Effect of Tomato Powder and Sepiolite to Liver Damage in Broiler Chickens Exposed to Aflatoxin

N W Purnama¹, O Sjofjan¹ and E Widodo¹
¹Faculty of Animal Science, University of Brawijaya, Jl. Veteran, Malang 65145, Indonesia

Abstract. The purpose of this research was to evaluate the effect of adding combination of tomato powder (TP) and sepiolite to feed with aflatoxin contaminated corn on liver damage of broiler chickens. The method was in vivo experiment by using a Completely Randomized Design with 7 treatments and 4 replications. The treatments consisted of T0= positive control (corn contaminated 10 ppb aflatoxin), T1= negative control (corn contaminated 102.5 ppb), P2= P1+0.25% sepiolite, P3= P2+0.5% TT, P4= T2+1% TP, T5= T2+1.5% TP, and T6= T2+2% TP. The measured variable were SGPT, SGOT and liver histopathological change according to Manja Roenigk scoring. The data were analysed by ANOVA and continued with Duncan’s multiple range test. The result showed that the highest SGPT level (P<0.01) was in T2 (8.13±0.12) and the lowest was in T4 (4.67±0.31). The treatments did not affect to SGOT levels. The lowest level of liver damage (P<0.01) according to Manja Roenigk score was in T5 (230±17.80). The conclusion of this research was addition of 0.25% sepiolite and 1% tomato powder in feed with aflatoxin contaminated corn decrease the liver damage in broilers.

1. Introduction
Poultry feed is generally based on cereal crops such as corn, soybean, and wheat which is easily contaminated by aflatoxin under certain conditions especially in tropical countries such as Indonesia. Aspergillus sp., Fusarium sp., and Penicillium sp. are three important types of molds producing aflatoxin that grow at temperatures 28-31°C and humidity 60-80%. There are several types of mycotoxin that are found to contaminate feed such as aflatoxin, ochratoxin, fumonisin, T2 toxin, zearalenone, and deoxynivalenol. Among these types, aflatoxin is the most often found. Aflatoxin significantly decrease production performance and cause high economical losses. SNI [1] determine the maximum level of aflatoxin contamination in corn was 50 ppb. However, Tangendjaja, Rachmawati, and Wina [2] reported that aflatoxin contamination in Indonesian local corn was seven times higher (58.8 ppb) than corn imported from US (8.8 ppb).

Liver as an organ which has a function to neutralize toxic substances, is responsible for neutralizing mycotoxin into substances that are not harmful to the body. In high levels of contamination, liver will work harder so it cause damage to cell structure and impaired liver function. Che et al. [3] stated that broilers exposed to mycotoxin showed liver damage according to histopathological analysis and increased enzyme activity in blood liver serum.

A technology has been developed to reduce and eliminate the impact of mycotoxin by using mycotoxin binder. Mycotoxin binder is an ingredient added to feed which does not affect the nutritional...
value, and able to binding mycotoxin in digestive tract to be excreted into faeces. Sepiolite is a type of mycotoxin binder which has been shown reduce the negative effect of aflatoxin in laying hens by increase the egg production and decrease the feed conversion ratio [4].

Combining mycotoxin binder with other feed additive such as phytobiotic rich in antioxidants might show a more beneficial effect. Tomato powder is a potential antioxidant source which contain lycopene as main phytochemical content. Lycopene has a high antioxidant activity to protect cells and tissues from oxidatives damage caused by reactive oxygen species [5]. Considering the benefits of tomato powder and sepiolite, as well as the negative effects of aflatoxin in feed, it is necessary to investigate the effects of adding sepiolite and tomato powder to liver damage of broiler chickens exposed to aflatoxin.

2. Materials and methods

2.1. Birds and dietary treatments

One hundred ninety six DOC Lohmann broilers with initial body weights 39.93±3.09 were randomly divided into 7 treatments, 7 birds per treatments, and replicated 4 times. Each replication reared in 1 m² litter cage. The brooding period was carried out for 14 days using heater gasolec in temperatures of 32-34°C. The lighting was used 10 watt LED lamp at 17.00-06.00. Feed and water was given ad libitum. The treatments given includes:

T0= positive control (basal feed with normal corn)
T1= negative control (basal feed with moldy corn)
T2= negative control + sepiolite 0.25%
T3= negative control + sepiolite 0.25% + tomato powder 0.5%
T4= negative control + sepiolite 0.25% + tomato powder 1%
T5= negative control + sepiolite 0.25% + tomato powder 1.5%
T6= negative control + sepiolite 0.25% + tomato powder 2%

Normal corn and moldy corn have different nutritional qualities which are described in the following table:

| Corn     | Moisture (%) | CP (%) | Fat (%) | CF (%) | Ash (%) | Aflatoxin (ppb) | GE (Kcal/kg) | ME (Kcal/kg) |
|----------|--------------|--------|---------|--------|---------|-----------------|--------------|--------------|
| Normal   | 13.81        | 8.92   | 2.01    | 2.43   | 1.19    | 10              | 3842.99      | 3144.73      |
| Moldy    | 18.50        | 8.11   | 2.74    | 1.80   | 1.07    | 102.5           | 3682.58      | 2876.01      |

Tomato powder obtained from UPT Materia Medica, Batu had nutritional contain: moisture 8.14%, crude protein 6.85%, crude fat 4.27%, crude fiber 15.50%, ash 0.73%, carotenoid total 180.04 µg/g, and estimated lycopene 140 µg/g. Sepiolite obtained from PT. Anpario Biotechnology Indonesia trade name sorbasafe contain 100% sepiolite, given 0.25% according to recommendation dosage of the product. Analysis of feed nutrient content was used near infrared spectroscopy. Analysis aflatoxin was used ultraviolet box aflatoxin detector. 200 g ground corn sample placed in dark box 20x20x20 cm. Corn highlighted use an ultraviolet flashlight in a dark room. Part of corn which shows a purplish green glow counted. 1 whole corn seed equal to 15 ppb, 1 seed with the size of a mungbean equal to 5 ppb.

The formulation and nutrient contents of basal diet explained in the following table:

| Raw Materials (%) | Starter (1-14 days old) | Grower-finisher (15-42 days old) |
|-------------------|-------------------------|----------------------------------|
| Corn              | 55                      | 57                               |
| Rice bran         | 8                       | 9.5                              |
| Palm oil          | 2.5                     | 3.5                              |
| SBM               | 27                      | 24                               |
| MBM               | 6                       | 4.5                              |
2.2. Measured variables
Variables measured includes liver histopathological changes and blood serum (SGPT and SGOT). Blood samples were taken using 2 ml injection syringe through the brachial vein. Each sample placed in yellow vacutainer (serum separator tube) containing silica as activator to separate serum from blood plasma. After that, samples were centrifugated 5000 rpm for 10 minutes. The serum obtained were analyzed using pentra C200 automatic biochemical analyzer.

Liver organ samples were prepared into liver tissue preparations with hematoxylin eosin. The condition of hepatocytes observed using a Miconos MCX50 LED light microscope, equipped with Optilab plus digital cameras and Optilab viewer 2.0 image processing software. Analysis level of liver damage was assessed by scoring according to Manja Roenigk scoring method [7]. Each preparations observed at 4 corner and at the middle of preparations. Each corner observed for 20 hepatocytes, for each normal hepatocyte multiplied by 1, parenchymous degeneration hepatocyte multiplied by 2, hydropic degeneration multiplied by 3, and necrosis hepatocyte multiplied by 4. Then the results obtained were calculate. Higher values indicate more severe liver damage.

2.3. Statistical analysis
The data obtained were tabulated in Microsoft Excel for one way analysis of variance. If there are significant differences between the treatments followed by Duncan’s multiple range test (DMRT).

3. Result and discussion

3.1. SGPT and SGOT
Data describing the effect of adding tomato powder and sepiolite to SGPT and SGOT levels of broiler chickens described in table 3.

| Table 3. SGPT and SGOT levels of broiler chickens with different treatments of tomato powder and sepiolite addition |
|---------------------------------------------------------------|
| Treatment          | SGPT (U/L)*          | SGOT (U/L)        |
|-------------------|----------------------|-------------------|
| T0                | 6.23±0.49<sup>a</sup> | 292.25±72.64      |
| T1                | 8.13±0.12<sup>b</sup>| 214.50±39.69      |
| T2                | 6.30±1.13<sup>a</sup> | 276.00±36.58      |
| T3                | 6.16±0.74<sup>a</sup> | 247.75±46.74      |
| T4                | 4.67±0.31<sup>a</sup> | 241.50±22.17      |
| T5                | 5.73±0.64<sup>a</sup> | 239.75±11.30      |
| T6                | 5.10±0.30<sup>a</sup> | 249.00±22.14      |

*different superscripts in the same column show significant differences (P<0.05)
Serum glutamic pyruvic transaminase (SGPT) and serum glutamic oxaloacetic transaminase (SGOT) are enzymes from the group of aminotransferases that have functions in the formations of amino acids in the liver. Liver damage causes the release of both intracellular enzymes, so the increase of SGPT and SGOT levels in the serum could be used as diagnosis of liver damage [7]. Based on statistical analysis, SGPT level in birds fed with corn containing high aflatoxin contamination without added feed additive (T1) showed the highest SGPT level. SGPT level decreased with addition 0.25% sepiolite (T2). Addition of tomato powder to diet that has been given sepiolite did not show significant differences in SGPT level compared to T2 and positive control (T0). Addition of sepiolite with or without combine with tomato powder did not show an effect on decrease SGOT levels. This results inversely to the results of study by Che et al. [3] that showed SGOT level in broilers exposed to aflatoxin were higher, but did not significantly influence SGPT level.

Hepatotoxicity mechanism caused by aflatoxin occurs by reducing glutathione peroxidase and superoxide dismutase that results in the formation of radical oxygen species (ROS). ROS that are not balanced with antioxidants can cause oxidative stress [9]. Free radicals will damage the cell membrane, mitochondria, and endoplasmic reticulum resulting in an increase in cytosolic Ca$^{2+}$. This condition causes activation of enzymes phospholipase, protease, endonuclease, and ATPase which results in decrease in phospholipids, disruption of membrane proteins, DNA fragmentation, and decreased ATP that causes necrosis in hepatocytes [10].

Tomato powder that rich in lycopene has high antioxidant activity. Antioxidants work by giving electrons to stop reaction of free radicals so the oxidation of fats and proteins could not be continued. Thus cell damage can be prevented [8]. Sepiolite is a natural mineral in the form of magnesium silicate complex (Mg$_4$Si$_6$O$_{15}$(OH)$_2$.6H$_2$O) found in clay. Sepiolite belongs to the phyllosilicate which have a sheet shape structure. The binding capacity of aflatoxin could be even greater because of sepiolite has large surface area. Cation of sepiolite Mg$^{2+}$ has an active position as a binder of carbonyl group in aflatoxin molecule [11].

3.2. Liver histopathology
Data describing the liver histopathological analysis showed in the following table:

**Table 4. Manja Roenigk score of broiler chickens with different treatments of tomato powder and sepiolite addition**

| Treatments | Manja Roenigk Score* |
|------------|----------------------|
| T0         | 252.50±19.36$^{\text{AB}}$ |
| T1         | 305.00±13.54$^{\text{C}}$ |
| T2         | 266.25±9.46$^{\text{B}}$ |
| T3         | 273.75±6.29$^{\text{B}}$ |
| T4         | 251.25±13.15$^{\text{AB}}$ |
| T5         | 230.00±17.80$^{\text{A}}$ |
| T6         | 250.00±14.14$^{\text{AB}}$ |

*different superscripts in the same column show very significant differences (P<0.01)

Based on statistical analysis, the result showed that T5 had the lowest level of liver damage, which means that addition sepiolite 0.25% and combined with 1.5% tomato powder was the most effective treatment to prevent liver damage in broiler chickens fed with high aflatoxin contaminated corn. Increasing the level of tomato powder to 2% (T6) did not show a better effect, and has the same results as bird fed with normal corn (T0). T1 showed the highest Manja Roenigk score that means showed the most severe liver damage. Addition 0.25% sepiolite and 0-0.5% tomato powder already show significant improvement.

The Manja Roenigk scoring system is a quantitative method to determining the level of liver damage by assessing histopathological changes in the liver. Changes in hepatocytes morphology the presence of toxins in early stage is degeneration that is still reversible. Degeneration consists of parenchymal degeneration and hydropic degeneration. Parenchymal degeneration is described by the swelling of hepatocytes due
to shifting extracellular water that enters to the cell. Hydropic degeneration is the level of continued damage after parenchymal degeneration which is characterized by the presence of small vacuoles in the cytoplasm. In continued damage, cell will exceed the point of no return which is irreversible called the stage of cell death or necrosis [12].

![Figure 1. Liver tissue of T1, white circle show local inflammation](image1)

![Figure 2. Liver tissue of T0, red arrow indicates karyorrhexis](image2)
Figure 3. Liver tissue of T1, black arrow indicate hydropic degeneration, green arrow indicate parenchymal degeneration, yellow arrow indicate picnosis, red arrow indicate karyorrhexis

Figure 4. Liver tissue of T5, black arrow indicate hydropic degeneration, green arrow indicate parenchymal degeneration, yellow arrow indicate picnosis

Degeneration is reversible, if the root cause which causing liver damage could be stopped, cells will return to normal. Necrosis is a degeneration process that occurs continuously due to exposure to toxic agents, chemical, free radicals, anemia, oxygen deprivation, and DNA and peptide synthesis disorders. Microscopically, necrosis could be picnosis (the nucleus appears denser and black), karyorrhexis (nucleus divided into fragments), and karyolysis (nucleus does not appear real). Necrosis is characterized by the presence of inflammatory cells whether local or diffuse (figure 1) [13].

4. Conclusions
Addition of 0.25% sepiolite and 1% tomato powder in diet containing corn with aflatoxin contamination showed the best results in decreasing liver damage in broiler chickens
References

[1] SNI 1998 *Jagung Bahan Baku Pakan SNI no. 01-4483-1998* (Indonesia: Badan Standarisasi Nasional Indonesia) (In bahasa Indonesia)

[2] Tangendjaja B, Rachmawati S and Wina E 2008 Mycotoxin contamination on corn used by feed mills in Indonesia. *Indonesian Journal of Agricultural Science* 9(2) 68-76

[3] Che Z, Liu Y, Wang H, Zhu H, Hou Y and Ding B 2011 The protective effects of different mycotoxin adsorbents against blood and liver pathologhical changes induced by mold-contaminated feed in broilers. *Asian-Australasian Journal of Animal Science* 24(2) 250-256

[4] Mizrak C, Yenice E, Kahraman Z, Tunca M, Yildirim U and Ceylan N 2014 Effects of dietary sepiolite and mannanoligosaccharide supplementation on the performance, egg quality, blood and digestion characteristics on laying hens receiving aflatoxin in their feed. *Ankara Univ Vet Fak Derg* 61 65-71

[5] Srivastava S and Kulshrestha K 2013 Nutritional content and significance of tomato powder *Annals of Arid Zone* 52(2), 121-124

[6] Sulaiman A 2019 *Penetapan Persyaratan Teknis Minimal Mutu dan Keamanan Pakan dan/atau Bahan Pakan Keputusan Menteri Pertanian Republik Indonesia nomor: 430/Kpts/KN.200/M/7/2019*

[7] Ramachandran R and Kakar S 2009 Histological patterns in drug-induced liver disease *Journal Clinical Pathology* 62 481-492

[8] Rafita I D, Lisdiana and Marianti A 2015 Pengaruh ekstrak kayumanis terhadap gambaran histopatologi dan kadar SGPT-SGOT hepar tikus yang diinduksi parasetamol *Unnes Journal of Life Science* 4(1) 29-37

[9] Wang R J, Fu S X, Miao C H and Feng, D Y 2006. Effect of different mycotoxin adsorbents on performance, meat characteristic, and blood profiles of avian broiler fed mold contaminated corn. *Asian-Australasian Journal of Animal Science* 19(1) 72-79

[10] Sulistyowati E, Purnomo Y, Nuri S and Audra F 2013 Pengaruh diet sambal tomat ranti pada struktur dan fungsi hepar tikus yang diinduksi tawas *Jurnal Kedokteran Universitas Brawijaya* 27(3) 156-161

[11] Widiyanti P M and Maryam R 2016 Pemanfaatan bahan pengikat mikotoksin untuk menanggulangi kontaminasinya dalam pakan *Wartazoa* 26(2) 91-101

[12] Rohmani A and Rakhmawatie M D 2015 Efek ekstrak kulit manggis terhadap gambaran histopatologi hepar tikus wistar yang diinduksi formalin *Jurnal berkala ilmiah kedokteran dan kesehatan* 1(2) 88-95

[13] Fahmi M, Fahrimal Y, Aliza D, Budiman H, Aisyah S and Hambal M 2015 Gambaran histopatologi hati tikus (*Rattus novergicus*) yang diinfeksi *Trypanosoma evansi* setelah pemberian ekstrak kulit batang jahol (*Salix tetrasperma* Roxb) *Jurnal Medika Veterinaria* 9(2) 141-145