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Status of cholesterol and lipase in *Ascaridia galli* nematode parasite parasitizing Domestic Fowl Host

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Abstract

In *A. galli* Schrank [17], Freeborn, [8] most of the lipids are located in the hypodermis, especially in the lateral cords, in the non-contractile part of the muscle cells, in the intestine of host. Cholesterol and lipase has a variety of functions in tissues. Cholesterol occurs in association with proteins, carbohydrates, lipo proteins and glycolipids. They are major structural components of cell membranes. Lipids include simple lipids, such as fats and oils and derived lipids such as fatty acids, cholesterol and ketone bodies. Although information regarding cholesterol and lipase from nematodes is very less. Further this analysis deals with to find out the content level of cholesterol and lipase in *A.galli* from naturally infected domestic fowl in Nanded region (M.S.) India. The data obtained from estimation of cholesterol, content level in male *A. galli* is 141 ± 15.77 and in female *A.galli* is 169 ± 27.38 $\mu\text{gm}/100$ mg tissue respectively and in estimation of lipase, content level in male *A.galli* is 0.97 ± 0.12 and in female *A.galli* is 1.27 ± 0.13 lipase units /24 hours/100 mg tissue respectively.

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Key Words: *Ascaridia galli*, Cholesterol, Nanded region

Introduction

In lipid metabolism cholesterol is an important fraction content. Cholesterol present in the nematode parasite is comparatively smaller portion of unsaponifiable lipid fraction, Ansari, *et al.*, [1]. Cholesterol is a precursor of lipids. It is a steroid and is widely distributed in all the tissues of animals. It may occur either in free form or as esters. As demonstrated by Von Brand, *et al.*, [20] in helminthes the free form of Cholesterol is present. Hence, it is proved that the nematodes are not capable of synthesizing cholesterol, although they can esterify it. Incubation with common cholesterol precursors such as acetate and mevalonate gave uniformly negative result in *Moniliformis dubius*, Barrett, *et al.*, [2]. Cholesterol was investigated in few cestodes also *H. diminuta*, [18] the cholesterol content in two cestodes *Hydatigera taeniaeformis* and *Moniezia expansa* reported that 98% and 85% of unsaponifiable matter contained cholesterol. In Microsomal fractions of the cells are responsible for the biosynthesis of cholesterol. Acetyl co-enzyme A is the source of all carbon atoms in the cholesterol metabolism.

The related literature citations are those of [8, 6, 2, 11]. In *Ascaridia galli* the chemical structure of sterols has been worked out with less in detail by Lopez-Gorg [12]. Therefore above literature does not clear content of cholesterol in helminths with the exception of Bhonsle [3].

In the present study, quantitative estimation of cholesterol content in male and female *Ascaridia galli* which was collected from naturally infected host, domestic fowl has been taken up since the same was not investigated earlier. Lipase is that

enzyme which take part in the process of hydrolysis of triglycerols, supportive to released energy in a utilizable form.

The diglycerides can be further hydrolysed to monoglycerides and finally to glycerol. Lipases are considered as carboxy esterases since they can hydrolyse only the carboxyl ester bonds, hence they are grouped among hydrolases. Lipases differ from esterases because it is unable to attack substrate molecule when fully dispersed in water. Lipases are detected in many tissues of vertebrates and invertebrates.

Some of the recent qualitative and quantitative studies of lipases in a number of parasitic helminths are those of [16, 4, 10]. Besides, Marzullo, *et al.*, [14] and Mondlowitz, *et al.*, (1960) have also reported tissue lipases though with unknown significance, in the tissues of nematode parasites. In *Ascaris lumbricoides*, Matsuura [15], reported that the lipolytic enzyme from the said parasite. Hence, there is some data on the content of this enzyme, which is nicely give some knowledge about the degradation of the lipid material and its subsequent utility to this parasites.

In the present quantitative estimation, an attempt has been made to study the content of the lipase in male and female nematode parasite *Ascaridia galli* which was collected from naturally infected host domestic fowl.

Materials and Methods

Cholesterol: Cholesterol content was estimated the method described by Crawford [7]. Homogenates of male and female worms of approximately 100-200 mg in weight were prepared

in 3 to 4 ml of alcohol, ether mixture (3:1). After filtering the homogenates through fat-free filter paper, 0.5 ml of each of them was taken as sample. To the samples, so obtained, 3 ml of glacial acetic acid, followed by 2 ml of colour reagent were added. The blank with alcohol-ether mixture and standard, with pure dry cholesterol (0.2 mg/ml) were treated as samples simultaneously. The colour developed in the samples and standard, was read at 540 m μ after half an hour, by adjusting the instrument to zero with the blank.

The quantitative expression for cholesterol is $\mu\text{gm}/100\text{ mg}$ tissue.

Lipase: The method of Cherry and Grandall [5] was followed for the determination of lipase activity. 20 to 30% homogenates of male and female worms, prepared in distilled water, were used for enzyme assay. After two-fold dilution of the supernatants, the test and the controls were prepared, each with 1 ml of the supernatant. The enzyme in the control tubes was denatured by boiling for one hour at 100°C in a

water bath. After they were cooled to room temperature 0.5 ml of (0.2M) phosphate buffer (pH 7.4) and 0.5 ml of olive oil emulsion were added to both control and test samples. Both the sets were left in an incubator, at 37°C, for 24 hours. At the end of incubation the reaction was stopped by the addition of 3 ml of 95% ethyl alcohol (v/v). Following this 2 drops of 1% phenolphthalein was added. The test and controls were titrated against 0.05N sodium hydroxide to a permanent pink colour. The difference in the quantity of sodium hydroxide, for each set of test and controls, was noted to calculate the lipase activity. The enzyme activity was expressed in lipase units/24 hours/100 mg tissue.

Observation

Table 1: Cholesterol content in male and female nematode parasites of *Ascaridia galli*.

Sex	CONTENT MEAN \pm S.D.	Male to female ratio (♂/♀)	Percentage difference (%)
Male (08) (♂)	141 \pm 15.77	0.834	16.56
Female (08) (♀)	169 \pm 27.38		

Values puted in parentheses showing number of samples estimated values expressed in $\mu\text{gms}/100\text{mg}$ tissue.

Table 2: Lipase content in male and female nematode parasites of *Ascaridia galli*.

Sex	MEAN \pm S.D.	Male to female ratio (♂/♀)	Percentage difference (%)
Male (07) (♂)	0.97 \pm 0.12	0.763	23.62
Female (07) (♀)	1.27 \pm 0.13		

Values puted in parentheses showing number of samples estimated, values expressed in lipase units/24hours/100mg tissue.

Results and Discussion

The values obtained from quantitative estimation are tabulated in the observation table and same are represented in the graph. They suggest that the content of cholesterol in male and female nematode parasites is 141 \pm 15.77 and 169 \pm 27.38 $\mu\text{gm}/100\text{ mg}$ tissue respectively. The male to female ratio is 0.83 $\mu\text{gm}/100\text{ mg}$ tissue which shows that the female consume 16.56% more cholesterol than males.

The above finding shows that cholesterol exist in male and female *A.galli* in large amount. The cholesterol activity in male and female *A.galli* slightly agree with the findings of Lopez-Gorge [12] in male and female *A.galli* which was infected by experimental condition. It proofs that avian parasite *A.galli* has been more cope-up with the intestinal micro-environmental condition compared with the experimentally infected *A.galli*. But, the role of cholesterol in the metabolism of nematodes is not understood clearly. The cholesterol helpful in the synthesis of bile acids, hormones and vitamin. The pathological conditions, the pathway of cholesterol production

is distributed and hence, the cholesterol content may either decrease or increase.

The values obtained from lipase activity are tabulated in observation table and same are represented in graph. The results indicate the content of lipase male and female nematode parasites is 0.97 \pm 0.12 and 1.27 \pm 0.13 lipase units/24 hours/100 mg tissue, respectively. Male to female ratio is 0.76 lipase units/24 hours/100 mg tissue, which suggest that the 23.62% more activity of lipase is recorded in female nematode parasite than male nematode parasite.

From above quantitative determination indicate that the female *A.galli* stored more cholesterol and lipase than male *A.galli* nematode parasite. The values obtained from metabolic differentiation shows variation due to species variation, variation in host, their metabolism and habitat of nematode parasite. The values of cholesterol are compared with Bhonsle, [3] in *T.tiara* i.e. in male 1.312 and in female 1.75 $\mu\text{gm}/100\text{ mg}$ which is slightly higher than *A.galli* and at the same the male to female ratio is 0.7492 in *T. tiara* is slightly near to male to female ratio of *A.galli* in lipase content level.

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