Animal Health Research Journal Vol. 6, No. 1, March 2018 pp. 19-38

ISSN: 2356-7767

Biochemical Effects of Saccharomyces Cerevisiae (Biological Product) and Hydrated Sodium Calcium Alimono-Silicate "HSCAS"(Chemical Compound) as Anti-Aflatoxin in Chicken Rations

Al-Afifi, SH.H.H.*; Manal, I. Abd El-Fadeel* and Bayomi, A.S.A.**

* Biochemistry, Feed Deficiency & Toxicology Dept., ** Kafr El-Sheikh Provisional Laboratory, Animal Health Research Institute, Dokki, Giza, Egypt.

Received in 4/3/2018
Accepted in 18/3/2018

Abstract

In the current study, ninety, one day old broiler chicks were randomly assigned into six equal groups; one control and five treatment groups with fifteen chicks per group. A basal diet was given to broiler chicks of the control group (control group). Groups 2&3 were given the basal diet supplemented with hydrated sodium calcium aluminosilicate (HSCAS) at 0.25% level and saccharomyces cerevisiae (SC) at 0.5 % level respectively. Group 4 was given the basal diet contaminated with Aflatoxin at 250 ppb. Groups 5&6 were given the basal diet supplemented with hydrated sodium calcium aluminosilicate (HSCAS) at 0.25% level and saccharomyces cerevisiae (SC) at 0.5 % level respectively and contaminated with Aflatoxin at 250 ppb level. The diets were given for six weeks to all studied groups.

The obtained results revealed that Aflatoxin toxicity at 250 ppb dose increased malondialdehyde (MDA), creatinine , uric acid concentrations, alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ-glutamyle aminotransferase (γGT), Creatine kinase (CK)& Lactate dehydrogenase (LDH) activities and decreased the reduced glutathione (GSH), total protein, albumin, globulin, calcium (Ca), phosphorus(P), magnesium(Mg), concentrations and superoxide dismutase (SOD) activity in broilers serum in comparison with control. As well as, dissemination of aflatoxin residue in broilers meat and liver was observed. Dietary addition of (HSCAS) at 0.25% level and (SC) at 0.5 % level mitigate the above mentioned changes and SC was more efficient than HSCAS.

Key words: *Aflatoxins, Hydrated Sodium Calcium Alumino-Silicate (HSCAS), Saccharomyces Cerevisiae (SC), Antioxidants, Metabolic Enzymes, Ca, P, Mg, Aflatoxin residue and Broilers Chickens.*

Introduction

Aflatoxins (AF) are potent toxic secondary metabolites produced mainly by *Aspergillus flavus, A. parasiticus* and rarely *A. nomius* **(Militа *et al*., 2010).** Aflatoxin contamination of crops is a worldwide food safety concern. More than 25% of the world cereals are contaminated with mycotoxins leading to significant economic losses **(Wu, 2007).** Mycotoxins are mainly produced by the fungal genera of Aspergillus, Fusarium and Penicillum during its growing on corps in the field, at harvest or during storage, as well as, during feed processing **(Devegowda *et al*., 1998).** A continuous increase of AF contamination is possible considering the impacts of global climate change, which favors AF production **(Streit *et al*., 2013).**

Occurrence of mycotoxin contamination in foods is more prevalent in the tropical and subtropical countries resulting in acute and chronic mycotoxicoses in humans and animals. Several approaches are available for mycotoxin decontamination but, most of them are not

widely available due to high cost or practical difficulties involved in detoxification process. Many of the developed countries have regulations for mycotoxins in food grains and its products **(Shetty and Jespersen, 2006)**. However, the risk of mycotoxin exposures continues in the developing countries due to lack of food security, poverty and malnutrition **(Williams *et al*., 2004)**.

Aflatoxin B1 (AFB1) is the most prevalent and toxic metabolite produced by *Aspergillus* fungi, primarily *A. flavus* and *A. parasiticus* **(Bhat *et al*., 2010)** which can contaminate corn, sorghum, oilseeds, and other food materials **(Alberts *et al*., 2006).** Various domestic and experimental species are sensitive to the carcinogenic, mutagenic, hepatotoxic and immunosuppressive effects of AFB1, in which the liver is the main affected organ **(Baptista *et al*., 2004). International Agency for Research on Cancer (2002)** classified AFB1 as belonging to Group 1, carcinogenic to humans. The major source of exposure to AF is via the ingestion of contaminated food **(Leeson *et al*., 1995)**.

Several strategies, including chemical, physical and biological control methods have been investigated to manage aflatoxins in foods **(Diaz *et al*., 2010** and **Reddy *et al*., 2010b**). Among them, biological control appears to be the most promising approach for control of aflatoxins **(Reddy *et al*., 2010a)**.

Enteroadsorption methods use nutritionally inert dietary compounds that prevent toxin absorption by the animal gastrointestinal tract **(Gratz *et al*., 2005).** Although several mineral adsorbents are available, their application is limited due to vitamin and mineral adsorption **(Hussein and Brasel, 2001).** An attractive alternative is the use of microorganisms to control or eliminate aflatoxins in food and feed, thus preserving their quality and safety **(Alberts *et al*., 2009).**

Inorganic materials such as aluminosilicates, bentonites, charcoal and zeolite, or clays in general, were early used for their adsorptive properties and efficient binders to reduce the toxic effect of aflatoxins. However, their efficacy against other mycotoxins such as ochratoxin, fusarium toxins and trichothecenes is very limited or near zero under field condition. Furthermore, it need to be incorporated at high levels and may have side effects on some dietary nutrients, thus reducing the nutritional value of animal diets. **(Galvano *et al*., 2001; Lemke *et al*., 2001; Santin *et al*., 2002 and Watts *et al*., 2003).**

Food and Drug Administration (FDA) stated that 'The use of sodium alumino-silicate and hydrated calcium sodium aluminosilicate as binders for mycotoxins is not considered to be generally recognized as safe' **(FDA, 1999)**. Additionally, clays accumulate in the manure and may have detrimental effects on the soils and pastures after the manure has been spread onto the field **(Mezes *et al*., 2010)**.

Therefore, several studies have been carried out on other types of binders during the last decade. Live yeast *(Saccharomyces cerevisiae; Sc)*, initially used as a performance promoter in the early 1990s, was found to have beneficial effects in broilers exposed to mycotoxins **(Newman, 1994)**.

Yeast is a promising candidate for AF decontamination **(Fruhauf *et al*., 2012).** Yeast contains various immune-stimulatory compounds such as beta glucans and mannan oligosaccharides which serve as an excellent health promoter. Live yeast is widely used as probiotic and toxin binder in animal feed industry **(Sreeparvathy and Anuraj, 2016)**. **Smits *et al*. (1999)** reported that 30 to 40 per cent of the yeast cell wall is composed of mannans and manno-proteins.

Esterified glucomannan (EG); a derivative of the SC yeast cell wall, is able to bind higher levels of several important mycotoxins as compared to the limited narrow range binding abilities of inorganic binders **(Mahesh and Devegowda, 1996 and Volkl and Karlovsky, 1998).** It has a large surface area of 22,000 m^2 per 1 kg and contains a large number of pores of different sizes to trap a wide range of mycotoxins **(Cole, 1999).**

Broilers are the major source of meat supply around the World. This massive growth and

spurt in poultry production has put a great pressure on proper feeding of poultry **(Manafi *et al*., 2014).**

Chickens are still quite susceptible to aflatoxicosis and suffer from reduced vitamin D synthesis in addition to the other effects. This leads to reduced growth, increased bruising and leg weakness with contamination as low as 14 µg/kg **(Devegowda and Murthy, 2005).**

The United States Food and Drug Administration (FDA) set a regulatory level of 20 ppb of aflatoxin in poultry feedstuffs **(Aravind *et al*., 2003).** While regulations are less strict for some ingredients intended for animals (100-300 µg/kg for corn intended for poultry). **(CFIA, 2009).**

The aim of the present work is to evaluate both Saccharomyces Cerevisiae (Biological Product) and HSCAS (Chemical Compound) as anti -mycotoxine feed additives against harmful effects of aflatoxin B_1 in chicken ration on light of their biochemical effects on the liver functio [aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyle aminotransferase (ɤGT) activities, total protein, albumine, globuline concentrations& A/G ratio], kidney function (uric acid and creatinine concentrations), muscles function [Creatine kinase (CK)& Lactate dehydrogenase (LDH) activities], some mineral concentrations [calcium (Ca), phosphorus (P)& magnesium (Mg)] in broilers serum, and determination of aflatoxin B_1 residue in broilers meat and liver.

Materials and Methods

Hydrated sodium calcium aluminosilicate (HSCA):

Hydrated sodium calcium aluminosilicate (HSCAS) was obtained from El Nasr for Pharmaceutical Chemicals Co. (ADWIK), Abu Zaabal, Egypt and thoroughly, mixed with diet mash at concentrations of 0.25 %, according to **Denli and Okan, (2006).**

Saccharomyces cerevisiae(SC):

Instant dry yeast of Saccharomyces cerevisiae (Angel®) was obtained from Angel Yeast (Egypt) Co., Ltd, Medium industries area, Eastern Nile, Beni Suef, Egypt, added to ration mash at concentrations of 0.5 %, according to **Eshak *et al*., (2010)**.

All other chemicals used were of highest analytical grade.

Aflatoxin B_1 (AFB_1) Production and quantification:

Aflatoxin was produced using a reference toxigenic strain of *Aspergillus parasiticus* obtained from mycology department, faculty of Veterinary Medicine, Cairo university as the method of **Shotwell *et al.* (1966)**.The fermented cultures were harvested (6^{th} day), autoclaved, oven dried, pulverized and AF was extracted **(Romer, 1975)** and quantified by the procedures of **Truckess *et al*., (1991) ,** using Vicam™ Fluorometer.

The culture material was mixed with the experimental crushed pellets to contain total AF concentration of 250.0 µg/Kg of ration.

Animals, Diets and Experimental Design

Ninety, one-day-old, Ross, male broiler chicks fed on a starting diet (23% Protein) for 3 weeks, growing diet (21% Protein) for 2 weeks and finishing diet (19% Protein) for 1 week. The ingredients and the nutrient composition of the basal diet were formulated to meet the requirements of the National Research Council **(NRC, 1994)** for broilers. Ration samples were analyzed for the detection of aflatoxins according to the procedures described by **Truckess *et al*., (1991)**.

Broiler chicks were randomly assigned into six groups; one control and five treatment groups with fifteen chicks per group. Basal diet was given to broiler chicks in the **control group**. Groups 2&3 were given the basal diet supplemented with hydrated sodium calcium aluminosilicate (HSCAS) at 0.25% level **(HSCAS group)** and saccharomyces cerevisiae (SC) at 0.5 % level **(SC group)** respectively. Group 4 was given the basal diet contaminated with Aflatoxin (AF) at 250 ppb **(AF group)**. Groups 5&6 were given the basal diet supplemented with hydrated sodium calcium aluminosilicate (HSCAS) at 0.25% level and Saccharomyces

cerevisiae (SC) at 0.5 % level respectively and contaminated with Aflatoxin at 250 ppb level; **(AF+ HSCAS group)** and **(AF+SC group)**. The diets were given for all groups for a period of six weeks.

Along the feeding period of 42 days, diets and water were provided *ad libitum.* Ten broilers were kept in pens (1m x 1.5m) in a ventilated broiler house containing wood shavings as litter material. All groups were kept under observation and were vaccinated against Newcastle disease virus (ND) at 8, 18 and 32 days of age and infectious bursal disease virus (IBD) at 14^{th} and 24^{th} days of age.

Samples:

Blood samples were collected from wing vein, two times at 3 weeks intervals. Serum was then separated by centrifugation of blood at 3000 rpm for 20 minutes and used for biochemical analysis.

At the end of experiment, liver and muscles samples were collected, washed with saline and kept at -20 ^{o}C until used for AF residues analysis.

Biochemical analysis:

Serum was used for determination of malondialdehyde concentration according to **Albro *et al*., (1986),** reduced glutathione content according to **Chanarin, (1989),** superoxide dismutase activity after **Minami and Yoshikawa, (1979).** Serum samples were subjected to determination of aspartate aminotransferase (AST), alanine aminotransferase (ALT) activities according to **Reitman and Frankel, (1957)** and γ-glutamyltransferase(γ GT) activity according to **Persijn,** and **Vander (1976),** Total protein (TP) as described by **Cannon *et al*., (1974)**, albumin assay after **Doumas *et al.,* (1971)**. Serum globulin level and albumin/globulin ratio (A/G ratio) were calculated mathematically according to **Coles (1986)**.

CK and LDH activities in serum were measured kinetically according to the method described by **Rosalki, (1967)** and **Buhl and Jackson, (1978)** respectively.

Serum creatinine concentration was measured by the method adopted by **Bowers and Wong, (1980)**. Determining serum uric acid concentration was assayed by the method of **Fossati *et al*., (1980)**.

Serum calcium and magnesium concentrations were determined according to **Barnett (1965)**. Inorganic phosphorus concentration was determined according to **Daly and Ertingshausen (1972)**.

Aflatoxin residue in broilers meat and liver was extracted and quantified by the procedures of **Truckess *et al*., (1991),** using Vicam™ Fluorometer.

Statistical analysis:

Data obtained were presented as mean ± standard error (SE). Significance of differences was evaluated using analysis of variance (ANOVA) and least significant difference (LSD) at $P \leq 0.05$ by the Statistical Package for the Social Sciences PC statistical program **(SPSS 14, 2006)**.

Results

Antioxidant status parameters.

The recorded results in tables **(1)** revealed a significant and time dependent increase in serum MDA concentration in AF intoxicated group in comparison with control. While, in SC group a significant decrease was observed at the 3^{rd}& 6^{Th} week without time effect. Concerning the effect of HSCAS and SC addition on AF toxicity, broilers of groups 5& 6 showed a significant decrease in serum MDA concentration after 3 weeks to reach the maximal decrease at the 6^{Th} week in SC group which was similar to value of control.

Serum GSH concentration was significantly decreased in all AF intoxicated groups (2, 5& 6) against control in a time dependent manner. The lowest concentration occurred in AF group (group 2) at the 6^{Th} week, followed by AF+ HSCAS group (group 5) then AF+ SC group (group 6). On the other hand in SC group (group 4) a significant elevation was observed at the 6^{Th} week.

Concerning serum superoxide dismutase

(SOD) activity, the data presented in table **(2)** showed a significant reduction in AF and AF+HSCAS groups (groups 2& 5) at the 3rd& 6Th week in comparison with control without time effect. Addition of SC alone increased SOD activity significantly than that of control in SC group at the 6Th week and kept its level as that of control when added with AF toxin in AF+SC group.

Liver enzymes activities and serum proteins concentrations.

As shown in table **(2)** all liver enzymes (ALT, AST & γGT) activities recorded a significant elevation in all AF groups (2, 5& 6) in a time dependent manner in comparison with control values. Dietary supplementation of HSCAS at 0.5% level and SC at 0.5% level alone have no effect on liver enzymes (ALT, AST & γGT).

Regarding serum proteins concentrations, the data presented in table **(3)** exhibited a significant decrease in total protein and albumin concentrations in AF group at the 3rd& 6Th weeks, in AF+HSCAS and AF+SC groups (groups 5& 6) at the 6Th week. Serum globulin concentration recorded a significant decrease in AF group only than control level at the 6Th week. Non-significant changes of A/G ratio in all groups were occurred.

Muscular enzymes activities, and kidney function markers.

From the results recorded in table **(4),** it is an evidenced that serum LDH & CK activities in AF group were significantly increased at the 3rd week and reached the maximum at the 6th week in comparison with control values. However, the activities of both enzymes were still significantly higher than control from the 3rdweek in AF+HSCAS group and at 6Th week in AF+SC groups.

As shown in table **(5)**, compared with control level Creatinine was significantly increased in AF group at the 3rd& 6Th weeks, in AF+HSCAS and AF+SC groups (groups 5& 6) at the 6Th week. Uric acid concentration was significantly elevated in AF, AF+HSCAS and AF+SC groups (groups 2, 5& 6) in comparison with control values at the 3rd& 6Th weeks.

Serum minerals profile.

It is obvious from the data presented in table **(6)** that the serum Ca concentration was significantly reduced in AF and AF+HSCAS groups at the 3rd week and reached the minimum with time, at the 6th week in comparison with control. However, a significant increase was observed in Sc group at 6Th week. Phosphorus (P) and magnesium (Mg) concentrations were significantly reduced in AF, AF+HSCAS, AF+SC groups at the 3rd& 6Th weeks and in HSCAS group at 6Th week. On the other hand, Phosphorus was significantly increased than control in Sc group at 6Th week. Phosphorus reduction was time dependent in AF, HSCAS and AF+HSCAS groups. However, magnesium changes didn't show any time effect.

Aflatoxin(AF) residues in liver and muscles tissues.

As shown in table **(7)**, AF residues were detected in all AF groups (groups 2, 5& 6) in both liver and muscles tissues with higher concentrations in liver than muscles. Addition of HSCAS and SC significantly reduced liver and muscles AF residues in comparison with AF group.

Table (1). Effect of dietary aflatoxin(**AF**), hydrated sodium calcium aluminosilicat (**HSCAS)** and saccharomyces cerevisiae **(SC**) on **MDA**, **GSH** concentrations and **SOD** activity in broiler serum, at 3& 6 weeks (**Ws**). (Mean ± SE).

Parameter / Group	**MDA** (nmol/ml)		**GSH** (µmol/ml)		**SOD** (U/ml)	
	3Ws	**6Ws**	**3Ws**	**6Ws**	**3Ws**	**6Ws**
1- Control	**0.74** ^{a} ±0.051	**0.86** ^{a} ±0.075	**2.22** ^{a} ±0.073	**2.12** ^{a} ±0.066	**9.20** ^{a} ±0.58	**8.80** ^{a} ±0.37
2- AF	**1.80** ^{b} ±0.1	**3.50** ^{b*} ±0.16	**1.38** ^{b} ±0.037	**0.96** ^{b*} ±0.081	**5.22** ^{b} ±0.29	**4.34** ^{b} ±0.32
3-HSCAS %	**0.82** ^{a} ±0.037	**0.90** ^{a} ±0.045	**1.96** ^{a} ±0.081	**1.92** ^{a} ±0.073	**9.80** ^{a} ±0.37	**9.60** ^{a} ±0.40
4- SC %	**0.52** ^{c} ±0.02	**0.58** ^{c} ±0.026	**2.36** ^{a} ±0.075	**2.58** ^{c*} ±0.11	**10.20** ^{a} ±0.37	**11.40** ^{c} ±0.25
5- AF+HSCAS	**1.06** ^{d} ±0.024	**1.02** ^{d} ±0.037	**1.68** ^{c} ±0.12	**1.42** ^{d*} ±0.086	**7.60** ^{c} ±0.68	**7.00** ^{d} ±0.71
6- AF+SC	**0.80** ^{a} ±0.071	**0.98** ^{a} ±0.11	**1.82** ^{c} ±0.058	**1.58** ^{d*} ±0.037	**8.88** ^{a} ±0.23	**8.34** ^{a} ±0.38

In **columns**, presence of different superscript small letters means significant variation between different groups, while presence of asterisk (*) means significant variation between the same groups at different times at (P≤0.05).

Table (2). Effect of dietary aflatoxin(AF), hydrated sodium calcium aluminosilicat (**HSCAS)** and saccharomyces cerevisiae **(SC)** on liver enzymes activities in broiler serum, at 3& 6 weeks (**Ws**). (Mean ± SE).

Parameter / Group	**ALT** (U/L)		**AST** (U/L)		**γGT** (U/L)	
	3 Ws	**6 Ws**	**3 Ws**	**6 Ws**	**3 Ws**	**6 Ws**
1- Control	**14.0** ^{a} ± 0.55	**15.4** ^{a} ± 0.75	**21.60** ^{a} ±0.98	**23.00** ^{a} ±1.48	**13.60** ^{a} ±0.81	**14.80** ^{a} ±0.37
2- AF	**31.0** ^{b} ± 1.05	**47.4** ^{b*} ± 1.43	**58.40** ^{b} ±2.16	**75.40** ^{b*} ±2.92	**20.0** ^{b} ±0.63	**28.20** ^{b*} ±1.11
3-HSCAS %	**15.8** ^{a} ± 0.66	**16.6** ^{a} ± 1.03	**22.80** ^{a} ±1.39	**23.60** ^{a} ±1.53	**13.80** ^{a} ±0.37	**15.00** ^{a} ±0.71
4- SC %	**12.6** ^{a} ± 0.98	**14.2** ^{a} ± 0.66	**19.20** ^{a} ±0.80	**21.20** ^{a} ±0.58	**12.40** ^{a} ±0.60	**13.80** ^{a} ±0.80
5- AF+HSCAS	**17.4** ^{c} ± 0.88	**19.6** ^{c} ± 0.51	**27.20** ^{c} ±0.86	**32.80** ^{c*} ±1.39	**17.60** ^{c} ±0.49	**18.40** ^{c} ±0.40
6- AF+SC	**16.6** ^{c} ± 1.21	**17.4** ^{ac} ± 0.88	**25.20** ^{c} ±1.39	**28.40** ^{d} ±0.75	**16.20** ^{c} ±1.03	**17.00** ^{c} ±0.89

In **columns**, presence of different superscript small letters means significant variation between different groups, while presence of asterisk (*) means significant variation between the same groups at different times at (P≤0.05).

Table (3). Effect of dietary aflatoxin(AF), hydrated sodium calcium aluminosilicat **(HSCAS)** and saccharomyces cerevisiae **(SC)** on serum proteins concentrations in broiler, at 3& 6 weeks (**Ws**). (Mean ± SE).

Parameter / Group	**TP** (g/dl)		**Alb.** (g/dl)		**Glob.** (g/dl)		**A/G**	
	3 Ws	**6 Ws**	**3 Ws**	**6 Ws**	**3 Ws**	**6 Ws**	**3 Ws**	**6 Ws**
1- Control	**7.60** ^{a} ±0.08	**7.68** ^{a} ±0.1	**3.92** ^{a} ±0.04	**3.80** ^{a} ±0.05	**3.68** ^{a} ±0.06	**3.68** ^{a} ±0.27	**1.065** ^{a} ±0.01	**1.033** ^{a} ±0.09
2- AF	**6.10** ^{b} ±0.07	**5.26** ^{b*} ±0.05	**2.94** ^{b} ±0.13	**2.34** ^{b*} ±0.12	**3.16** ^{a} ±0.07	**2.92** ^{b} ±0.08	**0.93** ^{a} ±0.07	**0.80** ^{a} ±0.06
3-HSCAS %	**7.52** ^{a} ±0.1	**7.46** ^{a} ±0.22	**3.82** ^{a} ±0.07	**3.64** ^{ad} ±0.15	**3.70** ^{a} ±0.15	**3.82** ^{a} ±0.21	**1.032** ^{a} ±0.05	**0.95** ^{a} ±0.04
4- SC %	**7.68** ^{a} ±0.07	**7.72** ^{a} ±0.10	**3.96** ^{a} ±0.05	**3.98** ^{ac} ±0.04	**3.72** ^{a} ±0.06	**3.74** ^{a} ±0.11	**1.065** ^{a} ±0.02	**1.064** ^{a} ±0.04
5- AF+HSCAS	**7.40** ^{a} ±0.13	**7.36** ^{c} ±0.06	**3.76** ^{a} ±0.07	**3.54** ^{d*} ±0.07	**3.64** ^{a} ±0.09	**3.82** ^{a} ±0.12	**1.033** ^{a} ±0.02	**0.93** ^{a} ±0.05
6- AF+SC	**7.42** ^{a} ±0.09	**7.38** ^{c} ±0.20	**3.80** ^{a} ±0.10	**3.66** ^{ad} ±0.10	**3.62** ^{a} ±0.17	**3.72** ^{a} ±0.24	**1.05** ^{a} ±0.07	**0.98** ^{a} ±0.08

In **columns**, presence of different superscript small letters means significant variation between different groups, while presence of asterisk (*) means significant variation between the same groups at different times at (P≤0.05).

Table (4). Effect of dietary aflatoxin(AF), hydrated sodium calcium aluminosilicat **(HSCAS)** and saccharomyces cerevisiae **(SC)** on muscles enzymes activities in broiler serum, at 3& 6 weeks (**Ws**). (Mean ± SE).

Parameter / Group	**LDH** (U/L)		**CK** (U/L)	
	3 Ws	**6 Ws**	**3 Ws**	**6 Ws**
1- Control	**142.0** ± 6.3 ^{a}	**145.0** ± 4.5 ^{a}	**33.8** ± 1.85 ^{a}	**34.0** ± 1.87 ^{a}
2- AF	**187.0** ± 2.0 ^{b}	**237.0** ± 5.6 ^{b*}	**63.0** ± 2.15 ^{b}	**91.5** ± 3.92 ^{b*}
3-HSCAS %	**141.0** ± 11.0 ^{a}	**144.0** ± 7.3 ^{a}	**31.0** ± 1.87 ^{ac}	**35.0** ± 2.24 ^{a}
4- SC %	**131.0** ± 2.9 ^{a}	**142.0** ± 3.7 ^{a}	**28.0** ± 1.22 ^{ac}	**31.0** ± 1.18 ^{a}
5- AF+HSCAS	**161.0** ± 8.4 ^{c}	**175.0** ± 7.1 ^{c*}	**42.0** ± 2.55 ^{d}	**45.0** ±3.54 ^{c}
6- AF+SC	**157.0** ± 7.1 ^{ac}	**167.0** ± 6.3 ^{c}	**39.0** ± 1.87 ^{ad}	**42.0** ± 2.55 ^{c}

In **columns**, presence of different superscript small letters means significant variation between different groups, while presence of asterisk (*) means significant variation between the same groups at different times at (P≤0.05).

Table (5) Effect of dietary aflatoxin(AF), hydrated sodium calcium aluminosilicat (**HSCAS)** and saccharomyces cerevisiae **(SC)** on kidney function markers in broiler serum, at 3& 6 weeks (**Ws**). (Mean ± SE).

Parameter / Group	**Creatinine** (mg/dl)		**Uric acid** (mg/dl)	
	3 Ws	**6 Ws**	**3 Ws**	**6 Ws**
1- Control	**0.90** ± 0.05[a]	**0.86** ± 0.05[a]	**4.18** ± 0.21 [a]	**4.5** ± 0.14 [a]
2- AF	**1.50** ± 0.07 [b]	**1.72** ± 0.06 [b*]	**7.34** ± 0.46 [b]	**8.36** ± 0.17 [b*]
3-HSCAS %	**0.82** ± 0.04 [a]	**0.84** ± 0.05 [a]	**3.90** ± 0.19 [a]	**4.16** ± 0.21 [a]
4- SC %	**0.78** ± 0.04 [a]	**0.82** ± 0.04 [a]	**4.10** ± 0.19 [a]	**4.40** ± 0.19 [a]
5- AF+HSCAS	**1.06** ± 0.05 [ac]	**1.22** ± 0.04 [c*]	**5.82** ± 0.14 [d]	**6.62** ± 0.25 [d*]
6- AF+SC	**1.02** ± 0.06 [ac]	**1.08** ± 0.04 [c]	**5.34** ± 0.21 [d]	**5.50** ± 0.14 [e]

In **columns**, presence of different superscript small letters means significant variation between different groups, while presence of asterisk (*) means significant variation between the same groups at different times at (P≤0.05).

Table (6). Effect of dietary aflatoxin(AF), hydrated sodium calcium aluminosilicat (**HSCAS)** and saccharomyces cerevisiae **(SC)** on **Ca**, **P** and **Mg** concentrations in broiler serum, at 3& 6 weeks (**Ws**). (Mean ± SE).

Parameter / Group	**Ca** (mg/dl)		**P** (mg/dl)		**Mg** (mg/dl)	
	3 Ws	**6 Ws**	**3 Ws**	**6 Ws**	**3 Ws**	**6 Ws**
1- Control	**12.02** [a] ± 0.21	**11.54** [a*] ± 0.33	**7.96** [a] ± 0.13	**8.04** [a] ±0.26	**3.38** [a] ± 0.12	**3.40**[a] ± 0.13
2- AF	**8.68** [b] ± 0.25	**8.18** [b*] ± 0.32	**6.40** [b] ± 0.20	**5.28** [b*] ±0.20	**2.24** [b] ± 0.06	**1.70**[b] ± 0.11
3-HSCAS %	**11.80** [a] ± 0.30	**11.40** [a] ± 0.32	**7.92**[a] ± 0.14	**7.04** [cd*] ±0.17	**3.28** [a] ± 0.18	**3.18** [a] ± 0.12
4- SC %	**12.18** [a] ± 0.09	**12.50** [c] ± 0.35	**8.24**[a] ± 0.21	**8.56** [f] ±0.20	**3.58** [a] ± 0.18	**3.68**[a] ± 0.15
5- AF+HSCAS	**11.24** [c] ± 0.38	**10.54** [d*] ± 0.34	**7.16**[c] ± 0.36	**6.56** [cd*] ±0.22	**2.96**[b] ± 0.68	**2.86**[c] ± 0.04
6- AF+SC	**11.96** [a] ± 0.21	**11.28** [a*] ± 0.13	**7.32**[c] ± 0.31	**7.48**[cde] ±0.14	**3.16**[b] ± 0.16	**2.96**[c] ± 0.11

In **columns**, presence of different superscript small letters means significant variation between different groups, while presence of asterisk (*) means significant variation between the same groups at different times at (P≤0.05).

Table (7). Effect of dietary, hydrated sodium calcium aluminosilicat (**HSCAS)** and saccharomyces cerevisiae **(SC)** on aflatoxin (AF) residues in liver and muscles tissues (ppb), at 6 weeks. (Mean ± SE).

Group / Tissue	Control	AF	HSCAS	SC	AF+ HSCAS	AF+ SC
Liver	**ND**	**3.4** [a] ±0.24	**ND**	**ND**	**1.0** [b] ±0.0	**0.4** [c] ±0.24
muscles	**ND**	**2.6** [a] ±0.51	**ND**	**ND**	**0.6** [b] ±0.24	**0.2** [b] ±0.2

In **rows**, presence of different superscript small letters means significant variation at (P≤0.05).

Discussion

Feeds contaminated with AFB1 can result in aflatoxicosis in poultry **(Rustemeyer *et al*., (2010)**. Feeding grains contaminated with AFB1, either natural or purified, can reduce growth performance and immunity, alter intestinal morphology and blood biochemistry parameters, and damage liver and kidney tissues in broilers **(Magnoli *et al*., (2011)**. Additionally, residues of aflatoxins and their metabolites may be present in meat and other products of animals fed rations contaminated with aflatoxins and potentially result in health problems in human **(Zhang *et al*., 2017)**

Nutrition, environment and toxins are involved in the occurrence of oxidative damage **(Shehata and Yosef, 2010).**

The significant increase in lipid peroxidation product; malondialdehyde (MDA) in serum of AF groups as shown in table **(1)** are corresponding with that reported by **Liu *et al*., (2016)** where, the contents of H_2O_2, and MDA in the liver and spleen of broilers fed low level of AFB1 (22.5–25.0 µg/kg) were significantly increased. Also, **Özen *et al*. (2009)** stated that AFB1 increased the (MDA) level, and induced vacuolar degeneration, necrosis, and bile duct hyperplasia in chicken liver.

Zhang *et al*., (2017) found that feeding diets contaminated with 22.44±2.46 µg/kg aflatoxins resulted in an increase in the MDA level in both serum and the liver of ducks. Additionally, **Essiz *et al*., (2006)** found that administration of aflatoxin at a dose of 2.5 ppm for a period of 21 days caused lipid peroxidation in quails.

Many mycotoxins alter the oxidative status of cells by adduction or creation of reactive oxidative species. Aflatoxin B1 is metabolized by P450; CYP3A4 to create AFB1-8, 9- reactive epoxide **(Resanovia *et al*., 2009).** It had been reported that the toxic effect elicited by AFB1 could be closely related to the generation of reactive oxygen species (ROS) mainly, superoxide (O2-), hydrogen peroxide (H_2O_2), and hydroxyl ($OH^{\cdot}$) **(Mehrzad *et al*., 2011)**. Elevated reactive oxygen species (ROS) levels damage cellular macromolecules like proteins, lipids, and DNA and induce a state of redox imbalance and oxidative stress (**Circu** and **Aw, 2010**). Lipid peroxidation could be cause direct or indirect by free radicals production of mycotoxins (**Walters, 2012)**.

Groups that were administered aflatoxin and HACAS or SC in their diet revealed that MDA levels of these groups were close to that of the control group. Such results are consistent with the findings of **Armando *et al*. (2011)** and **Bovo *et al*.., (2015).** They observed that in vitro using of intact and viable cells of *S. cerevisiae*, removed AFB1 (500 ng/mL) by 45.5 to 65.5% depending on the yeast strain. Also, **Diaz *et al*. (2002)** found that esterified glucomannan addition of 1% removed AFB1 by 96.6%. However, **Devegowda and Raju, (2002)** reported that using of esterified glucomannan, at a rate of 0.1% removed AFB1 (300 ppb) by 80.7% and 82.5%, at pH 3.0 and 6.0,

respectively. The main mechanism of this decrease is related directly to the binding of aflatoxin to these adsorbent substances in the digestive tract; and therefore, a decrease in the level of aflatoxin that passes into the systemic blood circulation.

The proposed mechanism of AF chemisorptions by HSCAS is the formation of a complex by the β-carbonyl system of the AF with uncoordinated edge site of aluminum ions in HSCAS **(Phillips *et al*., 1995).** AFB1 may react at surfaces and within the inter layers of HSCAS particles **(Phillips *et al*., 2008).** The protective effects of HSCAS also appear to involve sequestration of AFs so that they become not available for gastrointestinal tract absorption by chicks. The mechanism is probably either chemisorptions (i.e. strong bond formation) between HSCAS and AFs **(Phillips *et al*., 1988)** or an interaction between HSCAS and other food practices **(Kubena *et al*.,1990),** thereby reducing the bioavailabilty of AF **(Davidson *et al*., 1987).**

Yeast cell walls, in particular from *Saccharomyces cerevisiae*, have highly branched glucomannan polymers which contain a mix of positive and negative charges that may adsorb mycotoxins electrostatically **Walters, (2012)**.

Jouany *et al*. (2005) explained that the *S. cerevisiae* cell wall is composed mainly of polysaccharides (80-90%) and their mechanical strength is due to an inner layer formed by chains of β-D-glucans. These β-D-glucans are composed of a complex network of β-(1, 3)-D-glucans with high degree of polymerization, branched with β-(1,6)- D-glucans with a low degree of polymerization. β-D-glucans are the cell wall components responsible for complexation with the toxin, and the reticular organization of β-D-glucans and their distribution among β-(1, 3) and β-(1, 6)-D-glucans plays an important role in this efficacy. In addition, weak hydrogen bonds and vander Waals bonds are involved in the complex chemical formation between mycotoxins and β-D-glucans, leading to a chemical interaction of "adsorption" rather than "contact".

Regarding AFB1, the toxin is bound to the glucans due to the interaction between the aromatic ring and the lactone and ketone groups of the polar form of AFB1, as well as by chemical bonds with glucose units of the single helix β-D-glucans **(Jouany *et al*., 2005).** Also, **Joannis-Cassan *et al*., (2011); Pizzolitto *et al*., (2012)** and **Corassin *et al*. (2013)** reported the adsorption of AFB1 by *S. cerevisiae* yeast and yeast based products.

In the present study the mean values of broilers serum glutathione (GSH) concentrations, and superoxide dismutase (SOD) activity showed a significant reduction in all AF groups compared with control **(table 1)**.

Such results are consistent with the findings of **Zhang *et al*., (2017).** They reported a decreased activity of superoxide dismutase (SOD) in ducks fed diets contaminated with 22.44±2.46 µg/kg aflatoxins. The total antioxidant capacity, CAT enzyme activity and GSH level in the liver and spleen of broilers fed a diet contaminated with low level of AFB1 (22.5–25.0µg/kg) were significantly lower than control group **(Liu *et al*., (2016)**. Many studies observed that dietary AFB1 could decrease the activities of antioxidant enzymes and levels of non-enzymatic antioxidants in broilers **(Yang *et al*., 2012; Zuo *et al*., 2013**; **Li *et al*., 2014a&b;** and **Fan *et al*., 2015)**.

In the present study, dietary inclusion of HSCAS and SC ameliorated the decreased activity of SOD enzyme and GSH level induced by AF to be close to their control levels (**table 1**). These findings suggest that HSCAS and SC may have protective effects on the damages caused by AFB1.

The mechanism for the protective effects of *S. cerevisiae* cells against AF toxicity seems to involve the sequestration of the toxin in the gastrointestinal tract and chemisorption to yeast cells with subsequent elimination of the toxin (**Bovo *et al*., 2015)**.

Chen *et al*., (2014) found that Dietary HSCAS supplementation at 0.5% improved liver SOD activity for birds fed 1 mg of AFB1/kg for 21 days.

As shown in tables **2, 3, 4, 5& 6** dietary supplementation of HSCAS at 0.5% level and SC at 0.5% level alone have no effect on liver enzymes (ALT, AST& γGT), serum total protein, albumin, globulin, muscular enzymes (LDH&CK), kidney function markers (creatinine & uric acid concentration) and the measured minerals (Ca, P, & Mg) except Ca concentration which was significantly higher than control in SC supplemented group at 6th week only. However, phosphorus concentration was significantly decreased in HSCAS group at 6th week.

In AF group serum total protein, albumin, globulin, Ca, P and Mg concentrations were significantly decreased with time than control levels. Along with, liver and muscular enzymes activities (ALT, AST, γGT, LDH& CK), kidney function markers (creatinine & uric acid concentration) were significantly elevated with time than control **(tables 2, 3, 4, 5& 6)**. The aflatoxin effects on biochemical alterations in this study are in consistent with **Chen *et al*., (2014); Bovo *et al*., (2015); Chen *et al*., (2016); Liu *et al*., (2016); and Gómez-Espinosa *et al*., (2017).**

AFB1 is known to be the target of liver primarily by inducing oxidative damage and thus impairing its functions **(Rawal *et al*., 2010; Marin *et al*., 2013** and **Chen *et al*., 2014a**). AFB1 is extremely toxic to birds being rapidly absorbed from gastrointestinal tract. Once absorbed, AFB1 is immediately bound to albumin and in a lesser extent to other proteins, and then transported to the tissues, especially the liver **(Santurio, 2000)**. After being deposited in the liver, AFB1 is biotransformed by the hepatic microsomal cytochrome P450 (CYP) enzymes to toxic reactive metabolites with the ability to covalently bind to intracellular components, including DNA and RNA. The net result of these reactions is altering of protein synthesis and other liver functions **(Diaz *et al*., 2010 and Murcia *et al*., 2011)**.

AFB1 is known to be an immunosuppressive in birds, and it has been reported that diets containing 300 µg/kg of AFB1 significantly reduced the serum IgA, IgG and IgM of broilers **Chen *et al*., (2014)**. Broilers fed diets contaminated with a low level of AFB1 (22.5–25.0 µg/kg) had significantly lower serum TP, IgA and IgG **(Liu *et al*., 2016)**.

Elevated serum activities of AST, ALT, and γGT enzymes and the reduced serum level of total protein (TP) and albumin have been recognized as sensitive serological indicators in the impairment of the hepatic tissues and biliary system in broilers due to aflatoxin **(Abdel-Wahhab and Aly, 2005; Pasha *et al*., 2007; Gowda *et al*., 2008; Denli *et al*., 2009; Matur *et al*. 2010; Zhao *et al*., 2010; and Kumar *et al*., 2015).**

Exposure to AFB1 also altered renal functions in broilers **(Shi *et al*., 2006; Tessari *et al*. (2006) and Chen *et al*., 2014a).** AF contaminated diet Significantly increased serum creatinine in turkey poults **(Gómez-Espinosa *et al*., 2017)** and uric acid in Japanese quail fed AF contaminated diet (2.5 mg/kg) for 49 days **(Bagherzadeh Kasmani *et al*., 2012)**. The elevated serum creatinine in AF group indicates the increased transformation of phosphocreatinine to creatinine in the muscle, which might be due to a lesser utilization of phosphocreatinine during muscular contraction and/or decreased excretion from the kidney, suggesting that aflatoxins cause adverse changes in both skeletal muscle and kidney **(Mathuria and Verma, 2008)**.

Reduction of calcium, and phosphorus levels were observed in broilers fed AF-contaminated diet **(Stanley *et al*., 2004; Franciscato *et al*., 2006 and Chen *et al*., 2014).** Aflatoxicosis affects on excretion of phosphorous and Ca as a direct result of renal tubular damage, decreased Ca absorption from the gut, because of the altered circulating levels of parathyroid hormone(PTH), and possibly decreased renal sensitivity to PTH **(Glahn *et al*., 1991 and Kececi *et al*., 1998)**. Therefore, the decreased Ca and P concentration in our study may be attributed to the direct or indirect effects of aflatoxin on Ca and phosphorous metabolisms, and the effects may be related to altered vitamin D and parathyroid hormone metabolism.

In the present study, dietary addition of

HSCAS adsorbent at 0.25% concentration with Aflatoxin (250ppb) partially improved the adverse effects of AF on the above-mentioned parameters. SC supplementation at 0.5% concentration with Aflatoxin (250ppb) restored serum total protein, albumin, globulin and Ca concentrations and ALT activity to their control levels, prevent the deleterious effects of Aflatoxin on LDH, CK activities and creatinine concentrations at 3rd week and partially improved AST, γGT activities and uric acid concentration **(tables 2, 3, 4, 5& 6)**.

Dietary HSCAS supplementation at 0.5% improved serum albumin levels, restored alkaline phoshatase (ALP) activity reversed the increase of CK levels for birds fed 1 mg of AFB1/kg, reversed the decreased serum P and decreased AST activity for birds fed 2 mg of AFB1/kg **(Chen *et al*., 2014**).

The addition of beer fermentation residue containing *Saccharomyces cerevisiae* cells (BFR) to the AF-contaminated feed indicated that this compound ameliorated feed intake, body weight gain, serum biochemistry and completely prevented kidney lesions in broilers. BFR is rich in proteins, amino acids, and B vitamins and can contribute to the nutritional value of broiler diets **(Bovo *et al*., 2015)**.

The addition of yeast glucomannans at a rate of 0.5 to 1 g/kg decreased the severity of liver and kidney histological changes. The number of affected organs also decreased in the group receiving 1g/kg of glucomannans compared to the group that received only AF **(Karaman *et al*., 2005).**

Residues of aflatoxins and their metabolites may be present in meat, dairy products and eggs and other products of animals fed rations contaminated with aflatoxins **(Hussain *et al*., 2010)** and potentially result in health problems in human **Pandey and Chauhan, (2007)** and **Denli *et al*., (2009).** In many countries, the maximum tolerance level of AFB1 in human food products is 2 µg/kg **Zhang *et al*., (2017)**.

As shown in table **(7)**, AF residues were detected in both liver and muscles tissues in AF group (groups 2, 5& 6) with higher concentrations in liver (3.4 ppb) than muscles (2.6 ppb). Addition of HSCAS significantly reduced liver and muscles AF residues (1.0 and 0.6 ppb respectively**)** and addition of SC significantly reduced liver and muscles AF residues (0.4 and 0.2 ppb respectively).

Several studies indicate that, residue levels of AFB1 (0.05 and 0.13 µg/kg) were found in the livers of broilers given diets contain 50 and 100 µg of AFB1/kg for 42 days **(Bintvihok and Kositcharoenkul, 2006)**. AFB1 residue was also observed in livers of laying hens fed 2.5 mg/kg AFB1 diet for four weeks **(Zaghini *et al*., 2005)**. Low levels (0.12 and 0.10 µg/Kg) of AFB1 and AFM1 were retained in livers of ducks fed the aflatoxin-contaminated diet for 21 days. Residue levels may be different because of the type of bird and diet, the concentrations of AFB1, and the duration of exposure **(Zhang *et al*., 2017)**.

The protective effects of HSCAS and SC against aflatoxins may be due to their specific adsorption of aflatoxins in the intestinal tract, which leads to the reduction of aflatoxins absorbed by the intestinal tract and, consequently, a decrease in aflatoxin residues in the liver and muscles.

Conclusion

Dietary addition of (HSCAS) at 0.25% level and (SC) at 0.5 % level mitigate the adverse biochemical effects of aflatoxin toxicity at 250 ppb dose in broilers and SC was more efficient than HSCAS. Therefore, it may be concluded that due to the contributions of SC to antioxidant status and other metabolites activities, the addition of this biological product to chicken rations could be important to chicken and human health rather than chemical feed additives.

References

Abdel-Wahhab M.A. and Aly, S.E. (2005). Antioxidant property of Nigella sativa (black cumin) and Syzygium aromaticum (clove) in rats during aflatoxicosis. J. Appl. Toxicol., 25, 218–223.

Alberts, J.F.; Engelbrecht, Y.; Steyn, P.S.; Holzapfel, W.H. and van Zyl, W.H. (2006). Biological degradation of aflatoxin B1 by

Rhodococcus erythropolis cultures. Int. J. Food Microbiol. 109: 121–126.

Alberts, J.F., Gelderblom, W.C.A., Botha, A. (2009). Degradation of aflatoxin B1 by fungal laccase enzymes. Int J Food Microbiol 135:47-52.

Albro, P.W.; Corbett, J.T. and Schroder, J.L. (1986). Application of the thiobarbituric assay to the measurement of lipid peroxidation products in microsomes. J. Biochem. Biophys. Methods. 13(3): 185-194.

Aravind, K.L.; Patil, V.S.; Devegowda, G.; Umakantha, B. and Ganpule, S.P. (2003). Efficacy of esterified glucomannan to counteract mycotoxicosis in naturally contaminated feed on performance and serum biochemical and hematological parameters in broilers. Poult. Sci., 82 (4): 571-576.

Armando, M.R.; Dogi, C.A. and Pizzolitto, R.P. (2011). *Saccharomyces cerevisiae* strains from animal environment with *in vitro* aflatoxin B1 binding ability and antipathogenic bacterial influence. World Mycotoxin J 4: 59-68.

Bagherzadeh Kasmani, F.; Karimi Torshizi, M.A.; Allameh, A. and Shariatmadari, F. (2012). A novel aflatoxin-binding Bacillus probiotic: Performance, serum biochemistry, and immunological parameters in Japanese quail. Poult. Sci., 91, 1846–1853.

Baptista, A.S.; Horii, J.; Calori-Domingues, M.A.; Gloria, E.M.; Salgado, J.M. and Vizioli, M.R. (2004). The capacity of mannooligosaccharides, thermolysed yeast and active yeast to attenuate aflatoxicosis. World J. Microb. Biot. 20: 475–481.

Barnett, R.N. (1965). A scheme for the comparison of quantitative methods. Am. J. Clin. Pathol., 43: 562.

Bowers, L.D. and Wong, E.T. (1980). Kinetic serum creatinine assays. II. A critical evaluation and review. Clin. Chem., 26: 555-561.

Bhat, R.; Rai, R.V. and Karim, A.A. (2010). Mycotoxins in food and feed: Present status and future concerns. Compr. Rev. Food Sci. F. 9: 57–81.

Bintvihok, A. and Kositcharoenkul, S. (2006). Effect of dietary calcium propionate on performance, hepatic enzyme activities and aflatoxin residues in broilers fed a diet containing low levels of aflatoxin B1. Toxicon, 47, 41–46.

Bovo, F.; Franco, L.T.; Kobashigawa, E.; Rottinghaus, G.E.; Ledoux, D.R. and Oliveira C.A.F. (2015). Efficacy of beer fermentation residue containing *Saccharomyces cerevisiae* cells for ameliorating aflatoxicosis in broilers**.** Poultry Science. 94: 934–942.

Buhl, S.N. and Jackson, K.Y. (1978). Determination of serum lactate dehydrogenase (LDH). Clin. Chem., 24, 828.

Cannon, E.C.; Olitzky, I. and Inkpen, J.A. (1974). Proteins. In: Clinical chemistry principles and technics, 2nd ed. R.J. Henery, D.C. Cannon, J.W. Winkelman, editors, Harper & Row, New York, pp 407-421.

CFIA. (2009). Fact Sheet – Mycotoxins. Available:http://www.inspection.gc.ca/animals/feeds/regulatory-guidance/rg-1/chapter-7/mycotoxins/eng/1328860069173/1328860172463. Accessed last Sept. 6, 2012

Chanarin, I. (1989). Textbook of laboratory haematology: An Account of laboratory techniques. Churchill Livingstone, New York. Pp.107- 109.

Chen, K.J.; Fang, J.; Peng, X.; Cui, H.M.; Chen, J.; Wang, F.Y.; Chen, Z.L.; Zuo, Z.C.; Deng, J.L. and Lai, W.M. (2014).

Effect of selenium supplementation on aflatoxin B1–induced histopathological lesions and apoptosis in bursa of Fabricius in broiler. Food Chem. Toxicol., 74, 91–97.

Chen, X.; Horn, N. and Applegate, T.J. (2014a). Efficiency of hydrated sodium calcium aluminosilicate to ameliorate the adverse effects of graded levels of aflatoxin B1 in broiler chicks. Poult. Sci. 93:2037–2047.

Chen, X.; Naehrer, K. and Applegate, T.J. (2016). Interactive effects of dietary protein concentration and aflatoxin B1 on performance, nutrient digestibility, and gut health in broiler chicks. Poultry Science 95:1312–1325.

Circu, M.L. and Aw, T.Y. (2010). Reactive oxygen species, cellular redox systems, and apoptosis. Free Radic Biol. Med. 48: 749-762.

Cole, D. (1999). EGMs, the tool to heat mycotoxin contaminated feed. Asian Pork Magazine, 2: 30–35.

Coles, E.H. (1986). Veterinary Clinical Pathology, 4th Edition W.B. Sanders Company, Pliladelphia, 22-23.

Corassin, C.H.; Bovo, F.; Rosim, R.E. and Oliveira, C.A.F. (2013). Efficiency of Saccharomyces cerevisiae and lactic acid bacteria strains to bind aflatoxin M1 in UHT skim milk. Food Control. 31: 80–83.

Daly, J.A. and Ertingshausen, G. (1972). Direct method for determining inorganic phosphate in serum with the "CentrifiChem". Clin Chem. 18 (3): 263-265.

Denli, M.; Blandon, J.C.; Guynot, M.E.; Salado, S. and Perez, J.F. (2009). Effects of dietary AflaDetox on performance, serum biochemistry, histopathological changes, and aflatoxin residues in broilers exposed to aflatoxin B1. Poultry Science 88: 1444–1451.

Denli, M. and Okan, F. (2006). Efficacy of different adsorbents in reducing the toxic effects of AFB1 in broiler diets. South Afr J Anim Sci, 36, 222-228.

Devegowda, G.; Raju, M.V.L.N.; Afzali, N. and Swamy, H.V.L.N. (1998). Mycotoxins picture: worldwide, novel solutions for their counteraction. Biotechnology in the Feed Industry, edited by T.P. Lyons, and K.A. Jacques. Proceedings of Alltech's 14th Annual Symposium, Nottingham, U.K, pp 241-255.

Devegowda, G. and Murthy, T.N.K. (2005). Mycotoxins: Their effects in poultry and some practical solutions. In *The Mycotoxin Blue Book*. D. Diaz, ed. Nottingham University Press, Nottingham, United Kingdom. p. 25.

Devegowda, G. and Raju, M.V.L.N. (2002). Esterified-glucomannan in broiler chicken diets contaminated with aflatoxin, ochratoxin and T-2 toxin: evaluation of its binding ability (*in vitro*) and efficacy as immunomodulator. J Anim Sci., 15:1051-1056.

Diaz, G.; Murcia, H.W. and Cepeda, S.M. (2010). Cytochrome P450 enzymes involved in the metabolism of aflatoxin B1 in chickens and quail. Poultry Science 89: 2461– 2469.

Diaz, D.E.; Hagler, Jr. W.M.; Hopkins, B.A. and Whitlow, L.W. (2002). Aflatoxin binders I: *In vitro* binding assay for aflatoxin B1by several potential sequestering agents. Mycopathologia, 156: 223-226.

Doumas, B.T.; Watson, W.A. and Biggs H.C. (1971). Albumin standards and Management of serum albumin with bromocresol green. Chin. Chim. Acta., 31: 87-96.

Eshak, M.G.; Khalil, W.K.B.; Hegazy, E.M.; Ibrahim, M.F.; Fadel, M. and Stino, K.R.D. (2010). Effect of SCE on reduction of aflatoxicosis, enhancement of growth performance and expression of neural and go-

nadal genes in Japanese quail. J Am Sci., 6, 824-838.

Essiz, D.; Altintas, L. and Das, Y.K. (2006). Effects of aflatoxin and various adsorbents on plasma malondialdehyde levels in quails. Bull. Vet. Inst. Pulway, 50, 585–588.

Fan, Y.; Zhao, L.H.; Ji, C.; Li, X.Y.; Jia, R.; Xi, L.; Zhang, J.Y. and Ma, Q.G. (2015). Protective Effects of Bacillus Subtilis ANSB060 on Serum Biochemistry, Histopathological Changes and Antioxidant Enzyme Activities of Broilers.Fed Moldy Peanut Meal Naturally Contaminated with Aflatoxins. Toxins, 7, 3330–3343.

FDA (1999). CVM position on mycotoxin binding claims on anticaking agents. http:// www.fda.gov./cvm/mycotoxup.html

Fossati, P.; Prencipe, L. and Berti, G. (1980). Use of 3,5-dichloro-2-hydroxybenzene-sulfonic acid/4-aminophenazone chromogenic system in direct enzymatic assay of uric acid in serum and urine. Clin. Chem., 62: 227-231.

Franciscato, C.; Lopes, S.T.A.; Santurio, J.M.; Wolkmer, P.; Maciel, R.M.; Paula, M.T.; Garmatz, B.C. and Costa, M.M. (2006). Concentra¸coes sericas de minerais e fun¸coes hepatica e renal de frangos intoxicados com aflatoxina e tratados com montmorilonita s´odica. Pesq. Agropec. Bras. 41:1573–1577.

Fruhauf, S., Schwartz, H., Ottner, F. (2012). Yeast cell based feed additives: studies on aflatoxina B1 and zearalenone. Food Addit Contam Part A 29:217-231.

Galvano, F.; Piva, A.; Ritieni, A. and Galvano, G.(2001). Dietary strategies to counteract the effects of mycotoxins: A review. J. Food. Prot., 64: 120–131.

Glahn, R.P.; Beers, K.W.; Bottje, W.G.; Widerman, R.F.; Huff, W.E. and Thomas, W. (1991). Aflatoxicosis alters avian renal function, calcium, and vitamin D metabolism. J. Toxicol. Environ. Health. 34, 309–321.

Gómez-Espinosa, D.; Cervantes-Aguilar, F.J.; Río-García, J.C.D.; Villarreal-Barajas, T.; Vázquez-Durán, A. and Méndez-Albores, A. (2017). Ameliorative Effects of Neutral ElectrolyzedWater on Growth Performance, Biochemical Constituents, and Histo-pathological Changes in Turkey Poults during Aflatoxicosis. Toxins, 9, 104.

Gowda, N.K.S.; Ledoux, D.R.; Rottinghaus, G.E.; Bermudez, A.J. and Chen, Y.C. (2008). Efficacy of turmeric (Curcuma longa), containing a known level of curcumin, and a hydrated sodium calcium aluminosilicate to ameliorate the adverse effects of aflatoxin in broiler chicks. Poult. Sci. 87: 1125–1130.

Gratz, S.; Mykkänem, H. and El-Nezami, H. (2005). Aflatoxin B1 binding by a mixture of *Lactobacillus* and *Propionibacterium*: *in vitro* vs. *ex vivo*. J Food Prot 68: 2470-2474.

Hussain, Z.; Khan, A.; Khan, M.Z.; Javed, I.; Saleemi, M.K.; Mahmood, S. and Asi, M.R. (2010). Residues of aflatoxin B1 in broiler meat: Effect of age and dietary aflatoxin B1 levels. Food Chem. Toxicol., 48, 3304–3307.

Hussein, H.S. and Brasel, J.M. (2001). Toxicity, metabolism, and impact of mycotoxins on humans and animals. Toxicology 167: 101 -134.

International Agency for Research on Cancer. (2002). IARC Monograph on the evaluation of carcinogenic risk to humans. 82:171–300.

Joannis-Cassan, C.; Tozlovanu, M.; Hadjeba-Medjdoub, K.; Ballet, N. and Pfohl-Leszkowicz, A. (2011). Binding of zearalenone, aflatoxin B1, and ochratoxin A by yeast-based products: A method for quantification of adsorption performance. J. Food Prot. 74: 1175– 1185.

Jouany, J.P.; Yiannikouris, A. and Bertin, G. (2005). The chemical bonds between mycotoxins and cell wall components of *Saccharomyces cerevisiae* have been identified. Arch Zootec 8: 26-50.

Karaman, M.; Basmacioglu, H.; Ortatatli, M. and Oguz, H. (2005). Evaluation of the detoxifying effect of yeast glucomannan on aflatoxicosis in broilers as assessed by gross examination and histopathology. Br. Poult. Sci., 46: 394–400.

Kececi, T.; Oguz, H.; Kurtoglu, V. and Demet, O. (1998). Effects of polyvinyl poly pyrrolidone, synthetic zeolite and bentonite on serum biochemical and hematological characters of broiler chickens during aflatoxincosis. Br. Poult. Sci. 39: 452–458.

Kubena, L.F.; Harvey, R.B.; Huff, W.E. and Corrier, D.E. (1990). Efficacy of a hydrated sodium calcium aluminodilicate to reduce the toxicity of aflatoxin and T-2 toxin. Poult. Sci. 69: 1078–1086.

Kumar, D.S.; Rao, S.; Satyanarayana, M.L.; Kumar, P.G.P. and Anitha, N. (2015). Amelioration of hepatotoxicity induced by aflatoxin using citrus fruit oil in broilers (Gallus domesticus).Toxicology and Industrial Health, Vol. 31(11): 974–981.

Leeson, S.; Diaz, G.J. and. Summers, J.D. (1995): "Aflatoxins" in Poultry Metabolic Disorders and Mycotoxins, S. Leeson, J. D. G. Gonzala, and J. D. Summers, Eds., University Books, Ontario, Canada, pp. 249–298.

Lemke, S.L.; Ottinger, S.E.; Mayura, K.; Ake, C.L.; Pimpukdee, K.; Wang, N. and Phillips, T.D. (2001). Development of a multi-tierid approach to the *in vitro* prescreening of clay-based enterosorbents. Anim. Feed. Sci. Technol., 93: 17–29.

Li, Y.; Ma, Q.G.; Zhao, L.H.; Guo, Y.Q.; Duan, G.X.; Zhang, J.Y. and Ji, C.(2014a). Protective Efficacy of Alpha-lipoic Acid against AflatoxinB1-induced Oxidative Damage in the Liver. Asian Australas. J. Anim. Sci., 27, 907–915.

Li, Y.; Ma, Q.G.; Zhao, L.H.; Wei, H.; Duan, G.X.; Zhang, J.Y. and Ji, C.(2014b). Effects of Lipoic Acid on Immune Function, the Antioxidant Defense System, and Inflammation-related Genes Expression of Broiler Chickens Fed Aflatoxin Contaminated Diets. Int. J. Mol. Sci., 15, 5649–5662.

Liu, T.; Ma, Q.; Zhao, L.; Jia, R.; Zhang, J.; Ji, C. and Wang, X. (2016). Protective Effects of Sporoderm-Broken Spores of Ganderma lucidum on Growth Performance, Antioxidant Capacity and Immune Function of Broiler Chickens Exposed to Low Level of Aflatoxin B. Toxins, 8, 278.

Madheswaran, R.; Balachandran, C. and Murali Manohar, B. (2004). Influence of dietary culture material containing aflatoxin and T2 toxin on certain serum biochemical constituents in Japanese quail. Mycopathologia 158: 337–341.

Magnoli, A.P.; Monge, M.P.; Miazzo, R.D.; Cavaglieri, L.R.; Magnoli, C.E.; Merkis, C.I.; Cristofolini, A.L.; Dalcero, A.M. and Chiacchiera, S.M. (2011). Effect of low levels of aflatoxin B (1) on performance, biochemical parameters, and aflatoxin B(1) in broiler liver tissues in the presence of monensin and sodium bentonite. Poult. Sci., 90, 48–58.

Mahesh, B.K. and Devegowda, G. (1996). Ability of aflatoxin binders to bind aflatoxin in contaminated poultry feeds. An *in vitro* study. In: Proceedings of the 20th World's Poultry Congress, New Delhi, India, 4: 296-303.

Manafi, M.; Hedayati, M. and Yari, **M. (2014).** Effectiveness of Rosemary (*Rosmarinus officinalis* L.) Essence on Performance and Immune Parameters of Broilers during Aflatoxicosis. Advances in Life Sciences, 4(3): 166-173.

Marin, S.; Ramos, A.J.; Cano-Sancho, G. and Sanchis, V. (2013). Mycotoxins: Occurrence, toxicology, and exposure assessment. Food Chem. Toxicol., 60, 218–237.

Mathuria, N. and Verma, R.J. (2008). Ameliorative effect of curcumin on aflatoxin-induced toxicity in serum of mice. Acta Pol. Pharm., 65, 339–343.

Matur, E.; Ergul, E.; Akyazi, I.; Eraslan, E.and Cirakli, Z.T. (2010). The effects of Saccharomyces cerevisiae extract on the weight of some organs, liver, and pancreatic digestive enzyme activity in breeder hens fed diets contaminated with aflatoxins. Poult. Sci. 89: 2213–2220.

Mehrzad, J.; Klein, G.; Kamphues, J.; Wolf, P.; Grabowski, N. and Schuberth, H.J. (2011). In vitro effects of very low levels of aflatoxin B1 on free radicals production and bactericidal activity of bovine blood neutrophils. Vet. Immunol. Immunopathol., 141, 16 –25.

Mezes, M.; Balogh, K.; and Toth, K. (2010). Preventive and Therapeutic Methods Against the Toxic Effects of Mycotoxins – A Review. Acta Veterinaria Hungarica 58 (1): 1–17.

Miliţa, N.M.; Mihăescu, G.and Chifiriuc, C. (2010). Aflatoxins-Health risk factors. Bacteriol. Virusol. Parazitol. Epidemiol. 55: 19-24.

Minami, M. and Yoshikawa, H. A. (1979). Simplified assay method of superoxid dismutase. clinic, chimica. Acta: 29-337-342.

Murcia, H.W.; Dıaz, G.J. and Cepeda, S.M. (2011). Enzymatic activity in turkey, duck, quail and chicken liver microsomes against four human cytochrome P450 prototype substrates and aflatoxin B. Journal of Xenobiotics 1: e4, 17–21.

Nazar, F.N.; Magnoli, A.P.; Dalcero, A.M. and Marin, R.H. (2012). Effect of feed contamination with aflatoxin B1 and administrationof exogenous corticosterone on Japanese quail biochemicaland immunological parameters. Poult. Sci. 91: 47–54.

Newman, K. (1994). Mannan-oligosaccharides: Natural polymers with significant impact on the gastrointestinal microflora and the immune system. *In*: Biotechnology in the Feed Industry. Proceedings of Alltech's Tenth Annual Symposium. (Lyons, T. P. and Jacques, K. A., Eds.). Nottingham University Press, Nottingham, UK, pp. 167-174.

NRC; National Research Council. (1994). Nutrient Requirements of poultry. 9thRev. Edn., National Academy Science Press, Washington, DC.

Özen, H.; Karaman, M.; Ciğremiş, Y.; Tuzcu, M.; Özcan, K. and Erdağ, D. (2009). Effectiveness of melatonin on aflatoxicosis in chicks. Res. Vet. Sci., 86, 485–489.

Pandey, I. and Chauhan, S.S. (2007). Studies on production performance and toxin residues in tissues and eggs of layer chickens fed on diets with various concentrations of aflatoxin B1. British Journal of Poultry Science 48: 713–723.

Pasha, T.N.; M.U. Farooq; F.M. Khattak, M.A. Jabbar, and A.D. Khan. (2007). Effectiveness of sodium bentonite and two

commercial products as aflatoxin absorbents in diets for broiler chickens. Anim. Feed Sci. Technol. 132:103–110.

Persijn, J.P. and Vander Slik, W. (1976). A new method for the determin-ation of γ-glutamyltransferase in serum. J. Clin. Chem. Clin. Biochem. 14(9): 421: 427.

Pizzolitto, R.P.; M.R. Armando; M. Combina; L.R. Cavaglieri; A.M. Dalcero and M.A. Salvano (2012). Evaluation of *Saccharomyces cerevisiae* strains as probiotic agent with aflatoxin B1 adsorption ability for use in poultry feedstuffs. J. Environ. Sci. Health B. 47: 933–941.

Phillips, T.D.; Afriyie-Gyawu, E.; Williams, J.; Huebner, H.; Ankrah, N.A.; Ofori-Adjei, D. and Jolly, P. (2008). Reducing human exposure to aflatoxin through the use of clay: a review. Food Addit Contam A. 25 (2): 134–145.

Phillips, T.D.; Kubena, L.F.; Harvey, R.B.; Tayler, D.S.; and Heidelbaurgh, N.D. (1988). Hydrated sodium calcium aluminosilicate: A high affinity sorbent for aflatoxins. Poultry Science, 67: 243-247.

Phillips, T.D.; Sarr, A.B. and Grant, P.G. (1995). Selective chemisorptions and detoxification of aflatoxin by phyllosilicate clay. NaturalToxins, 3: 204-213.

Davidson, J.N.; Babish, J.G.; Delamey, K.A.; Taylor D.R. and Phillips, T.D. (1987). Hydrated sodium calcium aluminosilicate decreases the bioavailabilty of aflatoxins in the chicken. Poultry Science, 66: 89-93.

Rawal, S.; Kim, J.E. and Coulombe, Jr. R. (2010). Aflatoxin B1 in poultry: Toxicology, metabolism and prevention. Res. Vet. Sci. 89: 325–331b.

Reddy, K.R.N., Farhana, N.I., Salleh, B. and Oliveira, C.A.F. (2010a). Micro-biological Control of Mycotoxins: Present Status and Future Concerns. In Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology, A Mendez –Vilas (Ed) Pp: 1078-1086

Reddy, K.R.N.; Salleh, B.; Saad, B.; Abbas, H.K.; Abel, C.A. and Sheir, W.T. (2010b). An overview of mycotoxin contamination in foods and its implications for human health. *Toxin Rev*. 29: 3-26.

Reitman, S. and Frankel, S. (1957). A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Amer. J. Clin. Path., 28: 56.

Resanovia, R.; Nesicb, K.; Nesia, V.; Palia, T. and Jaaevia, V. (2009). Mycotoxin in poultry production. Matica Srpska Proceedings for Natural Sciences 116: 7–14.

Romer T.R. (1975). Screening method for the detection of aflatoxins in mixed feeds and other agricultural commodities with subsequent confirmation and quantitative measurement of aflatoxins in positive samples. J Assoc of Anal Chem., 58: 500-506.

Rosalki, S.B. (1967). An improved procedure for serum creatine phosphokinase determination. J. Lab. Clin. Med. 69: 696-705.

Rustemeyer, S.M.; Lamberson, W.R.; Ledoux, D.R.; Rottinghaus, G.E.; Shaw, D.P.; Cockrum, R.R.; Kessler, K.L.; Austin, K.J. and Cammack, K.M. (2010). Effects of dietary aflatoxin on the health and performance of growing barrows. J. Anim. Sci., 88, 3624–3630.

Santin, E.; Maiorka, A.; Krabbe, E. L.; Paulillo, A. C. and Alessi, A. C. (2002). Effect of hydrated sodium calcium aluminosilicates on the prevention of the toxic effects of ochratoxin. J. Appl. Poult Res., 11: 22–28.

Santurio, J.M. (2000). Micotoxin as e micotoxicoses naavi cultura. Rev. Bras. Cienc. Avıcola. 2: 1–12.

Shehata, A.M. and Yousef, O.M. (2010). Physiological studies on the risk factors responsible for atherosclerosis in rats. Nat. and Sci., 8(5): 144-151.

Shetty, P.H. and Jespersen, L. (2006). *Saccharomyces cerevisiae* and lactic acid bacteria as potential mycotoxin decontamination agents. *Trends Food Sci Technol*. 17: 48-55.

Shotwell, O.L.; Hesseltine, C.W.; Stubblefield, R.D. and Sorenson, W.G. (1966). Production of aflatoxin on rice. Appl Microbiol, 14: 425-428.

Shi, Y.H.; Z.R. Xu; J.L. Feng and C.Z. Wang (2006). Efficacy of modified montmorillonite nanocomposite to reduce the toxicity of aflatoxin in broiler chicks. Anim. Feed Sci. Technol. 129: 138–148.

Smits, G.J.; Kapteyn, J.C.; Endo, H.V.D. and Klis, F.M. (1999).Cell wall dynamics in yeast. *Curr. Opin. Microbiol.* 2: 348–352.

SPSS 14 (2006). Statistical package for social science, SPSS for windows release 14.0.0, Standard version, SPSS Inc.

Sreeparvathy, M. and Anuraj, K.S. (2016). Spent Brewer's yeast as a Protein supplement. Int. J. Curr. Res. Biol. Med. 1(4): 16-18.

Stanley, V.G.; Winsman, M.; Dunkley, C.; Ogunleye, T.; Daley, M.; Krueger, W.F.; Sefton, A.E. and Hinton, Jr. A. (2004). The impact of yeast culture residue on the suppression of dietary aflatoxin on the performance of broiler breeder hens. J. Appl. Poult. Res. 13: 533–539.

Streit, E.; Naehrer, K.; Rodrigues, I. and Schatzmayr, G. (2013). Mycotoxin occurrence in feed and feed raw materials worldwide: longterm analysis with special focus on Europe and Asia. J. Sci. Food Agr. 93: 2892–2899.

Tessari, E.N.C.; Oliveira, C.A.F.; Cardoso, A.L.S.P.; Ledoux, D.R. and Rottinghaus, G.E. (2006). Effects of aflatoxin B1 and fumonisin B1 on body weight, antibody titres and histology of broiler chicks. Br. Poult. Sci. 47: 357–364.

Truckess, M.W., Stack, M.E., Nesheim, S., Page, S.W., Albert, R.H., Hasen T.J. and Donahue, K.F. (1991). Immunoafffinity column coupled with solution fluorometry or liquid chromatography post column derivatization for determination of aflatoxins in corn, peanuts and peanut butter: collaborative study. Journal of the Association of the Official Analytical Chemistry, 74 (1): 81-88.

Volkl, A. and Karlovsky. P. (1998). Biological detoxification of fungal toxins and its use in plant breeding and feed and food production. Natural Toxins, 7: 1-23.

Walters, B.P. (2012). Efficacy of A Yeast Cell Wall-Derived Mycotoxin Adsorbent on Excretion of Aflatoxin B2 in Weanling Rats. Master of Science Thesis, University of Guelph, Ontario, Canada.

Watts, C.M.; Chen, Y.C.; Ledoux, D.R.; Broomhead, J.N.; Bermudez, A.J. and Rottingus, G.E. (2003). Effects of multiple mycotoxins and a hydrated sodium calcium aluminosilicate in poultry.

Williams, J.H.; Phillips, T.D.; Jolly, P.E.; Stiles, J.K.; Jolly, C.M. and Agarwal D. (2004). Human aflatoxicosis in developing countries: A review of toxicology, exposure, potential health consequences, and interventions. *American J Clinical Nutrition*.:80: 1106-1122.

Wu, F. (2007). Measuring the economic im-

pacts of Fusarium toxins in animal feeds. Anim Feed Sci. Technol. 137(3–4): 363–374.

Yang, J.; Bai, F.; Zhang, K.; Bai, S.; Peng, X.; Ding, X.; Li, Y.; Zhang, J. and Zhao, L. (2012). Effects of feeding corn naturally contaminated with aflatoxin B1 and B2 on hepatic functions of broilers. Poult. Sci., 91, 2792–2801.

Zaghini, A.; Martelli, G. and Roncada, P. (2005). Mannanoligosaccharides and Aflatoxin B1 in Feed for Laying Hens: Effects on Egg Quality, Aflatoxins B1 and M1 Residues in Eggs, and Aflatoxin B1 Levels in Liver. Poult. Sci., 84, 825–832.

Zhang, L.; Ma, Q.; Ma, S.; Zhang, J.; Jia, R.; Ji, C.; and Zhao, L. (2017). Ameliorating Effects of Bacillus Subtilis ANSB060 on Growth Performance, Antioxidant Functions, and Aflatoxin Residues in Ducks Fed Diets Contaminated with Aflatoxins. Toxins, 9: 1.

Zhao, J.; Shirley, R.B.; Dibner, J.D.; Uraizee, F.; Officer, M.; and Kitchell, M. (2010). Comparison of hydrated sodium calcium aluminosilicate and yeast cell wall on counteracting aflatoxicosis in broiler chicks. Poultry Science 89: 2147–2156.

Zuo, R.Y.; Chang, J.; Yin, Q.Q.; Wang, P.; Yang, Y.R.;Wang, X.;Wang, G.Q. and Zheng, Q.H. (2013). Effect of the combined probiotics with aflatoxin B1-degrading enzyme on aflatoxin detoxification, broiler production performance and hepatic enzyme gene expression. Food Chem. Toxicol., 59, 470–475.