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Short communication

Immunohistochemical study of proliferating cell nuclear antigen (PCNA) in duckling liver fed with aflatoxin B_1 and esterified glucomannan

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Abstract

The effect of esterified glucomannan on aflatoxin B_1 toxicity in ducklings was studied by immunohistochemical staining of proliferating cell nuclear antigen (PCNA) in hepatic cells on formalin-fixed paraffin-embedded liver samples. Cherry Valley ducklings were divided into five groups, 20 birds in each. One of the groups was fed with conventional feed, and the other groups were fed with diet containing 100 ppb aflatoxin B_1, that containing 0.05% esterified glucomannan, or that containing 100 ppb aflatoxin B_1 supplemented with 0.05 or 0.1% esterified glucomannan, from five days of age for one month, and subsequently all the groups were fed with conventional feed for 20 days. Four birds of each group were sacrificed on the 30th, 35th, 40th, 45th and 50th day of feeding, and PCNA on the liver tissue sections was quantitatively analyzed by immunohistochemical staining. The percentage of PCNA-positive hepatocytes was significantly higher in the group given diet containing aflatoxin B_1 than in the other groups, which were not significantly different from each other. The results demonstrate that supplementation of feed with esterified glucomannan is effective in reduction of aflatoxin B_1-induced hepatic injury in ducklings.

Keywords: Proliferating cell nuclear antigen (PCNA); Duckling; Aflatoxin B_1; Esterified glucomannan; Liver; Immunohistochemistry

Aflatoxin B_1 is a hepatotoxic and hepatocarcinogenic mycotoxin produced by *Aspergillus flavus* and *Aspergillus parasiticus*. The most common acute toxicity is hepatitis with microscopic changes including diffuse degeneration in parenchymal cells, enlarged nuclei and extensive bile duct proliferation (Norred, 1986; Bintvihok et al., 1991a,b, 1997). Natural contamination of crops with aflatoxin has caused severe problems in performance of farm animals including poultry in tropical and subtropical regions (Dalvi, 1986).

Esterified glucomannan is a toxin binder consisting of functional carbohydrates extracted from yeast cell walls of *Saccharomyces cervisiae*. It has a large surface area of 22,000 m^2 per 1 kg and contains a large number of pores of different sizes to trap a wide range of chemicals (Cole, 1999; Devegowda et al., 2000). Esterified glucomannan has been found to counteract the toxic effect of

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aflatoxin in feed in growing broiler chicken (Raju and Devegowda, 2000; Aravind et al., 2003). In consistent with these findings, we found previously that supplementation of aflatoxin B_1-contaminated diet with esterified glucomannan can reduce the liver injury such as bile duct proliferation and fatty degeneration in ducklings (Bintvihok et al., 2002).

The objective of the present study is to clarify whether esterified glucomannan in feed can prevent toxic effects of aflatoxins in hepatic cells of ducklings by using the cellular proliferation activity in the liver. The proliferation activity was assessed by counting proliferating cell nuclear antigen (PCNA) positive nuclei on

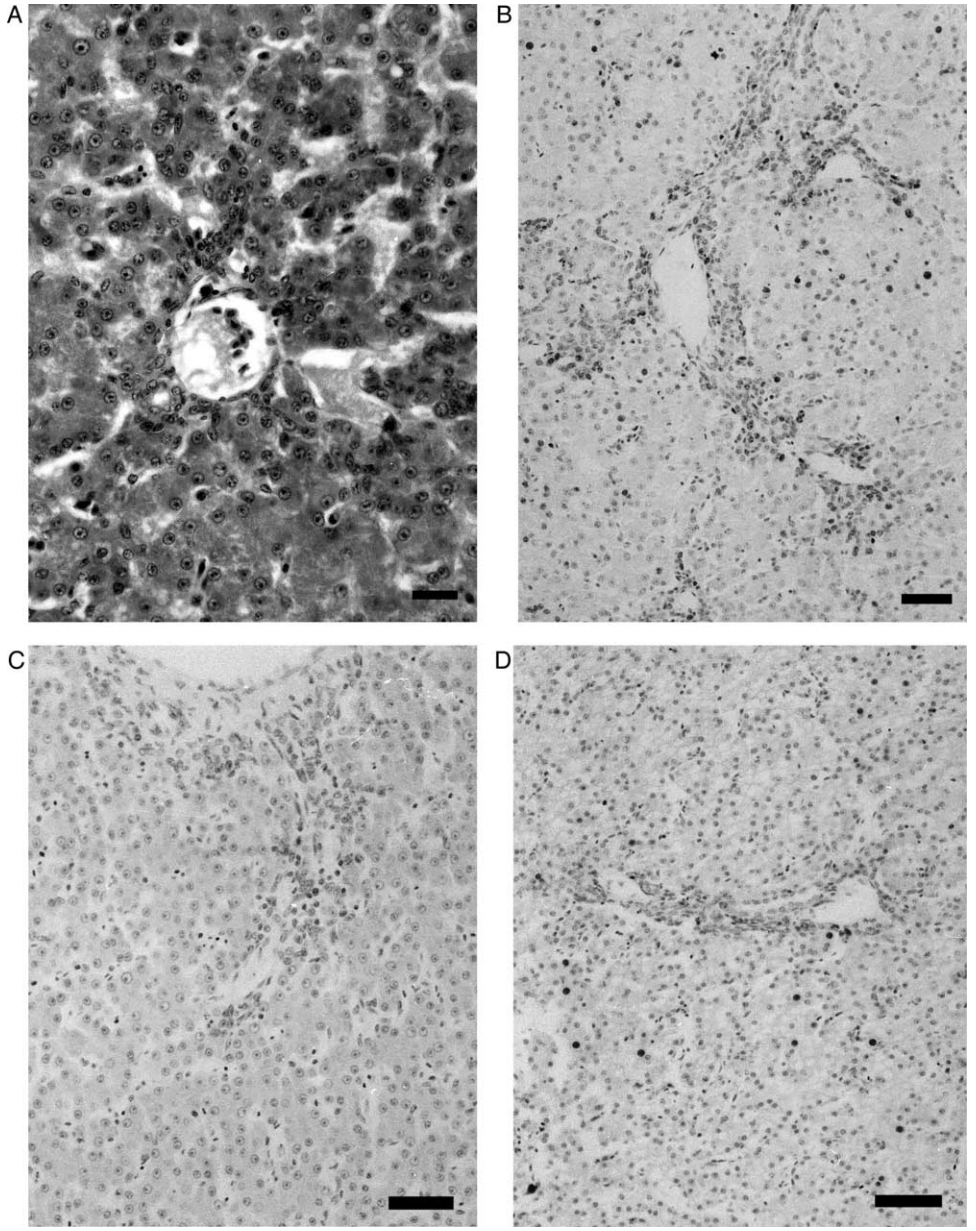

Fig. 1. Liver sections of ducklings fed with conventional feed, 100 ppb aflatoxin B_1 contaminated diet and 100 ppb aflatoxin B_1 contaminated diet supplemented with 0.1%esterified glucomannan on the 35th day of experiment. (A) Bile duct proliferation and intermediate morphology of duckling fed with 100 ppb aflatoxin B_1 contaminated diet. H&E, bar 35 μm. (B) High number of PCNA-positive nuclei in the periportal area of duckling fed with 100 ppb aflatoxin B_1 contaminated diet. ABC method, bar 70 μm. (C) Very low number of PCNA-positive nuclei of duckling fed with conventional feed. ABC method, bar 80 μm. (D) Low number of PCNA-positive nuclei, which scattered around the periportal area and liver lobules, of the duckling fed with 100 ppb aflatoxin B_1 contaminated diet supplemented with 0.1%esterified glucomannan. ABC method, bar 80 μm.