



EXPERIMENTAL STUDY OF THE EFFECTS OF AFLATOXICOSIS IN EWES

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ABSTRACT

The experiment was conducted to investigate the clinical effects of aflatoxin in ewes, in addition to its effects on leukogram. Twelve Iraqi local breed apparently healthy ewes of 2 – 4 years old were subjected to oral administration of 64 µg aflatoxin. Anorexia, depression, heavy diarrhea, dysentery, fever, tachycardia, tachypnea, rumen atony and cachexia leading to decrease body condition score were observed in all ewes belonged to treatment group. Two ewes in treatment group died and were subjected to postmortem examination which revealed swelling and congestion of liver, in addition to necrosis on liver and kidney as well as the gall bladder was markedly enlarged with edema of the bile duct, and congestion of lungs. Enteritis appeared markedly and accompanied by enlargement of mesenteric lymph nodes, and bleeding on heart. Total leucocyte count and lymphocyte count increased significantly ($P < 0.05$) two weeks after aflatoxicosis and then decreased significantly ($P < 0.05$) two other weeks later, while monocyte decreased significantly ($P < 0.05$) six weeks after aflatoxicosis. It is concluded that aflatoxin is dangerous food contaminant and a potent hematotoxic and hepatotoxic mycotoxin.

KEYWORDS: Aflatoxin, ewes, diarrhea, leukogram, hematotoxic, hepatotoxic *etc.*

INTRODUCTION

The aflatoxins (AFs) are a group of closely related, biologically active mycotoxins, produced by strains of *Aspergillus flavus*, *A. parasiticus* and *A. nomius* (Creppy, 2002), other fungi reported to produce AFS are *A. bombycis*, *A. ochraceoroseus* and *A. pseudotamari* (Klich *et al.*, 2000, Bennett & Klich, 2003, Mishra and Das, 2003). In sheep, a dose rate of 4 mg/kg is associated with death at 15-18 hours due to acute hepatic insufficiency at dose rates of 2 mg/kg there is an increasing of respiratory rate, a rise in temperature and diarrhea with blood and mucus, at a dose rate of 0.2 mg/kg there is anorexia and diarrhea (Radostits *et al.*, 2010). The occurrence of AFS contamination is global, with severe problems (Khanafari *et al.*, 2007) as it has been reported in most countries and many spoiled feeds, especially harvested peanuts, peanuts-in shells on hay, cottonseed meal, sorghum grain, corn, moldy bread, green chop sorghum, or rarely on a standing crop, *e.g.* ears of sweet corn as well as are found in soil used for growing crops (Hall *et al.*, 1989). Aflatoxicosis has a wide variety of effects on animals, including weight loss, decrease growth rate, and poor performance as well as an alteration in mineral metabolism coagulation profile, changes in the clinical biochemistry panel, and an increase in susceptibility to infectious diseases due to depression of the immune status (Sharma, 1993, Fernaindez *et al.*, 1996, Ramos *et al.*, 1996).

MATERIALS & METHODS

Animals

Twelve local breed apparently healthy ewes of 2 – 4 years old were identified by ear tags. The health of all animals was closely monitored before and during the study. All ewes were subjected to detailed clinical examination according to Jackson and Cockroft (2002) before and during the experiment and Total White Blood Cells (WBC) count ($10^9/l$), Lymphocytes count ($10^9/l$), Monocyte (MID) count ($10^9/l$) and Granulocytes count ($10^9/l$) by automated hematological analyzer (Abacus Vet Junior, Hungary) at zero day and (2, 4 and 6) weeks after the last dose of aflatoxin or PBS.

Grouping of the animals

The ewes will be divided into 2 groups as the following:

Group 1 which considered as negative control group (NC): Consist of 6 ewes was administered with PBS only without AF but they subjected to all tests mentioned at the same times of the second group.

Group 2 which considered as treatment group (TRT): Consist of 6 ewes administered orally with 64 µg AF for 14 consecutive days. All animals in this group were subjected to all tests mentioned previously.

Production of AF *Aspergillus flavus* isolate which used to produce the AF was obtained from the mycology laboratory /College of Veterinary Medicine – Mosul University, Iraq and cultured in sabouraud – dextrose agar and identified according to Quinn *et al.* (2004). The AF was produced according to Shotwell *et al.* (1966) and estimated by ELISA technique.

Statistical Analysis

Analysis of variance (ANOVA) was conducted and means were compared by t-test (Steel & Torrie, 1980).

RESULTS

Physical examination

From the eleventh day of administration of AF until the tenth day after the last dose of AF the following criteria were observed by the physical examination of the ewes: Anorexia, depression, Heavy diarrhea and dysentery

were observed in all ewes belonged to TRT group, while not observed in any ewe in NC group. Fever, tachycardia, tachypnea, rumen atony and cachexia leading to decrease body condition score was observed in TRT group, where the score of 4 ewes was declined from score 3 at the start of the experiment to score 1, while 2 ewes in the same group declined to score 2, whereas it was not decreased in ewes belong to NC. As shown in Fig. 1.

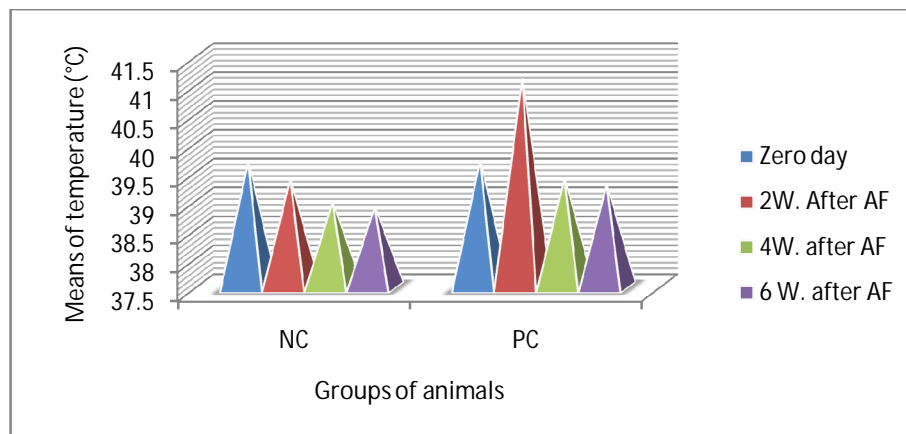


FIGURE 1. Histogram of means of the body temperature in both groups

The means of body temperature of ewes involved in NC group were 39.68 °C ranged between (39 – 40.2 °C) in zero day, 39.38 °C ranged between (39.2 – 39.9 °C) in the second period (2 weeks after administration of PBS), 38.98 °C ranged between (38 – 40.1 °C) in the third period (4 weeks after administration of PBS) and 38.9 °C ranged between (38.1 – 40.2 °C) in the fourth period (6 weeks after administration of PBS). In TRT group, the means of body temperature were 39.7 °C ranged between (38.5 – 40.2 °C) in zero day, then elevated significantly ($P < 0.05$) to 41.13 °C ranged between (39.7 – 42 °C) in the

second period, where 5 ewes in this group suffered from fever in this period, but resumed to 38.98 °C ranged between (37.9 – 40.8 °C) in the third period where only one ewe remained feverish 4 weeks after aflatoxicosis, while in the fourth period the mean of body temperature was 38.9 °C ranged between (38.8 – 40.8 °C). As shown in Fig. 2, the means of heart rate in NC group were 79.6 beat/minute (bpm) ranged between (75 – 90), 77 bpm ranged between (75 – 80), 73.4 bpm ranged between (70 – 76) and 76.25 bpm ranged between (75 – 78) in the four consecutive periods, respectively.

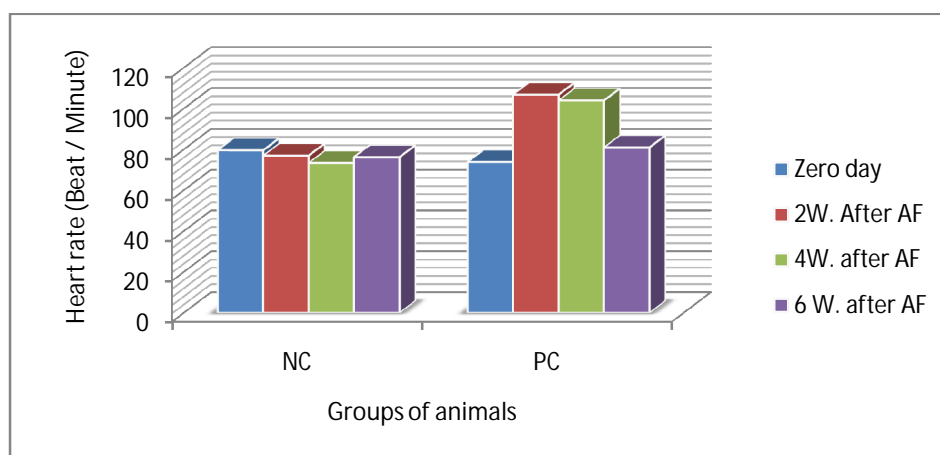


FIGURE 2. Histogram of means of the heart rate

In TRT group, the means of heart rate in zero day was 73.86 bpm ranged between (68 – 84) which increased significantly ($P < 0.05$) to 106.86 bpm ranged between (102 – 104) two weeks after aflatoxicosis and to 104 bpm ranged between (70 – 76) after two additional weeks,

whereas it resumed to 81 bpm ranged between (74 – 93) in the last period. Fig. 3. reveals the means of respiratory rate were also remained approximately in the normal levels in NC group, where they were 29.2 breath / minute (b/m) ranged between (26 – 31), 27 b/m ranged between (25 –

30), 28.4 b/m (ranged between 25 – 30) and 27.8 b/m ranged between (25 – 29) in the four consecutive periods, respectively. In TRT group the means were increased significantly ($P < 0.05$) from 26.43 b/m ranged between

(22 – 31) in zero day to 34 b/m ranged between (29 – 36) , 31.75 b/m ranged between (29 – 34), 30.5 b/m ranged between (28 – 35) b/m in the 2 , 4 and 6 weeks after aflatoxicosis, respectively.



FIGURE 4. Liver necrosis and enlargement of gall bladder with edematous bile duct in a dead ewe

Postmortem examination

Two ewes in TRT group died 2 and 4 days after the last dose of AF, these ewes were subjected to post mortem examination and revealed the following:

The liver was swollen, friable and congested, in addition to necrosis on liver and kidney as well as the gall bladder

was markedly enlarged with edema of the bile duct (Fig.4), in addition to, congestion of lungs. Enteritis was prominently appeared (Fig.5) accompanied by enlargement of mesenteric lymph nodes (Fig.6) and inflammation of rumen wall with engorged blood vessels and cyanosis, in addition to bleeding on heart.



FIGURE 5. Showing the marked enteritis in a dead ewe



FIGURE 6. Showing the marked enlargement of mesenteric lymph nodes in a dead ewe

The leukogram

As shown in Fig 7. the means of total leucocyte count ($10^9/l$) not revealed significant differences ($P < 0.05$) in NC group and they were 10.31 ranged between (4.87 – 13.22) , 9.89 ranged between (3.74 – 13.75), 11.3 ranged between (7.57 – 14.77) and 11.86 ranged between (9.52 – 13.66) , in the four consecutive periods, respectively.

Inversely, In TRT group, there were significant differences in total leucocyte count ($10^9/l$) between the different

periods, where it increased significantly ($P < 0.05$) from 11.21 ranged between (7.97 – 13.05) in zero day , to 19.78 ranged between (10.46 – 27.26) two weeks after aflatoxicosis, and then decreased significantly ($P < 0.05$) to 3.81 ranged between (0.7 – 6.72) four after aflatoxicosis and then retained to normal level of 9.72 ranged between (9.14 – 10.12) six weeks after exposure to AF .

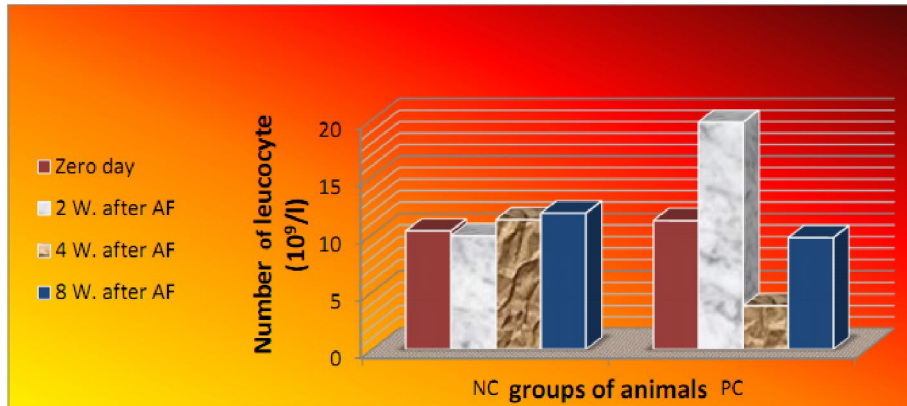


FIGURE 7. Histogram of means of the total leucocyte count in both groups

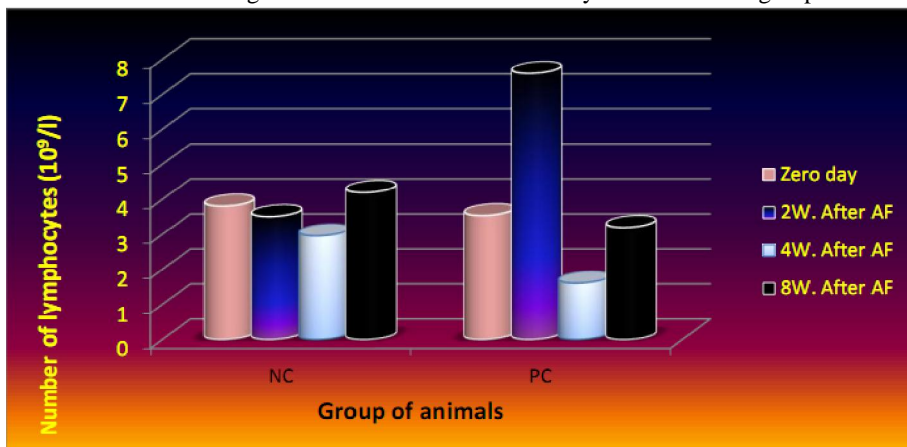


FIGURE 8. Histogram of means of the lymphocyte count in both groups

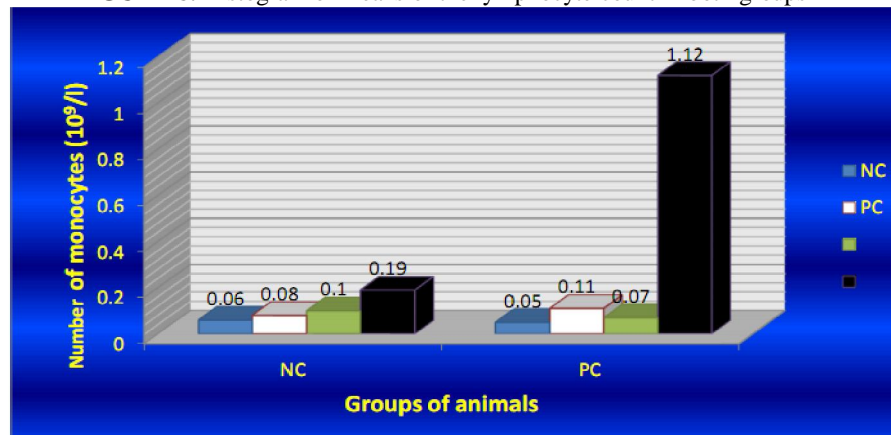


FIGURE 9. Histogram of means of the monocyte count in both groups

The lymphocytes count indicated prominent variation between TRT group and the NC group where , as appeared in Fig. 8. there were no significant differences ($P < 0.05$) in means of the lymphocytes count ($10^9/l$) in NC group in the different consecutive periods where they have 3.81

(ranged between 2.1 – 5.98) , 3.51 ranged between (2.16 – 9.83), 2.96 ranged between (1.69 – 7.45) and 4.2 ranged between (2.27 – 8.96) in the four consecutive periods, whereas in TRT group the mean in zero day was 3.54 ranged between (2.54 – 5.53) which increased

significantly ($P < 0.05$) to 7.6 ranged between (4.39 – 10.1) two weeks after exposure to AF then sharply decreased ($P < 0.05$) 1.61 ranged between (1.19 – 2.69) , four weeks after aflatoxicosis then after retained to 3.2 ranged between (2.43 – 4.25) six weeks after aflatoxicosis to become nearby the mean of zero day. Fig 9. reveals the means of monocyte count which remained in the normal levels in both groups except six weeks after aflatoxicosis in TRT group, where the means of monocyte count ($10^9/l$) in NC group were 0.06 ranged between (0.03 – 0.08), 0.08 ranged between (0.03 – 0.12), 0.1 ranged between (0.04 – 0.17) and 0.19 ranged between (1.01 – 1.16) in the four consecutive periods, respectively. In TRT group the means of monocyte ($10^9/l$) remained in the normal levels where they were 0.05 ranged between (0.02 – 0.09) in zero day to 0.11 ranged between (0.01 – 0.17), 0.07 ranged between (0.02 – 0.12) in two and four weeks after exposure to AF,

respectively but, the mean increased significantly ($P < 0.05$) to 1.12 ranged between (0.65 – 1.5) six weeks after aflatoxicosis. Fig.10. shows the means of granulocytes count ($10^9/l$) in which there are no significant differences ($P < 0.05$) in the four consecutive periods , where they were 6.44 ranged between (4.37 – 10.11) , 6.29 ranged between (2.35 – 8.45), 8.24 ranged between (5.92 – 14.96) and 7.46 ranged between (5.73 – 10.82), respectively. Conversely, In TRT group, mean of granulocyte count ($10^9/l$) was increased significantly ($P < 0.05$) from 7.61 ranged between (4.59 – 11.6) in zero day , to 12.07 ranged between (6.02 – 17.2) two weeks after aflatoxicosis, and then decreased significantly ($P < 0.05$) to 2.13 ranged between (1.92 – 3.56) four after aflatoxicosis then after elevated slightly to 5.4 ranged between (4.32 – 6.47) six weeks after exposure to AF.

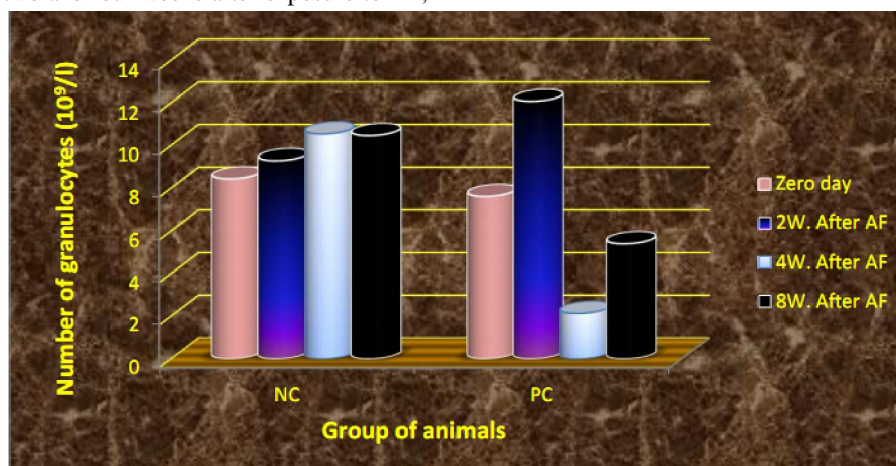


FIGURE 10. Histogram of means of the granulocytes count in both groups

DISCUSSION

The clinical signs observed in this study are in agreement with Abdelsalam *et al.* (1989) and Dhanasekaran *et al.* (2011). From these results, it is clear that the fever was common in the TRT group two weeks after administration of AF (Fig. 1), this result were in agreement with those obtained by Armbrecht *et al.* (1970) and Wylie and Morehouse (1978), who observed that the fever is one of the clinical signs of aflatoxicosis in sheep. The occurrence of fever may be attributed to the secondary infections which may follows the immune suppression resulted from aflatoxicosis that predisposed the animals to infection (Pier, 1992).The prominent elevation of heart rate in TRT group two weeks after aflatoxicosis may attributed to life threatening conditions (Radostitis *et al.*, 2000) due to aflatoxicosis and the secondary infections which predisposed by immunotoxic effects of AF, in addition to effect of stress and animals' fear. It is shown that tachypnea occurred in the TRT group after exposure to the AF and it is in agreement with Armbrecht *et al.* (1970) , while it was not observed in NC group. Two ewes in TRT group died and this result agree with Angus (2007).The mortalities that occurred in TRT group may not occurred due to AF alone but, the secondary infections also play an important role leading to death after predisposition by immunosuppressive effects of AF especially that *E. coli* and *Salmonella* spp. were isolated from 3 and 1 diarrheic

ewes in TRT group, respectively. The postmortem changes were in agreement with Angsubhakorn *et al.* (1981) and Coppock & Christian (2007). The increment means of total leucocyte count two weeks after aflatoxicosis in TRT are agreed with Lanza *et al.* (1980) , while they disagreed with Fernandez *et al.* (2000), and this increment level may attributed to effect of AF and to the fact that 2 weak ewes in this group died before taken the blood samples in the day 14 of clearance period, thus the more resistant ewes survived the lethal effect of AF but it respond to it at the level of cell counts leading to increased leucocytic count. In the third period , four weeks after exposure to AF, the count was decreased sharply in TRT group, this attributed to cytotoxic to effects of mycotoxins on a variety of cells including hematopoietic precursor cells and lymphocytes (Weiss, 2010) , in addition to the following causes which were suggested by Coles (1986):

- 1) Cachexia and debilitation which appeared markedly in all ewes belonged to this group (Fig. 4.3).
- 2) Endotoxins from gram negative bacteria, especially those secondary bacterial infections due to *E.coli* were identified in 3 diarrheic ewes in this group.
- 3) Overwhelming bacterial infections, *e.g.* salmonellosis, where *Salmonella* spp was isolated from a diarrheic ewe in this group.

The results revealed that the lymphocytes in TRT group increased during the second period, 2 weeks after aflatoxicosis, due to secondary bacterial infections which predisposed by immunosuppressive effects of AF, while this count was decreased significantly to a low level of $1.61 (10^9/l)$ due to the cytotoxic effects of AF on T-lymphocytes (Virdi *et al.*, 1989) leading to lymphopenia (Lanza *et al.*, 1980, Tornquist & Rigas, 2010) or due to septicemia which considered as one of causes of lymphopenia (Kauf *et al.*, 2007) in addition to effect of stress (Coal, 1986) and decreased recirculation of lymphocytes from intestinal lymphatics where the lymphadenopathy was observed in this group (Fig. 4).

Levels of monocyte in both groups were not increased significantly except in the last period, six weeks after AF, in TRT group and this result can be attributed to following causes:

- 1) Chronic disease inflammatory conditions e.g. fungal infections Coles (1986).
- 2) Monocytosis is sometimes seen as part of a stress response in ruminants (Tornquist & Rigas, 2010).
- 3) After development of leukopenia, in the third period in TRT group in this study, an increased blood concentration of monocytes occurred as favorable indicator for recovery from leukopenia (Gaunt, 2004). Elevated number of granulocytes especially the neutrophil (neutrophilia), which considered as the predominant granulocytic cells, two weeks after aflatoxicosis in TRT group attributed to the following causes:

- 1) Response to infection: Initially the demand for neutrophils is met from the bone marrow pool (Coles, 1986) where neutrophilia in ruminants is frequent in mild or moderate inflammation and in chronic inflammation, a neutrophilia may be observed (Dore *et al.*, 2007).
- 2) Bacterial and fungal infections of sheep may be associated with inflammatory neutrophilia (Summers *et al.*, 2002).
- 3) Neutrophilia is also variably reported in cases of toxicosis (Crowell *et al.*, 1979).
- 4) Immunological purposes where the neutrophil play a role in the following aspects (Weiss *et al.*, 2010):
 - a. Modulating the adaptive immune response.
 - b. Immunoregulation and are able to signal other members of the immune system. Through release of chemokines, and recruiting immune cells including T cells, monocytes, macrophages, and dendritic cells. In addition, antimicrobial peptides produced by neutrophils (e.g. alpha - defensins) can attract T cells and immature DCs.
 - c. Neutrophils may shuttle particulate antigens and microbes from the periphery to lymphoid tissue.

Conversely, regulatory T cells can secrete cytokines (e.g. IL - 17) that attract neutrophils leading to neutrophilia.

So eosinophilia may occurred to performed the function of eosinophils in detoxification by inactivation of histamine or histamine-like toxic materials, and due to tissue injury where chronic eosinophilia was common in diseases of tissues which contain large numbers of mast cells, such as skin, lungs, gastrointestinal tract and uterus. Tissue injury leads to degranulation of mast cells and histamine release,

and since histamine is chemotactic for eosinophils these are attracted from the bone marrow into circulation (Kerr, 2002) where this study was revealed a prominent gastrointestinal infection with AF. The basophils also increased during sensitization to an allergen or antigen (Coles, 1986), thus increased numbers of granulocytes may include neutrophilia, eosinophilia and basophilia. Significant decreased level of neutrophil (neutropenia) from 12.7 two weeks after exposure to AF to 2.3 four weeks later in TRT group may attributed to:

- 1) Increased movement of neutrophils into the marginal pool which occurs shortly after endotoxin ingestion and food toxicosis and the source of the toxin is generally the spores of bacteria or fungi in contaminated food (Kerr, 2002).
- 2) Effect of gram - negative sepsis (Santos *et al.*, 2002) which occurred in some ewes in this group,
- 3) Additional mechanisms of neutropenia may include direct infection of progenitor cells as well as loss of neutrophils into inflamed organs (Spanguolo *et al.*, 1997) where, bone marrow necrosis, fibrosis, or suppression will lead to neutropenia in association with other cytopenias, these have been reported in association with toxins (Tornquist & Rigas, 2010).

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