ENVIRONMENT, HEALTH, AND BEHAVIOR

Efficacy of Silymarin-Phospholipid Complex in Reducing the Toxicity of Aflatoxin B1 in Broiler Chicks

D. Tedesco,* S. Steidler,* S. Galletti,* M. Tameni,* O. Sonzogni,* and L. Ravarotto†

*Department of Veterinary Science and Technology for Food Safety, Via Celoria, 10, 20133 Milan, Italy; and †Istituto Zooprofilattico Sperimentale delle Venezie, Via dell’Università, 10, 35020 Legnaro, Italy

ABSTRACT Silymarin, the standardized extract of Silybum marianum, is used as a hepatoprotector in man, and is a potent antihepatotoxic agent. This study focused on the effects of a silymarin-phospholipid complex in reducing the toxic effects of aflatoxin B1 (AFB1) in broiler chickens. Twenty-one 14-d-old male commercial broilers were randomly allotted to 3 groups and treated as follows: basal diet alone [Group C (Control)]; AFB1 at 0.8 mg/kg of feed [Group B1]; AFB1 at 0.8 mg/kg of feed plus silymarin phytosome, a silymarin complexed form with phospholipids from soy, at 600 mg/kg of BW [Group B1+Sil]. Considering the whole growth cycle, BW gain and feed intake were lower in AFB1-treated birds with respect to controls (P < 0.05). In the B1+Sil group, BW gain and feed intake were higher with respect to birds receiving AFB1 alone (P < 0.05), and not different from the control birds. Serum biochemistry showed no difference among groups, except for a decrease of alanine amino transferase (ALT) in chicks treated only with AFB1. Alanine amino transferase activity in AFB1 plus silymarin phytosome treated birds was not different from the controls. No treatment differences were noted on liver weight. In conclusion, our results suggest that silymarin phytosome can provide protection against the negative effects of AFB1 on performance of broiler chicks.

(Key words: silymarin, phytosome, aflatoxin B1, broiler)

INTRODUCTION

Aflatoxins are secondary fungal metabolites produced by some strains of Aspergillus flavus and A. parasiticus. Aflatoxins B1, B2, G1, and G2 are natural contaminants of many animal feed ingredients, especially peanuts and cereals. Aflatoxin B1 (AFB1) is considered the most potent, having hepatotoxic, hepatocarcinogenic, mutagenic, and teratogenic effects in many animal species, and is classified as a Group I carcinogen in humans (IARC, 1987). Aflatoxin B1 is biotransformed in the liver by monoxygenases and then transformed by cytochrome P450 into aflatoxin 8,9 epoxide (Emerole et al., 1979), a highly active electrophilic compound that is inactivated by conjugation with glutathione and excreted through urine and bile (Essigmann et al., 1982).

Among birds, aflatoxicosis may affect turkeys, quail, ducklings, goslings, and chickens (Arafa et al., 1981). In these species, aflatoxins are claimed to be the cause of fatty liver syndrome (Hamilton and Garlich, 1971; Kubena et al., 1991). Aflatoxicosis in chickens is characterized by mortality, listlessness, anorexia, decreased growth rates, negative feed conversions, fatty liver, decreased egg production, poor pigmentation, and increased susceptibility to other diseases (Arafa et al., 1981; Doerr et al., 1983). Aflatoxicosis is a cause of economic losses in broiler production. Furthermore, small amounts of AFB1 and its metabolites can be found in several edible tissues (Micco et al., 1988), and risks for public health, related to the human consumption of exposed animals, are still to be properly defined. For these reasons, intensive research has been pursued to develop cost-effective and safe methods to reduce the effects of AFB1. Nutritional actions focus on the addition to feedstuffs of nonnutritive sorbents, such as bentonite or hydrated sodium aluminosilicate (Phillips et al., 1988; Oguz and Kurtoglu, 2000; Rosa et al., 2000). These compounds produce large molecules that cannot be carried across the intestinal wall. However, because the binding spectra of these substances are broad and nonspecific, nutritional components such as vitamins and minerals may also be removed (Ramos et al., 1996). Several authors have recently focused on the inhibition of aflatoxin biotransformation into its 8,9-epoxide constituents through interaction with cytochrome P450 enzymes using oltipraz (Kuilman et al., 2000) or natural compounds (Kim et al., 2000; Lee et al., 2001; Liu et al., 2001).

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To whom correspondence should be addressed: doriana.tedesco@unimi.it.

Abbreviation Key: AFB1 = aflatoxin B1; ALT = alanine amino transferase; GGT = γ-glutamyl transferase.
The bioactive extract from *Silybum marianum* (milk thistle), silymarin, contains a mixture of flavonolignans and a residual fraction that has not yet been defined chemically in detail (Skottova et al., 2003). Silymarin is used in humans for the treatment of numerous liver disorders characterized by degenerative necrosis and functional impairment (Luper, 1998). The beneficial effects of silymarin have been shown in dairy cows during peripartum, a period in which they are subject to fatty liver (Tedesco et al., 2004). Its mechanism of action still seems to be poorly understood, but data in the literature suggest that it acts as an antioxidant, cell membrane stabilizer and permeability regulator, as well as a promoter of DNA, RNA, and protein synthesis (Magliulo et al., 1973). Silybin, a major constituent of silymarin (Morazzoni and Bombardelli, 1995), has been shown to preserve the functional and structural integrity of hepatocyte membranes by preventing alterations of their phospholipid structure produced by carbon tetrachloride and by restoring alkaline phosphatase and γ-glutamyl transferase activities (Muriel and Mourelle, 1990). The hepatoprotective actions of silymarin and silybin have been studied in acute liver intoxication induced by toxic agents. Some authors reported that silymarin has hepatoprotective properties in experimental intoxication with *Amanita phalloides*, for a competitive inhibition of α-amanitin uptake (Kröncke et al., 1986). It is reported that silybin inhibits the murine hepatic cytochrome P450 detoxification system in the phase I metabolism (Baer-Dubowska et al., 1998). Rastogi et al. (2000) reported that silymarin reversed changes in liver and serum in AFB1 intoxicated rats, indicating that it has a hepatoprotective action in preventing AFB1 induced injury. An antioxidant effect is also suggested by a lower milk excretion of AFM1, an AFB1 metabolite, observed in dairy cows receiving silymarin (Tedesco et al., 2003). This suggests that silymarin may contribute to prevent the aflatoxicosis-induced damage. The present trial was conducted to evaluate the efficacy of a silymarin-phospholipid complex (silymarin phytosome) against aflatoxicosis in experimentally intoxicated broiler chickens.

**MATERIALS AND METHODS**

**Birds and Diets**

Twenty-one 14-d-old male commercial broilers (BW, 377 ± 34 g) were randomly allotted into 3 groups of 7 birds each. The birds of each group were randomly housed in individual suspended stainless steel cages. The dimensions of each cage were 55 × 60 × 58 cm (width × length × height). Feed (corn and soybean meal standard, 20.4% protein, 3,150 kcal of ME/kg) and water were given ad libitum. The birds were kept under artificial light with a 12 h photoperiod, and adapted for 5 d before treatment.

The birds used in this experiment were cared for in accordance with the guidelines established by the European Union and approved by the Italian Ministry of Health.

Treatment lasted for 35 d and consisted of: AFB1 at 0.8 mg/kg of feed (group B1); AFB1 at of 0.8 mg/kg of feed plus silymarin phytosome at 600 mg/kg of BW (group B1+Si); control group (group C) was fed on a basal diet alone. Silymarin phytosome is a complex of silymarin and phospholipids from soy in a molar ratio of 1:2; lipophilic complexes have shown, in vitro with biological membranes and in vivo in monogastric animals, a higher bioavailable rate for the active compound (Livio et al., 1990; Morazzoni et al., 1992). Treatment was administered daily by gavage to assure the correct dose administration and to ensure the safety of the operators. Feed intake and BW determined at the start of each experimental week were used to adjust the AFB1 and silymarin phytosome doses.

**Data Collection**

**Performance.** Chicks were weighed individually every week. Individual feed intake was recorded daily in the last 2 wk of the experimental period. Morbidity and mortality were recorded in each group.

**Necropsy Examination and Liver Histology.** At the end of the trial, chickens were killed by cervical dislocation and necropsied. Liver, small intestines, pancreas, kidney, spleen, and bursa were examined for gross pathology. Lesion scores were assigned as follows: the most severe lesion observed was assigned a score of 4 on a 4-point scale where normal = 0. Liver weight was recorded for each bird; 2 birds from each treatment group were randomly selected for histological studies of liver tissue. Samples were fixed in 10% neutral buffered formalin and kept at −4°C until histological examination. The samples were dehydrated through a graded series of ethanol, cleared in xylene, embedded in paraffin wax, and sectioned at 5 μm with a microtome. The sections were stained with hematoxylin and eosin and examined with an Olympus BX51 microscope.

**Serum Biochemistry.** Before slaughter, each bird had blood collected from the brachial vein. Blood samples were centrifuged and sera were stored at −20°C. Samples were submitted for analysis to measure total protein, albumin, globulin, glucose, urea, total bilirubin, direct bilirubin, indirect bilirubin, aspartate amino transferase, γ-glutamyl transferase (GGT), alanine amino transferase (ALT), calcium, and phosphorus, using an automatic analyzer.

**Statistical Analysis**

The data were subjected to ANOVA as a completely randomized design using the GLM procedure of SAS (SAS Inst., 2001). Growth parameters were analyzed as repeated measures. The model contained the effects of treatment, time, and their interaction, random effect of bird nested within treatment and residual error. Differ-
In this experiment, a dose of AFB1 (0.8 mg/kg of feed) was evident after 3 wk of treatment at the dosage used. Reduced by AFB1 alone, there were no differences in BW ±2,490 period (Table 1) was lower in AFB1-treated birds (Table 2). The initial BW in all 3 groups was 652.5 ± 16 g. In the first 3 wk of the experimental period, there were no differences in BW among the 3 groups (P > 0.05). After 3 wk of treatment, the weight of the AFB1 group was lower compared with the other groups, with a significant difference at 28 and 35 d of treatment (P < 0.05). In contrast with the significant effect on growth performance induced by AFB1 alone, there were no differences in BW between control and B1+Sil groups (respectively 2,875 ± 107.5 g vs. 2,871 ± 124 g at 28 d and 2,996 ± 78 g vs. 3,026 ± 89.5 g at 35 d).

As shown in Figure 1, the effect of AFB1 on performance was evident after 3 wk of treatment at the dosage used. In this experiment, a dose of AFB1 (0.8 mg/kg of feed) was chosen that represents a contamination level that can be found naturally in feed. Various authors have studied the effects of experimentally induced aflatoxicosis, and some have specifically examined its effects in broilers (Doerr et al., 1983; Fernandez et al., 1994; Quezada et al., 2000). Results from the present study agree with those that showed AFB1 severely affected the performance of chickens.

The mean feed intake in the last 2 wk of experimental period (Table 1) was lower in AFB1-treated birds (P < 0.05) compared with the other groups. Feed conversion ratio was not influenced by treatments. Considering the entire experimental period, aflatoxin adversely affected feed consumption and weight, but not feed conversion, suggesting that the primary effect of the toxin was on feed intake, and the effect on weight depression was secondary, as observed by Nandakumar Reddy et al. (1984). Despite this evident effect of AFB1 intoxication, there was no difference in performance when the birds received AFB1 plus silymarin phytosome. Silymarin action was constant and positive with respect to birds receiving only AFB1, considering BW and feed intake. These results suggest that treatment with silymarin can be effective in counteracting the negative effects of AFB1 intoxication on feed intake and BW in growing broilers.

### RESULTS AND DISCUSSION

#### Performance

No morbidity or mortality due to aflatoxin ingestion was recorded in this study. The average BW of each experimental group is shown in Figure 1. The initial BW in all 3 groups was 652.5 ± 16 g. In the first 3 wk of the experimental period, there were no differences in BW among the 3 groups (P > 0.05). After 3 wk of treatment, the weight of the AFB1 group was lower compared with the other groups, with a significant difference at 28 and 35 d of treatment (P < 0.05), when BW in the AFB1 group was 2,490 ± 115 g and 2,635 ± 83 g, respectively. In contrast with the significant effect on growth performance induced by AFB1 alone, there were no differences in BW between control and B1+Sil groups (respectively 2,875 ± 107.5 g vs. 2,871 ± 124 g at 28 d and 2,996 ± 78 g vs. 3,026 ± 89.5 g at 35 d).

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#### Necropsy Examination and Liver Histology

At necropsy, macroscopic observation of the organs showed individual rather than treatment-related variations. Liver weights in treated groups were not different from those of the control group (data are not shown). Histological sections from the livers of AFB1-treated birds showed multifocal portal infiltration composed of mononucleates, granulocytes, and eosinophils diffused in the parenchyma, especially at the portal areas, and necrosis in zone 1. These changes were less severe in the birds receiving AFB1 plus silymarin. Control birds presented modest vascular congestion and minimal focal infiltration. Our observations demonstrate the ability of silymarin to counteract the action of AFB1, reducing hepatic histopathological changes induced by this toxin.

#### Serum Biochemistry

Treatments did not influence the serum concentrations of proteins, glucose, urea nitrogen content, aspartate amino transferase, GGT, bilirubin, calcium, and phosphorus (Table 2). The serum activity of ALT was lower (P < 0.05) in AFB1-treated birds. In birds receiving AFB1 plus silymarin phytosome, there was no difference in ALT serum activity compared with controls.

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TABLE 1. Effects of aflatoxin B1 (AFB1) and silymarin phytosome on feed intake and feed conversion ratio in the last 2 wk of the experimental period

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Feed intake (g/d)</th>
<th>Feed conversion ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>211b</td>
<td>2.483</td>
</tr>
<tr>
<td>AFB1</td>
<td>157b</td>
<td>2.928</td>
</tr>
<tr>
<td>AFB1 + Sil</td>
<td>192e</td>
<td>2.527</td>
</tr>
<tr>
<td>SEM2</td>
<td>11.07</td>
<td>0.27</td>
</tr>
</tbody>
</table>

a,bMeans within a column lacking a common superscript differ significantly (P < 0.05).

1Each value represents the least square mean from 7 broilers per treatment. Control = basal diet alone; AFB1 = aflatoxin B1 at 0.08 mg/kg of feed; AFB1 + Sil = aflatoxin B1 at 0.08 mg/kg of feed plus silymarin at 600 mg/kg of BW.

2Pooled standard error of the mean.
Fernandez et al. (1994) found that cholesterol, triglycerides, calcium, phosphorus, ALT, GGT, and lactate dehydrogenase levels in serum did not vary in broilers fed aflatoxin contaminated feed, whereas they did in laying hens. Other authors reported a decrease in serum ALT content because of AFB1 intoxication in chickens (Stanley et al., 1993; Valdivia et al., 2001). Solter et al. (2000) observed a relationship between subchronic exposure to a hepatotoxic agent (microcystin-LR) and decreased hepatic ALT synthesis. From our serum biochemistry results, no differences were observed in any of the biochemical parameters evaluated, except for a lower level of ALT seen only in the AFB1-treated group (P < 0.05), but not in any other group. As reported before, this can be due to a recognized hepatotoxic effect of AFB1.

We can observe that treatment with silymarin prevented these changes in ALT activity, supporting the suggestion that these compounds may provide protection against toxic effects of AFB1.

The mechanism of silymarin action against AFB1 intoxication can only be hypothesized. Many authors have demonstrated that the activation of AFB1 in human and rat liver is a complex process controlled by multiple cytochrome P450 enzymes (Emerole et al., 1979; Forrester et al., 1990, Gallagher et al., 1996). As reported by Baer-Dubowska et al. (1998), silymarin can inhibit the cytochrome P450 system, and consequently inhibit AFB1 activation. Silymarin is known to be a potent antioxidant: it acts as a scavenger of free radicals (Mira et al., 1994) and can influence enzyme systems associated with glutathione and superoxide dismutase (Valenzuela et al., 1989). Either the action on cytochrome P450 or the inhibition of oxidative damage could be responsible for the protective effect against AFB1-induced alteration in liver, such as that observed in the present study.

**Conclusions**

In summary, these results suggest that treatment with silymarin phytosome decreases the toxic severity of AFB1 on serum ALT concentration, liver histology, feed intake, and BW gain in broilers. The protective action of this compound was particularly evident on BW gain and feed intake. These findings suggest that silymarin might be used in chickens to prevent the effects of AFB1 in contaminated feed and provide a basis for further studies on the relationship between silymarin and protection against AFB1 toxicity, to improve safety and quality of poultry products.

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