Effect of Fatty Acid Saturation in Broiler Diets on Abdominal Fat and Breast Muscle Fatty Acid Composition and Susceptibility to Lipid Oxidation

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ABSTRACT A total of 600 1-d-old Hybro N broiler chicks were randomly distributed into 24 litter pens and fed from 21 to 52 d of age isocaloric and isonitrogenous diets containing 8% of three different fat sources (tallow, lard or sunflower oil). Dietary treatment produced a significant effect ($P < 0.05$) in fatty acid composition of the intramuscular neutral lipids among all dietary treatments and for all lipid classes. A similar range of variation was obtained for abdominal fat fatty acid composition. In the intramuscular polar lipid fraction, dietary fat led to a narrow range in the variation of poly- and monounsaturated fatty acid composition, while the concentration of saturated fatty acids was maintained constant in all groups. A wide range in the melting point temperature of abdominal fat was observed (mean values of dietary treatments from 8.49 to 30.91 C), which indicates a major effect of dietary fatty acids on fat consistency. The variation of breast meat oxidation was small (mean values from 7.68 to 9.76 nmol malonaldehyde/mg protein). A dietary fat source rich in linoleic acid produces a marked effect on fat consistency, but only a moderately higher susceptibility to lipid oxidation of meat compared to tallow or lard.

(*Key words*: dietary fat, broiler, fatty acid, oxidation)

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INTRODUCTION

Fats are frequently included in broiler diets to increase the energy density. Several experiments have shown that an increase in energy concentration produces a decrease in feed intake but does not negatively affect daily gain, resulting in an improvement in feed efficiency (Hulan *et al*., 1984, Pinchasov and Nir, 1992). However, fat inclusion in broiler diets must take into account the effect on carcass fat quality, because dietary fatty acids are incorporated with little change into body fat (Olomu and Baracos, 1991; Scaife *et al*., 1994). Previous research indicates a marked effect of dietary fat on abdominal fat characteristics in broilers (Sklan and Ayal, 1989; Yau *et al*., 1991), but there is limited information on the effect of dietary fat on intramuscular fatty acids of neutral (mostly triglycerides) and polar (mostly phospholipids) lipids. Diets containing a relatively high concentration of linoleic acid ($C_{18:2}$) have been negatively associated with soft fat tissues and high susceptibility of meats to oxidation (Zollitsh *et al*., 1997; Lopez-Bote *et al*., 1997). However, the actual importance and the recommended limits of dietary linoleic acid in broiler feeding have not been clearly established, particularly in the relationship of susceptibility of meats to oxidation.

This research was conducted to assess the effect of three dietary fat sources that differ in fatty acid profile (tallow, lard, and sunflower oil) on muscle neutral and polar fatty acid composition, fat consistency, and susceptibility of meat to oxidation in broilers.

MATERIALS AND METHODS

Animals and Diets

A total of 600 1-d-old (300 male and 300 female) Hybro N broiler chicks were randomly distributed, by sex, into 24 litter pens and fed a standard broiler diet for 3 wk. At 21 d of age, the number of birds per pen were equalized to 20. The experimental arrangement consisted of a 2 × 3 factorial design (two sexes and three dietary fat sources) with four pens per sex and dietary treatment. From 21 to 52 d of age, birds were fed isocaloric and isonitrogenous diets containing 8% of three different fat sources (tallow, lard, or sunflower oil) (NRC, 1994). In addition, a total of eight birds per dietary treatment were placed in individual metabolic cages at 28 d of age. Metabolizable energy of the diets was determined following the European reference method (Bourdillon *et al*., 1990). Ingredients and chemical composition of diets are shown in Table 1.

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TABLE 1. Ingredients and analyzed nutrient content of broiler diets enriched in tallow, lard, or sunflower oil

Ingredients and content	Dietary added fat type		
	Tallow	Lard	Sunflower oil
	(g/kg)		
Ingredients			
Wheat	581	482	459
Barley	. . .	50	50
Soybean meal 44	228	238	239
Full-fat soybean	64	64	64
Wheat bran	. . .	20	36
Tallow	80	. . .	. . .
Lard	. . .	80	. . .
Sunflower oil	. . .	. . .	80
Sodium chloride	3	3	3
Calcium carbonate	8	14	14
Dicalcium phosphate	14	14	15
Lysine complement (20%)[1]	5	4	5
Methionine complement (20%)[2]	8	8	9
Sepiolite[3]	. . .	14	20
Vitamin mineral premix[4]	8	8	8
Analyzed nutrient content			
AME_n, kcal/kg	3,020	3,020	3,020
Crude protein	178	184	180
Ether extract	101	102	101

[1]80% carrier + 20% L-Lysine.

[2]80% carrier + 20% D-L Methionine.

[3]Hydrated magnesium trisilicate; $Si_{12}Mg_8O_{30}(4OH)(4H_2O)\cdot(8H_2O)$.

[4]Vitamin mineral premix provided (per kilogram of diet) = vitamin A, 7,500 IU; cholecalciferol, 1,500 IU; vitamin E, 7.52 mg; vitamin B_2, 5.28 mg; pantothenic acid, 8 mg; vitamin B_6, 1.84 mg; folic acid, 0.5 mg; vitamin B_{12}, 0.0125 mg; choline, 350 mg; Se, 0.15 mg; I, 1.9 mg; Co, 0.2 mg; Cu, 6 mg; Fe, 30.8 mg; Zn, 50 mg; Mn 80 mg; S, 232 mg.

Slaughter and Sample Collection

Broilers were stunned, slaughtered and bled at a local slaughter house. Two birds per pen (eight birds per sex and dietary treatment) were taken for analysis. The abdominal adipose tissue (from the proventriculus surrounding the gizzard down to the cloaca) and breast meat from each broilers were taken at slaughter. Lipid oxidation studies were carried out immediately after slaughter.

Chemical Analysis

Diet samples were analyzed as previously described (Lopez-Bote *et al*., 1997). For fatty acid determination in the feeds and in abdominal pad, fat was extracted by chloroform-methanol (Bligh and Dyer, 1959). Intramuscular neutral and polar lipids were extracted by consecutive solvent elution with dichloromethane and dichloromethane-methanol (90:10, vol/vol) respectively, on a glass column containing anhydrous sodium sulfate, celite 545, and bicalcium phosphate (Marmer and Maxwell, 1981). Fatty acids were methylated for gas chromatographic identification as previously described (Lopez-Bote *et al*., 1997). Fatty acid analysis was done on a 5890 Hewlett Packard gas chromatograph.[2] A 30 m × 0.32 mm × 0.25 μm cross-linked polyethylene glycol capillary column was used.[3] Analyses were performed with a temperature program from 170 to 245 C at a rate of 1 C/min. Injector and FID detector were maintained at 250 C. The carrier gas was helium at flow rate of 3 mL/min. Pentadecanoic acid[3] was used as internal standard. The melting point of abdominal fat was determined by the capillary tube method according to the procedure of the Association of Official Analytical Chemists (1990: reference 920.157).

The susceptibility of muscle homogenates to iron-induced lipid oxidation was determined by a modification of the method of Kornbrust and Mavis (1980) as previously described (Lopez-Bote *et al*., 1997). Approximately 1-g meat samples were homogenized in 10 mL of 0.04 mol/L Tris-maleate buffer (pH 7.4) for 10 s. Iron sulfate was added immediately after homogenization as a catalyst of lipid oxidation to reach a final concentration of 0.001 mol/L. Homogenates were incubated at 37 C. Every hour, aliquots were removed for measurement of thiobarbituric acid reactive substances, which were expressed as nanomoles of malonaldehyde per milligram of protein. Protein was measured by the procedure of Bradford (1976).

Statistical Analysis

Data were analyzed using the General Linear Models (GLM) procedure of SAS® (SAS Institute, 1988). Duncan's multiple range test was used to separate means. No sex or interaction effects were observed in none of the variables studied.

RESULTS AND DISCUSSION

There is a relationship in all lipid classes between dietary fatty acid composition and abdominal fat and intramuscular (neutral and polar) lipids, but the extent of the effect is different in each case and for each lipid class (Figure 1, 2, 3, and 4). The concentration of saturated fatty acids in the feeds, abdominal fat and intramuscular lipids is shown in Figure 1. Although abdominal fat and intramuscular neutral lipid fatty acid concentration reflected the differences between animal fat (tallow and lard) and sunflower oil in the concentration of total saturated fatty acids, the concentration of saturated in the polar lipid fraction was not affected by dietary fat source. Several authors reported a narrow limit of variation for the concentration of saturated fatty acids in the phospholipid fraction, which is attributed to the physiological need of the membrane to maintain its physical characteristics (Pan and Storlien, 1993; Lopez-Bote *et al*., 1997).

Although the concentration of saturated fatty acids was higher in the diet containing tallow than in that

[2]Hewlett-Packard Espanñola S.A., Las Rozas, 20230 Madrid, Spain.
[3]Sigma-Aldrich Quimica S.S., Alcobendas, 28100 Madrid, Spain.

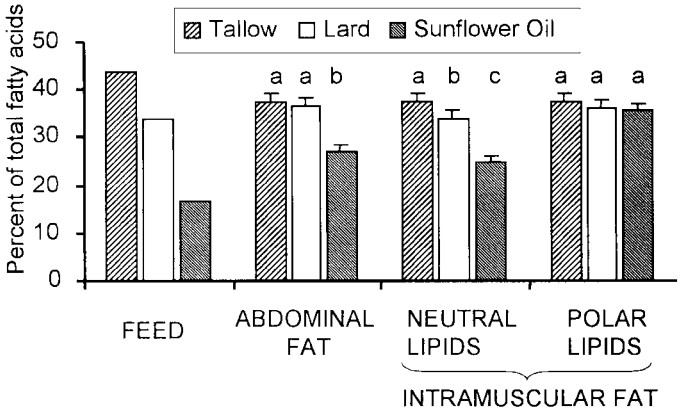

FIGURE 1. Effect of saturated fatty acids in feed on abdominal fat pad and intramuscular neutral and polar lipids of broilers fed diets containing tallow, lard, or sunflower oil ($\bar{x}$ ± SD).

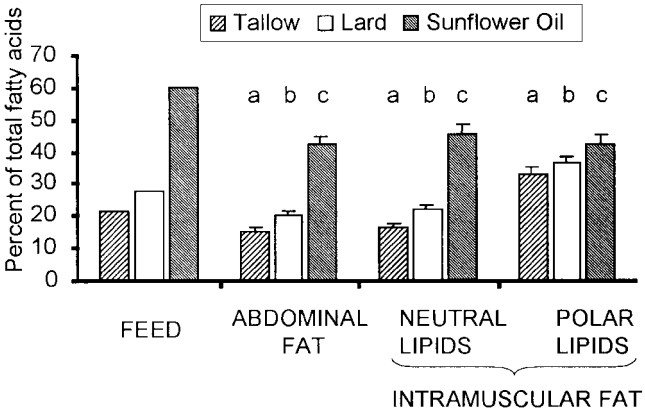

FIGURE 2. Effect of polyunsaturated fatty acids in feed on abdominal fat pad and intramuscular neutral and polar lipids of broilers fed diets containing tallow, lard, or sunflower oil ($\bar{x}$ ± SD).

containing lard, the differences in the concentration of saturated fatty acids between groups receiving a diet containing tallow or lard were not significant for the abdominal fat, and were of limited magnitude for the intramuscular neutral lipids (Figure 1). The limited differences in the concentration of saturated fatty acids between groups fed diets containing tallow or lard can be partially explained considering individual fatty acid composition. There were no differences in the concentration of stearic acid ($C_{18:0}$) among groups (10.25, 10.23, and 8.91 g/100 g total fatty acids) for diets containing tallow, lard, and sunflower oil respectively). Marion and Woodroof (1963) also reported that the $C_{18:0}$ level in body fat was relatively unchanged when dietary fat was administered, even when high levels of tallow were given. Valencia *et al.*, (1993) reported a minimal effect of feeding different fat sources on the monounsaturated fatty acids, whereas saturated fatty and polyunsaturated fatty acids were affected more severely. In addition, a correlation between individual dietary fatty acids and tissue fatty acids was carried out and showed a close relationship for all fatty acids ($r^2 > 0.75$) among dietary and abdominal or intramuscular neutral lipids, except for $C_{18:0}$, which may be partially attributed to the low digestibility of this fatty acid (Young and Garret, 1963). Moreover, it may also reflect decreased synthesis or increased desaturation of absorbed $C_{18:0}$ in response to dietary level (Fisher, 1984). Similar results were reported by Scaife *et al.* (1994) and Hrdinka *et al.* (1996).

The greatest differences among dietary treatments were found for the percentages of polyunsaturated fatty acids (Figure 2). This result was expected because in our experiment $C_{18:2\ (n\text{-}6)}$ was the main source of variation among diets. Differences in the concentration of polyunsaturated fatty acids among experimental groups are of different magnitude for each tissue or lipid fraction analyzed. Whereas in the abdominal fat and intramuscular neutral lipids, the concentration of polyunsaturated fatty acids ranged from 15.2 to 42.8 and from 16.5 to 45.6% respectively, in the polar fraction of intramuscular lipids, the concentration ranged only from 32.8 to 42.6%. Considering individual fatty acids of the polar fraction of intramuscular lipids, it is noteworthy that the broilers fed diets containing sunflower oil had a higher concentration of polyunsaturated (n-6) fatty acids, but the groups fed diets containing tallow or lard had a higher concentration of total (n-3) fatty acids. These results are in agreement with previous reports, which indicate little variation in the concentration of polyunsaturated fatty acids from experimental groups receiving different fat sources (Pan and Storlien, 1993; Lopez-Bote *et al.*, 1997).

Total monounsaturated fatty acids composition also varied depending on the dietary concentration of these fatty acids (Figure 3).

Yau *et al.* (1991) and Hrdinka *et al.* (1996) reported that the effect of dietary fat on the composition of breast muscle fat was less pronounced than that for abdominal fat. According to our results this is only true for the polar lipid fraction of intramuscular lipids, which is less dependent on the dietary fatty acid profile. Triglycerides (main constituents of abdominal fat and intramuscular

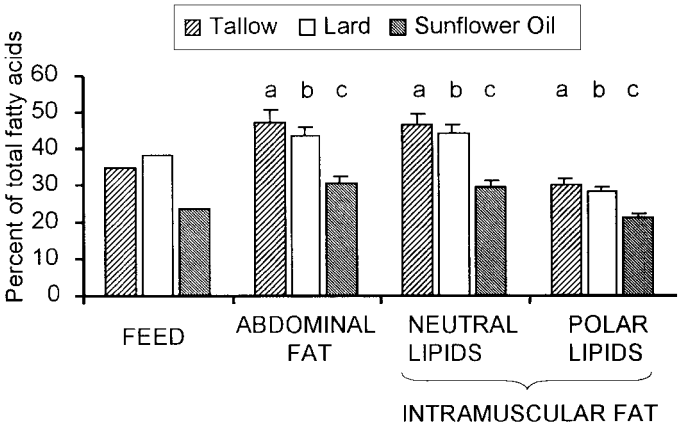

FIGURE 3. Effect of monounsaturated fatty acids in feed, on abdominal fat pad and intramuscular neutral and polar lipids of broilers fed diets containing tallow, lard, or sunflower oil ($\bar{x}$ ± SD).

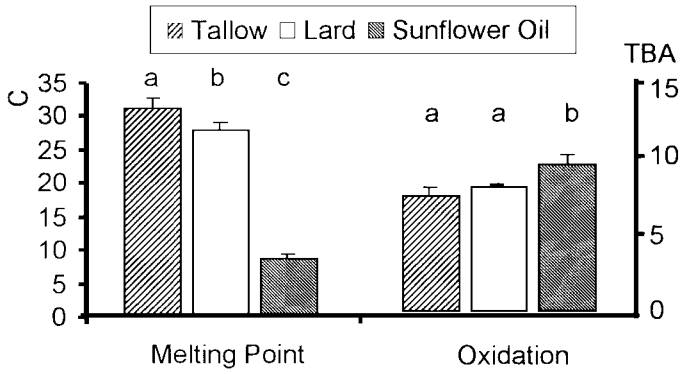

FIGURE 4. Melting point (degrees Celsius) of abdominal fat pad and thiobarbituric acid reactive substances (nanomoles of malonaldehyde per milligram of protein) in breast meat homogenates incubated at 37 C for 4 h from broilers fed diets containing tallow, lard, or sunflower oil ($\bar{x}$ ± SD).

neutral lipids) apparently respond to a similar extent in both tissues.

The effect of dietary treatment on abdominal fat melting point is shown in Figure 4. The group fed a diet containing tallow had the highest melting point of fat, whereas the group fed a diet containing sunflower oil had the lowest value. The group fed a diet containing lard showed intermediate values ($P < 0.05$). A wide range in the melting temperatures was found (mean value of different dietary treatments from 8.49 to 30.91 C), which indicate a major impact of dietary fat on fat consistency. We found a significant relationship between each fatty acid class and the melting point of the abdominal fat. The higher coefficient of determination for the melting point was found for total polyunsaturated fatty acids ($r^2 = 0.922$), which were negatively related to melting point ($P < 0.001$). Moreover, visual observation of polyunsaturated fatty acid concentration and melting point in abdominal fat evidence a close relationship in all groups (Figures 2 and 4).

The effect of dietary treatment on breast meat susceptibility to oxidation is also shown in Figure 4. After 4 h of incubation, the samples from broilers fed a diet containing sunflower oil had higher concentration of thiobarbituric acid reactive substances than the other two groups. The group fed a diet containing lard showed intermediate values, but were not significantly different from broilers fed a diet containing tallow. The range of lipid oxidation was narrow (from 7.68 to 9.76 nmol malonaldehyde/mg protein), indicating a limited effect of the dietary fats utilized in this experiment on the susceptibility of broiler meat to lipid oxidation. The low range of variability in oxidation may be attributed to the relative low effect of experimental diets on the polar lipid fatty acid composition. Several reports indicate that fatty acid from phospholipids present in the membranes play a key role in the development of oxidation (Ashgar *et al.*, 1990; Lopez-Bote *et al.*, 1997). Moreover, observation of polyunsaturated fatty acids of intramuscular polar lipids evidence a close relationship to breast lipid oxidation (variations among groups less than 21.3 and 23% respectively for oxidation values and concentration of polyunsaturated fatty acids, respectively) (Figures 2 and 4). Regression equation of intramuscular polar lipid fatty acid classes to oxidation of breast muscle showed the only significant difference for total polyunsaturated fatty acids ($P = 0.014$), which was positively related to lipid oxidation ($r^2 = 0.321$). Saturated and monounsaturated fatty acids were negatively related to lipid oxidation. This result is in agreement to that reported by Lin *et al.* (1989a,b), who found relatively low variation in the susceptibility to lipid oxidation of broiler meat when coconut, olive, hydrogenated soybean, or sunflower oils were used as a dietary fat source in comparison to the marked effect of oxidized oils, oils rich in (n-3) fatty acids (linseed oil), or supplemented levels of dietary vitamin E.

It is concluded that dietary fatty acid profile markedly influences abdominal fat and intramuscular neutral lipid fatty acid composition, but polar lipid fatty acids are less variable. A dietary fat source rich in linoleic acid produces a marked effect on fat consistency, but only a moderately higher susceptibility to lipid oxidation of meat than tallow or lard. Current restrictions of dietary $C_{18:2}$ concentration based on the effect on fat consistency are enough to maintain the susceptibility of meat from broilers within an acceptable range of meat oxidation acceptability.

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