Effect of Alfatoxin and Ninivite on Total and Differentlal Leukoceyte Counts in Broiler Chicks

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ABSTRACT

An experiment was conducted to investigate the effect of aflatoxin at a concentration 2,5 mg/kg diet alone or with different levels of Ninivite 0.5, 1, 1.5, 2 and 2.5% on total, differential and absolute leukocyte counts in broiler chicks. The addition of aflatoxin (AF) in broilers diet at a concentration of 2.5 mg/kg did not significantly alter leukocyte counts when compared with the control group. The addition of Ninivite at all doses to the AF contaminated diet were resulted in a significant increase in the total circulating leukocytes when compared with the control group.

Differential leukocyte counts revealed a significant increase in the percentages of both monocytes and heterophils, and a reduction in lymphocytes when Ninivite was added at all doses to AF contaminated diet. The differential leucocyte counts of the remaining cells (Basophils and Eosinophils) show no significant changes when compared with AF and control group. The heterophils lymphocytes (H/L) ratio was significantly increased with each increase in the level of Ninivite addition to AF contaminated diet when compared with AF and control group .The picture of the absolute and differential leukocyte counts were identical except that, the lymphocytes were significantly reduced when Ninivite was added to AF contaminated diet at concentrations more than 1.5% when compared with AF and control group.

تأثير الافلاتوكسين والنينافايت على اعداد خلايا الدم البيض الكلي والتفريقي في افراخ فروج اللحم

الملخص

أجريت تجربة لتحديد تاثير اعطاء الافلاتوكسين بمعدل 2,5 ملغم/كغم عليقة بمفرده او مع تراكيـز مختلفة من النينافايت 0,5، 1,5، 2، 2,5% على العد التفريقي والمطلق لخلايا الدم البـيض فـي أفـراخ فروج اللحم واتضح من النتائج ان اعطاء الافلاتوكسين بمفرده لم يحدث تأثيراً ملحوظاً على اعداد كريات الدم البيض عند مقارنته مع مجموعة السيطرة. أما عند اضافة النينافايت بجميع التراكيز للاعلاف الملوثة بالافلاتوكسين فقد ادى ذلك الى زيادة معنوية في العدد الكلي لكريات الدم البيض عند مقارنتها بمجموعـة

السيطرة. كما واظهر العد التفريقي لكريات الدم البيض زيادة معنوية في نسبة الخلايا العدلة وخلايا وحيدة النواة. وانخفاض في الخلايا اللمفية عند المقارنة مع المجموعة المستهلكة للافلاتوكسين بمفـرده او مـع مجموعة السيطرة. بينما لم يظهر أي تأثير على خلايا الدم البيض الحمضة والقعدة. اضافة الى ذلك فقـد ارتفعت نسبة خلايا الدم البيض القعدة/اللمفية بشكل معنوي مع كل اضافة اللنينافايت الى العلائق الملوثـة باللافلاتوكسين عند المقارنة مع المجموعة المستهلكة للافلاتوكسين بمفرده او مع مجموعة السيطرة، نتائج العد المطلق لكريات الدم البيض وسيلة الخلايا القعدة/اللمفية لم يختلف عن مثيلاته في العد التفريقي فبمـا عدا الانخفاض المعنوي للخلايا اللمفية الذي سجل عند اضافة النينافايت بتراكيز اعلى من 1,5% للعلائـق الملوثة بالافلاتوكسين بالمقارنة مع المجاميع المستهلكة للافلاتوكسين بمفرده او مع مجموعة السيطرة.

INTRODUCTTION

Aflatoxins (AF), are a group of closely related biologically acive mycotoxins produced by certain strains of *Aspergillus flavus* and *A. parasiticus*. They commonly occur us natural contaminants of poultry feeds (Edds and Borteel, 1983). The effects of aflatoxins on poultry health are quite numerous. These are ranged from sudden death due to acute toxicity (Spensly, 1963). To the formation of tumors after ingestion of small quantities of aflatoxin over an extended period of time (Hamilton, 1984). They are also responsible for an adverse effects on blood parameters (Ibrahim et al., 2001). They are also in weight gain and feed conversion: increase in the relative weights of several internal organs (Al-Jubory et al., 2001); impairment of the reticuloendothelial activity (Michael et al., 1973), inhibition of the primary immune response (Thaxton et al., 1974); complementary system (Richard and Thurston, 1973). Phagocytes activity of leukocytes and alveolar macrophages (Change and Humilton, 1997). Aflatoxin has been reported to increase the severity of an ian infection disease (Bocachuvit and Hanilton, 1985) and failure of vaccination programmes (Al-Jubory and Sharecf, 1997).

A variety of physical chemical and biological methods to counteract the mycotoxin problem have been reported (lbrahim et al., 1998). In a large scale, practical and cost-effective methods for detoxifying mycotoxin containing feed staffs are not currently available (Ramos et al., 1996). The more recent approach to the problem has been the addition to the animals feed of non-nuritive adsorbants that sequester mycotoxin and prevent their gastrointestinal absorption, thus reducing their toxic effects on livestock and poultry performance as well as toxin carry-over into their products. One of the several assayed and used adsorbent was hydrated sodium calcium aluminum silicate (HSCAS). This clay, when added to poultry feed a concentration of 0.5% was effective in reducing the detrimental effects of aflatoxin on broiler performance (Kubena et al., 1990; phillipxs et al., 1988); and had an ameliorative effect on the negative aflatoxin effect on phagocytosis and Newcastle antibody formation in broiler chickens (lbrahim et al., 2000) Recently, local activated sodium bentonite was experimentally proved to be effective in reducing aflatoxicosis in broiler chicks (Al-Jubory et al., 2001; lbrahim et al., 2001). The successes of this product under experimental and field conditions (personnel communications). Enhanced many feed additives producers to using different kinds of silicates as mycotoxins; Unfortunately most of these new silicates were merely silica

dioxide and were indeed faulty licensed by veterinarian and poultry produces to be used as mycotoxins adsorbent. In this context. we try to clueidate the negative additive effects of feeding siliceous aluminum silicate named Ninixite with aflatoxin on total. differential and absolute leukocyte counts. As well as their combined effect on the ratio of heterophils/ lymphocytes ratio in broiler chickens.

TERIALS AND METHODS

One hundred and forty, 1 day-old male chicks were individually weighed; wing banded and housed in heated battery brooders under continuous fluorescent lighting. Chicks were fed a corn-soybean meal based starter diet obtained from a commereial mill. According to the manufacturers. it contained 22% crude protein. 2950 keal/kg metabolisable energy. 1.1% lysine and 0.6% methionine.

Aflatoxin (AF) was prepared through inoculation of rice with *Aspergillus parasiticus* NRRL 2999 (0btained from the college of Agriculture and Forestry. Mosul University. Mosul-Iraq) as described (Shotwell et al., 1996) and modified previously (West et al., 1973).

Fermented rice was then autoclaved and grounded. the aflatoxin content was measured by spectrophotometric analysis (Nabney and Nesbit, 1965; Wiseman et al., 1967). Of the total AF content in the rice powder. 81% was AFB_1 14% was AFG_1 4% was AFB_2 and 1% was AFG_2. The rice powder was incorporated into the diet to provide the described level of 2.5 mg/hg.

Ninivite was collcctcd as relatively large whitc bodies from Salamyia at south of Mosul city . It was grind to pass 0.5 mm sieve.

The chemical analysis of Ninivite was as follows: SiO_2, 95.7%, Al_2O_3, 0.7%; CaO; 0.6% ; $Fe_2O_3$0.3% ; MgO, 0.07% ; Na_2O, 0.02% ; K_2O, 0.04% P_2O_3 , 0.01% ; CI, 0.06% ; O..

0-0.3% : 1.. 0.1. 2.1: SrO. 117 ppm cd.16pp V.5ppm: Ni 3ppm (Al.Nagib, 1993).

Feed (without added antibiotics . coccidiosias) or growth promoters) and water were vaibile and libiium. The chicks were assigned to the folloving treatment groups in a completty randomized design into replicates of chicks per replicate.

1. Control group . 0.0 Af. 0.0 Ninivine
2. 2.5 mg AF/kg diet.
3. 2.5 mg AF/kg + 0.5% Ninivite
4. 2.5 mg AF/kg + 1% Ninivite
5. 2.5 mg AF/kg + 1.5% Ninivite
6. 2.5 mg AF/kg + 2% Ninivite
7. 2.5 mg AF/kg + 2.5% Ninivite

At the end of the 3rd week of treatments, blood was taken from 5 birds from cach replicate and were bled cardiac puncture for total leukocyte count (Tung et al., 1975) Blood smears were prepared and stained with Wright's stain the difierential leukocyte count was determined (Jain, 1986). Data were statistically analysed using the general linear model procedure of SAS (Statistical Analysis System, 1986). Statistical significance was accepted at $P< 0.05$.

RESULTS

Figure 1 shows that Af alone at the level of 2.5 mg/kg did not significantly alter the total leukocyte counts. when compared with the control group. The addition of Ninivite at every dose level to the AF-contaminated diets resulted in a significant (P< 0.05) increase in the total circulating lcukocytes. At the highest level of Ninivite administration (2.5%) the count was (91%) higher than of the control group.

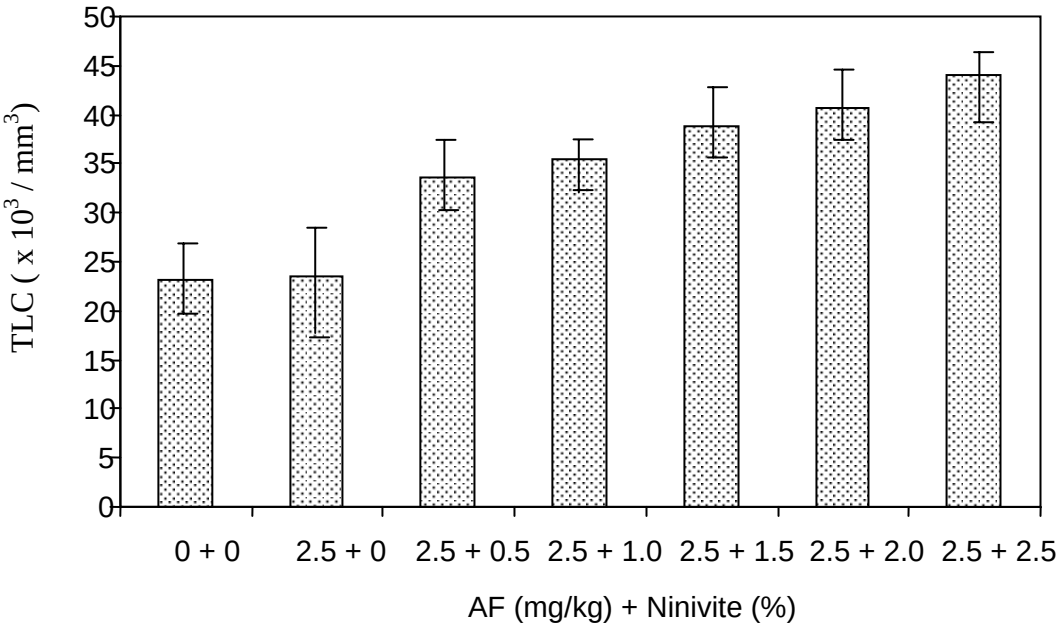

Fig. 1: The effect of AF , AF with Ninivite on the total Leucocytes count.

Figure 2 represeuts that AF thon no significant (p< 0.05) effect on the percentage of all lcucocyte. However, the addition of Ninivite at all donlevek to the AF contaminated diets resulted in a significant (p< 0.05) reduction in lymphocytes percentage and an increase in hetcrophils and monocytes percentage but no significant changes in basophils and eosinophils percentages was noticed when compared with the control group.

The contamination of the diet with 2.5 mg AF/kg feed had no detrimental effect on the H/L ratio, but a stressful condition was introduced in all group received Ninivite with AF, since H/L ratios w7ere significantly (p< 0.05) higher in these group when compared with the control group (Fig. 3).

The changes in relative percentages of different leukocytes was occurred in a progressive pattern with each increase in Ninivite level Figure 4 clears that when AF was given with all Ninivite levels there was a significant (p< 0.05) increase of the absolute number of heterophils and monocytes. And a significant reduction in lymphocytes particularly when Ninivite was incorporated at the higher levels (2 and 2.5%). but no significant changes in basophils and eosinophils were recorded.

The same picture of the H/L ratios was noted in the absolute count of leukocytes as that of the differential.

DISCUSSION

In the present study, chicks fed a diet containing only 2.5 mg aflatoxin kg feed did not exhibit lcukocytosis. Howevet, It was reported that leukocytosis could be induced when AF was included in broiler's diet at a rate of 3.5 mg/kg and more (Tung et al., 1975; Sharecf., 1999).

The addition of every dose level of Ninivite to AF contaminated diet was responsible for a consistent leukocytosis in broiler chicks. Although it was a non pan leukocytosis, but it was indeed a reflection to monocytosis and neutrophila. Monocytosis was so drastic than heterophila. Sice the absolute number of monocytes and netrophils were respectively (5-10) and (1-3) times more than those of control group. These result confirms the previous study of monocytosis induction in broiler chicks by feeding Ninivite (Shareef, 1999).

The consistent monocytosis induced by all Ninivite dietary levels, could be attributed to the high silicon dioxide content of Ninivite through the specific silica-macrophage-cytotoxic interaction. Which appeared to the rupture of the lysosomal membrane of the macrophage and the release of lysosomal enzymes into the cytoplasm (Jone et al., 1980). The macrophage is thus digesied by its own enzyme and free silica particles are once again relcased to be ingested by fresh macrophage in which the cycle is repeated. The damaged macrophage relcase a lipid factor (possibly a Lysolecithin). which is responsible for activation of the reticule-endothclial (R.E) system and felicitale the production of the more wondering blood monocytes that were converted to tissue macrophages replacing those which has been lysed dut to cytotoxic effect of cngulfed SiO_2 (Bruin, 1976). In this context silica could be regarded as an antimacrophageal agent (Rose, 1996).

Cross and siegelt (1983) regarded the ratio of H/L as an indicator of avian stress it is known that heterophilia did not indused in broiler chicks when AF was red at concentration of 2.5 mg/kg (Tung et al., 1975). Here the significantly higher H/L. Ratios in all broiler groups fed AFand Ninivite could be attributed to the stressful effect of Ninivite this result could give an explanation to the discrepancy between the low AF level inducing field afiatoxicosis due to the presence of mane stresses and that of the comparable more higher doses needed under laboratory conditions. The other alterative explanation of unexpectable heierophilia may be attributable to the additive effect of hemolytic anemia induced by both AF and dietary Ninivite (Shareef, 1999 ; Bruin, 1976). Although the negative in vivo studies on broiler performance induced by feeding siliceous Ninivite (Shareef., 2001) and the negative in vitro studies of SiO_2 cytoioxic effect on cultures of human umbilical vein endothelium, NIE-15 neuroblastoma and ROC1 oligodendoglial cells (Murphy et al., 1993), but the interaction between the haphazard addition of different aluminum silicate adsorbent containing different levels of SiO_2 to poultry feed and poultry welfare have as et not been extensively interconnected So. further studies should be performed to elucidate this interconnection and should also be stressed to select and to evaluate kind of aluminum silicate before its acceptance as mycotoxin adsorbent .

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