AMELIORATIVE IMPACTS OF GRAPE SEED EXTRACT ON GROWTH PERFORMANCE, IMMUNE RESPONSE, ANTIOXIDANT CAPACITY AND BIOCHEMICAL CONSTITUENTS IN BROILERS EXPOSED TO FUMONISIN B1

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SUMMARY

Fumonisin B1 (FB1) is a mold metabolite produced by Fusarium species that is frequently found in corn worldwide. It is toxic to both liver and kidney. This research aimed to assess the influence of grape seed extract (GSE) on reducing toxic influences of Fumonisin B1 (FB1) in broilers by examining the growth performance, immune response, antioxidant capacity and biochemical constituents. A total of 240 one-day-old Ross 308 chicks were randomly allocated into four treatments of six replicates each (10 birds per replicate), fed ad libitum for five weeks with the following dietary treatments: 1. Basal diet (control); 2. Basal diet + 400 mg/kg FB1 contaminated diet (FB1); 3. Basal diet + FB1 (400 mg/kg) + GSE (250 mg/kg); and 4. Basal diet + FB1 (400 mg/kg) + GSE (500 mg/kg). Results revealed that inclusion of FB1 in the diet of birds resulted in deleterious effects on all parameters traits included in this study. The addition of GSE at levels of 250 or 500 mg/kg of diet to the FB1–containing diet significantly improved the adverse effects of FB1 on growth performance, serum immunoglobulin contents, serum biochemical contents, and enzyme activities in the liver, malondialdehyde content and total antioxidant capacity and glutathione peroxidase concentration within the liver and serum. Moreover, 500 mg GSE / kg of diet surpasses the other level 250 mg GSE /kg diet concerning all above–mentioned the parameters. In conclusion, broilers performance, some blood constituents, oxidative stress and liver function were significantly affected by FB1 (400 mg) treatment; the addition of GSE (250 or 500 mg/kg of diet ) to the FB1–contaminated diet significantly recovered the adverse effects of FB1 on these traits; the protective effect of 500 mg GSE /kg of diet against the toxic effects of FB1 was greater than that of 250 mg GSE/kg of diet; and these improvements may contribute to a solution of FB1 problems in broiler chickens.

Keywords: Grape seed; Fumonisin B1; growth traits; oxidative stress; broilers.

INTRODUCTION

Fumonisins, which are secondary metabolites of Fusarium verticilloides, were discovered and chemically characterised for the first time in 1988 (Gelderblom et al., 1988). Since then, around 28 homologues have been found. However, Fumonisin B1 (FB) is the most typical and extensively researched homologue from a toxicological perspective. Others, like FB2, FB3, and FB4, are less common (Voss et al., 2007). The principal mode of action suggested for FB is that it inhibits the ceramide synthase enzyme, preventing the turnover of sphingoid molecules. The chemical structure of FB is similar to that of the sphingoid bases (sphinganine and sphingosine). Because ceramide synthase is inhibited, sphingoid bases—primarily sphinganine—accumulate and have proapoptotic, citotoxic, and growth-inhibitory effects. Therefore, in many animal species, the sphinganine-to-sphingosine ratio (Sa:So) is the most sensitive biomarker to FB intoxication (Voss et al., 2007). Fumonisin B1 (FB) contamination in feed is now linked to two significant animal diseases/syndromes: equine leukoencephalomalacia and swine pulmonary edoema (Marasas et al., 1988). According to several studies, FB may play a role in various human cancer instances, including esophageal (Dragan et al., 2001) and liver (Ueno et al., 1997) malignancies. There is general agreement that FB causes losses when contaminated feed is given to broilers (Del Bianchi et al., 2005), although there is disagreement about specific factors FB1 affects.

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Growing interest in plant-derived natural compounds as low harmful and prospective therapeutic approaches has recently been noted by researchers. Due to its strong antioxidant capacity, which is roughly 20 times stronger than that of vitamin E and 50 times greater than that of vitamin C, grape seed, a naturally occurring agricultural by-product of grapes, is a better source of antioxidative constituents than grape juice by-products (Abdou et al., 2021). Hepatic lipid peroxidation is prevented by grape seed extract (GSE) because it protects the cellular membrane from oxidative damage (Deng et al., 2020). In addition to its anti-microbial properties, GSE has been shown to have anti-cancer, anti-inflammatory, cardio-, neuro-, and hepatoprotective effects, as well as antidiabetic, anti-obesity, and lipid-lowering properties (Othman et al., 2020). Notably, GSE was demonstrated to reduce oxidative stress and liver damage in the liver of zearalenone-intoxicated patients (Taranu et al., 2020). This study's goal was to assess the toxic effects of FB1 and the protective effectiveness of GSE on the growth performance, serum biochemistry, serum immunoglobulins, serum, and liver antioxidant enzyme activities of broilers exposed to FB1-contaminated feed.

**MATERIALS AND METHODS**

**Experimental design and Management:**

For the trial, 240 one-day-old Ross-308 broilers were acquired from a local hatchery. Chicks with comparable body weight were randomly divided into four groups with six replicates per group (n = 60 per treatment) and grouped based on the next four dietary treatments; 1. A basal diet containing neither GSE nor FB1 (Control); 2. A basal diet containing 400 mg/kg FB1 (FB1), 3. Basal diet containing 400 mg/kg FB1 + 250 mg/kg GSE, and 4. Basal diet containing 400 mg/kg FB1 + 500 mg/kg GSE. Diets and water were provided *ad libitum* through the whole experimental time (5 weeks). The trial conducted under environmental controlled conditions.

**Experimental diets:**

A corn and soybean meal-based basal diet was formulated to meet the standard nutritional requirements of broilers in initial phase (NRC, 1994). The composition of the basal diet has been presented in Table 1.

**Table (1): Composition and calculated analysis of starter-grower and finisher diets.**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Starter-grower (1-21d)</th>
<th>Finisher (22-35d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yellow corn</td>
<td>54.40</td>
<td>62.00</td>
</tr>
<tr>
<td>Soybean meal, 44%</td>
<td>27.00</td>
<td>24.05</td>
</tr>
<tr>
<td>Corn Gluten meal, 60%</td>
<td>10.00</td>
<td>6.19</td>
</tr>
<tr>
<td>Soy bean oil</td>
<td>4.55</td>
<td>4.00</td>
</tr>
<tr>
<td>Limestone</td>
<td>1.10</td>
<td>1.00</td>
</tr>
<tr>
<td>Di-calcium phosphate</td>
<td>2.20</td>
<td>2.05</td>
</tr>
<tr>
<td>Vit &amp; min. premix*</td>
<td>0.30</td>
<td>0.30</td>
</tr>
<tr>
<td>DL-Methionine</td>
<td>0.05</td>
<td>0.01</td>
</tr>
<tr>
<td>L-lysine (HCl)</td>
<td>0.15</td>
<td>0.15</td>
</tr>
<tr>
<td>Na Cl</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

**Calculated analysis: **

- CP, %: 23.03, 20.02
- ME (Kcal/kg): 3004, 3201
- Calcium, %: 1.05, 0.97
- Available phosphorus, %: 0.45, 0.42
- Lysine, %: 1.14, 1.03
- Methionine, %: 0.52, 0.41
- TSAA, %: 0.90, 0.73

*Vitamin and mineral premix (Hero mix) produced by Hero pharm and composed (per 3 kg) of vitamin A 12,000,000 IU, vitamin D3 2,500,000 IU, vitamin E 10,000 mg, vitamin K3 2,000 mg, vitamin B1 1,000 mg, vitamin B2 5,000 mg, vitamin B6 1,500 mg, vitamin B12 10 mg, niacin 30,000 mg, biotin 50 mg, folic acid 1,000 mg, pantothenic acid 10,000 mg, manganese 60,000 mg, zinc 50,000 mg, iron 30,000 mg, copper 4,000 mg, iodine 300 mg, selenium 100 mg, and cobalt 100 mg. **According to Tables of NRC (1994).
**FB1 production:**

The National Institute of Animal Health, Dokki, Cairo, Egypt, was the site of fumonisin B1 production. 100 g of ground maize was placed to 0.946 -l jars along with 100 ml of distilled water, and the jars were autoclaved for 30 minutes at 121°C (Weibking et al., 1993). Fusarium verticilloides MRC-826 was added to sterile distilled water, and 2 ml of the resulting suspension was added to the autoclaved jars. After 24 h of incubation at 27°C in the dark, the jars were then shaken in a horizontal shaker at 350 rpm for 15 minutes. The jars were shaken again (350 rpm for 15 min) to achieve thorough dispersion of the fungal mycelium. The jars were incubated in the dark at 27°C for a total of 5 weeks. A 400 ml mixture of acetone: chloroform (75:25) was added immediately to each 5-week-old culture and allowed to stand overnight. The contents of each jar were placed in a blender for 30 s and then filtered. The solid culture residue was reextracted and allowed to air-dry overnight in the hood. The culture material was dried in a forced-air oven at 40°C for 48 h and ground to a soft powder in a grindery. The culture material was analyzed for FB1 by high performance liquid chromatography (Wilson et al., 1990), which found concentrations of 6000 mg FB1 /kg.

**Preparation of GSE Pre-extraction sample:**

Grape seeds were carefully separated from grape skin and stem, washed in tap water, and then allowed to dry outdoors out of the sun. Seeds were ground in a coffee grinder for two minutes at intervals of 15 seconds. To prevent the sample from heating up; the crushed seeds were covered and kept at -18°C until the extractions were carried out (Palma et al., 1999).

**Extraction process:**

To a thickness of 2 mm, the grape seed was ground. 25% dichloromethane and 75% methanol were used to extract the seeds from grapes. Grape seed extract's phenolic components were examined using chromatographic separation which was performed at 30°C using Iacopini et al. (2008) method. The amounts of the phenolic compounds in GSE are listed in Table 2.

**Collection of samples and measurements:**

Up to the completion of the trial (five weeks), feed consumption for each replicate was tracked weekly. Chickens were weighed on a weekly basis. Feed conversion ratio (FCR), total feed intake, average body weight gain, and final body weight were computed. Following a 12-hour fast, blood samples were obtained from the chickens during slaughtered and placed in tubes. The serum was isolated from the blood samples and stored at −20°C for biochemical, immunoglobulin, and serum antioxidant analyses. The blood samples were centrifuged at 1000× g for 10 min at 4°C. After slaughtered, the liver was taken out, and it was promptly weighed. To analyzing the antioxidants, a portion of liver was quickly frozen in liquid nitrogen and kept at −80 °C.

**Serum sample analysis:**

Using commercial kits (Biodiagnostic Company, Egypt) in a spectrophotometer, serum samples were examined for total proteins, albumin, total cholesterol, LDL, HDL, calcium (Ca), phosphorus (P), uric acid, alkaline phosphatase (AP), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) activity. According to Rauber et al. (2013), free sphinganine and sphingosine levels in serum were examined for the computation of the sphinganine-to-sphingosine ratio (Sa:So).

**Serum and liver antioxidant enzymes assays:**

Small liver tissue samples (0.5 g) were chopped up and homogenized in 4.5 ml ice-cold physiological saline. The homogenate was centrifuged at 1000 g for 15 min at 4°C. For the next analysis, the supernatant was gathered and kept at -80°C. Using commercially available assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China), the activities of glutathione peroxidase (GSH-Px), total antioxidant capacity (TAC), and malondialdehyde (MDA) concentration in the blood serum and hepatic supernatants were measured spectrophotometrically. The specifics of each determination procedure followed by the manufacturer's instructions for the commercial kits.

**Immune function:**

Using the ELISA method, the levels of immunoglobulin A, immunoglobulin M, immunoglobulin G (IgG), interleukin-2 (IL-2), interleukin-6, tumour necrosis factor- α (TNF-α), and interferon-γ (IFN-γ) in the serum were determined according to (Ao and Kim, 2019).
Jejunum morphology:
During slaughtered, tiny intestinal tissue samples were taken from the jejunum, which were about 2 cm long each, to determine the shape of the mucosa. The jejunum tissues were washed with saline before being preserved in 10% neutral formalin. For mucosal morphology and integrity, the fixed tissues were cut and embedded in paraffin. For histological analysis using an optical microscope (Olympus, Tokyo, Japan), thin sections (5 mm) were cut into slices, mounted on slides, and stained with hematoxylin and eosin. Villus height (VH) and crypt depth (CD) were morphologically examined in the jejunum that were measured. Accordingly, the villus height to crypt depth ratio (VH/CD) was determined. The methodology previously outlined was used to quantify these indices (Viveros et al., 2011). Within each section (10 villi per bird), the means for VH, CD, and VH/CD were computed.

Ileal microflora:
According to a prior study by Giannenas et al. (2010), fresh cecum digesta samples were obtained during slaughter and analyzed for microorganisms within one hour. Briefly, E. coli and Lactobacillus in digesta samples were counted by conventional microbiological methods and selective agar media after being serially diluted in 0.85 percent sterile saline solution. The average findings of each of the microbiological tests were used for statistical analysis after being performed in duplicate.

Statistical analysis:
Results from all response variables have been subjected to one-way variance analysis (SAS, 2004). Using Duncan's Multiple Range Test (Duncan, 1955), mean of variables with a significant F-test (p<0.05) were compared.

Model:
\[ X_{ij} = \mu + Ti + e_{ij} \]

Where: \( X_{ij} \) = Any observation; \( \mu \) = Overall mean; \( Ti \) = Treatments (i = 1, 2 . . . and 4); \( e_{ij} \) = Experimental error.

RESULTS AND DISCUSSION
Grape seed composition and extract analysis:
The chemical composition of the grape seed (crude fat, crude protein, crude fiber, calcium, and total phosphorus) and extract analysis (catechin, epicatechin and procyanidins) are shown in Table 2.

Table (2): Composition of the grape seed analyzed by AOAC (2005) methods and analysis of the grape seed extract by HPLC method.

<table>
<thead>
<tr>
<th>Grape seed</th>
<th>Grape seed extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude fat (%)</td>
<td>24.12</td>
</tr>
<tr>
<td>Crude protein (%)</td>
<td>10.25</td>
</tr>
<tr>
<td>Crude fiber (%)</td>
<td>34.22</td>
</tr>
<tr>
<td>Calcium (%)</td>
<td>0.62</td>
</tr>
<tr>
<td>Phosphorus (%)</td>
<td>0.36</td>
</tr>
</tbody>
</table>

Growth performance:
Table (3) illustrates the impact of adding grape seed extract (GSE) to a diet polluted with fumonisin to counteract the harmful effects of fumonisin B1 on growth performance in broilers fed for 35 days. The group given the FB1-contaminated meal (400 mg/kg) for the entire trial period had the lowest average final body weight, body weight gain, and cumulative feed intake (P<0.05) in comparison to other groups. This impact was alleviated by the addition of GSE (250 and 500 mg/kg) into diets contaminated with FB1, with a significant rise in final body weight, body weight gain and feed intake when compared with the FB1 group. Similarly, feed conversion ratio (FCR) of broilers was negatively influenced by the dietary FB1 group through the experimental period (P< 0.05). The addition of GSE (250 and 500 mg/kg)
resulted in markedly better FCR (P< 0.05) as compared with the FB1 group. However, 500 mg GSE /kg diet recorded the highest values of body weight gain and feed intake and better values of FCR, and mortality rate compared to 250 mg GSE/kg diet included in this study.

These findings showed how GSE helped to reduce negative effects of FB on growth efficiency. According to these findings, which are in line with those of Sharma et al. (2008), giving diets supplemented with 200 ppm of fumonisn B1 and 100 ppm of moniliformin (produce from Fusarum fujikuroi) caused anorexia, diarrhoea, and severe depression of body weight. According to Bermudez et al. (1997), dietary FB1 levels less than 325 mg/kg of feed had no effect on the feed intake and BW gain of chicks. Previous investigations using chicks, pouls, and ducklings fed 100 mg of moniliformin/kg of diet found reduced feed intake and BW gain (Morris et al., 1997). Birds receiving 80 to 330 mg/kg of moniliformin by day 8 had significantly lower body weights, according to Reams et al. (1997).

Table (3): Effects of grape seed extract (GSE) on growth performance of broilers fed diets contaminated with Fumonisin B1.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>FB1 (400mg/kg)</th>
<th>FB1+GSE (250mg/kg)</th>
<th>FB1+GSE (500mg/kg)</th>
<th>SEM</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IBW, (g)</td>
<td>40.2</td>
<td>40.2</td>
<td>40.3</td>
<td>40.2</td>
<td>1.26</td>
<td>0.962</td>
</tr>
<tr>
<td>FBW, (g)</td>
<td>2150&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1905&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2000&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2050&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>6.14</td>
<td>0.0001</td>
</tr>
<tr>
<td>BWG, (g)</td>
<td>2109.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1864.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1959.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2009.8&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>5.85</td>
<td>0.0001</td>
</tr>
<tr>
<td>TFI, (g/bird)</td>
<td>3405&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3380&lt;sup&gt;f&lt;/sup&gt;</td>
<td>3400&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3405&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.05</td>
<td>0.002</td>
</tr>
<tr>
<td>FCR</td>
<td>1.635&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.812&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.734&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.694&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.45</td>
<td>0.0001</td>
</tr>
<tr>
<td>Mortality rate</td>
<td>0.00&lt;sup&gt;c&lt;/sup&gt;</td>
<td>10.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.33&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.66&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.60</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

**Mean values within a row with different superscript letters are significantly different (p<0.05); SEM: Standard error of the mean; GSE: grape seed extract; FB1: Fumonisin B1; IBW: initial body weight; FBW: Final body weight; BWG: body weight gain; TFI: total feed intake; FCR: feed conversion ratio.

Body weight reduction in the FB1-fed group may be attributed to a lower feed utilization efficiency and may have been caused in part by the prevalence of diarrhoea and intestinal lesions (goblet cell hyperplasia) (Kubena et al., 1997), which was most likely brought on by FB1's disruption of sphingolipid biosynthesis (Wang et al., 1991). These sphingolipids have a role in the control of ion pumps, cell surface receptors, and other critical systems for cell survival and function (Merrill et al., 1996). Fumonisin B1 injury to the villus is anticipated to impair nutrient absorption, which will lower production efficiency (Grenier et al., 2016). According to Shanmugasundaram et al. (2023), the histomorphology examination of ileum samples revealed that Fumonisin B1 had harmed the villi's structural integrity, reduced nutrient absorption and resulted in reduced production capacity. According to Javed et al. (1993), 40 and 70% of chicks fed 27 and 154 mg of pure moniliformin/kg died, respectively.

Results obtained showed the positive effects of GSE against the negative effects of FB1 on growth and feed utilization. These enhancements may be attributable to the natural antioxidants in GSE, such as phenolic materials, which can safeguard the intestinal mucosa from oxidative damage and pathogens as well as limit peristaltic activity in digestive disorders and decrease intestinal movement, leading to better nutrient absorption (Ismail et al., 2003). These findings are in line with those of Wang et al. (2008) who found lower mortality for chicks fed diet supplemented with grape seed proanthocyanidin extract (10 to 20 mg/kg) than the control group. This could be because GSE plays a crucial role as antimicrobial in alleviates remarkably the intestine microbial populations and prevents the lysis of amino acids which is used in proteinic tissues (Lee et al., 2001). Also, it may be due to the biological properties of phenolic compounds in GSE as well that show an antioxidant activity which reduce oxidative damage (Xia et al., 2010).

Blood biochemistry:

The findings in Table (4) demonstrated that feed contaminated with FB1 (400 mg/kg) had a negative impact on the serum biochemical profile when compared to other groups (P< 0.05). When compared to the control group, feeding broiler chicks a food contaminated with fumonisin at a rate of 400 ppm led to a substantial (P< 0.05) rise in the levels of TP, albumin, globulin, AST, ALT, ALK, creatinine, uric acid, P, LDL, HDL and cholesterol, Ca, and Sa:So ratio. By supplementing with GSE (250 and 500 mg/kg), the toxic effects of FB1 have been lessened. This has led to a considerable drop in the levels of above-mentioned blood biochemical parameters when compared to the group that received only FB1. In comparison to fumonisin alone, adding 250 or 500 mg/kg of grape seed to a diet contaminated with...
fumonsin was successful in attenuating (P< 0.05) the detrimental effects of fumonsin on the blood biochemical markers. Moreover, when compared to a diet containing 250 mg of GSE per kg, the higher quantity of GSE (500 mg/kg) had the best benefits.

Broilers given hepatotoxic chemicals frequently have lower protein (TP and Alb) levels (Hochleithner, 1994). Although certain in vitro studies on the impact of FB on endothelial cells from swine pulmonary arteries revealed that this mycotoxin induces an increased permeability on the endothelium for Alb and other proteins (Ramasamy et al., 1995). Additionally, in vivo studies have showed this impact on total protein and Alb values in the serum of broilers fed contaminated diet with FB1 (Kubena et al., 1997). Dehydration or the synthesis of acute phase proteins, which takes place 2 to 5 days following tissue injury, inflammation, or necrosis, may therefore be responsible for a rise in total protein with somewhat greater levels in the FB group (Sharma et al., 2008).

A rise in the albumin fraction, whose values were higher in the group fed FB1 compared to the other groups up to 14 days after feeding, may also have contributed to an increase in total protein. This may have happened at the same time as a rise in globulin concentration due to dehydration (Sharma et al., 2008). This may have been brought on by the mycotoxins and ascribed to a decrease in feed consumption. FB1 or moniliformin contaminated diets have been shown to boost total protein levels in prior studies (Bermudez et al., 1997; Asrani et al., 2006). According to Hochleithner (1994) and Lumeij (2008), hyperalbuminemia is typically accompanied by elevated Ca levels.

This investigation revealed elevated levels of AST and ALT activity as well as cholesterol levels, all of which are frequently linked to liver injury while, increased creatinine, uric acid, and P levels signify renal lesions. According to (Lumeij, 2008), the liver and kidney are the key organs that FB targets. Previous studies on FB1-fed broiler chicks (Ledoux et al., 1992), turkeys (Bermudez et al., 1997), and quail (Asrani et al., 2006) found increases in serum AST activity.

A possible early indicator of FB-toxicosis is an increase in serum cholesterol (HDL, LDL). This rise has been observed in broilers (Ledoux et al., 1992). According to Edington et al. (1995), elevated blood cholesterol and triglycerides are likely linked to biliary blockage and acute hepatic damage. El-Nekeety et al. (2007) explained the rise in LDL and HDL levels to the need for more in lipoprotein to transport the water insoluble cholesterol which is elevated in FB-induced toxicity. Significant excess in LDL have also been observed previously in FB1- and Moniliformin -fed birds (Javed et al., 1995) and turkeys (Bermudez et al., 1997). Significant excess in Creatine Kinase activity in the groups fed FB1 and Moniliformin alone propose that both FB1 and Moniliformin are toxic to striated or cardiac muscle.

Table 4: Effects of grape seed extract (GSE) on some blood constituents of broilers fed diets contaminated with Fumonisin B1.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Dietary treatments</th>
<th>Control</th>
<th>FB1 (400mg/kg)</th>
<th>FB1+GSE (250mg/kg)</th>
<th>FB1+GSE (500mg/kg)</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP (g/dl)</td>
<td></td>
<td>5.42</td>
<td>5.86</td>
<td>5.55</td>
<td>5.40</td>
<td>0.586</td>
<td>0.065</td>
</tr>
<tr>
<td>Alb (g/dl)</td>
<td></td>
<td>2.53</td>
<td>2.80</td>
<td>2.61</td>
<td>2.43</td>
<td>0.854</td>
<td>0.125</td>
</tr>
<tr>
<td>Glo. (g/dl)</td>
<td></td>
<td>2.89</td>
<td>3.06</td>
<td>2.94</td>
<td>2.97</td>
<td>0.758</td>
<td>0.083</td>
</tr>
<tr>
<td>AST (IU/l)</td>
<td></td>
<td>37.65</td>
<td>50.22</td>
<td>40.45</td>
<td>38.24</td>
<td>2.658</td>
<td>0.002</td>
</tr>
<tr>
<td>ALT (IU/l)</td>
<td></td>
<td>25.17</td>
<td>54.15</td>
<td>38.9</td>
<td>27.2</td>
<td>1.365</td>
<td>0.0001</td>
</tr>
<tr>
<td>Chol.(mg/dl)</td>
<td></td>
<td>138.36</td>
<td>180.25</td>
<td>140.22</td>
<td>136.42</td>
<td>5.228</td>
<td>0.001</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td></td>
<td>24.44</td>
<td>30.32</td>
<td>20.55</td>
<td>18.28</td>
<td>1.685</td>
<td>0.004</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td></td>
<td>76.32</td>
<td>83.58</td>
<td>79.31</td>
<td>77.22</td>
<td>6.885</td>
<td>0.024</td>
</tr>
<tr>
<td>ALP (mg/dl)</td>
<td></td>
<td>41.57</td>
<td>46.0</td>
<td>45.0</td>
<td>40.8</td>
<td>2.687</td>
<td>0.035</td>
</tr>
<tr>
<td>Creat. (mg/dl)</td>
<td></td>
<td>0.660</td>
<td>1.16</td>
<td>0.852</td>
<td>0.700</td>
<td>0.052</td>
<td>0.001</td>
</tr>
<tr>
<td>Uric acid (mg/dl)</td>
<td></td>
<td>5.52</td>
<td>6.36</td>
<td>5.82</td>
<td>5.98</td>
<td>0.875</td>
<td>0.002</td>
</tr>
<tr>
<td>Ca (mg/dl)</td>
<td></td>
<td>9.41</td>
<td>10.65</td>
<td>9.84</td>
<td>9.38</td>
<td>1.668</td>
<td>0.038</td>
</tr>
<tr>
<td>P (mg/dl)</td>
<td></td>
<td>7.25</td>
<td>8.95</td>
<td>7.33</td>
<td>7.28</td>
<td>2.325</td>
<td>0.001</td>
</tr>
<tr>
<td>Sa (pmol/ml)</td>
<td></td>
<td>10.75</td>
<td>15.35</td>
<td>12.25</td>
<td>11.85</td>
<td>2.668</td>
<td>0.001</td>
</tr>
<tr>
<td>So (pmol/ml)</td>
<td></td>
<td>32.74</td>
<td>34.65</td>
<td>31.82</td>
<td>32.09</td>
<td>6.875</td>
<td>0.026</td>
</tr>
<tr>
<td>Sa : So ratio</td>
<td></td>
<td>0.328</td>
<td>0.443</td>
<td>0.385</td>
<td>0.369</td>
<td>0.060</td>
<td>0.002</td>
</tr>
</tbody>
</table>

**= Mean values within a row with different superscript letters were significantly different (P<0.05); SEM: Standard error of the mean; GSE: grape seed extract; FB1: Fumonsinin B1; TP: total protein; Alb.: Albumin; Glo: Globulin; AST: aspartate aminotransferase; ALT, alanine aminotransferase; Chol, cholesterol; ALP: alkaline phosphate; Sa:So: the sphinganine-to-sphingosine ratio.
GSE appeared to partially restore the liver to its normal state in the GSE group because GSE considerably decreased the rise of AST and ALT activity. This impact of the GSE pretreatment supports the notion that it is bioavailable and has substantial antioxidant and anti-inflammatory properties (Bagchi et al., 2002) as it considerably reduced the hepatotoxicity as an indirect target of cisplatin (Yousef et al., 2009). TC, HDL-C, and LDL-C values in the treated group were all considerably reduced by GSE. GSE may have a hypolipidemic impact by speeding up cholesterol catabolism by boosting the activity of the hepatic cholesterol 7-α-hydroxylase enzyme. This enzyme is the rate-limiting enzyme of bile acid biosynthesis, it is possible that GSE could prompt the conversion of cholesterol to bile acids, which is a crucial mechanism for elimination of cholesterol from body (Del Bas et al., 2005). The water-soluble antioxidant, proanthocyanidins in the GSE might trap ROS in aqueous series like plasma thereby preventing oxidation of LDL. GSE indirectly restores bodily homeostasis by enhancing renal function. According to Abd El-Wahab et al. (2008), GSE shows no nephrotoxicity. The current investigation demonstrated that GSE also has nephroprotective effects, restoring normal levels of uric acid and creatinine.

Fumonsin competitively inhibits the enzyme ceramide synthase because of the structural similarities between fumonsin and the sphingoid bases sphinganine (Sa) and sphingosine (So) and as a direct result, an accumulation of the substrates Sa and, to a lesser extent, So, occurs in tissues, serum, and urine (Voss et al., 2007). The biological response for this technique is an increase in Sa: So. Comparable impacts on Sa:So to the ones found in this experiment have been previously reported in numerous species, including broilers (Henry et al., 2000; Tran et al., 2003).

**Serum and hepatic antioxidant parameters:**

Table (5) provides a summary of the effects of GSE on the liver and serum antioxidant indices of broilers exposed to FB1. When compared to the control group, broilers fed a meal contaminated with FB1 had higher levels of serum and liver malondialdehyde (MDA). However, GSE considerably reduced the amount of MDA in diets tainted with FB1, and there was significant difference in the amount of MDA between the two GSE dose groups (250 and 500 mg/kg). When FB1 group was compared to the control group, the activities of glutathione peroxide (GSH-Px) and the concentration of total antioxidant capacity (TAC) in the serum and liver were both (P< 0.05) decreased. The activities of the antioxidant enzymes were dramatically increased when GSE (250 and 500 mg/kg) was added to the FB1-contaminated diet. These outcomes demonstrated that GSE greatly enhanced liver and serum antioxidant activities and reduced oxidative damage brought on by FB1. In addition, 500 mg/kg of GSE produced the best results when compared to a diet containing 250 mg/kg of GSE.

Oxidative stress is another side effect of FB1 poisoning. When compared to the control group, the FB1 group showed a significant increase in the concentration of the lipid peroxidation product MDA, which suggests that FB1 accelerates the formation of ROS. A significant decrease in GPX and TAC activity was seen in the FB1-group. These results were discriminatory of renal and hepatic damage.

The detoxification of the toxic of mycotoxins is significantly aided by glutathione. According to numerous research, liver necrosis starts as soon as the glutathione reserves are practically depleted (Abdel-Wahhab and Aly, 2005). Additionally, GPX’s association with FB1 or its metabolites may help to explain why the amount of GPX has decreased (Abdel-Wahhab et al., 2002).

**Table (5): Effects of grape seed extract (GSE) on serum and hepatic antioxidant parameters of broilers fed diets contaminated with Fumonsin B1.**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Dietary treatments</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>FB1</td>
<td>FB1+GSE</td>
</tr>
<tr>
<td></td>
<td>(400mg/kg)</td>
<td>(250mg/kg)</td>
<td>(500mg/kg)</td>
</tr>
<tr>
<td>Serum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GSH-Px (U/ml)</td>
<td>1544.3a</td>
<td>913.8d</td>
<td>1290.0b</td>
</tr>
<tr>
<td>TAC (U/ml)</td>
<td>8.22a</td>
<td>4.17c</td>
<td>7.65b</td>
</tr>
<tr>
<td>MDA (nmol/ml)</td>
<td>1.86c</td>
<td>3.06e</td>
<td>2.08b</td>
</tr>
<tr>
<td>Liver</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GSH-Px (U/mg protein)</td>
<td>51.65a</td>
<td>29.77d</td>
<td>40.66c</td>
</tr>
<tr>
<td>TAC (mmol/g protein)</td>
<td>5.61a</td>
<td>3.24b</td>
<td>4.58b</td>
</tr>
<tr>
<td>MDA (mmol/mg protein)</td>
<td>36.30c</td>
<td>56.58a</td>
<td>40.68b</td>
</tr>
</tbody>
</table>

Notes: a-d: Mean values within a row with different superscript letters were significantly different (P< 0.05); SEM: Standard error of the mean; GSE: grape seed extract; FB1: Fumonsin B1; GSH-Px, glutathione peroxidase; TAC: total antioxidant capacity; MDA, malondialdehyde.
The antioxidant and free radical scavenging properties of GSE may be due to the polyphenols in the extract, particularly flavonoids, which have the capacity to modulate the expression of gamma-glutamyl cystein synthetase, which catalyses the rate limiting step in the synthesis of endogenous antioxidant in cells, specifically glutathione. As a result, according to Moskaug et al. (2005), this enzyme is essential for both the detoxification of xenobiotics and the cellular antioxidant system.

Numerous research have suggested that grape seed byproducts may increase the antioxidant capacity of broilers (Abu Hafsa and Ibrahim, 2018). Our research further shown that GSE might have an antioxidative effects by elevating serum GSH-PX, T-AOC, and reducing serum MDA levels. This indicates that the GSE may be an effective antioxidant that, by triggering the antioxidant enzyme system, could reduce reactive free radicals and oxidative stress (Lipinski et al., 2017).

Comparing the control group to the FB1-group, it was found that MDA levels were considerably lower and GPx activity was higher. According to Yamanaka et al. (1997), eating foods and drinks high in flavonoids may assist to reduce the body's exposure to oxidative stress. Proanthocyanidins have been shown to have specificity for the hydroxyl radical in in vitro experimental results (Zayachkivska et al., 2006). The biochemical role of the selenium containing enzyme glutathione peroxidase is to decrease lipid hydroperoxides to their corresponding alcohols and to decrease free hydrogen peroxide to water (Ran et al., 2007). Therefore, any rise in the GPx activity results in a reduce in lipid peroxide values, so enhancing the lipid profile. This may interpret the decreased value of MDA and the near normal values of total cholesterol and LDL in the group giving GSE when compared to that of the FB1-group. The chemical characteristics of proanthocyanidins in terms of the availability of the phenolic hydrogens as hydrogen donating radical scavengers and singlet oxygen quenchers predict their antioxidant activity, which has been shown to be significantly more potent than that of beta-carotene or vitamins C and E (Joshi et al., 2001).

**Serum immunoglobin and inflammatory cytokines:**

In comparison to the control group, the FB1-treated birds displayed a substantial drop (P<0.05) in the immunoglobin (IgA, IgG, and IgM) levels and a significant increase (P<0.05) in the serum levels of IL-6 and TNF-γ (Table 6). Additional improvements in the previously described features were seen when GSE (250 or 500 mg/kg diet) was added to the FB1-containing diet. However, when compared to the 250 mg GSE/kg diet used in this study, the 500 mg GSE/kg diet showed the best outcomes regarding these characteristics. In particular, FB1 treatment significantly reduced immunoglobin while increased serum IL-6 and TNF-γ levels (P<0.05), while GSE supplementation alleviated the levels of these immunoglobin, and inflammatory markers compared to the FB1 birds (P<0.05).

**Table (6): Effects of grape seed extract (GSE) on serum immunoglobin and inflammatory cytokines of broilers fed diets contaminated with Fumonsinin B1.**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Dietary treatments</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>FB1</td>
<td>FB1+GSE</td>
</tr>
<tr>
<td>Serum immunoglobin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgA (µg/ml)</td>
<td>24.86</td>
<td>22.42</td>
<td>24.68</td>
</tr>
<tr>
<td>IgM (µg/ml)</td>
<td>42.36a</td>
<td>41.00c</td>
<td>42.10b</td>
</tr>
<tr>
<td>IgG (µg/ml)</td>
<td>82.60a</td>
<td>71.95c</td>
<td>82.25b</td>
</tr>
<tr>
<td>Serum inflammatory cytokines</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-2 (ng/ml)</td>
<td>120c</td>
<td>168a</td>
<td>142b</td>
</tr>
<tr>
<td>IL-6 (ng/ml)</td>
<td>21c</td>
<td>58a</td>
<td>38b</td>
</tr>
<tr>
<td>TNF-α (pg/ml)</td>
<td>20f</td>
<td>46a</td>
<td>28b</td>
</tr>
<tr>
<td>IFN-γ (ng/ml)</td>
<td>19c</td>
<td>36a</td>
<td>24b</td>
</tr>
</tbody>
</table>

<sup>a,b,c</sup> Mean values within a row with different superscript letters were significantly different (p<0.05); SEM: Standard error of the mean; GSE: grape seed extract; FB1: Fumonsinin B1; IL-2: interleukin-2; IL-6: interleukin-6; TNF-α: tumour necrosis factor-α; and IFN-γ: interferon-γ

The results demonstrated that GSE is ambidextrous to reduce the inflammatory response and enhance immune function induced by FB1. These results are in line with the earlier studies that, exposure to deoxynivalenol (DON) and fumonisins (FUM) can impair mucosal immunity (Bracarense et al., 2012). Shanmugasundaram et al. (2023) observed that, the fumonisins reduced the levels of total IgA and C. perfringens-specific IgA in bile. According to research by Wang et al. (1991), the mechanism of action of FB1 involves the disruption of complex sphingolipids and the buildup of free sphinganine and
sphingosine. It has been demonstrated that FB1’s inhibition of sphingolipid production correlates with repressed cell proliferation (Yoo et al., 1992). Sphingolipid breakdown products were recognized as antiproliferative lipids and were shown to reduce humoral and cell-mediated immunological responses (Martinova, 1996).

According to Kamboh et al. (2015), oxidative stress may have an impact on immunity, and enhanced antioxidant function may boost immunity in poultry. Polyphenols might enhance immune role by reducing the inflammatory process through nuclear factor-kappaB and nuclear factor-2–dominated passages in the small intestine (Paszkiewicz et al., 2012). The addition of GSE raised serum immunoglobulins in the present study. IFN-γ plays as a crucial regulator in the activation of lymphocytes and monococytes and serum IL-2 encourages the proliferation of activated natural killer cells, T lymphocytes, B lymphocytes, as well as the creation of antibodies (Ao and Kim, 2019). The current findings revealed that the supplementation of GSE improved serum IL-2, and INF-γ, suggesting that GSE could improve immune response by controlling antibodies, complements, and cytokines (Lipinski et al., 2017).

Serum concentrations of pro-inflammatory cytokines are regarded indicators of cellular immunity. Presently, FB1 group showed a significant elevation in serum pro-inflammatory cytokines such as IL-6 and TNF-γ. These findings are consistent with recent studies suggesting that FB1 might cause inflammation and change the immunological response (Hassan et al., 2020). In addition to, GSE supplementation significantly reduced elevated concentrations of IL-6 and TNF-γ in the serum of FB1-fed chicks. Accordingly, Rajput et al. (2019) observed that GSE could significantly alleviate the high concentrations of inflammatory cytokines in serum of broiler following ingestion of AFB1 diet. All of these findings also supported the antioxidant action and anti-inflammatory of GSE that could be responsible for its decisiveness effect.

**Jejunum morphology:**

In broilers receiving FB1, analysis of morphologic alterations in the small intestine showed a significant decrease in villus height (VH), crypt depth (CD) and villus-to-crypt ratio (VH:CD). Adding GSE (250 or 500 mg/kg diet) to the FB1-containing diet resulted in further improvements in the previously stated features. In contrast to the 250 mg GSE/kg diet used in this investigation, the 500 mg GSE/kg diet showed the highest outcomes in terms of these characteristics (Table 7). These findings are in line with those of Shanmugasundaram et al. (2023), who discovered that the histomorphology analysis of ileum samples revealed fumonisins (FUM) damaging the villi structure and possibly reducing nutrient absorption, which in term reduced growth performance.

The primary site of nutrient absorption in the chicken intestine is the jejunum, whose shape indirectly reflects feed efficiency (Leeson and Summers, 2001). According to Mahdavi et al. (2010), the VH:CD may provide a thorough indication of the villi’s absorption function. Flat crypts indicate typical cell turnover on the villus, while high villus indicates a bigger absorption surface. High V:C illustrates the relationship between these two desirable qualities (Gao et al., 2008). In broiler chickens (Rauber et al., 2013), FB has been shown to reduce VH.

In line with Viveros et al. (2011), the current investigation demonstrated that dietary GSE supplementation had a favourable impact on jejunum CD and VH:CD. They found that adding GSE (0.72%) to broilers’ diets reduced jejunum CD but raised VH:CD, suggesting that this may be good for gut health and nutrient absorption. Similar to this, dietary grape proanthocyanidins (0.00075–0.0015%) significantly influenced the shape of the jejunum in broilers (Yang et al., 2016), which may be related to their anti-inflammatory and antibacterial properties (Oliveira et al., 2013; Surai, 2014). Gallic acid, a phenolic substance found in grape seeds, lowered jejunum CD and enhanced VH:CD in broilers (Samuel et al., 2017). The enhanced jejunum morphology might partially reflect the improved FCR in the current investigation.

**Ileal microflora:**

When compared to the control group, the FB1-treated birds showed a substantial drop (P<0.05) in Lactobacillus and a significant increase (P<0.05) in E. coli (Table 7). Adding GS (250 or 500 mg/kg diet) to the FB1-containing diet caused the Lactobacillus count to increase while the E. coli count to drop. However, when compared to 250 mg GS/kg diet, 500 mg GSE/kg diet showed the best outcomes in terms of these characteristics. According to Shanmugasundaram et al. (2023), exposure to FB increased the Clostridia while decreasing the relative abundance of the Lactobacillus and Faecalibacterium species. Numerous proteolytic enzymes produced by Lactobacillus are essential for the detoxification of mycotoxins. Using polysaccharides and peptidoglycans on their bacterial cell wall to bind to the mycotoxins and increase their physical adsorption, the Lactobacillus metabolizes the FB (Zhao et al., 2017).
2016). Therefore, the diminishing in the relative abundance of Lactobacillus in birds is most likely linked to their disintegrated binding sites for FB1 and, as a result, their diminished capability to metabolize the toxins and higher accumulation of mycotoxin, predisposing chickens to necrotic enteritis. The relative abundances of Faecalibacterium and Clostridiaceae suggest that FB increased the inflammation and provided a favorable environment for Clostridia as a consequence of intestinal dysbiosis, and this most likely contributes to subclinical necrotic enteritis.

Table (7): Effects of grape seed extract (GSE) on jejunum morphology and ileal microflora of broilers fed diets contaminated with Fumonsin B1.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Dietary treatments</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (FB1)</td>
<td>400mg/kg</td>
<td>FB1+GSE (FB1+GSE (250mg/kg)</td>
</tr>
<tr>
<td>Jejunum morphology</td>
<td>VH (µm)</td>
<td>1662^a</td>
<td>690^b</td>
</tr>
<tr>
<td></td>
<td>CD (µm)</td>
<td>226^a</td>
<td>142^d</td>
</tr>
<tr>
<td></td>
<td>VH:CD ratio</td>
<td>7.35^a</td>
<td>4.86^c</td>
</tr>
<tr>
<td>Ileal microflora (log^{10} cfu/g)</td>
<td>E. coli</td>
<td>3.26^c</td>
<td>5.86^a</td>
</tr>
<tr>
<td></td>
<td>Lactobacillus</td>
<td>7.25^a</td>
<td>4.78^b</td>
</tr>
</tbody>
</table>

Similar to this, the stabilization of ileal microflora is essential to gut health and function (Song et al., 2014). Grape by-products might promote the growth of specific helpful bacteria strains in the intestinal tract while competitively excluding some harmful bacteria (Brenes et al., 2016). In the current study, the GSE supplementation exerted a favorable impact on ileal bacterial populations, which was agree with Abu Hafsa and Ibrahim (2018). They observed raised ileal Lactobacilli counts but reduced E. coli counts in broilers fed diets containing 1 to 4% grape seed. Similar to this, Viveros et al. (2011) demonstrated that GSE could successfully raise the populations of helpful bacteria in the ileum and lower the number of harmful bacteria in broilers. The antibacterial activity of the GSE against E. coli in vitro was previously verified by research (Baydar et al., 2006). According to a theory put up by Viveros et al. (2011), the ability of ileal Lactobacilli to utilize and metabolize phenolic substances as nutritional substrates may be the reason of the elevated ileal Lactobacilli counts. Furthermore, the raised immunity may also mirror the favorable impact of GSE on ileal microflora in our study because polyphenols could enhance the shedding of microbial beneficial bacteria (Lactobacillus) to indirectly improve immunity and gut health (Paszkiewicz et al., 2012).

CONCLUSION

These results, as a conclusion, clearly demonstrated that; 1) traits mentioned hereinbefore were significantly affected by FB1 (400 mg) treatment; 2) the simultaneous addition of GSE (500 mg / kg diet) to the FB1–containing diet provided great improvement in FB1 toxicity on broiler performance. These ameliorations should contribute to the FB1 problem in broiler chickens, when used with other management practices.

REFERENCES


