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Glycogen Content and *In Vitro* Glycogen Consumption in Sheep Cestode *Moniezia expansa* (Rudolphi, 1805)

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Abstract:
Moniezia expansa is common cestode in the small intestine of sheep and causes considerable damage to it. Therefore, an effort to study such biological aspects of *Moniezia expansa* which would help in its control is much needed. The glycogen content and *in vitro* consumption of endogenous glycogen was determined in immature, mature and gravid proglottids of *Moniezia expansa* by Anthrone method by Carroll *et al.* (1956). The total glycogen (on fresh weight basis) was found to be 3.03%, 4.26% and 3.85% respectively in immature, mature and gravid proglottids of unstarved (control) parasite. The glycogen content in immature, mature and gravid proglottids of parasites starved for 3 hrs. is 2.51%, 3.09% and 2.91% respectively, parasites starved for 6 hrs is 1.91%, 2.0% and 2.0% respectively, parasites starved for 9 hrs. 1.25%, 1.33% and 1.35 respectively and parasites starved for 12 hrs is 0.79%, 0.80% and 0.99% respectively (on fresh weight basis).The endogenous glycogen consumption (in percent of total glycogen content of control parasite) by immature, mature and gravid proglottid was found to be 17.16%, 27.46% 24.42% respectively in parasites starved for 3 hours, 36.96%, 53.05% and 48.05% respectively in parasites starved for 6 hours, 58.75% 68.78% and 64.94% respectively in parasites starved for 9 hours and 73.93%, 81.22% and 74.29% respectively in parasites starved for 12 hours. Rate of consumption of endogenous glycogen between different starvation time periods (in percentage per hour) for immature, mature and gravid proglottids is also calculated. Rate of consumption was maximum during early hours of starvation i.e. between 0-3 hrs and 3-6 hrs and was minimum at 9-12 hrs. Glycogen is the major energy reserve in parasitic helminths which live in low oxygen condition, as they are the best substrate for anaerobic energy production. The study of consumption of endogenous glycogen in cestode during starvation period may be quite helpful for their control in the host. The rate of endogenous glycogen consumption is helpful in understanding metabolic rate and physiological aspects of different proglottids of the parasite.

Keywords: *Moniezia*, Glycogen, Cestode, Helminth, Sheep

INTRODUCTION

Parasites leading more or less anaerobic life usually store large polysaccharide amounts because carbohydrates are the best substrate for anaerobic energy production. The main carbohydrate reserve in parasitic helminths is "glycogen" which is a typical energy reserve of helminths inhabiting biotopes with low oxygen tensions. This glycogen closely resembles with the mammalian glycogen. An energy reserve evidently is especially important to parasites of the intestinal lumen. They have to cope not only with the difficulties of an oxygen-poor environment, but must also be able to withstand periods of partial or even complete starvation which are unavoidably linked to the feeding habits of many hosts. Therefore the study of glycogen content as well as it's percentage of consumption in cestode during starvation might be usefully exploited in helminth control.

Moniezia expansa is the common cestode of sheep, the oldest domesticated animal. They are economically beneficial to human population and are considered important in uplifting the rural economy. The parasite inhabits the small intestine especially the lower portion, of the host. Heavy infections of the parasite causes intestinal obstruction, weight loss and absorbs the nutrition of host from the intestine and deprive it of many important nutrients. They also deteriorate the quality of meat, wool and skin of sheep. They also influence the microflora of the gastro intestinal tract of sheep. Thus, the cestode causes considerable damage to its host. Therefore, an effort to study such biological aspects of *Moniezia expansa* which would help in its control is much needed.

Investigations on studies of glycogen content and its metabolism in different cestodes have been carried by a number of workers. Some of the notable workers are Weinland (1901)[33], Von Brand (1933,1973,1979)[26-28], Wardle (1937)[32], Smorodinzew and Bebeschin (1935, 1936a)[21,22], Reid (1942)[18], Daugherty (1955)[4], Read (1956,1967)[14,15], Read and Rothman (1957)[16], Fairbairn *et al.* (1961)[6], Goodchild (1961)[7], Von Brand and Bowman (1961)[29] , Goodchild and Vilar-Alvarez (1962)[8] , Read and Simmons (1963)[17], Lopez-George and Monteoliva (1965)[9], Von Brand *et al.* (1968)[30], Smyth (1969)[24], Premvati and Tayal (1978,1982)[12,13], Chopra (1981, 1993) [2,3], Smyth and Mc Manus (1989)[25], Saikumari and Rao (1991)[20], Nanware *et.al*. (2010, 2011)[10,11], Waghmare and Chavan (2010)[31], Zainad *et.al*.(2017)[34].

Very less work has been done on *in vitro* endogenous glycogen consumption in cestodes under aerobic conditions *viz.* in *Hymenolepis diminuta* (Read, 1956)[14], in *Taenia taeniaeformis* (Von Brand and Bowman, 1961)[29], in *Raillietina cesticillus* (Smyth, 1962)[23], in *Stilesia globipunctata* by Premvati and Tayal (1978, 1982)[12,13] and in *Avitellina centripunctata* and *Moniezia expansa* by Premvati and Tayal (1982)[13].

The present work deals with the study of glycogen content in immature, mature and gravid proglottids of *Moniezia expansa* in unstarved and starved parasites. It also includes the study of *in vitro* endogenous glycogen consumed at various starvation period and also the rate of consumption of endogenous glycogen between different starvation time periods for immature, mature and gravid proglottids of *Moniezia expansa* during *starvation* periods.

So far no such study on monitoring the in vitro consumption of endogenous glycogen at different starvation periods as well as the rate of glycogen consumption between different starvation time periods has been done for different proglottids of the cestodes.

MATERIALS AND METHODS

The freshly collected parasites from the intestine of sheep, were washed in several changes of physiological saline until the debris was removed and divided into 5 groups with 5 parasites in each group. The parasites of group 1 were untreated controls and were not starved and used for the determination of glycogen content. For the study of consumption of endogenous glycogen in parasites, group 2, 3, 4 and 5 were starved for 3, 6, 9 and 12 hrs respectively in a glucose-free Tyrode's solution (8 g of sodium chloride, 0.02 g potassium chloride, 0.02 g of magnesium chloride, 0.06 mg disodium hydrogen phosphate, 1 gm of sodium bicarbonate were dissolved in 1000 ml distilled

water) without glucose and with antibiotics (1000 unit/ml penicillin and 100 µg/ml streptomycin). All parasites were maintained at temperature 37°C (±1°C).

The parasites of each group at the end of periods mentioned above, were washed with several changes of distilled water and separated into immature, mature and gravid regions. They were blotted with a filter paper to remove the moisture adhering on their body surfaces and were then quickly weighed and then processed for extraction of glycogen by the method of Roe *et al.* (1961)[19]. The glycogen was determined by Anthrone method by Carroll *et al.* (1956)[1].

RESULTS

Glycogen content (in percentage of fresh weight) in immature, mature and gravid proglottids of unstarved and starved *M.expansa* is given in table 1 and percentage endogenous glycogen consumption by immature, mature and gravid proglottidsof *M.expansa* during various starvation periods (in hours) is given in table 2. All the values are mean ± S.D of three samples in duplicate. The rate of endogenous glycogen consumption (in percentage per hour) between different starvation time periods 0-3 hrs, 3-6 hrs, 6-9 hrs, 9-12 hrs for immature, mature and gravid proglottids of *Moniezia expansa* is given in table 3.

Table 1: Glycogen content (in percentage of fresh weight) in immature, mature and gravid proglottids of unstarved and starved *M. expansa*

S. No.		Unstarved parasites	Starved Parasites Starvation period in hrs			
			3	6	9	12
1.	Immature proglottids	3.03±0.102	2.51± 0.071	1.91±0.035	1.25±0.021	0.79± 0.022
2.	Mature proglottids	4.26±0.136	3.09± 0.146	2.0±0.029	1.33± 0.083	0.80± 0.014
3.	Gravid proglottids	3.85±0.113	2.91± 0.117	2.0± 0.082	1.35± 0.031	0.99± 0=019

Table 2: Percentage endogenous glycogen consumption by immature, mature and gravid proglottids of *Moniezia expansa* during various starvation periods (in hrs)

S. No.		Starvation period in hrs			
		3	**6**	**9**	**12**
1.	Immature proglottids	17.16%	36.96%	58.75%	73.93%
2.	Mature proglottids	27.46%	53.05%	68.78%	81.22%
3.	Gravid proglottids	24.42%	48.05%	64.94%	74.29%

Table 3: Rate of endogenous glycogen consumption in percentage per hour between different starvation time periods 0-3 hrs, 3-6 hrs, 6-9 hrs, 9-12 hrs for immature, mature and gravid proglottids of *Moniezia expansa* .

S. No.		Rate of endogenous glycogen consumption in percentage per hour between different starvation time periods			
		0-3	**3-6**	**6-9**	**9-12**
1.	Immature proglottids	0.173	0.43	0.22	0.153
2.	Mature proglottids	0.39	0.363	0.223	0.177
3.	Gravid proglottids	0.31	0.30	0.216	0.12

DISCUSSION

In present study, the glycogen content is 3.03%, 4.26% and 3.85% in immature, mature and gravid proglottids of *Moniezia expansa* respectively. It is in accordance to the glycogen content in same parasite reported by earlier workers, *viz*. Weinland (1901)[33], Von Brand (1933)[26], Wardle (1937)[32], Lopez-Gorgé and Monteoliva (1965)[14], Chopra (1981, 93)[2,3], Premvati and Tayal (1982)[13] and Nanware *et.al.* (2011)[11]. The variation in glycogen content along the strobila of *Moniezia expansa* has been observed in the present study. It is maximum in mature proglottid followed by gravid proglottids and is minimum in immature proglottids. This observation is in accordance with that of Read (1956)[14], Daugherty and Taylor (1956)[5], Chopra (1981, 1993)[2,3] and Saikumari and Rao (1991)[20], Nanware *et.al.*(2011)[11], Zainad *et.al* (2017)[34] . Premvati and Tayal (1978)[12] also reported maximum glycogen in mature proglottids but they reported minimum glycogen in gravid proglottid. The variation in the glycogen content along the strobilae of cestodes may be a reflection of the differential rates of metabolism along the strobilae related to the regional differences in anatomy and permeability of tapeworms[5]. The maximum glycogen in mature proglottids may be due to the fact that mature proglottids are responsible for reproductive activity, thus require more glycogen to carry out reproductive functions[2,3].

The study of *in vitro* glycogen consumption by immature, mature and gravid proglottids of *Moniezia expansa* during 3 hrs of starvation is 17.16%, 27.46% and 24.42%, during 6 hrs of starvation is 36.96%, 53.05% and 48.05%, during 9 hrs of starvation is 58.75%, 68.78% and 64.94% and during 12 hrs of starvation is 73.93%, 81.22% and 74.29% respectively. Thus, the endogenous glycogen consumption was highest in mature proglottids during each period of starvation. This shows a high metabolic activity in mature proglottids. These studies have been performed earlier in few cestodes. Read (1956)[14] recorded consumption of endogenous glycogen as 4.41 g per 100 g fresh tissue during 20 hours in *Hymenolepis diminuta*. Von Brand and Bowman (1961)[29] recorded 5.40% fresh weight consumption in 20 hrs in *Taenia taeniaeformis* and Smyth (1962)[23] stated 3.95% of fresh weight consumption in 20 hours in case of *Raillietina cesticillus.* The total endogenous glycogen consumption during 14 hrs of starvation was found to be 78.64%, 85.72% and 71.91% by immature, mature and gravid proglottids respectively in *Stilesia globipunctata* by Premvati and Tayal (1978)[12]. Premvati and Tayal (1982)[13] studied the percentage of glycogen consumption during 14 hours of starvation by immature, mature and gravid proglottids of *Avitellina centyripunctata* as 88.44%, 88.34% and 77.82% of *Stilesia globipunctata* as 78.64%, 85.71% and 71.09% and of *Moniezia expansa* as 81.10%, 85.05% and 77.73% respectively.

Rate of consumption of endogenous glycogen studies have not been done earlier. These studies in *M.expansa* showed that rate of glycogen consumption were higher in mature and gravid proglottids due to high metabolic activities but lower in immature during 0-3 hrs. But during 3-6 hrs the rate was slightly higher in immature than mature and gravid. Thus due to continuous starvtion, glycogen breakdown occurred rapidly for maintenance of metabolic activities. During 6-9 hrs and 9-12 hrs, the rate of consumption was almost similar in all three proglottids and was decreasing with time. Thus, the metabolic activities in parasites were gradually slowing down. Rate of consumption was maximum during early hours of starvation i.e. between 0-3 hrs and 3-6 hrs and was minimum at 9-12 hrs. After 12 hrs of starvation parasites were metabolically inactive. This was also found by Premvati and Tayal (1978)[12] who observed that the longevity of worms *in vitro* condition, apart from other factors also depend on the stored glycogen and when 70% of the glycogen was utilized, the parasites become lethargic and gradually disintegrated.

Thus, these parasites loose appreciable amount of glycogen during starvation period *in vitro* conditions.

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CONFLICT OF INTEREST

No conflict of interest exists.

REFERENCES

1. Carroll, N.V.; Longley, R.W. and Roe, J.H. (1956). The determination of glycogen in liver and muscle by use of Anthrone Reagent. *J. Biol. Chem.* 220: 583-593.
2. Chopra, A.K. (1981) Glycogen content and its distribution in three cyclophyllidean cestodes of sheep. *Comp. Physiol. Ecol*. 6: 173-176.
3. Chopra, A.K. and Tayal, S. (1993 publ. 1994) Alkaline and acid phosphatase activities in relation to glycogen content in three cestodes of sheep. *Indian J. Helminthol*. 45(1/2): 100-103.
4. Daugherty, J.W. (1955). The effect of fasting and alloxan treatment of the host on the glycogen gradient in *Hymenolepis. J. Parasitol*. 41: 30.
5. Daugherty, J.W. and Taylor, D. (1956) Regional distribution of glycogen in the rat tapeworm, *Hymenolepis diminuta*. *Exp. Parasitol.* 5: 376-390.
6. Fairbairn, D.; Wertheim, G; Harpur, R.P. and Schiller, E.L. (1961). Biochemistry of normal and irradiated strains of *Hymenolepis diminuta*. *Exp. Parasitol.* 11: 248-263.
7. Goodchild, C.G. (1961a) Carbohydrate contents of the tapeworm *Hymenolepis diminuta* from normal, bile less and starved rats. *J. Parasitol*. 47: 401-405.
8. Goodchild, C.G. and Vilar- Alvarez C.M. 1962. *Hymenolepis diminuta*in surgically altered hosts. II. Physical and chemical charges in tapeworms grown in shortened small intestines. *J. Parasitol* 48: 379-383.
9. (Rudolphi, 1805): II: Glucidos. *Rev. Iber. Parasitol*. 25(3/4): 299-319.
10. Nanware, Sanjay Shamrao, Dhondge, R.M., Kadam, M.S. and Bhure, Dhanraj Balbhim. (2010) Studies on glycogen profile of avian cestode . The Asian Journal of Animal Science. 4(2); 170-173.
11. Nanware, Sanjay Shamrao and Bhure, Dhanraj Balbhim. (2011) Studies on glycogen profile of cestodes of *Capra hircus* . International Multidisciplinary Research Journal. 1(10); 22-24.
12. Premvati, G. and Tayal, S. (1978). Glycogen content and *in vitro* glycogen consumption in *Stilesia globipunctata* (Rivolta, 1874). *Indian J. Parasitol.* 2: 73-75.
13. Premvati, G. and Tayal, S. (1982).In-vitro estimation of glycogen content in three sheep cestodes. Folia Parasitol.29:259-263
14. Read, C.P. (1956). Carbohydrate metabolism of *Hymenolepis diminuta. Exp. Parasitol.* 5: 325-344.
15. Read, C.P. (1967). Carbohydrate metabolism of *Hymenolepis* (Cestoda). *J. Parasitol.* 53: 1023-1029.
16. Read, C.P. and Rothman, A.H. (1957b). The role of carbohydrates in the biology of cestodes II. The effects of starvation on glycogenesis and glucose consumption in *Hymenolepis*. *Exp. Parasitol*. 6: 280-287.
17. Read, C.P. and Simmons, J.E. Jr. (1963). Biochemistry and Physiology of tapeworms. *Physiol. Rev.* 43: 263-305.
18. Reid, W.M. (1942). Certain nutritional requirements of the fowl cestode, *Raillietina cesticillus* (Molin) as demonstrated by short periods of starvation of the host. *J. Parasitol.* 28: 319-340.
19. Roe, J.R.; Bailey, J.M.; Gray, R.R. and Robinson, J.N. (1961). Complete removal of glycogen from tissues by extraction with cold TCA. *J. Biol. Chem.* 236: 1244-1246.
20. Sai Kumari, M.R.S. and Rao, R. (1991). Glycogen content in the cestode *Neokrimia singhia* parasitic in *Peridcula asiatica*. *Indian J. Helminthol.* 43(2): 129-133.
21. Smorodinzew, I.A. and Bebeschin, K.W. (1935). The content of glycogen in tapeworms (Cestoids). *C.R. Acad. Sci. U.R.S.S.* 8:413-414.
22. Smorodinzew, I.A. and Bebeschin, K.W. (1936a). Bectragezurchemia der Heminthen (Sic). Mitteilung III. Die Chemische Zusammensetzung des *Taeniasolium*. *J. Biochem. (Tokyo)* 23: 19-20.
23. Smyth J.D. (1962) Studies on tapeworm physiology. X. Axenic cultivation of the hydatid organism *Echinococus granulosus*; establishment of a basic technique. *Parasitology*. 52: 441-457.

24. Smyth, J.D. (1969) The physiology of cestodes. Ist edition. Oliver and Boyd. Edinburgh.
25. Smyth, J.D. and Mc Manus, D.P. (1989) The physiology and biochemistry of cestodes. Cambridge University Press. Cambridge.
26. Von Brand, T. (1933) Untersuchungeniiber den stoff best and einigercestoden und den stoffwechsel von *Moniezia expansa*. *Z.Vergl. Physiol*. 18: 562-596.
27. Von Brand, T. (1973) Biochemistry of parasites. 2nd Edition. *Academic Press. N.Y.*
28. Von Brand, T. (1979) Biochemistry and physiology of endo parasites. Elsevier North Holland Biomedical Press: Amsterdam.
29. Von Brand, T. and Bowman. I.B.R. (1961) Studies on the aerobic and anaerobic metabolism of larval and adult *Taenia taeniaeformis*. *Exp. Parasitol*. 11: 276-297.
30. Von Brand, T.; Churchwell, F. and Eckert, J. (1968). Aerobic and anaerobic metabolism of larval and adult *Taenia taeniaeformis:* V. Glycogen synthesis, metabolic end products and carbon balances of glucose and glycerol utilization. *Exp. Parasitol*. 23: 309-318.
31. Waghmare, S. and Chavan, R.(2010).Some quantitative studies of carbohydrate metabolites in cestode parasite of *Gallus gallus domesticus*. Int. J. Parasitol. Res., 2, 1-4.
32. Wardle Wardle, R.A. (1937a) The physiology of the sheep tapeworm, *Moniezia expansa* Blanchard. *Can. J. Res. Sec. D. Zool. Sci.* 15(6): 117-126.
33. Weinland, E. (1901) Ueber den Glykogengehalteiniger parasitischerwurmer. *Z. Biol*. 41: 69-74.
34. Zainad, S., Shaikh, J.D. and Dama, L.B.(2017) A comparative study of *Raillietina echinobothria, Raillietina cesticillus, Raillietina Tetragonl* and *Choanotaenia infundibulum* the intestinal parasites of *Gallus domesticus* on the glycogen content. *Trends in Parasitology research* 6(2):5-8.