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Aminoacids in sheep cestode *moniezia expansa* (Rudolphi, 1805)

[*1] Archana Gupta, [2] Vinod gupta

[1] Department of Zoology, University of Lucknow, Lucknow, Uttar Pradesh, India
[2] Retd., Former head, Department of Zoology, University of Lucknow, Lucknow, Uttar Pradesh, India

Abstract
The aminoacids were studied qualitatively by thin layer chromatography (TLC) in the homogenates of Sheep cestode *Moniezia. Expansa*. TLC separations, in comparision to paper chromatography are extremely sharp and much smaller quantities can be separated. The aminoacids were studied in whole adult worm as well as their presence in immature, mature and gravid proglottids separately. The detection of aminoacids in immature, mature and gravid proglottid separately is done for the first time. The difference in number of aminoacids was observed in different proglottids. There were 23 a present in homogenate of whole worm whereas immature, mature and gravid proglottids were found to contain 16, 22 and 19 a respectively. The difference in number of a seems due to variation in metabolism in different regions of parasite and presence of highest number of a in mature proglottid may be due to its high metabolic activity. Amino acid nor-Leucine is reported for the first time in *M. expansa* in present analysis.

Keywords: amino acid, *moniezia expansa*, thin layer chromatography (TLC)

Introduction
All tissues contain free and a much large fraction of bound amino acids. In cestodes, the amino acids have qualitatively been analysed by paper chromatography in a number of cyclophyllidean cestodes but not much work has been done in *M. expansa*. All the earlier qualitative studies of aminoacids in *M. expansa* are done by paper chromatography. The notable contribution in this field are those of Kent (1947) [13], Campbell (1960) [7], Good child and Dennis (1966) [11] for *Moniezia expansa*. So, the present work of qualitative anlaysis of amino acids in *Moniezia expansa* is done by thin layer chromatography (TLC). Also there is no information about aminoacids of immature, mature and gravid proglottids separately. Hence, the present work is also an attempt to study qualitative analysis of free aminoacids of *Moniezia expansa* in whole worm as well as in its immature, mature and gravid proglottids separately.

Materials and methods
The parasites were collected from the infected sheep intestine, washed well in normal saline and in distilled water. Immature, mature and gravid proglottids were separated. Whole worm, as well as separated immature, mature and gravid proglottids were blotted on a hard filter paper, homogenates of whole body and different proglottids were prepared by homogenising the tissue in 2 ml of 70% alcohol, kept in refrigerator for 24-48 hrs, filtered with what mann No. 1 filter paper. The filtrate was concentrated either by evaporating the alcohol slowly in air or quickly with the help of hair dryer. The concentrated solution was spotted on TLC sheets (7x4 inches silica gel plates). The spot of the experimental material was placed at first position followed by spots of different known amino acids at the interval of approximately 2 cm. Each spot was dried with an air blower.

The solvent used for TLC analysis was n-propanol-water in the ratio of 7:3. The solvent was allowed to flow-up to the point near the top of plate. The developed plates were dried and sprayed lightly and evenly with the location reagent (0.2% Ninhydrin in 95% ethanol). Plates were heated for 2 minutes at 100^{o}C and the spots were visualized as purple, pink, yellow and orange spots. The unknown spots were identified from their standard chromatograms of known amino acids.

Results
Whole body of parasite has a total of 23 aminoacids and immature, mature and gravid proglottids have 16, 22 and 19 amino acids respectively. Amino acid nor- Leucine is reported for the first time in *Moniezia expansa* in present analysis.

Amino acids found in whole parasite, immature, mature and gravid proglottids of *Moniezia expansa* are shown in the following table 1.

Table 1: Amino acids in whole parasite, immature, mature and gravid proglottids of *Moniezia expansa*.

S. No.	Amimo acids	Whole Parasite	Immature Proglottid	Mature Proglottid	Gravid Proglottid
1.	DL-Alanine	✓	✓	✓	✓
2.	DL-2-Amino-n-butyric acid	✓	✓	✓	✓
3.	L-Arginine monohydrate	✓	✓	✓	X
4.	DL-Aspartic acid	✓	X	✓	X
5.	L-Cysteine	✓	✓	✓	✓
6.	L-Cystine	✓	X	X	✓
7.	L-Hydroxyproline	X	X	X	X
8.	L-Glutamic Acid	✓	✓	✓	✓

9.	Glycine	✓	X	✓	X
10.	L-Hystidine	✓	X	✓	✓
11.	L-Leucine	✓	✓	✓	✓
12.	DL-Isoleucine	✓	✓	✓	✓
13.	3 (3-4-Dihydroxy phenyl)- DL-alanine (DOPA)	X	X	X	X
14.	DL-Nor Leucine	✓	✓	✓	✓
15.	L-Lysine	✓	✓	✓	✓
16.	DL-Methionine	✓	✓	✓	✓
17.	L-Ornithine	✓	✓	✓	✓
18.	DL-Phenylalanine	✓	✓	✓	✓
19.	L-Proline	✓	X	✓	X
20.	DL-Serine	✓	X	✓	✓
21.	DL-Threonine	✓	X	✓	✓
22.	DL-Valine	✓	✓	✓	✓
23.	DL-Tryptophan	✓	✓	✓	✓
24.	L-Tyrosine	✓	✓	✓	✓
25.	Taurine	✓	✓	✓	✓

(✓) Present (X) Absent

Discussion

The presence of alanine, valine, leucine, iso-leucine, aspartic acid, glutamic acid, arginine,threonine, serine, proline, glycine, cysteine, tyrosine, phenylalanine, lysine,histidine,γ-aminobutyric acid, cystine, methionine, ornithine, taurine and tryptophan in *Moniezia expansa* in this study is in accordance to Kent (1947) [13], Campbell (1960) [7] and Good child and Dennis (1966) [11].

Amino acid nor-leucine has been reported in present analysis for the first time in *Moniezia expansa*. This aminoacid has been reported in other cestodes by various workers like Bhalya *et al* (1982, 1985) [4, 6], Misra *et al* (1988, 1990) [15, 16].

All amino acids reported in present study in Moniezia expansa are also confirmed in different cestodes by many workers (Good child and Wells, 1957; Good child and Dennis, 1966; Chappell and Read, 1975; Campbell, 1963; Bhalya *et al*. 1982, 1985, 1987; Malhotra, 1982) [12, 8, 11, 10, 4, 6].

The amino acid 3, (3-4-Dihydroxyphenyl) DL-alanine (DOPA) and hydroxyprolineis found to be absent in *Moniezia expansa* in present work which is similar to Kent, 1947; Campbell, 1963; Good child and Dennis, 1966) [13, 8, 11].

The present analysis of amino acids in immature, mature and gravid proglottids of *Moniezia expansa* is done for first time and showed highest number of amino acids in mature proglottids than in gravid and immature, proglottids. This may possibly be due to greater metabolic activity in mature than gravid or immature proglottids.

The free aminoacids comprises the aminoacid pool of parasites and are being utilized in the synthesis of proteins and energy production. Thus, the above amino acids present in *Moniezia expansa* are very important and are associated with various physiological and metabolic activities of the parasite (Von Brand, 1973; Bell *et al*., 1972; Chappell and Read, 1975; Chappell, 1974 and Arme, 1977) [17, 10, 9, 1].

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