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LIPID AND PROTEIN COMPOSITION AT DIFFERENT DEVELOPMENTAL STAGES OF *PEDICULUS CAPITIS* (ARTHROPODA, PHTHIRAPTERA)

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ABSTRACT: Protein and lipid compositions were studied at different developmental stages of *Pediculus capitis* De Geer 1778. Phosphatidylcholine was found to be the predominant lipid at all stages and in both sexes. Palmitic and oleic acids were the main fatty acids throughout the 3 stages studied. A marked decline was observed in the total lipid content and triacylglyceride concentration during development, suggesting that their consumption is an energy source. The electrophoretic mobility revealed the predominance of a 320-kDa protein in eggs and adult females, whereas 2 major proteins of 514 and 439 kDa were found in nymphs, as well as in male and female adults. Two very high density lipoprotein fractions were isolated by ultracentrifugation of egg cytosol in a density gradient of NaBr. Both reserve lipoproteins contained phospholipids and triacylglycerols as the predominant lipids and a protein band of around 320 kDa. The structure of this band is likely to be similar to that found in females in a vitellogenic state.

Pediculus capitis De Geer 1778 (Insecta: Phthiraptera: Anoplura) is a hematophagous ectoparasite that lives on the head of humans. It is a vector of *Rickettsia prowazecki,* like *P. humanus,* though its proliferation is a matter of controversy (Altschuler and Kenney, 1984). This louse may form, in severe infestations, caps or plates of hair mixed with tegumentary exudation, agglutinated lice, and secondarily infected crusts with a nauseous odor. This clinical manifestation, known as placa polonica (Gougerot, 1924), causes intense and uncomfortable infestations, accompanied by secondary infections and hypersensitivity reactions that are highly significant from both a medical and social point of view (Gillis et al., 1990). This louse is distributed worldwide, with a high degree of prevalence. In Argentina, annual prevalences of >38% have been reported (Castro et al., 1994; Abrahamovich et al., 1996; Ranalleta et al., 1998). Knowledge of the essential aspects of its biology is fragmentary or null with respect to lipid and protein composition and metabolism. Moreover, few data deal with other lice (Phthiraptera) that are parasites of veterinary importance in mammals (Muñoz-Parra et al., 1987, 1988, 1994). More recently, a complete picture has been painted for the domestic pig-louse (*Haematopinus suis*) (Vazquez et al., 1999; Santamaría et al., in press).

Insects have developed a specific system for lipid transport and utilization. Lipid transport via hemolymph is accomplished by a multifunctional lipoprotein–lipophorin (Soulages and Wells, 1994). Whereas lipophorin appears to be common to all insects studied, a number of lipid-poor, very high density lipoproteins (VHDL) were also isolated from insect hemolymph (Beenakkers et al., 1985). In addition to blood lipoproteins common to males and females, oviparous animals contain female-specific lipoproteins, which appear in the plasma during vitellogenesis. They are transferred to the developing oocytes and accumulated as lipovitellins, the egg lipoproteins that provide energy and nutrients for the developing embryos.

The lipid and protein content and composition were studied at each stage of development (eggs, nymphs, and adults of both sexes) of *Pediculus capitis* and are reported herein. Two reserve lipoproteins of the egg vitellus were isolated and characterized as well.

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MATERIALS AND METHODS

Sample collection and homogenate preparation

Ectoparasites were manually extracted from heads of heavily parasitized children, 6 to 10 yr old. Lice were microscopically classified into male and female adults, nymphs, and eggs, then were fasted for 24 hr at room temperature to eliminate blood in the gut. Samples were homogenized on ice manually in 0.25 M sucrose containing 1.4 mM *N*-acetyl-cysteine; 62 mM potassium phosphate buffer, pH 7.4; and 0.5% (v/v) Trasylol (FBA Pharmaceuticals, New York, New York) as a protease inhibitor using a glass and Teflon homogenizer. Cell debris and the unbroken tissue were removed by centrifugation at 1,500 *g* for 20 min. The supernatants were used to determine proteins and lipids.

Fractionation of soluble egg lipoproteins

Eggs from *P. capitis* were weighed, cooled on ice, and homogenized in a Potter–Elvehjem, homogenizer using Tris-HCl buffer 0.02 M, pH 7.5 and 0.2 % (v/v) aprotinine Trasylol (FBA Pharmaceuticals). The ratio of buffer : sample was 5:1 (v/v). Homogenate was centrifuged sequentially at 10,000 *g* for 30 min and at 100,000 *g* for 50 min. Both pellets were discarded, and the second supernatant was dialyzed for 24 hr against NaBr (1.017 g/ml). The dialyzed sample was layered over NaBr (1.26 g/ml) and ultracentrifuged at 207,000 *g* for 22 hr at 10 C in a Beckman L8 70M centrifuge using a SW60Ti rotor. A tube layered with NaBr (δ = 1.07 g/ml) in lieu of the sample was used as a blank for density calculations. Subsequently, aliquots of 200 μl were collected sequentially starting at the top of the tube. Absorbance of each aliquot was determined at 280 nm to obtain the protein profile. Refractive index of the blank tube aliquots was determined with a refractometer (Bausch and Lomb, New York, New York) and converted to density (g/ml) using tables from Lindgren (1975).

Lipid extraction and analysis

Total lipids were extracted with chloroform–methanol (Bligh and Dyer 1959). The qualitative and quantitative determination of the lipid classes was performed by thin layer chromatography coupled to a flame ionization detector in an Iatroscan apparatus model TH-10 (Iatcom Laboratories, Tokyo, Japan), after separation on Chromarods type S-III (Iatcom Laboratories). Ackman et al. (1990) described this technique thoroughly. Hydrocarbons were separated from the other neutral lipids by development in hexane–benzene (70:30, v/v). Polar lipids were resolved by developing the rods in chloroform–methanol–water (70:25:3, v/v/v) and benzene–chloroform–formic acid (70:25:1, v/v/v). All lipid classes were quantified by comparison with known amounts of standards run under the same conditions and using monoacylglycerol as the internal standard. Total lipids were calculated by the summation of individual weights. The general procedure for separation, identification, and quantification of lipid classes was similar to that described in a previous work (Cunningham and Pollero, 1996).

TABLE I. Determination of lipid and protein content in homogenates of *Pediculus capitis* at different developmental stages.*

	Protein (mg/g)	Lipid (mg/g)
Egg	38.7 ± 0.3	3.60 ± 0.07
Nymph	16.7 ± 1.9	1.84 ± 0.02
Female	24.2 ± 0.4	1.72 ± 0.05
Male	10.6 ± 1.8	3.09 ± 0.03

* Results are averages of 4 determinations ± SD.

TABLE II. Composition of the main fatty acids from different development stages of *Pediculus capitis* (as a percentage of total fatty acids).*

Fatty acid	Egg	Nymph	Male	Female
Palmitic	30.6	38.5	23.9	33.3
Palmitoleic	21.5	9.2	6.8	10.4
Stearic	4.2	13.8	6.9	6.4
Oleic	32.8	28.0	51.3	40.3
Linoleic	7.5	7.6	11.1	9.6

* Fatty acids from total lipids were derivatized and analyzed as methyl esters by gas–liquid chromatography. Other minor fatty acids complete for 100%.

Characterization of proteins

Total protein concentration in each homogenate was measured colorimetrically (Lowry et al., 1951). Aliquots for each homogenate were analyzed by native electrophoresis. Nondissociating electrophoresis analysis was done using 4–23% gradient polyacrylamide gel electrophoresis (PAGE). Proteins were visualized with Coomassie brilliant blue R-250 stain (Sigma Chemical, St. Louis, Missouri). Molecular weight standards (Pharmacia, Uppsala, Sweden) were run in adjacent lanes.

Fatty acids analysis

Aliquots of total lipids from homogenates were saponified and the fatty acids were extracted with hexane after acidification and esterified with 10% boron trifluoride in methanol. Fatty acid methyl esters were analyzed by gas–liquid chromatography on an Omegawax 250 (Supelco, Inc., Bellefonte, Pennsylvania) (30 m × 0.25 mm, 0.25 μm film) capillary column in a Hewlett Packard HP-6890, equipped with a flame ionization detector. The column temperature was programmed for a linear increase of 3 C/min from 175 to 230 C. Fatty acid identification was done as described previously (Gaspar et al, 1997).

RESULTS

The protein and lipid concentration in total homogenates of *P. capitis* at different developmental stages is shown in Table I. They were relatively high in eggs, but they sharply decreased in the nymph stage. Protein content increased in adult females, and lipids increased in adult males.

Figure 1 shows the quali-quantitative composition of the different lipid classes at different developmental stages. Phosphatidylcholine is the predominant lipid in each stage and sex, followed by triacylglycerols, cholesterol, phosphatidylethanolamine, and hydrocarbons. An esterified sterol fraction, mainly composed of cholesterol esters, is present in nymphs and adult males, but it was not detected in eggs and females.

Table II shows the fatty acid composition of total lipids extracted from lice at different developmental stages. Only the major fatty acids were considered. Oleic and palmitic acids were the predominant lipids at the 3 developmental stages, although it must be noted that oleic acid was markedly increased in adults.

Electrophoretic mobility of the proteins revealed, under native conditions, a predominant band of 320 kDa in eggs, as well as in adult females, and 2 prominent bands of 514 and 439 kDa, which were detected in nymphs and adult males and females (Fig. 2).

The soluble proteins in the cytosol fraction of the eggs were separated by ultracentrifugation in density gradients. Density and protein concentration in each fraction were determined (Fig. 3). The protein profile shows the presence of 2 peaks with densities of 1.22 and 1.23 g/ml, respectively. Fractions belonging to both maxima were joined and identified as lipovitellin I (LV-I) and lipovitellin II (LV-II), respectively. Protein and lipid classes found in each lipovitellin were determined. The native PAGE of the lipoprotein fractions isolated from the egg cytosol

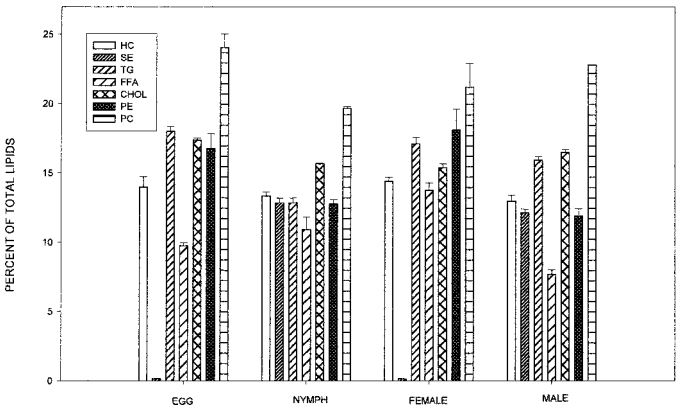

FIGURE 1. Lipid class composition of total homogenates from different development stages of *P. capitis*. HC, hydrocarbons; SE, sterol esters; TG, triacylglycerols; FFA, free fatty acids; CHOL, cholesterol; PE, phosphatidylethanolamine; PC, phosphatidylcholine. Results are averages of 3 determinations ± SD.

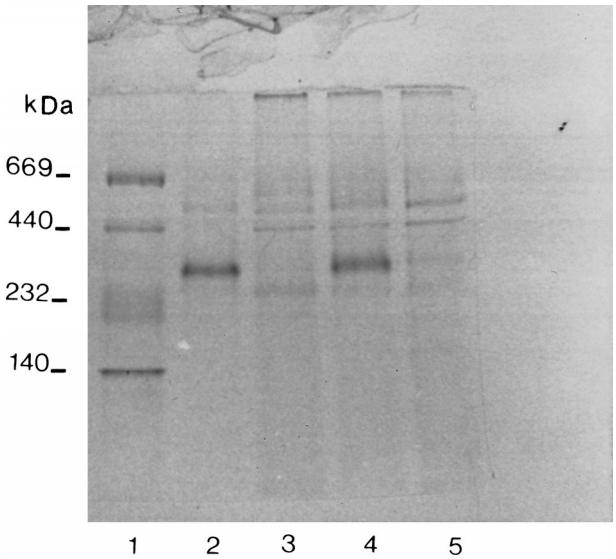

FIGURE 2. Native PAGE analysis (4–23% acrylamide) of total homogenate of egg, nymph, and males and females from *Pediculus capitis*. Lane 1: molecular weight standards (kDa). Lane 2: eggs. Lane 3: nymphs. Lane 4: female. Lane 5: male.

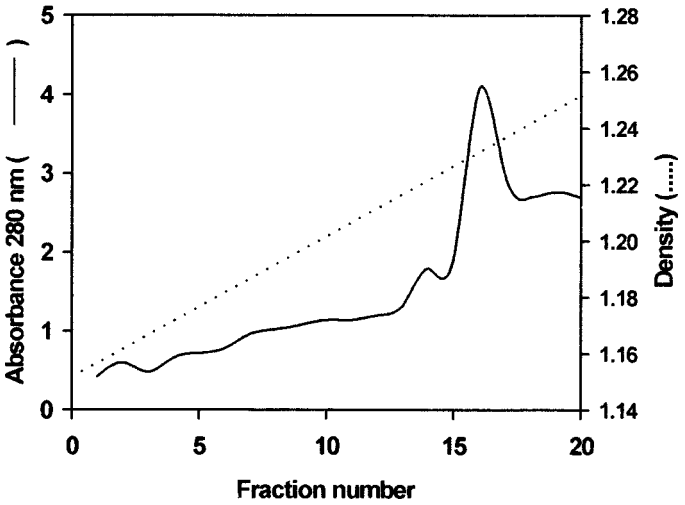

FIGURE 3. Protein and density profiles of egg cytosol fractions separated by ultracentrifugation in a density gradient of NaBr (1.26 g/ml). The absorbance was measured at 280 nm.

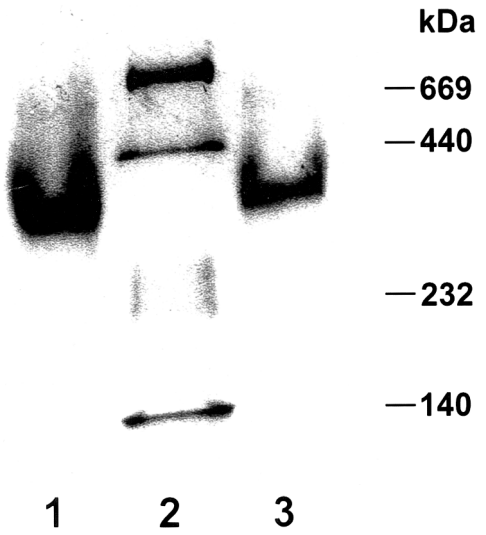

FIGURE 4. Native PAGE analysis (4–23% acrylamide) of isolated lipoprotein fractions of cytosol of *P. capitis* eggs obtained by ultracentrifugation in a density gradient. Lane 1: lipovitellin I. Lane 2: molecular weight standards. Lane 3: lipovitellin II.

is shown in Figure 4. Each isolated fraction contained a single protein; LV-I has a protein band of 320 kDa, and LV-II has a band of 370 kDa.

Table III shows the lipid composition of LV-I and -II. The prevailing lipid in both lipovitellins was phosphatidylcholine, followed by triacylglycerides in LV-I, and triacylglycerides and hydrocarbons in LV-II. Considerable quantities of phosphatidylethanolamine and small amounts of free fatty acids complete the total lipid spectrum. Both lipovitellins showed differences in their lipid : protein ratios.

DISCUSSION

The composition and metabolism of lipids, as well as their transport by means of lipoproteins, have been widely studied in insects that were large enough to allow the separation of organs and hemolymph. The present study was performed using only homogenates of total organisms because of the small size of the lice. Results revealed significantly higher total lipid and protein contents in eggs than in nymphs, which suggests an accumulation of energetic and structural reserve materials during vitellogenesis and a late consumption during embryonic development. However, these estimations of variations of total molecular groups are limited and only suggest general trends. Consequently, it was necessary to emphasize the analysis of the molecular classes from both proteins and lipids to obtain valid conclusions related to the physiology of these insects.

Triacylglycerides, the typical lipids of energy reserve, are the major lipids consumed before eclosion of the eggs, probably to support the metabolic effort implied in embryogenesis. The carbon needed in carbohydrate and protein synthesis during embryogenesis could also be supplied by degradation of triacylglycerides. These lipids are then maintained in a relatively low concentration during the nymph stage. In contrast, phosphoglycerides show a comparatively lesser decrease from egg to nymph stage because their function is mainly structural. Cholesterol, which plays several metabolic roles such as hormonal precursor or membrane constituent, is similar in behavior to phospholipids. At more advanced developmental stages, triacylglycerols increase again, mainly in adult females, which may be related to the need for accumulation of enough energy and of a carbon reservoir in the developing new vitellum. The variations of triacylglycerides from egg to nymph are coincident with those reported for *H. suis* (Santamaría et al., 1999), although they are not the same for phospholipids. These results are not similar to the triacylglyceride results in adults of *H. suis* because these lipids are scarce in female and male hog lice. Thus, the metabolic role of triacylglycerides can be different in both lice species. The fatty acid spectrum found in *P. capitis*, where oleic and palmitic acids predominate, is similar to that reported for adults of *H. suis*. However, unlike *H. suis*, the concentration of oleic acid was significantly lower in eggs of *P. capitis* and that of palmitoleic acid significantly higher than in adults. The low concentration of arachidonic acid found in both lice species is noteworthy because, as hematophagous parasites, the diet is abundant in this essential fatty acid. It may be that in lice, as has been described for other hematophagous insects (Brenner and Bernasconi, 1987), the synthesis of prostaglandins metabolizes the available arachidonic acid rapidly.

The analysis of tissue proteins performed under native conditions showed 2 strong protein bands of 439 and 514 kDa in adults of both sexes and in nymphs and a prominent band of

TABLE III. Lipid class composition of egg lipovitellins of *Pediculus capitis* (% wt/wt).

Lipid classes	LV-I	LV-II
Hydrocarbons	10.9 ± 0.2	17.1 ± 0.8
Triacylglycerides	13.7 ± 0.2	15.7 ± 0.2
Free fatty acids	5.6 ± 0.9	6.8 ± 0.9
Phosphatydilethanolamine	10.8 ± 0.4	14.0 ± 0.4
Phosphatydilcholine	59.1 ± 4.8	46.4 ± 3.5
Total lipids	14.6	7.1
Total proteins	85.4	92.9

320 kDa in adult females and eggs. This fact, together with the markedly high concentrations of triacylglycerides in eggs and adult females, suggested the existence of a vitellin lipoprotein that accumulates reservoirs of triacylglycerides in eggs and that contains an apoprotein similar to the one mentioned.

Lipovitellins are found in every oviparous species examined so far. They have been characterized in insects (Hagedorn and Kunkel, 1979), crustaceans, sea urchins, bivalve and cephalopod mollusks (Lee, 1991), and gastropods (Garin et al., 1996). Most are VHDL, and others can be classified as high-density lipoproteins (HDL). They usually contain more than a single apoprotein associated with variable quantities and qualities of lipids, with a predominance of phospholipids in some cases and triacylglycerides and cholesterol in others. Using density gradient ultracentrifugation, 2 vitellin fractions with VHDL characteristics were detected in cytosol isolated from *P. capitis* eggs. The major difference between both lipovitellins is found in the protein : lipid ratio, resulting in different densities in the gradient centrifugation. They contain the same lipid classes, but in different proportions. Although phosphoglycerides as a whole are the predominant lipids, triacylglycerides and hydrocarbons represent an important part of the neutral lipid portion. The function of these lipid reserves in the vitellum is to provide energy and the necessary materials for embryo development. Both lipovitellins contain a single apoprotein, which has a similar molecular weight to that of eggs and adult females. Its absence in nymphs suggests that it is consumed, probably providing structural precursors for the embryo tissues. The consumption of the protein moiety of the lipovitellins during embryogenesis has been demonstrated in other invertebrates during embryo development (Heras et al., 1998). Modifications of protein portions of native lipovitellins have also been reported in earlier stages of vitellogenesis (Giorgi et al., 1997).

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