Efficacy of N-Acetylcysteine to Reduce the Effects of Aflatoxin B_1 Intoxication in Broiler Chickens[1]

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ABSTRACT N-acetylcysteine (NAC) has been used safely in humans and in other mammals as an antidote against several toxic and carcinogenic agents, including aflatoxin B_1 (AFB_1). The aim of this study was to evaluate the capability of dietary supplementation with NAC to ameliorate the effects of subacute intoxication with AFB_1 in broiler chickens. One hundred twenty male Hubbard 1-d-old chickens were allocated into one of four dietary treatments: 1) control group without treatment, 2) purified AFB_1 added to diet (3 mg/kg of feed) for 21 d, 3) NAC (800 mg/kg BW, daily), or 4) AFB_1 plus NAC at the same doses as Groups 2 and 3. Broilers treated with AFB_1 plus NAC were shown to be partially protected against deleterious effects on BW (57.8%), daily weight gain (49.1%), feed conversion index (21.4%), plasma and hepatic total protein concentration (45.2, 66.7%), plasma alanine aminotransferase (67.4%), hepatic glutathione-S-transferase (18.8%), and reduced glutathione liver concentration (75.0%). In addition, they showed less intense liver fading, friable texture, and microvesicular steatosis. In the kidney, thickening of glomerular basement membrane was also less severe in NAC+AFB_1-treated chickens than in AFB_1-treated chickens. Our results suggest that NAC provided protection against negative effects on performance, liver and renal damage, and biochemical alterations induced by AFB_1 in broiler chickens. Effects of NAC alone on chick performance were also evaluated. Addition of NAC to diet (800 mg/kg BW) did not negatively affect feed consumption, conversion index, or serum chemistry and did not induce structural changes in the liver or kidney.

(*Key words*: aflatoxin B_1, N-acetylcysteine, plasma protein, nephrotoxicity, hepatotoxicity)

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INTRODUCTION

Aflatoxin B_1 (AFB_1) is a secondary metabolite of the *Aspergillus flavus* and *Aspergillus parasiticus* fungi, and it is found in grains and other foods and feeds as a natural contaminant. AFB_1 is the most potent of the naturally occurring mycotoxins; it is extremely toxic and a powerful carcinogen and, therefore, represents a serious risk to health in human populations (International Agency for Research on Cancer, 1993). In addition, AFB_1 causes various health effects in chickens in a dose-response pattern (Giambrone et al., 1985; Richardson et al, 1987). Hamilton (1984) has proposed an estimated minimum effective dose (10 μg/kg feed) based on the safety criterion of economic loss in broiler chickens.

Hepatic mixed-function oxydase system biotransforms AFB_1 and generates an aflatoxin metabolite or reactive epoxide. This intermediate molecule is inactivated by conjugation with reduced glutathione (γ-glutamil-cysteinyl-glycine, GSH) (Eaton and Gallagher, 1994). This reaction is catalyzed by glutathione-S-transferase (GST) to form a molecule that is eliminated as mercapturic acid-AFB_1 (8,9-dihidro-8-9-(S-cysteinyl-(N-acetyl))-9-hydroxy aflatoxin B_1) or N-acetylcysteine (NAC) bound to AFB_1 (Moss et al., 1985; Raney et al., 1992). When birds eat AFB_1, it is absorbed by the intestine and distributed by the bloodstream throughout the body; approximately 90% AFB_1 is removed through bile and renal secretion (Harland and Cardeilhac, 1975; Agacdelen and Acet, 1993). Studies on distribution of ^{14}C-AFB_1 in chickens revealed that 6.2% of radioactivity was retained in breast and leg meats (Mabee and Chipley, 1973).

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Abbreviation key: AFB_1 = aflatoxin B_1; ALT = alanine aminotransferase; AST = aspartate aminotransferase; DWG = daily weight gain; FCI = feed conversion index; GSH = reduced glutathione; GST = glutathione S-transferase; NAC = N-acetylcysteine.

In laying hens and broiler chickens, aflatoxin clearance times are 24 h for muscle and 8 d for eggs (Agacdelen and Acet, 1993; Fernandez et al., 1995).

For these reasons, intensive research has been pursued to develop cost-effective and safe procedures and agents that reduce the deleterious effects of AFB_1 (Kubena et al., 1993). Several approaches to avoid contamination, for decontamination or remediation of feedstuffs, have been proposed (Jones, 1987; Kubena et al., 1997; Bailey et al., 1998; McKenzie et al., 1998; Ledoux et al., 1999). Attention has been recently focused on the use of chemoprotective agents that act against AFB_1 that increase detoxification of mycotoxin by drug-metabolizing enzymes in human populations and in animal experimental models. (Larsen et al., 1985; Wang et al., 1997; Hayes et al., 1998; Wang et al., 1999).

Administration of NAC, the acetylated variant of the amino acid L-cysteine ($C_5H_9NO_3S$), to acquired immunodeficiency syndrome patients and to rats intoxicated with various xenobiotics increased the levels of cytosolic and mitochondrial GSH and decreased toxicity (Drogue, 1993; Kinscherf et al., 1994; Raju et al., 1994; Wispriyono et al., 1998). This compound is already commercially available for human consumption in several countries; it is well tolerated in daily doses as high as 500 mg/kg per os and has been successfully used as an antidote against various toxic agents and in several pathological disorders related to the GSH cellular homeostasis (Corcoran et al., 1985; Banner et al., 1986; Lancet, 1991; Kobrinsky, 1996). When NAC is given orally to healthy human volunteers, it is quickly absorbed and undergoes rapid and extensive metabolism in the gut wall and liver.

Approximately 10% of the unchanged drug reaches plasma and is transformed to cysteine and GSH (De Caro et al., 1989). It is possible that NAC has protective effects against the AFB_1 mutagenic attack by increasing intracellular glutathione levels in cells, by blunting the effects of activation of this mycotoxin, through stimulation of enzyme activities and by interacting with AFB_1 (De Flora et al., 1985). The aim of this study was to determine whether dietary supplementation with NAC prevented the appearance of negative effects during subacute AFB_1 intoxication in broiler chickens, because there are no indications in the literature for this therapeutic use of NAC.

MATERIALS AND METHODS

Animals and Diet

One-day-old male broiler chickens (58.8 ± 3.0 g BW), Hubbard × Hubbard, were obtained from a local hatchery,[3] individually weighed, wing-banded, and placed in electrically heated cages under continuous lighting with access to feed and water ad libitum. The basal sorghum-soybean meal starter diet, without added antibiotics, coccidiostats, or growth promoters, containing or exceeding the levels of critical nutrients recommended by NRC (1994) was purchased from Agricultural Sciences Centre Feed Mill.[4] Chicks were weighed on an individual basis, and feed consumption for each replicate was recorded daily.

NAC Addition. Purified NAC[5] was added daily to the basal diet (3.2 to 5.2 g/kg feed). Based on daily feed intake and BW records, it was assured that every bird consumed approximately 800 mg NAC/kg BW per day.

NAC and AFB_1

AFB_1 Administration. Pure crystallized AFB_1[5] solution in ethanol (1 mg/10 mL) was sprayed over a thin layer of the basal diet (<1-cm thickness), and a final concentration of 3.0 mg/kg of feed was achieved. The personnel that sprayed mycotoxin on the feed were protected with air filter masks, rubber overalls, and gloves; additional precautions were taken according to Shoemaker and Torchia (1995). The ethanol was thoroughly evaporated for 72 h; feed was mixed and then packed and stored until its use. Prior to the start of experiment, basal and contaminated whole diets where analyzed for aflatoxins by using solid-phase extraction tubes,[6] and the eluate extracted was derivatizated according to official method 990.33 of AOAC and by HPLC with fluorescence detection[7,8] (Scott, 1995). The basal diet did not contain detectable levels of AFB_1, AFB_2, AFG_1, or AFG_2 (detection limits <10 μg/kg).

AFB_1-NAC Protocol. A completly randomized design was used with 120 male 1-d-old chickens (58.8 ± 3.0 g BW) assigned to each of four dietary treatments. The groups were as follows: 1) control group with basal diet, 2) AFB_1 alone in the diet (3 mg/kg of feed) for 21 d, 3) NAC alone (800 mg/kg BW per day), and 4) AFB_1 plus NAC at the same doses as for Groups 2 and 3. Feed intake and BW were recorded daily. Chickens were slaughtered at 21 d of age, by cervical dislocation. Ten birds per group were randomly selected for biochemical assays and three more birds per group were used for histological studies.

Biochemical and Histological Analyses. Before slaughter, chicks were bled by cardiac puncture for biochemical analyses. Blood samples were obtained in EDTA-filled syringes, and plasma was collected by centrifugation (5,000 × g for 10 min). Plasma samples were labeled and stored (4 C for 24 h), until plasma total protein concentration, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured. After slaughter, the abdominal cavity was immediately opened, and the right liver lobe was removed. Hepatic tissue samples (100 mg) were stored in a cryogenic system in vials that contained 1.0 mL of buffer solution comprising 10 m*M* TRIS HCl, 5 m*M* EDTA, 10 m*M* KCl, and 250 m*M* sucrose, pH 7.4. Hepatic samples were processed in a Dunce-type homogenizer.

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[5]Sigma Aldrich, St. Louis, MO 63103.
[6]Supelclean LC-CN, Supelco Inc., Bellefonte, PA 16823-0048.
[7]Perkin Elmer Binary LC-1 250 pump, and Perkin Elmer LS-1 Fluorescence detector, Norwalk, CT.
[8]Supelcosil HPLC LC-18 column, Supelco, Bellefonte, PA 16823-0048.

TABLE 1. Effects of diets containing N-acetylcysteine (NAC), aflatoxin B_1 (AFB_1), or both, on body weight, daily weight gain, and efficiency of feed utilization at 3 wk of age[1]

Treatment		21-d Body weight		Daily weight gain		Feed:gain	
AFB_1 (μg/kg of feed)	NAC (mg/kg BW)	(g)	Change from control (%)	(g)	Change from control (%)	(g)	Change from control (%)
0.0	0.0	458 ± 7.4[a]	100	21.8 ± 0.4[a]	100	1.45 ± 0.02[b]	100
3.0	0.0	408 ± 6.4[b]	89.1	19.4 ± 0.3[b]	89.0	1.66 ± 0.02[a]	114
3.0	800	437 ± 9.9[ab]	95.4	20.8 ± 0.5[a]	95.4	1.61 ± 0.4[a]	111
0.0	800	449 ± 9.6[a]	98.0	21.4 ± 0.5[a]	98.2	1.51 ± 0.03[b]	104
LSD[2]		23.7		1.13		0.09	

[a,b]Means within a column with no common superscript differ significantly.

[1]Each value represents the mean ± SE from 30 broilers per treatment.

[2]LSD = least significant difference as determined by Fisher's protected LSD procedure.

The hepatic GSH was quantified by the method described by Hissin and Hilf (1976), using a refrigerated ultracentrifuge[9] and a spectrophotofluorometer[10] with 350 and 420 nm wavelengths excitation and emission, respectively. GSH experimental values were calculated by means of a GSH standard curve. GST was quantified by the method of Habig et al. (1974) by using a spectrophotometer[11] at 340 nm wavelength with a standard curve of 1-chloro-2,4-dinitrobenzene. Spectrophotometric analyses of hepatic and plasma total protein concentrations were conducted with a spectrophotometer[5] at 750 nm and a BSA standard curve, according to Peterson (1977). ALT and AST plasma activities were quantified by optimized UV procedures.[12] Briefly, serum ALT and AST catalyze the transfer of the amino group from added alanine or aspartate to form pyruvic or oxalacetic acid, respectively. The rate of decrease in absorbance for derivatives of these compounds is directly proportional to ALT or AST activity.

For histological studies, birds were anesthetized with pentobarbital sodium (25 mg/kg BW, i.v.). A cleanser solution (phosphate buffer, pH 7.4; heparin 500 U; and procaine, 1 g/L, 25 mL/min) was perfused into the left ventricle with a peristaltic pump, followed by a fixing solution (phosphate buffer, pH 7.4, and 10% formalin). Samples from liver and kidney were obtained and processed for periodic acid-Schiff staining.

NAC and Chick Performance

One relevant issue that arises from the use of NAC in feed for chickens is the palatability of feed that contains NAC, which may, therefore, negatively influence growth. To assess this issue, we performed the following experiments. Three groups (n = 10) of 21-d-old chickens were formed: control without any treatment; NAC-400, 400 mg/kg BW in feed per day for 7 d; and NAC-800, 800 mg/kg BW per day for the same period. Body weight and feed consumption were recorded through 28 d of age.

[9]Sorvall model RC5B, Dupont Co., Newtown, CT 06470-2337.

[10]Perkin Elmer LS50B, Buckinghamshire, UK HP91QA.

[11]Varian DMS 80, Varian Associates Inc., Australia.

[12]Procedures No. 15861 and 15860, Merck Co. Inc., State of Mexico, Mexico.

Statistical Analysis

Data for all variables in each experiment were subjected to one-way ANOVA using the general linear models procedure in the SAS® statistical software (SAS Institute, 1996). The means for treatments showing significant differences in the ANOVA were compared using the Fisher's protected least significant difference procedure (Snedecor and Cochran, 1967). A $P < 0.05$ was considered as significant.

RESULTS

NAC and AFB_1

AFB_1 and BW. The weights of the chickens that received AFB_1 in their feed were lower from 2 wk of age and later, as compared to controls. BW differences were more evident at Day 21, with losses attributable to intoxication (Table 1). When the birds were fed AFB_1 and NAC, mild growth depression was also detected; however, in contrast to the significant decrease ($P < 0.01$) induced by AFB_1 alone, the change in body weight was not significant when NAC was given simultaneously with aflatoxin. These findings suggested a protective effect of NAC on the deleterious effect of AFB_1. Other parameters such as average daily weight gain and feed conversion index (Table 1) were significantly altered in the chicks that received AFB_1. The mycotoxin elicited an increase in the feed conversion index (FCI) as compared to control group, whereas daily weight gain (DWG) decreased, showing the toxic effect of the mycotoxin. These effects were partially reversed by NAC administration, which did not change FCI or DWG as compared to control group.

Plasma and Liver Proteins. Plasma protein concentrations in AFB_1-fed chickens were lower (48.1%; $P < 0.01$) than in the control group (Figure 1). By contrast, birds fed AFB_1 plus NAC exhibited a less severe declilne in protein concentration (48.1% for AFB_1 vs. 26.4% for AFB_1 + NAC), affording a protection of 45.2%. In agreement with the findings for plasma proteins, the hepatic protein content of the group that consumed AFB_1 was also reduced (Table 2, $P < 0.01$) compared to the control group. The birds that received AFB_1 plus NAC exhibited a less marked decrease in hepatic protein concentrations (18.6 vs. 6.2%, respec-

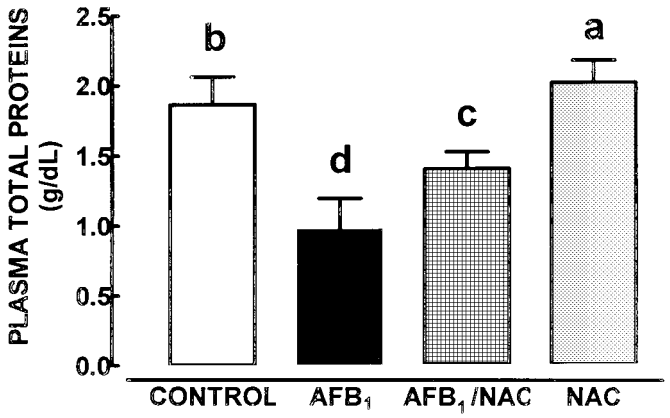

FIGURE 1. Plasma total proteins in aflatoxin B_1 (AFB_1) intoxicated broiler chickens. AFB_1: 3.0 mg/kg of feed in diet, to 21 d of age; N-acetylcysteine (NAC): 800 mg/kg BW daily. a–d, Fisher's protected LSD procedure; $\alpha = 0.05$.

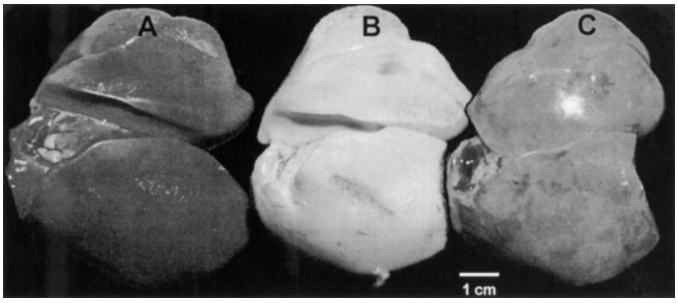

FIGURE 2. Morphologic changes in liver during subacute aflatoxin B_1 (AFB_1) intoxication. Panel A shows a liver with normal appearance from a bird that was fed only N-acetylcysteine (NAC) in the diet, without aflatoxins. Panel B shows the external appearance of a liver with pronounced color loss, obtained from a bird that consumed AFB_1 added to feed (3.0 mg/kg feed, for 21 d). Panel C shows a liver with less severe color loss than in Panel B, obtained from a chicken that consumed feed with AFB_1 (3.0 mg/kg) and 800 mg of NAC per kilogram of BW daily for 21 d.

tively), which was a protective effect of 66.7%. NAC alone did not change hepatic protein content.

GST and GSH. Hepatic GSH was increased by AFB_1 ingestion (40%); however, during simultaneous ingestion of NAC and AFB_1 this parameter increased only 10%, showing that NAC reduced the effects of AFB_1 on these biochemical parameters (Table 2). Increases of hepatic GST activity were detected in both groups: the one that consumed AFB_1 alone and the one that consumed AFB_1 + NAC (Table 2). Administration of NAC alone did not change GSH or GST as compared to the control groups.

ALT and AST. AFB_1 induced a divergent effect on the plasma activity of these enzymes. ALT decreased in the AFB_1-treated birds as compared to the activities in the control group (Table 3). Simultaneous administration of AFB_1 and NAC partially prevented the effect on ALT activity observed in the AFB_1-treated group (18.1 vs. 55.5%, respectively). NAC alone slightly but significantly increased ALT activity. AFB_1 increased the activity of AST by 65.0%. Administration of NAC with AFB_1 did not prevent this change in AST. The ratio of AST:ALT was increased 4.5 times in AFB-treated chickens as compared to the control group, whereas in the AFB_1/NAC group it was increased only 2.9-fold, suggesting a protective effect of NAC against mycotoxin action.

Morphological Findings. Histological studies provided additional evidence of the beneficial effect of NAC on suppressing the toxicity induced by AFB_1 to that provided by the biochemical and growth parameters. Livers from the AFB_1-treated chickens were friable and showed intense loss of color (Figure 2B) in all chickens studied. Absolute and relative weights were not different from those of the control group. In the AFB_1 + NAC-treated chicks, color loss was less severe (Figure 2C) and liver firmness was similar to that of the control group. Absolute and relative liver weights in this group were not different from those of the control group. NAC by itself did not change weight, color, or firmness of the liver (Figure 2A).

Histological sections from the livers of AFB_1-treated chickens showed severe microvesicular steatosis (Figure 3B), proliferation of bile ducts, degeneration of hepatocytes with pseudoacinar rearrangement, and multiple foci of lymphocyte infiltration. Notably, those changes were less severe in the AFB_1 + NAC-treated chickens than in the AFB_1-treated chickens (Figure 3C). Histology of the liver in the NAC-treated birds was not different from that of

TABLE 2. Effects of diets containing N-acetylcysteine (NAC) aflatoxin B_1 (AFB_1), or both, on total proteins, reduced glutathione (GSH), and glutathione S-transferase (GST) in liver tissue[1]

Treatment		Protein		GST		GSH	
AFB_1 (μg/kg of feed)	NAC (mg/kg BW)	(mg/100 mg tissue)	Change from control (%)	(mmol CDNB/min per mg protein)[2]	Change from control (%)	(μmol/g tissue)	Change from control (%)
0.0	0.0	19.4 ± 0.4[b]	100	4.4 ± 0.1[b]	100	3.0 ± 0.2[bc]	100
3.0	0.0	15.8 ± 0.3[c]	81.4	6.5 ± 0.1[a]	148	4.2 ± 0.2[a]	140
3.0	800	18.2 ± 0.4[b]	93.8	6.1 ± 0.2[a]	139	3.3 ± 0.1[b]	110
0.0	800	21.1 ± 0.7[a]	109	4.5 ± 0.2[b]	102	2.9 ± 0.1[c]	96.7
LSD[3]		1.33		0.46		0.43	

[a–c]Means within a column with no common superscript differ significantly.

[1]Each value represents the mean ± SE from duplicates of hepatic samples in 20 broilers per treatment.

[2]CDNB = 1-chloro-2, 4-dinitrobenzene used as indicator in standard curve.

[3]LSD = least significant difference as determined by Fisher's protected LSD procedure.

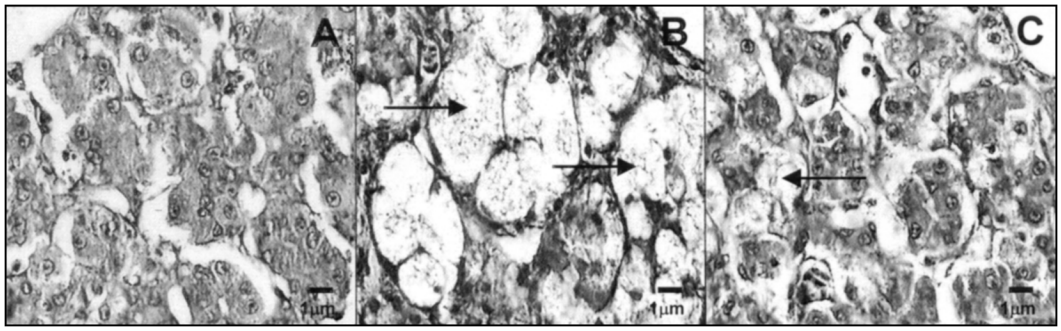

FIGURE 3. Photomicrographs (periodic acid-shift stain) of (A) liver sections from chicks fed control rations, (B) rations containing aflatoxin B_1 (AFB_1), and (C) rations containing AFB_1 and N-acetylcysteine (NAC). Liver sections from chickens fed AFB_1 had severe and widely distributed fatty infiltration with cytoplasmic vacuolation of hepatocytes (arrows). Hepatic appearance was preserved in broilers treated with AFB_1 plus NAC, and liver sections of the AFB_1 and NAC treatment had moderate lesions with scant fatty infiltration. Bars equal 1.0 μm.

control group (Figure 3A). In the kidney, significant changes in cellular architecture could not be identified in control or NAC chicks (Figure 4A). Severe changes were observed in chicks fed aflatoxin. The most consistent lesion was thickening of the glomerular basament membrane (Figure 4B). Glomerular changes similar to those observed in AFB_1 were observed in kidneys of broiler chickens fed AFB_1 plus NAC, although the frequency and severity of damage were lower (Figure 4C).

NAC and Chick Performance

Daily treatment with NAC (400 to 800 mg/kg BW for 7 d) did not alter BW, DWG, or FCI (Table 4). Carcass, muscle, or bone relative weight also was not altered as compared to control birds that did not receive any treatment (data not shown). These results suggested that the use of NAC by itself did not affect significantly the metabolism of broiler chickens that received this compound in their feed.

DISCUSSION

In this study, we show that the administration of N-acetylcysteine significantly prevented negative effects of AFB_1 on BW gain, hepatic glutathione, plasma, and liver proteins and diminished the severity of histological lesions induced by the mycotoxin. To our knowledge, the potentially therapeutic use of NAC represents the first report of this approach in chickens. It is highly relevant in the poultry industry to decrease the large and negative impact that this mycotoxin produces on growth and productive capacity.

NAC has been widely prescribed to humans in several countries, and therefore its safety and pharmacological properties are well established (De Caro et al., 1989). NAC is an excellent source of sulfhydryl groups and is capable of stimulating GSH synthesis, promoting detoxification, and acting directly as a free radical scavenger (De Flora et al., 1985; Lancet, 1991). Administration of NAC has been as a mucolytic drug in a variety of respiratory illnesses; however, it also appears to have beneficial effects in conditions characterized by decreased GSH or oxidative stress, such as human immunodeficiency viral infection, cancer, heart disease, and effects of cigarette smoking. NAC is currently the mainstay of treatment for acetaminophen-induced hepatotoxicity. This compound also appears to have some clinical usefulness in the treatment of acute heavy metal poisoning (Drogue, 1993; Kobrinsky et al., 1996; Kelly, 1998). For these reasons, we believe that NAC is a useful and available drug for controlling aflatoxicosis in broiler chickens.

Our studies agree with those that show that AFB_1 severely affects the performance of chickens (Bailey et al., 1998; Huff et al., 1988). Kubena et al. (1993) reported that AFB_1 (3.5 mg/kg feed) has a negative effect on body weight

TABLE 3. Effects of diets containing N-acetylcysteine (NAC) aflatoxin B_1 (AFB_1), or both, on plasma activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and AST:ALT ratio[1]

Treatment		ALT		AST		
AFB_1 (μg/kg of feed)	NAC (mg/kg BW)	(units/L)	Change from control (%)	(units/L)	Change from control (%)	AST:ALT ratio
0.0	0.0	49.2 ± 1.5[b]	100	150 ± 8.7[b]	100	3.1 ± 0.19[c]
3.0	0.0	21.9 ± 1.0[c]	44.5	297 ± 27.5[a]	165	14.0 ± 1.35[a]
3.0	800	40.3 ± 3.1[b]	81.9	312 ± 14.8[a]	173	9.0 ± 0.01[b]
0.0	800	64.5 ± 5.5[a]	131	262 ± 14.8[a]	146	4.8 ± 0.01[c]
LSD[2]		9.3		49.9		2.5

[a–c]Means within a column with no common superscript differ significantly.

[1]Each value represents the mean ± SE from duplicates of plasma samples in 20 broilers per treatment.

[2]LSD = least significant difference as determined by Fisher's protected LSD procedure.

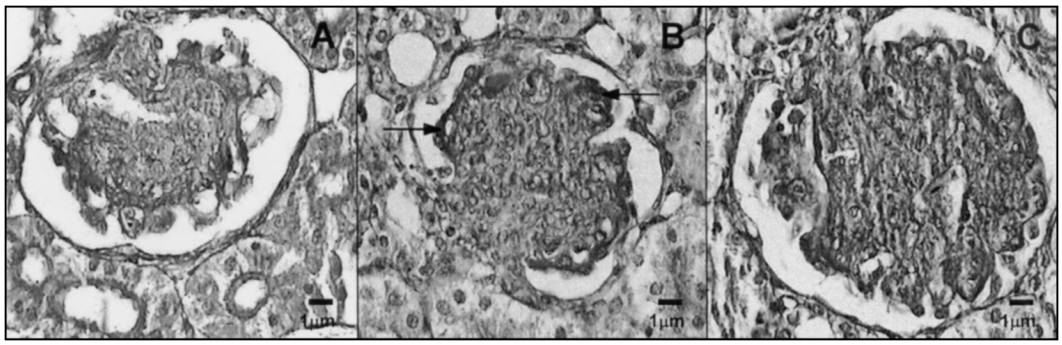

FIGURE 4. Photomicrographs (periodic acid-shift staining) of kidney sections from chicks fed (A) control rations; (B) rations containing aflatoxin B_1 (AFB_1), or (C) rations containing AFB_1 and N-acetylcysteine (NAC). Kidney sections from chickens fed AFB_1 had marked thickening of the glomerular basement membrane (arrows), whereas kidney sections in the two other treatments were normal. Bars equal 1.0 μm.

and feed conversion in broiler chickens. They found that BW decreased by 16%, and feed conversion increased (1.64 up to 1.81) at 21 d of age. In our study, these alterations were reduced by the simultaneous administration of NAC, suggesting a protective effect of this drug on the negative consequences of feeding this aflatoxin.

It must be stressed that there are large differences among species in the response of hepatic GSH to the exposure to xenobiotics. Previous observations have indicated that the GSH cycle in birds might differ substantially from that in mammals (Enkvetchakul and Bottje, 1995; Wang et al., 1998). For example, some studies have shown that, in broiler chickens, increments of hepatic GSH are induced by AFB_1 administration (Beers et al., 1992). Gawai et al. (1992) reported that AFB_1 administration resulted in a significant decrease in liver GSH content in rats, but this treatment augmented GSH in chickens (1.26 to 1.36 μmol/g). In this study, the marked increase of GSH hepatic concentration in response to AFB_1 intake was prevented by the simultaneous NAC intake. Comparable AFB_1-induced increment in hepatic GSH has been shown by Beers et al. (1992). They showed that the increment was attenuated by dietary methionine supplementation at 150% above requirements. In addition, it has been suggested (Reed et al., 1983) that the cystathionine pathway is responsible for methionine interconversion of cysteine into the sulfur pool for GSH synthesis. In broiler chickens, cysteine administration induces elevated GSH levels (Enktvetchakul and Bottje, 1995). Voigt et al. (1980) reported that aflatoxicosis in chickens induces strong reductions in plasma cysteine and cystine concentrations. A diet supplemented with cysteine produces a general increase in GSH, as shown by Enkvetchakul and Bottje (1995). The beneficial effect of NAC might be related to an increment of GSH availability, produced by an enhanced affluence of the forerunner cysteine (De Caro et al., 1989). Moss et al. (1985) suggested that AFB_1 is conjugated with GSH and is eliminated as a conjugate of mercapturic acid. In turn, elimination of the toxin would be favored (Drogue, 1993; Kinscherf et al., 1994; Wispriyono et al., 1998). In addition, the protective effect of NAC might be ascribed to the competitive binding of AFB_1 electrophilic metabolites with cysteine, the intracellular NAC derivative (De Flora et al., 1985).

Previous studies in chickens (Gawai et al., 1992), rats (Carrillo et al., 1990), and rat hepatocytes (Hayes et al., 1986) have shown a modest increase in GST activity mediated by AFB_1 exposure. In agreement with these findings, we found that GST was increased in aflatoxin-treated chickens, and this change was not inhibited by NAC in the AFB_1-NAC group.

Decreases in plasma total protein concentration are frequent findings in chickens with aflatoxicosis (Manning and Wyatt, 1990; Stanley et al., 1993; Kubena et al., 1997; Ledoux et al., 1999). In our study, this alteration was less severe

TABLE 4. Effects of diets containing N-acetylcysteine (NAC) on body weight, daily weight gain, and efficiency of feed utilization[1]

	28-d Body weight		Daily weight gain		Feed conversion index	
NAC treatment (mg/kg BW)[2]	(g)	Change from control (%)	(g)	Change from control (%)	(g)	Change from control (%)
0.0	915 ± 42[a]	100	36.3 ± 6.0[a]	100	2.02 ± 0.06[a]	100
400	901 ± 44[a]	98.5	34.7 ± 6.3[a]	89.0	1.97 ± 0.04[a]	97.5
800	921 ± 44[a]	101	35.9 ± 6.3[a]	95.4	2.01 ± 0.05[a]	99.5
LSD[3]	128		18.3		0.16	

[a]Means within a column with no common superscript differ significantly.

[1]Each value represents the mean ± SE from 10 birds per group.

[2]Daily dose.

[3]LSD = least significant difference as determined by Fisher's protected LSD procedure.

in the chickens that consumed AFB_1 simultaneously with NAC, as compared to those treated with the mycotoxin alone, which might be related to a reduction in liver and kidney damage, as was evident from our histological findings. The histological lesions of AFB_1-treated group are in agreement with previous reports (Mollenhauer et al., 1989; Espada et al. 1992; Ledoux et al., 1999).

Variations in the effect of AFB_1 on transaminase activity might be related to differences in gender and lines of the animals (Adav and Govindwar, 1997). Fernández et al. (1994) reported a decrease of 36% in ALT plasma activity in laying hens treated with AFB_1 (2.5 mg/kg), although they did not find changes in this enzyme in broiler chickens. Stanley et al. (1993) found an ALT decrease of 17 to 42% in AFB_1-intoxicated chickens. We found a decrease in ALT of 55.5%. NAC suppressed this change when administered simultaneously with aflatoxin and increased ALT activity when administered alone, supporting the suggestion that this compound could achieve a protective effect against the toxicity of AFB_1. Manning and Wyatt (1990) reported that AST was elevated in aflatoxin-intoxicated birds compared to controls. We also found an increase induced by the mycotoxin; this increase was not significantly modified by NAC ingestion. The use of the AST:ALT ratio has been used in some studies with human patients having nonalcoholic hepatitis (Sorbi et al., 1999). In our study, the AST:ALT ratio was a useful indicator of amelioration of aflatoxin effects by NAC in broiler chicks.

N-acetylcysteine can be useful alone or in complementary form with other methods to ameliorate the effects of aflatoxicosis in broilers. Previous studies have demonstrated that the use of aluminosilicates can be beneficial in reducing the deleterious effects of aflatoxins in broiler chicks; the protective effect of these adsorbents was ascribed to the sequestration of aflatoxins in the gastrointestinal tract, which reduces their bioavailability (Kubena et al., 1993; Bailey et al., 1988; Ledoux et al., 1999). Whereas NAC appears to be an agent capable of protecting the liver and kidney from damage and an intervention to enhance elimination of aflatoxins by binding this compound, or its intracellular tiol derivatives, to AFB_1 metabolites (De Flora et al., 1985; Lancet, 1991; Kelly, 1998).

We did not attempt to assess the protective effect of NAC on simultaneous exposure to several aflatoxins (as frequently occurs), or with concurrent combinations with others effective control methods, particularly aluminosilicates, because the therapeutic effect of NAC in this study represents the first report of this approach in chickens. Further studies will be required to examine these issues.

In summary, our results suggest that NAC intake decreased the severity of AFB_1 toxic effects. This protective action was evident on body growth, FCI, macro- and microscopic changes in liver and kidney, hepatic and plasma protein concentrations, GSH hepatic concentration, and ALT plasma activity. These data suggest that NAC might be used to prevent the effects of AFB_1 ingestion. NAC did not change the production parameters in control broiler chickens.

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