Comparison of fatty acid contents of the neutral and phospholipids of the trematode *Paramphistomum cervi* and liver of its host, *Capra hircus*

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Abstract The present observation records the lipid classes and their fatty acid composition of the neutral lipid and phospholipid of the trematode *Paramphistomum cervi* and liver of its host *Capra hircus*, the common Indian goat. Thin Layer Chromatography and Gas Liquid Chromatography were used to identify different neutral lipid and phospholipid components. The results show that among the neutral lipid fractions, the amount of combined hydrocarbon, wax ester and steryl ester is more or less equal in parasite and its host, but the percent of triacylglycerol is more in host liver than the parasite and the percent of total sterol is more in parasite than that of its host liver. Among the phospholipid fractions, phosphatidylinositol and phosphatidylethanolamine in the trematode parasite *P. cervi* and phosphatidylcholine and cardiolipin in the host's liver are the major components. The predominant fatty acids in the neutral lipid and phospholipid fractions of both the host liver and the trematode *P. cervi* include C_{16:0}, C_{18:0}, C_{18:1}, and C_{18:2}. The results reveal that the parasite *P. cervi* take up almost all the lipid and fatty acids from their host, which is required for their life cycle.

Keywords Cardiolipin · Cholesterol · Hydrocarbon · Esters · Trematode parasite · Triacylglycerol

Introduction

Trematodes are suctional feeders and show variations in their feeding mechanisms (Halton 1967; Smyth and Halton 1983). They acquire food materials from the fluids, semi-fluids, soft tissues or cell membranes of their hosts. Most trematodes suck in food through the action of their oral sucker, pharynx and also absorb food materials through their tegument (Smyth 1994). Lipids have some important role in trematode. Phospholipids form lipid anchors for protein antigen. Lipids in general probably function as replacement of surface membrane (Roberts et al. 2000). They also play various interesting roles during the life cycle of schistosomes and in their defense against the immune system of the host. They are used as energy source in developmental stages of the parasite *Fasciola hepatica* (Humiczewska and Rajski 2005). Trematodes are unable to synthesize most essential fatty acids and they have to acquire exogenous lipids from their host. The amount of total lipids, phospholipids, neutral lipids and fatty acids was determined in cattle parasites, namely the trematodes *Eurytrema pancreaticum*, *Calicophoron erschowi*, and in the free-living turbellarian *Penecurva sibirica* (Vykhrestyuk and Yarygina 1982). They observed neutral lipids of these flatworms contained sterols, sterol esters, triacylglycerol, and free fatty acids. *Penecurva sibirica* also contained diacylglycerols. Parasitic and free-living flatworms differed in phospholipid composition; the turbellarian did not contain phosphatidylserine, while the trematodes had practically no sphingomyelin or lysophosphatidylcholine. *E. pancreaticum*, *C. erschowi*, and *P. sibirica* contained high levels of phosphatidylcholine and phosphatidylethanolamine as well as lysophosphatidylethanolamine.

In our previous study (Ghosh and Misra 2011) we found that the level of total lipid (TL), neutral lipid (NL), glycolipid (GL) and phospholipid (PL) of both the host liver

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and the parasite *Paramphistomum cervi* were 4.56 and 1.31 %, 37.83 and 49.4 %, 7.80 and 8.07 %, 54.37 and 42.53 % respectively.

The aim of the present work, in continuation to previous work (Ghosh and Misra 2011), is to investigate the requirement of lipid fractions of the neutral and phospholipid for their life history strategy of the trematode in comparison to its host. The present work was designed to estimate the lipid fractions of the neutral lipid and phospholipid and their fatty acid compositions of both the parasite and liver of its host.

The hypothesis of the work is that parasite would absorb majority of the required neutral and phospholipid fractions and fatty acids from the rumen and reticulum fluid than those by the host. Probably the parasite would absorb more amount of sterol than the host for efficient reproductive strategy. The parasite needs to preserve energy for reproduction as they are unable to synthesize fatty acids *de novo*.

Materials and methods

The digenetic trematode parasite *P. cervi* were collected from rumen and reticulum of stomach of common Indian goat *Capra hircus*, from Khardah area, district North 24 Paraganas, West Bengal, India. The parasite samples were pooled from 10 hosts to achieve a wet weight of 100 g. Goat liver (from the same goat from which the parasites were isolated) was also pooled. In total, 40 g of goat liver was taken for analysis. The goat liver sample and the worms were washed in distilled water and kept in the freezer at -20°C for further analysis.

Total lipids were extracted from the goat liver and parasite samples following the method of Bligh and Dyer (1959). According to Rouser et al. (1976) a portion of the total lipid of the samples was subjected to Column chromatography to obtain lipid classes. Neutral lipid components are separated by thin layer chromatography. The reagents like, 2'-7'-dichlorofluorescein, Liebermann-Burchard were used to detect neutral lipid components. During fractionation of neutral lipid by preparative TLC hydrocarbon bands were overlapped by wax ester band. The overlapping hydrocarbon and wax ester bands were re-chromatographed by TLC using n-hexane: diethyl ether (98:2 v/v) as solvent system (Misra and Ghosh 1991). Phospholipid components are also separated by thin layer chromatography. Suitable staining reagents were used to identify the different classes of phospholipid. Specific spray reagents which were used to identify phospholipid components were Molybdenum blue reagent, Dragendorff's reagent, Ninhydrin reagent, etc. Fatty acid methyl esters (FAME) of the fractions of the lipid classes were prepared by the process of transmethylation according to

Christie (2003). According to the method of Mangold (1969) and Misra et al. (1984) fatty acid methyl esters were purified by TLC. GLC of fatty acid methyl esters were done on a Chemito 1000 instrument, equipped with Flame Ionisation Detector (FID). Quantization was done by computer using specific Clarity Lite Software (Data Apex Ltd., Praha, Czech Republic). Sterol samples were derivatised to their volatile trimethyl silylether derivatives for GLC analysis. Identification of fatty acids was done by comparing their retention times with those of standards and chromatograms were made under identical operational conditions of GLC. Confirmation of fatty acid identifications were done, using cod liver oil fatty acid methyl esters as secondary standard (Ackman and Burgher 1965). The alcohols obtained from the wax ester of NL were silylated according to Sweely et al. (1963). TMS derivatives of alcohol were preserved for GLC analysis. Hydrocarbons were analyzed by directly injecting the samples into the GLC machine, without derivatization. Identifications were made by comparison of retention times of the peaks with those of standards.

Results

Initially, total lipid was extracted from goat liver and parasite *P. cervi*. Different components of neutral lipids (NL) mainly, hydrocarbons (HC), wax esters (WE), sterol esters (SE), triacylglycerols (TG), total sterol (ST) and of phospholipids (PL) *i.e.* cardiolipin (CL), phosphatidylethanolamine (PE), phosphatidylcholine (PC), phosphatidylinositol (PI) phosphatidylserine (PS); and sphingomyelin (SPH), and their fatty acids of both the goat liver and the parasite were analyzed.

The percent of combined HC + WE + SE occupied major portion of the neutral lipid fractions in both the goat liver and the trematode *P. cervi*. Among the fractions of the phospholipid, PC, CL, and PE in goat liver and PI and PE in parasite are the major fractions (Table 1). Comparison of different NL and PL fractions of the goat liver and the trematode parasite are represented in Figs. 1 and 2, respectively.

Fatty acid composition of NL components of the host liver and the parasite

The hydrocarbon profile shows (Table 2) that among the saturated fatty acids, the amount of $\text{C}_{20:0}$ is highest in both the liver and the parasite. The percentages of saturate, *iso*-, and *anteiso*- acids in the parasite and in the host liver are more or less equal.

Wax Ester: Liver is rich in $\text{C}_{16:0}$, $\text{C}_{18:0}$, $\text{C}_{22:0}$, $\text{C}_{18:1}$, $\text{C}_{18:2}$, $\text{C}_{18:3n-3}$, and $\text{C}_{22:4}$ acids. However the parasite is rich

Table 1 Composition of various classes of lipids obtained from goat liver and the trematode parasite *P. cervi*

Lipids	Liver	Parasite
Neutral Lipids (NL) ^a	37.83	49.4
Phospholipids (PL) ^a	54.37	42.53
(HC + WE + SE) ^b	61.63	61.1
Triacylglycerol (TG) ^b	15.55	6.55
Total Sterol (ST) ^b	22.81	32.35
Hydrocarbon (HC) ^c	76.2	88.63
Wax Ester (WE) ^c	14.76	5.01
Steryl Ester (SE) ^c	9.04	6.36
Cardiolipin (CL) ^d	22.15	13.43
Phosphatidylethanolamine (PE) ^d	20.77	27.27
Phosphatidylcholine (PC) ^d	47.82	11.98
Phosphatidylinositol (PI) ^d	3.09	31.82
Sphingomyelin (SPH) ^d	4.37	10.23
Phosphatidylserine (PS) ^d	1.81	5.27

^a Expressed as % w/w of total lipids^b Expressed as % w/w of neutral lipids^c Expressed as % w/w of (HC + WE + SE)^d Expressed as % w/w of Phospholipid **Fig. 1** Comparison of different neutral lipid fraction of goat liver and the trematode parasite

in $C_{14:0}$, $C_{16:0}$, $C_{18:0}$, and $C_{16:1}$ acids. Interestingly, $C_{16:1}$ acid is not recorded in the liver whereas it is present in high amount in case of the parasite (Table 3).

Steryl Ester: Table 4 shows that the fatty acids $C_{16:0}$, $C_{18:0}$, $C_{18:1}$ and $C_{18:2}$ are present in considerable amount in both the parasite and the liver.

Triacylglycerol: Among the saturated fatty acids, $C_{16:0}$ and $C_{18:0}$ and among the unsaturated fatty acids, $C_{18:1}$ and $C_{18:2}$ are present in higher amount than others in both the samples (Table 5).

The comparative account of WE, SE and TG is represented in Fig. 3. The percentage of saturated fatty acid is higher in both the goat liver and the parasite in case of WE and SE but in case of TG liver is rich in saturated fatty acid whereas, parasite is rich in monoenoic acid.

The amount of saturated fatty acid of the parasite is just over 97.3 % whereas, it is 55.5 % in the liver in the wax ester/alcohol (Table 6). The parasite is rich in $C_{16:0}$, $C_{18:0}$, $C_{20:0}$, and $C_{22:0}$ acids of which the last named acid constitutes over 25 % of the fatty acids. In contrast, liver exhibits much higher percent (35.3 %) of $C_{18:0}$ (*iso*). The amount of *iso*-acids in the parasite is very small. The amount of saturated acids is higher than the *iso*- (branched) acids in both the liver and the parasite.

Composition of free sterol and sterol from steryl ester of host's liver and parasite

The composition of free sterol and sterols from steryl ester of host's liver and parasite are presented in Table 7.

Among the free sterols coprostanol is mainly found in the liver and *Cis*-22-dehydrocholesterol and *Trans*-22-dehydrocholesterol are present in high amount in the liver but absent in the parasite. Cholesterol and campesterol are present in good amount in the parasite but present in very

Fig. 2 Comparison of different phospholipid fractions of goat liver and the trematode parasite

Table 2 Fatty acid composition of hydrocarbon of NL of goat liver and the trematode parasite *P. Cervi*

Saturates	Liver	Parasite	Iso	Liver	Parasite	Anteiso	Liver	Parasite
12:0	7.90	1.70	12:iso	–	0.90	21:anteiso	0.40	0.40
14:0	1.00	4.60	14:iso	–	0.30	22:anteiso	1.00	0.70
16:0	1.00	2.20	16:iso	0.10	0.10	23:anteiso	0.80	0.80
17:0	3.20	4.30	17:iso	0.80	1.30	24:anteiso	0.60	0.50
18:0	7.70	7.40	18:iso	1.90	1.60	25:anteiso	0.20	0.30
19:0	6.80	7.20	19:iso	2.00	2.10	26:anteiso	0.20	0.10
20:0	9.50	9.30	20:iso	2.50	2.30	27:anteiso	0.10	0.03
21:0	8.50	9.20	21:iso	0.30	0.20			
22:0	8.30	9.00	22:iso	0.20	0.20			
23:0	6.60	7.60	23:iso	0.30	0.20			
24:0	6.40	6.80	24:iso	0.10	0.10			
25:0	5.70	5.90	25:iso	0.10	0.03			
26:0	4.20	4.00	26:iso	0.03	0.10			
27:0	3.10	2.80	27:iso	0.02	0.05			
28:0	2.30	1.90	28:iso	0.10	0.02			
29:0	1.70	1.10						
30:0	1.90	1.10						
31:0	1.30	0.60						
32:0	0.50	0.50						
33:0	0.50	0.30						
34:0	0.20	0.10						
35:0	0.20	0.05						
Σ	88.50	87.65	Σ	8.45	9.50	Σ	3.30	2.83

Table 3 Fatty acids composition of wax ester (WE) of NL of liver and trematode parasite *P. cervi*

Components	Saturates		Components	Monoenes		Components	Dienes		Components	Polyenes	
	Liver	Parasite		Liver	Parasite		Liver	Parasite		Liver	Parasite
14:0	2.4	7.7	14:1	–	Tr	16:2	Tr	2.7	18:3n-3	12.7	0.01
15:0	1.9	4.2	15:1	Tr	Tr	18:2	11	3.4	18:3n-6	0.7	0
16:0	23.2	33.9	16:1	–	15.3	26:2	–	–	20:3n-3	–	–
17:0	1.3	Tr	17:1	–	0.3				20:4n-3	–	2.8
18:0	14.2	13.5	18:1	7.1	8.6				20:4n-6	1.2	0.5
22:0	11.9	0.2	22:1	–					20:5n-3	–	3.9
24:0	5.5	0.5	24:1	–					22:4	6.9	0.1
26:0	–	–	26:1	–					22:5n-3	–	0.5
28:0	–	–	27:1	–					22:5n-6	–	0.1
30:0	–	–	28:1	–					22:6n-3	–	1.7
			30:1	–							
Σ	60.4	60	Σ	7.1	24.2	Σ	11	6.1	Σ	21.5	9.61

small quantity in the liver. The parasite is rich in stigmaterol, which is absent in the liver.

Four classes of sterols from steryl ester are recorded from both the liver and the parasite. The liver is rich in cholesterol (64 %) followed by Trans-22 dehydrocholesterol (34.4 %), stigmaterol and campesterol. The parasite contains equal amount of campesterol and stigmaterol followed by Trans-22-dehydrocholesterol and cholesterol (Table 7).

Fatty acid composition of PL components of host's liver and parasite

Fatty acid composition of PL components of both the liver and the parasite are presented in Table 8. It is interesting to note that the number of fatty acids in all the classes i.e. CL, PE, PC, PI, SPH, and PS is more in the parasite than that of the liver. Among the fatty acid classes either C_{16:0} or C_{18:1}

Table 4 Fatty acids composition of steryl ester (SE) of NL of liver and trematode parasite *P. cervi*

Components	Saturates		Components	Monoenes		Components	Dienes		Components	Polyenes	
	Liver	Parasite		Liver	Parasite		Liver	Parasite		Liver	Parasite
14:0	0.3	9.2	14:1	0.1	3	16:2	1.2	2.5	18:3n-3	2.8	1.2
15:0	1.4	6.2	15:1	0.4	1.3	18:2	13.8	12.7	18:3n-6	0.2	
16:0	17.7	33	16:1	2	1.8	26:2	0.01	–	20:3n-3	0.8	0.3
17:0	1.8	0.7	17:1	0.8	–				20:4n-3	0.2	
18:0	15	14.9	18:1	22.6	11.2				20:4n-6	1.5	
22:0	5.8	0.2	22:1	0.05	–				20:5n-3	6.4	0.3
24:0	0.2	0.1	24:1	0.2	0.05				22:4	0.7	0.1
26:0	–	0.1	26:1	0.01	–				22:5n-3	0.1	0.1
28:0	–	0.5	27:1	0.4	–				22:5n-6	0.6	0.2
30:0	–	–	28:1	0.01	0.1				22:6n-3	0.1	0.1
			30:1	–	–						
Σ	42.2	64.9	Σ	26.57	17.45	Σ	15.01	15.2	Σ	13.4	2.3

Table 5 Fatty acids composition of triacylglycerol (TG) of NL of liver and trematode parasite *P. cervi*

Components	Saturates		Components	Monoenes		Components	Dienes		Components	Polyenes	
	Liver	Parasite		Liver	Parasite		Liver	Parasite		Liver	Parasite
14:0	0.30	0.80	14:1	Tr	Tr	16:2	1.30	1.90	18:3n-3	0.40	4.10
15:0	0.40	1.20	15:1	Tr	0.50	18:2	7.80	17.20	18:3n-6	0.10	0.10
16:0	31.90	25.90	16:1	2.50	0.20	26:2	–	–	20:3n-3	0.30	0.10
17:0	1.20	0.20	17:1	0.70	Tr				20:4n-3	0.60	0.10
18:0	15.80	5.20	18:1	35.00	36.70				20:4n-6	0.20	0.90
22:0	0.40	0.30	22:1	0.04	0.10				20:5n-3	0.10	0.40
24:0	0.02	0.60	24:1	0.10	–				22:4	0.10	0.10
26:0	–	0.03	26:1	–	0.60				22:5n-3	0.10	0.01
28:0	–	2.00	27:1	–	–				22:5n-6	0.03	0.02
30:0	–	–	28:1	–	–				22:6n-3	0.03	0.05
			30:1	–	–						
Σ	50.02	36.23	Σ	38.34	38.1	Σ	9.1	19.1	Σ	1.96	5.88

is the chief component for the parasite in all the PL classes. However, C_{16:1} is the major fatty acid found in the liver, while this occurs in very little amount in the parasite.

Discussion

Helminths take up lipids from external sources, either as fatty acids or sterols, because they have limited ability in the biosynthesis of lipids. Parasitic worms display selective mechanisms for absorption of fatty acids from their environment and incorporate these absorbed fatty acids into various lipid fractions. Undissociated fatty acids diffuse through the absorptive surface of the membrane lipid bilayer and dissociated fatty acids enter by mediated

transport. The tegument helps in absorption of cholesterol and fatty acid (Dalton et al. 2004).

The result of HC, WE and SE shows that the parasites uptake more hydrocarbon than that of the host but not WE and SE which means that the parasite utilizes/requires more hydrocarbons than the host.

The TG content is more in the liver than the parasite. In majority of the helminths TG is the major portion of the NL. However, in *P. cervi* TG, SE and WE are very low in amount. Some of the components of free sterol occur in higher percentage in the parasite than that of the liver. According to Barrett (1981), free sterols are predominant in helminths and probably one kind of steroid fraction is being esterified. The level of sterol component of *P. sibirica*, *E. pancreaticum*, and *C. erschowi* are 19.2, 43.6 and

Fig. 3 Comparison of fatty acid class composition of triacylglycerol, wax ester and steryl ester of neutral lipid from goat liver and trematode parasite

Table 6 Fatty acid composition of wax ester/alcohol of NL of goat liver and trematode parasite, *P. cervi*

Components	Saturates		Components	Iso	
	Liver	Parasite		Liver	Parasite
12:00	1.4	–	16:0 (iso)	1.1	–
14:0	1.5	1.9	18:0 (iso)	35.3	0.6
15:0	–	3.1	19:0 (iso)	0.5	–
16:0	1.2	17.5	20:0 (iso)	–	0.6
18:0	1.5	20.5	23:0 (iso)	6.1	–
19:0	6.4	0.6	24:0 (iso)	–	0.3
20:0	4.7	16.4	25:0 (iso)	1.5	0
21:0	6.3	–	26:0 (iso)	–	0.7
22:0	6	25.9			
24:0	1.3	6.3			
26:0	1.3	3.9			
27:0	–	0.1			
28:0	12.1	0.1			
29:0	2.5	–			
30:0	6.5	0.4			
31:0	–	0.4			
32:0	2.8	0.2			
Σ	55.5	97.3	Σ	44.5	2.2

68.6 % respectively (Vykhestyuk and Yarygina 1982). This shows that the percentage of total sterol among the trematodes is not comparable. It is known that helminths' eggs contain substantial amount of sterols (Smith et al. 1977). Vykhestyuk and Yarygina (1982) opined that the reason for high percentage of sterol accounts for the presence of higher number of eggs. In the present study, the trematodes were pooled from ten hosts and all the parasites were adults and were engorged with eggs. The percentage

of SE in both the parasite and liver is close. According to Gallo and Fried (1984) the major NL obtained from all system of *Echinostoma revolutum* was free sterol and this excretory-secretory product of free sterol are involved in pheromone communication in this adult trematode. Marsit et al. (2000) commented that neutral lipids are used in some form during the transformation from cercariae to metacercaria. Waxes play an important role as water barrier for insects, birds and animals such as sheep (Conn et al. 2003). Wax esters of helminths are esterified as long chain alcohol (Barrett 1981). Wax ester of *Ascaris columnaris* consists of large amount of C_{16:0} and C_{18:0} alcohols but *Ascaridia galli* contains long chain acids and alcohols (Tarr 1973). The amount of WE in *P. cervi* is least among the neutral lipids. This species of trematode lives in the lumen of rumen and reticulum, they need at least some amount of wax ester, which is probably used for water barrier.

According to Grunwald (1978), sterol synthesis and its balance is not static in plants and have the ability to synthesize sterols. Stigmasterols are one of the predominant sterol in plant plasma membrane (Wu et al. 2006). In the present study, *P. cervi* possesses high percentage of campesterol and stigmasterol in both the free sterol and sterol from SE, whereas, these two components are found negligible in amount in the liver of the host. High amount of campesterol and stigmasterol of the present parasite might be related with the reproduction. Barrett et al. (1970) compared the sterols of *Ascaris lumbricoides*, *Macracanthorhynchus hirudinaceus*, *Moniliformis dubius*, and *E. revolutum* with that of their hosts' intestinal content and found cholesterol as the major component. They suggested that the intestinal parasites obtain cholesterol from their host diet or host secretion. Parasitic helminths modify cholesterol obtained from the host which is used as their membrane component and also used as a substrate for the synthesis of other

Table 7 Composition of free sterols and sterols obtained from steryl ester of NL of goat liver and the trematode parasite *P. cervi*

Components	Free sterol		Sterol from steryl ester	
	Liver	Parasite	Liver	Parasite
Coprostanol	13.20	1.00	–	–
Cis-22-Dehydrocholesterol	21.60	–	–	–
Trans-22-Dehydrocholesterol	42.30	–	34.40	26.9
Cholesterol	0.10	10.40	64.00	10.3
Campesterol	0.50	38.20	0.30	31.4
Stigmasterol	–	47.00	1.30	31.4
Unidentified (RT-1.0)	0.80	0.50		
Unidentified (RT-1.7)	2.20	2.80		
Unidentified (RT-2.1)	0.50	–		
Unidentified (RT-2.6)	2.80	–		
Unidentified (RT-3.0)	–	0.10		
Unidentified (RT-3.7)	0.10	–		
Unidentified (RT-4.0)	8.50	–		
Unidentified (RT-4.5)	5.80	–		
Unidentified (RT-6.3)	0.50	–		
Unidentified (RT-6.9)	1.00	–		
Σ	99.90	100.00	100.00	100.00

compounds such as ecdysteroids, hydroxylated steroids, etc. (Tielens, 1997). Cholesterol might also play the same role in *P. cervi*. According to Awharitoma et al. (1988), cholesterol among the neutral lipid components and phosphatidylcholine and phosphatidylethanolamine among the phospholipid components were the major component in four species of amphistomes *Carmyerius minutus*, *Ceylonocotyle dicranocoelium*, *Cotylophoron cotylophorum*, and *Paramphistomum microbothrium*. The findings of Barrett et al. (1970) also revealed that the amount of cholesterol in the free sterol component is high in the parasite than that of its host. Apart from cholesterol, campesterol, β -sitosterol and their reduction product campestanol and stigmasterol were found in the above mentioned three nematodes (Barrett et al., 1970). Phytosterol is obtained by these three helminths probably from the host's intestinal content (Barrett 1981). The liver of the host possesses Cis-22-dehydrocholesterol and Trans-22-dehydrocholesterol of the free sterol, which is not found in *P. cervi*. These components are not absorbed by the parasite because these might have no role in maintenance of their life cycle.

Phospholipids play an important role in defense mechanism and act as membrane component in *Schistosoma mansoni* (Furlong 1991). It is considered that molecular species of phospholipids are important mediators of signal transduction, immune response modulation and membrane fusion. These are all important aspects of schistosomes' membrane (Brouwers et al. 1998). *S. mansoni* is capable of synthesizing phospholipids and triacylglycerol from the precursors obtained from the host (Brito et al. 2002).

Similar mechanism of phospholipid utilization might takes place in gut dwelling trematodes like *P. cervi*.

In the present observation PI and PE in the parasite *P. cervi* and PC and CL in the host's liver are the major components in the phospholipid fractions. In mammals, the function of PE, PC and PI is that they are always associated with the membranes (Conn et al. 2003).

The amount of four fractions, i.e., PE, PI, PS, and SPH is higher in the parasite than those of host's liver. This indicates that these fractions are absorbed more by the parasite than that of the host. The function of this large amount of these fractions of PL might be related with the membrane restructuring.

Phosphatidylinositol, an anionic phospholipid, is an important lipid which acts both as a key membrane constituent and as a participant in essential metabolic processes (Payraastre 2004). It is the main source of diacylglycerols that serve as signaling molecules in membrane (Gardocki et al. 2005). Among the PL components, phosphatidylinositol content is highest in the trematode *P. cervi*. Pearce and Sher (1989) identified three glycosylphosphatidylinositol anchored surface antigens (mw. 38,000, 32,000 and 18,000) in *S. mansoni*, which serve as targets for protective immunity. Thus the high amount of PI in *P. cervi* may be involved in the production of various surface antigens to evade host's immune response.

Inter-conversion of sphingomyelin and ceramide is reported in *S. mansoni* (Redman et al. 1997). Similar function of sphingomyelin may be attributed in case of *P. cervi*. It was reported that unsaturated fatty acids are capable of activating neutral sphingomyelinase, which

Table 8 Fatty acid compositions of phospholipid components of goat liver and parasite, *P. cervi*

Components		CL		PE		PC		PI		SPH		PS	
		Liver	Parasite	Liver	Parasite	Liver	Parasite	Liver	Parasite	Liver	Parasite	Liver	Parasite
Saturates	14:0	–	1.50	–	0.50	–	0.50	–	1.50	–	1.40	–	0.90
	15:0	–	4.10	–	1.50	–	1.60	0.60	4.40	–	3.70	–	1.70
	16:0	7.80	30.20	7.30	25.10	12.80	33.80	23.10	36.40	4.30	40.30	2.60	31.10
	17:0	0.20	0.80	–	0.02	0.20	0.70	0.50	1.00	–	0.90	–	–
	18:0	8.20	10.10	16.90	9.80	26.30	9.60	19.60	11.80	5.20	11.50	5.90	7.50
	22:0	0.10	0.10	0.20	0.40	1.00	0.60	0.40	0.40	0.40	0.30	–	10.20
	24:0	–	0.10	0.03	0.40	0.30	–	0.10	0.70	1.10	3.10	–	0.40
	Σ	16.30	46.90	24.43	37.72	40.60	46.80	44.30	56.20	11.00	61.20	8.50	51.80
Monoenes	14:1	–	–	–	0.20	–	Tr.	–	0.10	–	0.10	–	Tr.
	15:1	–	4.60	–	5.00	–	Tr.	0.10	0.80	–	0.40	–	Tr.
	16:1	64.80	0.40	50.90	0.10	25.10	0.70	19.20	0.10	80.30	0.30	87.40	2.10
	17:1		0.10		0.20	0.04	Tr.	–	0.04	–	Tr.	–	–
	18:1	4.80	20.50	9.30	30.50	12.40	30.70	25.40	20.50	2.20	18.70	2.30	19.30
	22:1	1.30	–	6.30	–	7.20	–	1.70	–	1.30	–	0.70	–
	24:1	–	0.03	–	0.10	–	–	–	–	–	0.50	–	–
	27:1	–	–	–	–	–	–	0.01	–	0.20	–	–	–
	Σ	70.90	25.63	66.50	36.10	44.74	31.40	46.41	21.54	84.00	20.00	90.40	21.40
Dienes	16:2	0.40	2.00	0.60	0.70	1.00	–	1.00	0.10	0.40	0.10	–	0.20
	18:2	9.70	21.90	3.30	20.80	4.60	17.50	5.40	8.60	0.40	9.40	0.70	18.40
	Σ	10.10	23.90	3.90	21.50	5.60	17.50	6.40	8.70	0.80	9.50	0.70	18.60
Polyenes	18:3n-3	1.50	2.10	0.50	2.10	0.70	1.60	0.60	0.50	0.20	0.60	–	1.30
	18:3n-6	–	0.04	–	0.10	0.20	0.10	0.20	–	0.20	–	–	–
	20:3n-3	–	0.30	0.20	0.40	0.03	0.20	0.03	1.10	–	0.90	–	0.40
	20:4n-3	0.03	0.10	–	0.10	0.02	0.10	–	1.60	–	0.05	–	0.30
	20:4n-6	–	0.20	0.10	0.70	0.10	1.10	0.20	2.60	–	2.10	–	0.80
	20:5n-3	0.20	–	1.30	0.10	2.20	0.20	0.40	0.01	2.30	0.70	–	0.30
	22:4	–	0.10	–	0.20	0.10	0.10	0.02	0.90	0.10	1.30	–	0.30
	22:5n-3	0.40	0.01	1.40	0.04	3.00	0.30	0.50	0.02	0.60	0.04	0.30	0.10
	22:5n-6	–	0.03	0.10	0.40	0.20	0.04	0.05	3.50	0.10	0.02	–	0.10
	22:6n-3	0.20		1.10	0.02	1.70	0.20	0.20	0.20	0.10	0.20	0.10	0.40
	Σ	2.33	2.88	4.70	4.16	8.25	3.94	2.20	10.43	3.60	5.91	0.40	4.00
Sum total		99.63	99.31	99.53	99.48	99.19	99.64	99.31	96.87	99.40	96.61	100.00	95.80

leads to the break down of sphingomyelin at neutral pH (Robinson et al. 1997; Worgall et al. 2002). Primordial role of sphingolipids in surface membrane antigen confinement in *S. mansoni* and *S. haematobium* is envisaged by Tallima and Ridi (2005). A review on sphingomyelin synthesis in helminths is reported by Bankov et al. (1998).

Continuous reshuffling of membrane in parasitic trematodes is essential and is, perhaps, helped both by cardiolipin and phosphatidylserine. It is to be mentioned that concentration of phosphatidylserine is more in trematodes than that of their hosts as observed in *F. hepatica* (Oldenborg et al. 1975) and in *P. cervi*.

Parasites living in anaerobic condition usually needs to limit its energy consumption in order to adapt to its

environment. This is the reason why the parasite might restrict itself for *de novo* synthesis of fatty acids.

The predominant fatty acids of NL and PL fractions of the liver and the parasite include C_{16:0}, C_{18:0}, C_{18:1}, and C_{18:2}. Most C₁₈ acids are the major components of total fatty acids. The reason behind is that C₁₈ acids are the main fatty acid of the parasite and thus required for the chain elongation to form C₂₀ and C₂₂ acids. Sato et al. (2008) showed relatively high weight-percentage of C_{16:0}, C_{18:1(n-9)}, C_{18:0}, and C_{22:6n-3} acids in adult monogenean, *Neobenedenia girellae* and skin and mucous of its fish host *Seriola dumerili*. In various parasite lipids such as phospholipids, triacylglycerol, and cholesterol esters host's fatty acids are incorporated. *F. hepatica* and *S. mansoni* synthesize specific unsaturated fatty acids such as

eicosanoids, ecdysteroids, and quinones by elongation of host's fatty acids. Acyltransferases of the trematodes might help in exchanging fatty acids between NL and PL (Tielens, 1997, 1999). According to Tielens (1997) the modification of lipids in the parasite which are obtained from the host might be related with the fitness of the parasite. *S. mansoni* is unable to synthesize fatty acids and sterols *de novo* and host's lipid is required for their survival (Furlong et al., 1992). Mechanism of chain elongation has been demonstrated in *Spirometra mansonioides* for C₁₆, C₁₈, C_{18:1}, C_{18:2}, C_{18:3} to C₂₀ and C₂₂ acids (Meyer et al. 1966). It has also been reported that the cestode, *Hymenolepis diminuta*, can convert palmitate and stearate into saturated fatty acid chains as long as 26 carbons and *Fasciola hepatica* can produce C_{20:1} and C_{20:2} acids from C_{18:1} and C_{18:2} acids (Barrett 1981). Brouwers et al. (1997) mentioned that efficient conversion of oleate (C_{18:1}) to eicosanoids (C_{20:1}) took place by chain elongation in adult *S. mansoni* but palmitate was not elongated at an appreciable rate. Awharitoma et al. (1988) analyzed the lipid and fatty acid profile of four amphistomes, *Carmyerius minutus*, *Ceylonocotyle dicranocoelium*, *Cotylophoron cotylophorum*, and *Paramphistomum microbothrium* from rumen of cattle. They found that the fatty acid profile of these four amphistomes was similar and their chain length varied from 12 carbon atoms to 20 carbon atoms. The percentage of unsaturated fatty acid was 50 %. They also found that qualitative similarity in the lipid classes and fatty acid composition of host rumen tissue and rumen content and those of the parasites, thus emphasizing nutritional dependence of parasites on their host. In case of *P. cervi* majority of the lipids and fatty acids of the parasite are absorbed from the rumen fluid, and some might be absorbed by sucker from the host's gut wall (rumen). Besides, the parasite is also involved in the chain lengthening process of absorbed fatty acids.

The results obtained from the present work corroborate with the stated hypothesis that the parasite take up fatty acids and free sterol more in amount than that of the host as a part of their reproductive strategy and inability to synthesize fatty acids. The present study would enlighten host-parasite interaction on the basis of their nutritional requirement. The result would also enrich the parasite biology, trematode in particular. Further studies on other helminth parasite should be undertaken to understand parasite biology.

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