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Qualitative and quantitative changes in lipids along the length of female reproductive system of the poultry nematode *Ascaridia galli* (Schrank 1788)

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Abstract. Qualitative and quantitative studies have been made of lipids along the length of the female reproductive system of the poultry nematode, *Ascaridia galli*. Adult specimens were collected in 0·9% saline from the intestines of naturally infected fowls. The quantitative studies have revealed significant ($P = 0{\cdot}05$) differences of total lipids, phospholipids, sterols, free fatty acids and glycerides and significantly ($P < 0{\cdot}001$) higher amounts of non polar than polar lipids in different regions of the reproductive system. The qualitative studies have showed that the germinal, growth and maturation regions of the ovary, oviduct and uterus contained 7, 7, 6, 6, 10 fractions respectively of polar and 12, 12, 12, 11, 10 fractions respectively of non polar lipids.

Keywords. *Ascaridia galli*; lipids; nematode; ovary; oviduct; oogenesis; uterus.

1. Introduction

The female reproductive system of ascarid nematodes contains high amounts of lipids (Fairbairn 1955, 1957). These lipids undergo marked changes in their amount and localization along with the length of *Ascaridia galli* (Schrank 1788) as revealed by histochemical techniques (Parshad and Guraya 1982). But no detailed quantitative and qualitative studies have been made previously of lipids during oogenesis of nematodes. Their aspect in germinal, growth and maturation regions of the ovary, oviduct and uterus have been studied for the poultry nematode *Ascaridia galli*. The possible physiological significance of various lipid changes is also discussed.

2. Materials and methods

The adult specimens of *A. galli* were collected in 0·9% saline from the intestines of naturally infected fowls, *Gallus domesticus*. The worms were cut longitudinally and their isolated reproductive systems were divided into germinal, growth and maturation regions of the ovary, oviduct and uterus. Confirmation of these regions of the reproductive system was based on their histological examination (Parshad and Guraya 1982). The different regions of the female reproductive system from a large number of worms were pooled separately and subjected to the extraction of total lipids in chloroform: methanol (2:1) (Folch *et al* 1957). For the quantitative determination of sterols, free fatty acids and phospholipids, the methods of Stadtman (1957), Mahadevan *et al* (1969) and Ames (1966) were used respectively. The remaining part of the lipids was considered to be the glycerides. The difference between the lipid fractions of various regions were compared by student's '*t*' test and analysis of variance.

Fractionation of lipids was carried out by thin layer chromatography (TLC) using chloroform; methanol: 7N NH_3 (75:25:4) for non polar (Horrocks 1963) and petroleum ether:ethyl ether:acetic acid (80:20:1) for polar lipids (Williams *et al* 1960). The colouring reagents used were iodine vapours (Sims and Larose 1962), sulphuric acid (Heftman *et al* 1966) and cupric acetate phosphoric reagent (Fewster *et al* 1969). The different fractions have been identified from their R_f values (Work and Work 1972).

3. Results

The amounts of total lipids, phospholipids, sterols and free fatty acids of the ovary were significantly ($P=0.05$) more and of glycerides less than that of the uterus (table 1). The oviduct contained significantly ($P=0.05$) more amounts of total lipids and free fatty acids than any other part of the female reproductive system (table 1). The female system contained significantly ($P<0.001$) higher amounts of non-polar than the polar lipids (table 2). The germinal, growth and maturation regions of the ovary contained significantly ($P=0.05$) different amounts of total lipids, sterols and free fatty acids. The amount of phospholipids of growth and maturation regions did not differ significantly but was significantly ($P=0.05$) less than that of the germinal region. The amount of glycerides of the germinal and growth regions, was significantly ($P=0.05$) less than that of the maturation region.

The germinal, growth and maturation regions of the ovary, oviduct and uterus contained 7, 7, 6, 6, 10 fractions respectively of polar lipids (table 3) and 12, 12, 12, 11, 10 fractions respectively of non-polar lipids (table 4).

Table 1. Lipid composition ($\bar{x} \pm SE$) of the female reproductive systems of *A. galli* (mg/100 mg tissue on wet weight basis).

Parts of reproductive system	Amount of lipids				
	Total lipids	Phospholipids	Glycerides	Sterols	Free-fatty acids
Ovary	6·60 ± 0·04	1·51 ± 0·11 (22·88)	2·44 ± 0·03 (36·97)	1·43 ± 0·006 (21·67)	1·22 ± 0·01 (18·48)
Germinal	7·16 ± 0·06	1·89 ± 0·01 (26·39)	2·89 ± 0·06 (40·36)	1·17 ± 0·02 (16·34)	1·20 ± 0·01 (17·76)
Growth	8·10 ± 0·08	1·27 ± 0·04 (15·68)	2·53 ± 0·13 (31·23)	2·49 ± 0·02 (30·74)	1·80 ± 0·02 (22·22)
Maturation	4·54 ± 0·10	1·36 ± 0·01 (29·96)	1·89 ± 0·11 (41·63)	0·63 ± 0·002 (13·88)	0·66 ± 0·01 (14·57)
Oviduct	18·00 ± 0·08	NR	NR	NR	4·07 ± 0·01 (22·61)
Uterus	5·56 ± 0·12	0·35 ± 0·01 (6·9)	4·29 ± 0·12 (77·16)	0·47 ± 0·01 (8·45)	0·45 ± 0·01 (8·09)
CD* ($P=0.05$) n = 5	0·2794	0·0917	0·3803	0·0583	0·0201
CD** ($P=0.05$) n = 5	0·3025	0·0347	0·3867	0·0408	0·1021

Numbers in parentheses represent percentages of the respective fractions with respect to total lipids.
NR, Not recorded.
*CD between different regions of the ovary.
**CD between different parts of the reproductive system.

Table 2. Non-polar and polar lipid composition ($\bar{x} \pm SE$) of the female reproductive system of *A. galli* (mg/100 mg tissues of wet weight basis).

Parts of reproductive system	Amount of lipids		*t*-test between non-polar and polar lipids	
	Non-polar	Polar		
Germinal	5·26 ± 0·07	1·89 ± 0·01	$t_8 = 48{\cdot}44$	$P < 0{\cdot}001$
Growth	6·82 ± 0·09	1·27 ± 0·04	$t_8 = 54{\cdot}85$	$P < 0{\cdot}001$
Maturation	3·18 ± 0·01	1·36 ± 0·01	$t_8 = 17{\cdot}99$	$P < 0{\cdot}001$
Uterus	5·20 ± 0·13	0·35 ± 0·001	$t_8 = 38{\cdot}34$	$P < 0{\cdot}001$

Table 3. Polar lipids of female reproductive system of *A. galli*.

Spot No.	Regions of ovary					Inference
	Germinal	Growth	Maturation	Oviduct	Uterus	
1	+	+	−	−	+	Phosphatidyl serine, phosphatidic acid, lysophosphatidic acid
2	−	−	+	+	−	Lysolecithin
3	+	+	+	+	+	Phosphatidyl inositol
4	−	−	+	+	+	Cerebroside sulphate
5	+	+	+	−	+	Sphingomyelin
6	−	−	+	+	−	Unidentified
7	−	−	−	−	+	Lecithin
8	−	−	−	−	+	Phosphatidyl ethanolamine
9	−	−	−	−	+	Cerebrosides
10	+	+	−	−	+	Unidentified
11	+	+	+	+	+	—"—
12	+	+	−	+	+	—"—
13	+	+	−	−	−	—"—

Table 4. Non-polar lipids of female reproductive system of *A. galli*.

Spot No.	Regions of ovary					Inference
	Germinal	Growth	Maturation	Oviduct	Uterus	
1	+	+	+	+	+	Monoglycerides
2	+	+	+	+	+	1-0-Monoalkyl glycerol ethers
3	+	+	+	−	+	Unidentified
4	+	+	+	−	+	—"—
5	−	−	−	+	−	—"—
6	+	+	+	+	+	1,2-Diglycerides
7	+	+	+	+	+	Sterols
8	+	+	+	+	−	1,3-Diglycerides
9	+	+	+	+	−	0-Dialkyl glycerol ethers
10	+	+	+	+	+	Fatty acids
11	+	+	+	+	+	Triglycerides
12	+	+	+	+	+	Aldehyde dimethyl acetals/long chain aldehydes
13	+	+	−	−	−	Alk-1-enyl diglycerides
14	−	−	−	−	+	Tri-alkyglycerol ethers
15	−	−	+	+	−	0-Dialkyl monoglycerates

4. Discussion

The amount of total lipids of the female reproductive system of *A. galli* estimated quantitatively ranged from $5{\cdot}56 \pm 0{\cdot}12\%$ in the uterus to $18 \pm 0{\cdot}08\%$ in the oviduct. These studies as well as Fairbairn (1955), Lee (1960), Parshad and Guraya (1982) and Frayha and Smith (1983) have clearly revealed that the nematode female reproductive system is an important site for accumulation of large amount of lipids, which are utilized in egg production (Krusberg *et al* 1973). This is supported by the observation that the enzymes of β-oxidation are present in embryonating eggs of *Ascaris* sp. (Barrett *et al* 1970; Ward and Fairbairn 1970). The morphology, distribution and the amount of lipid inclusions showed progressive increase in the oocytes from the anterior germinal to the growth zone of the ovary (Anya 1964; Parshad and Guraya 1982). The mature ova contained largest amount of lipids. The present biochemical estimates of the total lipids of the germinal and growth regions of the ovary agreed with the previous histochemical findings but differed in case of the maturation region.

The amount of non-polar lipids of all the regions of the ovary of *A. galli* was significantly ($P = 0{\cdot}001$) more than the polar lipids. The female reproductive systems of nematodes contained greater amount of neutral lipids which served as substrates for carbohydrate synthesis in the developing embryos (Fairbairn 1955; Tarr 1972). The growth region of the ovary of *A. galli* contained the maximum amount of sterols which might be used for the development of cellular organelles in growing oocytes (Krusberg 1967; Hirsch and Rothstein 1968; Barrett *et al* 1970) along with phospholipids in synthesis of cellular and subcellular membranes (Fairbairn 1957).

The growth region of the ovary of *A. galli* contained maximum amount of free fatty acids which decreased in the maturation region. Possibly larger amounts of free fatty acids are utilized in the synthesis of ascaroside esters in the oocytes. In the maturation region of the *Ascaris* almost all of the ascarosides of the maturing oocytes get esterified and these esters possibly form the precursors of the ascaroside layer of the egg shell (Fairbairn and Passey 1955; Wharton 1980).

The qualitative differences of the polar and non-polar lipid fractions between the ovary and the uterus are attributed to the different metabolic needs of the oocytes and the eggs.

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