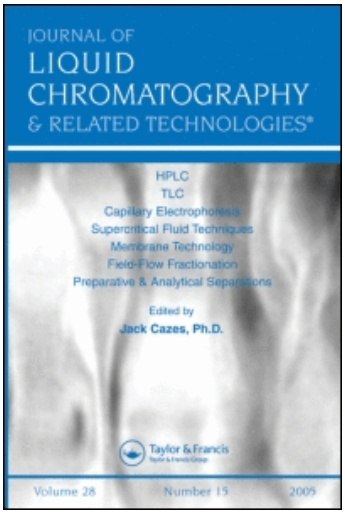

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

HPTLC ANALYSIS OF NEUTRAL LIPIDS IN THE CECA OF MICE AND CHICKS, AND IN HICK CECA INFECTED WITH *ZYGOCOTYLE LUNATA* (TREMATODA)

Meredith S. Lee[a]; Bernard Fried[a]; Joseph Sherma[b]
[a] Department of Biology, Lafayette College, Easton, PA, U.S.A. [b] Department of Chemistry, Lafayette College, Easton, PA, U.S.A.

Online publication date: 13 January 2005

To cite this Article Lee, Meredith S. , Fried, Bernard and Sherma, Joseph(1999) 'HPTLC ANALYSIS OF NEUTRAL LIPIDS IN THE CECA OF MICE AND CHICKS, AND IN HICK CECA INFECTED WITH *ZYGOCOTYLE LUNATA* (TREMATODA)', Journal of Liquid Chromatography & Related Technologies, 22: 1, 119 — 124
To link to this Article: DOI: 10.1081/JLC-100101647
URL: http://dx.doi.org/10.1081/JLC-100101647

J. LIQ. CHROM. & REL. TECHNOL., 22(1), 119–124 (1999)

HPTLC ANALYSIS OF NEUTRAL LIPIDS IN THE CECA OF MICE AND CHICKS, AND IN CHICK CECA INFECTED WITH *ZYGOCOTYLE LUNATA* (TREMATODA)

Meredith S. Lee,[1] Bernard Fried,[1] Joseph Sherma[2]

[1] Department of Biology
[2] Department of Chemistry
Lafayette College
Easton, PA 18042, USA

ABSTRACT

High-performance thin layer chromatography (HPTLC) was used to determine neutral lipids in the cecal contents and mucosa of domestic chicks and ICR mice, and in the cecal mucosa of chicks infected with *Zygocotyle lunata* (Trematoda). Silica gel plates were developed with petroleum ether-diethyl ether-acetic acid (80:20:1) mobile phase, and neutral lipids were detected using phosphomolybdic acid reagent. The cholesteryl ester fraction was quantified by HPTLC-densitometry. HPTLC analysis showed differences in the neutral lipid composition of the cecal contents of chicks, compared with the cecal contents of ICR mice. There were differences in neutral lipid profiles between the uninfected cecal mucosa of chicks and chick cecal mucosa infected with *Z lunata*. These findings showed that this parasite can alter the neutral lipid composition of the host cecal mucosa.

INTRODUCTION

Several parasitic flatworms (Trematoda) infect the intestinal ceca of their vertebrate hosts. One of the best known trematodes that lives in this site is the amphistome, *Zygocotyle lunata*. This trematode is not specific to the types of definitive hosts it infects and lives in the ceca of various avian and mammalian hosts.[1]

Fried and Nelson[2] described the histopathological effects of *Z lunata* on the cecum of the domestic chick, but pathobiochemical effects of the this trematode on the cecum are not known. Moreover, information on the biochemistry of the cecum is sparse, and a computer-aided literature search on this topic, using Chemical Abstracts, revealed only one paper describing the effects of diet on the chemical composition of cecal contents in chicks.[3] The purpose of this study was to use high performance thin layer chromatography (HPTLC) to determine neutral lipids in the cecal mucosa and cecal contents of domestic chicks and ICR mice. Additionally, wherever possible, we examined cecal sites of hosts infected with *Z lunata*.

EXPERIMENTAL

Preparation of Samples

To obtain cecal contents and cecal mucosa, 24 day-old domestic chicks (uninfected) were maintained for 2 weeks and fed, ad libitum, on a standard laboratory diet (Pacemaker Starter Medicated, Agway Inc., Syracuse, NY). In addition, 24 6-8 week-old female ICR mice were maintained for 2 weeks and fed, ad libitum, on a standard diet (Prolab Rat, Mouse, Hamster 3000, PMI Feeds, St. Louis, MO). At necropsy, the ceca were removed from these animals and opened longitudinally. Cecal contents were removed from both mice and chicks using a metal spatula, weighed, and prepared for HPTLC as described below. Cecal mucosa from the chicks were scraped, collected, weighed, and prepared for HPTLC as described below. Because of the small size of the mouse cecum, insufficient cecal mucosa was obtained to prepare HPTLC samples.

In addition, 24 chicks were each infected with 25 metacercarial cysts of *Zygocotyle lunata* as described earlier in Fried and Nelson. The chicks were necropsied at 2 weeks postinfection. Cecal contents were not obtained from the infected chicks because most of the contents were depleted, as described previously in chicks infected with *Z lunata*.[2] However, cecal mucosa was obtained from infected chicks as described above.

For HPTLC, each cecal sample was homogenized in a glass tube and extracted with 3 mL of chloroform-methanol (2:1). The extracts were filtered through a plug of glass wool contained in a Pasteur pipet. Non-lipid contaminants were removed by adding 1.5 mL Folch wash (0.88% KCl) and discarding the aqueous phase. The lipid extract was dried under a stream of nitrogen at 22ºC, and the sample was prepared by reconstituting with 200 μL of chloroform-methanol (2: 1).

HPTLC Analysis

Analyses were performed on Whatman (Clifton, NJ, USA) 20 x 10 cm high performance silica gel plates containing pre-adsorbent sample application area and 19 scored lanes (LHP-KDF, catalog number 4806-711). Plates were pre-cleaned by development with dichloromethane-methanol (1: 1) and dried in an oven for ca. 10 min at 115ºC. The standard used was a non-polar lipid mix-B (Matreya, Pleasant Gap, PA, USA) which was diluted with chloroform-methanol (2:1) to contain a concentration of 0.20 mg mL^{-1} each of cholesterol, oleic acid, triolein, methyl oleate, and cholesteryl oleate.

The standard and reconstituted samples were spotted in 2.0, 4.0, 8.0, and 16.0 μL aliquots on separate lanes of each plate with a 25 μL Drummond (Broomall, PA, USA) digital micro-dispenser. Plates were developed in the Mangold solvent system, composed of petroleum ether-diethyl ether-acetic acid (80:20:1), to a distance of 7.5 cm past the pre-adsorbent-silica gel interface in a glass, paper-lined Camag (Wilmington, NC, USA) twin-trough HPTLC chamber (24 x 8 x 14 cm). After development, plates were dried using a hair dryer, and lipids were detected as blue spots on a yellow background by spraying with 5% phosphomolybdic acid (PMA) in ethanol and heating for 20 min at 110ºC.

Quantitative analysis of the cholesteryl ester fraction was performed with a Camag TLC Scanner II using the tungsten source (k = 700 nrn), slit dimension settings of length 3 and width 4, and a scanning rate of 4 mm s^{-1}. The CATS-3 software program controlled the automated scanning of standard and sample zones in each lane, calculation of a calibration curve relating standard zone weights to their optimized scan areas, and interpolation of the weight of cholesteryl ester from the curve for the sample zone having an area closest to that of the middle standards.

The percentage of cholesteryl ester was calculated by dividing the interpolated weight by the ratio [reconstitution volume (μL) / spotted volume (μL)], dividing the result by the wet weight of sample (μg), and multiplying by 100. Three replicate samples were analyzed in each case.

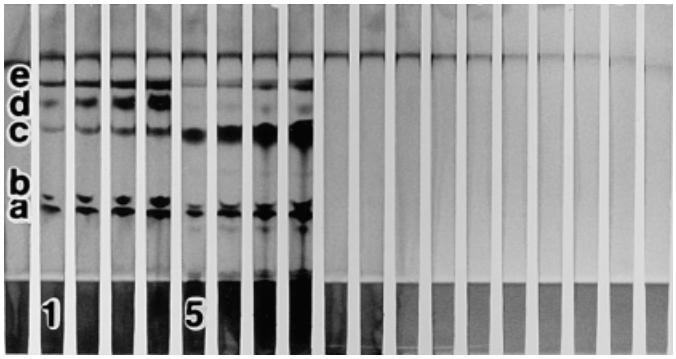

Figure 1. Chromatograms of neutral lipids in the cecal mucosa of domestic chicks. The standard and samples were spotted in 2.0, 4.0, 8.0, and 16.0 μL aliquots. The standard was spotted in lanes 1-4. Lanes 5-8 contain the cecal mucosa extracts. Abbreviations: a, free sterols; b, free fatty acids; c, triacylglycerols; d, methyl esters; e, cholesteryl esters.

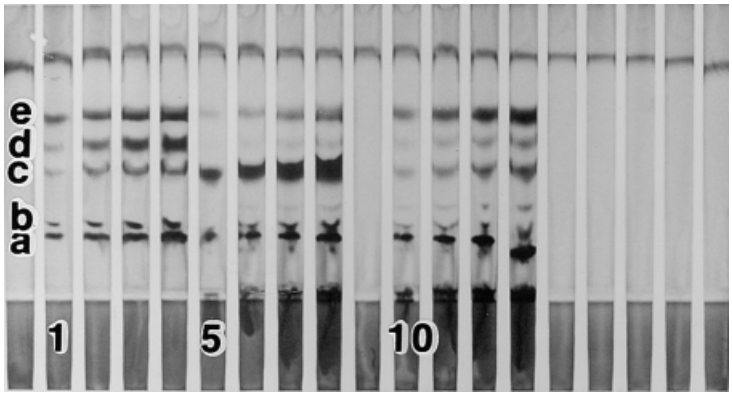

Figure 2. Chromatograms of neutral lipids in uninfected cecal mucosa of domestic chicks and cecal mucosa infected with Zygocotyle lunata. The standard and samples were spotted in 2.0, 4.0, 8.0, and 16.0 μL aliquots. The standard was spotted in lanes 1-4. Lanes 5-8 contain uninfected cecal mucosa extracts, and the infected cecal mucosa extracts were spotted in lanes 10-13. Abbreviations: see Figure 1.

RESULTS AND DISCUSSION

Qualitative analysis of the neutral lipids in uninfected chick cecal mucosa showed that the major fractions were triacylglycerols, free sterols, and free fatty acids. There was a less abundant cholesteryl ester fraction, and only trace amounts of methyl esters (Fig. 1). Quantitative analysis was done on the cholesteryl ester fraction. The mean percentage wet weight of cholesteryl esters + SE in the chick cecal mucosa was 0. 13 + 0.02.

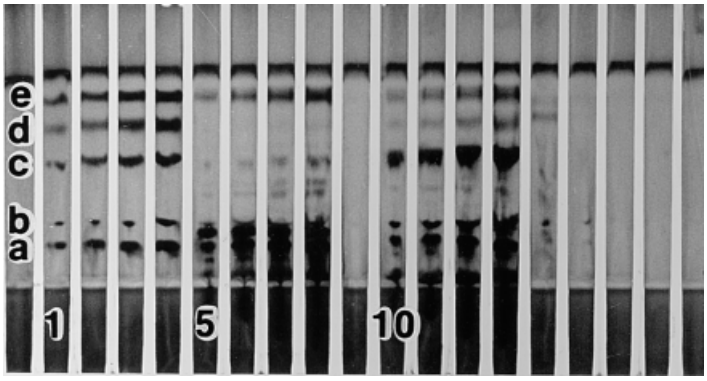

Figure 3. Chromatograms of neutral lipids in the cecal contents taken from domestic chicks and ICR mice. The standard and samples were spotted in 2.0, 4.0, 8.0, and 16.0 μL aliquots. The standard was spotted in lanes 1-4. Lanes 5-8 contain extracts from the cecal contents of chicks, and lanes 10-13 contain extracts from the cecal contents of ICR mice. Abbreviations: see Figure 1.

Visual comparison of the chromatograms of cecal mucosa showed differences in samples obtained from chicks infected with *Z lunata* compared with those from uninfected chicks. The mucosa from infected chicks had large cholesteryl ester and free sterol fractions, less abundant fractions of triacylglycerols, and only trace amounts of methyl esters (Fig. 2). There was a dramatic difference between the abundant triacylglycerol fraction of the uninfected-chick mucosa and the less abundant triacylglycerol zone of the infected-chick mucosa.

There were major visual and textural differences between the cecal contents of the mouse and chick. When removed from the chick, the cecal contents were seen as a homogenous, dark-brown to black substance. The mouse cecal contents were lighter in color (tan to brown) and appeared gritty in texture.

Semi-quantitative visual comparisons of the HPTLC plates also showed differences in neutral lipids between chick and mouse cecal contents. The most dramatic difference between the two was in the triacylglycerol fraction. This fraction was much greater in the mouse cecal contents than in the chick. The methyl ester fraction was also greater in the mouse cecal contents than in the chicks (Fig. 3). Cholesteryl esters appeared more abundant in the chick cecal contents compared with the mouse cecal contents, but when this fraction was quantified no significant difference was found (Student's t-test, $P = 0.17$). The mean percentage wet weight ± SE of cholesteryl esters was 0.19 ± 0.06 in the cecal contents of the domestic chick and 0.06 ± 0.01 in the cecal contents of the ICR mouse.

This study has provided information on the lipid biochemistry of the ceca of the uninfected domestic chick and ICR mouse. It also provided information on the pathobiochemical effects of *Z lunata* infection on the cecal mucosa in chicks. The infected cecal mucosa was depleted of triacylglycerols, probably because the worm was ingesting and then utilizing this lipid. However, the cholesteryl ester and methyl ester fractions were more abundant in the infected cecal mucosa than the uninfected mucosa, probably because the parasite altered the biochemical composition of the cecal mucosa and enhanced those lipid fractions.

REFERENCES

1. C. H. Willey. *Zoologica*, **26**, 65-88 (1941).

2. B. Fried, P. D. Nelson. *Parasitology*, **77**, 49-55 (1978).

3. Y. Isshiki, Y. Nakahiro. *Nippon Chikusan Gakkai-Ho*, **47**, 532-536 (1976).

Received May 23, 1998
Accepted June 19, 1998
Manuscript 4826-TLC

Request Permission or Order Reprints Instantly!