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INVITED REVIEW

A review from experimental trials on detoxification of aflatoxin in poultry feed

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Özet

Oğuz H. Kanatlı yemlerindeki aflatoksinlerin etkisizleştirilmesine yönelik deneysel çalışmalardan bir derleme. ***Eurasian J Vet Sci, 2011, 27, 1, 1-12***

Bu meta-analitik derlemede kanatlı yemlerindeki aflatoksinlerin etkisizleştirilmesi ile ilgili genel bilgiler verildikten sonra bu amaçla yem katkısı şeklinde kullanılan adsorbanlar ve biyolojik ürünlerle yapılan *in vivo* deneysel çalışmalar özetler halinde sunuldu. Bu amaçla 33 farklı ülkede gerçekleştirilen toplam 135 araştıma tarandı ve sonuçları ana hatlarıyla verildi. Bu derleme ile ilgili konuda yapılan tüm çalışmalar ve sonuçlarının konu ile ilgilenen sektör ve bilim temsilcilerine ülkeler bazında toplu halde sunulması ve onların bütüncül bir değerlendirme yapmalarına imkan sağlanması amaçlandı.

Üreticiler ve araştırıcılar için yeme aflatoksin bağlayıcı olarak katılan yem katkısının etkinliğini belirlemede en iyi yol, aflatoksin ve aflatoksin + koruyucu madde grubunu karşılaştırılarak elde edilen sonuçların performans, biyokimyasal-hematolojik, immunolojik ve makroskobik-histopatolojik verilerin hepsinin "bir bütün olarak" ele alınması ve değerlendirilmesidir. Üretici ve araştırıcılar denemelerin bütününe ulaşıp koruyucu maddenin pratikte kullanılabilirliğini değerlendirmek için makale başlıklarını, ortak yazarları ve/veya makalenin materyal ve metodunu takip edebilirler.

Abstract

Oguz H. A review from experimental trials on detoxification of aflatoxin in poultry feed. ***Eurasian J Vet Sci, 2011, 27, 1, 1-12***

In this meta-analytic review, *in vivo* experimental trials on inactivation of aflatoxins by using adsorbents and biological products as a feed additive in poultry feed were briefly summarized after given the general information about this subject. For this purpose, 135 study performed in 33 different countries were examined and classified according to countries and their results were presented. The aim of this review is to present the results of the experiments to the producers and scientists and to provide a total evaluation possibility to them on the basis of the countries.

The best way for the producers and scientists to assess the performance of preventive efficacy of used feed additive is to evaluate the results "as total" in terms of performance, biochemical-hematological, immunological and gross and histopathologic parameters by comparing the aflatoxin groups with aflatoxin plus feed additive groups. The producers and scientists can reach to the total experiment for assessing preventive efficacy and practical usability of feed additives by following the titles of articles, associate authors and/or materials and methods of articles.

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► Introduction

Aflatoxins (AF) are a major concern in poultry production and public health because of serious economic losses and health problems. AF contamination causes reduced feed quality and reduced animal efficiency either through poor conversion of nutrients or problems such as reproductive abnormalities (Oguz and Kurtoglu 2000, Ortatatli et al 2002). Aflatoxicosis in poultry also causes listlessness, anorexia with lowered growth rate, poor feed utilization, decreased egg production and increased mortality (Miazzo et al 2000). Additionally, anemia (Oguz et al 2000), reduction of immune function (Gabal and Azzam 1998, Oguz et al 2003), hepatotoxicosis, hemorrhage (Ortatatli and Oguz 2001), teratogenesis, carcinogenesis and mutagenesis are associated with aflatoxicosis. The toxicity of AF in poultry has been widely investigated by determining their teratogenic (Sur and Celik 2003), carcinogenic, mutagenic and growth inhibitory (Oguz and Kurtoglu 2000) effects. The biochemical-hematological (Basmacioglu et al 2005), immunological (Qureshi et al 1998), gross and histopathological (Ortatatli and Oguz 2001) toxic effects of AF have also been well described.

Preventing of mould growth and AF contamination in feed and feedstuffs is very important but when contamination cannot be prevented, decontamination of AF is needed before using these materials. Producers, researchers and governments aim to develop effective prevention management and decontamination technologies to minimize the toxic effects of AF.

Practical and cost-effective methods of detoxifying AF-contaminated (AF-CT) feed are in great demand. Besides of the preventive management, approaches have been employed including physical, chemical and biological treatments to detoxify AF in contaminated feeds and feedstuffs. An approach to the problem has been the usege of non-nutritive and inert adsorbents in the diet to bind AF and reduce the absorption of AF from the gastrointestinal tract. Since the early 1990s, experiments with adsorbents such zeolites and aluminosilicates have proven to be successful, but high inclusion rates and possible potential interactions with feed nutrients are causes for concern (Dwyer et al 1997, Phillips 1999, Rosa et al 2001). Also, possible dioxin contamination may be a risk factor for using of natural clays in case of forest and trash fire near the source of them (Abad et al 2002, Feidler 2002, Trckova et al 2004, Arikan et al 2009).

Some studies suggested that the best approach for decontamination would be biological degradation such as yeast and yeast components which could allow removal of AF under mild conditions, without using harmful chemicals or causing appreciable losses in nutritive value and palatability (Bata and Lasztity 1999). Also, a successful detoxication process must be economical, must be capable of eliminating all traces of toxin without leaving harmful residues and must not impair the nutritional quality of the commodity (Bailey et al 1998, Kubena et al 1998, Parlat et al 1999). As a result, researchers have directed efforts towards finding effective means of biological degradation of AF.

Most studies have used greater concentrations of AF than can naturally occur in the field condition. The AF concentrations in these experiments ranged from 2 to 5 ppm (Kubena et al 1990, 1993, 1998, Kiran et al 1998, Ibrahim et al 2000, Oguz et al 2000a, Miazzo et al 2000, Rosa et al 2001) because these high concentrations may help to elicit the toxic effects of AF and also any effects of the feed additive would be easily seen in a shorter experimental period.

The in vivo experimental trials performed by using adsorbents and biological products as a feed additive in poultry are briefly given below. A total of 135 study (made as in vivo and in poultry species only) were examined and listed in 33 different countries according to the first author's country.

► Countries and Studies

• *Argentina*

Miazzo et al (2000) added synthetic zeolite (1%) to AF-CT (2.5 ppm) broiler diet and zeolite significantly diminished the adverse effects of AF on performance and reduced the incidence and/or severity of hepatic histopathology lesions caused by AF.

Miazzo et al (2005) added sodium bentonite (SB; 0.3%) to AF-CT (2.5 ppm) broiler diet and SB provided significant improvements in liver histopatholgy and biochemistry.

Magnoli et al (2008) added natural bentonite (0.3%) to AF-CT (30-135 ppb) broiler diet and bentonite reduced severity of hepatic histopathology changes associated with aflatoxicosis.

• *Belgium*

Schwarzer and Baecke (2009) reviewed inactivators for mycotoxins (based on botanicals, yeast and clay-minerals) on animal performance.

• *Brazil*

Santurio et al (1999) supplemented SB (0.25 and 0.5%) to AF-CT (3 ppm) broiler diet and SB partially neutralized the effects AF on broiler chickens when included at 0.5% in the diet.

Rosa et al (2001) added SB (0.3%) to AF-CT (5 ppm) broiler diet and SB in the diets significantly improved the adverse effects of AF on performance, biochemistry and gross and histopathology of liver.

Santin et al (2003) added *Saccharomyces cerevisiae* (SCE; 0.2%) to the broiler diet and SCE did not improved the suppressive effects of AF on performance and immunity.

Batina et al (2005) added sodic montmorillonite (MNT; 0.25 and 0.5%) to AF-CT (5 ppm) broiler diet and addition of 0.5% level MNT provided partial improvements in biochemical changes associated with AF.

Franciscato et al (2006) added sodic MNT (0.25 and 0.5%) to AF-CT (3 ppm) broiler diet. Addition of 0.5% sodic MNT provided significant improvements in biochemistry.

Santin et al (2006) incorporated yeast cell wall (0.1%) into AF-CT (250 and 500 ppb) broiler diet and yeast cell wall was found to be effective in preventing the detrimental effects of AF on performance.

• *China*

Shi et al (2009) added MNT (0.3%) and MNT nanocomposite (0.3%) to AF-CT (110 ppb) broiler diet and MNT nanocomposite significantly diminished the effects of AF on performance and biochemistry.

Juan-juan et al (2010) incorporated yeast cell extracts, HSCAS and a mixture of yeast product and HSCAS at the levels of 1.5% into AF-CT (100 ppb) broiler diet and HSCAS effectively prevented the toxic effects of AF on performance and biochemistry.

• *Colombia*

Diaz et al (2009) added some feed additives (containing aluminosilicate and phytogenic substances) to AF-CT (250 and 500 ppb) turkey diet and used feed supplements partially diminished the negative effects of AF on performance and immunology by the supplements.

• *Croatia*

Peraica et al (2002) reviewed prevention of mycotoxin production and methods of decontamination including adsorbents, with related 68 references.

• *Cuba*

Rivera and Farias (2005) reviewed clinoptilolite (CLI)-surfactant composites as a drug support and their mechanism, with related 52 references.

• *Czech Republic*

Trckova et al (2004) reviewed kaolin, bentonite and zeolites, their binding properties and their usage as feed supplements for animals, with related 108 references.

• *Denmark*

Shetty and Jespersen (2006) reviewed SCE and lactic acid bacteria for decontamination of mycotoxins. The authors also noted the binding mechanism of the them, with related 84 references.

• *Egypt*

Matari (2001) incorporated SB (0.5 and 1%) into AF-CT broiler diet and SB significantly restored the adverse effects of AF.

Eshak et al (2010) added SCE (0.5, 1, 2, 2.5%) to AF-CT (0.5 ppm) quail diet and addition of SCE to quail diets suppressed the aflatoxicosis in quail tissues leading to improvement of growth performances and enhancement of expression levels of neural and gonadal genes.

• *France*

Guerre (2000) reviewed the physical and chemical methods used for inactivation of mycotoxins. The adsorbents including aluminosilicates were also explained in detail, with the results of related 128 references.

Jouany (2007) reviewed the methods for preventing, decontaminating and minimizing the toxicity of mycotoxins including aluminosilicates and yeast derivates, with related 165 references.

• *Germany*

Danicke (2002) reviewed prevention of control of mycotoxins in the poultry feed the results of the researches in detail, with related 128 references.

• *Hungary*

Bata and Laztity (1999) reviewed physical and chemical methods and biolgical adsorbents recommended for detoxification of mycotoxin-contaminated feed. The present state of research in this field and the perspectives of such procedures were also discussed, with related 42 references.

• *India*

Jindal et al (1994) added activated charcoal (200 ppm) to AF-CT (0.5 ppm) broiler diet and the results showed that activated charcoal provided protection to the broilers against harmful effects of AF on performance and biocemistry.

Raju and Devegowda (2000) incorporated EGM (0.1%) into AF-CT (300 ppb) broiler diet and addition of EGM significantly decreased the detrimental effects of AF on performance parameters, biochemistry and organ morphology.

Girish and Devegowda (2004) added EGM (0.1%) and hydrated sodium calcium aluminosilicate (HSCAS; 1%) to AF-CT (2 ppm) broiler diet and both adsorbents provided significant improvements in performance and relative organ weights associated with aflatoxicosis.

Gowda et al (2008) added turmeric powder (0.5%) and HSCAS (0.5%) to AF-CT (1 ppm) broiler diet and the adsorbents demonstrated protective action in the deleterious effect of AF on performance, biochemistry, antioxidant functions and histopathology.

• *Indonesia*

Sjamsul et al (1990) supplemented activated charcoal (1.5 and 3%) to AF-CT (150 ppb) duck diet and addition of charcoal alleviated the detrimental effects of AF on gross and histopathology of the livers of ducks. 3% activated charcoal was found to be more effective.

• *Iran*

Modirsanei et al (2004) added SCE (0.5%) and natural zeolite (0.75%) to AF-CT (1 ppm) broiler diet and addition of 0.75% zeolite did not reduce any of the adverse effects, whereas, supplemention of SC moderately ameliorated the effects in respect of performance and biochemistry.

Safameher et al (2004) administrated ammonia to AF-CT (1 ppm) broiler diet and they provided significant improvements in performance and hematology by treating ammonia in contaminated feed.

Abousadi et al (2007) incorporated SB (0.5%), SCE (0.2%), HSCAS (0.5%), ammonia (0.5%), formycine (0.1%), and toxiban (0.1%) into AF-CT (125 ppb) broiler diet. Generally addition of the compounds made an improvement against negative effects of AFB1 on performance an biochemistry in broiler chickens. Formycine was recognized to be the best additive in this respect.

Modirsanei et al (2008) added diatomaceous earth (30 ppm) to AF-CT (1 ppm) broiler diet and the added adsorbent provided the negative changes in performance and biochemistry associated with aflatoxicosis.

Safameher (2008) supplemented CLI (2%) to AF-CT broiler diet to ameliorate the toxic effect of AF (0.5 ppm) and CLI provided significant improvements against AF toxicity in performance, biochemistry and liver histopathology.

Ghahri et al (2009) added esterified glucomannan (EGM; 0.1%), SB (0.5%) and humic acid (0.2-1%) to AF-CT broiler diet to ameliorate the toxic effect of AF (254 ppb) against humoral immunity. The addition of EGM, SB and humic acid to the AF-CT diet ameliorated the negative effects of AF on ND antibody titers, but humic acid proved to be more effective in the amelioration of the detrimental effect of AF on humoral immunity against ND.

Kamalzadeh et al (2009) added yeast glucomannan (0.5, 1 and 1.5%) to AF-CT (184 ppb) broiler diet and yeast glucomannan significantly decreased the negative effects of AF on performance. 1% glucomannan was found more effective than other concentrations.

Kermanshahi et al (2009) supplemented SB (0.5 and 1%) to AF-CT (0.5 and 1 ppm) broiler diet and SB significantly improved the effects of AF on performance and biochemistry.

Manafi et al (2009) added high-grade SB (1%) to AF-CT (500 ppb) broiler diet and SB reduced the toxicity of AF on some parameters.

Shabani et al (2010) incorporated nanozeolite (0.25-1%) into AF-CT (500 ppb) broiler diet and nanozeolite significantly restored the toxic effects of AF in performance and biochemistry.

• *Iraq*

Ibrahim et al (2000) added SB (0.2, 0.4 and 0.6%) to AF-CT (2.5 ppm) broiler diet and the addition of SB was significantly effective in ameliorating deleterious effect of AF on humoral immunity. SB also improved the adverse effects of AF on performance and hematology (Ibrahim et al 1998) and carry-over of AF from feed to eggs (Ibrahim and Al-Jubory 2001).

• *Italy*

Rizzi et al (1998) supplemented EGM (0.11%) to the layer diet and EGM provided significant improvements in the detrimental effects of AF.

Galvano et al (2001) reviewed dietary strategies to counteract the toxic effects of mycotoxins and in this review feed additives and binding agents were discussed in detail, with the results of related 113 references.

Rizzi et al (2003) added CLI (2%) to AF-CT (2.5 ppm) layer diet and CLI provided no improvements in egg quality.

Tedesco et al (2005) added silymarin-phospholipid complex (600 mg/kg BW) to AF-CT (800 ppb) broiler diet and they provided significant improvements in performance parameters by adding feed additive.

Zaghini et al (2005) added mannanoligosaccharide (MOS; 0.11%) to AF-CT (2.5 pmm) layer diet and MOS decreased the gastrointestinal absorption of AF and its level in tissues.

• *Korea*

Kim et al (2003) incorporated soybean paste (doenjang; 0.5, 1 and 5%) into AF-CT (500 ppb) layer diet and the addition of 5% soybean paste significantly reduced the effects of AF on performance, biochemistry, gross and histopathology of liver, egg production and accumulation of AF in hens' eggs.

• *Mexico*

Mendez-Albores et al (2007) treated AF-CT (110 ppb) duck feed with citric acid (1N for 15 min, 3 ml/g feed) and citric acid significantly ameliorated negative effects of AF on mutagenity, carcinogenity and toxicity in respect of performance, biochemistry and pathology.

• *Pakistan*

Musaddeq et al (2000) added Myco-Ad, Sorbatox and Mycofix-Plus to AF-CT (8 and 60 ppb) broiler diet and the adsorbents recovered the negative effects of AF on performance of chicks.

Hashmi et al (2006) supplemented yeast sludge (1%; 0.26% mannan oligosaccharide) to AF-CT (100, 200 and 300 ppb) broiler diet and 1% yeast sludge act as toxin binder effectively at 100 and 200 ppb AF, but its efficiency was reduced at 300 ppb AF level. So, it was observed that higher levels of yeast sludge would effectively improve the aflatoxicosis condition.

Pasha et al (2007) added SB (0.5 and 1%), SB+gention violet, SB+acetic acid, Sorbatox and Klinofeed to AF-CT (100 ppb) broiler diet. Addition of indigenous 0.5% SB gave overall better results than the market products and provided significant improvements in performance, organ weight and immunology.

• *Poland*

Kolacz et al (2004) reviewed the use of syntetic aluminosilicates in decontamination of mycotoxins including AF. They also noted the characteristics of aluminosilicate and the decontaminating effect of them, with related 43 references.

• *Saudi Arabia*

Teleb et al (2004) added kaolin and activated charcoal (0.5%) to AF-CT (30 ppb) broiler diet and two adsorbents ameliorated the toxic effects of AF on performance but did not reduce the histopathological changes associated with aflatoxicosis.

• *Serbia*

Zekovic et al (2005) largely reviewed natural and modified glucans and their using in health promotion and diseases including their immunomodulator effects and mycotoxin adsorption ability, with related 245 references.

• *Slovak Republic*

Iveta et al (2000) added CLI and cephalite (0.5%) to AF-treated (0.5 mg/kg BW) broilers and long term per oral administration of two sorbents caused an increase in CD3+ cells in lamina of duodenum. AF did not cause significant changes in the number of CD3+ lymphocytes.

• *South Africa*

Rensburg (2005) incorporated humic acid (0.35%) into AF-CT (1 and 2 ppm) broiler diet and partial improvements have been shown in performance, hematology and biochemistry associated with AF toxicity.

Rensburg et al (2006) also added humic acid (0.35%) and dried brewer yeast (0.35%) to AF-CT (1 and 2 ppm) broiler diet and they provided significant improvements by humic acid in performance, biochemistry and hematology. Humic acid was found to be much more effective than brewer yeast.

• Spain

Marquez and Hernandez (1995) added two Mexican aluminosilicates (Atapulgita and Füller earth) at the levels of 0.5 and 1% to AF-CT (200 ppb) broiler diet and the results showed that both aluminosilicates were as efficient as the commercial material in protecting chicks against the AF toxicity on performance and gross and histopathology.

Ramos et al (1997) reviewed the nonnutritive adsorbent compounds used for prevention of toxic effects of mycotoxins, with related 111 references.

Denli et al (2009) added AflaDetox (1, 2 and 5%) AF-CT (1 ppm) broiler diet and the addition of AflaDetox prevented all of the toxic effects on performance and serum biochemistry and reduced the accumulation of AFB1 residues in the livers.

• *Switzerland*

Huwig et al (2001) reviewed nonnutritive clay-based adsorbents used in the poultry feed and their respective mechanism of adsorption. They also listed the adsorption capacity of used compounds particularly, with related 73 references.

• *Thailand*

Banlunara et al (2005) supplemented EGM (0.05 and 0.1%) to AF-CT (100 ppb) duck diet. The results demonstrated that supplementation EGM is effective in reduction of AFB1-induced hepatic injury in ducklings.

Bintvihok and Kositcharoenkul (2006) added Ca propionate (0.25 and 0.5%) to AF-CT (100 ppb) broiler diet and the results indicated that addition of Ca propionate appears to be effective in reducing toxicity of AF on performance and hepatic enzyme activities in broilers.

Bintvihok (2010) reported that using EGM (0.05% and 0.1%) to AF-CT (60 and 120 ppb) duck diet and EGM provided significant improvements in performance, histopathology and leg deformity caused by AF (Khajarern and Khajarern 1999). The addition of 0.05% EGM also recovered the adverse effects of AF (100 ppb) on serum biochemistry and in ducklings (Bintvihok et al 2002).

• *Turkey*

In Turkey, AF was produced on rice by using *Aspergillus parasiticus* culture in October 1994 by Oguz (1997) with minor modification of Shotwell's method (1966) for using in feeding trials. After production of AF, fermented rice was steamed to kill the fungus, dried and ground to a fine powder. The rice powder was then analyzed for AF content. Then it became useful rice powder which was possible to be incorporated into the basal diet to provide desired amounts of AF levels in feed for experimental feeding trials in animals for any purposes.

Kececi et al (1998) incorporated synthetic zeolite (0.5%) into AF-CT (2.5 ppm) broiler diet and synthetic zeolite provided significant improvements in the adverse effects of AF on performance, hematology and biochemistry.

Oguz and Kurtoglu (2000) added CLI (1.5 and 2.5%) to AF-CT (2.5 ppm) broiler diet and CLI provided significant improvements in performance. Addition of 1.5% CLI also ameliorated the toxic effects of AF (2.5 ppm) on hematology-biochemistry (Oguz et al 2000a) and reduced the number of affected broilers and the severity of gross and histopathological lesions caused by AF (Ortatatli and Oguz 2001).

Oguz et al (2000b) also incorporated CLI (1.5%) into lower levels AF-CT (50 and 100 ppb) broiler diet and CLI significantly recovered the negative effect of AF on performance of broilers. Adding 1.5% CLI also improved the changes in gross and histopathology of target organs (Ortatatli et al 2005) and humoral immunity (Oguz et al 2003) associated with aflatoxicosis.

Parlat et al (2001) added SCE (0.1%) to AF-CT (2 ppm) quail diet and SCE provided significant improvements the effect of AF on performance. SCE (0.2%) was also added to AF-CT (5 ppb) quail diet and the negative changes in the performance, egg production and egg quality were significantly ameliorated by adding of SCE (Acay 2006).

Celik et al (2001) added SCE (0.1%) to AF-CT (100 ppb) quail diet and SCE partially neutralized some toxic effects of AF.

Denli et al (2003) supplemented vitamin A (15.000 IU) to AF-CT (100 ppb) quail diet and vitamin A partially decreased the negative effects of AF on performance, biochemistry and pathology.

Denli et al (2004) added conjugated linoleic acid (CLA; 0.2 and 0.4%) to AF-CT (200 and 300 ppb) broiler diet and CLA provided a partial improvement in performance and biochemistry parameters. CLA also decreased the detrimental effects of AF on liver pathology (Denli et al 2005).

Eraslan et al (2004a) incorporated SB (0.25 and 0.5%) into AF-CT (1 ppm) broiler diet and SB provided a partial improvement in lipid peroxidation in the liver and kidneys of broilers.

Eraslan et al (2004b) also added HSCAS (0.5 and 1%) to AF-CT (2.5 ppm) quail diet and HSCAS provided a moderate amelioration the negative effects of AF on performance and biochemistry.

Oguz and Parlat (2004) added MOS (0.1%) to AF-CT (2 ppm) quail diet and MOS significantly improved the adverse effects of AF on performance of quail.

Yildiz et al (2004) added SCE (0.2%) to AF-CT (2 ppm) quail diet and the addition of SCE significantly recovered the deleterious effects of AF on performance, egg production and egg weight. The addition of 0.2% SCE also provided significant improvements in hatchability and fertility of quails (Yildirim and Parlat 2003).

Basmacioglu et al (2005) supplemented EGM (0.1%) to AF-CT (2 ppm) broiler diet and EGM significantly ameliorated the toxic effects of AF on hematology and biochemistry. Addition of 0.1% EGM also reduced the number of affected broilers and the severity of lesions in the target organs caused by AF (Karaman et al 2005).

Celik et al (2005) added tribasic copper chloride (200 ppm) to AF-CT (1 ppm) broiler diet and tribasic copper chloride significantly improved the effects of AF on performance and biochemistry.

Sehu et al (2005) incorporated Mycotox (0.5%) into AF-CT (2.5 ppm) quail diet and adsorbent was found no effective in the toxic effects of AF.

Denli and Okan (2006) added HSCAS, diatomite and activated charcoal (0.25%) to the AF-CT (40 and 80 ppb) broiler diet. HSCAS was the most effective adsorbents among them to ameliorate the toxic effects of AF in performance and biochemistry.

Essiz et al (2006) supplemented HSCAS (0.5%) and yeast wall (0.5%) and to AF-CT (2.5 ppm) quail diet and they restored plasma malondialdehyde levels altered by AF. The addition of 0.5% HSCAS also moderately decreased the toxic effects of AF (2.5 ppm) in quail in terms of performance, histopathology and immunology parameters (Sehu et al 2007).

Kabak et al (2006) largely reviewed the strategies to prevent contamination of animal feed. They also listed all of the detoxification methods made in vivo and in vitro and used for mycotoxins decontamination by giving the results, with related 276 references.

Cinar et al (2008) added yeast glucomannan (0.075%) to AF-CT (2 ppm) broiler diet and yeast glucomannan was not sufficient to ameliorate the oxidative damage caused by AF in broilers in this level.

Keser and Kutay (2009) reviewed chemical methods including adsorbents and biological methods for preventing of mycotoxins, with 40 related references.

Ozen et al (2009) added melatonin (10 mg/kg/bwt) to AF-CT (150 and 300 ppb) broiler diet and melatonin supplementation greatly reduced the nitrosative tissue degeneration caused by AF.

Demirel et al (2010) reviewed the usage of natural zeolites in animal production including poultry, with 49 related references.

Karaman et al (2010) added lipoic acid (60 mg/kg/bw) to AF-CT (150-300 ppb) broiler diet they and lipoic acid provided moderate improvements in lipid peroxidation and histopathology of target organs.

Matur et al (2010) supplemented SCE extract (0.1%) to AF-CT (100 ppb) hen diet and their results showed that addition of SCE extract reduces the toxic effects of AF on pancreatic lipase and chymotrypsin activity.

• *United States*

In United States, AF was produced on rice by using *Aspergillus flavus* culture in 1966 by Shotwell et al (1966) for using in feeding trials in poultry and other animals practically. This method has become a preferential method in the experiments for investigation of AF toxicity and/or evaluation of preventive efficacy of feed additives against AF so far.

Kubena et al (1990) supplemented HSCAS (0.2%) and activated charcoal (0.5%) to AF-CT (5 and 7.5 ppm) Leghorn chicks' diet and HSCAS significantly diminished the adverse effects of AF on performance, organ weights and biochemistry but these effects were not alleviated by adding activated charcoal.

Araba and Wyatt (1991) added SB, HSCAS and ethacal (0.5 and 1%) to AF-CT (5 ppm) broiler diet. Addition of 0.5% SB and HSCAS significantly reduced the deleterious effects of AF on performance, liver weights and liver lipids.

Kubena et al (1991) added HSCAS (0.5%) to AF-CT (0.5 and 1 ppm) turkey diet and HSCAS neutralized the effects of AF performance, relative organ weights, hematological and biochemical values associated with 0.5 ppm AF.

Huff et al (1992) incorporated HSCAS (0.5%) into AF-CT (3.5 ppm) broiler diet and HSCAS effectively recovered the detrimental effects of AF on serum biochemistry.

Harvey et al (1993) added zeolites (CLI, zeomite and mordenite) (0.5%) to AF-CT (3.5 ppm) broiler diet; zeomite and mordenite decreased the toxicity of AF to growing chicks as indicated by weight gains, liver weight, and serum biochemical values.

Kubena et al (1993) added HSCAS (0.5%) to AF-CT (2.5 and 5 ppm) broiler diet. The addition of 0.5% of the HSCAS compounds significantly recovered the growth inhibitory effects caused by AF. The increases in relative organ weights and the decreases in serum biochemical values caused by AF were significantly alleviated to differing degrees by HSCAS compounds and HSCAS was found to be protective against the effects of AF in young growing broilers.

Scheideler (1993) incorporated Ethacal, Novasil, zeobrite and perlite (1%) into AF-CT (2.5 ppm) broiler diet. Initial three adsorbents provided significant improvements in performance and liver lipid, and partial improvements in mineral status.

Abo-Norag et al (1995) added HSCAS (0.5%) to AF-CT (3.5 ppm) broiler diet and HSCAS effectively restored the negative effects of AF on performance and serum biochemistry.

Edrington et al (1997) supplemented super activated charcoal (0.5%) to AF-CT (4 ppm) broiler diet and active charcoal moderately alleviated the toxic effects of AF on performance, hematology and biochemistry.

Bailey et al (1998) added three different adsorbents (0.5%) to AF-CT (5 ppm) broiler diet the adsorbents showed to offer some protection against AF toxicity in chickens.

Kubena et al (1998) added HSCAS (0.25%) to AF-CT (5 ppm) broiler diet and HSCAS significantly prevented the reduced performance and serum biochemistry observed in chicks fed AF.

Ledoux et al (1999) added HSCAS (Milbond-TX; 1%) to AF-CT (4 ppm) broiler diet and HSCAS completely prevented the improved performance, changes in organ weights, serum chemistry changes, and gross pathology observed in chicks fed AF. HSCAS also effectively reduced the incidence and severity of the hepatic and renal histopathology changes associated with aflatoxicosis.

Phillips (1999) reviewed dietary clay used in the prevention of aflatoxicosis. In this review AF prevention strategies, chemoprevention, HSCAS and possible nutrient interaction with adsorbents were expressed, with related 70 references.

Stanley et al (2003) added SCE (0.05 and 0.1%) to AF-CT (5 ppm) broiler diet and the addition of 0.1% SCE significantly improved the changes in performance, relative organ weights and serum biochemistry associated with aflatoxicosis.

Stanley et al (2004) also added yeast culture residue (2 lb/ton) to AF-CT (3 ppm) breeder hen diet and the inclusion of yeast culture in the AF-treated diet raised the level of hatchability, egg production, and lowered embryonic mortality significantly. Serum globulin and albumin were partially restored with the addition of yeast.

Bailey et al (2006) incorporated MNT clay (0.5%) into AF-CT (4 ppm) broiler they reported that MNT clay in broiler diets provided significant protection on growth performance, serum biochemistry, and the relative organ weight associated with aflatoxicosis.

Fairchild et al (2008) added bentonite based Astra-Ben (1 and 2%) to AF-CT (4 ppm) broiler diet and the adsorbent provided significant improvements in performance and liver lipid content.

Rawal et al (2010) reviewed toxicology, metabolism and prevention of AF and in this review clay-based inorganic adsorbents and their effects were also given, with related 121 references.

Zhao et al (2010) supplemented HSCAS and yeast cell wall component with two doses (0.1 and 0.2%) to AF-CT (1 and 2 ppm) broiler diet and they provided significant improvements by adding of HSCAS and less improvements by yeast cell wall components in performance, biochemistry and histopathology changes associated with aflatoxicosis.

• *Venezuela*

Marin et al (2003) added SCE (0.1%) and selenium (2.5 ppm) to AF-CT (70 ppb) broiler diet and they provided no improvements in biochemistry and hematology by addition of supplements.

Arrieta et al (2006) incorporated SCE (0.1%) and selenium (2 ppm) into AF-CT (70 ppb) broiler diet and no improvements were seen in biochemical parameters. Also no significant changes were seen by adding low levels of AF in parameters.

Gomez et al (2009) supplemented SCE (0.1%) and Se (2 ppm) to AF-CT (70 ppb) broiler diet and the results suggested that the ingestion during 42 days period with 70 ppb AFB1 on diet of broiler could have some effects on production parameters.

• *Vietnam*

Kinh et al (2010) added Mtox (0.25%) to AF-CT (31-44 ppb) broiler diet and Mtox improved significantly the performance (growth rate and feed efficiency) of broiler chicken.

► Conclusion

The evaluation of preventive efficacy of protective agent is possible by determining significant statistical differences between parameters of AF and AF plus additive groups in the target organs and key parameters in favor of AF plus feed additive groups. In my opinion, the best way to assess the performance of feed supplements against AF toxication for producers and scientists is to evaluate the results “as total” in terms of performance, biochemical-hematological, immunological and gross pathologic and histopathological parameters by comparing the AF groups with AF plus feed additive groups.

Sometimes evaluation of the experiments “as total” may be difficult. Because in some cases the editors of the scientific journals may ask to divide the total experiment into different disciplines for publication because of extent of articles and they may be published separately. Similarly the authors from different departments involved in the work also want to divide the total expirement for publication according to the kinds of paramerets obtained. In these cases, the producers and the scientists can reach to the total experiment for assessing preventive efficacy and practical usability of used toxin binders by following the titles of articles and/or associate authors and/or materials and methods of articles.

It is cleary seen that above evaluated studies made with poultry feed have been mainly performed by zeolites and bentonites such as HSCAS, CLI and SB or biological matters such as yeast (SCE) and yeast derivates (EGM). The producers and scientists can examine the results and decide to use the protective agent by taking account the AF doses in feed, levels of protective agent, the experimental period and the species/variety of poultry species. Feed supplements must be inert and non-toxic and have no pharmacological and toxicological effects themselves in the organisms of animals. Possible nutrient interaction and dioxin contamination should also be regarded for using natural clays.

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