Major lipid classes and their fatty acids in a parasitic nematode, *Ascaridia galli*

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Abstract The paper presents major lipid classes and their fatty acids investigated from *Ascaridia galli*, a nematode parasite of country fowl. Thin layer chromatography (TLC) reveals that the percent of total lipid, neutral lipid, phospholipids, and glycolipids are 1.94, 54.39, 26.95 and 18.66, respectively. Gas–liquid chromatography (GLC) analysis shows that the saturated fatty acids are the major components in all the lipid fractions followed by monoenes and dienes. Polyunsaturated fatty acids (PUFA) were present in low amount. Stearic acids (C₁₈) were the chief components among all the fatty acids in all the lipid fractions.

Keywords *Ascaridia galli* · Fatty acids · Fowl · Glycolipid · Neutral lipid · Phospholipid

Introduction

Nematodes and cestodes parasites, which live in the intestine of host, obtain their food directly from host's nutrients. *Ascaridia galli* is a common large parasite of the small intestine of domestic and wild birds. It has a worldwide distribution. Lipid synthesis is an important process in most organisms and the same is true for

helminthes. Lipid and fatty acid requirement are different in different animals and are probably related to the need of the animal concerned. Parasitic stages of helminthes do not use lipids normally as energy reserve (Barrette 1981; Tielens 1997; Dalton et al. 2003). The adult worms take large amount of lipids to obtain sufficient amount of specific fatty acids and fat-soluble vitamins and the excess lipid is stored and eventually excreted (Cheng 1986). Since nematodes, trematodes, and cestodes are unable to synthesize most essential lipids they have to acquire exogenous lipids from tissues, fluids or cell membrane of the host (Smyth 1994). Helminthes have higher percentages of unsaturated C₁₈ acids (Barrette 1981). The major fatty acids in nematodes are usually C₁₆ and C₁₈. In mammals unsaturated C₁₈ acids are usually 30–40% of the total acids whilst in helminthes it is 40–70%. Usually helminthes contain a small proportion of odd numbered C₁₅ and C₁₇ (Barrette 1981). The principal fatty acid at all evolutionary and trophic levels is mainly C_{16:0} (Ackman 2000).

A very few studies on the lipid classes and fatty acids have been made on the parasitic nematode species (Beames 1965; Subrahmanyam 1967; Ansari et al. 1973; Hutchinson et al. 1976; Lee et al. 1977; Furukawa et al. 1985; Liu and Weller 1989; Maloney and Semprevivo 1991; Smith et al. 1996). Lipids and fatty acids of roundworm, *Ascaridia galli*, were not investigated so far. This parasite causes serious disease in poultry birds (Berger et al. 1958; Roberts and Janovy 2000). The present work is, thus, designed to investigate the major lipid classes and their fatty acid composition in an intestinal nematode *Ascaridia galli* inhabiting small intestine of country fowl. The result will help us in understanding the biochemical aspects of this nematode species of veterinary importance.

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Materials and methods

Material

Ascaridia galli Schrank (Freeborn 1923), (Family: Ascaridiidae), a large roundworm parasite, was collected from the upper portion of the small intestine of country fowl from Titagarh, North 24 Parganas, West Bengal, India.

The parasites were kept in 0.85% saline. Living nematodes were carefully soaked in tissue paper and kept in -4°C . The parasites were pooled from at least 30 hosts to a wet weight of approximately 7 g.

Methods

Extraction of lipids

The total lipids were extracted from the samples following the method of Bligh and Dyer (1959) using methanol–chloroform (2:1 v/v), methanol–chloroform–water (2:1:0.8, v/v) and then again with the first solvent system. Sample was ground with the solvent, filtered and residue was extracted with the next solvent system. The process was repeated. Finally, the three extracts were pooled, diluted with water and layer is allowed to separate in a separatory funnel. The chloroform layer at the bottom was withdrawn and dried over anhydrous sodium sulphate. The chloroform solution of lipid was evaporated under vacuum, redissolved in distilled n-hexane and kept at -20°C for future use. BHT (butylated hydroxy toluene) was added at a level of 100 mg/l to the solvent as antioxidant.

Thin layer chromatography for lipid class separation

A portion of the total of the samples was subjected to TLC using silica gel G (SLR) (Rouser et al. 1976). The glycolipids were eluted by the chloroform, and acetone, respectively by successive developments. The glycolipid band was marked, scrapped of the plate and extracted by chloroform. The residual band at the bottom of the plate consisting of phospholipids was also extracted by methanol. The solvent was evaporated and fractions were kept in redistilled hexane at -20°C . Each class of lipid was estimated by weighing in a microbalance.

Preparation of methyl esters of fatty acids

A portion of total lipid (TL), glycolipid (GL) and phospholipid (PL) of parasite samples were dissolved in anhydrous methanol containing concentrated Sulfuric acid (1%, v/v) and the mixture was refluxed for 2 h. Methanol was evaporated to a small volume and cooled. Distilled water was added to the cooled mixture and the methyl

esters of fatty acids were extracted three times with aliquots of diethyl ether. The ethereal extracts were dried over anhydrous sodium sulphate, filtered, vacuum dried, redissolved in n-hexane and kept for future use.

Purification of fatty acid methyl esters by thin layer chromatography

Fatty acid methyl esters (FAME) were purified in thin layer chromatography (TLC) using n-hexane–diethyl ether (90:10, v/v) as a solvent. A standard methyl ester was also run on the same plate in a separate lane. The location of methyl ester bands were done by placing the TLC plate in iodine vapour chamber. The methyl ester bands corresponding to the standard marked and then scrapped off the plate. Methyl esters were recovered by extracting the bands in a mini column with chloroform, the later was evaporated and the methyl esters were kept in n-hexane till analyzed by GLC. Fatty acid methyl esters (FAME) of total lipid (TL), neutral lipid (NL), phospholipid (PL), and glycolipid (GL) were prepared by transmethylation (Christie 2003).

Gas liquid chromatography

GLC of fatty acid methyl esters were done on a Gas liquid chromatography (GLC) (Chemito 1000), with Flame ionization Detector (FID). Quantitation was done by computer using specific software (Clarity Lite).

Analysis of fatty acid methyl esters

GLC of analysis of fatty acid methyl esters (FAME) was done on a BPX—70 megabore capillary column of 30 m length and 0.53 mm i.d. obtained from SGE, Australia. Oven temperature was programmed from 150 to 240°C and 300°C , respectively. Injection port detector temperatures were 250 and 300°C , respectively. Nitrogen gas was used as carrier gas and its flow rate was 6.44 ml/min. Identification of fatty acids was done comparing their retention times with those of standard.

Results

The total lipid of *Ascaridia galli* is 1.94% of the wet weight of the body tissue. Percentage of neutral lipid, phospholipid, and glycolipid are 54.39, 26.95, and 18.66%, respectively (Table 1). Altogether 20 fatty acids are identified from this nematode parasite (Table 2). The percent total saturated fatty acid (SFA) is highest than those of other groups of fatty acids in all the lipid classes. Palmitic (16:0) and stearic (18:0) acids dominate the SFA while 18:1 ω 9 present as highest percent among monoenes

Table 1 Composition of major lipid classes obtained from *Ascaridia galli* inhabiting small intestine of country fowl

Lipid classes	% Of wet weight
Total lipid (TL)	1.94
Neutral lipid (NL)*	54.39
Phospholipid (PL)*	26.95
Glycolipid (GL)*	18.66

* Expressed as % of wet weight of total lipid

Table 2 Fatty acid composition of total lipid, neutral lipid, glycolipid, and phospholipid of *Ascaridia galli* infecting country fowl

Fatty acid	% w/w Of each component			
Components	Total lipid	Neutral lipid	Glycolipid	Phospholipid
Saturates				
14:0	1.2	0.6	1.1	0.5
15:0	–	–	0.1	–
16:0	19.7	21.3	25.7	19.1
17:0	0.4	0.5	0.9	0.4
18:0	13.1	9.8	14.98	23.1
20:0	0.4	0.2	0.4	0.8
22:0	0.5	0.4	0.3	0.5
24:0	0.3	0.2	0.1	0.3
Σ	35.6	32.4	43.5	44.7
Monoene				
14:1	0.8	0.3	1.2	1.1
16:1	0.5	0.7	0.4	–
17:1	1.2	0.1	–	4.6
18:1 ω 9	30.6	28.9	32.8	33.3
20:1	1.0	0.6	0.4	1.6
22:1	–	0.3	0.1	0.2
24:1	0.1	0.1	0.01	–
Σ	34.2	31.0	34.91	40.8
Dienes				
18:2 ω 6	26.1	31.5	20.1	12.6
Σ	26.1	31.5	20.1	12.6
Polyenes				
18:3 ω 6	–	–	–	0.2
18:3 ω 3	2.4	3.3	0.8	0.3
20:4 ω 3	1.6	1.3	0.4	1.1
22:4 ω 6	0.1	0.05	0.1	0.2
Σ	4.1	4.65	1.3	1.8
Total	100.0	99.55	99.81	99.9

The ω values represent the methyl end chain from the centre of double bond furthest removed from the carboxyl group

(MUFA) and 18:2 ω 6 is the only dienes (DUFA) recorded. Polyunsaturated fatty acids (PUFA) are present in low amount in the lipid classes. Comparative assessment of occurrence of fatty acids of lipid fractions of *A. galli* is

Fig. 1 Comparison of occurrence of fatty acids in lipid classes recorded from *Ascaridia galli*

shown in Fig. 1. Figure 2 represents comparative account of unsaturated fatty acids (UFA), total C₁₈ and C₁₈ UFA in the major lipid fractions of *A. galli*.

Discussion

Nematodes, which live under anaerobic condition, are not normally able to utilize lipids although a slight increase in total lipids in *Ascaris* during starvation has been reported (Beames et al. 1968). Parasitic worms display selective mechanisms for absorption of fatty acids from their environment and incorporate these absorbed fatty acids into various lipid fractions (Barrette 1981). Undissociated fatty acids diffuse through the absorptive surface of the membrane lipid bilayer and dissociated fatty acids enter by mediated transport.

Total lipid content of nematodes varies among species. The lipid content of male and female *Ascaris lumbricoides* is 1.3 and 1.6% of the wet tissue respectively (vonBrandt 1973), 2.1% in *Dirofilaria immitis* (Hutchinson et al. 1976), whereas it is 1.94% in *Ascaridia galli*. Total lipid of adult *Nippostrongylus brasiliensis* is 11.9% of the dry mass (vonBrandt 1973); however, it is 15–20% of the dry mass of the larvae (Wilson 1965). The larvae of *Eustrongylides ignotus* contain 1.1% of the wet weight and 4.4 of the dry

Fig. 2 Comparison of unsaturated fatty acids (UFA) and C18 acids among the lipid fractions *Ascaridia galli*

mass (vonBrandt 1973). Total lipid as well as neutral and phospholipid contents of *A. lumbricoides* vary in different tissues; it is highest in reproductive tissue over the muscle and cuticle (Fairbairn 1955, 1956; Beames 1965). In the present study the abundance of recorded lipid classes in the whole body of the poultry roundworm is NL > PL > GL.

Smith et al. (1996) identified 18 fatty acids from the surface and internal lipids of the filarial worm, *Brugia malayi*. Out of 20 fatty acids recorded from *A. galli*, TL, NL, GL and PL contain 17, 18, 18 and 17 fatty acids. It is interesting to note that both SFA and MUFA are much more in PL and GL fractions than NL in *A. galli*. However, percent of DUFA is more in NL and TL than PL and GL. Percent occurrence of SFA, MUFA, DUFA of *B. malayi* are less than those obtained from *A. galli*, whereas percent of PUFA is much higher in *B. malayi* (16.92) than *A. galli* (4.1). The total percent of the C₁₈ acids of TL in *A. galli* is 72.2 and 51.83 in *B. malayi*. Helminthes possess 40–70% unsaturated C₁₈ acids (Barrette 1981) which are 59.1 in *A. galli* and 40.74 in *B. malayi*. Most parasitic helminthes exhibit 50–60% of unsaturated fatty acids and *A. lumbricoides* possess 75% (Barrette 1981), whereas this is 64.4 and 68.67 in *A. galli* and *B. malayi*, respectively. The three adult filarial worms, *Dirofilaria immitis*, *Dipetalonema viteae*, and *Litomosoides carinii* contain 72.42, 72.82 and

78.25% UFA, respectively (Hutchinson et al. 1976). The cestode parasite of domestic fowl, *R. (F.) echinobothrida*, possesses 51.8% unsaturated fatty acids of which 49.1% are unsaturated C₁₈ acids (Mondal et al. 2009). It is very difficult to assume the reason behind such variation in fatty acid concentrations among the parasites inhabiting different microhabitat. One of the reasons might be the non-availability of the fatty acids in last part of the intestine where the nematodes live. Among the SFA of the lipid fractions, C₁₆ and C₁₈ acids are the chief components recorded in *A. galli*. The major fatty acid among the lipid fractions in *A. galli* is C_{18:1ω9} followed by linoleic (C_{18:2ω6}) acid. Comparative study among UFA, C₁₈ total and C₁₈UFA (Fig. 2) of *A. galli* shows that the amount of total C₁₈ acid is more than that of the UFA and C₁₈UFA is the major component of UFA. Similar findings were made in a trematode *Paramphistomum cervi* (Ghosh and Misra 2010). It was reported that parasitic flatworms especially *F. hepatica* can form C_{20:1} and C_{20:2} fatty acids from C_{18:1} and C_{18:2} acids (Barrette 1981; Tielens 1997). It is probable that the nematode in question is also able to modify C_{18:1} and C_{18:2} acids to C_{20:1} and C_{20:2} fatty acids. Thus stearic acid in parasitic helminthes is unique in their presence as precursor of other fatty acids required for chain elongation.

PUFA is found in low amount in *A. galli*. Comparatively high amount of PUFA is recorded from blood or tissue dwelling helminthes (Liu and Weller 1989) than those living in GI tract. It might be that PUFA is required for the defense purposes of those living in blood-reach microhabitat. Bennett and Caulfield (1991) believed that lipids taken from the host play an important role in immuno-protection in helminthes. Thus it can be assumed that this gut dwelling nematode utilizes mostly SFA, MUFA, and DUFA concentrating on C₁₈ acids.

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