour using a Testo 205 pH meter which was preliminary calibrated with buffer solutions with known pH (pH 4 and 7). A minimum of three measurements in different areas of the sample were made and the average of the 3 determinations was retained as ultimate value.

Meat colour analysis

Muscle colour (breast fillet; breast tenderloin; thigh) was determined in the CIE L^*a^*b system with Minolta CR-400 colorimeter (Konica Minolta, Osaka, Japan), in D65 illuminant and observation angle 2°. The values of coordinates L^* (lightness), a^* (redness) and b^* (yellowness) were determined on 24th *post mortem* hour on the cut surface of the respective muscle. Five measurements were made on all samples and the average of the five measurements was used as L^* , a^* and b^* values.

Table 1. Composition of feed

Ingredient, %	Starter (1-10 day)			Grower (11-24 day)			Finisher I (25-42 day)		
	G ₁	G ₂	G ₃	G ₁	G ₂	G ₃	G ₁	G ₂	G ₃
Corn	40.83	40.83	40.83	43.18	43.18	43.18	48.89	48.89	48.89
Wheat	20.0	13.5	9.0	21.95	15.51	10.0	22.05	15.1	7.96
Soybean meal, 46%	35.0	35.0	35.0	31.2	31.2	31.2	25.7	25.7	25.7
Wheat bran	–	5.0	8.0	–	5.0	9.0	–	5.5	11.2
Flaxseed oil	–	1.5	3.0	–	1.5	3.0	–	1.5	3.0
L-Lysine	0.18	0.18	0.18	0.14	0.13	0.13	0.17	0.16	0.15
DL-Methionine	0.32	0.32	0.32	0.27	0.27	0.28	0.24	0.25	0.25
L-Threonine	0.14	0.14	0.14	0.10	0.10	0.10	0.09	0.09	0.09
NaCl	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Sodium bicarbonate	0.13	0.13	0.13	0.16	0.16	0.16	0.16	0.16	0.16
Calcium carbonate	1.10	1.10	1.10	0.90	0.95	0.95	0.85	0.90	0.90
Dicalcium phosphate	1.55	1.55	1.55	1.35	1.25	1.25	1.15	1.05	1.00
Mineral and vitamin premix*	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Immunobeta**	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Coxidin***	0.05	0.05	0.05	0.05	0.05	0.05	–	–	–
Tox-Aid****	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Nutrient specification									
Metabolizable energy, MJ/kg	11.8	11.8	11.9	12.0	12.0	12.0	12.2	12.2	12.2
Crude Protein, %	23.0	23.0	23.0	21.5	21.5	21.5	19.5	19.5	19.5
Crude fiber, %	3.6	4	4.2	3.5	3.8	4.1	3.3	3.6	4
Digestible Lysine, %	1.4	1.4	1.41	1.26	1.26	1.27	1.13	1.13	1.13
Digestible Methionine, %	0.62	0.62	0.62	0.56	0.56	0.57	0.51	0.52	0.52
Digestible Threonine, %	0.97	0.97	0.97	0.87	0.87	0.88	0.78	0.78	0.79
Calcium, %	1	1	1	0.87	0.87	0.87	0.79	0.79	0.79
Available phosphorus, %	0.48	0.49	0.49	0.44	0.44	0.44	0.4	0.4	0.4

* Mineral and vitamin premix: TB 102, Nurscience; Vitamins – Vitamin A – 5000000 IU/kg; Vitamin D3 – 1500000 IU/kg; Choline chloride – 150000 mg/kg; Vitamin E – 15000 mg/kg; Niacin amide – 14000 mg/kg; Calcium D- pantothenate – 4500 mg/kg; Vitamin B2 (riboflavin) – 2500 mg/kg; Vitamin B6 – 1500 mg/kg; Vitamin K3 – 1500 mg/kg; Vitamin B1 – 1000 mg/kg; Folic Acid – 1000 mg/kg; Vitamin B12 – 13 mg/kg; Biotin/D – 1 mg/kg. Trace elements: Manganese oxide – 45000 mg/kg; Zinc oxide – 25000 mg/kg; Ferrous sulphate (monohydrate) – 13000 mg/kg; Copper sulfate (pentahydrate) – 8000 mg/kg; Calcium iodate (anhydrous) – 500 mg/kg; Sodium selenite – 240 mg/kg.

** Immunobeta – prebiotic, Chemifarma, Italy

*** Coxidin – anticoccidial additive (monensin sodium), Huvepharma, Belgium

**** Tox-Aid – mycotoxin binder, E.F.S., Holand

Determination of the water – holding capacity (WHC) of meat

Water holding capacity of meat (WHC) was determined using the classic method of Grau and Hamm (1953), described by Zahariev and Pinkas (1979) with modifications of Petrov (1982). The method was based on determination of the amount of water in the sample by pressing about 5 g of meat sample between two acrylic plates for 5 minutes with a 5 kg weight.

Determination of cooking loss percentage

Cooking loss (CL) was determined by roasting a meat sample at 150° C for 20 min in a convection oven. CL at roast was determined as difference in weight of sample before and after roasting in percents.

Determination of meat tenderness

Meat tenderness was determined with PA VEB Feinmess penetrometer (Dresden, Germany) equipped with a penetration cone (needle). The principle of the technique is based on

Table 2. Average live body weight, g (n = 60 in group).

Age, days	Groups			SEM*	P-Value
	G ₁	G ₂	G ₃		
1	41.48 ± 0.26	41.48 ± 0.26	41.52 ± 0.26	0.15	0.99
11	190.43 ± 4.08 ^b	205.57 ± 4.86 ^a	203.02 ± 4.44 ^a	2.61	0.04
25	874.48 ± 20.36	890.93 ± 24.95	936.20 ± 21.13	12.92	0.13
42	2485.33 ± 49.14	2500.52 ± 49.09	2551.10 ± 47.38	27.96	0.61

the depth of needle penetration in a meat sample under the pressure exerted by the cone (equal to 103.3 g for this appliance). Tenderness values were reported in penetrant degrees – °P, one °P being equal to 0.1 mm.

Chemical and fatty acid composition of breast and thigh muscles

Chemical composition of thigh and breast fillet meat and dry matter (DM) were determined according to Association of Official Analytical Chemists (AOAC, 2016). Crude protein (CP) was calculated from the nitrogen content via the Kjeldahl method using factor 6.25. Ash content was evaluated by incinerating at 550 °C in a muffle furnace for 6 h. Crude fat (CF) was extracted in Soxhlet apparatus with hexane for 8 h. Subsequently, the solvent was removed by a rotary evaporator and the residue was weighed to determine the oil content of each sample. Determination of nitrogen free extract (NFE) was calculated by taking the difference of all the other items measured in the proximate analysis:

$$\text{NFE (\%)} = 100 - (\% \text{ moisture} + \% \text{ crude protein} + \% \text{ crude fat} + \% \text{ ash})$$

The isolated lipids were subjected to transesterification with methanol in the presence of sulfuric acid (ISO 12966-2:2011) and the obtained fatty acid methyl esters (FAMES) were determined by gas chromatography (GC) (ISO 12966-1:2014). Determination was performed on Agilent 8860 gas chromatograph equipped with a capillary column DB – Fast FAME 30 m x 0.25 mm x 0.25 µm (film thickness) and a flame ionization detector (FID). The column temperature was programmed from 70°C (1 min), at 6°C/min to 180°C (0 min), and at 5°C/min to 250°C; the injector and detector temperatures were 270°C and 300°C and the split ratio was 50:1. Identification was carried out by comparison of the retention times with those of a standard mixture of FAME.

Statistical analysis

One-way analysis of variance and Tukey's Post Hoc Test were used to assess the differences at a significance level of

$P \leq 0.05$. The IBM Statistics SPSS 24 statistical software product (Landau & Everitt, 2004) was used for the mathematical data processing.

Results and discussion

At 1 day of age, the chickens from the control and both experimental groups were with uniform body weight. At the end of the starter period, chickens from G₂ and G₃ had a higher live body weight than those from G₁ by 7.95% and 6.61% respectively ($P < 0.05$) – Table 2. The higher body weight of birds from the experimental groups G₂ and G₃ vs G₁ was preserved until the end of the trial – by 0.61% and 2.65% ($P > 0.05$). Similar data were reported by Olomu and Baracos (1991), observing no statistically significant differences in the final live weight between control and flaxseed oil-supplemented broilers: 1.5 % and 4.5%.

During the starter period (day 1-10) the FCR in the three groups was higher than the recommendations of the manufacturer of the hybrid (<https://en.aviagen.com>). FCR of birds from G₁ for the other two periods – grower (day 11 – 24) and finisher (day 25 – 42) was also higher – Fig. 1. Throughout the entire rearing period, feed conversion was better in chickens from G₂ and G₃ as compared to those of G₁ ($P < 0.05$). FCR in groups G₂ and G₃ was in line with manufacturer's

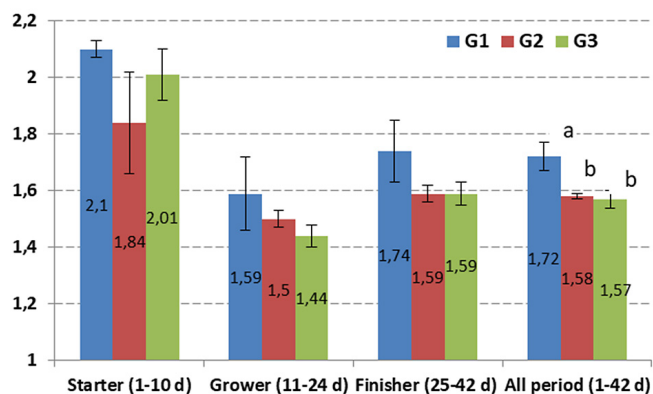


Fig. 1. Feed conversion ratio (FCR), kg diet /kg live weight gain

recommendations- 1.61 kg diet/kg live weight gain (<https://en.aviagen.com>).

During the experiment, no fatal outcome was recorded in all three groups – survival of 100%. According to Al-Khalifah et al. (2020) the good liveability of chickens may be attributed to flaxseed oil, which enhanced the immunity. Coccidiosis, induced by several *Eimeria* species (protozoa) is a problem of poultry farming. In their study with broiler chickens, Allen et al. (1996, 1997) found out that offering feeds supplemented with omega-3 fatty acids, suppressed the development of *E. tenella* in chicken ceca. A number of other researchers reported that flaxseed had also antifungal activity against *Alternaria solani*, *Candida albicans* and *Aspergillus flavus* (Xu et al., 2007; Guilloux et al., 2009; Kaithwas et al., 2011; Abdelillah et al., 2013).

The slaughter analysis at 42 days of age demonstrated that the slaughter yield in broilers from group G_1 was the highest, yet no statistically significant differences were found out with the other two groups – $P > 0.05$ (Table 3). Carcass conformation in the three groups did not show significant differences. The proportion of breast meat (breast fillet and breast tenderloin) in G_1 was 30.98%, in G_2 – 30.52% and in G_3 – 29.53% of carcass weight. In a previous study, Mridula et al., (2015) reported lack of considerable differences in slaughter carcass traits and a significantly higher proportion of breast meat in control chickens compared to flaxseed oil-supplemented ones. The thigh and drumstick proportions were as followed: G_1 – 18.12% and 14.44%, G_2 – 18.57% and 14.65%, G_3 – 18.54% and 14.22% vs carcass weight. The differences were slight and insignificant ($P > 0.05$). Only the weight of drumettes in G_1 and G_3 exceeded statistically significantly that of G_2 ($P < 0.05$). The average edible offal share (neck, gizzard, liver, heart) vs the carcass weight

was: for G_1 – 10.39%, G_2 – 10.86% and G_3 – 10.56%. In accordance with our results, Lopes et al., (2013) have neither demonstrated significant differences between slaughter traits of broiler supplemented with 3%, 4% and 5% flaxseed oil through the compound feed.

The proximate composition of the two breast muscles and the thigh muscle are present in Table 4.

The pH_{24} *post mortem* values of all three muscles in all groups varied within very close ranges, showing that the tested dietary flaxseed oil levels had no influence on this parameter.

The best water holding capacity of breast muscle was found out in birds from group G_2 , followed by G_1 . There was a statistically significant difference between values of this parameter in G_1 and G_2 vs G_3 – $P < 0.05$ both for breast fillet and breast tenderloin. For thigh muscle, G_2 broilers showed lower meat WHC than respective values in G_1 and G_3 – $P < 0.05$. At this stage, no categorical conclusion on the effect of flaxseed oil on hydrophilic traits of meat should be made. It may be, however, concluded convincingly that thigh muscles had substantially lower WHC values than breast muscles.

Culinary processing of poultry meat and preservation of its juiciness and tenderness is at the background of consumer's final evaluation. These traits are directly dependent on cooking loss (CL, %) (Barbantia & Pasquini, 2005; Küçüközet & Uslu, 2018). The meat CL and WHC in the three analysed muscles had similar values. The CL of *thigh muscle* was statistically significantly higher in G_3 vs both G_1 and G_2 – $P < 0.05$. Increased CL parallelly to increase in dietary flaxseed oil level was observed in the three types of muscles. Previously reported data by Betti et al., (2009 a), stating that CL values increased from 27.4% in controls to 31.6% in broilers fed feed supplemented with 17% flaxseed

Table 3. Slaughter characteristics (n = 8 in group)

Traits	G_1	G_2	G_3	SEM	P-Value
Live body weight, g	2727.63 ± 6.26	2716.50 ± 35.53	2761.38 ± 38.84	13.49	0.67
Carcass weight, g	1931.63 ± 32.00	1861.88 ± 24.16	1904.25 ± 21.91	20.29	0.20
Slaughter yield, %	70.81 ± 0.71 ^a	68.54 ± 0.27 ^b	68.99 ± 0.54 ^b	0.34	0.01
Breast, g	484.67 ± 17.16	459.43 ± 9.13	451.41 ± 8.77	10.02	0.81
Breast tenderloin, g	113.80 ± 3.02	108.75 ± 2.79	110.82 ± 1.32	1.47	0.32
Thighs, g	350.08 ± 9.95	345.76 ± 8.56	353.13 ± 5.94	2.14	0.87
Drumsticks, g	278.89 ± 5.39	272.72 ± 3.76	270.81 ± 6.67	2.44	0.55
Drumettes, g	116.17 ± 1.71 ^a	106.56 ± 1.52 ^b	114.22 ± 2.11 ^a	2.93	0.03
Wings, g	101.26 ± 2.34	97.59 ± 1.57	100.46 ± 1.48	1.11	0.35
Edible offal					
Neck, g	101.65 ± 4.24	97.26 ± 3.86	99.30 ± 1.44	1.27	0.64
Gizzard, g	34.21 ± 1.33 ^b	40.89 ± 1.68 ^a	38.14 ± 1.91 ^a	1.94	0.03
Liver, g	53.69 ± 3.57	53.56 ± 1.10	51.81 ± 2.30	0.61	0.84
Heart, g	11.12 ± 0.18	10.43 ± 0.44	11.93 ± 0.82	0.43	0.18

oil from 11 to 35 days of age, were in agreement with our results.

Meat tenderness or texture is probably the most important characteristics for consumers, associated with its satisfaction with poultry meat and meat products consumption (Fletcher, 2002). The analysis of our results allowed affirming that the dietary addition of 1.5% and 3.0% flaxseed oil had no effect on meat tenderness for each of muscles – $P > 0.05$.

The analysis of meat colour by the 24th post-mortem hour using the L^* , a^* , b^* colour coordinates exhibited uneven pigment saturation in relation to the dietary flaxseed oils level. The analysis of meat colour by the 24th post-mortem hour using the L^* , a^* , b^* colour coordinates exhibited uneven pigment saturation in relation to the dietary flaxseed oils level. For the L^* coordinate, higher values in G_2 and G_3 vs G_1 ($P < 0.05$) were found out only for the breast fillet. For breast tenderloin and thigh muscles, between-group differences were insignificant. With respect to the a^* coordinate, it was considerably higher (about 4.3 times) in the meat of group G_1 compared to G_2 and G_3 – $P < 0.05$, and conversely, lower in breast tenderloin of G_1 vs G_2 (by 34.09%) and G_3 (by 36.26%) – $P < 0.05$. For the b^* coordinate, higher saturation of the breast tenderloin was observed in G_2 and G_3 vs G_1 – $P < 0.05$, and in thigh muscle: in G_3 vs both G_1 and G_2 – $P < 0.05$. In an earlier study, Betti et al. (2009 a) reported that the prolonged feeding diets containing flaxseed oil had a significant effect on meat colour characteristics both of breast and thigh muscles.

The results from the chemical analysis of breast and thigh muscles are present in Fig. 2. In general, in all three groups, thigh muscles had a lower crude protein content than breast muscles – $P < 0.05$. No statistically significant differences were found out within a specific muscle among the three groups, despite the tendency towards slight increase in dry matter percentage and respectively, crude protein proportion in both experimental groups fed flaxseed oil-containing diets.

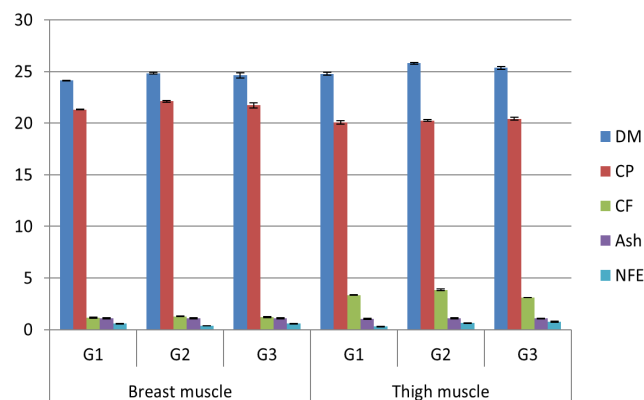


Fig. 2. Chemical analysis of breast and thigh muscles, %; dry matter (DM), crude protein (CP), crude fat (CF), nitrogen free extract (NFE)

Table 4. Physicochemical characteristics in the muscle samples at 24th hour.

Group	pH ₂₄	WHC, %	CL, %	Tenderness, °P	L*	a*	b*
Breast Fillet							
G ₁	5.80 ± 0.03	21.39 ± 0.89 ^b	27.16 ± 0.83	384.04 ± 5.56	56.84 ± 0.57 ^b	6.70 ± 2.78 ^a	13.71 ± 2.28
G ₂	5.78 ± 0.02	20.36 ± 0.74 ^b	27.88 ± 0.97	380.91 ± 5.32	58.44 ± 0.47 ^a	1.56 ± 0.20 ^b	10.09 ± 0.34
G ₃	5.79 ± 0.01	24.23 ± 0.90 ^a	29.12 ± 1.11	382.24 ± 6.07	58.81 ± 0.54 ^a	1.55 ± 0.19 ^b	10.93 ± 0.51
SEM	0.00	1.16	0.57	0.91	0.61	1.71	1.10
P-Value	0.94	0.01	0.37	0.92	0.02	0.05	0.16
Breast tenderloin							
G ₁	5.81 ± 0.02	19.02 ± 0.90 ^b	28.28 ± 0.71	400.00 ± 0.00	56.39 ± 0.57	2.32 ± 0.15 ^b	9.54 ± 0.52 ^b
G ₂	5.79 ± 0.01	21.59 ± 0.83 ^b	29.43 ± 1.12	400.00 ± 0.00	56.41 ± 0.39	3.52 ± 0.24 ^a	11.19 ± 0.26 ^a
G ₃	5.82 ± 0.01	23.88 ± 0.84 ^a	31.07 ± 1.00	400.00 ± 0.00	55.31 ± 0.58	3.64 ± 0.23 ^a	11.22 ± 0.32 ^a
SEM	0.01	1.40	0.81	0.00	0.36	0.42	0.55
P-Value	0.43	0.001	0.14	–	0.24	0.00	0.003
Thigh							
G ₁	6.04 ± 0.04	16.36 ± 1.99 ^a	25.70 ± 0.98 ^b	313.32 ± 12.23	55.93 ± 0.74	8.13 ± 0.53 ^b	11.32 ± 0.42 ^b
G ₂	5.95 ± 0.02	11.57 ± 0.68 ^b	27.26 ± 0.89 ^b	313.07 ± 11.86	57.44 ± 0.62	8.22 ± 0.61 ^b	11.66 ± 0.54 ^b
G ₃	5.96 ± 0.04	15.22 ± 1.07 ^a	31.30 ± 1.34 ^a	304.28 ± 14.91	55.56 ± 0.59	11.27 ± 0.66 ^a	13.21 ± 0.34 ^a
SEM	0.03	1.44	1.67	2.97	0.57	1.03	0.58
P-Value	0.16	0.04	0.002	0.867	0.10	0.00	0.01

Table 5. Effects of flaxseed oil supplement on fatty acid composition (%) of thigh and breast fillet meat in broiler at 42 day of age

Fatty Acids	Breast Fillet Meat					Thigh Meat				
	G ₁	G ₂	G ₃	SEM	P-Value	G ₁	G ₂	G ₃	SEM	P-Value
C _{12:0} Lauric acid	0.03 ± 0.03	0.00 ± 0.00	0.00 ± 0.00	0.01	0.48	0.00 ± 0.00	0.07 ± 0.03	0.07 ± 0.03	0.02	0.22
C _{14:0} Myristic acid	0.50 ± 0.00	0.40 ± 0.17	0.50 ± 0.06	0.06	0.79	0.60 ± 0.12	0.50 ± 0.00	0.50 ± 0.00	0.04	0.52
C _{14:1} Myristoleic acid	0.07 ± 0.03	0.07 ± 0.03	0.07 ± 0.03	0.02	1.00	0.13 ± 0.03	0.17 ± 0.03	0.10 ± 0.00	0.02	0.30
C _{15:0} Pentadecanoic acid	0.10 ± 0.00	0.10 ± 0.00	0.10 ± 0.00	0.00	–	0.10 ± 0.00	0.20 ± 0.00	0.10 ± 0.00	0.02	–
C _{15:1} Pentadecenoic acid	0.17 ± 0.09 ^b	1.00 ± 0.06 ^a	0.15 ± 0.03 ^b	0.14	0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	–	–
C _{16:0} Palmitic acid	37.13 ± 1.85 ^a	37.37 ± 0.85 ^a	29.47 ± 0.78 ^b	1.44	0.00	36.47 ± 0.78 ^a	32.77 ± 0.20 ^{ab}	29.93 ± 1.70 ^b	1.09	0.02
C _{16:1} Palmitoleic acid	7.57 ± 0.32 ^a	4.30 ± 0.40 ^b	4.07 ± 0.15 ^b	0.59	0.00	8.50 ± 0.46 ^a	7.17 ± 0.32 ^a	5.33 ± 0.32 ^b	0.5	0.003
C _{17:0} Margaric acid	0.00 ± 0.00	0.10 ± 0.00	0.10 ± 0.00	0.02	–	0.07 ± 0.03	0.10 ± 0.00	0.10 ± 0.00	0.01	0.42
C _{17:1} Heptadecenoic acid	0.00 ± 0.00 ^b	0.17 ± 0.03 ^a	0.13 ± 0.03 ^a	0.03	0.01	0.10 ± 0.00	0.20 ± 0.00	0.23 ± 0.09	0.03	0.24
C _{18:0} Stearic acid	2.33 ± 0.71	4.60 ± 0.78	3.10 ± 0.64	0.49	0.15	1.80 ± 0.35	1.90 ± 0.46	1.97 ± 0.90	0.31	0.98
C _{18:1} Oleic acid	47.13 ± 0.74 ^a	44.77 ± 0.15 ^b	41.85 ± 0.29 ^c	0.80	0.001	45.53 ± 1.01	43.90 ± 0.06	44.33 ± 1.41	0.56	0.53
C _{18:2} Linoleic acid	4.60 ± 0.81 ^b	5.80 ± 0.87 ^b	13.27 ± 0.15 ^a	1.40	0.00	6.47 ± 0.03 ^b	10.20 ± 0.12 ^{ab}	11.23 ± 1.59 ^a	0.86	0.02
C _{18:3} Linolenic acid	0.07 ± 0.03 ^b	0.80 ± 0.23 ^b	6.77 ± 0.55 ^a	1.07	0.00	0.10 ± 0.06 ^b	2.60 ± 0.23 ^b	5.67 ± 1.13 ^a	0.87	0.003
C _{21:0} Heneicosanoic acid	0.07 ± 0.03	0.03 ± 0.03	0.00 ± 0.00	0.02	0.30	0.00 ± 0.00 ^b	0.03 ± 0.01 ^a	0.00 ± 0.00 ^b	0.02	0.004
C _{20:3} Eicosatrienoic acid	0.13 ± 0.03	0.13 ± 0.03	0.13 ± 0.03	0.02	1.00	0.03 ± 0.03	0.03 ± 0.03	0.13 ± 0.09	0.03	0.42
C _{22:0} Behenic acid	0.00 ± 0.00 ^b	0.00 ± 0.00 ^b	0.13 ± 0.03 ^a	0.02	0.00	0.00 ± 0.00	0.00 ± 0.00	0.10 ± 0.00	0.02	–
C _{23:0} Tricosanoic acid	0.07 ± 0.03 ^{ab}	0.13 ± 0.03 ^a	0.00 ± 0.00 ^b	0.02	0.04	0.00 ± 0.00	0.03 ± 0.03	0.07 ± 0.03	0.02	0.32
C _{20:5} Eicosapentaenoic acid	0.00 ± 0.00 ^b	0.07 ± 0.03 ^{ab}	0.10 ± 0.00 ^a	0.02	0.03	0.07 ± 0.03	0.03 ± 0.03	0.07 ± 0.03	0.02	0.73
C _{24:1} Tetracosenoic acid	0.03 ± 0.03	0.17 ± 0.12	0.07 ± 0.03	0.04	0.47	0.03 ± 0.03	0.00 ± 0.00	0.07 ± 0.03	0.02	0.30

Fatty acid composition of lipids from breast fillet and thigh meat of broilers (with and without supplementation of flaxseed oil) are given in Table 5.

The main fatty acid in the lipids of breast fillet and thigh meat of G₁ was oleic acid (47.13% and 45.53%, respectively), followed by palmitic (37.13% and 36.47%). Significantly lower contents of linoleic (4.60% and 6.47%) and palmitoleic (7.57% and 8.50%) acids were detected. The amount of the polyunsaturated linolenic acid was negligible in both control samples – 0.07% and 0.10%.

Significant changes were observed in the main fatty acids in the breast fillet and thigh meat of broilers fed diets supplemented with 1.5% and 3.0% flaxseed oil. In the lipids from breast fillet the content of oleic (44.77% in G₂ and 41.85% in G₃), palmitic (37.37% and 29.47%) and palmitoleic (4.30% and 4.07%) acids decreased with increasing the dietary amount of flaxseed oil, while those of linoleic (5.80% and 13.27%) and linolenic (0.80% and 6.77%) acids increased drastically.

A significant decrease of the amount of palmitic (32.77% in G₂ and 29.93% in G₃) and palmitoleic (7.17% and 5.33%) acids, an increase of the content of the polyunsaturated linoleic (10.20% and 11.23%) and linolenic (2.60% and 5.67%) acids were noticed in the lipids from thigh meat of broil-

ers, but the levels of oleic acid remained unchanged (from 45.53% in G₁ to 44.33% in G₃).

Overall, the most significant changes were observed in the fatty acid composition of the third group for both meat samples which was an indicator that supplementation of the diet with 3.0% flaxseed oil had the greatest influence on the lipid composition of the broiler's meat.

Conversely, Anjum et al. (2013) reported that the supplementation of the broilers with extruded flaxseed meal caused increase of the amount of palmitic (from 55.87% to 60.91%), stearic (from 27.65% to 37.59%) and oleic (from 56.22% to 66.57%) acids in the lipids of breast meat, alongside with increase of the content of linoleic (from 55.81% to 74.14%) and linolenic (from 1.96% to 3.24%) acids. On the other hand, the levels of linolenic acid in the meat lipids of broilers fed flaxseed oil-supplemented diet in the present study were higher than these in the above mentioned research. The reason for that is probably that the broilers were fed forage with addition directly of flaxseed oil, instead of extruded flaxseed meal (Anjum et al., 2013).

The contents of saturated (SFA), unsaturated (UFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids are presented in Figure 3.

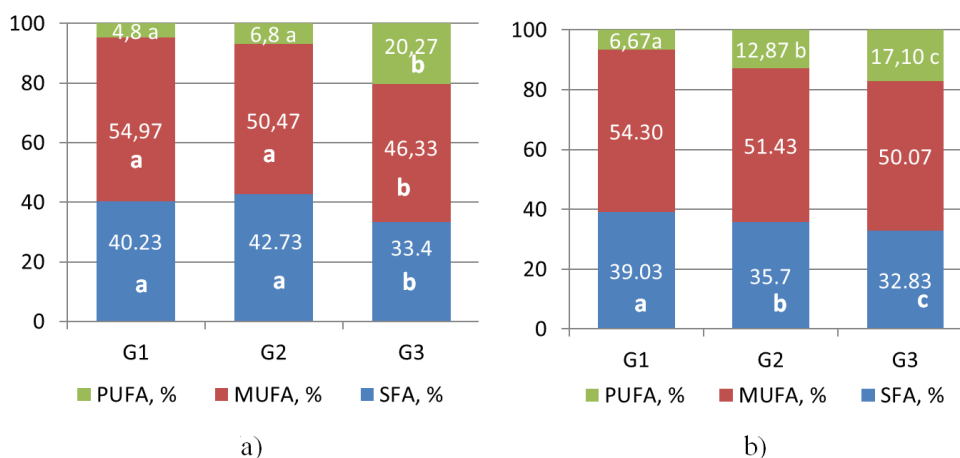


Fig. 3. Content of saturated (SFA), unsaturated (UFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids of lipids from breast fillet (a) and thigh meat (b) in broiler at 42-day of age

UFA predominated in the lipids isolated from the breast fillet and thigh meat of the control broiler group (G₁) – 59.77% in breast fillet meat and 60.97% in thigh meat. Among the UFA, the content of MUFA was higher (54.97% and 54.30%) than that of PUFA (4.80% and 6.67%).

Significant changes were noticed in the content of SFA, UFA, MUFA and PUFA in the lipids from breast fillet of broilers fed flaxseed oil supplemented diets. The amount of UFA, especially that of PUFA, increased drastically, which was due to the increased content of linoleic and linolenic acids in these samples. On the other hand, the content of SFA and MUFA decreased simultaneously with increasing the concentration of flaxseed oil in the diet.

The lipids from thigh meat of broilers in G₂ and G₃ demonstrated significant changes in the levels of SFA, UFA and PUFA, while the differences in the content of MUFA were insignificant (P > 0.05). The amount of SFA decreased with addition of flaxseed oil to the feed, while those of UFA and PUFA in particular, increased, which once again was due to the increased proportions of linoleic and linolenic acids in the lipids.

Similar results are reported by Anjum et al. (2013) who established that the amount of PUFA significantly increased in the meat lipids of broilers whose diet was supplemented with higher levels of flaxseed.

Conclusions

No statistically significant differences were demonstrated in growth performance and slaughter traits of the different groups of broiler chickens.

Feed conversion rate was substantially lower (P < 0.05)

in broilers supplemented with 1.5% (G₂) and 3.0% (G₃) flaxseed oil compared to the control birds (G₁).

There were no consistent differences with respect to meat pH and tenderness unlike the water holding capacity and some colour characteristics of the meat. The higher dietary flaxseed oil level resulted in reduction of saturated fatty acids proportion and respective increase in that of unsaturated fatty acids. The highest PUFA content was found out in the meat of G₃ birds – supplemented with 3.0% flaxseed oil; the difference vs the other two groups were statistically significant (P < 0.05).

Acknowledgments

The study is a part of the PhD thesis of Petra Ivanova supported by Grant № D-102 at Agricultural University funded by the Ministry of Education and Science, Republic of Bulgaria.

References

- Abbasi, F., Samadi, F., Jafari, S. M., Ramezanzpour, S., & Shargh, M. S. (2019). Ultrasound-assisted preparation of flaxseed oil nanoemulsions coated with alginate-whey protein for targeted delivery of omega-3 fatty acids into the lower sections of gastrointestinal tract to enrich broiler meat. *Ultrasonics Sonochemistry*, 50, 208-217.
- Abdelillah, A., Houcine, B., Halima, D., Meriem, C. S., Imane, Z., Eddine, S. D., Abdallah M. & Sari Daoudi, C. (2013). Evaluation of antifungal activity of free fatty acids methyl esters fraction isolated from Algerian *Linum usitatissimum* L. seeds against toxigenic *Aspergillus*. *Asian Pacific Journal of Tropical Biomedicine*, 3(6), 443- 448.

- Aguillón-Páez, Y. J., Romero, L. A. & Diaz, G. J.** (2020). Effect of full-fat sunflower or flaxseed seeds dietary inclusion on performance, egg yolk fatty acid profile and egg quality in laying hens. *Animal Nutrition*, 6(2), 179-184.
- Al-Khalaifah, H., Al-Nasser, A., Givens, D. I., Rymer, C. & Ya-qoob, P.** (2020). Comparison of different dietary sources of n-3 polyunsaturated fatty acids on immune response in broiler chickens. *Heliyon*, 6(1), e03326.
- Allen, P. C., Danforth, H. A. R. R. Y. & Levander, O. A.** (1997). Interaction of dietary flaxseed with coccidia infections in chickens. *Poultry Science*, 76(6), 822-827.
- Allen, P. C., Danforth, H. D. & Levander, O. A.** (1996). Diets high in n-3 fatty acids reduce cecal lesion scores in chickens infected with *Eimeria tenella*. *Poultry Science*, 75(2), 179-185.
- Anjum, F. M., Haider, M. F., Khan, M. I., Sohaib, M. & Arshad, M. S.** (2013). Impact of extruded flaxseed meal supplemented diet on growth performance, oxidative stability and quality of broiler meat and meat products. *Lipids in Health and Disease*, 12(1), 1-12.
- AOAC** – Association of Official Analytical Chemists, AOAC International, Official Methods of Analysis, 20th edition. Washington, DC, (2016).
- Barbantia, D. & Pasquini, M.** (2005). Influence of cooking conditions on cooking loss and tenderness of raw and marinated chicken breast meat. *LWT – Food Science and Technology*, 38(8), 895-901.
- Betti, M., Schneider, B. L., Wismer, W. V., Carney, V. L., Zuidhof, M. J. & Renema, R. A.** (2009 a). Omega-3-enriched broiler meat: 2. Functional properties, oxidative stability, and consumer acceptance. *Poultry Science*, 88(5), 1085-1095.
- Betti, M., Perez, T. I., Zuidhof, M. J. & Renema, R. A.** (2009 b). Omega-3-enriched broiler meat: 3. Fatty acid distribution between triacylglycerol and phospholipid classes. *Poultry Science*, 88(8), 1740-1754.
- Calder, P. C.** (2014). Very long chain omega-3 (n-3) fatty acids and human health. *European Journal of Lipid Science and Technology*, 116(10), 1280-1300.
- Choo, W. S., Birch, J. & Dufour, J. P.** (2007). Physicochemical and quality characteristics of cold-pressed flaxseed oils. *Journal of Food Composition and Analysis*, 20(3-4), 202-211.
- De Lorgeril, M., Salen, P., Laporte, F. & De Leiris, J.** (2001). Alpha-linolenic acid in the prevention and treatment of coronary heart disease. *European Heart Journal Supplements*, 3 (suppl D), D26-D32.
- Fletcher, D. L.** (2002). Poultry meat quality. *World's Poultry Science Journal*, 58(2), 131-145.
- Grashorn M. A.** (2005). Enrichment of eggs and poultry meat with biologically active substances by feed modifications and effects on the final quality of the product. *Polish Journal of Food and Nutritional Science*, (14) 1, 15-20.
- Grashorn, M. A.** (2007). Functionality of poultry meat. *Journal of Applied Poultry Research*, 16(1), 99-106.
- Grau, R., Hamm, R.A.** (1952) Simple method for the determination of water absorption in the meat. *Fleischwirtschaft*, 4, 295–297, (De).
- Guilloux, K., Gaillard, I., Courtois, J., Courtois, B. & Petit, E.** (2009). Production of arabinoxylanoligosaccharides from flaxseed (*Linum usitatissimum*). *Journal of Agricultural and Food Chemistry*, 57(23), 11308-11313.
- Harris, W. S., Poston, W. C. & Haddock, C. K.** (2007). Tissue n-3 and n-6 fatty acids and risk for coronary heart disease events. *Atherosclerosis*, 193(1), 1-10.
- Haug, A., Eich-Greatorex, S., Bernhoft, A., Wold, J. P., Hetland, H., Christophersen, O. A. & Sogn, T.** (2007). Effect of dietary selenium and omega-3 fatty acids on muscle composition and quality in broilers. *Lipids in Health and Disease*, 6(1), 1-9.
- <http://eu.aviagen.com> (Accessed 15 March 2019)
- ISO 12966-1:2014.** Animal and vegetable fats and oils – Gas chromatography of fatty acid methyl esters – Part 1: Guidelines on modern gas chromatography of fatty acid methyl esters
- ISO 12966-2:2011.** Animal and vegetable fats and oils – Gas chromatography of fatty acid methyl esters – Part 2: Preparation of methyl esters of fatty acids.
- Kaithwas, G., Mukerjee, A., Kumar, P. & Majumdar, D. K.** (2011). *Linum usitatissimum* (linseed/ flaxseed) fixed oil: antimicrobial activity and efficacy in bovine mastitis. *Inflammopharmacology*, 19(1), 45-52.
- Kanakri, K., Carragher, J., Hughes, R., Muhlhauser, B. & Gibson, R.** (2017). A reduced cost strategy for enriching chicken meat with omega-3 long chain polyunsaturated fatty acids using dietary flaxseed oil. *British Poultry Science*, 58(3), 283-289.
- Konieczka, P., Czauderna, M. & Smulikowska, S.** (2017). The enrichment of chicken meat with omega-3 fatty acids by dietary fish oil or its mixture with rapeseed or flaxseed – Effect of feeding duration: Dietary fish oil, flaxseed, and rapeseed and n-3 enriched broiler meat. *Animal Feed Science and Technology*, 223, 42-52.
- Küçükközet, A.O. & Uslu, M.** (2018). Cooking loss, tenderness, and sensory evaluation of chicken meat roasted after wrapping with edible films. *Food Science and Technology International*, 24(7), 1-9.
- Lalev, M., Hristakieva, P., Oblakova, M. & Mincheva, N.** (2021). Effect of polyunsaturated fatty acids dietary supplementation of broiler turkeys on growth performance and fatty acid content of meat. *Journal of Hygienic Engineering and Design*, 35, 48-55.
- Landau, S. & Everitt, B.** (2004). A Handbook of Statistical analyses using SPSS, Charman and Hall/CRC, London, 145.
- Lopes, D. C. N., Xavier, E. G., Santos, V. L., Goncalves, F. M., Anciuti, M. A., Roll, V. F. B., Del Pino F. A. B., Feijó, J. O. & Catalan, A. A. S.** (2013). Growth performance, carcass traits, meat chemical composition and blood serum metabolites of broiler chicken fed on diets containing flaxseed oil. *British Poultry Science*, 54(6), 780-788.
- Mridula, D., Kaur, D., Nagra, S. S., Barnwal, P., Gurumayum, S. & Singh, K. K.** (2015). Growth performance and quality characteristics of flaxseed-fed broiler chicks. *Journal of Applied Animal Research*, 43(3), 345-351.
- Olomu, J. M. & Baracos, V. E.** (1991). Influence of dietary flaxseed oil on the performance, muscle protein deposition, and fatty acid composition of broiler chicks. *Poultry science*, 70(6), 1403-1411.
- Qi, K.K., Chen, J. L., Zhao, G. P., Zheng, M. Q. & Wen, J.** (2010). Effect of dietary x6/x3 on growth performance, carcass traits, meat quality and fatty acid profiles of Beijing-you

- chicken. *Journal of Animal Physiology and Animal Nutrition*, 94, 474-485.
- Petrov, Y.** (1982). Specific features of species and breed on micro-structure of skeletal muscles during ontogenesis in productive animals DSc thesis, VIZVM, Stara Zagora, Bulgaria, (Bg).
- Rahimi, S., Kamran, A. S. & Karimi, T. M.** (2011). Omega-3 enrichment of broiler meat by using two oil seeds. *Journal of Agriculture Science and Technology*, 13, 353-365.
- Salamatdoustnobar, R., Aghdamshahriar, H. & Gorbani, A.** (2008). Enrichment of broiler meat with n-3 Polyunsaturated Fatty Acids. *Asian Journal of Animal and Veterinary Advances*, 3(2), 70-77.
- Singh, K. K., Mridula, D., Rehal, J. & Barnwal, P.** (2011). Flaxseed: a potential source of food, feed and fiber. *Critical Reviews in Food Science and Nutrition*, 51(3), 210-222.
- Xu, Y., Hall, C. & Wolf-Hall, C.** (2007). Antifungal activity stability of flaxseed protein extract using response surface methodology. *Journal of Food Science*, 73(1), 9-14.
- Zahariev, Z. & Pinkas, A.,** (1979). Methods about leading of experiments, slaughtering analysis and quality evaluation of the meat. – NAPS, Sofia, (Bg).
- Zduńczyk, Z. & Jankowski, J.** (2013). Poultry Meat as Functional Food: Modification of the Fatty Acid Profile—A Review. *Annals of Animal Science*, 13(3), 463-480.
- Zuidhof, M. J., Betti, M., Korver, D. R., Hernandez, F. I. L., Schneider, B. L., Carney, V. L. & Renema, R. A.** (2009). Omega-3- enriched broiler meat: 1. Optimization of a production system. *Poultry Science*, 88(5), 1108-1120.