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Life stage-related differences in fatty acid composition of an obligate ectoparasite, the deer ked (*Lipoptena cervi*)—Influence of blood meals and gender

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ABSTRACT

Metamorphosis and diet often influence fatty acid (FA) signatures (FAS) of insects. We investigated FAS in a hematophagous ectoparasite, the deer ked (*Lipoptena cervi*). Deer keds shed their wings upon attachment on the host and, thus, the FAS of an individual blood-fed imago/pupa in the fur of its host can be traced back to the blood FA profile of a single moose (*Alces alces*). Host blood and different life stages of deer keds were investigated for FA by gas chromatography. The FAS of life stages resembled each other more closely than the diet. Blood meals modified the FAS of both sexes but the FAS of the blood-fed females were closer to those of the prepupae/pupae. The parasitizing males had higher proportions of major saturated FA (SFA) and polyunsaturated FA (PUFA) than the females, which contained more monounsaturated FA (MUFA) with higher ratios of n-3/n-6 PUFA and unsaturated FA (UFA)/SFA. The proportions of 16:1n-7 were <1% in the blood but 18% (males) and 29% (females) in the blood-fed keds. Allocation of lipids to offspring by the females and possible accumulation of PUFA in male reproductive organs may have induced these sex-related differences. MUFA percentages and UFA/SFA ratios increased while SFA and many PUFA decreased from the reproducing females to the pupae. The diapausing pupae displayed lowered n-3/n-6 PUFA ratios and could have mobilized 16:0 and 18:3n-3 for the most fundamental metabolic processes. In conclusion, FAS are modified through the life stages of the deer ked possibly due to their different FA requirements.

1. Introduction

Lipids serve as energy stores and structural components of biomembranes in insects (Stanley-Samuelson et al., 1988). In addition, fatty acids (FA) are involved in the formation of eicosanoids, pheromones as well as cuticular and defensive secretions. Hematophagous insects can ingest large amounts of blood per meal and, during digestion, free FA are released in the midgut lumen (Atella et al., 2005). The absorbed FA are used in the synthesis of lipids (triacylglycerols [TAG], phospholipids [PL], etc.) in the midgut epithelium. Lipids are transferred to circulating lipophorin and transported to organs, such as the fat body and ovaries, to be stored or utilized. The resulting body FA signatures (FAS) of insects generally reflect their diet (Hood-Nowotny et al., 2012; Sighinolfi et al., 2013). Regarding fat utilization, most of the lipids in the fat body are in the form of TAG, and during fat mobilization lipids are released as diacylglycerols (Canavoso et al., 2001).

In addition to Δ9-, Δ6- and Δ5-desaturation, insect species of several orders are capable of Δ12-desaturation transforming 18:1n-9 into 18:2n-6, which is dietarily essential to vertebrates (Stanley-Samuelson et al., 1988). At present, however, there are no reports of dipterans having verified biosynthesis of 18:2n-6. Some polyunsaturated FA ([PUFA]; 20:4n-6, 20:5n-3, 22:6n-3) are required for normal growth and flight of mosquitoes (Moribayashi et al., 2004), and eicosanoids derived from certain C20 PUFA, especially from 20:4n-6, may control oogenesis and egg-laying behavior in insects (Atella et al., 2005). Some dipterans do not seem to require or biosynthesize C20–22 PUFA (Shen et al., 2010).

The previously reported changes in FA composition during insect metamorphosis have been variable. In some species, more unsaturated FA (UFA) than saturated FA (SFA) accumulate during larval growth but later the proportion of SFA increases (Fast, 1971). In the dipteran *Agria affinis*, 16:0, 16:1n-7 and 18:1n-9 decreased in proportion during pupation, while 18:2n-6 increased from pupae to adults (Barlow, 1965). Changes reminiscent of these were also observed in other dipterous insects (Takata and Harwood, 1964; Teague et al., 1986; Moribayashi

Abbreviations: ANOVA, analysis of variance; DBI, double bond index; Δ9-DI, Δ9-desaturation index; FA, fatty acid; FAME, fatty acid methyl ester; FAS, fatty acid signature; FID, flame ionization detector; MUFA, monounsaturated fatty acid; PCA, principal component analysis; PL, phospholipid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; T_a, ambient temperature; TACL, total average chain length; TAG, triacylglycerol; UFA, unsaturated fatty acid.

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et al., 2004). In some dipterans, the FA composition of TAG remained relatively unchanged during metamorphosis (Madariaga et al., 1974), while the proportions of long-chain PUFA could increase in the PL fraction (Moribayashi et al., 2004).

The deer ked (*Lipoptena cervi*, Hippoboscidae, Diptera) is an obligate ectoparasite infecting cervids (Hackman et al., 1983; Paakkonen et al., 2014). Winged imagines emerge in late summer–autumn and wait for a potential host to pass by. Both sexes are blood-feeders and shed their wings upon attachment on the host. In eastern Finland, moose (*Alces alces*) have harbored up to 17,500 deer keds (Paakkonen et al., 2010). According to laboratory experiments in the former Soviet Union (Ivanov, 1981; but see also Paakkonen et al., 2012), blood-feeding starts in 0.5–1 h after attachment and 2–3 mg of blood is consumed 15–21 times within the 24-h cycle. The ingested volume increases to 4–5 mg after a few days. After mating, hippoboscid larvae develop one at a time in the uterus nourished by the milk-like secretion of the accessory reproductive glands (Metcalf and Metcalf, 1993). The female deposits a total of 20–25 pupae (Popov, 1965) that drop from the fur to the forest floor, often on bedding sites of the host animal (Kaunisto et al., 2009). In winter, the fallen pupae are eventually deposited within the snow cover. The freezing point of non-acclimated diapausing pupae can be −26 °C enabling survival during serious frosts (Härkönen et al., 2012). The off-host life stages do not feed and, thus, all energy reserves required for metamorphosis originate from maternal provisioning during the larval stages. In addition to diapausing pupae, winged imagines can encounter subfreezing ambient temperatures (T_a) in autumn between emergence and host location, and the contribution of FA to their autumnal cold-hardening was examined previously (Nieminen et al., 2013). The other life stages have not been examined for FAS.

The deer ked offers a unique model to investigate the modifications of FAS from the host blood through the different life stages of the parasite, as the FA composition of an individual deer ked (when extracted from the fur as an imago or a pupa) can be traced back to the blood FA profile of a single moose. We hypothesized that FAS of the deer ked would differ between the life stages. *i*) Winged, unfed imagines fast and wait for a potential host. As the host location and attachment flight is short (Hackman et al., 1983) and presumably fueled by carbohydrates (Yuval et al., 1994), winged adults probably require FA to sustain basic metabolic functions and sufficient cold tolerance. They should have a high degree of unsaturation in the acyl moieties of their membrane lipids to survive freezing nights and to be able to fly during daytime in late autumn. *ii*) On-host deer keds feed regularly on blood, mate and produce offspring in a warm environment. Unsaturation of lipids is probably less important during this stage, but there is presumably a major effect of blood FA composition on the FAS of fed keds. Females are likely to have specific requirements for FA during oogenesis and intrauterine feeding of larvae, while spermatogenesis in males could influence their FAS. *iii*) Diapausing pupae do not operate actively in the environment but overwinter in a metabolically frugal way. They presumably still need some FA oxidation to produce energy for the maintenance of the most fundamental metabolic processes but, at the same time, the physical properties of membrane and storage lipids need to be adjusted to low T_a by structural modifications of the acyl chains.

2. Material and methods

The flying deer keds were collected by hand with rubber gloves in Liperi commune, eastern Finland (6932946N, 606525E) on September 4, 2012, and frozen at −70 °C. The on-host life stages were extracted from skins of moose (1 cow, 2 male calves, 3 female calves) harvested in the same area (within the coordinates: 6936862N, 610850E; 6935553N, 610544E; 6931932N, 609873E; 6930372N, 616878E; 6935374N, 615543E) during the hunt on October 15–17, 2012, and the diapausing pupae were collected from the topmost layer of snow on 1–3-day-old moose bedding sites (6936600N, 610193E) on February 18, 2013. The T_a varied from −3 to −7 °C in the area between the falling of the pupae from moose and their collection (http://www.wunderground.com/history/airport/EFJO/2013/2/18/DailyHistory.html). The moose blood samples were obtained immediately after killing from cut jugular blood vessels into BD Falcon™ test tubes containing ethylenediaminetetraacetic acid. The whole blood was stored at −70 °C until lipid transmethylation producing derivatized FA for gas chromatography (GC). After skinning, the anterior back that contains a large proportion of deer keds (Paakkonen et al., 2010) was sealed in a plastic bag and frozen at −20 °C for 7–19 days. In the laboratory, the hair was cut with scissors and the keds were extracted and frozen at −70 °C. The sex of the imagines was determined microscopically according to Paakkonen (2012). To obtain adequate concentrations of FA for analyses, 3–4 imagines, prepupae or pupae were combined for each final sample. The categories and numbers of final samples were as follows: moose blood (n = 6), winged unfed adult males (n = 5) and females (n = 5), blood-fed adult males (n = 18) and females (n = 18), prepupae inside females (n = 18), pupae inside females (n = 18), pupae in fur (n = 11) and pupae on snow (n = 9).

The samples were transmethylated in methanolic H_2SO_4 under nitrogen atmosphere (Christie, 1993). The formed FA methyl esters (FAME) were extracted with hexane and the dried and concentrated solutions were injected for quantitative analysis into a Shimadzu GC-2010 Plus gas chromatograph (Shimadzu, Kyoto, Japan) employing a ZB-WAX capillary column (length 30 m, internal diameter 0.32 mm, film thickness 0.25 μm; Phenomenex, Torrance, CA, USA) and a flame ionization detector (FID). The temperatures used for the GC were as follows: injector 250 °C, detector 280 °C, column T-program 180 °C for 8 min, and rate 3 °C/min to 210 °C, held until 40 min. Sample solutions (2 μl) were injected in split mode (1:35) using helium as the carrier gas with a column flow of 1.3 ml/min. The integrated areas of the resulting chromatographic peaks were checked and corrected manually by using the GCsolution software by Shimadzu. The peak areas of the FID chromatograms were converted to mol% by using the theoretical response factors for FID (Ackman, 1992) and calibrations with quantitative authentic standards (Supelco, Bellefonte, PA, USA). The results represent the FA composition of the total lipids. The identification of the FAME was based on the use of several authentic standard mixtures (Supelco) and natural standards of known composition, which together covered most FAME identified in the deer ked samples. In addition, the identifications were further confirmed by mass spectrometry by using a 6890N network GC system with a 5973 mass selective detector (Agilent Technologies, Santa Clara, CA) equipped with a DB-WAX capillary column (J&W Scientific, Folsom, CA). The spectra were compared with those of the standards and published reference spectra (Christie W.W., http://lipidlibrary.aocs.org/ms/masspec.html). The total average chain length (TACL), Δ9-desaturation index (Δ9-DI) and double bond index (DBI) were calculated as previously (Nieminen et al., 2013). Fractionation coefficients between the dietary and body levels of each FA were calculated as: (mol% in a blood-fed deer ked)/(mol% in blood of its host).

Multiple comparisons between the values of the moose blood and the deer ked life stages were analyzed with the nonparametric Kruskal–Wallis analysis of variance (ANOVA) and the post hoc Dunn's test (SPSS *v*19 software package, IBM, Armonk, NY, USA). Comparisons between two study groups (e.g., blood-fed male keds vs. blood-fed female keds) were performed with the Mann–Whitney U test. The p value <0.05 was considered statistically significant. The results are presented as the mean ± SE. To analyze the relationships between the FAS of the blood and different life stages, the data were subjected to the multivariate principal component analysis (PCA) using the Sirius *v*6.5 software package (Pattern Recognition Systems AS, Bergen, Norway; Kvalheim and Karstang, 1987). In PCA, the data were standardized and the relative positions of each sample and FA were plotted by