Effect of yeast (*Saccharomyces cerevisiae*) on reduction of aflatoxicosis, enhancement of growth performance and expression of neural and gonadal genes in Japanese quail

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Abstract: The present investigation was designed to evaluate the role of yeast, *Saccharomyces cerevisiae* (SC) in the reduction of aflatoxicosis induced by aflatoxin B_1 (AFB_1) in Japanese quail. Sixty male quail were used and distributed into six groups. The first group received basal diet. The other five groups received the basal diet plus 0.5 mg AFB_1/kg diet. Four of them received increasing levels of SC (0.5, 1.0, 2.0 and 2.5 gm/kg diet, respectively). All groups received their prospective diets for 35 days. The birds were weighed weekly to determine body weight (BW) and body weight gain (BWG). The results showed that addition of the SC to AFB_1-containing diet significantly reduced the adverse affect of AFB_1 on quail BW and BWG. The concentrations of AFB_1 had been lowered in the breast muscle and liver samples of quail fed diet containing AFB_1 plus SC than those found in such quail organs of AFB_1 group. The expression levels of neural and gonadal genes were significantly up-regulated in quail fed diet containing AFB_1 plus high levels of SC compared to those of AFB_1 group. It could be concluded that SC supplementation 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. Thus, the use of HPLC and gene expression analysis might contribute in detecting aflatoxin contamination in the poultry industry in Egypt.
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Key words: Quail; body weight; growth rate; yeast; aflatoxin B_1; sqRT-PCR; gene expression.

1. Introduction:

Aflatoxins are a group of closely related, biologically active mycotoxins which are produced by storage fungi during growth on a number of foods and feed materials (Abo *et al.*, 1995 and Oliveria *et al.*, 2002). Aspergilla are the most common fungal species that can produce aflatoxins (AFs) in food and feedstuffs (Oliveria *et al.*, 2002 and Abousadi *et al.*, 2007). Among the different types of AFs produced, AFB_1 is the most prevalent and potent and is often found in high concentrations in cereal grains and peanut meal, which comprises between 50 and 60 percent of many poultry diets (Stanley *et al.*, 1993; Miazzo *et al.*, 2000; Parlat *et al.*, 2001 and Gowda *et al.*, 2004). Aflatoxicosis due to AFB_1 in poultry, causes listlessness, anorexia with lowered growth rate, immunosupression, decreased body weight gain, poor feed utilization, reduced egg production and increased mortality (Oguz and Kurtoglu, 2000; Oliveria *et al.*, 2002 and Abousadi *et al.*, 2007).

Removing AF from contaminated food and feedstuffs remains a major problem and there is a great demand for effective decontamination technology. These procedures have focused on degrading, destroying, inactivating or removing AF by physical (heat, irradiation), chemical (ammoniation, sulphites, hypochlorides, ozone), nutritional (vitamins, minerals) or biological (bacteria, yeast) methods (Stanley *et al.*, 1993; El-Nezami *et al.*, 2000; Raju and Devegowda, 2000; Galvano *et al.*, 2001; Abousadi *et al.*, 2007 and Tovar-Ramirez *et al.*, 2010). A successful detoxification process must be economically capable of eliminating all traces of a toxin without having harmful residues and must not impair the nutritional quality of the commodity (Leeson *et al.*, 1995 and Kubena *et al.*, 1998). One approach of many to this problem is the use of the yeast *Saccharomyces cerevisiae* (SC) and its cell wall component (manner oligosaccharide) for minimizing the adverse effects of AF in poultry on the basis of biological degradation (Parlat *et al.*, 2001).This approach will not only benefit human health but also result in increased profit and productivity of poultry (Galvano *et al.*, 2001). The inclusion of SC (1gm/kg) to the AFB_1-containing diet provided significant improvements on the adverse effects of AFB_1 (5 mg/kg) in broiler chicks fed for 28 days (Stanely *et al.*, 1993). Also, Raju and Devegowda (2000) extracted mannan oligosccharide, which was believed to be responsible for the beneficial effect against AFB_1 from the wall of SC. They added (1gm/kg) to AFB_1-containnated feed and reported significant amelioration on the adverse effect of AFB_1 (0.3mg/kg) in broiler fed for 35 days. Moreover, Abousadi *et al.* (2007) observed that the addition of SC (0.2%) to AFB_1-containing diet significantly improved the adverse effect of AFB_1 (125 ppb) on growth performances in broiler chicks fed for 21 days. In quail, Parlat *et al.*, (2001) reported that the supplementation of SC (1gm/kg) to the AF-containing diet significantly reduced the deleterious effect of AFB_1 (2.5mg/kg diet) on body weight and body weight gain in birds fed for 35 days (Parlat *et al.*, 2001)

The present study was designed to investigate the role of SC in reducing aflatoxicosis caused by AFB_1 in Japanese quail. Therefore, the measurements of growth performances and the concentrations of AFB_1 in some quail organs were determined. Moreover, the expression levels of some neural(Glycerinaldehyde-3-phosphate ehydrogenase, GAPDH, neural cell adhesion molecule, NCAM, and Cadherin-2, CDH2) and gonadal (Cytochrome P450 cholesterol side chain cleavage, P450scc) genes were studied.

2. Materials and Methods

Birds and diet:

Sixty unvaccinated 14 days old male Japanese quail chicks were obtained from the quail project, Faculty of Agriculture, Cairo University. The quail were divided into six groups. Each group of 10 quail, were weighted and placed in a heated wooden brooder (battery cage). The birds received 24 h of light per day (continues light) for the duration of the experiment. They also received *ad lib*., water and a commercial growing quail ration (basal diet) containing 24% protein and 2,900 Kcal ME/Kg diet. The diet also contained all the required amino acids, vitamins and minerals according to the recommendations of the National Research Council (NRC, 1994), without adding antibiotics, coccidiostats or growth promoters. All animals received humane care in compliance with the guidelines of the Animal Care and Use Committee of Faculty of Agriculture, Cairo University, Egypt.

Aflatoxin B_1 (AFB_1):

AFB_1 used in this experiment was purchased from Sigma (Buchs, Switzerland). Twenty five milligrams of pure crystalline of AFB_1 were dissolved in chloroform. The chloroform solution was placed in a flask, and kept in a water bath at 60°C until complete evaporation of the solvent. Then AFB_1 was dissolved in 500 ml sterile maize oil. This solution was then added to 50 kg basal diet and homogenized to obtain the required final levels of AFB_1 which was 0.5mg of AFB_1/kg diet according to procedure of Soares and Rodrigues-Amaya (1989).

The appropriate experimental doses of AFB_1 that affect quail organs were determined previously by Sinsek *et al.* (2007). These appropriate doses ranged from 0.5 to 6.0 mg of AFB_1/kg diet.

Saccharomyces cerevisiae (SC):

SC was obtained from Microbial Chemistry Department, National Research Centre, Giza, Egypt. The SC culture was supplied at four rates, 0.5 gm, 1.0 gm, 2.0 gm and 2.5 gm/kg of feed. The live yeast was added to the appropriate diets mixed in cooking grade soybean oil. The soybean oil-SC suspension was added to the feed in the final mix as the last step in mixing, such as would occur commercially according to the procedure of Parlat *et al.* (2001). The AFB_1 was incorporated into the mixed feed before SC was added.

Experimental design:

Six groups of quail were supplemented with six dietary treatments as follows; (1) control, basal diet; (2) basal diet plus 0.5 mg of AFB_1/kg diet; (3) basal diet plus 0.5 mg of AFB_1 plus 0.5 gm of SC/kg diet; (4) basal diet plus 0.5 mg of AFB_1 plus 1.0 gm of SC /kg diet (5) basal diet plus 0.5 mg of AFB_1 and 2.0 gm of SC/kg diet (6) basal diet plus 0.5 mg of AFB_1 plus 2.5 gm of SC /kg diet.

Performance parameters:

a) Growth performance

The trial period was carried out for 5 weeks. During the experiment, the birds were weighed weekly to determine their body weight (BW) and body weight gain (BWG) at 14, 21, 28, 35, 42 and 49 days of age after fasting for 8 hours, to the nearest gram using a digital scale. The cumulative body weight gains were calculated by subtracting W2-W1.

After the end of the experiment, all birds were euthanized. Liver, brain, testis and breast muscle were collected, in order to (i) determine the aflatoxin concentrations in breast muscle and liver samples by using HPLC analysis and (ii) to evaluate the expression levels of some neural and gonadal genes by using sqRT-PCR method.

b) Analysis of toxin residues in quail organs:

Samples of liver and breast muscle were collected, squashed, homogenized and extracted by using acetonitrile-water solution (85/15) (v/v). The sample extraction was filtered and diluted (5ml) with 95 ml of phosphate buffer saline (PBS). The filtrated solution was applied onto the immuno-affinity column (Alfa BG. 1003, VICAM). The column was rinsed twice with 10 ml of deionized water and the toxin was eluted from the column with 1.0 ml of acetonitrile-methanol mixture (3+2). The column was subsequently washed with 1ml of deionized water and the washing was combined with the acetonitrile-methanol elute. AFB_1 in the acetonitrile-methanol-water mixture was determined by an HPLC method using a Lich rospher 100 PR-18 ECO Pack column (5µm, 25 x 4.6 mm i.d., Merek, Portugal), with post-column derivatization involving bromination with pyridinum hydrobroide perbromide (PBPB, Sigma P-3179, Quimica S.A., Spain) and fluorescence detection (Merck Hitachi, excitation and emission wavelengths were 360 nm and 420 nm, respectively). The mobile phase was water-acetonitrile-methanol solution (6/2/3) (v/v/v) and flow rates were 1.00 ml/min for mobile phase and 0.30 ml/min for the PBPB reagent (Martins *et al.*, 2008).

c) Gene expression Analysis:

1. Extraction of total RNA

Liver, brain and testis tissues of quail of all groups were used individually to extract total RNA using TRIzol® Reagent (cat#15596-026, Invitrogen, Germany). Total RNA of each tissue was treated individually with 1 U of RQ1 RNAse-free DNAse (Invitrogen, Germany) to digest DNA residues, re-suspended in DEPC-treated water and photospectrometrically quantified at A_{260}. Purity of total RNA was assessed by the 260/280 nm ratio (between 1.8 and 2.1). Additionally, integrity was assured with ethidium bromide-stain analysis of 28S and 18S bands by formaldehyde-containing agarose gel electrophoresis. Aliquots were used immediately for reverse transcription (RT), otherwise stored at -80°C.

2. Synthesis of the cDNA using reverse transcription (RT) reaction

The complete Poly(A)$^+$ RNA isolated from quail tissues was reverse transcribed into cDNA in a total volume of 20 µl using RevertAid^{TM} First Strand cDNA Synthesis Kit (MBI Fermentas, Germany). An amount of total RNA (5µg) was used with a reaction mixture, termed as master mix (MM). The MM was consisted of 50 mM $MgCl_2$, 5x reverse transcription (RT) buffer (50 mM KCl; 10 mM Tris-HCl; pH 8.3), 10 mM of each dNTP, 50 µM oligo-dT primer, 20 U ribonuclease inhibitor (50 kDa recombinant enzyme to inhibit RNase activity) and 50 U M-MuLV reverse transcriptase. The mixture of each sample was centrifuged for 30 sec at 1000 g and transferred to the thermocycler (Biometra GmbH, Göttingen, Germany). The RT reaction was carried out at 25°C for 10 min, followed by 1 h at 42°C, and finished with a denaturation step at 99°C for 5 min (Khalil *et al.*, 2008a,b). Afterwards the reaction tubes containing RT preparations were flash-cooled in an ice chamber until being used for DNA amplification through sqRT-PCR.

3. Semi Quantitative Real Time-Polymerase Chain Reaction (sqRT-PCR)

An iQ5-BIO-RAD Cycler (Cepheid. USA) was used to determine the quail cDNA copy number. PCR reactions were set up in 25 µL reaction mixtures containing 12.5 µL 1× SYBR® Premix Ex TaqTM (TaKaRa, Biotech. Co. Ltd.), 0.5 µL 0.2 µM sense primer, 0.5 µL 0.2 µM antisense primer, 6.5 µL distilled water, and 5 µL of cDNA template. The reaction program was allocated to 3 steps. First step was at 95.0°C for 3 min. Second step consisted of 40 cycles in which each cycle divided to 3 steps: (a) at 95.0°C for 15 sec; (b) at 55.0°C for 30 sec; and (c) at 72.0°C for 30 sec. The third step consisted of 71 cycles which started at 60.0°C and then increased about 0.5°C every 10 sec up to 95.0°C. At the end of each sqRT-PCR a melting curve analysis was performed at 95.0°C to check the quality of the used primers. Each experiment included a distilled water control.

The quantitative values of RT-PCR of Glyceraldehyde Phosphate dehydrogenase (GAPDH-F: 5′-GGT GAA AGT CGG AGT CCA-3′, GAPDH-R: 5′-TTC TGT GTG GCT GTG ATG -3′, (SQUITTI *et al.*, 1999); N-cadherin (N-cadherin-F: 5′-GAT GTC AAT GAC AAT CCT CC-3′, N-cadherin-R: 5′-CAT CCT AGT TGC GTC TTC AAA G -3′, (Squitti *et al.*, 1999); Neural cell adhesion molecule (NCAM-F: 5′-GCC TGA AAC CTG AGA CAA C-3′, NCAM -R: 5′- CTT ACG AAC TGG CTG TGT TC -3′, (Squitti *et al.*, 1999); and Cytochrome P450 cholesterol side chain cleavage (P450scc-F: 5′- ACA GCA GTT CAT CGA CGC CG -3′, P450scc-R: 5′-AAG GAG GCT GAA GAG GAT G -3′, (KANDA *et al.*, 2000) genes were normalized on the bases of β-actin (β-actin-F: 5′- TGT GAT GGT GGG AAT GGG TCA G -3′, B-actin-R: 5′- TTT GAT GTC

ACG CAC GAT TTC C -3′, (Hwang *et al.*, 2009) expression.

The selected genes are responsible for different functions during cell differentiations. Where, GAPDH is a gene coding an enzyme that catalyzes the sixth step of glycolysis and thus serves to break down glucose for energy and carbon molecules. NCAD is a gene coding a protein that has been implicated as having a role in cluster of differentiations. Also, NCAM is a gene coding a hemophilic binding glycoprotein expressed on the surface of neurons, ganglia, skeletal muscle and natural killer cells. P450scc is a gene coding a mitochondrial enzyme associated with the conversion of cholesterol to pregnenolone.

At the end of each sqRT-PCR a melting curve analysis was performed at 95.0°C to check the quality of the used primers.

4. Calculation of Gene Expression

First the amplification efficiency (Ef) was calculated from the slope of the standard curve using the following formulae (BIO-RAD, 2006):

$Ef = 10^{-1/slope}$

Efficiency (%) = (Ef – 1) x 100

The relative quantification of the target to the reference was determined by using the ΔC_T method if E for the target (GH, IGF-1) and the reference primers (β-Actin) are the same (Bio-Rad, 2006)

$Ratio_{\ (reference/\ target\ gene)} = Ef^{C_T(reference)\ -\ C_T(target)}$

Statistical analysis:

Body weight (BW), body weight gain (BWG) and gene expression data were analyzed as a one-way analysis of variance using the General Linear Model, SAS software (SAS INSTITUTE, 2004). Weight data were reported as least square means (LSM) ± standard errors (SEM). Gene expression data are expressed as means ± SEM. Mean values were separated, when significance is present, using Duncan′s Multiple Range Test (Duncan, 1955). Significance level was set at 5%.

3. Results

Effect of AFB_1 and yeast on growth performances:

Data presented in Table (1) showed the effect of dietary treatments on body weight (BW). Feeding AFB_1 alone suppressed the BW from the 3rd week (BW3) to week 7th week (BW7) compared to control. This deleterious effect of AFB_1 on BW was significant ($P \leq 0.05$) in the 6th week and the 7th week. The addition of (SC) at levels of 0.5-2.5 gm/kg to AFB_1-containing diets had ameliorated the adverse effect on BW. However, it was only significant during the 6th and 7th week of age.

Feeding AFB_1 alone also suppressed the BWG (Table 2) from the first week onwards and increased progressively until the end of the experimental period compared to controls. This suppression in weight gain was significant ($P \leq 0.05$) from the 6th to the 7th week of age and highly significant ($P \leq 0.01$) from 2nd to the 7th week of age (overall). The addition of SC to AFB_1 -containing diets improved the adverse effect of AFB_1 on BWG. This improvement was significant ($P \leq 0.05$) in group fed diet containing AFB_1 plus SC at 2.5 gm/kg diet level for the overall period.

Toxin analysis in some quail organs by using HPLC method:

As shown in Table (3), the concentrations of AFB_1 were higher in breast muscle (Figure 1[a-f]) and liver (Figure 2[a-f]) samples of quail fed diet containing AFB_1 than those found in samples of quail fed basal diet alone (control). The liver samples had the highest concentration of AFB_1. On the other hand, the amount of AFB_1 were reduced in breast muscle and liver samples that were collected from quail fed diets containing AFB_1 plus SC compared to those observed in samples of quail fed diets containing AFB_1 alone. The concentrations of AFB_1 in breast muscle and liver samples of quail fed diets containing AFB_1 plus high levels of SC (2.5 gm/kg) had the lowest amount of AFB_1 followed by the group that was fed diet containing AFB_1 plus 2.0 gm SC/kg diet.

Gene expression analysis:

Determination of the linear range of PCR amplification

The optimum values for the oligonucleotide primer concentrations were performed by using quail first strand cDNA as a template. The relationship between the CT value and the logarithm of the dilution factor of cDNA was evaluated under conditions that optimized the amplification of the target genes (GAPDH, N-cadherin, NCAM and P450scc). This evaluation was shown to be linear with a correlation coefficient > 0.99.

Gene expression of GAPDH, N-cadherin, NCAM and P450scc genes

The present results (Figures 3-5) revealed a significant ($p \leq 0.01$) decrease of gene expression levels of GAPDH, N-cadherin and NCAM genes in quail fed diet containing AFB_1 compared with those of the control group.

In contrary, the expression levels of GAPDH, N- cadherin and NCAM genes in quail fed diet containing AFB_1 plus low levels of SC (0.5 gm or 1.0 gm SC/kg diet) were higher than those found in quail fed diet containing AFB_1 alone. However, these differences were not statistically significant. On the other hand, the expression levels of GAPDH, N-cadherin and NCAM genes in the brain and liver samples collected from quail fed diet containing AFB_1 plus high levels of SC (2.0 gm or 2.5 gm SC/kg diet) were significantly ($p \leq 0.01$) higher than those observed in quail fed diet containing AFB_1 alone.

Table (1): Effect of different levels of *Sccharomyces cerevisiae* on body weight (BW) of Japanese quail fed diets containing aflatoxin B_1 from 14 to 49 days of age.

Treatments	BW2 LSM±SEM	BW3 LSM±SEM	BW4 LSM±SEM	BW5 LSM±SEM	BW6 LSM±SEM	BW7 LSM±SEM
BD (C)	49±2.3	102±3.7	161±4.5	207±5.5	241±7.2 ^{a}	268±10.3^{a}
BD + T	50±2.8	96±5.5	149±7.1	191±6.7	215±6.6 ^{b}	224±5.3 ^{c}
BD + T + SC_1	51±2.9	88±5.3	147±5.1	196±5.8	221±6.8 ^{ab}	228±8.1 ^{bc}
BD + T + SC_2	48±2.7	100±5.2	154±8.5	193±7.8	222±9.8 ^{ab}	232±9.4 ^{bc}
BD + T + SC_3	50±1.4	96±4.7	155±6.5	202±8.9	225±10.4 ^{ab}	232±10.6 ^{bc}
BD + T + SC_4	48±2.4	97±4.4	157±4.1	208±4.9	238±6.4 ^{ab}	253±7.2 ^{ab}

a-c means, within age group, followed by different superscripts, differ significantly ($P \leq 0.05$); BD (C) = Basal diet (control); T = AFB_1 (0.5mg/kg diet); SC_1 = 0.5 gm SC/kg diet; SC_2 = 1.0 gm SC /kg diet; SC_3 = 2.0 gm SC/kg diet; SC_4 = 2.5 gm SC /kg diet.

Table (2): Effect of different levels of *Sccharomyces cerevisiae* on body weight gain (BWG) of Japanese quail fed diet containing aflatoxin B_1 from 14 to 49 days of age.

Treatments	BWG2 LSM±SEM 2wks-3wks	BWG3 LSM±SEM 3wks-4wks	BWG4 LSM±SEM 4wks-5wks	BWG5 LSM±SEM 5wks-6wks	BWG6 LSM±SEM 6wks-7wks	BWG7 LSM±SEM 2wks-7wks
BD (C)	53±2.7^{a}	59±1.5	45±2.5^{ab}	34±5.3	26±6.0 ^{a}	218±10.3^{a}
BD + T	46±3.7^{ab}	53±3.0	43±3.0^{ab}	23±2.6	9±3.0 ^{b}	174±5.4 ^{c}
BD + T + SC_1	37±4.3^{b}	59±2.3	50±3.0^{a}	24±3.0	7±3.2 ^{b}	177±8.2 ^{c}
BD + T + SC_2	52±5.0^{a}	54±6.0	40±9.6^{b}	29±4.0	10±3.4 ^{b}	189±7.6 ^{bc}
BD + T + SC_3	48±4.0^{ab}	60±3.1	47±3.1^{ab}	24±3.7	6±2.6 ^{b}	183±9.7 ^{bc}
BD + T + SC_4	48±2.5^{ab}	59±3.6	51±2.7^{a}	31±2.5	15±2.7 ^{b}	203±6.8 ^{ab}

a-c means, within age group, followed by different superscripts, differ significantly ($P \leq 0.05$); BD (C) = Basal diet (control); T = AFB_1 (0.5mg/kg diet); SC_1 = 0.5 gm SC/kg diet; SC_2 = 1.0 gm SC /kg diet; SC_3 = 2.0 gm SC/kg diet; SC_4 = 2.5 gm SC /kg diet.

Table (3): Concentrations of AFB_1 in breast muscle and liver samples of quail fed different diets.

Quail Diets or treatments	Aflatoxin B_1 ppb µg/kg	
	Breast muscle	Liver
BD (C)	0.43	0.07
BD + T	4.37	8.64
BD + T + SC_1	4.01	5.93
BD + T + SC_2	2.19	2.74
BD + T + SC_3	1.37	1.95
BD + T + SC_4	0.86	0.94

BD (C) = Basal diet (control); T = AFB_1 (0.5mg/kg diet); SC_1 = 0.5 gm SC/kg diet; SC_2 = 1.0 gm SC /kg diet; SC_3 = 2.0 gm SC/kg diet; SC_4 = 2.5 gm SC /kg diet.

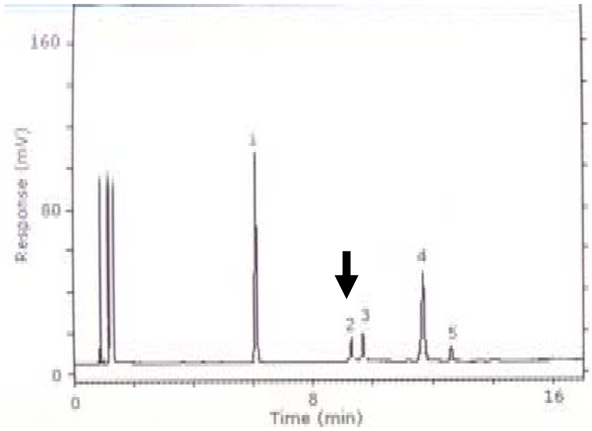

Fig. (a). Concentration of AFB$_1$ (0.43/ppb) in control group

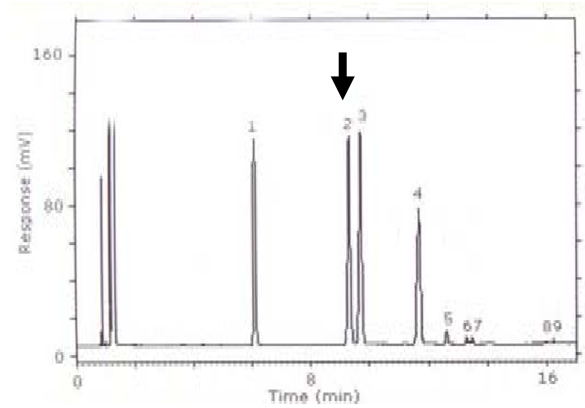

Fig. (b). Concentration of AFB$_1$ (4.37 ppb) in quail fed diet containing AFB$_1$ (0.5 mg/kg)

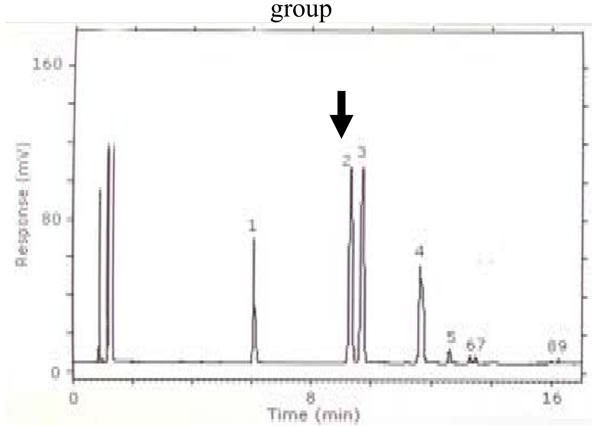

Fig. (c). Concentration of AFB$_1$ (4.01 ppb) in quail fed diet containing AFB$_1$ plus 0.5 gm SC/kg.

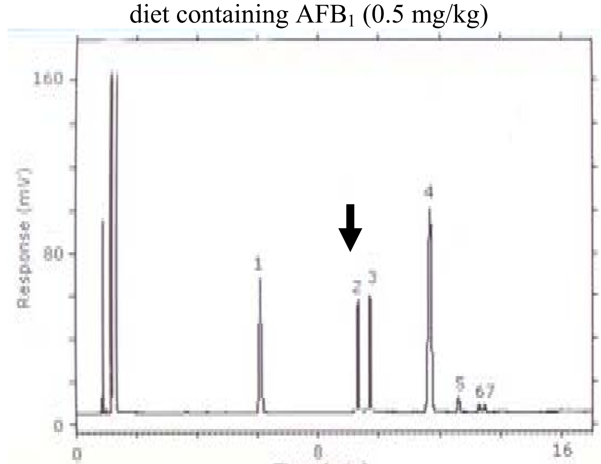

Fig. (d). Concentration of AFB$_1$ (2.19 ppb) in quail fed diet containing AFB$_1$ plus 1.0 gm SC/kg.

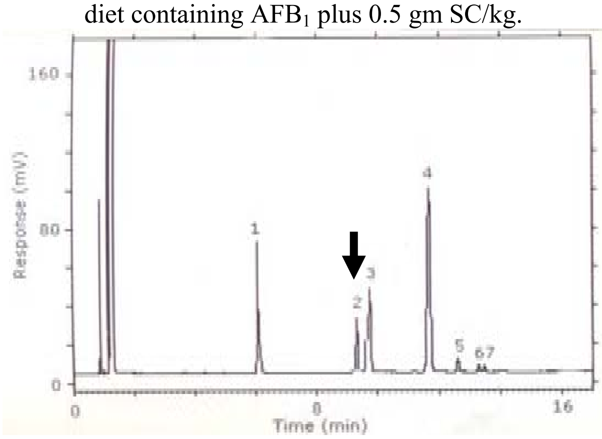

Fig. (e). Concentration of AFB$_1$ (1.37 ppb) in quail fed diet containing AFB$_1$ plus 2.0 gm SC/kg.

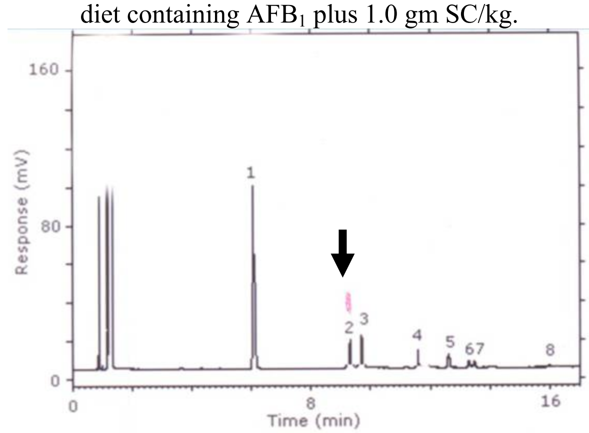

Fig. (f). Concentration of AFB$_1$ (0.86 ppb) in quail fed diet containing AFB$_1$ plus 2.5 gm SC/kg.

Fig. 1: Concentrations of AFB$_1$ as confirmed by HPLC method in quail breast muscle.

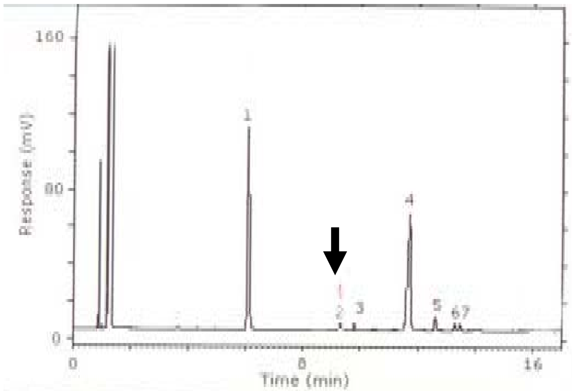

Fig. (a). Concentration of AFB_1 (0.071 ppb) in quail control group

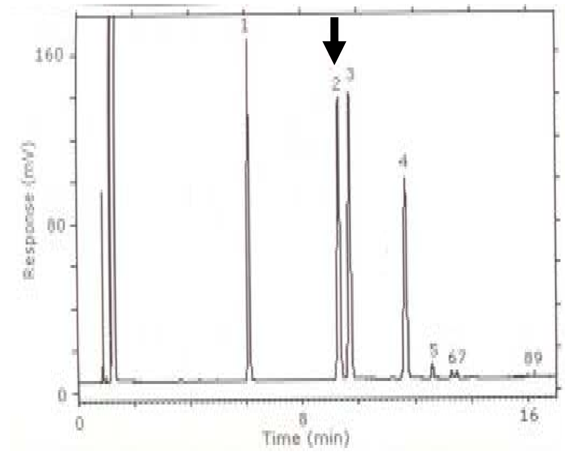

Fig. (b). Concentration of AFB_1 (8.639 ppb) in quail fed diet containing AFB_1 (0.5 mg/kg)

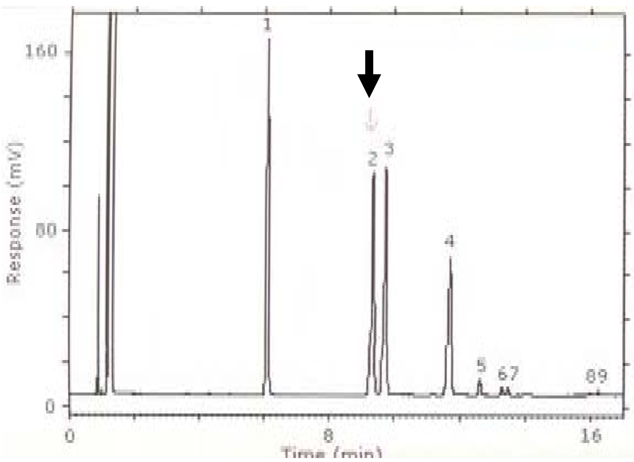

Fig. (c). Concentration of AFB_1 (5.93ppb) in quail fed diet containing AFB_1 plus 0.5 gm SC/kg.

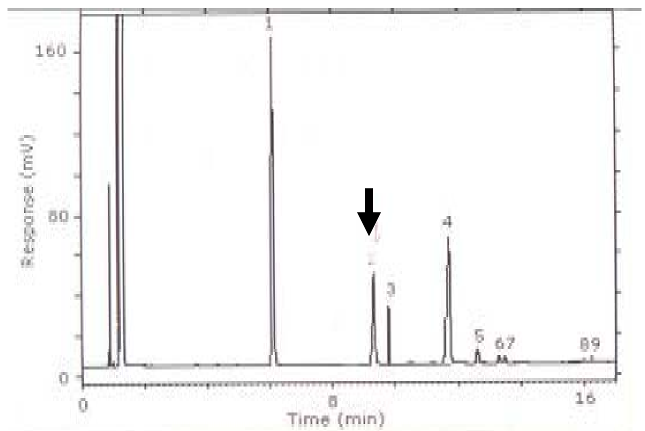

Fig. (d). Concentration of AFB_1 (2.74 ppb) in quail fed diet containing AFB_1 plus 1.0 gm SC/kg.

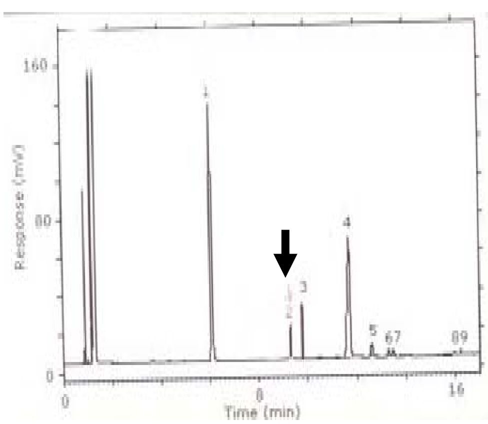

Fig. (e). Concentration of AFB_1 (1.95 ppb) in quail fed diet containing AFB_1 plus 2.0 gm SC/kg.

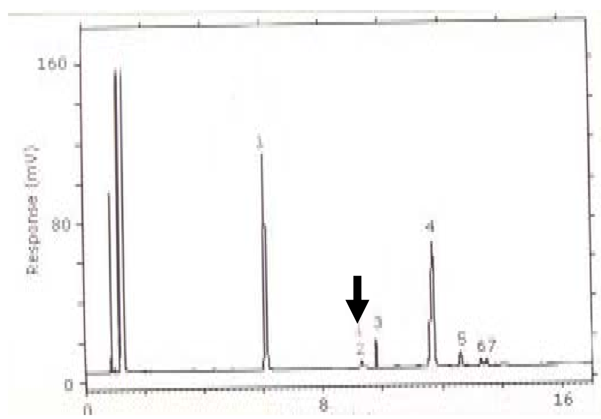

Fig. (f). Concentration of AFB_1 (0.935 ppb) in quail fed diet containing AFB_1 plus 2.5 gm SC/kg.

Fig. 2: Concentrations of AFB_1 as confirmed by HPLC method in quail liver.

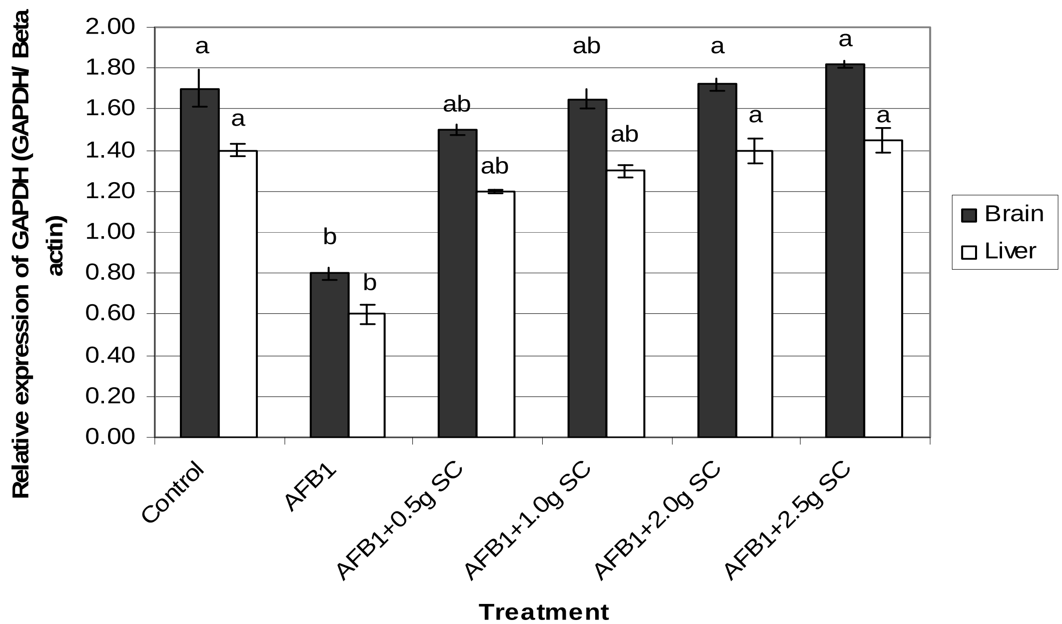

Fig. 3: Semi-quantitative Real Time-PCR analysis of GAPDH-mRNAs in brain and liver tissues collected from male quail (n=10) fed standard diet containing AFB_1 alone or AFB_1 combined with different concentrations of SC. Means with different letters, within tissue, differ significantly ($p \leq 0.05$).

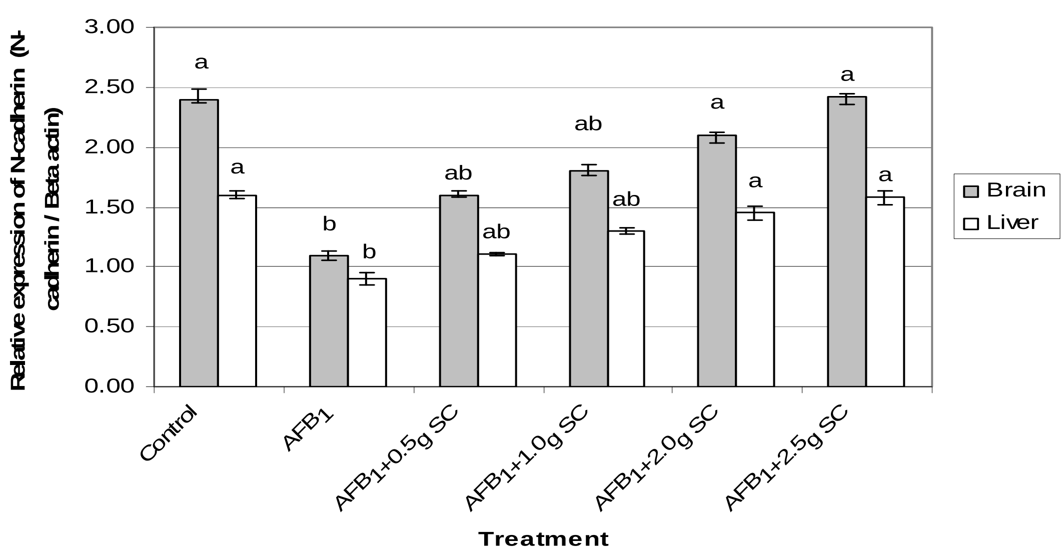

Fig. 4: Semi-quantitative Real Time-PCR analysis of N-cadherin-mRNAs in brain and liver tissues collected from male quail (n=10) fed standard diet containing AFB_1 alone or AFB_1 combined with different concentrations of SC. Means with different letters, within tissue, differ significantly ($p \leq 0.05$).

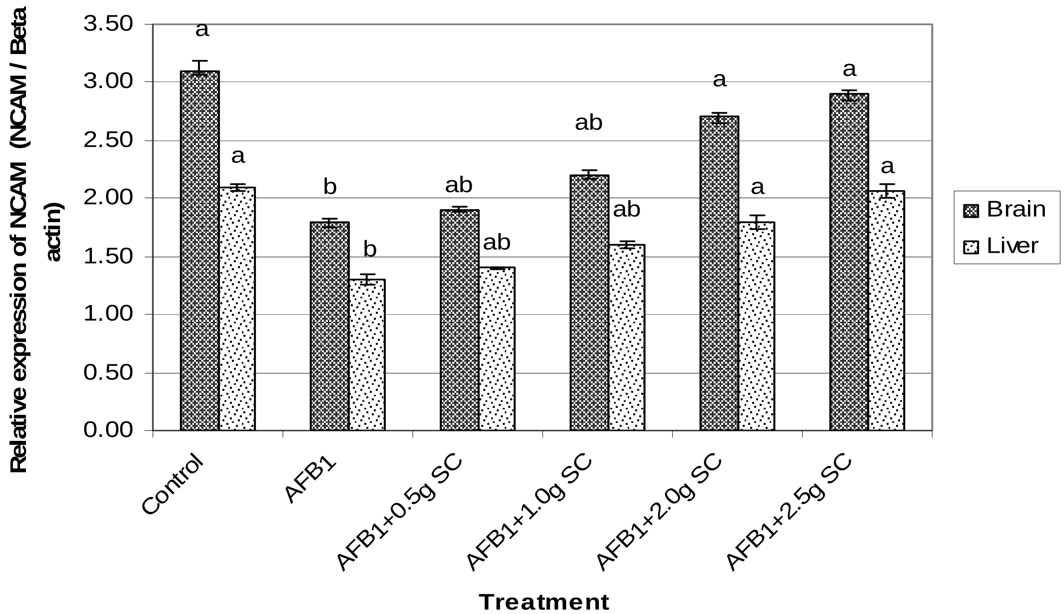

Fig. 5: Semi-quantitative Real Time-PCR analysis of NCAM-mRNAs in brain and liver tissues collected from male quail (n=10) fed standard diet containing AFB_1 alone or AFB_1 combined with different concentrations of SC. Means with different letters, within tissue, differ significantly ($p \leq 0.05$).

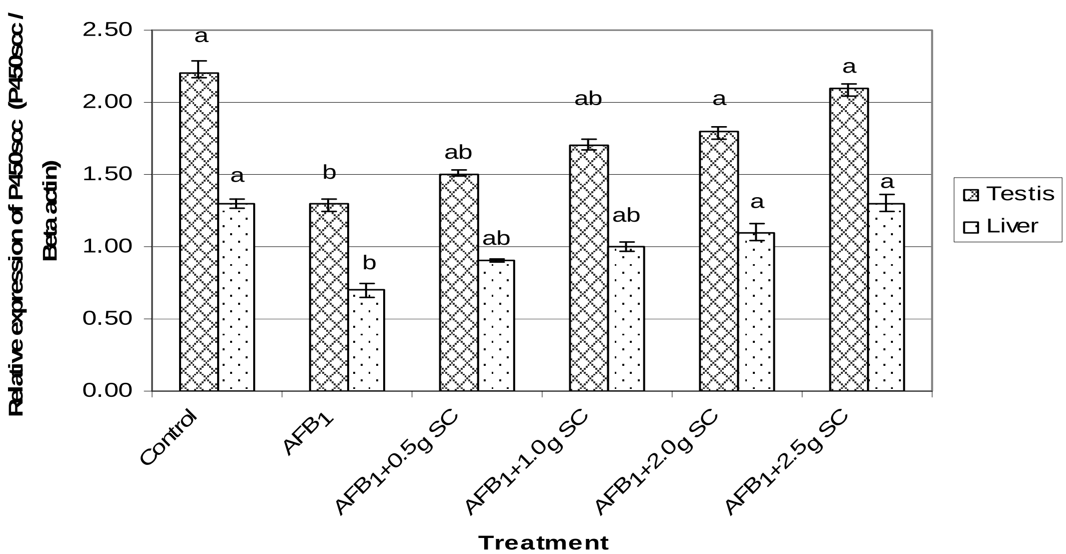

Fig. 6: Semi-quantitative Real Time-PCR analysis of P450scc-mRNAs in testis and liver tissues collected from male quail (n=10) fed standard diet containing AFB_1 alone or AFB_1 combined with different concentrations of SC. Means with different letters, within tissue, differ significantly ($p \leq 0.05$).

4. Discussion:

Regarding to P450scc gene, the present results (Figure 6) revealed that the expression level of this gene in testis and liver samples of quail fed diet containing AFB_1 was significantly ($p \leq 0.05$) low compared to those of the control group.

The expression level of P450scc gene in quail fed diet containing AFB_1 plus low level of SC (0.5 gm or 1.0 gm SC/kg diet) were higher than those detected in quail fed diet containing AFB_1 alone. However, these increases were not statistically significant. On the other hand, the gene expression level of P450scc in quail fed diet containing AFB_1 plus high levels of SC (2.0 gm or 2.5 gm SC/kg diet) were significantly higher ($p \leq 0.05$) compared with those found in quail fed diet containing AFB_1 alone.

In the present study, the quail consumed AFB_1 (0.5 mg/kg) containing diet showed poor body weight (BW) and body weight gain (BWG). These deleterious effects of AFB_1 on BW were statistically significant at 6 weeks ($P \leq 0.05$) and 7 weeks ($P \leq 0.01$) of age compared to control. Also the suppression of BWG was statistically significant ($P \leq 0.05$) from 6 to 7 weeks of age and highly significant ($P \leq 0.01$) from 2 to 7 weeks of age (overall). These results agree with other research on experimental aflatoxicosis in quail (Parlat *et al.*, 1999; Celik *et al.*, 2001 and Denli *et al.*, 2003); in broiler (Kubena *et al.*, 1998 and Celik *et al.*, 2001); and other poultry species (Kubena *et al.*, 1991 and Stewart *et al.*, 1998). These adverse effects of AFB_1 on BW and BWG may be due to anorexia, listlessness, inhibition of protein synthesis and lipogenesis (Campbell *et al.*, 1983; Oguz and Kurtoglu, 2000; Oguz *et al.*, 2000a,b and Parlat *et al.*, 2001). Moreover, Campbell *et al.* (1983) reported that the AF- contaminated feed decreased the activities of several enzymes, which are important to the digestion of carbohydrates, proteins, lipids and nucleic acid in broiler chicks. Also, Boden and Jensen (1985) stated that toxic effect induced by AF could have disrupted the activity of the digestive enzymes and the absorption of essential nutrients.

On the other hand, the present results showed that the addition of SC to AFB_1–containing diet significantly improved the adverse effect of AFB_1 on BW and BWG in quail. These findings were similar to that reported in quail by Parlat *et al.* (2001). They found that the addition of SC (1.0 gm/kg) to the AFB_1-containing diet, significantly elevated the adverse effects of AFB_1 (2.5 mg/kg diet) on BW and BWG in quail fed for 35 days. Our results were also supported by the study of Stanley *et al.* (1993) on broiler chicks, who observed significant amelioration of the adverse effects of AFB_1 (0.5 mg /kg) on performance in broilers fed for 28 days. Moreover, Abousadi *et al.* (2007) reported that the addition of SC (0.2%) to AFB_1-containing diet significantly decreased the adverse effect of AFB_1 (125 ppb) on BW and BWG in broiler chicks fed for 21 days.

The role of SC on the detoxification were attributed to its ability to produce biological enzymes that interacts with the AF molecules (Stanley *et al.*, 1993) and other growth promoting effects (Raju and Devegowda, 2000). It was also reported that yeast has been known to alter stress in animals by providing a source of vitamins, enzymes and growth protein for reducing stress, to enhance the biological value of nitrogen compounds along the digestive tract (Stanley *et al.*, 1993). Moreover the additional benefits of SC which were observed in the present study may be due to stimulation of the immune response (Savage *et al.*, 1996), alteration of intestinal microbial environment (Newman, 1994) and producing enzymes for gut micro flora to enhance the nutrients bioavailability (Stanley *et al.*, 1993; Raju and Devegowda, 2000; Parlat *et al.*, 2001 and Abousadi *et al.*, 2007).

The mode of action of yeast or its constituents (antioxidant compounds) against mutagenic or toxic effect of AFB_1 in animal cells (*in vivo*) may be due to binding with the mutagens or inhibition of activation of enzymes of cytochrome system-mediated N-hydroxylation with consequently enhancement of liver and kidney functions and reduction of abnormalities of genetic materials (Wang *et al.*, 2004 and Devaraj *et al.*, 2008).

As known, the liver is considered the principal target organ for Aflatoxins (Heathcote and Hibbert, 1978 and Phillips *et al.*, 1995). From the present results it was observed that the concentrations of AFB_1 were higher in liver samples than those found in breast muscle samples. These results are similar to those reported by Bintvihok *et al.* (2002) who found that the levels of AFB_1 and its metabolites, including acid-hydrolysable metabolites, were much higher (about 10-fold) in liver than those observed in muscle cells in all species of treated domestic fowls, with such toxin. Our findings were also supported by those results reported by Howard and Eaton (1990); Eaton and Groopman (1994); Cullen and Newberne (1994) and Smele and Curier (2001) who observed that the liver is considered the main organ in which the AFB_1 are metabolized by enzymes of cytochrome P450 group and converted to many metabolic product such aflatoxins Q_1, P_1 and M_1 and also Aflatoxin 8, 9 epoxide.

The present results showed that the concentrations of AFB_1 have been reduced in liver and breast muscle samples that were obtained from quail groups receiving AFB_1+SC than those found in the liver and breast muscle samples from quail receiving AFB_1 alone. As discussed above, the SC or its constituents (some of the carotenoids and vitamins) have the ability for detoxification of AFB_1 through interacting or binding with AFB_1 molecule (Stanley *et al.*, 1993 and Raju and Devegowda, 2000). Also, Gradelet *et al.* (1998) reported that carotenoids exert their protective effect through the deviation of AFB_1 metabolism towards detoxification pathways in rats. In quail, Denli *et al.* (2003) observed that the dietary vitamin A reduced the toxic effects of AFB_1 so yeast addition caused less toxicity in the liver and kidney than the AFB_1 group.

The present results revealed that the gene expression of neural cell adhesion molecule (GAPDH, N-cadherin- and NCAM) genes were down-regulated in the quail fed diet containing AFB_1. Such changes of gene expression of neural genes are characteristic due to the AFB_1 according to Kreutzberg (1995). The present findings are in agreement with those reported by Ahmed and Singh (1984) in chickens and by Ibegwuonu (1983) and Llewellyna *et al.* (1988) in rats. Those authors found that the concentrations of RNA in the nervous tissues were depressed by AFB_1 treatment. The breakdown of gene expression of neural genes which was observed in the present study may be due to retrograde signals from the nervous terminal as a result of AFB_1 treatment (Pershon *et al.*, 1989; Pahl and Baeuerle, 1996 and O′Neill and Kaltschmidt, 1998).

The present results also showed that the expression level of P450scc gene was down-regulated in quail fed diet containing AFB_1. These findings are supported by Macé *et al.* (1997) who found that treatment with AFB_1 induced DNA adduct formation and P^{53} mutations in CYP450 in human liver cell lines. Epidemiological studies by Bressac *et al.* (1991); Harris (1996) and Soini *et al.* (1996) showed a positive association between AFB_1 intake and a hot-spot guanine to thymine transversion mutation at the third base in the codon 249 of the P^{53} tumor suppressor gene. Moreover, some *in vitro* studies have provided indirect evidence for a mutative role of aflatoxin exposure in p^{53} mutagenesis by showing that AFB_1 causes primarily G-T transversions in bacteria (Foster *et al.*, 1993) or human cells (Trottier *et al.*, 1992 and Cariello *et al.*, 1994). Aflatoxins have been found to be a potent mutagenic food component (Hall *et al.*, 1988). This component is metabolized by the mixed function oxidase system to a number of hydroxylated metabolites and to aflatoxin 8, 9 epoxide which binds to DNA forming covalent adducts (Bushy and Wogan, 1984). The DNA adduct formation causes genomic instability including gene expression of animal genes (Soini *et al.*, 1996; Harris, 1996; Macé *et al.*, 1997; Huang and Kolodner, 2005 and Guardiola *et al.*, 2008).

On the other hand, the present results showed that SC was able to prevent the genetic alterations induced by AFB_1 in the quail tissues. Where, the mRNA concentrations were significantly increased in AFB_1 + SC groups compared to AFB_1 group in quail brain, liver and testis. To our knowledge, there is no available information about the use of SC as protective agents on gene expression of animal genes against the toxic effect of mycotoxins. However, some studies reported that the yeast cells contain high amounts of carotenoids, vitamins, minerals, and essential amino acids as well as the presence of B-D glucans on the cell wall of yeasts (Hussein *et al.*, 1996; Vetvicka, 2001; Brown and Gordon, 2003). These constituents are considered as antioxidant agents, that interrupts the free radical-initial chain reaction of oxidation or scavenge and disable free radicals (ROS) and reduced DNA-oxidative damage (Vasankari *et al.*, 1997; Sener *et al.*, 2007 and Oliveria, *et al.*, 2009) leading to genomic stability including gene expression of animal genes (Vetvicka, 2001; Brown and Gordon, 2003 and Van Breda *et al.*, 2005).

Furthermore, in a previous study, Park *et al.* (2000) identified five peroxiredoxins in the SC that were named Tsa1 (CTPX1), Tas2, AhP1, Dot5 and Prx1, of which Tsa1 possesses the most potent ability to scavenge H_2 O_2. Also, Huang and Koshland, (2003) reported that the Tsa1 is the most potent protector of genomic stability and prevents a broad spectrum of mutations.

In Conclusion; *Sccharomyces cerevisiae* yeast has the ability to reduce the toxic effect of AFB_1 in quail. It was also apparent that the higher the inclusion rates of SC in the diet of quail (2.5 mg/kg) the more the effective it is. This was apparent from the BW, BWG data and the level of AFB_1 in the different quail tissues and the gene expression data.

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