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Phospholipids and Their Fatty Acid Composition in the Muscle of Trout Fed on Diets Supplemented with Olive Oil Bagasse or Technical Rendered Fat

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A study to determine the effects of two by-products from the food industry (olive oil bagasse or technical rendered fat) on the phospholipid content and the fatty acid composition of the muscle of rainbow trout (*Salmo gairdneri*) has been made. Three batches of 150 trout were given for 100 days a commercial diet alone or supplemented either with 11 % olive oil bagasse or technical rendered fat. The phospholipid content in the muscle of the three batches of trout ranged from 0.70 to 0.93 % (wet weight). In this fraction, six different phospholipid classes were detected, phosphatidylcholine and phosphatidylethanolamine achieving average values of 55 and 25 % of total phospholipids. Although differences in the fatty acid composition of the diet were observed, the only clear influence of diet was on the fatty acid C-22:6 of muscular phosphatidylethanolamine.

Key words: Trout, Phospholipids, Fatty acids.

It is well known that diet composition affects the total muscle fatty acids. In the case of *Salmo gairdneri*, some authors (3, 17) have studied the fatty acid composition of the lipids of several tissues of trout fed on diets containing different levels of the essential linoleic and linolenic fatty acids. From these studies it may be deduced that, in general, trout may elongate and desaturate these fatty acids to synthesize phospholipids with an appropriate fatty acid composition. This effect is not clearly reflected on apolar lipids, in which several unsaturated long chain fatty acids (e.g. C-20:3, C-20:4, C-22:5 and C-22:6) are detected at only a very low percentage. Therefore, when an attempt is made to study the effect of a given diet on the lipids and fatty acid composition of the muscle of trout it seems to be appropriate to determine separately the apolar and polar lipid fractions.

The effects of diets supplemented with

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olive oil bagasse or technical rendered fat on the apolar lipids and their fatty acid composition of trout muscle have been previously reported (8). The clearest effect was observed on C-16:1 and C-18:1 in muscle triglycerides. The present work deals with the effect of those byproducts of the food industry on phospholipids and their fatty acid composition.

Materials and Methods

Three batches of 150 rainbow trout (*Salmo gairdneri*) of initial average weight of 28.6 g were used. Each batch was fed to satiation twice daily with the appropriate dry pelleted feed. Two batches were fed with a commercial diet supplemented with 11 % of either olive oil bagasse (batch OD) or technical rendered fat (batch RD). As a control, one batch (CD) was fed on the commercial diet. The final chemical compositions of the three different diets are shown in table I and their fatty acid contents in table II.

Chemical composition of diets (protein, fat, crude fibre and ash) were determined according to AOAC (1) methods.

The experiments were performed in tanks (9 × 1 × 0.6 m) at a fish farm located at about 800 m above the sea level. Identical water temperatures were recorded for the three batches. The temperature throughout the experiment was slowly increasing from 5° to 9° C although an important fluctuation was recorded during the period 23rd-31st days: the temperature first decreased to 3° C, then increased to 10° C (27th day) and finally (31st day) reached the normal temperature (6° C).

For analysis, 50 animals of each batch were randomly taken from the corresponding tank. The head, tail, fins, viscera and skin of each fish were immediately removed and fillets were obtained from the fish by removing carefully the flesh from the backbone. The meat obtained from the 50 animals of each batch was finely minced in a blender (Sorvall, Omni-mixer 17106). The final sample was composed of a homogenate of the meat from the 50 animals. Sampling was made at 45, 70 and 100 days.

Lipids were extracted and purified from the former homogenate according to BLIGH and DYER (2). To fractionate the total lipids into apolar and polar lipids, glass columns (1 × 20 cm) were packed with a slurry of a mixture of silicic acid and celite (1/1, w/w) in chloroform. Lipid samples (15 mg · g^{-1} of silicic acid) in the same solvent were applied to the column, which was successively eluted with chloroform (10 ml · g^{-1} of silicic acid), acetone (6 ml · g^{-1} of silicic acid) and methanol (10 ml · g^{-1} of silicic acid). With this chromatographic system it is possible to obtain three separate fractions: apolar lipids, glycolipids and phospholipids (15). All lipids extracted were kept at −20° C until analysis.

Total lipids and phospholipids were determined by weighing the lipids extracted from the initial homogenate and the lipids eluted by methanol from the silicic acid-celite column, respectively.

Thin layer chromatography (TLC) of polar lipids was developed on 0.25 mm silica gel G-60 plates with chloroform-methanol-water (65/25/4, by volume). The plates were sprayed with iodine vapour as general reagent and Schiff periodate (13), molybdenum blue (5), ninhydrin (16) and Dragendorff (16) as specific reagents. Purification of the different phospholipid classes was achieved on preparative 0.50 mm plates developed with the same solvents mixture. Samples were applied as a line and the phospholipid classes were extracted with chloroform-methanol (2/1, v/v) from silica gel G-60 scraped from the plates. To visualize the different spots the plates were stained with iodine vapour prior to quantitative analysis or with 0.15 % (w/v) dichlorofluorescein in 95 % methanol when fatty acids were to be analyzed.

The estimation of the individual phos-

pholipids amount was based on its phosphorus content (4). For gas-liquid chromatography (GLC) analysis of fatty acids, individual phospholipids were methylated by the method of SHEATA *et al.* (14): Methyl esters were analyzed with a Perkin-Elmer 910 chromatograph equipped with a dual flame-ionization detector. The glass columns (200 cm, internal diameter 0.2 cm) were packed with Chromosorb W coated with 10 % DEGS and the fatty acid methyl esters were separated using a temperature gradient from 80 to 180° C programmed at 8° C min^{-1}; N_2 flow, 35 ml · min^{-1}. For quantitative analysis, a Perkin-Elmer Minigrator M-2 integrator was used. The identification of different fatty acid methyl esters was made by comparison with standards (Sigma).

Results and Discussion

The total phospholipid content in the muscle of the three batches of rainbow trout is shown in table III. The percentage of muscle phospholipids of the groups tested ranged from 3.12 to 4.35 % of dry weight. These results agree with those reported by other authors in *Salmo gairdneri*, e.g. KINSELLA *et al.* (9) who found

Table I. *Percentage composition of diets (dry weight) and weight of trouts.*

	Diet*		
	CD	OD	RD
Crude protein	48.74	44.26	42.70
Crude fat	8.37	9.59	19.04
Crude fibre	5.50	5.47	3.59
Ash	12.57	14.20	11.31
Average initial fish weight (g)	28.60	28.60	28.60
Average final fish weight (g)	87.30	79.21	83.44

* CD: Commercial diet (Control). OD and RD: Commercial diet (CD) supplemented with 11 % of olive oil bagasse and 11 % technical rendered fat, respectively.

Table II. *Percentage fatty acid composition of dietary lipids.*

	Diet*		
Fatty acid	CD	OD	RD
C-14	3.67	3.51	2.84
C-16	22.65	23.82	24.68
C-16:1	4.52	3.90	3.90
C-17	0.55	0.39	0.57
C-18	6.30	7.30	13.99
C-18:1	28.61	30.06	33.52
C-18:2	12.43	14.41	10.99
C-20	0.24	0.32	0.26
C-18:3	3.88	4.05	2.82
C-20:1	0.58	0.54	0.25
C-20:2	0.04	0.16	0.21
C-20:3	0.10	0.35	0.21
C-22:1	3.24	3.17	1.38
C-22:2	0.47	0.26	0.11
C-20:5	3.45	2.73	1.35
C-24	1.16	0.35	0.05
C-24:1	0.39	0.74	0.31
C-22:5	0.58	0.75	0.17
C-22:6	6.79	3.87	1.76
Others	1.64	1.59	1.49

* Legend as in table I.

Table III. *Phospholipids in* Salmo gairdneri *muscle, fed on three different rations.*
45, 70 or 100: number of days the trout were fed with the different rations. Abbreviations as in table I.

	Phospholipids (%)		
Population	Wet weight	Dry weight	Total lipid
Initial*	0.70	3.48	29.46
CD-45	0.93	4.35	42.08
CD-70	0.83	3.82	35.62
CD-100	0.75	3.44	27.57
OD-45	0.76	3.77	33.48
OD-70	0.91	4.27	34.46
OD-100	0.68	3.12	28.10
RD-45	0.91	4.26	31.05
RD-70	0.89	4.10	28.99
RD-100	0.74	3.22	25.43

* Initial: initial population.

Table IV. *Behaviour of different phospholipids isolated from* Salmo gairdneri *muscle against Iodine (I), Molybdenum blue (MB), Ninhydrin (N), Dragendorff (DR) and Schiff-periodate (SP) reagents.*

Spot No	Rf	I	MB	N	DR	SP	Characterization
1*	0.20	+	+	−	+	−	Lysophosphatidylcholine
2*	0.32	+	+	−	+	−	Sphingomyelin
3**	0.35	+	+	+	−	−	Phosphatidylserine
4*	0.47	+	+	−	+	−	Phosphatidylcholine
5*	0.77	+	+	+	−	−	Phosphatidylethanolamine
6*	0.95	+	+	−	−	−	Cardiolipin

* Eluted from silicic acid column by methanol.
** Eluted from silicic acid column by acetone.

values of 0.87 % (wet weight) and CASTELL *et al.* (3) who reported levels ranging between 21.6 and 35.4 % of total body lipids.

No clear differences were found in the muscle phospholipid content of the three batches. In this aspect, it has been reported that diets containing 0.5 % or more linolenic acid increased the percentage of phospholipids by 28 to 59 %, with higher percentages directly related to increased amounts of C-18:3 (3). However, from tables II and III it can be deduced that although the C-18:3 content of the diets was the lowest on the RD diet, it was not reflected in the respective muscle phospholipids.

TLC revealed the presence of 6 phospholipids in the polar lipids extracts of all batches. According to their Rf and behaviour against general and specific reagents were characterized (table IV) as phosphatidylserine (eluted from silicic acid column by acetone), lysophosphatidylcholine, sphingomyelin, phosphatidylcholine, phosphatidylethanolamine and cardiolipin (all eluted by methanol). In general, the same phospholipids have been reported by different authors in fish muscles. However, GRAY and MACFARLANE (7) found phosphatidylinositol but not lysophosphatidylcholine in trout and FERNÁNDEZ and BURGOS (6) did not detect phosphatidylserine in *Salmo fario*. In other marine fishes as *Sardina ocellata* (11) and *Merluccius capensis* (10) lysophosphatidylethanolamine and phosphatidylinositol have also been observed.

Table V. *Phospholipid composition of trout muscle (% of total phospholipids).* Legend as in table III.

	Population									
Component*	Initial**	CD-45	CD-70	CD-100	OD-45	OD-70	OD-100	RD-45	RD-70	RD-100
LPC	3.31	3.34	3.33	5.11	3.22	5.66	3.99	3.82	4.33	3.68
S	5.09	4.62	6.40	7.12	4.98	6.95	5.60	8.04	5.73	6.55
PS	2.45	1.77	1.97	1.72	2.28	2.26	2.59	1.94	1.51	2.96
PC	57.84	55.68	53.87	52.51	56.76	57.71	57.97	61.11	60.93	51.83
PE	26.58	29.81	26.28	26.83	27.09	20.91	23.71	19.42	20.28	29.42
CL	4.71	4.78	8.13	6.70	5.64	6.48	6.12	5.65	7.25	5.55

* LPC: lysophosphatidylcholine; S: sphingomyelin; PS: phosphatidylserine; CL: cardiolipin; PC: phosphatidylcholine; PE: phosphatidylethanolamine.
** Initial: initial population.

Table VI. *Fatty acid composition of phosphatidylcholine (weight %).* Legend as in table III.

	Population									
Fatty acid	Initial*	CD-45	CD-70	CD-100	OD-45	OD-70	OD-100	RD-45	RD-70	RD-100
C-14	2.27	1.96	2.06	2.70	2.15	1.70	1.67	1.68	2.42	1.44
C-16	25.84	37.17	34.97	33.33	29.97	37.73	30.55	30.35	28.79	35.43
C-16:1	4.27	7.77	7.04	4.42	6.45	8.52	3.49	5.92	6.91	3.37
C-17	0.13	0.17	0.23	0.45	0.09	0.28	0.52	0.13	0.23	0.24
C-18	2.06	2.07	1.87	2.24	1.14	2.05	2.30	1.55	1.61	1.68
C-18:1	16.33	16.10	16.82	15.41	16.78	16.63	18.64	18.67	19.00	18.80
C-18:2	5.79	5.50	6.02	5.80	7.15	5.71	4.97	7.03	6.35	5.78
C-20	1.24	0.08	0.22	0.41	0.18	0.28	0.35	0.13	0.46	0.04
C-18:3	0.43	1.24	1.17	1.56	0.71	1.24	0.52	1.54	1.49	0.60
C-20:1	1.15	0.84	0.61	0.39	1.29	0.59	1.39	0.54	0.57	0.48
C-20:2	1.36	0.20	0.47	0.46	0.35	0.17	1.04	0.39	0.34	0.24
C-20:3	0.48	0.67	0.78	1.09	0.64	0.68	0.52	1.09	1.03	0.78
C-22:1	3.99	1.99	2.34	2.43	2.51	2.63	2.79	2.38	2.82	2.95
C-20:5	4.65	3.67	4.13	3.75	4.37	3.12	4.50	4.15	4.14	3.56
C-24	2.34	0.12	0.23	0.31	0.18	0.17	1.39	0.39	0.34	0.20
C-24:1	2.58	1.03	1.42	0.52	1.57	1.19	2.09	1.90	1.49	1.80
C-22:5	1.51	0.53	0.54	0.44	1.07	0.44	1.04	1.99	0.69	0.60
C-22:6	22.26	17.10	17.70	19.14	21.52	15.87	19.34	17.51	18.43	20.25
Others	1.21	2.01	1.28	5.06	1.81	0.91	2.80	2.61	2.80	1.67

* Initial: initial population.

The amount of each phospholipid was not the same in the different batches of trout but no consistent pattern was clearly observed (table V). Phosphatidylcholine (PC) was found in the highest concentration followed by phosphatidylethanolamine (PE), totalling an average of 55 % and 25 %, respectively. PC plus PE represents an 80 % of total phospholipids. Thus, the polar lipid fatty acid composition, as a whole, is mainly due to the summation of the fatty acid of each particular phospholipid. For this reason, only these major phospholipid classes have been considered.

GLC analysis of methyl esters from phospholipids revealed the presence of more than 25 fatty acids, the predominant ones being shown in tables VI and VII. The fatty acids C-16, C-18:1 and C-22:6 in PC and PE were the major ones representing together more than 60 % of total fatty acids. It is interesting to observe that the C-18:1 percentage of both phospholipids was similar but an inverse relationship between C-16 and C-22:6 may be deduced. The values found for the C-22:6 contents ranged from 22.63 to 33.80 % in PE and from 15.87 to 22.26 % in PC. Although these levels are high, other authors (7) have reported even higher percentages up to 50 % in trout muscle PC. The main difference observed in the fatty acid contents of phospholipids (table VI and VII) and triglycerides (8) was in this fatty acid. The concentration of C-22:6 in phospholipid classes was 2-8 fold higher than in triglycerides. This fact appears to indicate the important function of polyunsaturated fatty acids, especially C-22:6, in fish physiology. In this sense all fatty acids have been reported to be subjected to metabolism except those fatty acids incorporated rapidly into phospholipids (12) which

Table VII. *Fatty acid composition of phosphatidylethanolamine (weight %).* Legend as in table III.

	Population									
Fatty acid	Initial*	CD-45	CD-70	CD-100	OD-45	OD-70	OD-100	RD-45	RD-70	RD-100
C-14	0.76	0.72	1.35	3.98	1.07	2.50	2.79	1.11	0.57	0.39
C-16	19.20	18.07	10.72	9.93	21.81	16.75	15.80	22.27	16.23	13.23
C-16:1	3.53	4.13	2.90	1.13	2.90	2.25	1.42	3.04	1.62	2.81
C-17	0.76	0.08	1.13	0.51	0.18	0.27	0.72	0.74	2.02	1.34
C-18	4.68	4.13	4.52	3.62	3.78	3.33	4.30	3.71	3.66	5.62
C-18:1	17.66	18.79	19.63	15.96	22.26	20.49	16.52	22.58	16.63	16.43
C-18:2	9.21	8.51	5.45	6.20	8.34	6.08	6.98	9.72	6.37	6.74
C-20	0.02	0.36	0.97	0.61	0.07	0.10	0.85	0.03	0.12	1.49
C-18:3	1.84	1.58	1.20	0.43	0.78	0.30	0.32	1.48	0.37	0.27
C-20:1	0.61	0.92	1.16	2.64	0.89	1.94	1.50	0.37	2.76	0.10
C-20:2	0.96	1.39	0.45	0.61	1.18	0.73	0.20	0.55	0.15	0.15
C-20:3	0.61	1.13	1.20	1.43	0.96	0.91	1.42	0.89	0.90	2.02
C-22:1	3.66	4.25	3.25	5.78	3.47	3.24	4.59	2.97	5.12	5.20
C-20:5	3.58	4.47	3.48	3.10	4.45	4.38	4.73	2.67	2.61	3.59
C-24	0.47	0.90	1.69	1.02	1.06	0.33	2.29	1.11	2.28	2.91
C-24:1	0.96	2.94	0.90	3.10	1.78	0.46	2.72	1.41	4.92	6.54
C-22:5	2.37	1.08	1.42	1.23	1.33	0.93	0.54	1.18	0.61	0.23
C-22:6	26.62	24.55	30.14	33.80	22.85	29.57	27.03	22.63	26.85	25.24
Others	2.42	1.89	5.49	4.54	0.75	4.93	4.92	1.46	6.14	5.60

* Initial: initial population.

has been demonstrated for C-22 : 6 (18).

In a previous work, the influence of the fatty acid composition of the diet on the fatty acid composition of the apolar lipids has been studied (8), where it was observed that the higher the C-16:1 and C-18:1 percentages on the diets, the richer are the contents of these fatty acids in muscle triglycerides. However, a comparison of table II and tables VI and VII reveals that in phospholipid classes the most evident effect of the fatty acid of the diet was on the C-22:6 content of PE. The higher the C-22:6 content of the diet the greater is the concentration of this fatty acid in the muscle PE of trout. No consistent effects were observed in the other fatty acids in either PC or PE. Since it has been reported that diet fatty acid composition influences the muscular phospholipids (3) it was expected that some effects would be observed in PC. However, the levels of fatty acids in this phospholipid show different patterns (tables II and VI) but no relation to the fatty acid composition of the diet. No explanation has been found on the different behaviour of PC and PE.

Resumen

Se estudia la influencia de dos subproductos de la industria alimentaria (orujo de aceituna y grasa técnica de matadero) en el contenido de fosfolípidos del músculo de trucha (*Salmo gairdneri*) y en su composición en ácidos grasos. Tres lotes de 150 truchas recibieron durante 100 días una dieta comercial sola o suplementada con 11 % de orujo de aceituna o de grasa técnica de matadero. La tasa de fosfolípidos en el músculo de los tres lotes de truchas varió entre 0,70 y 0,93 %. En esta fracción se detectan seis fosfolípidos distintos, siendo la fosfatidilcolina y la fosfatidiletanolamina los mayoritarios; sus valores medios son, respectivamente, 55 y 25 % del contenido fosfolipídico total. A pesar de las diferencias en la

composición de ácidos grasos de las dietas, la única influencia clara se manifiesta en el ácido C-22:6 de la fosfatidiletanolamina muscular.

Palabras claves: Truchas, Fosfolípidos, Acidos grasos.

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