Aflatoxin B₁ in Affecting Broiler’s Performance, Immunity, and Gastrointestinal Tract: A Review of History and Contemporary Issues

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Abstract: Aflatoxin B₁ is a common contaminant of poultry feeds in tropical and subtropical climates. Research during the last five decades has well established the negative effects of the mycotoxin on health of poultry. However, the last ten years of relevant data have accentuated the potential of low levels of aflatoxin B₁ to deteriorate broiler performance. In this regard, any attempt to establish a dose-effect relationship between aflatoxin B₁ level and broiler performance is also complicated due to differences in types of broilers and length of exposure to the mycotoxin in different studies. Contrary to the prevalent notion regarding literature saturation with respect to aflatoxicosis of chicken, many areas of aflatoxicosis still need to be explored. Literature regarding effects of the mycotoxin on the gastrointestinal tract in this regard is particular scanty and non-conclusive. In addition to these issues, the metabolism of aflatoxin B₁ and recently proposed hypotheses regarding biphasic effects of the mycotoxin in broilers are briefly discussed.

Keywords: aflatoxin; broiler; chicken; hormesis
1. Introduction

Aflatoxins, secondary metabolites of various *Aspergillus* spp., commonly contaminate a wide variety of tropical and subtropical food/feed stuffs. These mycotoxins are known to have strong hepatotoxic and carcinogenic effects and are regulated by feed/food law in at least 100 countries [1]. Chemically, aflatoxins are difuranocoumarin compounds and include B₁, B₂, G₁, G₂, M₁, and M₂ [2] (Figure 1). These mycotoxins contaminate a wide variety of agricultural commodities including oilseed meals, dried fruits, spices, and cereals [3]. Aflatoxins M₁ and M₂ however, mainly occur in milk (AFM₁ in small quantities also reported in eggs) as metabolites of the B₁ and B₂. Among the various types of aflatoxins, aflatoxin B₁ (AFB₁) is most commonly encountered and it is also considered to have higher toxicity than other aflatoxins.

![Figure 1. Structure of aflatoxins.](image_url)

The discovery and isolation of aflatoxins is well known to be a result of investigations on the mysterious Turkey-X disease of 1960 which resulted in loss of several thousand turkey poults in the United Kingdom. The cause of enormous mortality in turkey poults and of similar outbreaks in other farm animals could be linked with the use of moldy Brazilian peanut meal in the diet of affected animals [4]. The suspected toxic factor was found to be extractable by using chloroform [5]. Its
association with *Aspergillus flavus* could then be established in the year 1961 [6]. In 1962, the name “aflatoxin”, using first letter from “Aspergillus” and the first 3 letters from “flavus” was proposed [7]. Aflatoxin was in the same year isolated in crystalline form in the Netherlands [8], and separated into two components, B and G in the United Kingdom [9]. This was followed by a further division of the aflatoxin B into B₁ and B₂ and later their chemical characterization by Asao *et al.* [10]. Details of these landmarks and other studies have been reported in earlier reviews [7,11].

Since the discovery of aflatoxins, their negative effects on animal health have been an active area of research. In this regard, research during the last five decades has well elucidated the negative effects of aflatoxins on animal performance and immunity. To date, various aspects of the aflatoxicosis in farm animals including effects on animal performance and metabolism, metabolism of the toxin, and carryover of toxic residues to animal products have been the subjects of several comprehensive reviews [12]. However, some aspects of aflatoxicosis, particularly effects on gastrointestinal tract (GIT), are not well documented. The present review therefore intends to encompass these areas of aflatoxicosis in broilers. Furthermore, contemporary issues regarding dose-effect relationship between dietary levels of AFB₁ and broiler performance [13], and recently proposed biphasic effects of the toxin on broiler’s weight gain [14] are discussed. The latter hypothesis regarding aflatoxicosis is extended to other variables of broiler health wherever sufficient data are available.

### 2. Metabolism of Aflatoxin B₁

Relative sensitivity of various animal species to AFB₁ has been presented in Table 1. The sensitivity of chicken is comparative to that of rats, and both species appear to be insensitive on a comparative scale. The difference regarding sensitivity of various animal species towards AFB₁ is thought to be linked with differential state of the toxin’s metabolism and the types of metabolites formed [15]. However, many aspects of metabolism of AFB₁ in chickens need to be investigated.

| Species         | LD₅₀ (mg/kg) | Necrosis and Hemorrhage | Fibrosis | Regeneration of Nodules | Bile Duct Proliferation/Hyperplasia | Vacuolation and Fatty Infiltration | Enlarged Hepatic Cells |
|-----------------|-------------|-------------------------|----------|-------------------------|-----------------------------------|-----------------------------------|------------------------|
| Rabbit          | 0.4         | +                       | -        | +                       | +                                 | -                                 | +                      |
| Duckling        | 2.8         | +                       | -        | +                       | +                                 | +                                 | +                      |
| Pig             | 3.9         | +                       | +        | +                       | +                                 | +                                 | +                      |
| Dog             | 6.3         | +                       | +        | +                       | +                                 | +                                 | +                      |
| Guinea pig      | 10.6        | +                       | -        | +                       | +                                 | +                                 | +                      |
| Sheep           | 12.5        |                         |          |                         |                                   |                                    |                        |
| Mouse           | 56.3        | -                       | -        | -                       | -                                 | +                                 | +                      |
| Chicken         | 72.0        | -                       | -        | -                       | +                                 | +                                 | +                      |
| Rat             | 73.3        | +                       | -        | +                       | +                                 | +                                 | +                      |

1 Modified from Patterson [7], with data on chicken from Miazzo *et al.* [16], and Denli *et al.* [17]. LD₅₀ in mg/kg body weight. 2 Data not available, however metabolism of AFB₁ is slower in sheep [15]. Sufficient data indicate reduced weight of liver [18], and hepatic carcinoma [19] in sheep. Abbrev.: + noted effects; - effects not noted; empty cells indicate lack of data.
2.1. Absorption and Excretion

Work done utilizing murine models indicate that absorption of aflatoxins is a very fast process that follows first order kinetics [20,21]. Approximately all of the orally administered AFB1 has been noted to be absorbed in rats [22,23]. Absorption is followed by an extensive transformation into metabolites primarily in liver [24]. However, the elimination of aflatoxins from body is slower as compared to the case of other mycotoxins especially trichothecenes. Wong and Hsieh [25] investigated the excretion of 14C-labelled AFB1 in mice, rats, and monkeys. These authors found the excretion of AFB1 to be high during initial 24 h of the i.v. injection. However, the total recovery of the administered AFB1 was between 72 and 80% during the first 100 h after the i.v. injection. In case of laying hens, 71% of the 14C-labelled AFB1 administered into crop could be recovered within 7 days post-administration [26]. In this study, only 28% of the administered dose of AFB1 could be recovered during first 24 h. On day 1, 4, and 7 of post-administration of 14C-labelled AFB1, the accumulation of radioactivity was estimated by these authors to be 1.3, 1, and 1.1% of the total administered dose. Liver and reproductive organs were found to be the main sites for accumulation of radioactivity. In a contemporary study, Mabee and Chipley [27] investigated the metabolism of AFB1 during continuous exposure. These authors administered 14C-labelled AFB1 to laying hen by using crop intubation tube for 14 consecutive days. At 5 h post-intubation of the last dose, the total radioactivity in hens was approximately equal to the daily dose of the toxin. It was therefore concluded that most of the 14C-labelled AFB1 administered during first 13 days was excreted before administration of the final dose on 14th day—providing a clue that elimination AFB1 is faster during continuous exposure. Wolzak et al. [28] have reported that tissue residues of aflatoxins were highest in kidney, gizzard, and liver (average conc. 3 µg/kg mass) when broilers were exposed for 4 weeks to a mixture of AFB1 and AFB2. After 7 days of removal of the contaminated feed, aflatoxin residues could not be detected in aforementioned tissues. In this regard, a recent study by Hussain et al. also indicates that the elimination of AFB1 in chicken increase during longer exposure to AFB1 [29]. These authors fed broiler chicks on rations containing 0, 1.6, 3.2, and 6.4 µg AFB1/kg for 7, 14, or 28 days age. After 2 to 3 days of exposure, AFB1 could be detected in livers of the birds exposed to 1.6 µg AFB1/kg and higher dietary levels of the toxin. After cessation of toxin feeding, AFB1 residues decreased in livers and muscles of all the birds, with lower levels at 10 days post-cessation in the birds exposed to higher toxin levels. These authors concluded that the residues of AFB1 in tissues increase with increase in dietary concentration of the toxin but decrease with increase in age (or after longer exposure) of broiler chicks. The elimination of AFB1 from tissues was rapid in older birds than in younger birds.

2.2. Metabolism

Besides being the primary organ of AFB1 accumulation and metabolism, liver is also the main site where AFB1 is metabolized and where the metabolites bind with nucleic acids and proteins. Kidneys also take part in detoxification of aflatoxins and are also among the organs where most of the aflatoxin residues are detected [26,30]. The metabolism of AFB1 after absorption has been previously reviewed in detail [7,24,31–35]. In summary, cytochrome P450 enzymes (CYP) (including CYP1A2, CYP3A4 and CYP2A6) in the liver and other tissues convert AFB1 to epoxides (AFB1-8,9-exo-epoxide, and
AFB$_1$-8,9-endo-epoxide), and to AFM$_1$, AFP$_1$, AFQ$_1$, and its reduced form aflatoxicol (Figure 2). Of the epoxides, the AFB$_1$-8,9-exo-epoxide (and not the AFB$_1$-8,9-endo-epoxide) can form covalent bonds with DNA and serum albumin resulting in AFB$_1$-N7-guanine and lysine adducts, respectively. Like AFB$_1$, AFM$_1$ can also be activated to form AFM$_1$-8,9-epoxide that binds to DNA resulting in AFM$_1$-N7-guanine adducts. These guanine and lysine adducts have been noted to appear in urine. The metabolites AFP$_1$, AFQ$_1$, and aflatoxicol are thought to be inactive and are excreted as such in urine, or in the form of glucuronyl conjugates from bile in feces.

In case of chicken exposed to AFB$_1$ contaminated rations, AFB$_1$, AFM$_1$, and aflatoxicol have been detected in liver, kidneys, and thigh muscles [36]. Besides these, AFB$_{2a}$ has also been detected in livers of both broilers and layers on a ration contaminated with a mixture of aflatoxins (AFB$_1$ 80%; AFB$_2$ 2.6%; AFG$_1$ 16.8%; and AFG$_2$ 0.1%) [30]. Recent studies have shown that CYP2A6 and to a lesser extent YP1A1 are responsible for bio-activation of AFB$_1$ into epoxide form in the liver of chicken and quail [37]. More data are however needed to fully understand the differences in metabolism of chicken with species which are comparatively more sensitive to AFB$_1$.

**Figure 2.** Mechanisms of AFB$_1$ toxicity [32]. In the endoplasmic reticulum, AFB$_1$ is converted to hydroxylated metabolites (via monooxygenases) which are then metabolized to glucuronide and sulfate conjugates. An alternate pathway is the oxidation of AFB$_1$ to form AFB$_1$-8,9-epoxide which can further undergo hydrolysis to form AFB$_1$-8,9-dihydrodiol. The epoxide can also be conjugated (to form GSH-conjugate) and thus detoxified by glutathione S-transferases.
3. Effects of Aflatoxin B1 on Performance and Serum Chemistry

Various reports on effects of aflatoxins on bird performance and serum chemistry have been previously reviewed by Patterson [7], Dersjant-Li et al. [13], and Devegowda and Murthy [38]. There is a general agreement that dietary aflatoxins reduce weight gain, feed intake, and increase feed conversion ratio. Information from the aforementioned reviews and some recent studies is summarized in Table 2. These data indicate that AFB1 has the capability to reduce broiler performance and increase the incidence of bruising in carcass when present at levels of more than 0.5 mg/kg diet. Dersjant-Li et al. in this regard concluded in their review that each mg of AFB1/kg diet would decrease the growth performance of broilers by 5% [13]. However, data published during last decade regarding effect of low doses of AFB1 on weight gain is not consistent with this generalization. For instance, Raju and Devegowda [39] noted 21% decrease in final body weight at 35 days age in broilers fed on 0.3 mg AFB1/kg diet. Contrary to this, Tedesco et al. [40] noted only 10% reduction in weight gain of broilers at 28 days of exposure to 0.8 mg AFB1/kg diet. For levels of AFB1 of 1 mg/kg diet, 10% reduction in weight gain was noted by Zhao et al. [41] at 21 days of exposure while 15% reduction at 42 days exposure was noted by Denli et al. [17]. At further higher levels of 3 mg AFB1/kg diet, only 11% reduction in weight gain at 21 days exposure was noted by Valdivia et al. [42]. Similarly, Miazzo et al. [16] found 11% reduction in weight gain when 2.5 mg AFB1/kg diet was fed to broilers from 21 to 42 days of age. From these reports, it is evident that both the level and length of AFB1 exposure affect the amount of reduction in weight gain of broilers. Furthermore, different type of and rations used in different studies make it impractical to generalize the dose-response relationship regarding weight gain.

Table 2. Summary of effects of AFB1 on gross performance variables in chicken.

| AFB1 (mg/kg) | Performance | Bruising | Liver ¹⁴ | Spleen ¹⁴ | Bursa and Thymus ²⁵ | Serum ¹³,⁴ |
|-------------|-------------|----------|----------|-----------|-------------------|-------------|
| ≤0.1        | ~           | ~        | ~        | ~         |                   | ~           |
| 0.5         | ↓           | ↑        | ~        | ~         | ↓                 | ~           |
| 1.0         | ↓           | ↑        | ↑        | ↓         | ↓                 | ↓           |
| 2.5         | ↓           | ↑        | ↑        | ↑         | ↓                 | ↓           |
| ≥5.0        | ↓           | ↑        | ↑        | ↑         | ↓                 | ↓           |

* Bird performance variables include body weight gain, feed consumption. Abbrev.: empty cells indicate lack of effect; ~ indicates inconsistent data; ↑ indicate increase; ↓ indicate decrease; ? indicates lack of data; enzyme activity in terms of lysosomal enzyme activity; TP, total protein; wt., weight. ¹[7]; ²[13]; ³[38]; ⁴[16–17,39–48]; ⁵[49,50].

It is interesting to mention that many authors who reviewed the studies conducted prior to the 1980s considered 1.25 mg AFB1/kg diet as not having any negative effects on broiler performance [7]. Recent literature, as briefly reviewed in the preceding paragraph, on the other hand documents negative effects of lower levels of the toxin on broiler performance. Even the levels of AFB1 as low as 0.02 mg/kg diet have been indicated to decrease weight gain of broilers by 5% (P < 0.05) in a 3 weeks feeding study [48]. One explanation of these differences in earlier and recent reports could be the difference in
the performance of broilers available at the time of study. Modern broiler in this connection is known to gain more weight by utilizing less feed in shorter time [51–53]. As AFB₁ is known as hepatotoxic, it might result in more profound negative effects in birds with more efficient nutrient conversion demanding faster hepatic metabolism. Differences in the susceptibility of broilers and layers in this regard have been already postulated to be due to differences in metabolic rate of these bird types. Yet another possible cause of these differences could be sensitivity of analytical methods available at the time of previous and present studies.

In a recent review, Diaz et al. proposed that the effects of AFB₁ on weight gain in broilers could be of biphasic nature (hormesis), i.e., improvement at low doses while reduction at high doses [14]. In the review of Diaz et al., the maximum improvement in weight gain of broilers was stated to be 3 to 4% during exposure to low levels of AFB₁. In the aforementioned report of Tedesco et al. [40] these authors however noted 13% improvement in weight gain of broilers during 2nd week of exposure to 0.8 mg AFB₁/kg diet. After 2nd week of exposure the weight gain of broilers started to decline under AFB₁ diet with statistically significant effects apparent during 4th week of exposure. It therefore seems that the length of exposure to AFB₁ besides its level could also influence the type of response regarding weight gain. However these improvements in weight gain, though might be of economic importance, were never reported to be of any statistical significance.

Studies conducted during last decade on effects of AFB₁ on serum chemistry are summarized in Table 3. From the presented data, it is apparent that AFB₁ at levels of up to 0.3 mg/kg decreases serum cholesterol levels. As the dietary level of AFB₁ increases to 1 mg/kg, total serum protein and albumin contents are decreased. At further higher levels of 2 mg/kg diet, lower serum glucose, Ca, and inorganic P levels are recorded. Though Raju and Devegowda [39] reported lower total serum protein in broilers exposed to 0.3 mg AFB₁/kg diet, several other authors including Tedesco et al. [40] could not note any effects of higher doses of AFB₁ on this variable. From the presented data, it is also not possible to draw a dose-effect relationship for levels of serum enzymes including alkaline phosphatase, alanine transferase, γ-glutamyl transferase. However, altered concentrations of these enzymes are usually noted at 1 mg AFB₁/kg diet. Besides these effects, AFB₁ is also known to induce glutathione depletion and result in lipid peroxidation [54].

Table 3. Effect of AFB₁ on hematology and serum chemistry, as noted in recent studies.

| AFB₁ Level (ppm) | Bird Type, and Age | n # | Hematology and Serum Chemistry | Year of Study and Reference |
|------------------|-------------------|-----|--------------------------------|-----------------------------|
|                  |                   |     | Effects                        |                             |
| 0, 0.1           | ♀Ross308, 427–457 | 4   | ↓ AP                           | AST, γ-GT, TP, Chl, BUN, creatinine 2010 [55] |
| 0, 0.3           | Broilers, 1–35    | 12  | ↓ TP and Chl at 21 days        | BUN, ALT, γ-GT, AST at 21 days. 2000 [39] |
|                  |                   |     | ↓ TP, Chl, γ-GT, AST at 35 days| BUN, ALT, Hb at 35 days       |
| 0, 0.8           | ♀Broilers, 14–49  | 7   | ↓ ALT                          | TP, albumin, globulin, Glc., AST, γ-GT, Ca, P 2004 [40] |
| 0, 1.0           | ♀Cobb, 1–21       | 4   | ↓ TP, albumin, Chl, Ca         | Uric acid, γ-GT, P 2008 [47] |
| 0, 1.0           | ♀Ross308, 1–42    | 10  | ↑ AP                           | TP, albumin, AST, γ-GT, uric acid, Chl, triglyceride 2009 [17] |
Table 3. Cont.

| AFB<sub>1</sub> Level (ppm) | Bird Type, and Age (days) | n * | Hematology and Serum Chemistry | Year of Study and Reference |
|-----------------------------|---------------------------|-----|---------------------------------|----------------------------|
|                             |                           |     | Effects                         |                            |
| 0, 1.0                      | broilers, 1–21            | 5   | ↓ TP, albumin, globulin         | BUN, Glc., AP, AST, γ-GT, CK, Na, K, Cl, Ca, P | 2010 [41] |
|                             |                           |     |                                 |                            |
| 0, 2                        | broilers, 1–21            | 5   | ↓ TP, albumin, globulin, Glc., Ca, P | BUN, AST, γ-GT, CK, uric acid, Na, K, Cl | 2010 [41] |
|                             |                           |     |                                 |                            |
| 0, 3                        | Hubbard, 1–21             | 20  | ↓ TP, ALT                       | -                           | 2001 [42] |
|                             |                           |     | ↑ AST                           |                            |
| 0, 3.5                      | broilers, 1–21            | 6   | ↓ TP, albumin, Chl, creatinine, Ca, MCV | AP, ALT, P, RBC, MCH, MCHC | 1997 [45] |
|                             |                           |     |                                 |                            |
| 0, 4                        | PetxHubb, 1–21            | 6   | ↓ TP, BUN, Chl, PMCV, hematocrit | -                           | 1997 [43] |
|                             |                           |     | %                               |                            |
|                             | broilers, 1–21            | 5   | ↓ TP, albumin, globulin, Chl, Glc., Ca, P | -                           | 1998 [46] |
|                             |                           |     | ↑ Na, Cl                        |                            |
| 0, 5                        | AAxPet, 1–21              | 6   | ↓ TP, albumin, Chl, uric acid, AP, Ca. | P                           | 1998 [56] |
|                             |                           |     | ↑ CK                            |                            |
|                             | broilers, 1–21            | 6   | ↓ TP, albumin, Chl               | -                           | 1998 [45] |

* Number of replicates. The figure in parenthesis indicates number of animals per replicate. Abbrev.: AA, Arbor Acres; ALT, alanine transferase; AP, alkaline phosphatase; AST, aspartate amino transferase; BUN, blood urea nitrogen; Chl, cholesterol; CK, creatinine kinase; conc., concentration; Glc, glucose; Hb, hemoglobin; Hubbard; Pet, Peterson; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RBC, red blood cell; γ-GT, γ-glutamyl transferase.

4. Effects of Aflatoxin B<sub>1</sub> on Adaptive Immunity

Secondary to the effects on liver, the immunosuppressive nature of AFB<sub>1</sub> is the best documented area of its toxicity. Recent epidemiological data also indicate high correlation between outbreaks of Newcastle disease (ND) and aflatoxin contamination of broiler rations [57,58].

Generally, the immunotoxic dose of AFB<sub>1</sub> is considered as less than the dose required eliciting a reduction in bird performance. Selected studies on the effects of AFB<sub>1</sub> on response from vaccines (humoral immunity), and cell mediated immunity are presented in Table 4. Though several contradictory reports are available, the threshold dose of AFB<sub>1</sub> may be generalized to be 0.4 and 1 mg/kg for the negative effects on cell mediated and humoral immunity, respectively. However, the question regarding susceptibility of modern broiler regarding immunotoxicity remains yet to be answered. Furthermore, there is evidence regarding biphasic nature of the effects of AFB<sub>1</sub> on humoral immunity. In this regard our recent data (Table 5) indicate that humoral immune response from broilers could increase and decrease depending upon the level and length of exposure to the toxin.
Table 4. Effects of AFB<sub>1</sub> on humoral and cell mediated immunity in chicken.

| AFB<sub>1</sub> Level (ppm) | Vaccine | Bird Type, Age (days) | Age | Effects | No Effects | Year of Study and Reference |
|-----------------------------|---------|-----------------------|-----|---------|------------|-----------------------------|
| Humoral immunity:           |         |                       |     |         |            |                             |
| 0.1, 0.2, 0.4, 0.5, 1.0     | ?       | Broiler, 14–49        |     | -       | Titors to ND and fowl cholera | 1985 [59]                   |
| 0.1, 0.2, 0.4, 0.8          | ?       | broiler, 14–49        |     | -       | Titors to ND and fowl cholera | 1985 [60]                   |
| 1 (AF)                     | ?       | Broiler, 7–49         | 14  | ↓ ND titers at 1 and 3 weeks post vaccination | ND titers at 2, 4, and 5 weeks post vaccination | 2003 [61]                   |
| 2.5 (AF)                   | 7 + 21  | Faobro, 1–21          |     | ↓ ND titers at 28 days age | - | 2000 [62] |
| 0, 0.6, 1.2, 2.5           | ?       | Broiler, 1–42         |     | ↓ total complement activity at 2.5 ppm | total complement activity at 0.6 and 1.2 ppm | 1985 [63] |
| 5                          | 1 + 21  | Broiler, 1–35         |     | ↑ secondary antibodies against IBD at 28 and 35 days | - | 1997 [64] |
| ♀ Leghorn, 126–280         | ?       | 2.5                   |     | ↓ antibody titers to ND, IB, and IBD | - | 1998 [65] |
| 2.5                        | 21      | ♀ Leghorn, 1–28       |     | -       | ND, IB titers; at 35 days susceptibility to ND | 1978 [66] |
| 2.5                        | 21      | ♀ Leghorn, 1–49       |     | -       | ND titers; susceptibility to ND at 35 days | 1978 [66] |
| Cell mediated immunity:    |         |                       |     |         |            |                             |
| 0, 0.1, 0.2, 0.4, 0.8      | -       | Broiler, 14–49        |     | ↓ DHST from 0.2 ppm | - | 1985 [60] |
| 0.1, 0.2, 0.4, 0.5, 1.0    | -       | Broiler, 14–49        |     | ↓ DHST at 0.4 ppm AFB<sub>1</sub> + AFB<sub>2</sub> | DHST on AFB<sub>1</sub> alone | 1985 [59] |
| 1                          | -       | Broiler, 7–49         |     | ↓ DHST     | - | 2003 [61] |
| 0.3                        | -       | Leghorn, 1–42         |     | ↓ DHST at 30, 45, and 60 days age | - | 1988 [67] |

Abbrev.: ↓ reduction; ↑ increase; ? not specified; - data not relevant; DHST, delayed hypersensitivity skin test; IB, infectious bronchitis; IBD, infectious bursal disease; ND, Newcastle disease.

The data presented in Table 5 are not the first observations of increase in humoral immune response during initial stages of exposure to low levels of AFB<sub>1</sub>. Similar results, i.e., an initial increase followed by a decrease in humoral immune response, have been documented in at least two previous reports. However, these effects of AFB<sub>1</sub> were not discussed in any of these reports and thus had remained overlooked. For instance, Giambrone et al. [59] who conducted two separate experiments in 1985 on Hubbard broilers, noted a non-significant increasing trend in ND titers with increase in the AFB<sub>1</sub>
content of ration from zero to 0.5 mg/kg in one of these experiments. Also, a higher ($P < 0.05$) response from fowl cholera vaccine was noted in the birds fed 0.5 mg AFB$_1$/kg diet. In the other experiment, higher ($P < 0.05$) ND, and fowl cholera titers were noted in birds fed 0.1 mg, and 0.2 mg AFB$_1$/kg diet, respectively. The increase in titers against ND and fowl cholera in birds fed on AFB$_1$ contaminated ration was not seen in the birds fed on rations containing mixtures of AFB$_1$ and AFB$_2$. In a latter study, these authors reported non-significantly higher titers against ND and fowl cholera in birds fed on 0.1 to 0.8 mg AFB$_1$/kg rations as compared to the birds fed on control ration [60]. The underlying mechanisms for this temporary increase in humoral immune response are not known. As a matter of fact, the exact mechanisms of even immunosuppression during aflatoxicosis are not clearly understood in spite of 50 years of research on the mycotoxin. In this regard, Corrier [68], and Surai and Dvorska [54] have reviewed some aspects of AFB$_1$-induced immunotoxicity. A brief but comprehensive discussion on the subject can also be found in an article by Celik et al. [49]. It is a general observation that size of lymphoid organs is not normal in birds exposed to AFB$_1$ (Table 2). In such animals, lymphoid cell depletion in thymus, spleen, and bursa of Fabricius has been described [61]. Thus one explanation of immunotoxicity of AFB$_1$, as also proposed by Azzam and Gabal [65,69], could be inhibition of antibody production through the toxin’s effects on lymphocytes leading to enhanced turnover of serum antibodies and consequently to decreased antibody half-life.

Table 5. Effects of level and length of AFB$_1$ exposure on ELISA titers against Newcastle disease and serum protein in Ross 308 broilers $^1$.

| Item                  | 2nd Week Exposure | 4th Week Exposure | 5th Week Exposure |
|-----------------------|-------------------|-------------------|-------------------|
| Titers against ND:    |                   |                   |                   |
| 0.07 mg AFB$_1$/kg diet | 33% *             | 407%              | −27%              |
| 0.75 mg AFB$_1$/kg diet | 127% *            | 594%              | −28%              |
| Serum protein:        |                   |                   |                   |
| 0.07 mg AFB$_1$/kg diet | 5%                | −2%               | 2.6%              |
| 0.75 mg AFB$_1$/kg diet | −32% **           | −32% *            | −21% *            |

Significant differences with regards to control with * at $P < 0.05$, ** at $P < 0.01$. Data presented as percentage change over control. $^1$ Experiment conducted in 2010 (author’s unpublished data). Statistical analysis by using ANOVA and LSD ($n = 7$/treatment).

During earlier studies on effects of AFB$_1$, Tung et al. [70] described the toxin-induced increase in lysosomal enzyme activity in liver and skeletal muscles of chicken. These authors postulated that this increase in lysosomal activity, besides other factors, could negatively affect tissue integrity during aflatoxicosis. In this regard, dietary AFB$_1$ has been found by Çelik et al. [49] to result in degeneration of follicle associated epithelium (FAE) in bursa of Fabricius and destruction of thymic cortex in chicken. On the grounds of the report of Tung et al., it was therefore urged that any impaired function of FAE might result in serious deficiencies in both cellular and antibody responsiveness of the chicken immune system [49]. This is because FAE of bursal follicles play a crucial role in antigen presentation to the lymphoid cell population. Besides the effects on lymphocytes, non-specific effects of the toxin on protein synthesis through inhibition of RNA polymerase, lipid peroxidation, and liver injury are also considered to result in reduced immunoglobulin production. The data presented in Table 5 however indicate modulation of serum protein and antibody titers in different directions. This indicates
that AFB$_1$-induced modulation of humoral immunity in broilers may not be a result of the toxin’s non-specific effects on protein metabolism.

5. Effects of Aflatoxin B$_1$ on Gastrointestinal Tract

Gastrointestinal tract is the main site where conversion and absorption of food components takes place. The host-derived physiological processes, the residing microorganisms, and healthy absorptive surfaces are all equally important to ensure normal nutrient supply. Gastrointestinal tract is the first organ coming into contact with mycotoxins of dietary origin and should be expected to be affected by AFB$_1$ with greater potency as compared to other organs. However, this aspect of aflatoxicosis is the often neglected area of mycotoxin research and available literature is non-conclusive.

5.1. Aflatoxin B$_1$ and Gut Morphology

Various studies documenting effects of AFB$_1$ on weight and histological characteristics of different segments of GIT are summarized in Table 6. The weights of proventriculus, gizzard, and pancreas relative to body weight of broilers have not been reported to be affected at levels of AFB$_1$ up to 3.5 mg/kg diet [39,44,48]. However, at a dietary level of 4 mg AFB$_1$/kg or higher, the relative weight of these organs has been noted to decrease by some authors [45,56], while increase by other [46]. However, Edrington et al. could not find any effect of 4 mg AFB$_1$/kg diet on the relative weight of gizzard and pancreas [43].

Literature on the effects of AFB$_1$ on histology of GIT is scanty and not conclusive. In this regard, the density of whole intestine (weight/length) has been reported to decrease after 3 weeks of dietary exposure to AFB$_1$ at levels as low as 0.02 mg/kg [48] and 0.7 mg/kg [71]. As the width of muscularis tends of be relatively constant, the density of intestine could be a good indicator of unit absorptive area. On this variable, the effects of higher AFB$_1$ dosage in broilers are not known. At higher levels of 1 mg AFB$_1$/kg diet, Kumar and Balachandran however noted catarrhal enteritis with lymphocytic or mononuclear cell infiltrations in the intestine of broilers fed on the toxin contaminated ration for 4 weeks [72]. Contrary to these reports, no histopathological changes in duodenum, jejunum, cecum, and ileum could be noted by Ledoux et al. when male broilers were exposed to 4 mg AFB$_1$/kg diet for 3 weeks [46]. Similarly, breaking strength, size, and collagen content of large intestine was not found to be affected in broilers (male Cobb × Cobb; exposure age 1 to 21 days) exposed to 0, 0.6, 1.2, 2.5, 5.0, and 10 mg AFB$_1$/kg diet in the earlier report by Warren and Hamilton [73]. Lipid content of large intestine was decreased only at the highest level of AFB$_1$ (10 mg/kg) in that report.

From the aforementioned studies, it is difficult to draw a dose-effect relationship between AFB$_1$ and histological changes in the GIT. This is because specific sections of GIT, studied variables, and length of exposure were different in the aforementioned studies. Furthermore, the type and specific line of chicken used in various studies may also affect the reaction of intestine towards chronic aflatoxicosis. This hypothesis is supported by the recent observations regarding aflatoxicosis in layers (Hyline W36; exposure age from 140 to 154 days) by Applegate et al. [74]. Contrary to the observations in broilers, these authors noted a linear increase in the crypt depth in distal jejunum with the increasing levels of AFB$_1$ in the diet as 0, 0.6, 1.2, and 2.5 mg/kg, but no effect of the toxin on villus height and number of
goblet cells. However, the duration of exposure to AFB₁ was short as compared with other studies and may not be long enough to provoke morphological changes in jejunum of layers.

### Table 6. Weight and histology of individual segments of gut in chicken during exposure to AFB₁.

| AFB₁ Level (ppm) | Bird Type, Age (days) | n * | Characteristics of Gut | Year of Study and Reference |
|------------------|-----------------------|-----|------------------------|-----------------------------|
| 0.07, 0.7        | ♂ Ross308, 7–29       | (7) | Density of duodenum and jejunum ↓ | 2011 [71] |
|                  |                       |     | Length of duodenum and jejunum ↑ | Weight of proventriculus and gizzard |
| 0.02             | ♂ Hybro, 21–49        | 5   | Density of intestine ↓ | 2010 [48] |
| 0.1              | ♂ Ross308, 427–457    | (3) | -                       | 2010 [57] |
| 0.3              | Broilers, 1–35        | (12)| -                      | 2000 [39] |
| 1                | Broiler, 1–28         | (2) | Necrosis/fibrosis in crop and proventriculus. Catarrhal enteritis in intestine | 2009 [72] |
| 0.6, 1.2, 2.5    | ♂ W36, 140–154       | (8) | linear effect: ↑ crypt length in distal jejunum | 2009 [74] |
|                  | Broilers, 1–21        | (6) | -                      | Number and density of goblet cell in jejunum |
|                  |                       |     | Gizzard weight         | 1997 [44] |
| 4                | PetxHubb, 1–21        | (3) | -                      | 1997 [43] |
| 5                | Broilers, 1–21        | (3) | ↑ Proventriculus and pancreas weight | Microscopic evaluation of pancreas and whole GIT |
|                  |                       |     | -                      | 1998 [46] |
| 5                | AA x Pet, 1–21        | (2) | ↑ Gizzard and pancreas weight | Proventriculus weight |
|                  | Broilers, 1–21        | (2) | ↑ Proventriculus and pancreas weight | 1998 [56] |
| 0, 0.6, 1.2, 2.5, 5, 10 | ♂ CobbxCobb, 1–21 | (10)| -                      | Breaking strength and size of large intestine |
|                  |                       |     | -                      | 1980 [73] |

* Number of replicates. The figure in parenthesis indicates number of animals per replicate.

AA, Arbor Acres; Hubb, Hubbard; Pet, Peterson; W36, Hyline W36.

From the recent studies of Kana et al. [48], Yunus et al. [71], and Kumar and Balachandran [72] in broilers, it appears that the unit absorptive surface of small intestine would deteriorate during a chronic exposure to low levels of AFB₁. However, broilers have been noted to compensate the reduced unit absorptive surface by increasing the length of small intestine in one study [71]. Such a reaction of intestinal tissues to low levels of AFB₁, if also proven in future studies, would certainly add to the present understanding regarding intestinal adaptability to chronic AFB₁ exposure.
5.2. Aflatoxin B₁ and Active Transport of Nutrients

After a thorough search of various databases only two reports could be found in which the issue of intestinal active transport of nutrients during aflatoxicosis was addressed. In this connection Ruff and Wyatt showed that 3 weeks feeding of 1.25 to 5 mg AFB₁/kg diet has no effect on in vitro absorption of glucose and methionine in the intestine of broilers [75]. However, a high dose of 10 mg AFB₁/kg diet, for more than 1 week, increased both the mediated and diffusion components of glucose and methionine absorption. Absorption of glucose and methionine was not affected in broilers exposed to these high amounts of AFB₁ for only one week in the study of Ruff and Wyatt. In the second study which utilized murine in vitro model, acute exposure to AFB₁ (5 µg/mL of buffer) was not found to affect glucose uptake in everted rat jejunum [76].

In some studies, active nutrient uptake was not addressed but movement of ions across intestinal epithelia and activity of ion transporters was studied. These studies may give some insight into the possible mechanisms of effects of AFB₁ on active transport of nutrients including glucose absorption. This is because the active absorption of glucose through sodium glucose co-transporter (SGLT1) is influenced by intracellular levels of Na⁺ and movement of other ions across a cell. In this regard, Chotinski et al. [77] studied the effects of 7 weeks of dietary exposure to 0.25 and 0.6 mg AFB₁/kg diet on activity of Mg²⁺(Na⁺/K⁺)-ATP in small intestinal mucosa of broilers. In this study, 0.6 mg AFB₁/kg diet was found to suppress the activity of Mg²⁺(Na⁺/K⁺)-ATP in small intestinal mucosa. Recently, acute AFB₁ exposure has been reported to evoke acetylcholine-sensitive contractions in the rat ileum [78]. One effect of acetylcholine and other cholinergic secretagogues is to increase basolateral K⁺ efflux and apical Cl⁻ secretion in epithelia [79]. During higher outgo of anions from epithelia, a lower absorption of Na⁺ and consequently lower absorption of glucose is expected. In this regard in vitro AFB₁ has been found to evoke cholinergic secretion of Cl⁻ and negatively affect glucose absorption in broiler’s jejunum [80]. However, these effects of acute exposure could not be established for a chronic exposure of broiler’s to low levels of AFB₁ [71]. It therefore seems that intestinal tissues may adapt to an on-going dietary challenge to low levels of AFB₁ as far as active transport of nutrients is concerned.

5.3. Aflatoxin B₁, and Digestibility and Activity of Digestive Enzymes

Aflatoxin B₁ is widely believed to result in malabsorption syndrome regarding macro nutrients and also in reduced activity of digestive enzymes [38,54]. However, many reports contrary to this notion are available. For instance, Nelson et al. did not find any effect of AFB₁ (natural contamination of corn with A. flavus) on dry matter (DM), and amino acid digestibility, and energy utilization in chicken [81]. Applegate et al. did not find any effect of 0.6, 1.2, and 2.5 mg AFB₁/kg diet on digestibility of DM and nitrogen (N) per hen/day [74]. At 0.6 and 1.2 mg AFB₁/kg diet, the apparent metabolizable energy (AME) was however found to be reduced in their study. Regarding the activity of pancreatic enzymes, Mathur et al. found higher amylase and chymotrypsin activity, while lower lipase activity after exposure of Ross 308 female birds to 0.1 mg AFB₁/kg diet (at 427 to 457 days age) [55]. The activity of trypsin in pancreas was not affected by AFB₁ treatment. These results, except for reduction in lipase activity, are supported by earlier work of Richardson and Hamilton on layers [82]. These authors
reported that 4 mg AFB1/kg diet increases the activity of pancreatic chymotrypsin, amylase, and lipase. Pancreatic trypsin was not affected by AFB1 in their study and the noted changes in the pancreatic secretions were also not reflected in the lipid content of the feces. Contrary to these two reports, Osborne and Hamilton noted lower activity of pancreatic amylase, trypsin, lipase, RNase, and DNase when broilers were exposed to 1.25 and 2.5 mg AFB1/kg diet [83].

Regarding activity of intestinal enzymes, Mathur et al. in their aforementioned report found lower lipase activity in duodenum after exposure of Ross 308 female birds to 0.1 mg AFB1/kg diet [55]. These authors found that AFB1 at the tested low level had no effect on amylase and chymotrypsin activity in duodenum and jejunum, and lipase activity in jejunum. The activity of trypsin in duodenum, and jejunum was also not affected by the AFB1 treatment. Contrary to the case of broilers, Applegate et al. found the intestinal maltase activity to increase quadratically up to the doses of 1.2 mg AFB1 while decrease at 2.5 mg AFB1 with the exposure of layers (from 140 to 154 days age) to 0, 0.6, 1.2, and 2.5 mg AFB1/kg diet [74]. These changes were however not found to affect digestibility and retention of DM and N.

From the presented literature, it is impractical to draw any conclusions regarding effects of a certain level of AFB1 on digestive functionality in broilers. Some studies, in the past decade however indicate that the decreased nutrient utilization observed in those studies might be a factor of the effects of the toxin on systemic metabolism rather than an effect on digestive functionality [84,85]. This notion is also supported by the fact that apparent metabolizable energy of AFB1-contaminated rations was noted to be negatively affected by all the authors who included this variable in their studies and did not find any effect of the toxin on nutrient digestibility. More studies are no doubt needed in this direction.

5.4. Aflatoxin B1 and Intestinal Innate Immunity

The innate immune system of intestine plays a vital role in maintaining the integrity of the intestine and also participates with adaptive immune system in ensuring the subtle equilibrium between immune tolerance and immune response in the GIT [86]. Intestinal intraepithelial cells (IEC) in this regard produce a diversity of antimicrobial peptides and enzymes that protect intestinal mucosa and crypts against microbes. Some of these molecules also function in alarming the adaptive immune system. Contrary to many other mycotoxins, AFB1 has not been considered to date for possible effects on these peptides and enzymes. Furthermore, the barrier function of IEC during aflatoxicosis has not been subjected to extensive research. This passive barrier is formed by the IECs themselves, the tight junctions sealing the intercellular spaces, and the mucus secreted by them. This barrier provides a passive means to prevent most bacteria and antigens entering the body, and at the same time minimizes electrolyte and fluid loss into the intestinal lumen. Transepithelial electrical resistance (TEER) is an important indicator of barrier function of IEC. Limited data related with intestinal health suggests that AFB1 can only moderately affect TEER during acute exposure to the toxin [80]. In the report of Warren and Hamilton, mentioned under Section 5.1, a 3 weeks administration of AFB1 at the levels of 0, 0.6, 1.2, 2.5, 5.0, and 10 mg/kg diet to broiler chicks however did not affect the gross variable of breaking strength of large intestine [73].

In murine models, AFB1 has been found in some studies to result in morphologically damaged intestinal mucosal linings [87]), and in decreased cell proliferation [88]). In this regard, Watzl et al.
found AFB$_1$ to induce genotoxicity (comet assay) in isolated rat jejunal epithelial cells. However, oral exposure of rats to moderate doses of AFB$_1$ (100 μg/kg body weight once a week for 5 consecutive weeks), in the same study, was not found to induce DNA damage in jejunal epithelium [89]. Most recent report in this connection is of Garcia et al., who reported that AFB$_1$ acts in synergy with fumonisins in affecting intestinal barrier function as determined by cellular proliferation, cellular damage, and synthesis of IL-8 in porcine intestinal epithelial cell line [90]. Aflatoxin B$_1$ alone was found in this report to only affect the morphological characteristics of the cells and not other variables. From reports on species other than chicken, moderate and indirect effects (secondary to systemic) of AFB$_1$ on TEER of small intestine may be speculated. The practical significance of any such effect has been a subject of three different studies. In this connection, Rao et al. studied the clinical signs and gross lesions caused by *Eimeria uzura* in Japanese quail during intercurrent dietary aflatoxicosis [91,92]. In these studies, no significant differences in the mucosal morphology of the intestine were evident histologically. However, these authors found that the combination of *E. uzura* infection and aflatoxicosis causes reduced packed cell volume and hemoglobin, weight loss, increased coccidian oocyst production, and higher morbidity (60 vs. 8.3%) and mortality (28.3 vs. 6.6 and 21.6%) as compared to the coccidia or toxin alone. It was concluded that aflatoxicosis may influence the course of coccidial infection due to additive effects. In an earlier study on broiler’s exposure to 2.5 μg AFB$_1$ and/or *Eimeria acervulina*, Ruff et al. also concluded that the birds exposed to combined treatment gain significantly less weight with greater plasma depigmentation (deduced from plasma β-carotene level), but without apparent differences in gross lesions in intestine caused by the coccidian [93].

5.5. *Interaction of Aflatoxin B$_1$ with Gut Microbes*

Since the 1940s various studies have shown antimicrobial potential of several mycotoxins [94–98]. Regarding aflatoxins, the study of Burmeister and Hesseltine in 1966 was probably the first comprehensive study in which several microorganisms (329 spp.) were tested for their sensitivity against AFB$_1$ [99]. Among the strains tested in that investigation, 12 species of genus *Bacillus*, a *Streptomyces* sp. and *Clostridium sporogenes* were inhibited when various levels of AFB$_1$ (15–30 μg/mL) were incorporated into the growth substrate. None of the yeast strains tested in the study was affected by AFB$_1$ even at 40 μg/mL concentration. *Bacillus megaterium* and *B. brevis* were most susceptible to AFB$_1$, and many of the subsequent studies demonstrated the extreme sensitivity of *B. megaterium* to AFB$_1$ (as low as 1 μg AFB$_1$/mL) [100–102]. Contrary to the study of Burmeister and Hesseltine, inhibitory effects of AFB$_1$ on many fungal strains including *A. flavus* itself, *A. awamori*, *Penicillium chrysogenum*, and *P. duclauxii* were reported later [103]. Similarly, 10 ppm AFB$_1$ was found to inhibit the enzyme activity of *Mucor hiemalis* [104]. In other contemporary studies *A. niger*, *A. parasiticus*, *P. expansum*, *Cladosporium herbarum*, *Rhizopus nigricans*, *Thamnidium elegans*, and *Neurospora crassa* were also identified as being sensitive to 50 to 100 μg AFB$_1$/mL [105–108]. A comprehensive review of these studies has been presented earlier by Reiss [109]. In recent studies, AFB$_1$ was found to selectively inhibit *Streptococcus agalactiae*, *S. aureus*, and *Yersinia enterocolitica* [110].

Most of the earlier literature was dedicated to finding suitable bacterial test strains for mycotoxin bioassays. However, the biological methods for detection of aflatoxins were found to be of little use in the surveillance of the toxin [111]. Several *E. coli*, *Salmonella* typhimurium, and *Bacillus* strains on
the other hand have found uses as testers in genotoxic studies [112–116]. Other than the genotoxic effects, the toxic effects of aflatoxin on various microbes have been proposed to be as inhibition of oxygen [117] and inulin uptake [118], generation of oxygen radicals [119] and formaldehyde and its reaction products [120], and damage to cell membrane causing leakage of cell contents [118,121]. An interesting feature of these antimicrobial effects is that during continuous exposure to AFB1, some sensitive bacterial species (B. cereus, Proteus mirabilis) are able to survive the toxic effects to the extent that their growth is enhanced by presence of the mycotoxin [122] indicating ability to metabolize AFB1.

In spite of the indicated antimicrobial potential of AFB1, data regarding effects of the toxin on gut microbial population and fermentation are scanty. Kubena et al. in this regard performed two, 10-days experiments to study cecal VFA’s and broiler chick susceptibility to Salmonella typhimurium colonisation as affected by 2.5 and 7.5 mg aflatoxins/kg diet [123]. In one of these experiments no effects of aflatoxins were found on Salmonella colonization and on cecal VFA production. However, in the second experiment, both dietary levels of aflatoxins resulted in significant increase in total VFA’s at 5 days age. The lower aflatoxin dose (2.5 mg/kg diet) appeared to be more effective as it also resulted in significantly higher total VFA’s at 7 days age.

Related to the issue of effects of AFB1 on gut microbes, interesting data were presented in an earlier study of Larsen et al. [124]. These authors studied the effect of AFB1 on susceptibility of hamsters to orally administered Mycobacterium paratuberculosis. In the negative control group, the bacillus passed the epithelial barrier of the intestine and infection was established in small intestine and mesentric lymph nodes. In the positive control and test groups, aflatoxin-treated hamsters grew slowly and showed signs of AFB1 toxicity. Interestingly, the addition of AFB1 to the rations did not increase the susceptibility of hamsters to M. paratuberculosis, rather it decreased susceptibility to the bacillus. Hamsters not treated with AFB1 and infected with M. paratuberculosis had higher intestinal bacterial counts than did infected hamsters that had been treated with AFB1. These results are substantiated by the study of Abdelhamid et al. who found that effects of AFB1 on rumen fermentation may be like antibiotics: Affecting the harmful flora and encouraging the rumen microflora as noted by slight improvements regarding in vitro rumen fermentation of wheat straw and berseem (Trifolium alexandrinum) hay after dietary AFB1 exposure [125]. In this regard, fermentation patterns of Saccharomyces cerevisiae, and several Lactobacillus spp. have been noted to change under the influence of AFB1 [126,127]. Sutic and Banina in this regard reported that under the influence of AFB1, Lactobacillus casei, L. plantarum, and Streptococcus lactis, well known as not producing gas from glucose and other sugars, became heterofermentative and started producing significant amount of gas [128]. However, these studies do not warrant any positive effects of AFB1 on intestinal microbial population.

6. Conclusions

Recent literature documents the negative effects of those low dietary levels of aflatoxins which were previously thought to have no impact on broiler performance. Furthermore, available data indicate that both the level and the length of exposure influence the response of broilers towards chronic aflatoxin challenge. Therefore any attempt to establish dose-effect relationship between dietary
aflatoxin level and broiler’s performance would be influenced by these factors. Scanty data also indicate that some variables including bird performance and humoral immunity might improve during initial phases of exposure to aflatoxin.

In spite of 50 years of continuous research on aflatoxins, several areas of aflatoxicosis remain yet to be explored. These areas, as discussed in the present attempt, include comparative hepatic metabolism of aflatoxin, and response of gastrointestinal tract to the toxin. Literature available, regarding effects of the toxin on gastrointestinal tract, is particularly non-conclusive. However, there is evidence that gastrointestinal tract may adapt in some ways to a chronic aflatoxin challenge. As gastrointestinal tract is the first organ coming into contact with dietary aflatoxin challenge, its response toward the toxin may yield interesting data regarding tissue adaptability during chronic aflatoxicosis.

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Toxins 2011, 3

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