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ScienceDirect
International Dairy Journal 17 (2007) 854–859
INTERNATIONAL DAIRY JOURNAL
www.elsevier.com/locate/idairyj

Evaluation of the efficacy of whey protein to ameliorate the toxic effects of aflatoxins in rats

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Received 17 December 2005; accepted 22 September 2006

Abstract

The present study was conducted to assess the ability of whey protein concentrate (WPC) to protect against aflatoxicosis. Three groups of Sprague–Dawley male rats were used. The control (a) was fed on casein diet, the control (b) received the same diet contaminated with aflatoxins (AFT; 2.5 mg $AFB_1\,kg^{-1}$). The AFT-contaminated diet was supplemented with WPC and fed to the third group for 9 weeks. Compared with control (a) rats, those fed the contaminated diet (control b) showed significant drop ($p<0.05$) in body weight gains, food efficiency ratio (FER), an increase in the liver function enzymes, malondialdehyde level (MDA) and a decrease in blood glutathione (GSH), plasma calcium, magnesium and potassium. Addition of WPC to the AFT-contaminated diet realized better growth rate and FER and improved the above biochemical parameters. This study indicates that WPC supplementation appears beneficial to compensate AFT toxicity.

Keywords: Whey protein; Aflatoxins; Malondialdehyde–glutathione

1. Introduction

Aflatoxins (AFTs) are secondary metabolites of some mold strains of *Aspergillus flavus* and *A. parasiticus* that frequently contaminate cereal crops (Eaton & Gallagher, 1994). Reduced feed intake, lowered daily weight gains, and, in some cases, reduced feed efficiency have been observed for swine fed contaminated feed (Harvey, Kubena, Huff, Corrier, & Phillips, 1988). The physiological effects of AFT consumption include liver damage characterized by enlargement, release of enzymes into the blood (e.g., aspartate-aminotransferase, γ-glutamyltransferase, and alkaline phosphatase), and impaired protein synthesis (CAST, 1989). In combination with hepatitis, aflatoxin B_1 (AFB_1) is thought to be largely responsible for the high incidence of hepatocellular carcinoma in some parts of the world (Groopman, Cain, & Kensler, 1988).

Investigations into the metabolic basis for the natural variation in sensitivity of laboratory animals to AFB_1 suggest that the high expression of glutathione S-transferase (GST) what inactivates the exo-8,9-epoxide plays an indispensable role in protection against carcinogens (Monroe & Eaton, 1987). Also, depletion of glutathione (GSH) can result in a 25-fold increase in the sensitivity of murine liver to form AFB_1–DNA adduct (Monroe & Eaton, 1988).

In addition to GST-catalyzed conjugation of AFB_1 with GSH, other detoxification mechanisms exist that probably contribute to resistance to mycotoxin (Hayes, Judah, & Neal, 1993). The ability of natural antioxidants to prevent the development of cancer has provoked much interest as a mean of reducing the incidence of neoplastic diseases in human population. Because it is highly improbable that AFB_1-producing moulds can be eradicated from the environment, dietary antioxidants are an attractive strategy to protect individuals from the risk of liver cancer caused by exposure to the mycotoxins. The P450 1A inducer carotenoids (canthaxanthin, astaxanthin, beta-apo-8′-carotenal) exerted protective effect

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doi:10.1016/j.idairyj.2006.09.014

from hepatocarcinogenesis by AFB_1 through deviation of AFB_1 metabolism towards detoxification pathways, while beta-carotene decreased AFB_1 carcinogenicity but did not alter its metabolism (Gradelet, Astorg, LeBon, Berges, & Suschetet, 1997).

Whey protein concentrates (WPCs) and isolates are considered as functional food ingredients of important nutritional and health effects (McIntosh et al., 1998). They represent a mixture of secreted proteins with wide range of chemical, physical and functional properties. These proteins have been implicated in a number of biological effects observed in human and animal studies particularly their anti-cancer activity (Bounous, Batist, & Gold, 1991; McIntosh, Regester, LeLeu, Royle, & Smithers, 1995). A substantial increase in liver GSH was observed when rats were fed whey proteins (McIntosh et al., 1995). Based on the GST-conjugation of GSH to AFB_1, feeding whey proteins would prevent AFB_1 induced carcinogenesis. However, no cited studies have dealt with the ability of whey protein products to ameliorate the toxic effects of AFT in rats.

The present paper describes the effect of feeding rats on AFT-contaminated rations with and without whey proteins.

2. Materials and methods

2.1. Preparation of WPC

Sweet whey from Ras cheese manufacture (pH 6.0–6.2) was obtained from Arab Dairy Co., Kaha, Egypt. Fat and curd fines were removed from the whey by a cream separator (Alfa-Laval Lund, Sweden). Whey contained 7.5% total solids (TS), 0.75 % total protein and 4.5% lactose.

Ultrafiltration of Ras cheese whey was carried out using a Carbosep 252 UF system (SFEC, Bollene, France) equipped with zirconium oxide membranes (Mol. cut off 50,000). Ultrafiltration was carried out in batch mode at 50 °C and inlet and outlet pressure of 6 and 4 bar, respectively. Ultrafiltration was continued to a concentration factor of 20. The gross characteristics of the WPC were: TS 20.54 %, ash 2.53 %, total protein 14.58%, lactose 3.52%, fat 0.10%, pH 6.21, and acidity 0.47.

2.2. Aflatoxins

AFTs were produced by the method of Davis, Dioner, and Dridge (1966) using liquid medium, yeast extract sucrose (YES) as substrate. The AFT concentration of the crude extract (B_1, B_2, G_1 and G_2) were determined and calculated according to Shannon, Shotwell and Kwolek (1983). The AFTs were thoroughly mixed with the diet at a level of 2.5 mg kg^{-1} diet. This level of aflatoxin was selected based on a previous study made by Saleh, Abdel-kader, El-Shobaki, & El-Hawary, 2000. In this study, they used a dose of 2.5 mg kg^{-1} diet, which was suitable to produce biochemical symptoms in rats due to toxicity of the AFT.

2.3. Animals and diets

Male, Sprague–Dawley rats (average body weight 153 g) were obtained from the animal house of the National Research Center, Dokki, Cairo. Animals were randomly divided into three groups of seven animals each and maintained individually in stainless steel cages. A slightly modified AIN-93M semi-purified diet recommended by the American Institute of Nutrition (Reeves, Nielson, & Fahey, 1993) was used for the control in the present study (Table 1). The same diet was given to the experimental group, but after replacing 50% of casein with WPC.

The AFT were incorporated into the diet by dissolving a known amount of the crude extract that provided the desired level of 2.5 mg kg^{-1} diet in a minimal amount of ether and mixed well into 100 g of the diet. This was mixed again with the rest of the diet to ensure equal distribution of the AFT. The diet of the control group was mixed with the same amount of ether. The solvent was evaporated in open air for 30 min.

A group of animals (control a) given AFT-free diet was pair-fed with an amount of diet similar to that consumed by the second group that was given AFT-contaminated diet ad libitum and used as control (b). The third group continued to be fed ad libitum the same contaminated diet but with WPC providing 50% of protein (the remainder was casein). To avoid the fermentation of diets due to addition of WPC, fresh diets were prepared every day. The feeding period lasted for 9 weeks during which food consumption and body weight were regularly recorded twice a week.

At the end of the experiment the rats were fasted over night and blood samples were collected in heparinized tubes under slight diethyl ether anesthesia by open-heart puncture. Samples were centrifuged at $1348 \times g$, for 10 min and plasma samples stored in Ependorf vials at −20 °C until biochemical analysis.

Feed efficiency ratios of the different diets were calculated as the gain in body weight (g) per feed intake (g) according to Smith and Circle (1971).

The activity of GSH peroxidase (GSH-Px) was determined in the whole blood using kits provided by Randox

Table 1
Composition of the diet

Ingredient	Content (g kg^{-1})
Casein	140
D, L-methionine	2.5
Sucrose	100
Cotton seed oil	50
Fibre	40
AIN-93M vitamin mixture[a]	10
AIN-93M mineral mixture[a]	35
Corn flour starch	621.5
Choline chloride	1

[a]Vitamin and mineral mix (AIN-93, American Institute of Nutrition, 1993).