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# Incorporation of DHA and EPA Fatty Acids into Broiler Meat Lipids

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**Abstract:** One of the most famous omega 3 fatty acids source is canola oil. The aim of this study was to evaluate the effects on omega 3 fatty acids include of Docosahexaenoic (DHA) and Eicosapentaenoic (EPA) content when Canola Oil (CO) was included in broiler chicks rations. Ninety one day old male broiler chicks (Ross- 308) were randomly distributed into 3 treatment: Control (0% CO), 2% CO and 4% CO for 5 week. These diets were isonitrogenous and isoenergetic were given to broiler chickens throughout a 42 days growth period. Eicosapentaenoic and docosahexaenoic acids and other fatty acids were analyzed by gas chromatography. This trial was conducted in completely randomized design. Birds were slaughtered at 56 days of age. Breast and thigh meat samples were separated and frozen at -20°C until to determine as fatty acid profile. Data was analyzed with one way ANOVA and means compared with Duncan test. Results show that using CO with high level of omega 3 fatty acids could influence fatty acid profile and improved meat quality. With increasing dietary canola oil level in diet (from 0-4 g $kg^{-1}$ diet) omega 3 contents that were significantly ($p<0.05$) increased in thigh and breast meat. N-3 fatty acids, DHA and EPA content were significant among treatment ($p<0.05$).

**Key words:** Broiler, meat, omega-3, DHA, EPA

## INTRODUCTION

There are two reasons for the increasing level of polyunsaturation in chicken meat. First, human nutritionists recommend reducing the intake of Saturated Fatty Acids (SFA) because of its relationship with the development of cardiovascular diseases (Krauss *et al*., 2001). Secondly, the use of animal fats has been reduced approximately in world, in favor of vegetable oils that are more polyunsaturated. Many authors have studied how the inclusion of different fat sources in the broiler's diet affect the proportion of Fatty Acids (FA), mainly Polyunsaturated Fatty Acids (PUFA), in meat (Salamatdoustnobar *et al*., 2007; Scaife *et al*., 1994; Hrdinka *et al*., 1996; Lo´pez-Ferrer *et al*., 1999a, b) and the amount of fat deposited by the birds (Sanz *et al*., 1999, 2000; Crespo and Esteve-Garcý´a, 2001, 2002a, b). However, there are few reports on the effect of increasing levels of dietary PUFA on the amount and type of FA deposited in chicken tissues, especially in the edible portions. An increase in the degree of polyunsaturation of meat may enhance the development of organoleptic problems (Ajuyah *et al*., 1993; Gonza´lez-Esquerra and Leeson, 2000; Bou *et al*., 2001) and lead to an increased susceptibility to lipid oxidation (Klaus *et al*., 1995; Cortinas *et al*., 2001; Grau *et al*., 2001a, b). Most of the reports do not express the FA composition of chicken meat as amount but rather as profile (percentage of total FA). The objective of this trial was to determine the rate of incorporation of dietary fatty acids from feeding a PUFA-rich diet to broiler meat lipids.

## MATERIALS AND METHODS

**Animals and diets:** Experiment was conducted of the Ross 208 strain were obtained from a commercial hatchery (90 one day old male broiler chicks) and were placed in 9 floor pens of 2×2 m with 10 birds per pen. All chicks were fed a starter diet from 0-21 d and were ad libitum access to water and feed. The experimental design consisted in a completely randomized design with 3 treatments [T1 Control (Soybean + corn), T2 (2% CO) and T3 (4% CO)] with three replication. The treatments diets of were isonitrogenous and isoenergetic. Diets were formulated by adding 0, 2 and 4% canola oil to basal diet (corn and soybean meal) that met the requirements recommended by the National Research Council (1994). The control diet, which was not enriched with canola oil and was administered throughout the 21 days of experimental period (starter). The levels of canola oil were replaced with corn in diets during 2 different periods (grower and finisher). Ingredient composition and nutrient analysis for each treatment is described in (Table 1-3). At the age of 8 weeks, all the birds were weighed before being

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Table 1: Percentage composition of experimental diet in starter period

| Ingredients | (%) |
|---|---|
| Corn | 53.50 |
| Soybean | 34.00 |
| Canola oil | 0.50 |
| Starch | 8.00 |
| Wheat bran | 0.00 |
| DL-Methionine | 0.54 |
| Lysine | 0.00 |
| DCP | 1.38 |
| Oyster | 1.33 |
| Vitamin 1 | 0.25 |
| Mineral 2 | 0.25 |
| Salt | 0.25 |
| Coccidiostat | 0.00 |
| Sand | 0.00 |
| | 100.00 |
| **Calculated nutrient content** | |
| ME kcal $kg^{-1}$ | 2920.00 |
| Crude protein (%) | 21.00 |
| Calcium (%) | 0.94 |
| Available P (%) | 0.43 |
| ME/CP | 139.70 |
| Ca/P | 2.10 |

[1]: Vitamin content of diets provided per kilogram of diet: vitamin A,D, E and K, [2]: Composition of mineral premix provided as follows $kg^{-1}$ of premix: Mn, 120,000mg; Zn, 80,000 mg; Fe, 90,000 mg; Cu, 15,000 mg; I, 1,600 mg; Se, 500 mg; Co, 600 mg

Table 2: Percentage composition of experimental diets in growth period

| | Experimental diets | | |
|---|---|---|---|
| Ingredients | T1[3] | T2 | T3 |
| Corn | 64 | 60 | 55 |
| Soybean | 27.4 | 28 | 27.1 |
| Canola oil | 0 | 2 | 4 |
| Starch | 3.74 | 2.06 | 1.22 |
| Wheat bran | 1 | 2 | 5.5 |
| DL-Methionine | 0 | 0 | 0 |
| Lysine | 0 | 0 | 0 |
| DCP | 1.13 | 1.14 | 1.16 |
| Oyster | 1.5 | 1.48 | 1.46 |
| Vitamin[1] | 0.25 | 0.25 | 0.25 |
| Mineral[2] | 0.25 | 0.25 | 0.25 |
| Salt | 0.25 | 0.25 | 0.25 |
| Coccidiostat | 0.15 | 0.15 | 0.15 |
| Sand | 0.33 | 2.42 | 3.66 |
| | 100 | 100 | 100 |
| **Calculated nutrient content** | | | |
| ME kcal $kg^{-1}$ | 2920 | 2920 | 2920 |
| Crude protein (%) | 18.2 | 18.2 | 18.2 |
| Calcium (%) | 0.9 | 0.9 | 0.9 |
| Available P (%) | 0.35 | 0.35 | 0.35 |
| ME/CP | 160.1 | 160.8 | 160.7 |
| Ca/P | 2.5 | 2.5 | 2.5 |

[1]: Vitamin content of diets provided per kilogram of diet: vitamin A, D, E and K, [2]: Composition of mineral premix provided as follows $kg^{-}$ of premix: Mn, 120,000mg; Zn, 80,000 mg; Fe, 90,000 mg; Cu, 15,000 mg; I, 1,600 mg; Se, 500 mg; Co, 600 mg.3 T1 = 0%
[3]: T1= 0 % canola oil (CO); T2= 2%CO; T3=4% CO

slaughtered and then eviscerated. Weights air-chilled carcasses after cutting off their heads and feet and after removing abdominal fat (considered as the fat extending within the ischium, surrounding the cloaca and adjacent to the abdominal muscle) to obtain ready-to-cook carcasses were recorded.

Table 3: Percentage composition of experimental diets in finisher period

| | Experimental diets | | |
|---|---|---|---|
| Ingredients | T1[3] | T2 | T3 |
| Corn | 66.5 | 57.5 | 56 |
| Soybean | 24.1 | 25.85 | 24 |
| Canola oil | 0 | 2 | 4 |
| Starch | 3.81 | 4.34 | 1.94 |
| Wheat bran | 0 | 5 | 6 |
| DL-Methionine | 0.44 | 0.45 | 0.45 |
| Lysine | 0.043 | 0.015 | 0.08 |
| DCP | 0.89 | 0.92 | 0.89 |
| Oyster | 1.38 | 1.36 | 1.31 |
| Vitamin[1] | 0.25 | 0.25 | 0.25 |
| Mineral[2] | 0.25 | 0.25 | 0.25 |
| Salt | 0.25 | 0.25 | 0.25 |
| Coccidiostat | 0.15 | 0.15 | 0.15 |
| Sand | 1.937 | 1.665 | 4.43 |
| | 100 | 100 | 100 |
| **Calculated nutrient content** | | | |
| ME kcal $kg^{-1}$ | 2920 | 2920 | 2920 |
| Crude protein (%) | 16.5 | 16.4 | 16.5 |
| Calcium (%) | 0.79 | 0.79 | 0.77 |
| Available P (%) | 0.3 | 0.3 | 0.3 |
| ME/CP | 176.8 | 177.4 | 176.6 |
| Ca/P | 2.6 | 2.6 | 2.6 |

[1]: Vitamin content of diets provided per kilogram of diet: vitamin A, D, E and K, [2]: Composition of mineral premix provided as follows $kg^{-1}$ of premix: Mn, 120,000mg; Zn, 80,000 mg; Fe, 90,000 mg; Cu, 15,000 mg; I, 1,600 mg; Se, 500 mg; Co, 600 mg.3 T1 = 0%
[3]: T1= 0 % canola oil (CO); T2= 2%CO; T3 = 4% CO

In order to reduce variation in the cutting procedure, all dissections were carried out by one operator. After weighing the eviscerated carcass, it was apportioned into commercial cuts as back, 2 leg-thigh, 2 wings and breast (Hudspeth *et al*., 1973; Orr *et al*., 1984). Breast was obtained after removing wings by cutting through the shoulder joint at the proximal end of humerus and by cutting through the ribs, thereby separating the breast from the back (excluding skin). The resulting cut pieces (breast meat, wings and thighs with drumsticks) were then weighed. After quartering, breasts and thighs were separated and frozen at -20°C until to determine as fatty acids profile. The lipid composition was determined by gas chromatography (Model 6890N American Technologies Agilent). The composition of meat samples fatty acid of supplemented lipids s (Table 4-7) data were statistically analyzed using one-way ANOVA and means with significant F ratio were compared by Duncan multiple range test.

**Gas chromatography of fatty acids methyl esters**

**Sample preparation**

**Fatty acids:** Total lipid was extracted from breast and thigh according to the method of Folch *et al*. (1957). Approximately 0.5 g of meat weighed into a test tube with 20 mL of (chloroform: methanol = 2:1, vol/vol) and homogenized with a polytroon for 5-10 sec at high speed. The BHA dissolved in 98% ethanol added prior to

Table 4: Least square means for fatty acid profiles in broilers breast meat fed canola oil

| | Treatments | | | | |
|---|---|---|---|---|---|
| | T1 | T2 | T3 | SEM | p>F |
| C14:0 | 0.59$^{a}$ | 0.51$^{a}$ | 0.54$^{a}$ | 0.016883 | 0.1101 |
| C14:1n5 | 0.11$^{b}$ | 0.10$^{b}$ | 0.54$^{a}$ | 0.009156 | <0.0001 |
| C16:0 | 28.50$^{a}$ | 27.01$^{a}$ | 22.71$^{b}$ | 0.765465 | 0.0262 |
| C16:1n7 | 6.47$^{a}$ | 6.60$^{a}$ | 5.26$^{b}$ | 0.176499 | 0.0218 |
| C18:0 | 6.60$^{a}$ | 6.21$^{a}$ | 6.34$^{a}$ | 0.18724 | 0.4380 |
| C18:1n9 | 33.65$^{a}$ | 30.00$^{a}$ | 30.81$^{a}$ | 0.920539 | 0.1299 |
| C18:1n7 | 2.40$^{b}$ | 2.93$^{a}$ | 2.73$^{ab}$ | 0.078816 | 0.0379 |
| C18:2n6cis | 15.35$^{a}$ | 13.53$^{ab}$ | 12.33$^{b}$ | 0.404577 | 0.0295 |
| C18:3n3 | 0.72$^{b}$ | 0.75$^{b}$ | 0.87$^{a}$ | 0.02186 | 0.0295 |
| C20:0 | 0.75$^{a}$ | 0.24$^{b}$ | 0.23$^{b}$ | 0.013268 | 0.0002 |
| C20:5n3 | 0.37$^{c}$ | 1.18$^{b}$ | 2.03$^{a}$ | 0.040638 | 0.0002 |
| C20:1n9 | 0.17$^{c}$ | 0.23$^{b}$ | 0.31$^{a}$ | 0.007092 | 0.002 |
| C22:6n3 | 0.61$^{b}$ | 0.62$^{b}$ | 0.75$^{a}$ | 0.01854 | 0.0228 |
| C22:0 | 0.93$^{b}$ | 1.96$^{a}$ | 1.93$^{a}$ | 0.050233 | 0.0011 |

Table 5: Least square means for fatty acid profiles in broilers thigh meat fed canola oil

| | Treatments | | | | |
|---|---|---|---|---|---|
| | T1 | T2 | T3 | SEM | p>F |
| C14:0 | 0.60$^{a}$ | 0.14$^{c}$ | 0.17$^{b}$ | 0.0122 | 0.0002 |
| C14:1n5 | 0.195$^{c}$ | 0.87$^{a}$ | 0.47$^{b}$ | 0.0171 | 0.0002 |
| C16:0 | 26.21$^{a}$ | 22.37$^{b}$ | 21.88$^{b}$ | 0.6878 | 0.0370 |
| C16:1n7 | 6.20$^{b}$ | 7.83$^{a}$ | 6.17$^{b}$ | 0.1973 | 0.0149 |
| C18:0 | 8.280$^{b}$ | 8.96$^{ab}$ | 10.07 $^{a}$ | 0.2667 | 0.0393 |
| C18:1n9 | 35.32$^{a}$ | 37.25$^{a}$ | 35.76$^{a}$ | 1.0534 | 0.4686 |
| C18:1n7 | 2.52$^{a}$ | 2.48$^{a}$ | 2.27$^{a}$ | 0.0703 | 0.1453 |
| C18:2n6cis | 13.14$^{a}$ | 11.53$^{b}$ | 12.0$^{ab}$ | 0.3565 | 0.1002 |
| C18:3n3 | 0.52$^{b}$ | 0.66$^{a}$ | 0.74$^{a}$ | 0.0185 | 0.0085 |
| C20:0 | 0.81a | 0.54$^{c}$ | 0.63$^{b}$ | 0.0185 | 0.0041 |
| C20:5n3 | 0.34$^{c}$ | 1.43$^{b}$ | 2.36$^{a}$ | 0.0461 | 0.0002 |
| C20:1n9 | 0.12$^{b}$ | 0.21$^{a}$ | 0.14$^{b}$ | 0.0041 | 0.0013 |
| C22:6n3 | 0.25$^{b}$ | 0.50$^{a}$ | 0.57$^{a}$ | 0.0126 | 0.0016 |
| C22:0 | 0.76$^{b}$ | 1.94$^{a}$ | 1.96$^{a}$ | 0.04656 | 0.0005 |

Table 6: Least square means for different traits in broilers breast meat fed canola oil

| | Treatments | | | | |
|---|---|---|---|---|---|
| | T1 | T2 | T3 | SEM | p>F |
| Satur f.a | 37.37$^{a}$ | 35.94$^{ab}$ | 31.76$^{b}$ | 0.96900 | 0.0534 |
| MUFA | 42.80$^{a}$ | 39.87$^{a}$ | 39.65$^{a}$ | 0.80700 | 0.1166 |
| PUFA | 16.33$^{a}$ | 15.33$^{a}$ | 15.11$^{a}$ | 0.39000 | 0.2061 |
| Total n-6 | 15.35$^{a}$ | 13.53$^{b}$ | 12.33$^{b}$ | 0.40300 | 0.0292 |
| Total n-3 | 1.70$^{c}$ | 2.55$^{b}$ | 3.66$^{a}$ | 0.04300 | 0.0002 |
| EPA | 0.37$^{c}$ | 1.18$^{b}$ | 2.03$^{a}$ | 0.040638 | 0.0002 |
| DHA | 0.61$^{b}$ | 0.62$^{b}$ | 0.75$^{a}$ | 0.01854 | 0.0228 |

Table 7: Least square means for different traits in broilers thigh meat fed canola oil

| | Treatments | | | | |
|---|---|---|---|---|---|
| | T1 | T2 | T3 | SEM | p>F |
| Satur f.a | 36.65$^{a}$ | 33.94$^{a}$ | 34.73$^{a}$ | 0.970 | 0.2709 |
| MUFA | 44.28$^{b}$ | 48.62$^{a}$ | 44.79$^{ab}$ | 0.910 | 0.0761 |
| PUFA | 13.74$^{a}$ | 13.45$^{a}$ | 14.81$^{a}$ | 0.330 | 0.1163 |
| Total n-6 | 13.14$^{a}$ | 11.52$^{b}$ | 12.00$^{ab}$ | 0.360 | 0.1008 |
| Total n-3 | 1.12$^{c}$ | 2.58$^{b}$ | 3.56$^{a}$ | 0.053 | 0.0001 |
| EPA | 0.34$^{c}$ | 1.43$^{b}$ | 2.36$^{a}$ | 0.0461 | 0.0002 |
| DHA | 0.25$^{b}$ | 0.50$^{a}$ | 0.57$^{a}$ | 0.0126 | 0.0016 |

homogenization. The homogenate filtered through a Whatman filter paper into a 100 mL graduated cylinder and 5 mL of 0.88% sodium chloride solution added, stopper and mixed. After phase separation, the volume of lipid layer recorded and the top layer completely siphoned off. The total lipids converted to Fatty Acid Methyl Esters (FAME) using a mixture of boron-trifluoride, hexane and methanol (35:20:45, vol/vol/vol). The FAME separated and quantified by an automated gas chromatography equipped with auto sampler and flame ionization detectors, using a 30 m 0.25 mm inside diameter fused silica capillary column, as described. A (Model 6890N American Technologies Agilent) (USA) Gas chromatography used to integrate peak areas. The calibration and identification of fatty acid peak carried out by comparison with retention times of known authentic standards. The fatty acid results form gas chromatography with Chem Station software analyzed and expressed as weight percentages.

**Statistical analyses:** Data were analyzed in a complete randomized design using the GLM procedure of SAS version 12 (SAS Inst. Inc., Cary, NC).

$$y_{ij} = \mu + a_i + \varepsilon_{ij}$$

Where:

$y_{ij}$ = All dependent variable
$\mu$ = Overall mean
$a_i$ = The fixed effect of oil levels (i = 1, 2, 3)
$\varepsilon_{ij}$ = The random effect of residual

Duncan multiple range test used to compare means.

## RESULTS

We used various rations with different levels of canola oil to study of it effects on the meat fatty acid profiles. Results of breast meat samples quality parameters (Table 4-7). With increasing levels of dietary polyunsaturation caused the higher accumulation of n-3 (C18:2n6cis, C18:3n3, C20:5n3 and C22:6n3) in thigh and breast meat. The n-3 fatty acids content in thigh and breast meat was significantly affected by canola oil and treatment with 4% CO (T3) was higher level of PUFA fatty acids. According to results with increased canola oil level in all experimental diets, content of omega 6 fatty acids have decreased. T3 and T2 treatment was the lowest omega 6 fatty acids in breast and thigh meat respectively. Result show that the omega 36 fatty acids contents in breast meat was higher than thigh meat. The content of omega 3 fatty acid in T2, T3 (C18:3n3, C20: 5n3 and C22:6n3) for breast and thigh meat is higher than T1

(control) treatment and was significant ($p<0.0001$). EPA and DHA fatty acids content of breast and thigh meat samples significantly affected by dietary polyunsaturation level and were significant ($p<0.002$) (Table 4). Comparisons show that T3 treatment (4% CO) with 2.03 and 0.75% for breast meat and 2.36 and 0.57% of thigh total fatty acids is best treatment.

## DISCUSSION

Depending on dietary polyunsaturation level, EPA and DHA proportions changed for breast and thigh meat samples from 0.37-2.03% and 0.61-0.75% in breast and 0.34-2.36% and 0.25-57% in thigh respectively. Thus, increasing the level of dietary polyunsaturation caused an increase in the accumulation of PUFA in thigh and breast meat lipids, which was according to Sanz *et al.* (1999), results. Furthermore, both tissues accumulated a similar proportion of total omega 3 fatty acids (C18:3n3, C20:5n3, C22:6n3), respectively 1.70, 1.12 and 2.55, 2.58 and 3.66, 3.56 for breast and thigh meat samples. There are contradictory reports on fatty acids content in breast. Some authors showed that the dietary polyunsaturation level of fat does not influence intramuscular lipid content of breast (Scaife *et al.*, 1994; Crespo and Esteve-Garcý´a, 2001),but Kirchgessner *et al.* (1993) and Ajuyah *et al.* (1991) found a higher fat content in breast muscle with increasing levels of PUFA in the diet that according with this research finding. However, other authors found lower lipid content of breast of chickens fed diets enriched with polyunsaturated oils (Sanz *et al.*, 1999). Such discrepant findings in intramuscular fat content of breast muscles may be attributed to several factors, such as the analytical procedure used to extract fat from samples. Recent studies showed that fat content of tissues in more polyunsaturated treatments was underestimated when lipid contents were analyzed using AOAC (1995) methodology, suggesting total FA content as an estimator of crude fat in highly polyunsaturated samples (Villaverde *et al.*, 2003b). In general, modification of FA composition of intramuscular fat seems to be more limited (Pan and Storlien, 1993; Lo´pez-Bote *et al.*, 1997). It may be due to the fact that FA in intramuscular fat are used mainly as components of cellular membranes and the cell has to maintain its physical characteristics to ensure fluidity and permeability of different compounds. As expected, when the dietary polyunsaturation level increased, PUFA content in the tissues also increased (Table 4-7). EPA and DHA contents in breast and thigh samples with increased canola oil from, 0-4% were affected and those differences were 1.66 and 0.14 for breast and 2.02 and 0.32 is for thigh. In this research T3 with 4% canola oil is best treatment and results show that canola oil could influence meat quality and DHA and EPA contents in broiler meat. (Hulan *et al.*, 1988; Lo´pez-Ferrer *et al.*, 1999a; Gonza´lez Esquerra and Leeson, 2000; Crespo and Esteve-Garcý´a, 2001).

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