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Enrichment of Broiler Meat with n-3 Polyunsaturated Fatty Acids

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Abstract: One hundred twenty male broiler chickens were randomly distributed into three experimental treatments to determine of the amount Canola Oil (CO) fatty acids deposited in raw chicken tissues. These diets were isonitrogenous and isoenergetic were given to broiler chickens throughout a 42 day growth period. This trial was conducted in 3×3 factorial experiment. Birds were slaughtered at 56 days of age. After weighing the eviscerated carcass was apportioned into commercial cuts (back, two leg-thigh, two wings and breast). 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 ω-3 fatty acids could influence fatty acid profile and improved meat quality. Means of fatty acids percent for meat samples showed the quality of fatty acid composition improved with increase CO. The increase in dietary canola oil (from 0 to 4 g kg^{-1} diet) resulted in levels of ω-3 that approximately were 2 times higher in thigh and breast meat. Total n-3 content of breast and thigh meat significantly ($p<0.05$) was affected by canola oil in the diet and that contents were 1.70 and 1.12% for control group reached to 3.66 and 3.56% in T3 (4% CO) in breast and thigh, respectively.

Key words: Broiler, breast, thigh, fatty acid, ω-3

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 researchers 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 (Scaife *et al*., 1994; Hrdinka *et al*., 1996; Lopez-Ferrer *et al*., 1999a, b) the amount of fat deposited by the birds (Sanz *et al*., 1999, 2000; Crespo and Esteve-Garcýa, 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). For a healthy diet, there should be a consumption of canola oil and some vegetable oils. For this reason, numerous research activities have been devoted to increasing the levels of these Fatty Acids (FA) in widely consumed products of animal origin whose lipid composition is easily modified (Hargis *et al*., 1991; Huyghebaert *et al*., 1995). Specifically, the use of vegetable oils in the diet of poultry makes it possible to increase the level of long-chain n-3 FA in meat (Chanmugam *et al*., 1992; Pinchasov *et al*., 1992).

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Vegetable sources, such as canola oil and Linseed Oils (LO), may clearly increase the n-3 FA content in the form of Linolenic Acid (LNA), the precursor of the whole n-3 family. To enhance the conversion to longer-chain n-3 FA from their precursors and to increase the nutritional quality of poultry meat.

MATERIALS AND METHODS

Animals and Diets

The experiment was conducted of which Ross 208 strain were obtained from a commercial hatchery (270 one day old male broiler chicks) and were placed in 12 floor pens of 2×2 meters with 10 birds per pen. All chicks were fed a starter diet from 0 to 21 day and were ad libitum access to water and feed. The experimental design consisted in a completely randomized design with 3 treatments [T_1 Control (Soybean +corn), T_2 (2% CO) and T_3 (4% CO)] with four 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 two different periods (grower and finisher). Ingredient composition and nutrient analysis for each treatment is described in Table 1-3.

At the age of 8 week, all the birds were weighed before being 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. 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, two leg-thigh, two 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

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
Total	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 per kilogram 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	T_1[3]	T_2	T_3
Corn	6.04	60.00	55.00
soybean	27.40	28.00	27.10
Canola oil	0.00	2.00	4.00
Starch	3.74	2.06	1.22
Wheat bran	1.00	2.00	5.50
DL-Methionine	0.00	0.00	0.00
Lysine	0.00	0.00	0.00
DCP	1.13	1.14	1.16
Oyster	1.50	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
Total	100.00	100.00	100.00
Calculated nutrient content			
ME kcal/kg	2920.00	2920.00	2920.00
Crude protein (%)	18.20	18.20	18.20
Calcium (%)	0.90	0.90	0.90
Available P (%)	0.35	0.35	0.35
ME/CP	160.10	160.80	160.70
Ca/P	2.50	2.50	2.50

1: Vitamin content of diets provided per kilogram of diet: vitamin A, D, E and K; 2: Composition of mineral premix provided as follows per kilogram 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: T_1 = 0% canola oil (CO); T_2 = 2%CO; T_3 = 4% CO

Table 3: Percentage composition of experimental diets in finisher period

	Experimental diets		
Ingredients	T_1[3]	T_2	T_3
Corn	66.500	57.500	56.00
soybean	24.100	25.850	24.00
Canola oil	0.000	2.000	4.00
Starch	3.810	4.340	1.94
Wheat bran	0.000	5.000	6.00
DL-Methionine	0.440	0.450	0.45
Lysine	0.043	0.015	0.08
DCP	0.890	0.920	0.89
Oyster	1.380	1.360	1.31
Vitamin1	0.250	0.250	0.25
Mineral2	0.250	0.250	0.25
Salt	0.250	0.250	0.25
Coccidiostat	0.150	0.150	0.15
Sand	1.937	1.665	4.43
Total	100.000	100.000	100.00
Calculated nutrient content			
ME kcal kg^{-1}	2920.000	2920.000	2920.00
Crude protein (%)	16.500	16.400	16.50
Calcium (%)	0.790	0.790	0.77
Available P (%)	0.300	0.300	0.30
ME/CP	176.800	177.400	176.60
Ca/P	2.600	2.600	2.60

1: Vitamin content of diets provided per kilogram of diet: vitamin A, D, E and K; 2: Composition of mineral premix provided as follows per kilogram 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: T_1 = 0% canola oil (CO); T_2 = 2%CO; T_3 = 4% CO

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 is shown in 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 for Fatty Acids Determine

Breast and thigh weight was expressed as percentage of the carcass weight. 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

Table 4: Least square means for fatty acid profiles in broilers breast meat without skin fed canola oil[1]

	Treatments				
Parameters	T_1	T_2	T_3	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}	0.187240	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}	0.021860	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	0.0020
C22:6n3	0.61^{b}	0.62^{b}	0.75^{a}	0.018540	0.0228
C22:0	0.93^{b}	1.96^{a}	1.93^{a}	0.050233	0.0011

1: Values given in this table are means of 3 dietary treatments with different levels of canola oil

Table 5: Least square means for fatty acid profiles in broilers thigh meat without skin fed canola oil[1]

	Treatments				
Parameters	T_1	T_2	T_3	SEM	P>F
C14:0	0.60^{a}	0.14^{c}	0.17^{b}	0.01220	0.0002
C14:1n5	0.195^{c}	0.87^{a}	0.47^{b}	0.01710	0.0002
C16:0	26.21^{a}	22.37^{b}	21.88^{b}	0.68780	0.0370
C16:1n7	6.20^{b}	7.83^{a}	6.17^{b}	0.19730	0.0149
C18:0	8.280^{b}	8.96^{ab}	10.07^{a}	0.26670	0.0393
C18:1n9	35.32^{a}	37.25^{a}	35.76^{a}	1.05340	0.4686
C18:1n7	2.52^{a}	2.48^{a}	2.27^{a}	0.07030	0.1453
C18:2n6cis	13.14^{a}	11.53^{b}	12.0^{ab}	0.35650	0.1002
C18:3n3	0.52^{b}	0.66^{a}	0.74^{a}	0.01850	0.0085
C20:0	0.81^{a}	0.54^{c}	0.63^{b}	0.01850	0.0041
C20:5n3	0.34^{c}	1.43^{b}	2.36^{a}	0.04610	0.0002
C20:1n9	0.12^{b}	0.21^{a}	0.14^{b}	0.00410	0.0013
C22:6n3	0.25^{b}	0.50^{a}	0.47^{a}	0.01260	0.0016
C22:0	0.76^{b}	1.94^{a}	1.96^{a}	0.04656	0.0005

1: Values given in this table are means of 3 dietary treatments with different levels of canola oil

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

	Treatments				
Parameters	T_1	T_2	T_3	SEM	P>F
Satur f.a	37.37^{a}	35.94^{ab}	31.76^{b}	0.969	0.0534
MUFA	42.80^{a}	39.87^{a}	39.65^{a}	0.807	0.1166
PUFA	16.33^{a}	15.33^{a}	15.11^{a}	0.390	0.2061
Total n-6	15.35^{a}	13.53^{b}	12.33^{b}	0.403	0.0292
Total n-3	1.70^{c}	2.55^{b}	3.66^{a}	0.043	0.0002

Values in the same row with no common superscript are significantly different; Satur f.a; saturated fatty acids, MUFA; Monounsaturated fatty acid, PUFA; Polyunsaturated fatty acid

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

	Treatments				
Parameters	T_1	T_2	T_3	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

Values in the same row with no common superscript are significantly different; Satur f.a; Saturated fatty acids, MUFA; Monounsaturated fatty acid, PUFA; Polyunsaturated fatty acid

with a polytroon for 5 to 10 sec at high speed. The BHA dissolved in 98% ethanol added prior to 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 previously. A (Model 6890 N 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

μ = Overall mean

a_i = The fixed effect of oil levels ($^{i = 1, 2, 3}$)

ε_{ij} = The random effect of residual

Duncan multiple range test used to compare means.

RESULTS

Fatty acids of thigh and breast meat with out skin were modified by dietary polyunsaturation level. Total ω-3 FA content in breast and thigh meat, were significantly ($p>0.01$) increased (from 1.70% for control group to 3.66% T_3 and 1.12% for control group to 3.56% for T_3, respectively) with usage canola oil in diets. (Table 6 and 7). Recent studies showed that fatty acids content of this tissues influence by usage canola oil in diets and C18:3n3, C20:5n3, C20:1n9 and C22:6n3 contents in T_3 significantly ($p<0.05$) was higher(Table 4 and 5). As expected, when the dietary polyunsaturation level increased, PUFA content in the tissues also increased (Table 6 and 7). The increased dietary canola oil (from 0 to 4 g kg^{-1} diet) resulted in levels of ω-3 fatty acids that approximately were 2 times higher in thigh and breast meat and that was significant ($p<0.05$). However, the Saturated FA and MUFA contents of thigh and breast were reduced as the dietary degree of polyunsaturation increased and for thigh meat this condition for myristic acid (C14:0) and stearic

acid not significant but for palmitic acid (C16:0), arachidic acid (C20:0) and behenic acid(C22:0) was significant ($p<0.05$) also for breast meat the contents C14:0, C16:0, C18:0, C20:0 and C22:0 all was significantly ($p<0.05$) reduce in T_2 and T_3. This reduction was more marked in saturated FA (1.92 and 5.61% in thigh and breast, respectively, between control group and T_3). Increasing the level of dietary polyunsaturation caused an increase in the accumulation of PUFA in thigh and breast. Results showed approximately same content of PUFA in breast and thigh respectively 15.11 and 14.81% but not significant (Hulan *et al*., 1988; Lopez-Ferrer *et al*., 1999a; Gonza´lez-Esquerra and Leeson, 2000; Crespo and Esteve-Garcýa, 2001). The relationship between dietary PUFA content and the content of the different families of FA in chicken tissues supports the idea that the FA composition in chicken tissues is a combination of direct deposition from dietary FA and endogenous fat synthesis.

DISCUSSION

In the percent study, effects of different levels of canola oils for enrichment of breast and thigh meat were investigated. This study suggests that ω-3 FA content in breast and thigh meat increased and reached 1.70 and 1.12% to 3.66 and 3.56%, respectively. A similar difference in thigh and breast content was reported by Lopez-Ferrer *et al*. (1999b and 2001) who observed increases of 2.7 and 2.3% in thigh proportion when fish oil replaced rapeseed oil or tallow, respectively. Other authors did not find any differences in thigh proportion between chickens fed tallow or different vegetable oils with a lower content of very long chain ω-3 PUFA (Olomu and Baracos, 1991; Crespo and Esteve-Garcýa, 2001).These results agree with other studies showing that separable fat depots (Crespo and Esteve-Garcýa, 2002a) and specifically abdominal fat (Vila and Esteve-Garcýa, 1996; Sanz *et al*., 1999; Crespo and Esteve-Garcýa, 2001, 2002a, b; Villaverde *et al*., 2003a), are reduced with the addition of unsaturated oils to the diets. It seems that fat deposits may easily be influenced by the polyunsaturation level of the diet. The mechanism by which dietary polyunsaturation modifies body fat deposition is not completely understood. Some authors have suggested that the lower fat deposition in broilers fed polyunsaturated fats compared with those fed saturated fats was, in part, explained by an increased rate of lipid catabolism and by a decrease of FA synthesis (Sanz *et al*., 2000). Similarly, other authors reported significantly lower metabolic oxidation of lipids and consequently a lower thermogenesis in tissues of rats fed saturated fats than in rats fed unsaturated fats (Shimomura *et al*., 1990; Wilson *et al*., 1990). Some authors showed that the dietary polyunsaturation level of oils does not influence intramuscular fatty acids content of breast and thigh (Scaife *et al*., 1994; Crespo and Esteve-Garcýa, 2001), but Kirchgessner *et al*. (1993) and Ajuyah *et al*. (1991) found a higher lipid content in breast and thigh muscle with increasing levels of PUFA in the diet. However, other authors found lower lipid content of breast and thigh of chickens fed diets enriched with unsaturated oils (Sanz *et al*., 1999). Similar to the results of the percent study and such discrepant findings in intramuscular fat content of breast and thigh muscles may be attributed to several factors, such as the analytical procedure used to extract fat from samples. In general, modification of FA composition of intramuscular fat seems to be more limited (Pan and Storlien, 1993; Lopez-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. Few differences in this tissues PUFA profile could be attributed to different roles of FA in these tissues or to their different phospholipid contents. The PUFA are preferentially incorporated into phospholipids (Hulan *et al*., 1988) and phospholipids are in a higher proportion in breast than in thigh muscles (Ratnayake *et al*., 1989). These results agree with other authors who observed that the PUFA content in chicken tissues depends more on the variation in dietary FA (Lopez-Ferrer *et al*., 2001). Results of the percent study conclude that a 4 percent canola oil an provide optimal performance according to ω-3 fatty acids in breast and thigh meat and could usage this oil in broiler chicks diets and could replace with energetic ingredients in diet with any problems.

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