Effect of vitamin E and selenium supplement in reducing aflatoxicosis on performance and blood parameters in broiler chicks

A. A. Shlig

College of Agriculture, Tikrit University, Salah Al-deen, Iraq

Abstract

This study was aimed to investigate the sufficiency of Vitamin E and Selenium Supplementation to Diets against containing aflatoxins on the relative organ weights and various structural blood parameters. One hundred and twenty unsexed Ross birds were used from 3 to 7 weeks of age. Birds were randomly distributed and subjected to five nutrition treatments as follows: (T1. Control Group: 0.0 AF + 0.0 Se +0.0 Vit. E, T2. 2.5 mg AF/kg diet, T3. 2.5 mg AF /kg diet + 0.18mg/kg Se +10 IU Vit. E, T4. 2.5 mg AF/kg diet + 0.32mg/kg Se +30 IU Vit. E, T5. 2.5 mg AF /kg diet + 0.50mg/kg Se +50 IU Vit. E) pollution by aflatoxins causes a significant increase in relative weights of liver, heart, gizzard, abdomen fat, and spleen. A significant decrease in total body and carcass weight gastrointestinal tract long. Moreover existence of aflatoxins caused a significant decrease in values of Packed cell volume (PCV). Red blood cells counts (RBC), Hemoglobin (Hb), mean corpuscular hemoglobin (MCH) mean corpuscular hemoglobin concentration (MCHC) significantly moreover, it has been noticed a significant increase occurrence in values of total white blood cell (WBC) and ratio of Heterophils to lymphocytes in second treatment birds influenced by Aflatoxins. The addition of graded levels of Vit. E and Se to the AF containing diets (T4, T5) improvement total body weight and carcass weight and internal organs studied. The addition of both Vit. E and Selenium (T3, T4, T5) to the provender containing by aflatoxins, had no effects on blood parameters to their natural averages at control group. The existence of the two Vit. E and Selenium in polluted provender doesn't prevent a decrease in these values with a significant improvement (up to 10 IU Vit. E and 0.18mg/kg Se) occurrence from birds group values which had been fed by polluted provender only.

Keywords: Vitamin E, Selenium, Aflatoxins, Broiler.

Available online at http://www.vetmedmosul.org/ijvs

Correo Elías

 تمام اضافة فيتامين E وعناصر السيلنيوم لتبديل تأثير سموم الأفلا على الإداء

معاير الدم في فروج اللحم

عقل عبد شليج

قسم الثروة الحيوانية، كلية الزراعة، جامعة تكريت، صلاح الدين، العراق

الخلاصة

قدت الدراسة إلى معرفة تأثير إضافة فيتامين E وعناصر السيلنيوم إلى علاقق فروج اللحم في الحد أو التقليل من تأثير سموم الأفلا على الأوزان النهائية والنسبية للأعضاء الداخلية ونسبة التصاصي وصورة الدم الكاملة والعد التفريقي للكريات الدم البيضاء. استخدمت في الدراسة (120) طائرًا من مجمعة من سلالة روس 3 أصابع قسمت على خمس معاملات، وزعت الطيور عشوائياً بواقع (20) طائرًا لكل معاملة وتضمن كل مكرر منها (8) طيور. وكانت المعاملات التغذوية كالآتي (T1: معاملة السيطرة، T4: 1.88 ملغ/كغم علف السيلنيوم، T5: 0.18 ملغ/كغم علف السيلنيوم، T2: 2.5 ملغ سموم الأفلا/كغم علف، T3: 2.5 ملغ سموم الأفلا/كغم علف + 2.5 ملغ سموم الأفلا/كغم علف + 30 ملغ/كغم علف السيليكون، T4: 2.5 ملغ سموم الأفلا/كغم علف + 30 ملغ/كغم علف السيليكون) أشارت النتائج التحليل الإحصائي إلى أن تكون على الطيور بسموم الأفلا 2.5 ملغ سموم الأفلا/كغم علف أدى إلى انخفاض معنوي (0.05) في الوزن الحي ووزن الذبيحة وطول الأمعاء ونسبة التصاصي وقيم خضاب الدم.
Aflatoxins (AF) are a secondary fungal metabolites produced by some strains of Aspergillus flavus and Aspergillus parasiticus. Aflatoxin B1, B2, G1 and G2 are natural contaminants of many animal feed ingredients. AFB1 is the most abundant and toxic form of all naturally occurring aflatoxins also represents 75% of all aflatoxins found in contaminated food and feeds. It is hepatotoxic (1), hepa-tocarcinogenic (2), and teratogenic (3) to various animal species. They were classified as a group I carcinogen in humans (4) Aflatoxin B1 is biotransformed in the liver by monooxygenases and then transformed by cytochrome P450 into aflatoxin 8-9 epoxide and hydroxylated metabolites (AFM1, AFM1, AFQ1 and aflatoxicol) (5). Both Vitamin E and Selenium are essential nutrients for humans and animals. They are involved in the protection of biological membranes against lipid peroxidation and preventing the free radicals damage to phospholipids membranes and enzymes (6). Vitamin E is the major lipid soluble antioxidant that is present in biomembranes, scavenges free radicals in the early stages of lipid peroxidation and Selenium is essential to the activity of glutathione peroxidase (GSH –Px) which reduces H2O2 and lipid hydroxides to less reactive products (7). It is well understood that Selenium has a strong interaction with vitamin E in the protection of cell from important molecules (6). Alpha – tocopherol and glutathione peroxide protect tissues from oxidative damage associated with the free radicals generated during the respiratory burst of macrophages and neutrophils (experienced during immune response) (8) and improvement the bird health and immune status, Vitamin E and Se are antioxidants essential for cell survival in environments containing peroxides (9,10).

The response of broilers to Se supplementation could vary greatly with the level of Vit. E in the diet (6) Selenium is between 50 and 100 times more effective as an antioxidant than vitamin E. while Se supplementation rate of 0.1-0.5 mg/kg is satisfactory for most animals including poultry (11) Vitamin E was reported as an excellent biological chain – breaking antioxidant that protects cells and tissue from lipoperoxidative damage induced by free radicals (12,13), Chickens, however can not synthesize Vit. E such requirements must be met from dietary sources (14) Supplementation with 100 IU of Vit. E/kg feed has been shown to decrease the concentration of malondialdehyde a product of lipid peroxidation in the livers of chickens fed ochratoxin A and T-2 toxin compared to dietary level of 10 IU of Vit. E/kg feed (15). A highly active electrophilic compound that is inactivated by conjugation with glutathione –s- transferase (GST) to the AFB – glutathione metabolite was produced in bile acid from rats fed diets containing aflatoxin with Se. Thus dietary addition of Vit. E and Se may alleviate the aflatoxicosis by increasing GSH-Px activity from 144.5 IU/g Hb to186.2 IU/g Hb and eventual changes of toxic substances to inert metabolites (6) The objectives of the present study was to evaluate the effect of dietary aflatoxin contamination alone or with the addition of Vitamin E and Se on broiler performance and blood parameters.

Materials and methods

The experiment was conducted at the poultry production farm, College of Agriculture, Tikrit University during the period from 4th March to 2nd April -2009. The total number birds was 120 twenty one day-old Ross unsexed chicks were individually weighed, wing – banded, and housed in 15 pens under continuous lighting. Chicks divided at random into 5 treatment groups. Each one contains 3 replicates. Eight broiler chicks for each replicate group were fed corn – soybean meal based finisher diet was obtained from it contained 18.3 % crude protein, 1.30 % lysine and 0.52% methionine, 3100 Kcal /Kg metabolizable energy. The feed and water were ad-libitum. Chicks were randomly assigned to the following treatment groups (T1, T2, T3, T4, T5).

Control group: Corn – soybean basal diet+ 0.0 AF + 0.0 Se
increased relative weights internal organs. The grade from the data of the present study that feeding the liver is considered the principle target organ for AF it was significant enlargement in the size of internal organs like heart kidneys gizzards proventriculus spleen and diet containing AF alone resulted in a significant increase in findings agreed with those obtained previously (26-28) the liver, kidney, spleen, proventriculus, gizzard and heart close to those of the control group. No significant differences were observed in relative weight of the above mentioned organs between those of the control group and those fed the AF containing diets amended with 0.32mg/kg Se +30 IU Vit. E and 0.50 mg/kgSe+50 IU Vit. E. The toxin fed birds treated with vitamin E and Se showed a significant gain of body weight as compared to toxin alone fed birds indicating the beneficial effect of vitamin E and Se on total body weight gain during aflatoxicosis.

The effect of aflatoxin with combination with Vitamin E and Se on selected blood parameters is presented in table (2). Values of hemoglobin, haematocrit, mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration were significantly (P<0.05) decreased in chicks fed the diet containing AF alone. Blood picture of treatment three was significantly decreased in Hb, RBC, PCV, MCH and MCHC value when compared with control group. Except MCV value which no significant differences were observed these findings are in agreement with those obtained previous (29,30). The addition of Vit. E and Se at the two stated levels (T4 and T5) was similarly effective in alleviating the negative effect of AF on all blood parameters Hb, RBC, PCV, MCH and MCHC significantly improved the values of when compared with those of birds fed the diet containing AF alone but they were still significantly lower then those of the control group. Data presented in table (3) show the effect of dietary treatment on the results indicated that the presences of Aflatoxin alone in the diet caused a significant enlargement in the size of internal organs like liver, kidney, spleen, gizzard, heart and abdomen fat. These findings agreed with those obtained previously (26-28) the liver is considered the principle target organ for AF it was evident from the data of the present study that feeding the diet containing AF alone resulted in a significant increase in the relative weight of liver as well as other internal organs including heart kidneys gizzards proventriculus spleen and pancreas. The results of third treatment differ significantly from that of the control group total body, carcass weight and gastrointestinal tract long with a significant (P<0.05) increased relative weights internal organs. The graded addition of Vit. E and Se at the two stated levels T4 and T5 ameliorated parameters mentioned above in comparison those of the control group or those of birds fed the diet containing AF alone. It is thought that the addition of Vit. E and Se (T4,T5) to the AF containing diets helped in restoring the relative weights of liver, kidney, spleen, proventriculus, gizzard and heart close to those of the control group.

Results

The effect of AF alone and when combination with graded addition of Vitamin E and Se on performance of growing chicks and the relative weight of internal organs from 21 day –old to the age of 46 day –old show in table (1). Chicks fed the diet containing 2.5mg AF/kg had a significantly (P<0.05) lower total body and carcass weight with length of the gastrointestinal tract. The results indicated that the presence of AF alone in the diet caused a significant enlargement in the size of internal organs like liver, kidney, spleen, gizzard, heart and abdomen fat. These findings agreed with those obtained previously (26-28) the liver is considered the principle target organ for AF it was evident from the data of the present study that feeding the diet containing AF alone resulted in a significant increase in the relative weight of liver as well as other internal organs including heart kidneys gizzards proventriculus spleen and pancreas. The results of third treatment differ significantly from that of the control group total body, carcass weight and gastrointestinal tract long with a significant (P<0.05) increased relative weights internal organs. The graded beneficial effect of vitamin E and Se on total body weight gain during aflatoxicosis.
Table (1) Effect of graded addition of Vitamin E and Se on relative organ weights of chicks fed diets containing 2.5mg AF/kg diet.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>AF mg/kg</th>
<th>Vit.E IU</th>
<th>Se mg/kg</th>
<th>Final body weight</th>
<th>Carcass weight</th>
<th>Gastro-intestinal tract</th>
<th>Proventriculus</th>
<th>Gizzard</th>
<th>Liver</th>
<th>Pancreas</th>
<th>Spleen</th>
<th>Heart</th>
<th>abdomen fat</th>
<th>dressing percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2222.5 ± 28.7</td>
<td>1672 ± 21.7</td>
<td>180.5 ± 4.51</td>
<td>0.378 ± 0.02</td>
<td>1.180 ± 0.01</td>
<td>1.722 ± 0.02</td>
<td>0.210 ± 0.02</td>
<td>0.112 ± 0.01</td>
<td>0.045 ± 0.03</td>
<td>1.442 ± 0.05</td>
<td>75.55 ± 1.08</td>
</tr>
<tr>
<td>T2</td>
<td>2.5</td>
<td>0</td>
<td>0</td>
<td>1682.5 ± 35.9</td>
<td>1182 ± 42.2</td>
<td>162.2 ± 2.05</td>
<td>0.379 ± 0.07</td>
<td>1.202 ± 0.01</td>
<td>1.857 ± 0.03</td>
<td>0.227 ± 0.01</td>
<td>0.185 ± 0.01</td>
<td>0.045 ± 0.09</td>
<td>1.655 ± 0.05</td>
<td>70.15 ± 1.26</td>
</tr>
<tr>
<td>T3</td>
<td>2.5</td>
<td>10</td>
<td>0.18</td>
<td>1773.8 ± 55.9</td>
<td>1250 ± 59.5</td>
<td>163 ± 2.58</td>
<td>0.376 ± 0.04</td>
<td>1.197 ± 0.01</td>
<td>1.850 ± 0.02</td>
<td>0.242 ± 0.01</td>
<td>0.122 ± 0.01</td>
<td>0.032 ± 0.12</td>
<td>1.640 ± 0.10</td>
<td>70.80 ± 1.14</td>
</tr>
<tr>
<td>T4</td>
<td>2.5</td>
<td>30</td>
<td>0.32</td>
<td>2180 ± 167.9</td>
<td>1623 ± 126.9</td>
<td>170.7 ± 3.1</td>
<td>0.376 ± 0.02</td>
<td>1.185 ± 0.01</td>
<td>1.772 ± 0.02</td>
<td>0.205 ± 0.01</td>
<td>0.122 ± 0.01</td>
<td>0.040 ± 0.09</td>
<td>1.490 ± 0.09</td>
<td>75.02 ± 0.73</td>
</tr>
<tr>
<td>T5</td>
<td>2.5</td>
<td>50</td>
<td>0.50</td>
<td>2344 ± 175.9</td>
<td>1756 ± 118.5</td>
<td>178.7 ± 4.91</td>
<td>0.376 ± 0.04</td>
<td>1.192 ± 0.01</td>
<td>1.720 ± 0.02</td>
<td>0.207 ± 0.01</td>
<td>0.112 ± 0.01</td>
<td>0.041 ± 0.07</td>
<td>1.467 ± 0.05</td>
<td>74.92 ± 0.76</td>
</tr>
</tbody>
</table>

Means within a column with different letters differ significantly (P<0.05).

Discussion

There are many reports on the effects of various foods or nutrients and xenobiotics on AFB1-macromolecule adducts formation. Obviously the major objectives of these studies were to determine if and how those nutrients or xenobiotics could affect adducts formation especially DNA adduct. This study was undertaken to evaluated the effect of feeding AF containing diet amended with Vit. E and Se. Increasing Vit. E level in the diet from minimum requirement recommended by (33) 10 IU to 50 IU/kg diet and Se from 0.18 to 0.50 mg/kg diet influence important production parameters such as performance of growing chicks, relative weight of internal organs, hemoglobin, haematocrit, mean corpuscular volume, mean corpuscular hemoglobin, and the mean corpuscular hemoglobin concentration. (34) reported that the combined Se (0.2 mg/kg as Na2 SeO3)-VE (100 IU/kg) deficiency enhances activation or inhibits detoxification of aflatoxin B1,1mg/kg (decreased AFB1 binding to DNA, RNA, and protein) in white leghorn chicks which was completely effective in preventing oxidative diathesis and death. (35,36) reported that high concentration of aflatoxin B1-GSH–Px conjugate non toxic metabolite was produced in bile acid from rats red diets containing aflatoxin with Se. Thus dietary addition of Se may alleviate the aflatoxicosis by increasing GSH–Px activity and eventual changes of toxic substances to inert metabolites. They also reported that the Vit. E reduced the formation of AFB1 adducts in the liver. The observation that the rats fed AF free diet containing 120 IU VitE/kg showed significantly enhanced blood GSH – Px activity and eventual changes of toxic substances to inert metabolites. The feeding of AF diets containing 60 IU Vit. E/kg induced a significant decrease in the liver lipid contents (37).
Table (2) Effect of graded addition of Vitamin E and Se on hematological values of chicks fed diets containing 2.5mg AF/kg diet.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>AF mg/kg</th>
<th>Vit.E IU</th>
<th>Se mg/kg</th>
<th>Hemoglobin g/dl</th>
<th>R.B.C (10^6/mm³)</th>
<th>P.C.V%</th>
<th>MCV (µm³)</th>
<th>MCH pg</th>
<th>MCHC g/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>9.62± 0.22</td>
<td>3.12± 0.12</td>
<td>35.75± 0.95</td>
<td>114.65± 2.40</td>
<td>31.27± 0.97</td>
<td>26.87± 0.17</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
</tr>
<tr>
<td>T2</td>
<td>2.5</td>
<td>0</td>
<td>0</td>
<td>5.05± 0.19</td>
<td>2.27±0.05</td>
<td>26.75± 0.95</td>
<td>90.82± 5.40</td>
<td>22.24± 0.34</td>
<td>18.82± 0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>b</td>
<td>c</td>
<td>c</td>
</tr>
<tr>
<td>T3</td>
<td>2.5</td>
<td>10</td>
<td>0.18</td>
<td>5.12± 0.09</td>
<td>2.31± 0.04</td>
<td>26.50± 0.57</td>
<td>114.61± 1.87</td>
<td>22.16± 0.38</td>
<td>19.32± 0.18</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>c</td>
<td>c</td>
</tr>
<tr>
<td>T4</td>
<td>2.5</td>
<td>30</td>
<td>0.32</td>
<td>8.12± 0.66</td>
<td>2.78± 0.11</td>
<td>31.00± 1.41</td>
<td>111.51± 0.55</td>
<td>29.19± 1.18</td>
<td>26.12± 0.97</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>b</td>
<td>b</td>
<td>b</td>
<td>a</td>
<td>b</td>
<td>b</td>
</tr>
<tr>
<td>T5</td>
<td>2.5</td>
<td>50</td>
<td>0.50</td>
<td>8.13± 0.31</td>
<td>2.79± 0.07</td>
<td>31.25± 0.95</td>
<td>112.19± 1.31</td>
<td>29.16± 0.55</td>
<td>25.95± 0.26</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>b</td>
<td>b</td>
<td>b</td>
<td>b</td>
<td>a</td>
<td>b</td>
</tr>
</tbody>
</table>

Means within a column with different letters differ significantly (P<0.05).

Table (3) Effect of graded addition of Vitamin E and Se on Differential count of normal leukocytes chicks fed diets containing 2.5mg AF/kg diet.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>AF mg/kg</th>
<th>Vit.E IU</th>
<th>Se mg/kg</th>
<th>Leukocytes (10^6 / mm³)</th>
<th>Monocytes %</th>
<th>Lymphocytes %</th>
<th>Heterophil %</th>
<th>Basophile %</th>
<th>Eosinophil %</th>
<th>H/L ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>24.40± 0.07</td>
<td>6.51± 0.09</td>
<td>61.32± 0.30</td>
<td>25.17± 0.21</td>
<td>4.93± 0.13</td>
<td>2.04± 0.05</td>
<td>0.40± 0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>c</td>
</tr>
<tr>
<td>T2</td>
<td>2.5</td>
<td>0</td>
<td>0</td>
<td>32.29± 0.50</td>
<td>3.97± 0.16</td>
<td>47.14± 0.12</td>
<td>44.98± 3.64</td>
<td>0.24± 0.05</td>
<td>0.20± 0.05</td>
<td>0.95</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>a</td>
<td>c</td>
<td>a</td>
<td>c</td>
<td>a</td>
<td>a</td>
<td>c</td>
</tr>
<tr>
<td>T3</td>
<td>2.5</td>
<td>10</td>
<td>0.18</td>
<td>32.12± 0.48</td>
<td>4.02± 0.11</td>
<td>47.06± 0.18</td>
<td>43.71± 4.06</td>
<td>1.13± 0.01</td>
<td>0.37± 0.01</td>
<td>0.92</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>a</td>
<td>c</td>
<td>a</td>
<td>a</td>
<td>b</td>
<td>b</td>
<td>a</td>
</tr>
<tr>
<td>T4</td>
<td>2.5</td>
<td>30</td>
<td>0.32</td>
<td>29.34± 0.38</td>
<td>6.09± 0.16</td>
<td>52.98± 0.22</td>
<td>36.72± 3.33</td>
<td>0.87± 0.69</td>
<td>0.02± 0.01</td>
<td>0.69</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>b</td>
<td>b</td>
<td>b</td>
<td>b</td>
<td>a</td>
<td>b</td>
<td>b</td>
</tr>
<tr>
<td>T5</td>
<td>2.5</td>
<td>50</td>
<td>0.50</td>
<td>28.73± 0.43</td>
<td>6.25± 0.09</td>
<td>52.77± 0.41</td>
<td>35.62± 3.70</td>
<td>1.48± 0.66</td>
<td>0.51± 0.01</td>
<td>0.66</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>b</td>
<td>b</td>
<td>b</td>
<td>b</td>
<td>a</td>
<td>a</td>
<td>b</td>
</tr>
</tbody>
</table>

Means within a column with different letters differ significantly (P<0.05).
The inhibition of body growth and the decline in carcass muscles weight, muscle protein and RNA synthesis appear to be solely the result of the profound effect of AF. Inhibition of cellular processes the most prevalent symptom of aflatoxicosis in poultry is reduced growth rate and poor performance. These adverse effects of AF due to anorexia, listlessness, inhibition protein and DNA synthesis and lipogenesis (38) AF appears to exert its negative effect on cells chiefly by depressing DNA and RNA synthesis and protein synthesis which eventually reflected in the final body weight and later carcass yields (49). (40) find out the influences of 2 mg/kg of aflatoxin B1 and immunomodulators on the performance of broiler, aflatoxin B1 alone had significantly reduced the body weight feed efficiency carcass yield immune development against Newcastle disease in broilers supplementation of lactobacilli, Vit. E and Se had improved the body weight, feed efficiency, carcass yield and immune against Newcastle disease. Feeding of aflatoxin B1 (1 mg/kg) to 2-week old Japanese quail for a period of 8 weeks indicating that supplementation of selenium selenite 5 mg/kg had some protective action against the toxic effect microscopic changes in the liver heart and bursa of fabricius lymphoid aggregation in liver aflatoxin B1 (41). An improved antioxidant status was observed in chicks of second group with α-tocopherol and selenium supplementation including higher concentration vitamin E, increased activity of glutathione peroxidase and lower levels of lipid peroxidation (42).

Acknowledgments

This study was supported by the College of Agriculture, Tikrit University, and the state company for drugs industry and medical appliances, Samarra.

References

25. Duncan DB. Multiple range and F. test Biometric. 1955;11:82.
42. Sodhi, S. A. Sharma A.P.S. Brar R. S. Effect of a tocopherol and Selenium on antioxidant status, lipid peroxidation and hepatopathy induced by malathion in chicks. pesticide Biochemistry and physiology.2007; 90:82-86.