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 B₁ (AFB₁) 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 AFB₁/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 AFB₁-containing diet significantly reduced the adverse affect of AFB₁ on quail BW and BWG. The concentrations of AFB₁ had been lowered in the breast muscle and liver samples of quail fed diet containing AFB₁ plus SC than those found in such quail organs of AFB₁ group. The expression levels of neural and gonadal genes were significantly up-regulated in quail fed diet containing AFB₁ plus SC than those found in such quail organs of AFB₁ 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.

Key words: Quail; body weight; growth rate; yeast; aflatoxin B₁; 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, AFB₁ 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 AFB₁ in poultry, causes listlessness, anorexia with lowered growth rate, immunosuppression, 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 (mannan oligosaccharide) for minimizing the adverse effects of AF in poultry on the basis of biological
amelioration on the adverse effect of AFB1 contained feed and reported significant improvements on the adverse effects of AFB1 (5 mg/kg) in broiler chicks fed for 28 days (Stanely et al., 1993). Also, Raju and Devegowda (2000) extracted mannan oligosaccharide, which was believed to be responsible for the beneficial effect against AFB1 in Japanese quail. Therefore, the inclusion of SC (1gm/kg) to the AFB1-containing diet provided significant improvements on the adverse effects of AFB1 (0.3mg/kg) in broiler fed for 35 days. Moreover, Abousadi et al. (2007) observed that the addition of SC (0.2%) to AFB1-containing diet significantly improved the adverse effect of AFB1 (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 AFB-containing diet significantly reduced the deleterious effect of AFB1 (2.5mg/kg diet) on body weight and body weight gain in birds fed for 35 days (Parlat er al., 2001).

The present study was designed to investigate the role of SC in reducing aflatoxicosis caused by AFB1 in Japanese quail. Therefore, the measurements of growth performances and the concentrations of AFB1 in some quail organs were determined. Moreover, the expression levels of some neural genes were incorporated into the mixed feed before SC was added. These appropriate doses ranged from 0.5 to 6.0 mg of AFB1/kg diet.

Saccharomyces cerevisiae (SC): SC was obtained from Microbial Chemistry Department, National Research Centre, Giza, Egypt. SC 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 AFB1/kg diet; (3) basal diet plus 0.5 mg of AFB1 plus 0.5 gm of SC/kg diet; (4) basal diet plus 0.5 mg of AFB1 plus 1.0 gm of SC/kg diet (5) basal diet plus 0.5 mg of AFB1 and 2.0 gm of SC/kg diet (6) basal diet plus 0.5 mg of AFB1 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. AFB1 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 elute. AFB1 in the acetonitrile-methanol mixture was determined by an HPLC method 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 (P450ccc-F: 5′-ACA GCA GTT CAT CGA CGC CG -3′, P450ccc-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 qRT-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 ΔCT method if E for the target (GH, IGF-1) and the reference primers (β-Actin) are the same (Bio-Rad, 2006)

\[ \frac{C_{\text{reference}}}{C_{\text{target}}} \]

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 AFB1 and yeast on growth performances:

Data presented in Table (1) showed the effect of dietary treatments on body weight (BW). Feeding AFB1 alone suppressed the BW from the 3rd week (BW3) to week 7th week (BW7) compared to control. This deleterious effect of AFB1 on BW was significant (P ≤ 0.05) in the 6th week and the 7th week. The addition of (SC) at levels of 0.5-2.5 gm/kg to AFB1-containing diets ameliorated the adverse effect on BW. However, it was only significant during the 6th and 7th week of age.

Feeding AFB1 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 ≤ 0.05) from the 6th to the 7th week of age and highly significant (P ≤ 0.01) from 2nd to the 7th week of age (overall). The addition of SC to AFB1-containing diets improved the adverse effect of AFB1 on BWG. This improvement was significant (P ≤ 0.05) in group fed diet containing AFB1 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 AFB1 were higher in breast muscle (Figure 1[a-f]) and liver (Figure 2[a-f]) samples of quail fed diet containing AFB1 than those found in samples of quail fed basal diet alone (control). The liver samples had the highest concentration of AFB1. On the other hand, the amount of AFB1 were reduced in breast muscle and liver samples that were collected from quail fed diets containing AFB1 plus SC compared to those observed in samples of quail fed diets containing AFB1 alone. The concentrations of AFB1 in breast muscle and liver samples of quail fed diets containing AFB1 plus high levels of SC (2.5 gm/kg) had the lowest amount of AFB1 followed by the group that was fed diet containing AFB1 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 ≤ 0.01) decrease of gene expression levels of GAPDH, N-cadherin and NCAM genes in quail fed diet containing AFB1 compared to those of the control group.

In contrary, the expression levels of GAPDH, N-cadherin and NCAM genes in quail fed diet containing AFB1 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 AFB1 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 AFB1 plus high levels of SC (2.0 gm or 2.5 gm SC/kg diet) were significantly (p ≤ 0.01) higher than those observed in quail fed diet containing AFB1 alone.

Table (1): Effect of different levels of *Saccharomyces cerevisiae* on body weight (BW) of Japanese quail fed diets containing aflatoxin B1 from 14 to 49 days of age.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>BW2 LSM±SEM</th>
<th>BW3 LSM±SEM</th>
<th>BW4 LSM±SEM</th>
<th>BW5 LSM±SEM</th>
<th>BW6 LSM±SEM</th>
<th>BW7 LSM±SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>BD (C)</td>
<td>49±2.3</td>
<td>102±3.7</td>
<td>161±4.5</td>
<td>207±5.5</td>
<td>241±7.2</td>
<td>268±10.3</td>
</tr>
<tr>
<td>BD + T</td>
<td>50±2.8</td>
<td>96±5.5</td>
<td>149±7.1</td>
<td>191±6.7</td>
<td>215±6.6</td>
<td>224±5.3</td>
</tr>
<tr>
<td>BD + T + SC1</td>
<td>51±2.9</td>
<td>88±5.3</td>
<td>147±5.1</td>
<td>196±5.8</td>
<td>221±6.8</td>
<td>228±8.1</td>
</tr>
<tr>
<td>BD + T + SC2</td>
<td>48±2.7</td>
<td>100±5.2</td>
<td>154±8.5</td>
<td>193±7.8</td>
<td>222±9.8</td>
<td>232±9.4</td>
</tr>
<tr>
<td>BD + T + SC3</td>
<td>50±1.4</td>
<td>96±4.7</td>
<td>155±6.5</td>
<td>202±8.9</td>
<td>225±10.4</td>
<td>232±10.6</td>
</tr>
<tr>
<td>BD + T + SC4</td>
<td>48±2.4</td>
<td>97±4.4</td>
<td>157±4.1</td>
<td>208±4.9</td>
<td>238±6.4</td>
<td>253±7.2</td>
</tr>
</tbody>
</table>

a-c means, within age group, followed by different superscripts, differ significantly (P≤0.05); BD (C) = Basal diet (control); T = AFB1 (0.5mg/kg diet); SC1 = 0.5 gm SC/kg diet; SC2 = 1.0 gm SC /kg diet; SC3 = 2.0 gm SC/kg diet; SC4 = 2.5 gm SC /kg diet.

Table (2): Effect of different levels of *Saccharomyces cerevisiae* on body weight gain (BWG) of Japanese quail fed diet containing aflatoxin B1 from 14 to 49 days of age.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>BWG2 LSM±SEM 2wks-3wks</th>
<th>BWG3 LSM±SEM 3wks-4wks</th>
<th>BWG4 LSM±SEM 4wks-5wks</th>
<th>BWG5 LSM±SEM 5wks-6wks</th>
<th>BWG6 LSM±SEM 6wks-7wks</th>
<th>BWG7 LSM±SEM 2wks-7wks</th>
</tr>
</thead>
<tbody>
<tr>
<td>BD (C)</td>
<td>53±2.7ab</td>
<td>59±1.5</td>
<td>45±2.5ab</td>
<td>34±5.3</td>
<td>26±6.0ab</td>
<td>218±10.3ab</td>
</tr>
<tr>
<td>BD + T</td>
<td>46±3.7ab</td>
<td>53±3.0</td>
<td>43±3.0ab</td>
<td>23±2.6</td>
<td>9±3.0b</td>
<td>174±5.4c</td>
</tr>
<tr>
<td>BD + T + SC1</td>
<td>37±4.3b</td>
<td>59±2.3</td>
<td>50±3.0a</td>
<td>24±3.0</td>
<td>7±3.2b</td>
<td>177±8.2c</td>
</tr>
<tr>
<td>BD + T + SC2</td>
<td>52±5.0a</td>
<td>54±6.0</td>
<td>40±9.6b</td>
<td>29±4.0</td>
<td>10±3.4b</td>
<td>189±7.6bc</td>
</tr>
<tr>
<td>BD + T + SC3</td>
<td>48±4.0ab</td>
<td>60±3.1</td>
<td>47±3.1ab</td>
<td>24±3.7</td>
<td>6±2.6b</td>
<td>183±9.7bc</td>
</tr>
<tr>
<td>BD + T + SC4</td>
<td>48±2.5ab</td>
<td>59±3.6</td>
<td>51±2.7ab</td>
<td>31±2.5</td>
<td>15±2.7b</td>
<td>203±6.8ab</td>
</tr>
</tbody>
</table>

a-c means, within age group, followed by different superscripts, differ significantly (P≤0.05); BD (C) = Basal diet (control); T = AFB1 (0.5mg/kg diet); SC1 = 0.5 gm SC/kg diet; SC2 = 1.0 gm SC /kg diet; SC3 = 2.0 gm SC/kg diet; SC4 = 2.5 gm SC /kg diet.

Table (3): Concentrations of AFB1 in breast muscle and liver samples of quail fed different diets.

<table>
<thead>
<tr>
<th>Quail Diets or treatments</th>
<th>Aflatoxin B1 ppb µg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Breast muscle</td>
</tr>
<tr>
<td>BD (C)</td>
<td>0.43</td>
</tr>
<tr>
<td>BD + T</td>
<td>4.37</td>
</tr>
<tr>
<td>BD + T + SC1</td>
<td>4.01</td>
</tr>
<tr>
<td>BD + T + SC2</td>
<td>2.19</td>
</tr>
<tr>
<td>BD + T + SC3</td>
<td>1.37</td>
</tr>
<tr>
<td>BD + T + SC4</td>
<td>0.86</td>
</tr>
</tbody>
</table>

BD (C) = Basal diet (control); T = AFB1 (0.5mg/kg diet); SC1 = 0.5 gm SC/kg diet; SC2 = 1.0 gm SC /kg diet; SC3 = 2.0 gm SC/kg diet; SC4 = 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$_1$ (5.93 ppb) 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 ≤ 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 ≤ 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 AFB1 alone or AFB1 combined with different concentrations of SC. Means with different letters, within tissue, differ significantly (p ≤ 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 AFB1 alone or AFB1 combined with different concentrations of SC. Means with different letters, within tissue, differ significantly (p ≤ 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 AFB1 was significantly (p ≤ 0.05) low compared to those of the control group.

The expression level of P450scc gene in quail fed diet containing AFB1 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 AFB1 alone. However, these increases were not statistically significant. On the other hand, the gene expression level of P450scc in quail fed diet containing AFB1 plus high levels of SC (2.0 gm or 2.5 gm SC/kg diet) were significantly higher (p ≤ 0.05) compared with those found in quail fed diet containing AFB1 alone.

In the present study, the quail consumed AFB1 (0.5 mg/kg) containing diet showed poor body weight (BW) and body weight gain (BWG). These deleterious effects of AFB1 on BW were statistically significant at 6 weeks (P ≤ 0.05) and 7 weeks (P ≤ 0.01) of age compared to control. Also the suppression of BWG was statistically significant (P ≤ 0.05) from 6 to 7 weeks of age and highly significant (P ≤ 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 AFB1 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 AFB1-containing diet significantly improved the adverse effect of AFB1 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 AFB1-containing diet, significantly elevated the adverse effects of AFB1 (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 AFB1 (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 AFB1-containing diet significantly decreased the adverse effect of AFB1 (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 AFB1 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 AFB1 in breast muscle samples were significantly 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 AFB1 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 AFB1 are metabolized by enzymes of cytochrome P450 group and converted to many metabolic product such aflatoxins Q1, P1 and M1 and also Aflatoxin 8, 9 epoxide.

The present results showed that the concentrations of AFB1 have been reduced in liver and breast muscle samples that were obtained from quail groups receiving AFB1+SC than those found in
the liver and breast muscle samples from quail receiving AFB1 alone. As discussed above, the SC or its constituents (some of the carotenoids and vitamins) have the ability for detoxification of AFB1 through interacting or binding with AFB1 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 AFB1 metabolism towards detoxification pathways in rats. In quail, Denli et al. (2003) observed that the dietary vitamin A reduced the toxic effects of AFB1 so yeast addition caused less toxicity in the liver and kidney than the AFB1 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 AFB1. Such changes of gene expression of neural genes are characteristic due to the AFB1 according to Kreutzberg (1995). The present findings are in agreement with those reported by Ahmed and Singh (1984) in chickens and by Ibegwunu (1983) and Llewellyna et al. (1988) in rats. Those authors found that the concentrations of RNA in the nervous tissues were depressed by AFB1 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 AFB1 treatment (Pershon et al., 1989; Pahl and Baeuerele, 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 AFB1. These findings are supported by Macé et al. (1997) who found that treatment with AFB1 induced DNA adduct formation and P53 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 AFB1 intake and 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 AFB1 treatment (Pershon et al., 1989; Pahl and Baeuerele, 1996 and O’Neill and Kaltschmidt, 1998).

On the other hand, the present results showed that SC was able to prevent the genetic alterations induced by AFB1 in the quail tissues. Where, the mRNA concentrations were significantly increased in AFB1 + SC groups compared to AFB1 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-initiation chain reaction of oxidation or scavenges 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), Tsa2, AhP1, Dot5 and Prx1, of which Tsa1 possesses the most potent ability to scavenge H2O2. 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 AFB1 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 AFB1 in different quail tissues and the gene expression data.

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