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LIPIDS AND FATTY ACIDS IN ACTIVE AND AESTIVATED HERMAPHRODITE PULMONATE GASTROPOD *ACHATINA FULICA* (BOWDICH)

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ABSTRACT
Some pulmonate gastropods undergo aestivation for a long period. A hermaphrodite pulmonate gastropod, *Achatina fulica*, was selected for the study of some of the lipid components in active state and during aestivation of the snail. Thin layer chromatography and gas liquid chromatography were employed in the present study. Total lipid, phospholipid, cholesterol and triacylglycerol and the fatty acids of total lipid were studied in the digestive gland, ovotestis, mantle and foot of active and 7, 15, 30, 45, 60 and 90 days aestivated male and female *A. fulica*. The size of the albumen gland was the criteria of the identification of the sex and was confirmed through the present study. Percent lipid components in all the four organs exhibit clear male/female biasness. The digestive gland is the major lipid storage site over the other three organs and also plays important role in transportation of lipid to other organs during aestivation and maintains higher fatty acid percent than other organs. The other organs (albumen gland, mantle and foot) studied in this snail maintain a more or less steady lipid pool during aestivation. The fatty acid data also clearly indicate male/female dimorphism, and also emphasizes monoene to polyene conversion in this hermaphrodite gastropod.

KEYWORDS: Aestivation, Cholesterol, Fatty acid, Phospholipid, Snail, Triacylglycerol.

INTRODUCTION

Lipids are an integral part of molluskan tissues. Almost all data included in molluskan lipid study concern the entire organism and only scanty reports on the anatomical distribution of total lipids are available (Rakshit *et al*., 1997; Misra *et al*., 2002). Many mollusks store complex carbohydrates, in the form of glycogen, in the mantle and adductor muscle while lipids are accumulated in the digestive gland (Napolitano & Ackman, 1992). Contrarily several workers have proposed accumulation of lipids in other organs apart from digestive gland (Mitra & Sur, 1989; Wenne & Polak, 1989). Lipids from marine gastropods and bivalves are more extensively studied than freshwater or land counterparts as reviewed by a number of authors (Viarengo *et al*., 1994; Abad *et al*., 1995; Rakshit *et al.*, 1997; Kraffe *et al*., 2004, 2005; Vale, 2010). Nevertheless, freshwater and land mollusks are important from the point of their evolutionary relationship within the sequence: marine mollusks-freshwater mollusks-land mollusks (Dembitsky *et al*., 1992). The basic types of lipids found to occur in mollusks include sterols, phospholipids, triacylglycerols, glycolipids to a lesser extent and fatty acids (Voogt, 1972). Major sterols occurring in gastropods include cholesterol, $\Delta^{5,7}$sterols, brassicasterol and β sitosterol in different quantities (Bradshaw *et al*., 1991; Fried *et al*., 1998). Sterols in gastropods have been reported in two species of *Cerethidea* (Dutta *et al*., 1986; Misra *et al*., 1986). However it needs mention that almost all the data for sterol composition of mollusks concern the entire organism (Frazer *et al*., 1997) and only few reports on the anatomical distribution of total lipids and sterols (Swift *et al*., 1980; Gordon & Collins, 1982; Klingensmith & Stillway, 1982; Stoilov *et al*., 1984) remain available. Dembitsky *et al*. (1992, 1993b,c, 1994) provide some information on phospholipid and fatty acid composition in pulmonates belonging to river Volga and Lake Baikal and also from some invertebrates (Dembitsky *et al*., 1993a). Triacylglycerols play an important role in reproductive strategy of mollusks and are incorporated into developing eggs (Barber & Blake, 1985; Napolitano *et al*., 1992) that are crucial for increasing larval survival and successful metamorphosis (Gallager & Mann, 1986). Dietary influence on sterol, triacylglycerols, and neutral lipids was indicated in studies with gastropod *Biomphalaria glabrata* fed with restricted diet (Conaway *et al*., 1996). Fatty acids have been studied in marine bivalves particularly because they serve as important source of polyunsaturated fatty acids (PUFAs) that are important from the standpoint of human nutrition and health (Johns *et al*., 1980; Jarzebski *et al*., 1986). Recently, studies on the lipids and fatty acids of fresh water mussels were also done (Ekin, 2012; Ekin *et al*., 2012). Fatty acid composition of freshwater gastropod mollusks of river Volga and Lake Baikal have been provided (Dembitsky *et al*., 1992, 1993 a,b,c). Biochemistry of PUFAs was studied in land snail *Cepaea nemoralis* by Van der Horst *et al*. (1973); Van der Horst & Oudejans (1976).and summarized in Ackman (1982). Zandee *et al*. (1980) indicated conversions of C_{18} fatty acids to unsaturated ω6 and ω3 acids belonging to C_{20} and

C_{22} series in terrestrial herbivore snails. Aestivation is a simple physiological phenomenon exhibited by gastropods as a result of "low water content" which may be brought about either by "dryness of the environment" or "by the natural hydration cycle of the animal" (Swami & Reddy 1978). Chaki *et al*. (2008) studied activities of 13 metabolic enzymes in digestive gland, ovotestis and mantle of the active and aestivated *Achatina fulica.* The result showed that the enzymes activities were higher in the ovotestis during aestivation followed by digestive gland, while no remarkable change was observed in the mantle. The effects of aestivation on the phospholipid-specific fatty acid composition of mitochondrial membranes in the hepatopancreas of the terrestrial snail *Cepaea nemoralis* were investigated by Stuart *et al.* (1998). Some information about depletion of free fatty acids during aestivation in giant African snail *Achatina achatina* is provided by Umezurike & Iheanacho (1983). Pulmonates are hermaphrodites (Hyman, 1967; Parivar 1978), most species functioning as males in the early part of their life and females later on (Luchtel, 1972; Chétail & Fournié, 1986; South, 1992; Tomiyama, 1995). It is observed that *Achatina fulica* undergoes prolonged periods of aestivation and some of them lay eggs just after awakening at the onset of monsoon. It is obvious that both testis and ovary (within a single follicle) should not develop at a time because of hormonal regulation, and they mature at different age classes. This is evident through the development of albumen gland which is now considered as diagnostic marker of male-female distinction (Cunha *et al*., 1998). Mukai *et al.* (2004) confirmed that albumen gland is an accessory female reproductive structure of pulmonate (Egonmwan, 2007). This observation led the authors to think that prospective egg layers (females?) should exhibit a different physiological state than the others (males?). Thus a possible functional male/female distinction is possible on the basis of lipid and fatty acid storage. The present paper aims in obtaining a comprehensive knowledge on: a) lipid profiles in various organs viz., digestive gland, albumen gland, mantle and foot of active and aestivating hermaphrodite pulmonate gastropod, *Achatina fulica*, b) information about the lipid and fatty acid storage pattern, transportation and its utilization in these tissues during prolonged aestivation period based on male/female differentiation, and c) throw some light on the physiology of aestivation in *A. fulica* in relation to preparation for fecundity. The hypothesis of the present work is that male/female bias exists in *A. fulica*, which can be explained through the studies on lipid and fatty acid composition during active and aestivation.

MATERIALS & METHODS

Collection of specimen: Healthy active snails, *Achatina fulica* (Bowdich), were collected from some selective fields around Calcutta. A total of 400 snails of an average size group (6.2 - 8.2 cm) were taken and 200 snails were allowed to aestivate. The snails selected for the experiment were more or less of similar in size and weight. The snails were induced to aestivate by placing them in an aestivation chamber under controlled temperature (20°C ± 2°C) and humidity (45 ± 10%) coinciding the time of their natural aestivation. The control specimens were kept active by providing them with leafy vegetables and water *ad libitum* throughout the experimental period. The control snails were also kept under controlled temperature (27°C ± 2°C) and humidity (90 ± 10%) to minimize seasonal stress.

Male/Female identification

Achatina fulica is a hermaphrodite snail and is very difficult to categorize them into male and female groups. In this study male and females were separated based on the size of their albumen glands during aestivation as it was observed that those with large albumen glands lay eggs (female) and those with smaller glands are males. Because it was observed that the snails having much larger albumen gland lay eggs after completion of aestivation and those with smaller albumen glands do not lay eggs. Thus during aestivation snails with larger albumen gland are supposed to act as females while others with small albumen gland remain as male (non-female).

Collection of animal tissue

Control and aestivating gastropods were sacrificed at intervals of 7, 15, 30, 45, 60 and 90 days of experimentation. On each autopsy approximately 25 snails were sacrificed of which 10 males (with small albumen gland) and 10 females (with larger albumen gland) were considered. During autopsy, the snails were de-shelled and following tissues of the animal (*viz*., digestive gland, albumen gland, foot and mantle) were carefully dissected out, excess mucous and water were wiped and weighed to a constant weight.

Extraction and analyses of lipid classes and fatty acids

Tissue lipid was extracted following the procedure of Bligh & Dyer (1959). The total lipid extracted from various tissues such as digestive gland, albumen gland, mantle and foot were weighed and residue was dissolved in known volume of fresh chloroform and nitrogen was bubbled through it (Kates, 1972). It was then stored in a freezer until further analysis.

Thin layer chromatoplates were prepared according to the general procedure described by Mangold (1969). Phospholipids were separated using solvent system of acetone-methanol-acetic acid-water (6: 8: 2: 1 v/v) (Rouser *et al*., 1967). Neutral lipids were separated using hexane-diethyl ether-acetic acid (80: 20.1 v/v). All the lipids were identified by comparing Rf values with those of known standards and spraying with specific reagents.

Total cholesterol in tissue lipid was estimated according to Zlatkis *et al.* (1953). Phospholipids of tissue sample were determined by estimating phosphorus in the lipid according to standard procedure of Chen *et al*. (1956). Estimation of tissue triacylglycerol was carried out according to the standard procedure of Van Handel *et al*. (1957) after isolating pure triacylglycerol from total lipid. The fatty acid composition of the tissue lipids was determined by converting the lipids into methyl esters, separating the esters by thin layer chromatography and then analyzing it by gas liquid chromatography. Analysis of methyl ester was carried out by gas liquid chromatographic technique (Ghosh Choudhuri *et al*., 1983) in GC (Hewlett Packard Model 5890 A) with Flame ionization detector. The oven, injection port and the detector block temperatures used were 190^0C, 230^0C and 240^0C respectively. The sample of methyl ester (0.1 - 1

μg) was injected from a Hamilton syringe and the respective chromatogram was obtained. The fatty acids were identified by comparing the retention time of GLC grade standard saturated and unsaturated acid esters of varying chain length and unsaturation. The percentage composition of the component fatty acids was determined by measuring the areas of the peaks with the help of integrator (Hewlett Packard - 3390A).

RESULTS

The data on total lipid, phospholipid, cholesterol and triacylglycerol (TG) of active and aestivated *Achatina fulica* are presented in Table 1 (male) and Table 2 (female). Percent composition of total lipid in all the organs of active male of *A. fulica* are in following order: albumen gland (31.6%), digestive gland (31%), mantle (20.7%) and foot (16.7%) in comparison to female which shows; digestive gland (35.02), albumen gland (25.04), mantle (23.17) and foot (16.75) respectively (Figs. 1-4). Students '*t*' test indicate that the average total lipid of digestive and albumen glands in active male and female differ significantly at 0.5% level. In general mantle and foot contain lesser amount of total lipid in comparison to two other organs. Total lipid percent in digestive gland of male decreases at 45 and 90 days of aestivation; however, in female the variation is little.

TABLE 1. Total lipid, phospholipid, cholesterol and triacylglycerol (expressed in mg/g of wet tissues) in the different organs of male active and aestivating *Achatina fulica*. (mean±S.D.)

Days of aestivation →		0 day	7 days	15 days	30 days	45 days	60 days	90 days
Total lipid	Digestive gland	39.08±8.93	39.06±5.77	32.52±7.54	25.65±3.41	26.15±3.26	35.15±0.60	25.37±5.18
	Albumen gland	39.86±1.03	36.17±3.89	25.27±8.70	29.79±6.82	29.65±2.81	31.67±4.01	36.73±3.09
	Mantle	26.11±1.94	20.06±3.45	21.63±2.62	21.40±5.43	19.23±2.28	23.67±3.11	20.27±4.04
	Foot	21.07±4.61	12.33±2.18	10.88±1.49	12.13±1.72	16.78±6.88	14.07±3.43	15.44±0.87
Phospholipid	Digestive gland	14.27±2.82	11.86±0.82	7.37±0.90	7.07±0.94	6.95±0.52	4.99±1.99	2.82±0.91
	Albumen gland	14.05±1.54	12.30±1.55	8.16±2.13	13.26±2.57	13.59±5.48	8.27±1.68	9.95±2.40
	Mantle	11.20±0.59	10.77±1.88	11.38±6.57	11.43±1.64	7.11±1.57	5.22±3.08	4.86±1.68
	Foot	6.27±0.23	5.85±1.59	5.24±0.53	5.62±0.94	4.94±0.55	3.02±1.69	3.13±1.57
Total cholesterol	Digestive gland	9.42±2.06	4.10±1.22	4.14±0.82	4.23±0.38	4.41±1.29	4.68±1.98	3.14±0.74
	Albumen gland	12.08±1.53	5.45±2.05	3.45±1.89	3.81±1.75	2.95±1.15	3.09±1.25	2.65±0.83
	Mantle	2.08±0.93	3.10±1.07	3.16±1.08	2.65±0.85	3.51±0.55	4.23±1.21	2.63±0.72
	Foot	2.67±0.92	2.48±0.97	2.53±1.31	2.60±0.61	2.59±1.04	2.88±0.95	2.15±0.99
Triacylglycerol	Digestive gland	3.61±0.95	3.68±0.58	2.43±0.32	3.13±0.18	4.47±0.40	2.99±0.56	2.23±0.10
	Albumen gland	1.52±0.21	1.54±0.47	1.34±0.13	2.19±0.09	1.10±0.02	0.62±0.18	0.39±0.05
	Mantle	0.35±0.001	0.34±0.01	0.34±0.01	0.29±0.02	0.30±0.02	0.34±0.09	0.22±0.01
	Foot	0.24±0.01	0.24±0.11	0.09±0.00	0.32±0.01	0.06±0.02	0.14±0.01	0.20±0.06

TABLE 2. Total lipid, phospholipid, cholesterol and triacylglycerol (expressed in mg/g of wet tissues) in the different organs of female active and aestivating *Achatina fulica.* (mean±S.D.)

Days of aestivation →		0 day	7 days	15 days	30 days	45 days	60 days	90 days
Total lipid	Digestive gland	41.17±5.40	35.83±2.51	35.05±2.24	29.24±2.90	29.31±2.44	26.27±4.61	35.02±2.07
	Albumen gland	17.51±2.26	26.47±3.50	21.09±2.79	16.50±4.86	18.52±4.38	14.38±4.18	12.45±2.60
	Mantle	26.99±1.49	20.37±2.24	22.20±5.04	19.94±4.66	20.13±6.14	20.98±3.50	21.11±3.94
	Foot	17.30±2.76	15.03±3.75	13.73±3.03	12.03±1.84	15.72±4.85	13.96±3.42	14.12±1.25
Phospholipid	Digestive gland	15.12±3.44	13.75±3.11	11.81±4.71	9.21±3.63	8.32±3.58	6.24±3.34	3.93±1.34
	Albumen gland	6.05±2.16	8.62±1.88	8.28±1.29	6.54±2.37	5.20±1.78	5.86±1.40	4.44±1.82
	Mantle	13.83±2.40	9.48±3.18	6.37±1.86	6.16±1.99	6.68±2.33	7.15±1.99	6.96±3.17
	Foot	6.43±2.59	5.76±1.41	4.83±1.08	4.50±1.05	4.49±0.87	3.62±1.32	4.00±1.40
Total cholesterol	Digestive gland	5.90±1.17	4.21±1.74	4.12±0.88	4.97±0.54	3.99±1.39	4.78±1.61	3.11±0.78
	Albumen gland	3.63±1.72	3.75±1.65	3.28±1.63	3.11±1.39	3.74±0.85	3.58±1.74	4.17±0.52
	Mantle	3.03±1.58	2.50±1.07	2.15±0.94	2.24±1.10	3.15±0.79	3.87±1.38	2.93±1.45
	Foot	2.75±1.37	2.20±0.33	2.50±0.15	2.31±0.92	2.41±1.45	2.98±1.05	2.53±1.14
Triacylglycerol	Digestive gland	2.32±0.89	2.35±0.01	1.68±0.26	4.47±0.40	3.17±0.50	1.83±0.04	1.08±0.07
	Albumen gland	0.59±0.03	0.58±0.02	0.68±0.08	0.74±0.14	0.77±0.02	0.35±0.08	0.30±0.05
	Mantle	0.27±0.02	0.27±0.00	0.23±0.05	0.31±0.01	0.18±0.01	0.19±0.23	0.22±0.03
	Foot	0.09±0.00	0.11±0.00	0.08±0.00	0.24±0.02	0.20±0.02	0.32±0.08	0.17±0.11

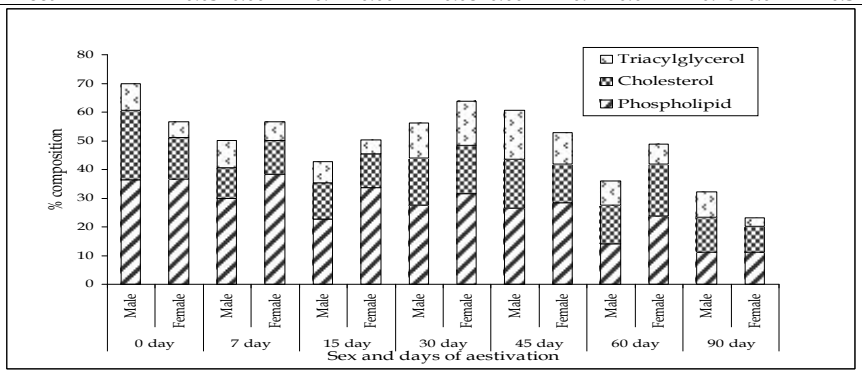

FIGURE 1. Percent composition of phospholipid, cholesterol and TG in total lipid of the digestive gland of active and aestivated *Achatina fulica*

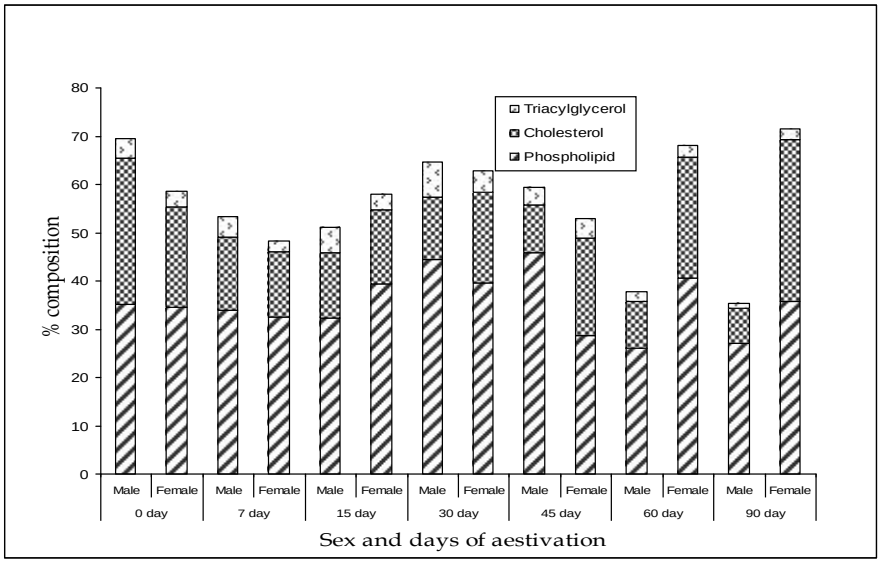

FIGURE 2. Percent composition of phospholipid, cholesterol and TG in total lipid of albumen gland of active and aestivated *Achatina fulica*.

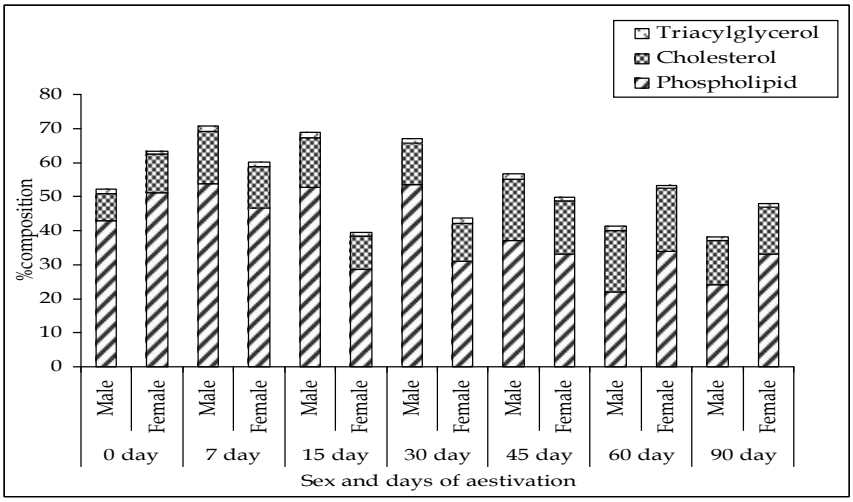

FIGURE 3. Percent composition of phospholipid, cholesterol and TG in total lipid of mantle of active and aestivated *Achatina fulica.*

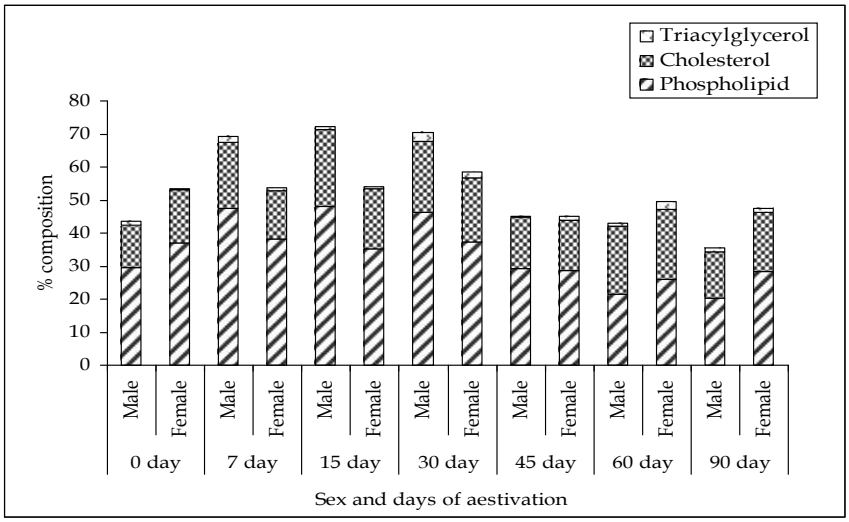

FIGURE 4. Percent composition of phospholipid, cholesterol and TG in total lipid of foot of active and aestivated *Achatina fulica*

It is observed that the percentage of total lipid in albumen gland is always lower in female than that of the male during aestivation and the amount of phospholipid in the digestive gland of female is always higher than that of the male; though this amount decreases gradually with the progress of aestivation. The amount of phospholipid in albumen gland is higher in males than that of the female. The amount decreases little in aestivating snails of both the sex. Phospholipid content in both mantle and foot show slow decreases in amount as aestivation proceeds. Percent of phospholipid in total lipid also show similar pattern. Drop in phospholipid percent in 60 and 90 days aestivated male snail is lower than that of the female. The cholesterol level in digestive and albumen gland (it is obvious this is in the snail!) in both sexes reduces gradually throughout aestivation. Cholesterol content in mantle and foot shows fluctuation during aestivation. Percent of cholesterol in total lipid in all the organs is provided in Figs. 1-4. Table 3 tabulates ANOVA results of all the lipid classes during the progression of aestivation. Phospholipid/cholesterol ratio in digestive and albumen glands of control snail is always higher in female than that of the male (Table 4). However it is almost equal in the mantle and foot of control snails of both the sexes. During aestivation, this ratio is higher in female. Profile of TG in digestive gland of active and aestivating snail is more or less similar in both the sex and shows no significant difference. Percent of TG in total lipid fluctuates in digestive and albumen gland throughout the aestivation period. However, it remains fairly constant in mantle and foot (Figs. 3-4).

Classes of fatty acids and their composition in digestive gland, albumen gland, mantle and foot of active and aestivated snails of both sexes is represented in Tables 5-8 respectively. Pearson correlations coupled with p-value among fatty acid classes in digestive and albumen glands of active and aestivated snails show that male/female distinction is statistically significant (Table 9). Detailed account of all the fatty acid (percent composition w/w) classes in all the four organs with the ratios of saturated fatty acids (SFA), monoenes (M) and polyenes (P) is presented in Table 10.

TABLE 3. Analysis of variance of total lipid, phospholipid, cholesterol and triacylglycerol of all the organs of active and aestivated *Achatina fulica* of both sex

0 day					
	Sum of sq's	D.F.	Mean sq.	F(D.F./1)	Prob.
Regression	263.461	2	131.73	76.6371	0.08051
Residual	1.7188	1	1.7188		
Total	265.179	3			

7 day					
	Sum of sq's	D.F.	Mean sq.	F(D.F./1)	Prob.
Regression	491.514	2	245.76	181.419	0.05243
Residual	1.3546	1	1.3546		
Total	492.869	3			

15 day					
	Sum of sq's	D.F.	Mean sq.	F(D.F./1)	Prob.
Regression	243.657	2	121.829	697.482	0.02676
Residual	0.17467	1	0.17467		
Total	243.832	3			

30 day					
	Sum of sq's	D.F.	Mean sq.	F(D.F./1)	Prob.
Regression	169.829	2	84.9144	49.3823	0.10012
Residual	1.71953	1	1.71953		
Total	171.548	3			

45 day					
	Sum of sq's	D.F.	Mean sq.	F(D.F./1)	Prob.
Regression	106.253	2	53.1265	67.7434	0.08559
Residual	0.78423	1	0.78423		
Total	107.037	3			

60 day					
	Sum of sq's	D.F.	Mean sq.	F(D.F./1)	Prob.
Regression	261.688	2	130.844	70.3964	0.08398
Residual	1.85867	1	1.85867		
Total	263.547	3			

90 day					
	Sum of sq's	D.F.	Mean sq.	F(D.F./1)	Prob.
Regression	250.242	2	125.121	2248.37	0.01491
Residual	0.055649	1	0.05565		
Total	250.297	3			

TABLE 4. Phospholipid : cholesterol ratio in four organs of active and aestivating *Achatina fulica* of both sex.

Different organs		0 day	7 day	15 day	30 day	45 day	60 day	90 day
Digestive gland	Male	1.5 : 1	2.8 : 1	1.7 : 1	1.6 : 1	1.6 : 1	1.2 : 1	0.8 : 1
	Female	2.5 : 1	3.2 : 1	2.9 : 1	1.8 : 1	2.1 : 1	1.3 : 1	1.3 : 1
Albumen gland	Male	1.2 : 1	2.2 : 1	2.4 : 1	3.5 : 1	4.6 : 1	2.7 : 1	3.7 : 1
	Female	1.7 : 1	2.4 : 1	2.5 : 1	2.1 : 1	1.4 : 1	1.6 : 1	1.1 : 1
Mantle	Male	5.4 : 1	3.5 : 1	3.6 : 1	4.3 : 1	2.0 : 1	1.2 : 1	1.8 : 1
	Female	4.6 : 1	3.8 : 1	3.0 : 1	2.8 : 1	2.2 : 1	1.8 : 1	2.4 : 1
Foot	Male	2.3 :1	2.3 : 1	2.1 : 1	2.1 : 1	1.9 : 1	1.1 : 1	1.4 : 1
	Female	2.3 : 1	2.6 : 1	1.9 : 1	1.9 : 1	1.9 : 1	1.2 : 1	1.6 : 1

TABLE 5. Principal fatty acids of total lipid of the digestive gland of active and aestivated male and female *Achatina fulica*. (mean±S.D.)

Fatty acids	Control		7 days		15 days		30 days		45 days		60 days		90 days	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Saturated														
14:0	3.66±0.54	3.16±0.65	5.25±0.30	2.34±0.31	3.69±0.22	4.49±0.10	7.05±1.92	4.44±3.61	3.35±0.45	5.78±0.14	1.57±0.05	2.71±0.08	4.52±2.33	3.56±1.80
15 ;0	-	0.70±0.32	-	-	-	-	-	-	-	-	-	-	-	-
16:0	6.66±0.54	6.59±0.31	8.06±0.88	5.74±0.26	14.27±0.62	8.57±0.41	12.61±2.91	10.73±2.92	8.22±0.62	9.62±0.25	13.64±0.11	9.41±2.51	5.88±0.29	11.06±2.73
17:0	1.5±0.65	4.61±0.95	1.86±0.06	3.57±0.59	3.31±1.04	3.51±0.30	2.12±0.56	2.98±2.26	2.94±0.80	1.69±0.44	1.15±0.14	1.34±0.38	11.99±0.81	1.23±0.24
18:0	11.48±0.67	8.30±0.75	7.86±0.15	6.61±0.35	8.81±0.16	5.4±0.28	10.52±1.36	7.45±0.33	7.10±0.67	4.55±0.15	7.88±0.54	9.36±2.84	7.75±0.58	5.99±1.60
24:0		2.41±0.81		1.12±0.92	-	0.98±0.2	-	0.54±0.12	-	0.78±0.55	-	2.62±0.51	-	5.96±1.56
Monoenes														
14:1	0.51±0.10	0.61±0.1	0.43±0.04	0.62±0.07	0.48±0.04	0.48±0.07	1.16±0.53	1.02±0.22	1.13±0.18	0.41±0.11	0.49±0.34	0.48±0.04	0.49±0.03	0.14±0.04
15:1	0.07±0.04	0.73±0.08	0.88±0.03	1.27±0.12	1.63±0.41	0.76±0.14	0.84±0.15	1.08±0.27	0.96±0.08	0.71±0.11	0.80±0.09	0.41±0.22	0.63±0.17	0.28±0.06
16:1	0.52±0.03	0.84±0.07	0.81±0.07	1.30±0.16	4.33±0.46	-	2.99±1.29	-	4.96±0.16	-	0.73±0.11	-	1.32±0.03	-
18:1	9.48±0.59	13.94±1.27	11.89±0.1	9.06±1.07	16.05±0.33	15.04±1.53	14.21±1.59	17.51±1.00	15.52±0.37	14.43±1.10	15.02±2.01	13.32±1.16	13.81±1.00	14.10±1.0
22:1	0.13±0.08	0.04±0.03	2.76±0.06	1.82±0.61	2.54±0.37	0.75±0.14	1.94±0.81	-	3.08±0.14	-	1.44±0.01	-	2.49±0.80	-
Polyenes														
18:2w6	14.59±0.36	10.63±0.57	13.68±0.32	12.10±0.18	16.1±0.17	14.45±0.93	15.0±1.63	15.80±2.52	16.50±0.44	16.65±0.36	17.83±0.70	17.21±1.03	13.52±0.77	13.78±1.43
18:3w3	20.09±0.1	21.02±0.74	18.07±0.74	16.63±1.12	10.27±0.98	16.96±0.50	8.43±0.98	15.85±0.58	9.16±0.06	14.55±0.11	11.62±3.46	16.89±0.68	8.63±3.03	12.84±0.73
18:4	0.02±0.02	-	0.54±0.11	2.08±0.02	0.36±0.07	5.02±0.53	1.29±0.69	2.78±0.67	4.23±1.03	4.25±0.12	4.23±1.17	5.38±1.89	7.48±0.86	4.77±0.49
20:3w3	19.15±0.21	18.32±0.51	12.23±0.48	13.41±1.83	12.38±0.42	16.34±1.02	15.78±2.36	11.55±1.82	14.49±0.51	13.34±0.51	12.93±4.99	14.77±0.54	17.23±2.34	9.27±1.1
20:4w6	8.33±1.3	9.54±0.20	2.73±0.40	1.87±0.60	1.36±0.45	1.51±0.61	2.47±0.27	1.31±0.17	1.73±0.51	1.61±0.10	5.07±1.62	3.49±1.20	-	5.16±0.66
20:5w3	0.06±0.03	0.01±0.0	0.39±0.03	0.21±0.09	0.38±0.09	-	0.27±0.2	-	-	-	-	-	-	-
22:4w3	-	0.31±0.03	-	-		-		-	1.71±0.91	-	-	-	1.49±0.83	-
22:5w6	0.04±0.04	0.03±0.01	0.47±0.03	1.53±0.36	0.88±0.03	-	-	-		-	-	1.10±0.02	-	1.28±0.41
24:2	-	-	-	-	-	-	-	-	2.91±0.89	-	-	-	2.49±1.48	-

TABLE 6. Principal fatty acids of total lipid of the albumen gland of active and aestivated male and female *Achatina fulica*. (mean±S.D.)

Fatty acids	Control		7 days		15 days		30 days		45 days		60 days		90 days	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Saturated														
14:0	0.87±0.05	0.54±0.11	0.84±0.05	0.65±0.03	0.71±0.02	0.91±0.02	0.99±0.02	0.93±0.25	3.09±0.17	1.28±0.06	1.16±0.1	1.77±0.72	1.53±0.49	0.90±0.08
16:0	17.98±0.18	19.40±0.16	14.4±0.46	22.02±4.28	14.26±0.08	20.40±1.30	19.36±0.02	17.99±1.85	16.62±0.1	12.71±0.17	15.04±3.41	17.92±1.49	15.38±1.89	16.96±2.12
17:0	0.36±0.06	0.32±0.08	-	-	-	-	-	-	-	-	-	-	-	-
18:0	7.5±0.51	7.81±0.34	9.23±0.63	8.39±0.46	6.54±0.41	12.31±0.36	9.330.89	8.41±0.23	7.79±0.17	5.36±0.30	8.57±0.27	4.81±1.20	7.26±0.42	4.33±2.02
24:0	-	0.92±0.31	-	1.21±0.43	-	0.98±0.39	-	1.12±0.84	-	2.30±0.09	-	1.98±0.80	-	2.39±0.42
Monoenes														
14:1	0.58±0.06	0.37±0.01	1.52±0.34	1.54±0.66	0.13±0.1	0.53±0.04	0.62±0.01	0.34±0.01	1.78±0.11	0.81±0.02	1.60±0.08	0.35±0.13	5.47±1.11	0.38±0.19
15:1	0.57±0.06	0.70±0.08	0.49±0.2	1.39±0.17	0.14±0.02	0.56±0.02	0.67±0.04	1.11±0.21	1.91±0.03	2.14±0.24	1.23±0.05	0.55±0.22	1.03±0.14	0.38±0.03
16:1	15.06±0.62	13.74±0.24	11.65±0.06	14.11±0.19	12.10±0.02	13.06±0.18	9.64±0.01	6.26±1.63	12.14±0.15	1.93±0.05	17.78±0.24	-	16.0±2.24	-
18:1	11.09±0.17	11.11±0.20	13.66±0.64	12.11±0.99	10.56±0.39	13.63±0.05	10.19±0.24	12.46±0.32	11.76±0.14	9.67±0.31	13.57±0.51	11.95±1.28	13.25±2.03	15.02±1.28
22:1	2.75±0.08	1.75±0.55	2.45±0.29	1.80±0.09	-	2.04±0.16	-	0.98±0.25	-	0.32±0.01	-	1.04±0.15	-	-
Polyenes														
18:2w6	6.17±0.25	9.64±0.45	9.97±0.06	10.38±0.63	9.96±0.05	10.38±0.03	8.09±0.17	11.01±0.21	7.48±0.08	11.13±0.13	10.05±0.13	9.80±2.74	6.60±2.02	12.28±3.47
18:3w3	7.11±0.21	1.94±0.72	8.39±0.20	8.96±0.82	6.35±0.30	8.18±0.56	6.12±0.08	7.45±0.25	7.08±0.06	5.44±0.41	-	5.24±2.93	-	7.26±1.77
18:4	-	6.24±0.63	-	1.98±0.03	-	-	-	-	-	-	-	-	-	-
20:3w3	8.07±0.15	9.99±0.07	6.66±0.06	7.54±0.56	5.18±0.06	4.80±0.09	7.92±0.04	12.99±0.45	4.70±0.09	7.63±0.03	8.70±0.03	8.52±0.10	5.89±0.89	8.30±0.51
20:4w6	6.3±0.11	6.67±6.46	5.45±0.12	3.87±0.29	5.26±0.06	5.08±0.19	4.33±0.36	7.58±2.60	4.35±0.04	8.87±0.14	3.04±0.68	4.54±0.14	3.55±1.17	6.68±1.96
20:5w3	4.35±0.09	2.34±0.18	1.81±0.02	-	-	-	-	-	-	-	-	-	-	-
22:4w3	-	3.17±0.84	-	3.71±0.43	-	-	1.35±0.04	-	1.09±0.03	1.18±0.16	1.05±0.06	1.88±0.06	1.14±0.15	1.60±0.51
22:5w6	-	0.72±0.03	-	-	-	-	-	-	-	-	-	-	-	-

TABLE 9. Correlations among three fatty acid classes in digestive and albumen glands of active and aestivating *Achatina fulica* of both sex.

	DgMs	DgFs	AlMs	AlFs	DgMm	DgFm	AlMm	AlFm	DgMp	DgFp	AlMp
DgFs	0.076										
	0.871										
AlMs	0.245	0.439									
	0.596	0.324									
AlFs	-0.468	-0.140	-0.313								
	0.289	0.765	0.494								
DgMm	0.373	0.056	0.481	0.395							
	0.409	0.906	0.275	0.381							
DgFm	0.508	-0.483	-0.391	0.027	0.331						
	0.244	0.272	0.385	0.953	0.469						
AlMm	0.094	0.431	-0.145	0.175	-0.205	-0.412					
	0.841	0.335	0.756	0.708	0.660	0.358					
AlFm	-0.019	-0.631	-0.612	0.054	-0.098	0.801	-0.566				
	0.967	0.128	0.144	0.908	0.834	0.03	0.185				
DgMp	-0.128	-0.358	-0.350	-0.423	-0.824	-0.29	0.246	-0.036			
	0.784	0.430	0.441	0.344	0.023	0.528	0.595	0.939			
DgFp	-0.288	-0.826	-0.476	0.072	-0.405	0.025	-0.129	0.258	0.717		
	0.531	0.022	0.280	0.879	0.367	0.958	0.782	0.576	0.070		
AlMp	-0.168	-0.584	-0.222	-0.064	0.031	0.557	-0.932	0.780	-0.115	0.300	
	0.718	0.169	0.633	0.891	0.948	0.194	0.002	0.039	0.805	0.513	
AlFp	0.145	0.414	0.733	-0.526	-0.070	-0.698	0.172	-0.823	0.270	-0.076	-0.434
	0.757	0.355	0.061	0.225	0.881	0.081	0.712	0.023	0.557	0.871	0.331

Cell contents : Pearson correlation
P- value

Dg = digestive gland
Al = albumen gland
M = male
F = female

s = saturated fatty acid
m = monoene fatty acid
p = polyenefatty acid

TABLE 10. Principal fatty acid of total lipid composition (%w/w) in all the organs of active and aestivating *Achatina fulica* of both sex

Days of		Digestive gland			Albumen gland			Mantle			Foot			Total			
aestivation	Sex	Saturated	Monoene	Polyene	Saturated	Monoene	Polyene	Saturated	Monoene	Polyene	Saturated	Monoene	Polyene	Saturated	Monoene	Polyene	*SFA:M:P* ratio
0 day	*Male*	24.20	11.12	64.68	30.05	33.87	36.07	35.26	17.17	47.48	30.40	20.29	48.96	119.91	82.45	197.21	1.45 : 1 : 2.39
	Female	23.56	16.30	60.14	29.77	28.42	41.81	22.53	22.11	55.35	22.93	28.02	49.04	100.59	94.85	206.34	1.06 : 1 : 2.17
7 day	*Male*	26.20	19.08	54.73	28.05	34.40	37.93	30.27	23.45	46.28	26.70	27.08	46.22	111.22	104.01	185.16	1.07 : 1 : 1.78
	Female	23.84	21.00	58.85	32.38	31.05	35.56	24.54	24.62	50.84	25.67	24.25	48.77	106.43	100.92	190.02	1.05 : 1 : 1.88
15 day	*Male*	31.06	25.85	43.09	30.21	32.21	37.58	26.46	24.21	49.33	25.53	28.10	46.36	113.26	110.37	176.36	1.03 : 1 : 1.60
	Female	23.71	2.23	56.05	37.26	32.11	30.63	36.47	27.50	36.00	28.71	29.13	42.08	126.15	108.99	164.76	1.16 : 1 : 1.55
30 day	*Male*	33.41	21.87	44.72	33.16	28.85	37.99	30.25	22.70	47.04	34.00	24.48	38.49	130.82	97.90	166.24	1.34 : 1 : 1.70
	Female	29.83	16.21	53.96	32.10	23.86	44.04	31.50	22.36	46.09	30.37	26.45	43.17	123.80	88.88	187.26	1.39 : 1 : 2.11
45 day	*Male*	22.05	26.18	51.77	34.47	34.58	30.96	28.67	20.97	50.36	32.35	24.74	39.92	117.54	106.47	173.01	1.10 : 1 : 1.62
	Female	25.37	17.61	57.03	30.59	21.01	48.40	27.92	20.58	51.50	27.60	24.99	47.40	111.48	84.19	204.33	1.32 : 1 : 2.43
60 day	*Male*	24.76	19.82	55.42	30.28	41.79	27.92	27.00	22.33	50.67	32.40	26.60	41.00	114.44	110.54	175.01	1.04 : 1 : 1.58
	Female	25.83	14.43	59.74	37.64	19.74	42.62	32.28	23.80	43.93	32.05	26.26	41.67	127.80	84.23	187.96	1.52 : 1 : 2.23
90 day	*Male*	30.22	18.79	50.98	31.35	46.37	22.26	24.48	25.43	49.76	27.73	24.35	47.92	114.12	112.94	170.94	1.01 : 1 : 1.51
	Female	31.91	16.24	52.67	32.14	20.63	42.23	24.19	27.56	48.25	28.66	22.37	48.95	116.90	86.80	192.10	1.35 : 1 : 2.21

DISCUSSION

Achatina fulica being herbivorous pulmonate gastropod, the lipid classes and fatty acids in this snail are probably exogenous in origin as true in the case of certain bivalves (Beninger & Stephan, 1985). The snails starve during aestivation and during this period females (?) make preparatory steps for future egg laying. It is evident that total lipid profiles of all the organs of *A. fulica* show distinct male/female differentiation. Total lipid of the digestive gland in both male/female groups reduces up to 30-45 days of aestivation after which it stabilizes in female while it increases in male, which is indicative of more lipid utilization in female than male during aestivation. In a study conducted without sex bias, Mitra and Sur (1989) record decrease of total and neutral lipid in *A. fulica* from 60 days of aestivation. Higher amount of lipid in digestive gland is reported in active snails and bivalves (Wenne & Polak, 1989; Napolitano & Ackman, 1992; Da Silva & Zancan, 1994; Rakshit *et al*., 1997). Lower amount of lipid in albumen gland, compared to digestive gland can be explained by the fact that the former acts as a reservoir of carbohydrate and protein (Chaki, 1987). A stable level of lipid in the albumen gland is indicative of lipid transportation from digestive gland which is reported in case of pectinid bivalves as important in reproduction (Vassallo, 1973; Robinson *et al*., 1981; Pazos *et al*., 1997). Lipid profiles of mantle and foot exhibit initial decline and then maintains stability throughout aestivation indicating the relative non-involvement of these organs in egg production. It was found that the activities of metabolic enzymes during aestivation were higher in digestive gland than of the mantle (Chaki *et al*., 2008).

In animals phospholipids serve as a membrane component and play a major role in signal transduction. Phospholipids also participate in the transport of triacylglycerol and cholesterol in animals (Conn *et al*., 2003). The amount of phospholipid gradually decreases to $1/4^{th}$ at 90 days of aestivation in both the sexes. Albumen gland of male exhibits higher amounts of phospholipids than female, but the female albumen gland shows less fluctuation and maintains more or less the same amount of phospholipid during aestivation. This indicates the involvement of this lipid class in egg production, which plays a dynamic role during aestivation. As an alternative explanation, it could be that a small proportion of the highly unsaturated fatty acids are converted to prostacyclins or prostaglandins (steroid hormones) to function in directing the assembly of the ovum. The conversion to steroid hormones is probably achieved by the hydrolysis of phospholipids into free fatty acids whenever required. Cytomorphological studies on the ovotestis of *A. fulica* show well developed gonads (Rakshit *et al*., 2005), which are attributed to the availability of steroid hormones Seasonal variations in phospholipid are documented in a terrestrial pulmonate gastropod by (Da Silva & Zancan, 1994). Higher amount of phospholipid in mantle and foot and steady decrease of phospholipid in mantle and foot of both sexes is probably due to maintenance of homeostasis and membrane fluidity (Alexandrov, 1977; Cossins & Prosser, 1978). Phytophagous snails contain cholesterol as the major component of sterols (Idler & Wiseman, 1972). In the present experiment the digestive gland of active male *A. fulica* shows higher amount of cholesterol than female and this trend is maintained throughout aestivation. Cholesterol shows higher levels in active male snails and may be associated with spermatogenesis as reported in case of bivalves (Napolitano *et al*., 1993). The results on the fluctuation level can be due to cessation of supply from the digestive gland at later stages of aestivation. Mantle being the membranous part interacts more with the environment and exhibits greater fluctuation in cholesterol which may be attributed to influence of environment as in the case of bivalve *Macoma* sp. (Jarjebski & Wenne, 1990), however, these results need further clarification.

Triacylglycerol (TG) plays an important role in reproductive strategy of mollusks (Napolitano *et al*., 1992). Triacylglycerols in digestive gland of male and female *A. fulica* is mostly above 10% level in both active and aestivated stages, the level being higher in males than female although the pattern of fluctuation appears same. In albumen gland of both sexes TG level is lower compared to digestive gland but no difference in pattern of fluctuation was noted. Mantle and foot contain least amounts of TG. The results are in accordance with the findings of Wenne & Polak (1992). To summarize, the highest amount of TG is found in digestive gland, which is probably because this gland appears to be the most active organ during aestivation. Lustrino *et al.* (2010) reported that triglycerides metabolism in *A. fulica* is more influenced by photoperiod than cholesterol metabolism. It is interesting to observe that some organs *viz*., digestive gland, albumen gland and mantle of both sexes show increment of certain lipid classes. This may be due to lipid transportation from a compensating organ or lipid biosynthesis by that organ. There is no existing information regarding existence of such compensating organ. According to previous workers (Hegg, 1977; Mitra & Sur, 1989) lipid and glycogen metabolism provide energy during aestivation in gastropod *Pila globosa*. Biosynthesis of lipids in active mollusks has been established but in aestivating snails information is scanty. Studies on metabolic adaptation in aestivating *Achatina achatina* by Umezurike & Iheanacho (1983) revealed that in short-spell forced starved and aestivating snails glycogen degradation and lipid mobilization takes place in foot, digestive gland and heart muscle at the beginning of aestivation. Such mechanism also takes place in other pulmonate (Van der Horst, 1974; Chaki *et al*., 2008). The above statement can explain depletion of TG in *A. fulica* up to 15 days of aestivation, but further studies need to be conducted to explain the surge in later stages of aestivation. Basic types of fatty acids occurring in mollusks include C_{14}, C_{16}, C_{17}, C_{18}, C_{20}, C_{22} and C_{24} (Misra *et al.*, 2002; Kraffe *et al*., 2008). Palmitic (16:0), stearic (18:0) and eicosanoic (20:0) acids are predominant saturated fatty acids in terrestrial snails (Voogt 1972; Van der Horst & Zandee 1973; Catalan *et al*., 1977), freshwater (Dembitsky *et al*., 1994) and marine (Saito & Hashimoto, 2010) snails. Fluctuation in saturated fatty acids (SFA) is noted in pulmonate *A. fulica*, which can be attributed to the fact SFA serves as energy source in bivalves and gastropods (Polak *et al*., 1987). Variation in 14:0 and 16:0 acids in *A. fulica* in active as well as aestivating stages can be explained as the result of *de novo* synthesis which occurs in bivalves (Sprecher & James, 1979). Higher percent of 16:0 and 18:0 acids in albumen gland of female

throughout aestivation is possibly related to vitellogenesis. It appears interesting that 18:0 is the predominating fatty acid in digestive gland while 16:0 is predominating in other organs. Sum of 16:0 acids of the four organs of the active snail shows higher value than 18:0 acids, which can be explained by Ackman's (1982) observations which inferred that 16:0 is the principal fatty acid at all evolutionary and trophic levels. This is true in case of freshwater gastropods (Dembitsky *et al*., 1993b, c, 1994; Misra *et al*., 2002). Monoenoic constitutes the least amount of fatty acid classes in all the organs of both sexes. Increase in 18:1 acid probably is the reason for increase in monoenoics in digestive gland in male during mid-aestivation stages. This fact is contradictory to Ackman (1980). In female 18:1 appears to be predominant in contrast to 16:1, which is undetectable after 7^{th} day of aestivation. In male albumen gland, 16:1 and 18:1 are the major monoenoics, while in female 18:1 appears to be the chief one and 16:1 drops down at 30^{th} day and is undetectable in the later stages. Most probably 18:1 plays a major role in vitellogenesis. Ackman (1980) provided several plausible methods of bioconversion of fatty acids in mollusks, of which 16:1ω7 + 2C→18:1ω7 seems the most appropriate mechanism in this gastropod. Similar bioconversion pathways are noted in active freshwater snails (Dembitsky *et al*., 1993b, c; 1994). All these factors point to a clear male/female distinction from the point of bioconversion and utilization of monoethylenic acids (Tables 5-8, 10). Polyunsaturated fatty acids (PUFA), particularly C_{18} and C_{20} contribute a major amount of fatty acids. PUFAs show maximum amount in digestive gland, lowest in albumen gland and similar amounts in mantle and foot. Predominating polyenes in active and aestivating stages include 18:2ω3 and 20:3ω3 acids in varying amounts. Most of these acids can be assumed to be dietary in origin as noted in bivalves (Ackman 1982). Arakelova *et al.* (2009) are of the opinion that the occurrence and fluctuation of ω3 fatty acids is contingent upon the habitat and motor activity of sea and freshwater mollusks. Significant amount of 20:5ω3 acid is observed in foot of female and albumen gland of male while lesser amount is recorded in albumen gland of female and this can be attributed to bioconversion or biosynthesis. Thus it can be inferred that digestive gland acts as the source of free fatty acids, which can be correlated, to trophic metabolic function of midgut (digestive) gland (Catalan *et al*., 1977). Low level of 20:3ω6 acid is observed in female digestive gland from 7^{th}–60^{th} day of aestivation while higher amounts are recorded in albumen gland of the same. This is contrary to the levels of 18:3ω3 acid in female. This is a strong indication of bioconversion through chain elongation process. Steady levels of 18:2ω6 acids in mantle of aestivating snails of both sexes can be attributed to an adaptation for minimization of water loss (Wertz & Downing 1990). Linolenic acid (18:3ω6) is probably the precursor for PUFA synthesis in this gastropod. Considerable amounts of 20:4 acid is detected in both digestive and albumen glands in both sexes which is probably dietary in origin. According to Sepe *et al.* (1998), 20:4 is of dietary origin in edible bivalves and indicator of salinity in marine bivalves (Wenne & Polak, 1992). Occurrence of 20:3ω3 acid appears to be high in this pulmonate as compared to marine bivalves (Napolitano *et al*., 1992) and freshwater gastropods (Misra *et al.*, 2002), but their exact role needs to be ascertained. Thus, total amount of fatty acids in all four organs of *A. fulica* shows that SFA in male is higher than that of the female up to 45^{th} day of aestivation; monoenoics are lower than female in active snails but higher in aestivating stages (Table 10). Polyenes are always higher in female than male except the 15^{th} day of aestivation. The fluctuation in the levels of polyenes can be explained due to decreased biological activity of membrane-related processes which occur in conjunction with the reduction of mitochondrial aerobic metabolism observed during aestivation (Stuart *et al*., 1998). The fatty acid data clearly indicate male/female dimorphism, and also emphasizes monoene to polyene conversion in this gastropod. Monoene: polyene ratio is higher in females after 15^{th} day of aestivation whereas higher amounts of SFA in females were observed in contrast to males where it got depleted (Table 10). It is an interesting finding that female snails derive more SFA through conversion from glycogen (neolipogenesis) to maintain supply of polyenes, which is important for vitellogenesis Ratio of SFA: MFA: PUFA (Table 10) supports this hypothesis. According to previous workers (George and Desai, 1954; Krishnamoorthy 1968; Swami & Reddy, 1978; Mitra & Sur, 1989) glycogen depletion during aestivation is related with energy requirement (*cf.* Chaki *et al*., 2008) as against lipid metabolism. Thus, it can be stated that glycogen depletion is also important for fatty acid conversion for vitellogenesis. Ovotestis requires more enzymatic activities in metabolic front for gametogenesis during aestivation over other organs (Chaki *et al.*, 2008). This fatty acid pool probably comes through glycogenolysis in the digestive gland. It is to be mentioned that all the above workers carried out the studies without male/female bias.

It can thus be inferred that, the digestive gland is the major lipid storage site and also plays important role in transportation of lipid to other organs during aestivation and maintains higher fatty acid percent than other organs. The other organs studied in this snail maintain a lipid pool. During aestivation, males utilize less amount of lipid for physiological maintenance as compared to females which utilize it for vitellogenic purposes. Phospholipid/cholesterol ratio is always higher in digestive gland and albumen gland of active and aestivating females. In aestivating female, monoenes of digestive and albumen glands are depleted to yield more polyenes, a phenomenon probably required for egg formation. This observation is also supported by higher SFA: MFA: PUFA ratio in female. Overall depletion in fatty acid level during aestivation in both male and female is due to slow and gradual use of these components towards maintenance of the minimum physiological demand. Mantle and foot of both sexes show similar and little variation in lipid and fatty acid storage pattern. Therefore, it is probable that the major site of bioconversion of lipids is digestive gland and there is clear indication of a sexual dimorphism from the point of lipid requirement and metabolism in this hermaphrodite snail. That the hypothesis of the present work that a male/female functional distinction in hermaphrodite snail exists.

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