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PHOSPHOLIPID AND FATTY ACID COMPOSITIONS OF THE LIPIDS OF THE EGGS AND CATERPILLARS OF THE SILKWORM, AND OF ARTIFICIAL DIETS AND THEIR COMPONENTS

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The lipids of silkworm eggs in the period of diapause and embryonic and postembryonic development, of artificial feeds, and of the main lipid-containing components of artificial nutrient media have been investigated.

The role of lipids in the metabolism of living organisms, including the silkworm, is exceptionally important. In view of this, the elucidation of the role of lipids in nutrition, and of the biotransformation of lipids in the processes of their digestion and absorption has particular importance. Literature information on the phospholipid and fatty acid compositions of the lipids of the silkworm in the early stages of development is sparse and fragmentary [1, 2].

In the present paper we give the results of an investigation of the composition of the fatty acids of the lipids of silkworm eggs in the periods of diapause and embryonic and postembryonic development and of artificial diets of UzNIISh and the Japanese industry, and also the main lipid-containing components of artificial nutrient media for the silkworm. The results obtained are of general biological and biochemical interest and can be used in the fodder industry, in the processing of mulberry seeds and cocoons for obtaining valuable lipid and biopreparations, the preservation of resources, and in the development of low-waste and waste-free technologies.

Table 1 shows the change in the acid composition of the total lipids of silkworm eggs according to the period of the diapause. On the day of egg-laying, among the saturated fatty acids (FAs) palmitic acid ($C_{16:0}$) stands out, and among the unsaturated acids oleic ($C_{18:1}$) and linolenic ($C_{18:3}$) with a ratio of the total saturated and total unsaturated FAs of 1:3. Attention is attracted by the presence of higher polyunsaturated FAs — arachidonic and eicosapentaenoic. The appearance of the latter is apparently a consequence of marked biochemical rearrangement in the composition of the FAs observed 2-3 days after the laying of the eggs. The importance of these FAs not only for embryogenesis but also for the postembryonic stage of development of the silkworm is difficult to overestimate, since, for example, arachidonic acid is a precursor in the biosynthesis of the prostaglandins [6]. As follows from Table 1, no pronounced differences in the compositions of the FAs connected with sex are observed apart from the results obtained for eicosapentaenoic acid on the 35th day.

On a chromatogram of the polar lipids (Fig. 1), in addition to the main phospholipids (PLs) an unidentified PL, X, was observed which, in its reactions with revealing agents (for inorganic phosphorus, for lipid phosphorus, and, very weakly, with the Dragendorff reagent) resembled sphingomyelin.

Table 2 shows the change in the amounts of PLs in silkworm eggs during diapause. As can be seen from the chromatograms and Table 2, the main PLs among the polar lipids are phosphatidylcholine (PC) and phosphatidylethanolamine (PE). As also in the case of the change in the FAs during the 2-3 days after egg-laying, the amount of PLs during this time undergoes short-term changes. It is precisely this period that is characterized by the beginning of a change in the color of the eggs and in other biochemical and physiological parameters. In the creation of artificial nutrient media (ANMs) for silkworms,

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TABLE 1. Dynamics of the Amounts of FAs in the Total Lipids of Silkworm Eggs in Various Periods of the Diapause

FA	Date of laying	Day of the diapause										
		1	2	3	6 ♀	6 ♂	10 ♀	10 ♂	35 ♀	35 ♂	110 ♀	110 ♂
12:0	0.4	0.4	0.3	0.4	0.4	0.4	0.5	0.4	0.8	0.8	0.9	0.8
14:0	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.2	0.3	0.3	0.3
16:0	20.9	20.3	21.4	19.3	19.9	19.7	19.6	19.4	19.3	18.4	18.6	19.1
16:1	1.2	1.2	1.3	1.3	1.5	1.4	1.5	1.4	1.5	1.6	1.6	1.6
17:0	0.1	0.1	0.1	1.4	0.1	0.1	0.1	0.1	—	0.1	0.1	0.1
18:0	3.2	3.1	3.9	2.2	2.9	3.1	3.0	3.0	3.0	2.8	3.0	3.0
18:1	26.9	26.4	29.0	26.1	26.6	27.6	27.8	27.8	28.0	26.0	28.0	28.1
18:2	9.5	9.7	9.5	9.9	10.0	9.8	10.0	10.5	10.3	10.4	10.6	10.5
18:3	37.1	38.2	34.0	38.5	37.4	36.9	36.8	37.2	36.5	38.5	36.3	35.9
20:1	—	—	—	—	0.1	—	—	—	—	0.1	—	—
20:4	0.6	0.5	0.4	0.7	0.5	0.5	0.4	0.4	0.3	0.6	0.4	0.4
20:5	—	—	—	0.1	0.4	0.4	0.2	0.2	0.1	0.4	0.2	0.2
ΣS	24.7	24.0	25.8	23.4	23.5	23.4	23.3	23.0	23.3	22.4	22.9	23.3
ΣU	75.3	76.0	74.2	76.6	76.5	76.6	76.7	77.0	76.7	77.6	79.1	76.7

TABLE 2. Phospholipid Composition of Silkworm Eggs during Diapause

Phospholipid	Day of laying	Day of diapause										
		1	2	3	6 ♀	6 ♂	10 ♀	10 ♂	35 ♀	35 ♂	110 ♀	110 ♂
PC	37.3	39.5	45.4	57.2	49.4	49.2	51.8	49.0	45.8	52.5	54.5	47.4
LPC	6.1	6.0	4.6	—	3.3	3.3	4.1	3.9	—	3.78	—	—
PE	30.3	25.3	28.6	24.5	25.1	28.0	24.4	24.0	30.6	24.2	25.0	32.0
LPE	4.3	3.9	3.0	—	3.0	3.3	3.7	3.6	—	2.1	—	—
PS	3.8	4.9	3.4	3.6	5.0	3.4	3.0	4.5	3.8	3.2	3.0	3.9
PI	8.5	7.3	6.1	7.7	7.3	5.9	4.6	6.0	7.6	6.8	5.7	5.8
LPC	9.6	13.2	8.9	7.0	6.8	6.8	8.4	9.0	12.0	7.3	12.0	10.9
Total PLs / Total lipids 100%	8.4	7.6	8.1	20.1	9.6	10.2	6.0	6.2	7.1	8.3	11.2	8.6

TABLE 3. Comparative Analysis of the Compositions of the FAs of Oils from Chinese Sybeans and a Local Soybean Variety

Soybean oil	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	Σ_{sat}	Σ_{unsat}	$\Sigma_{sat}/\Sigma_{unsat}$
Chinese product	12.8	3.7	19.1	51.1	13.3	16.5	83.5	1:5
Local variety	7.5	2.7	19.7	55.8	14.3	10.2	89.9	1:8.5

the lipid component is absolutely necessary, since on ANMs without lipid additions the caterpillars do not survive to the 2nd instar. Plant oils (soybean, cottonseed, sunflowerseed, etc.) and β-sitosterol are used as the lipid components of artificial diets.

Table 3 gives the FA compositions of oils obtained by the petroleum ether extraction of flour from Chinese soybeans and from the local soybean variety "Yulduz" grown in interrow plantations of UzNIISh. Both oils, but particularly the oils from the local variety, are rich in unsaturated FAs.

The amounts of PLs in the nonpolar fraction of the lipids of Chinese soybeans, of the local variety, of Argentinian soybeans, and of Argentinian soybean meal are given in Table 4. The main PL in all the soybean sources is lecithin (PC). The meals of industrial samples of both foreign and domestic production contain fairly high levels of phosphatidic acid (PA), which is apparently the result of the action of an endogenous phospholipase D [7].

TABLE 4. Phospholipid Compositions of the Lipids of Soybeans and Soybean Meal

Source	PC	LPC	PE	N-acyl-PE	PI	PA
Chinese soybeans	48.2	6.2	13.5	5.6	16.4	10.1
Local soybeans	35.6	9.1	20.6	9.8	11.4	13.5
Argentinian soybeans	33.4	15.9	13.4	—	20.1	17.2
Argentinian soybean meal	30.5	8.3	20.8	7.1	14.4	18.9

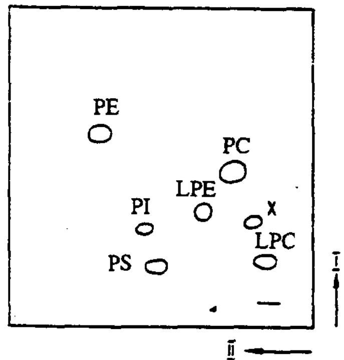

Fig. 1. Two-dimensional thin-layer chromatography of lipid extracts of silkworm eggs: systems: 1) $CHCl_3-CH_3OH-C_6H_6-28\%$ NH_4OH (65:30:10:6); 2) $CHCl_3-CH_3OH-C_6H_6-CH_3COCH_3-CH_3COOH-H_2O$ (70:30:10:5:4:1). PC — phosphatidylcholine; LPC — lysophosphatidylcholine; PE — phosphatidylethanolamine; LPE — lyso-PE; PS — phosphatidylserine; PI — phosphatidylinositol; X — unidentified phospholipid.

Table 5 shows the FA compositions of the phospholipid fractions of polar lipids in soybean preparations. Among the saturated FAs palmitic acid ($C_{16:0}$) predominates in all the PLs of the soybean preparations, and among the unsaturated acids linoleic ($C_{18:2}$).

The FA compositions of the components of artificial diets for silkworms and of the diet mixtures themselves are given in Table 6. In all the components of the diets unsaturated FAs predominated: linolenic and linoleic (in mulberry leaves), oleic and linolenic (in the lipids of the silkworm pupae and caterpillars), oleic and linolenic (in industrial plant oils). A very striking fact is the presence of more than 80% of linoleic acid in mulberry seeds. In characterizing artificial diets it can be stated that with respect to their FA content the Japanese formulations greatly resemble the natural food — mulberry leaves — while those developed in UzNIISh have a deficiency of saturated FAs and a considerable excess of unsaturated FAs.

Thus, comparative results have been obtained on the distribution of PLs and FAs both in living objects (silkworm eggs and larvae) and in specially prepared and commercial components (mulberry leaf powder, lipid preparations of silkworm pupae and seeds, industrial cottonseed and soybean oils) and also in compositions artificially balanced in accordance with the food demands of the silkworm (Japanese and UzNIISh artificial diets). The information obtained in the dynamics of the PLs and FAs of the compositions of a number of components of plant and animal origin permits, in the first place, an evaluation of their biological food value and their use for balancing and making up new diet formulations not only for silkworms but also for other agricultural animals; in the second place, the revelation of sources of polyunsaturated FAs "refined" by their very nature (mulberry seeds); and, in the third place, the monitoring of the FA compositions of lipids and phospholipids on storage and the determination of the biodegradation of agricultural raw material and products obtained from it. The use of the approaches developed in the present paper for comparison and correction and then for the construction of compositions (combined diets and biopreparations) with orientation towards the best natural or previously prepared samples will promote the creation of new highly effective technologies.

TABLE 5. Fatty Acid Compositions of the Phospholipids of Soybean Preparations from Various Sources

Source		12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3	ΣΠ	ΣH
Chinese soybeans	PC	—	—	12.4	0.8	2.7	4.2	71.4	8.5	15.1	84.9
	LPC	2.3	1.0	42.2	3.9	12.1	16.9	18.9	2.7	57.6	42.4
	PE	0.8	0.6	23.7	2.3	6.2	9.6	49.2	7.6	31.3	68.7
	N-acyl PE	—	—	22.7	0.8	2.3	4.4	62.2	7.6	25.0	75.0
	PI	0.6	0.4	37.3	—	7.8	6.8	41.8	5.3	46.1	53.9
	PA	1.3	0.9	38.4	3.5	14.2	18.6	20.4	2.7	54.8	45.2
Local variety	PC	—	0.2	18.7	0.6	4.5	6.0	64.3	5.7	23.4	76.6
	LPC	—	0.8	25.7	—	7.9	22.7	42.1	0.8	34.4	65.7
	PE	—	—	22.0	—	1.6	2.0	66.5	7.9	23.6	76.4
	N-acyl PE	0.4	0.4	17.5	—	8.0	19.3	47.1	7.3	26.3	73.7
	PI	—	—	29.2	—	6.5	13.2	46.1	5.2	35.7	64.3
	PA	0.9	0.6	27.4	1.9	6.99	10.2	45.5	6.6	35.8	64.2
Argentinian soybeans	PC	—	—	13.3	—	3.0	6.5	67.2	10.0	16.3	83.7
	LPC	1.7	1.4	30.9	0.7	9.5	13.9	37.2	4.7	43.5	56.5
	PE	—	—	18.8	—	2.1	5.5	64.3	9.3	20.9	79.1
	N-acyl PE	0.3	0.6	20.4	—	4.3	11.4	51.6	11.4	25.6	74.4
	PI	—	—	31.8	—	9.0	6.6	43.0	8.8	40.8	59.2
	PA	1.2	1.2	21.2	2.4	6.5	8.2	53.4	5.9	30.1	69.9
Argentinian soybean meal	PC	—	—	11.8	—	3.5	6.2	67.3	11.2	15.3	84.7
	LPC	—	—	20.7	—	6.6	12.4	50.4	9.9	27.3	72.7
	PE	—	—	18.6	—	3.6	5.7	61.2	10.9	22.2	77.8
	N-acyl PE	0.8	0.8	25.6	1.5	5.7	9.5	48.5	7.6	32.9	67.1
	PI	—	—	29.4	—	4.1	4.0	54.3	7.5	34.2	65.8
	PA	0.5	0.6	15.8	1.1	4.0	7.8	61.4	8.8	20.9	79.1

EXPERIMENTAL

Eggs of the strain Sovetskaya-5 from the beginning of egg laying and during the various days of diapause of caterpillars and pupae of the silkworm "ripe" for reeling (dried in an IR dryer with the use of a functional ceramic) soybean flour and soybean meals, mulberry leaves of the variety "Tadzhikskaya bessemyannaya" (TB) and variety mixtures employed for seasonal fattening, oils — cottonseed, soybean, and mulberryseed oils — and also samples of the standard UzNIISh diet and those of some Japanese firms for comparison were used. After homogenization in porcelain mortars with quartz sand, samples were

TABLE 6. Analysis of the FAs in Lipid Preparations of Larvae and Pupae and Their Components and in Artificial Diets

Fatty acid	Powder of mulberry leaves			Lipids		Oil			Artificial fodder			
	spring, fodder mixture	autumn, fodder mixture	Autumn, TB*	Pupae	Caterpillars of the 5th instar	Cottonseed	Soybean	Silkworm seeds	Kiodo Sire	Sirkumeita netting	Sirkumeita powder	UzNIISh
12:0	—	—	0.1	—	—	—	—	—	—	0.2	—	—
14:0	0.2	0.6	0.2	0.2	—	—	0.8	—	0.9	0.5	0.5	0.4
14:1	1.2	1.9	0.4	—	—	—	—	—	0.5	0.5	1.0	—
15:0	0.6	0.5	0.5	—	—	—	—	—	0.3	—	0.2	—
16:0	28.2	24.8	18.5	9.9	28.6	11.4	25.4	9.3	28.1	24.3	27.1	15.9
16:1	0.2	0.5	0.2	0.3	—	—	—	—	1.9	1.8	0.8	0.5
16:2	1.0	—	1.4	—	—	—	—	—	0.6	0.6	1.2	—
17:0	—	—	0.1	—	—	—	—	—	—	—	2.2	—
18:0	—	—	—	—	—	—	—	—	—	—	7.7	—
18:0	2.2	2.9	2.4	31.5	0.9	3.1	2.4	3.6	4.1	2.5	3.7	3.9
18:1	2.2	2.2	2.0	19.8	41.1	22.8	18.1	5.8	11.8	5.4	4.3	16.1
18:2	18.9	17.2	18.6	9.3	3.6	54.5	52.2	81.3	20.8	19.3	17.2	40.4
18:3	41.9	49.4	53.6	29.0	25.8	8.2	0.5	—	27.3	44.9	32.1	19.0
20:0	1.2	—	0.8	—	—	—	0.2	—	0.6	—	0.4	0.7
20:2	2.2	—	1.2	—	—	—	0.4	—	3.1	—	1.6	3.1
Σ П	32.4	28.8	22.6	41.6	29.5	14.5	28.8	12.9	34.0	27.5	41.8	20.9
Σ Н	67.6	71.2	77.4	58.4	71.5	85.5	71.2	87.1	66.0	72.5	58.2	79.1

*The Tadzhikskaya bessemyannaya variety

extracted by the Bligh–Dyer method [3]. The separation, identification, and qualitative analysis of the phospholipids were carried out as described in [4, 8]. The FA methyl esters were obtained as in [5] and were analyzed on a Shimadzu CC5A (Japan) and Chrom-41 (Czechoslovakia) chromatographs. The FAs were identified from their retention times and their "carbon numbers."

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