ESTIMATION OF ALKALINE PHOSPHATASE AND PROTEIN CONTENT IN DIFFERENT SPECIES OF CESTODE PARASITES OF DOMESTIC PIGEONS (*COLUMBA LIVIA*)(GMELIN,1789)

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خلاصہ

Cotugnia streptopell, Raillietina galeritae (Cestodes) گھریلو کبوتر *(Columba Livia)* میں پائے جانیوالے فیتے *Raillietina flaccida* اور میں الکلائین فاسفاٹیز اور پروٹین مندرجات *(Protein Content)* کی مقدار کا مشاہدہ کیا گیا الکلائین فاسفاٹیز کی اوسط مقدار کا تخمینہ *R.galeritae, C.streptopell* اور *R.flaccida* میں بالترتیب 19.228 *U/l,19.504,22,908* جبکہ پروٹین مندرجات کی اوسط مقدار کا تخمینہ 2.677,0.950 اور 4.117 *g/dl* بالترتیب لگایا گیا۔ الکلائین فاسفاٹیز کی زیادہ سے زیادہ اوسط مقدار *C.Streptopell* میں جبکہ کم ترین مقدار *R.flaccida* میں دیکھا گیا۔ پروٹین مندرجات *(Protein Content)* کا تخمینہ اس کے بالکل برعکس حاصل ہو ا زیادہ سے زیادہ پروٹین مندرجات *R.flaccida* میں اور کم سے کم کا اندازہ *C.Streptopell* میں لگایا گیا۔

Abstract

Level of alkaline phosphatase and protein content were observed in *Cotugnia Streptopell, Raillietina galeritae* and *Raillietina flaccida* in whole cestode from domestic pigeons (*Columba livia*). Mean values of alkaline phosphatse in C. *streptopell, R.galeritae and R. flaccida* were observed as 22.908, 19.504, 19.228 U/l respectively, while mean values of protein content were observed as 0.950, 2.677, 4.117g/dl respectively. The maximum values of alkaline phosphatase was obtained in *C. streptopell* while *R. flaccid* showed lowest mean values. The reverse result was observed in protein content estimation. The highest protein content was observed in *R. flaccida* while reduced protein content was observed in C. *streptopell.*

Key words: Cestodes, Pigeon, Alkaline phosphatase, protein content

Introduction

Biochemistry of parasites has great importance and some work has been done on helminth's physiology. Maximum parasites exist in connotation of animals, birds, and fishes of economic significance. Parasitic biochemistry has abundant practical significance through chemotherapy and vaccine manufacture and in considerate of the multifarious link involved in the host parasite relationship with helminth parasites enzyme (Alkaline phosphatase) is significant enzyme that are originated to be allied with consumption of nutriment ingredients. Some intervention to this enzyme might cause decrease and paralysis of the worms (Swargiary *et al*., 2013). The enzyme is established in the extracellular liquids and cells of a comprehensive variety of worms with helminths. (Kar and Tendon 2004) made histochemical and biochemical observations which confirmed the occurrence of alkaline phosphatse in adjacent relations with somatic musculature, sub-tegument, tegument, cuticle and gut of numerous helminths, mainly in the adhesive structures like oral suckersand acetabulum.The farthest body layer of trematode and cestodes Tegument parasites through which absorption and digestion of several food stuff takes place (Roy 1982). In cestodes, alkaline phosphatase is present in walls of excretory ducts and subcuticula (Bogitsh 1963). Protein content of the cuticles of cestodes is synthesize in subcuticular matrix (Lumsden 1966). (Rogers 1969) studied on the metabolism of protein, amino acids and other nitrogen compounds in nematode. . Protein and enzyme activity differs in different parasitic species due to capability of absorption of food from host and intestinal environment (Akhter *et al.*, 2006). Diet protein effects the growth of parasites and involve in protein metabolism of cestodes. (Mettrick and and Munro 1965) observed free nutritional amino acid supplements effects on the *Hymenolepis diminuta* growth.

Materials and Methods

Cotugnia Streptopell, Raillietina galeritae and *Raillietina flaccid* of different size were obtained from intestine of infected pigeons. Level of alkaline phosphatase and protein content estimated in these worms. 2g samples were crushed in 2ml of distilled water and homogenized in Teflon Pyrex soft tissue grinder for 5 min at 1000 rpm. The homogenates were centrifuged at 3500 rpm in Labofuge 15000 for 15 min. Supernatants were used for biochemical analysis.

Estimation of Alkaline phosphatase (ALP): Alkaline phosphatase was determined by the colorimetric kit method of Randox cat No. 307. One test tube was taken and add 0.02 ml of homogenate/supernatant and then 1ml of substrate was added and mixed. Reading was taken after 1, 2 and 3 minutes against the air blank at 405nm on Schimadzu spectrophotometer UV-120. Finally these values were kept in the following formula to calculate the alkaline phosphatase activity.
U/l =2760 x ΔA 405nm/min

Estimation of Total Protein: Total protein was estimated by Biuret method using Randox diagnostic kit Cat No. TP 245. Three test tubes were taken and marked as sample, standard and reagent blank. 1 ml of solution was added to each test tube then 0.02 ml of supernatant was added to their respective test tube and 0.02 ml of distilled water to the reagent blank's test tube and 0.02 ml standard in respective test tube. Test Tubes were incubated for 30 min. in water bath at 25 ^{0}C. Absorbance of sample was measured against reagent blank at 546 nm on schimadzu spectrophotometer U V-120.
For the estimation of total protein concentration, following formula was used.

Total protein concentration = 190 x A_{sample}(g/l)

Results and Discussion

Biochemical estimation in cestode parasites are shown in Table.1 and Table.2 Estimation of alkaline phosphatase and protein content were observed in *C. Streptopell, R.galeritae and Raillietina flaccida* in whole body. Mean values of alkaline phosphatse in *C.streptopell, R.galeritae* and *R.flaccida* were observed as 22.908, 19.504, 19.228U/l respectively. The maximum value of was obtained in *C. Streptopell*while *R.flaccida* and *R.galeritae* showed very close levels of alkaline phosphatase (Table 1).

Table 1. Level of Alkaline Phosphatase (ALP) in different cestode parasites.

	Mean (U/l)	S.D	S.E	Range(U/l)
C.streptopell	22.908	0.730	0.422	22.082-23.734
R.galeritae	19.504	0.575	0.332	18.854-20.154
R.flaccida	19.228	1.149	0.663	17.928-20.528

Mean values of protein content in *C.streptopell, R.galeritae* and *R.flaccida* were observed as 0.950, 2.677 and 4.117 g/dl respectively. The maximum value of protein content was obtained in *R.flaccida* while*C. Streptopell* showed lowest mean value of protein contents (Table 2).

Table 2. Level of protein content in different cestode parasites.

	Mean(g/dl)	S.D	S.E	Range(g/dl)
C.streptopell	0.950	0.380	0.219	0.520-1.380
R.galeritae	2.677	0.366	0.211	2.263-3.090
R.flaccida	4.117	0.611	0.353	3.426-4.808

Biochemical estimation in cestode parasites i.e *C.streptopell, R. galeritae* and *R. flaccida* are expressed in terms of unites. *Swargiary et al.,* (2013) observed alkaline phosphatase's characterization in an intestinal fluke *fasiolopsis buski* treated with alpinianigra'scrude extract Temperature, pH and incubation time effect alkaline phosphatse activity. Enzyme activity showed negative effect with increasing pH. By the temperature fluctuation activity of enzyme can be controlled. A huge quantity of chemicals have been seen to modify alkaline phosphatse activities. According to Mahanty *et al*., (2011)in the culture medium alkaline phosphates secretion found to be reduced when albendazole and PZQ was used to treatTaeniasolium Cysts. Pappas 1991observed divalentcations was used to check inhibition and activation brush-border membrane-bound alkaline phosphatase activity on *Hymenolepis diminuta* (Cestoda). Dusanic (1959) demonstrated the alkaline phosphatase histochemical localization in different stages of parasitic trematode, *Schistosomamansoni to*observed significant

difference from stage to stage. Krasnosnoschchekov and Tomilovskala (1975) observed distribution of alkaline phosphatase by means of the incubation of whole cestode. Alkaline phosphatase was associated with calcareous corpuscles, integumental tissue and excretory system.

Rothman (1966) observed alkaline phosphatase in the cestode *Hymenolepis citelli*, on outer membrane of the proximal microthrix. In present investigation highest level of alkaline phosphatse observed in *C.streptopell* and almost same level of alkaline phosphatase observed in *R. galeritae* and *R. flaccida.* Many factors like absorption of nutrients, pH and temperature effect the level of alkaline phosphatase in different cestodes. Proteins consume various altered biological functions. They are ubiquitous in their distribution and there is certainly no suitable outline of categorizing them. The principal group of proteins are the enzyme proteins that offer ridiculous setting for the nourishment of cestodes parasites (Sonune 2012).The cestodes consume diverse grades of protein for generating energy. Literature has exposed that the parasites are capable of adopting themselves to the parasitic manner of life, only due to protein. In helminths parasites, the protein generally present between 20 – 40 % of the dry weight (Sharma, 1979) but protein content value, as high as 70% of the aridmass have been observed for *Nippostrongylusbrasiliensis* (infective larvae) and *Macrachanthorhynchushirudinaceus* (Barrett, 1981). Jadhav *et al*. (2008) reported biochemical contents from Davaineashindei. Amount of protein present in Davaineashindei 13.20 mg/gm wt. of tissue. Bhure *et. al*.(2016) studied protein's amount present in nematode parasites is 24.80 mg / 100mg collected from Wallagoattu. Bhure et al., (2012) observed reduced amount of protein (15.88 mg/gm) in Ascardiagalli as associated to healthy intestine (19.77 mg/gm) and diseased host intestine (19.33 mg/gm). Pallewad *et al*. (2015) reported Proteinin Cotylophoron cotylophorum 23.60 mg / 100 mg in Capra hircus. Nanware*t.al*.(2012) observed protein's amount present in Cestode Cotugnia sp. is 5.77mg/gm. Dhondge *et al*.(2010) reported amount of Protein was lower in the body of the parasites than infected and normal intestinal tissue of host. In present study showed that highest protein content found in *R. flaccida.* Pathan *et al*., 2011 were observed worms were skilled of extracting nutritious material from their hosts and thus characterized elevated level in protein.

Conclusion

Different species have different protein content depend on parasites capability to absorb protein from nourishment of host. In present study the highest protein content was found in *R. flaccid.* Hence there is relationship between the protein content of parasites and nutrient protein content of host. Parasites depend on protein consumptions from host for energy. Many factors can effect the level of alkaline phosphatase in parasites.

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