APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Sept. 1976, p. 443-444 Vol. 32, No. 3
 Printed in U.S.A.

# Degradation of Ochratoxin A by a Ruminant

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Received for publication 18 May 1976

The fate of ochratoxin A during incubation with contents from the four stomachs of the cow was studied. It was concluded that ochratoxin A was cleaved into the nontoxic ochratoxin α and phenylalanine by the contents from all but the abomasum.

Ochratoxin A is thought to be the cause of porcine nephropathy (4, 6; L. Rutqvist, N.-E. Björklund, K. Hult and S. Gatenbeck, in press). If pigs are kept on ochratoxin A, contaminated feed ochratoxin A can be isolated from kidney, liver, and fat (4). This shows that ochratoxin A can be taken up from the alimentary canal without prior degradation. In contrast to pigs, ruminants expose their feed to microbial degradation before any essential uptake; therefore it is possible that ochratoxin A is degraded by the microorganisms before reaching the blood stream. The initial microbial degradation of ochratoxin A should be a hydrolysis of the susceptible peptide bond by a peptidase forming ochratoxin α and phenylalanine (2, 5). Ochratoxin α is nontoxic (17), and if this degradation actually takes place, ruminants should be less sensitive to ochratoxin A toxicosis.

Fresh contents from the four stomachs of the cow were obtained from a local slaughterhouse in Stockholm. To samples of 1.5 g of stomach contents, 2 ml of 0.04 M tris(hydroxymethyl)-aminomethane-hydrochloride buffer, pH 7.5, was added. The samples were incubated with 4 nmol of ochratoxin A at 37°C. After appropriate times the incubations were ended by addition of 1 ml of 1 M HCl, and the samples were extracted with 4 ml of chloroform. The chloroform was washed with water to neutrality and extracted twice with 2 ml of 0.04 M tris(hydroxymethyl)aminomethane-hydrochloride, pH 7.5. The buffer solutions were cooled in an ice bath, and fluorescence spectra were recorded from 320 to 400 nm of excitation at a 450-nm emission. Ochratoxin A and α have excitation maxima at 380 nm and 340 nm, respectively (3).

Six rumina were investigated. The disappearance of ochratoxin A from the free solution was at first very rapid. Fifty percent of added ochratoxin A disappeared in less than 15 min. A slower phase followed, and after 4 h, less than 5% of added ochratoxin A remained in the solution. In two cases about 20% ochratoxin A remained unaffected.

It was possible to detect the formation of ochratoxin α in the solution after 1 to 4 h. In two samples 40% of added ochratoxin A was found as ochratoxin α, but in the other cases only about 10% was observed. It is thought that ochratoxin α was further degraded and that the detected amounts only reflected the steady-state concentrations.

The ochratoxin A and α bound in one way or the other to microorganisms and other solids in the samples were analyzed after extraction of the solids with chloroform-methanol (1:1 by volume). It was found that the concentration of ochratoxin A rose very rapidly in the initial phase with a corresponding decrease of the concentration in the free solution. Thereafter the concentration in the solids declined in the same manner as in the free solution. Ochratoxin α had a maximum concentration in the cells before it appeared in the free solution.

Two reticula and two omasa were investigated. Those showed the same pattern of ochratoxin A disappearance as the rumina. Ochratoxin α appeared in higher concentrations as compared with the rumina. Sixty percent of ochratoxin A was detected as ochratoxin α when all ochratoxin A was consumed.

Two abomasa that were investigated failed to degrade ochratoxin A. The results show that the contents from the three first stomachs of the cow are able to hydrolyze ochratoxin A into the nontoxic ochratoxin α and phenylalanine.

In the reported experiments, 4 nmol of ochratoxin A was degraded by 1.5 g of rumen content in 4 h. Assuming that the same reaction velocity is obtained in the living cow and that the stomach content has a retention time of 48 h, every kilogram of content will degrade 30

μmol or 12 mg of ochratoxin A. This means that the cow should be able to degrade ochratoxin A in feed contaminated up to the level of 12 mg/kg.

We thank Lars Rutqvist for help and suggestions he gave during this work.

This work was supported by a grant from The Bank of Sweden Tricentenary Foundation.

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