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HYDROLYTIC AND OXIDATIVE CHANGES IN THE LIPIDS OF CHICKEN BREAST AND THIGH MUSCLES DURING

Abstract: *The changes in free fatty acid (FFA) amount and in Thiobarbituric acid reactive substances (TBARS) were simultaneously determined in chicken breast and thigh muscles at intervals between 1 and 14 d of storage at 4 °C (1,3,7,10,14). The rates of lipid hydrolysis were fast in the first 3 d and then slowed until day 14 ; phospholipids showed the same pattern but hydrolysis of triacylglycerols was linear at least in thigh muscles. Oxidation increased linearly during storage. Thigh muscles contained 3 times more FFAs than breast muscles and 2 to 4 times less TBARS suggesting that lipolysis did not favor lipid oxidation although both increased concomitantly.*

Key words: *lipolysis, free fatty acids, oxidation, muscles, refrigerated storage.*

Introduction.

Poultry meat is very sensitive to the development of oxi active rancidity because of its higher content in polyunsaturated fatty acids (PUFAs) (Wilson and others 1976; Igene and others 1980; Melton 1983). Numerous studies have been devoted

to oxidative changes in poultry meat showing an increase in the amount of Thiobarbituric acid reactive substances (TBARS)

PUFAs. during refrigerated storage and cooking (Sharma and others1982; Lin and others 1989). On the contrary, few studies have been done on lipolysis. Even so, lipolysis is suspected to promote in lipid oxidation because free fatty acids (FFAs) are often regarded were significant, but they accounted for less than 2% for most of as more sensitive to oxidation than esterifies ones (Labuza and others 1969; Nawar, 1996), particularly free long-chain PUFAs which arise from phospholipid hydrolysis (Moerck and Ball 1974; Gandemer 1990). However, in muscle, the impact of lipolysis on lipid oxidation remains a controversial topic. Thus, in fish muscles, several studies showed a positive correlation between FFA production and lipid oxidation (Shewfelt 1981), while others indicated a negative one (Shewfelt and others 1981; Shewfelt and Hultin 1983). In chicken muscles, FFA amount and oxidation level increased simultaneously and both were higher in thigh muscles than in breast muscles suggesting that lipolysis could promote lipid oxidation (Sklan and others 1983). However, their study was performed during 60 d on sterile meat, and the first examination was after 14 d of storage. So up to now, no data are available on lipolysis

during the first days of refrigerated storage accounted for covering the delay during which carcasses are cold-stored in the supermarket (less than 11 d). Our study deals with the simultaneous changes in FFA amount and composition and edin TBARS amount during refrigerated storage of both breast and thigh muscles to establish a possible relationship between lipolysis and oxidation.

Results

As expected, total lipids, triacylglycerol, and phpspholipipid contents were strongly related to muscle. Breast muscle contained less total lipids, triacylglycerols, and phospholipids than thigh muscles (Table 1). Phospholipids of breast muscle contained more monounsaturated fatty acids (19.0% to 20.7% vs 14.2% to 16.2%) and more long-chain PUFA (30.9% to 29.4% 29.0% to 27.9%) than those of thigh muscles but less saturatedand 18:2 n-6 (33.9% to 33.8% vs 35.7% and 16.1% vs 21% to 21.0%, respectively). In contrast, fatty acid composition of triacylglycerols was similar in both muscles. Triacylglycerols of breast and thigh muscles contained 32% to 35% saturated fatty acids, 36% to 40% monounsaturated fatty acids and 285 to 29% PUFAs.

Total lipid, triacylglycerol, and phospholipid contents and fatty acid composition of triacylglycerols did not change signif cantly in both muscles during 14 d of storage at +4 °C (Table 1). Differences in fatty acid composition of phospholipids between days 1 and 14 were significant, but they accounted for less than 2% for most of the fatty acids, including long-chain PUFAs. More than a storage effect, these differences could be related to the animals analyzed at days 1 and 14. Indeed, if the differences in PUFA proportions in phospholipids between days 1 and 14 were related to lipid oxidation during storage, they should be more pronounced in long-chain PUFAs that are very sensitive to oxidation (Moerck and Ball1974). That was not observed in our study.

Lipolysis and oxidation as related to muscles

Thigh muscles contained significantly 3 to 5 times more FFA amounts than breast muscles at all times of refrigerated storage(Fig. 1). At day 1, FFA amounts were 11.7 mg/100 g muscle in thigh muscles and 3.2 mg/100 g muscle in breast muscles and at day 14, they represented 51.0 and 15.5 mg/100 g muscle in thigh and breast muscles, respectively (Table 3). However, the rate of lipid hydrolysis was similar in both muscles: FFAs accounted for 0.4% of total lipids at day 1 and 1.9% to 2.0% at day 14 in thigh and breast muscles. FFA composition depended on muscle. The proportions of monounsaturated FFAs and free 18:2 n-6 were higher in thigh than in breast muscles whilst those of free long -chain PUFAs were lower in thigh than in breast muscles (Table 2).-

Lipid oxidation depended on muscles. Whereas lipids were oxidized to a similar level in both muscles at day 1, thigh con- tained 2 to 4 times less TBARS than breast at all other times of refrigerated storage (Fig. 2).

Table 1 – Lipid contents of breast and thigh muscel in chickens after 1 and 14 d of storage at 4 C

Muscles	Breast		Thigh		Stutistical effects			
Time (days)	1	14	1	14	M	T	M+T	SEE
Number of chickens	6	6	6	6				
Total lipids (g/100 g muscles)	0.87[a]		2.60[b]	2.73	***	n.s.	n.s	0.250
0.74[a]			1.84[b]	1.99	***	n.s.	n.s	0.250
Tryacylglycerol (g/100 g muscles)	0.35[a]		0.76[b]	0.74	***.	n.s.	n.s	0.066
			5.6[b]	5.4	***	n.s.	n.s	0.746
			0.74[b]	0.75	***	n.s.	n.s.	0.156
			31.8[a]	34.9	n.s	n.s	***	1.48
			38.8[ab]	37.1	n.s	n.s	**	2.34
[b]Phospholipids (g/100 g muscles)	0.52[a]	0.50[a]	29.2	28.0	n.s	n.s	n.s	2.10
Vitami E (mg/kg muscel)	2.2[a]	2.2[a]						
Vitami E/Phospholipids	0.42[a]	0.44[a]	35.7[c]	34.8	***	*	n.s	0.59
Fatty acids composition (%)			14.2 [a]	16.2	***	***	n.s	1.01
Tryacylglycerol			50.1 [a]	49.0	***	**	n.s	1.98
Saturated	35.2[b]	31.9[a]	21.1 [c]	21.0	***	n.s.	n.s	0.49
Monaunsaturated	36.4a	40.3[b]	29.0[b]	27.9	***	*	n.s.	1.17
Polyunsaturated	28.4	27.8						
Phospholipids								
Saturated	33.9[a]	33.8[a]						
Monaunsaturated	19.0 [c]	20.7[d]						
Polyunsaturated (PUFA)	47.1[b]	45.5[a]						
18:2 n-6	16.1[a]	16.1[a]						
Long-chain (FUFA)	30.9[b]	29.4[ab]						

On the same line, means superscripted by different letters significantly different n.s= not significant ($p>0.05$); $p<0.05$; $p<0.01$; $p<0.001$; M-muscle; T-time; SEE-stand error of estimation.

Long-chian FUFA=20:2 n-6+20: 3n-6+20: 4n-6+22: 4n-6+22: 5n-6+20: 5n-3+22: 6n-3

Time course of lipolysis and oxidation

Lipolysis. Refrigerated storage significantly affected FFA amounts in both muscles (Fig. 1). Between days 1 and 14, FFA amounts were multiplied by 4 to 5 in both muscels. The time of course of FFA formation during refrigerated storage not linear (Fig, 1). The rate of lipid hydrolysis was fast in the first 3 d of refrigerated storage and then it decreased slowly until day 14. The rate of phospholipid hydrolysis showed a similar pattern (Fig. 3).

Phospholipid hydrolysis provided between 2.4 and 11.7 mg/100 g muscle in breast and between 5.7 and 30.5 mg/100 g muscle in thigh muscles (Table 3). During the first days of storage, hydrolysis of phospholipids was faster than hydrolysis of

triacylglycerols (Fig. 3). Consequently, most of the FFAs arose from phospholipid hydrolysis, which provided 53% to 78% of total FFAs in breast muscle and 50% to 70% of total FFAs in thigh muscles, according to the time of storage (Table 3). In thigh muscles, the rate of triacylglycerol hydrolysis was linear throughout storage, and after 14 d of refrigerated storage, triacylglycerols provided an equal amount of FFAs as compared to phospholipids (Table 3). In breast muscle, the hydrolysis rate of triacylglycerols showed a pattern similar to that of phospholipids (Fig. 3).

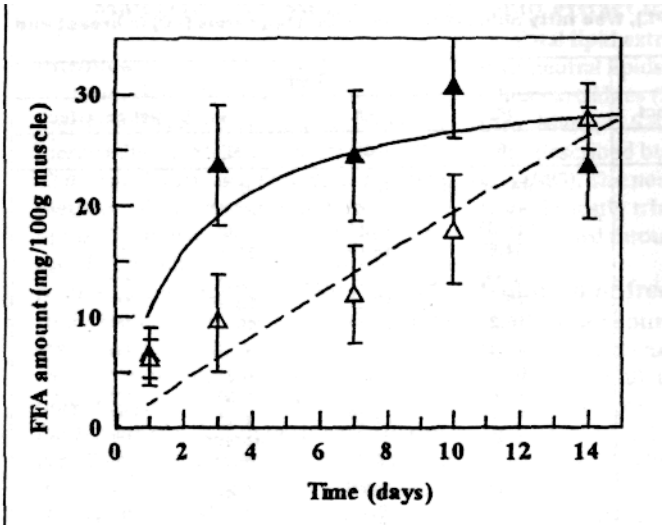
Changes in FFA composition were consistent with the changesin the ntime cours of hydrolysis of phospholipids and triacylglycerols in both muscles (Table 2). So in the first stage of storage (1 to 7 d), when the hydrolysis of phospholipids was faster that of triacylglycerols, the proportion of PUFAs increased from 31.9% to 40.0% in breast and from 37.0% to 39.4% in thigh), while the proportion of saturated fatty acids decreased (from 54.0% to 41.5% in breast and from 39.6% to 34.0% in thigh). In the second stage of storage, when the rate of phospholipids hydrolysis slowed down, the proportion of saturated fatty acids increased (from 41.5% to 44.4% in breast and from 34.0% to 45.0% in thigh) at the expense of the PUFA proportion (from 40.0% to 36.5% and from 39.4% to 34.9% in breast and thigh muscles, respectively), Hence, after 14 d of refrigerated storage, FFA composition was closer to the fatty acid composition of phospholipids than to that of triacylglycerols (Table 4). For example, in thigh muscles, the proportion of monounsaturated fatty acids was 20.1% in FFAs, 16.2% in phospholipids, and 40.4% in that of 20:4 n-6 was 13.4% in FFAs, 17.1% in phospholipids, and 1.1% in triacylglycerols.

Table 2 – Changes in FFA composition in chicken thigh and breast muscela during 14 d of storage at 4 ^{0}C

Muscles	Breast					Thigh					Statistical effects			
Time (days)	1	3	7	10	4	1	3	7	10	14				
Numb. of chick.	6	6	6	6	6	6	6	6	6	6	M	T	M=T	SEE
Saturated	54.0^{f}	48.1^{e}	41.5^{ed}	40.8^{od}	44.4^{de}	39.6^{bc}	36.3^{ad}	34.0^{a}	43.2^{od}	45.0^{de}	***	***	***	3.75
Monousaturated	14.1^{a}	17.3^{b}	18.4^{bc}	17.5^{b}	19.1^{bc}	23.4^{d}	22.7^{d}	26.6^{e}	20.0^{c}	20.1^{c}	***	***	***	1.86
Polyunsaturated (PUFA)	31.9^{bc}	34.6^{ab}	40.0^{c}	41.7^{od}	36.5^{abc}	37.0^{bxc}	41.0^{c}	39.4^{c}	36.8^{bc}		n.s.	***	***	3.83
18:2 n-6	9.0^{a}	12.2^{b}	14.2^{bc}	16.3^{od}	15.0^{bc}	34.9^{ab}					***	***	***	1.98
Long-chain PUFA [1]	22.6^{cde}	22.2^{cd}	25.5^{e}	25.5^{e}	21.6^{bcd}	17.9^{de} 18.6^{ab}	20.8^{f} 19.7^{abc}	20.3^{f} 19.0^{ab}	18.7^{ef} 17.8^{a}	17.9^{de} 16.8^{a}	***	n.s.	n.s.	2.91

On the same line, means superscripted by different letters are significantly different n.s.=not significant (P>0.05); *** = (P>0.05). M=muscle: T=time: SEE= standard error of estimation/

[1]long-chain PUFA = 20:2n-6 + 20:3n-6+22:4n-6+22:5n-3+22:5n-3+22:6n-3



Oxidation. The amount of TEARS increased linearly during refrigerated storage (y = 0.02 x + 0.02 and R^2 = 0.94 in breast muscles ; y = y=0.004 x +• 0.02 and R^2 = 0.92 in thigh muscles) (Fig. 2). The initial level of lipid oxidation was low in both muscles (0.03 mg eq. MDA/kg muscle at day 1). The level of lipid oxidation reached 0.10 and 0.30 mgeq. MDA/kg muscle at day 14 in breast and thigh muscles, respectively.

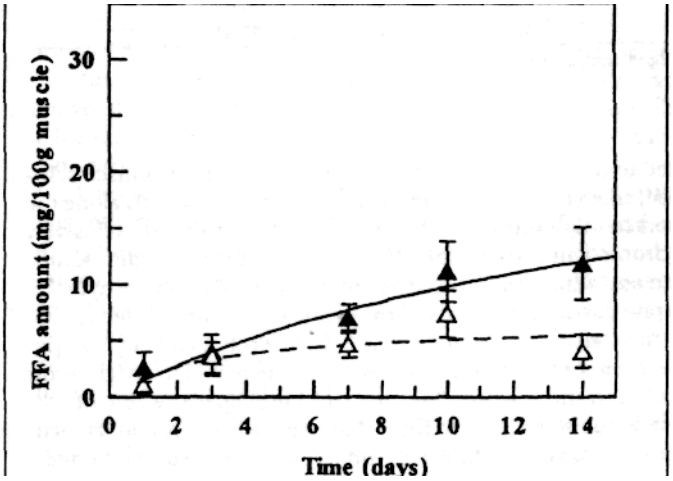


Figure *1*`—Changes in the amounts of FFAs arising from phospholipid hydrolysis (A) and from triacylglycerol hydrolysis (A) in chicken thigh muscles (upper) and breast muscle (lower) during 14 d of storage at 4 °*C*. Each point is the mean of 6 analysis. Error bars represent standard deviations.

Discussion

Lipolytic and oxidative changes as related to muscles

Thigh muscles contained more FFAs than breast muscles at all times of refrigerated storage (Fig. 1). These results confirmed those obtained in chicken (Currie and Wolfe 1977) and in turkey (Sklan and others 1983; Sklan andTenne 1984) and are in good agreement with those obtained in rabbit muscles, indicating that FFA amounts were higher in oxidative muscles than in glycolytic muscles (Alasnier and others 2000).

Lipids oxidized faster in breast than in thigh muscles as indicated by the slope of the curve (0.02 vs 0.004), although the initial level of lipid oxidation was similar in breast and thigh muscles). This result could be explained by the lower ratios of vitamin E to phospholipids and vitamin E to long-chain PUFAs in breast than in thigh muscles (0.42.10 $\sim^3$ vs 0.74.10$\sim^3$ and 1.9.10-^{3} vs 2.7.10$\sim^3$, respectively) (see Table 1). Indeed, vitamin E, which is a potent antioxidant in muscle food, is mainly stored in membrane and prevents oxidation of long-chain PUFAs of phospholipids (Machlin and Bendich 1987; Gray and others 1996). These results suggested that, during the first stage of storage, the antioxidant status of thigh muscles represented by vitamin E and the antioxidant system (such as glutathione, antioxidant enzymes) remained efficient to slow down the rate of lipid oxidation, despite the fact that thigh muscles contained more prooxi-dant agents, such as iron and potential substrate, for lipid oxidation than breast muscles (Lin and others 1989; Pikul and Kum-merow 1989; Mercier and others 1998). This hypothesis is strongly supported by the fact that we did find that vitamin E was not degraded during refrigerated storage (Table 1).

Time course of lipolysis

The time course of FFA formation during refrigerated storage was curvilinear in breast and thigh muscles . It was mainly related to the time course of phospholipid hydrolysis, which was faster in the first 3 d of storage than thereafter (Fig. 1). Phospholipid hydrolysis is catalyzed by phospholipases and lysophos-pholipases. Up to now, very little is known about the activities of these enzymes in skeletal muscles and about the regulation of their activity postmortem. Recently, we have established that rabbit muscles contain both phospholipases A and lysophospho-lipases (Alasnier and Gandemer 2000). Even if the postmortem regulation of the phospholipases is not known, it changed during storage. The fast rate of phospholipid hydrolysis during the first days should have several explanations. The first could be related to the Postmortem formation of membrane vesicles that occurred with the onset of death and increased the contact surface between membrane phospholipids and phospholipases (Stanley 1991). The second was the release of acid phospholipases located in lysosomes whose membrane was disrupted in the few hours following death (Stanley 1991). The third was the possible activation of phospholipases related to the postmortem release of calcium from sarcoplasmic reticuium and/or to the phosphorylation of the phospholipases by ATP. Indeed, calcium as low as 100 nM enhanced myocardial phospholipase A_2 activity (Wolf 100 nM enhanced myocardial phospholipase A_2byphosphorylation (Hazen and Gross 1991). The second stage of phospholipid hydrolysis was slower the first. The cause of this slowing-down

remained to be established. This could be related to the quick depletion of some cofactors essential for the activation of phospholipases, such assential for the activation of phospholipases, such as ATF! which is required for phosphorylation and whose concentration decreases very fast with death (Renou and others 1986).In thigh muscles, the rate of triacylglycerol hydrolysis was linear during all the storage periods whereas in breast muscles it was curvilinear like the rate of phospholipid hydrolysis (Fig. 1).

This result suggests that the triacylglycerol lipases in thigh muscles should not be affected by physicochemical changes occurring in skeletal muscle cells after death while the triacylglycerol lipases in breast muscle should be. This difference could be related to the location of triacylglycerols in these two muscles. Indeed, triacylglycerols are mainly stored in adipose cells along the fibers in thigh muscles, while they are located almost exclusively and Gross 1996) and ATP activated intracellular phospholipases in droplets in the cytosol of the fibers in breast muscles (KauffanandSafani 1967; Cassens and Cooper 1971). So the hydrolysis rate of triacylglycerols in thigh muscles corres-ponded to that of triacylglycerols in fat cells and the hydrolysis rate of triacylglycerols in breast muscles corresponded to that of triacylglycerols located in the cytosol. The triacylglycerol lipase system of adpose tissue was different from that in muscle fibers and its regulation could also be different. Indeed, adipose tissue contained 2 main triacylglycerol lipases: a lipoprotein lipase (LPL) and a hor-mono-sensible lipase (HSL) (Osca'i'and others 1990) and muscles contained an acid lysosomal lipase in addition of the 2 lipases cit--ed above (Motilva and others 1992). Postmortem, in fat cells, HSL should prevail as its potential activity was higher than that of LPL (Motilva and others 1993). In muscles, the acid lysosomal lipase may be the most prominent as its optimum pH was close to the ultimate pH of muscles. Hence, the regulation of the rate of triacylglycerol hydrolysis was c ifferent in thigh and breast muscles because the prevailing ti lacylglycerol Upases remaining postmortem in these muscles were different.

This higher contribution of phospholipids to FFA formation in chicken muscles is consistent with the increase in long-chain PU- FAs amounts in FFAs. These results are in good agreement with those previously published which indicated that phospholipids were the main source of FFAs in the muscles of turkey and chicken (Currie and Wolfe 1977; Sklan and others 1983; Sklan and Tenne, 1984) and in dry-cured hams (Buscailhon and others

Lack of relationship between lipolysis and oxidation

The increase in TEARS amount during storage at 4 °C is in good agreement with results previously published on TEARS values in chicken muscles, even if the values varied largely from one laboratory to another, according to the alternative methods used for TBARS measurement (Sharma and others 1982; Lin and others 1989; Pikul and Kummerow, 1989).

Although lipolysis (enzymatic) and oxidation (chemical) increased concomitantly during refrigerated storage at +4 °C

(Fig1), our results strongly suggest that lipolysis did not promote lipid oxidation in raw chicken muscel. Hence, compared to breast muscles, the rate of lipid oxidation was 5 times lower in thigh muscles, despite the fact that amounts of FFAs and free long-chain PUFAs were higher in thigh than in breast muscles. The fact that long-chain PUFAs arising from phospholipid hydrolysis did not promote lipid oxidation in raw meat could be ex plained by the fact that FFAs would remain in the membrane and be protected against oxidation by vitamin E included in the bilayers of the membranes. Indeed, FFAs remain in the membrane when their amounts did not exceed a few percent of total mernbrane lipids (Zeng and others 1998).

Conclusion

Lipid hydrolysis and phospholipid hydrolysis proceeded rapidly in the first 3d and then slowed down until day 14, suggesting changes in the regulation of phospholipases and lyso phospholipases during storage. Triacylglycerol hydrolysis was linear in thigh and curvilinear in breast muscles, suggesting that the regulation of triacylglycerol Upases was different in fat cells along the fibers and in the cytosol in the fibers. Oxidation increased lin early during storage. Lipolysis did not promote lipid oxidation.

Materials and Methods

Animals

Thirty male chickens of a commercial breed (GA557) were fed 3 successive commercial diets (0 to 21 d: starter diet; 22 to diet) containing between 3.9% and 4.6% lipids and between 25 and 34 ppm vitamin E. The birds were slaughtered in a private slaughterhouse (SOPARVOL,Ancenis, France) at 82 d of age having an anerage carcass weight of 1.5 kg. The car- casses wereimmediately packed in polypropylene tubs surrounded by a food-grade plastic film permeable to oxygen. The carcasses were stored at +4 °C in the dark for 14 d. Breast and thigh muscles were cut from 6 carcasses after 1,3,7,10, and 14 d of refrigerated storage.

Lipid extraction

Muscles were carefully trimmed to remove adipose tissue and were minced in a blender. Lipids were extracted from 10 g of muscle with chloroform/methanol (2:1), according to the method of Folch and others (1957). The extracts were dried under vacuum on a rotary evaporator. Lipids were weighed and lipid content was expressed in g/100 g muscle. The phospholipid content was calculated (P x 25) after phosphorus was determined in the total lipid extract by the method of Bartlett (1959). The neutral lipid content was estimated to be the dif-

ference between total lipid and phospholipid contents. Phospholipid and neutral lipid contents were also expressed ing/100 g muscle.

Lipid extract fractionation

Total lipid extracts were fractionated into neutral lipids and phospholipids onsilica cartridges (Sep-Pack, Waters, Milford, Mass., U.SA.) following the proceldure described by Juaneda and Rocquelin (1985). The neutral lipid fraction contained mostly triacylglycerols, and this term is used throughout the text.

Isolation of free fatty acids (FFAs)

FFAs wre purified from the neutral lipids using an anionic exchange resin (Amberlyst A26), according to the method of Gandermer and others (1991). An aliquor of 40 to 50 mg of neutral lipids was dissolved in 20 mL of a mixture of acetone/methanol 2:1 (v:v). After addition of 100 to 200 mg of resin and heptadecanoic acid as internal stand shken for 30 min.

Nonresinbound lipids were removed by washing the resin with acetone/methanol 2:1 (v:v). Resin was then transferred into a dry tube for FFA methylation.

Fatty acid Fatty acid composition

The fatty acid composition of triacylglycerols, FFA, and phospholipids was determinated by gas chromatography. Triacylglycerol, phospholipids, and free fattyacid methyl esters were prepared following the method of Morrison and Smith (1964). The gas chromatograph (Hewlett-Packard 5890) was equipped with a split injector and an on-column injector and a flame ionization detector. The derivatives were separated on a capillary column (DB 225, J&W, 30 m long, 0.32 mm internal diameter, 0.25 m film tickness, coated with a polar stationary phase (cyanopropylphenyl-methylpolysiloxane).Triacylgycerol methyl esters were injected in split mode. The split flow rate was set at 30 mL/min. The oven temperature was held at 150 ^{0}C for 4 min, increased 200 0C to at 10 ^{0}C/min, and then maintained at 200 ^{0}C. Methyl esters of phospholipids and FFA were injected in on-column mode, The oven was first held at 50 ^{0}C for 3 min and then increased from 180 to 210 ^{0}C at 20^{0}C/min and then maintained 210 ^{0}C for 10 min. The detector temperature was set at 250 ^{0}C. In both cases, the flow rate of the carrier gas (H_2) was set at 2 mL/min and kept constant. Data were collected with an Apex workstation including an acquisition interface, software, and a computer (Apex, Milford, Mass., U.S.A.). The individual fatty acid esters were indentified by mass spectrometer (Hewlett-Packard MSD 5971A). Fatty acid compositions were expressed as percent of total fatty acid methyl esters.

Determination of the origin of FFAs

The relative contribution of phospholipids and triacylglicerols to lipolysis was calculated as previously reported (Alasnier and others 2000). Considerring that long-chain PUFAs are almost exclusively found esterified in phospholipids and, consequently, that those found in FFA fractions arose from phospholipids hydrolysis, we can estimate the proportion of phospholipids hydrolyzed (Table 3 (3)) for each period of refrigerated storage by the ratio of the amount of long-chain PUFA in FFAs to that in phospholipids. This ratio (3) was used to calculate the total amount of FFAs arising from phospholipid hydrolysis. (Table 3 (4)) by

Multiplying (3) by the total amount faqtty acids esterified in phospholipids, wich accounted for 70% of the amount of phospholipids in muscle. The amount of FFAs provided by triacylglycerol hydrolysis (Table 3 (6)) were estimated to be the difference between the amount of total FFAs measured in musceles (5) and (4). The proportion of triacylglycerol hydrolyzed for each period (7) was calculated by the

ratio of (6) total fatty acids esterified in triacylglycerols, which accounted for 99.5% of

Vitamin E content __

Vitamin E contents of diet and muscles (№40). were determined according to the method of Butriss and Diplock (1984). Briefly, after saponification of samples and extraction of vitamin E with hexane, the extracts analyzed by normal-phase high-performance liquid chromatography (HPLC) (Lichrospher Si 60,5 m, Merck) fitted with a flurorimeter detector (excitation wavelength: 292 nm; emission wavelength: 330 nm). Vitamin E was quantified using alfa-tocoferol as an extermal stsndard. The result were expressed as mg vitamin E/kg diet or muscle

Oxidation measurement

Thiobarbituric acid reactive substances (TBARS) were evaluated using a method adapted from that of Salin and others (1987) and Botsoglou and others (1994) by Genot and others (1998). Two g of ground muscele were mixed with butyl hydroxyl touluene (BHT) in ethaqnol (10 g BHT. Of lipids) and 18 mL of trichloroacetic acid (TCA 5%). Samples were homogenized for 20 s at 20,000 rpm and then filtered through Whatman filter (N^0 40). Two tightly closed tubes were heated at 70 ^{0}C for 30 min. The absorbance was read against water at 508,532,, and 600 nm with a double beam spectrophotometer (lamba 12, Perkin Elmer). The absorbance measured at the maximum (532 nm) was corrected for the baseline drift as follows:

$\mathbf{A_{532}}$ corrected=A_{532} – ((A_{532} --- A_{600}) + (600 --- 532)/ (600 --- 508)) - A_{600}

Results were expressed as mg eq. MDA/kg of muscele ising the molar extinction coefficient of MDA – TBA adduct at 532 nm ($1.56.10^5 M^{-1}cm^{-1}$) (Buedge and Aust 1978).

Statistical analysis

Data on lipid composition and on FFA and TBARS amounts in breast and thigh musceles were subjected to a two-way vbariance analysis, according to the General Linear Model procedure, The model used, with fixed effects, included time ctorage (5 level) and musceles (2 level) and the interaction time storage = muscle. Data on FFA composition were subjected to a one –way variance analysis. The model included time storage (5 levels). The calculations were performed with Statgraphics software.

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