

AFLATOXICOSIS IN POULTRY:

2- EFFICACY OF HYDRATED SODIUM CALCIUM ALUMINOSILICATE AND YEAST CELL WALL TO AMELIORATE THE ADVERSE EFFECTS OF AFLATOXIN ON BROILER TISSUES

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ABSTRACT

Hydrated Sodium Calcium Aluminosilicate (HSCAS) and Yeast (*Saccharomyces cerevisiae*) cell wall (YCW) were evaluated for their ability to reduce the deleterious effects of aflatoxin (AFT) in broiler diets. They were incorporated singly or in combination into a diet containing total AFT 211.88 µg / kg feed. Aflatoxin induced vacuolar degeneration of hepatocytes, thickening in the wall of bile duct associated with leucocytic cells infiltration in the portal triad, kidneys showed necrobiotic changes of renal tubular epithelium with pyknosis of their nuclei and hypercellularity of glomerular tufts. Brain of chickens showed more or less similar histopathological changes described as necrosis of neurons , neuronophagia of necrotic neurons and necrosis of purkinje cells of the cerebellum . The levels of AFT residue in the treated groups were varied in type and amount from group to group as well as from tissue to tissue. Also aflatoxin induced an elevation in the levels of the elements AS , Pb and Zn in the tested tissues. Slight histopathological improvement was recorded in the previous tissues after the contaminated diet was incorporated singly or in combination with HSCAS and YCW. The most noticeable record was the drastic elevation in level of Al in the tissues of groups which were treated with HSCAS. The same groups revealed higher levels of AFT residue in meat and liver .The addition of YCW didn't induce noticeable improvement. Data concluded that, no clear improvements in the tested parameter were recorded after the contaminated diets were incorporated with the recommended dose of HSCAS and YCW , singly or in combination, during aflatoxicosis. More specific research is needed to clarify their unknown undesired effects on broiler.

INTRODUCTION

Aflatoxin contamination is a serious health hazard , it is extremely toxic and hepatocarcinogenic for animals and humans. Aflatoxins are toxic compounds produced by molds (*Aspergillus flavus*, *A. parasiticus* and *Penicillium puberulum*) and naturally occur in almost all feeding stuffs especially under tropical and subtropical conditions (Abdelhamid, 1993). Aflatoxin B₁ has been reported to be the parent compound of the common toxins (Mirocha, 1983). Contamination of feed and feed-stuffs with aflatoxin in particular has caused severe economic problems for livestock producers and may create potential health risks by the transmission of aflatoxin and/or their metabolites from livestock to human through consumption of meat, milk and eggs (Hsieh, 1983; Abdelhamid *et al.*, 1999 and Allam *et al.*, 1999). In some countries, control of aflatoxin often means balancing between a certain risk of

starvation against an uncertain risk of cancer (Abdelhamid, 1990,1993; Badria, 1996). When aflatoxin prevention fails, removal or destruction must be considered if the product is to be used for food or feed purposes (Park, 1993). Prevalence of high range of aflatoxin (AFT), mainly B₁, in some Egyptian food and feed stuffs are common (Badria, 1996; Selim *et al.*, 1996; El-Tahan *et al.*, 2000). Many practical approaches were carried out to solve this problem (Abdelhamid *et al.*, 2005) , the most famous one is to use smectite clay as an amendment to animal feed . At present, one of the more practical approaches is the use of adsorbents. Selected adsorbents added to AFT-contaminated feeds can sequester AF during the digestive process, allowing AFT to pass harmlessly through the animal. Major advantages of these adsorbents are that they are relatively inexpensive. It should be noted that most of these products haven't been approved for commercial use by FDA (Battacone *et al.*, 2009) . Hence, the present study was carried out in order to investigate the effect of aflatoxin - contaminated ration on broiler, and the efficacy of 2 adsorbents to ameliorate the adverse effects of aflatoxin on carcass quality of broiler.

MATERIALS AND METHODS

Animals:

Total number of 224 one-day old unsexed Ross chicks were obtained from a commercial hatchery and were randomly distributed among eight treated groups (each of 28 chicks) , in a washed fumigated batteries .

Groups:

- Group 1 : control -v (AFT free normal diet)
- Group 2 : control -v + HSCAS^{*}
- Group 3 : control -v + YCW^{**}
- Group 4 : control -v + HSCAS + YCW
- Group 5 : control +v (AFT ^{***} contaminated diet)
- Group 6 : control +v + HSCAS
- Group 7: control +v + YCW
- Group 8: control +v + HSCAS + YCW

* **HSCAS** : Hydrated Sodium Calcium Aluminosilicate 100 % (Origen -USA)
Regesterated in Ministry of Agriculture, Egypt (No.: 1661-26/8/2008), and given at a dose 2 kg/ ton feed.

** **YCW**: Yeast (*Saccharomyces cerevisiae*) cell wall 98 % contains:
Mannan - oligosaccharides 10 % Beta - Glucans 24 %
Regesterated in Ministry of Agriculture, Egypt (No: 9764 - 18/9/2007), under commercial name ALPHAMUNE (origen -USA)
and given at a dose 0.5 kg/ ton feed.

*** **AFT** :(aflatoxin) tested dose was(50B₁ + 18.85B₂ + 140.3 G₁ + 3G₂) µg,
give a total AFT 211.88 µg / kg feed.

Feed and water were provided *ad-libitum* . Feed was formulated in Regional Center for Food and Feed to be isonitrogenous , isocaloric and aflatoxin-free .Light was provided 24 hrs daily through out the period(40 days). Temperature keep to the required during brooding period. The chicks were weighed individually on 40 day old. Feed intake was recorded

throughout the period on a group basis. The feed conversion ratio (unit feed / unit gain) was calculated.

Sampling:

At the end of the experiments, five birds from each group were selected randomly and slaughtered after they were prevented from feed for 12 hr. Weights of hot carcass and liver of each animal were recorded. Carcasses of all groups were observed for post mortum examination just after slaughtering. Samples from breast and thigh muscles and liver were collected to assay the residue of AFT and levels of some elements in this tissues. Specimens from brain, liver and kidneys of the slaughtered animals were collected in buffer formalin for histopathological examination.

Aflatoxin production and assessment:

Aflatoxin production was carried out according to Davis *et al.*(1966) using liquid yeast medium and *Aspergillus flavus* strain (NRRL 3145). The media which contain detectable amount of aflatoxin was mixed well with the basal diet to get the aflatoxin - contaminated diet.

Aflatoxin in liquid medium , diet , tissues and excreta were determined according to Roos *et al.*,(1997) and A.O.A.C (2005) using HPLC technique (Agilent 1100 Series U.S.A. with column C₁₈ ,Lichrospher 100 RP-18 ,5µm x 25cm).

Micro- and macro-elements Assessment:

Assessment of micro-elements (Al , As , Cd , Pd , Se , Cu, Fe ,Mn and Zn) and macro-elements (K, Na and Mg) was done in both breast and thigh muscles, and liver tissues according to Agemian *et al.*(1980), using ICP- OE Plasma, optima DV 2000.

Histopathological examination:

Brain, liver and kidneys specimens of the slaughtered animals of each group were fixed in 10% buffered neutral formalin, then they were dehydrated, cleared and embedded in paraffin wax. Paraffin sections obtained at 4-5 µm and routinely stained with Haematoxyline and Eosin (Bancroft *et al.*, 1996).

Statistical analysis :

Statistical analysis was carried out according to Heath (1995) in one way analysis of variance. Data represented as means ± SD , for n = 3 .The difference was considered significant only at P < 0.05.

RESULTS AND DISCUSSTION

I- Histopathological examination:

Microscopically, liver of chicken from group 1 revealed no histopathological changes. Meanwhile, liver of chicken from group 2 showed vacuolar degeneration of hepatocytes, thickening in the wall of bile duct associated with leucocytic cells infiltration in the portal triad (Figure. 1). On the other hand, examined sections from group 3 revealed apparent normal hepatic tissues. Conversely, liver of chicken from group 4 showed hyperplasia of billiary epithelium, focal necrosis and desquamation of billiary epithelium, chronic cholangitis associated with pericholangiolar inflammatory cells infiltration (Figure. 2). Moreover, sections of liver from group 5 revealed

vacuolar degeneration of hepatocytes (Figure. 3), focal hepatic hemorrhage dispersed the hepatocytes far away from each other (Figure. 4), focal hepatic necrosis associated with leucocytic cells infiltration. Hyperplasia of biliary epithelium and portal infiltration with leucocytes were also noticed in all examined sections from this group. Slight improvement in the histopathological picture was observed in liver of chicken from group 6. Examined sections from this group showed slight hyperplasia of biliary epithelium as well as appearance of newly formed bile ductules (Figure. 5). However, liver of chicken from group 7 revealed hyper activation of epithelial lining bile duct, necrosis of biliary epithelium associated with edema and thickening in bile duct wall (Figure. 6). Meanwhile, liver of chicken from group 8 showed severe histopathological changes which confined as dissociation of hepatic cords, pyknosis of hepatocytic nuclei, dilatation and congestion of hepatic sinusoids (Figure. 7). In addition to the previous changes, examined sections also revealed hyperplasia and focal necrosis of biliary epithelium as well as massive leucocytic cells infiltration in portal triad (Figure. 8).

Kidneys of chicken from group 1 revealed no histopathological changes. Whereas, kidneys of chicken from groups 2, 3 and 4 showed necrobiotic changes of renal tubular epithelium with pyknosis of their nuclei and hypercellularity of glomerular tufts (Figure. 9). Some examined sections from those groups showed atrophy of glomerular tuft associated with distension of Bowman's space (Figure. 10). Microscopically, kidneys of chicken from group 5 revealed vacuolations of renal tubular epithelium with pyknosis of their nuclei (Figure. 11) as well as focal renal hemorrhage (Figure. 12). Meanwhile, examined sections from group 6 showed cytomegaly of renal tubular epithelium and karyomegaly of their nuclei as well as peritubular leucocytic cells infiltration (Figure. 13). Kidneys of chicken from group 7 revealed necrobiotic changes of renal tubular epithelium, congestion of intertubular blood capillaries associated with focal renal hemorrhage (Figure. 14). Examined sections from group 8 showed necrobiotic changes of renal tubular epithelium, pyknosis of some nuclei and karyomegaly of other nuclei (Figure. 15).

Histopathologically, brain of chicken from group 1 revealed apparent normal structure. However, examined sections from groups 2, 3 and 4 showed pyknosis of neurons (Figure.16). Meanwhile, brain of chickens from groups 5, 6, 7 and 8 showed more or less similar histopathological changes. Those changes described as necrosis of neurons (Figure. 17), neuronophagia of necrotic neurons (Figure.18) and necrosis of purkinje cells of the cerebellum (Figure.19).

Most of the previous findings are in synchronization with those recorded in different animal species during aflatoxicosis (Abdelhamid *et al.*, 1995 ; Lye *et al.*, 1995; Shebl ,1999; Abd-El-Rahman *et al.*, 2002; Shebl *et al.*, 2004 a,band 2007 and Tejada-Castañeda *et al.*, 2007) , where they recorded congestion and atrophy in blood vessels of brain ,liver and kidney of different animal species during aflatoxicosis. That concomitant with different degree of degenerative changes in neuronal cells , liver heptaocytes and renal cells.

Histopathological Figures

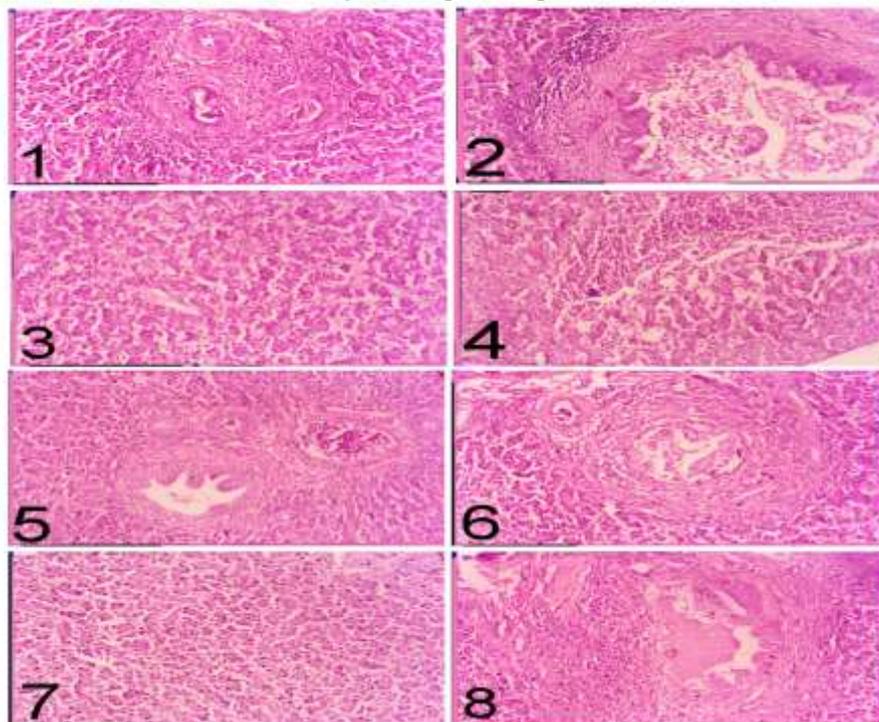


Figure.(1): Liver of chicken from group 2 showing thickening in the wall of bile duct associated with leucocytic cells infiltration in the portal triad. (H and E stain X 200)

Figure.(2): Liver of chicken from group 4 showing hyperplasia of biliary epithelium, focal necrosis and desquamation of biliary epithelium, chronic cholangitis associated with pericholangiolar inflammatory cells infiltration. (H and E stain X 200)

Figure.(3): Liver of chicken from group 5 showing vacuolar degeneration of hepatocytes (H and E stain X 200)

Figure.(4): Liver of chicken from group 5 showing focal hepatic hemorrhage dispersed the hepatocytes far away from each other (H and E stain X 200)

Figure.(5): Liver of chicken from group 6 showing slight hyperplasia of biliary epithelium as well as appearance of newly formed bile ductules. (H and E stain X 200)

Figure.(6): Liver of chicken from group 7 showing necrosis of biliary epithelium associated with edema and thickening in bile duct wall (H and E stain X 200)

Figure.(7): Liver of chicken from group 8 showing dissociation of hepatic cords, pyknosis of hepatocytic nuclei, dilatation and congestion of hepatic sinusoids (H and E stain X 200)

Figure.(8): Liver of chicken from group 8 showing hyperplasia and focal necrosis of biliary epithelium as well as massive leucocytic cells infiltration in portal triad. (H and E stain X 200) pyknosis of some nuclei and karyomegaly of other nuclei. (H and E stain X 400)

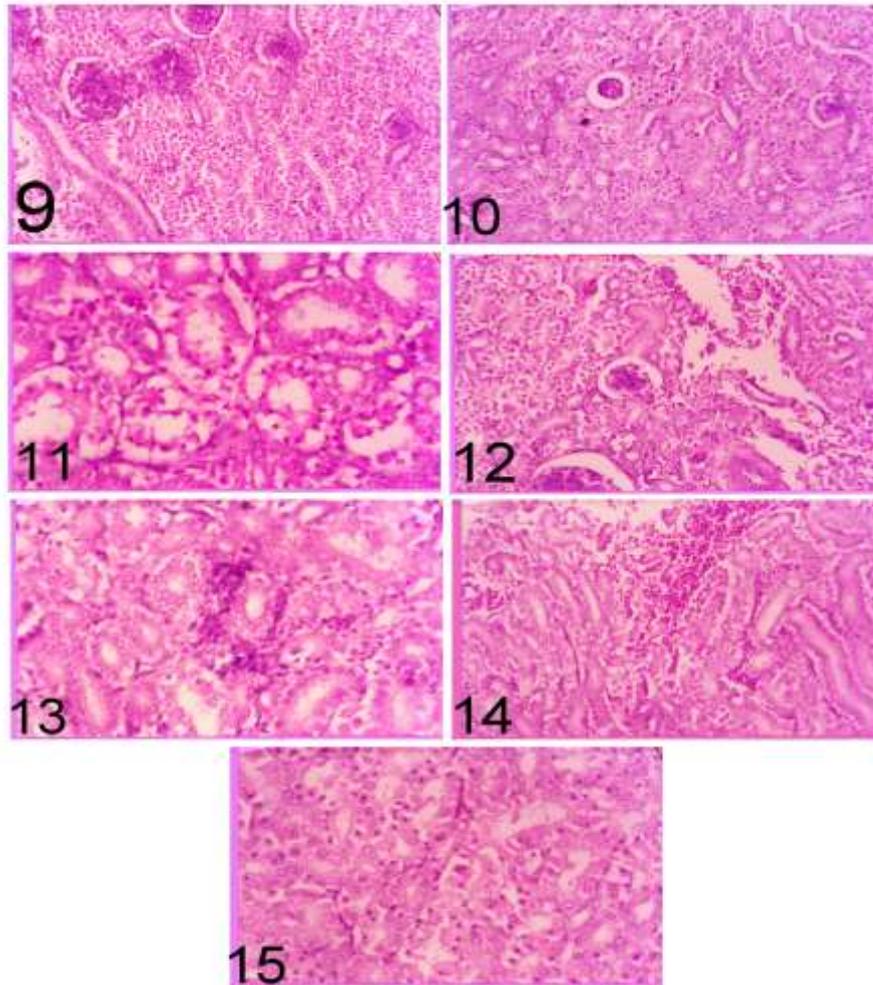


Figure.(9): Kidneys of chicken from group 3 showing necrobiotic changes of renal tubular epithelium with pyknosis of their nuclei and hypercellularity of glomerular tufts. (H and E stain X 200)

Figure.(10): Kidneys of chicken from group 4 showing atrophy of glomerular tuft associated with distension of Bowman's space (H and E stain X 200)

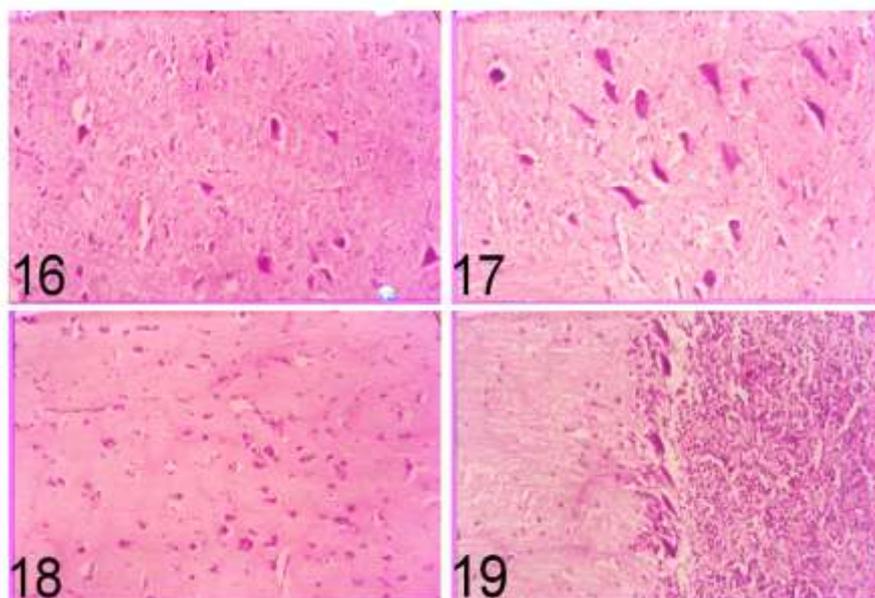
Figure.(11): Kidneys of chicken from group 5 showing vacuolations of renal tubular epithelium with pyknosis of their nuclei (H and E stain X 200)

Figure.(12): Kidneys of chicken from group 5 showing focal renal hemorrhage (H and E stain X 200)

Figure.(13): Kidneys of chicken from group 6 showing cytomegaly of renal tubular epithelium and karyomegaly of their nuclei as well as peritubular leucocytic cells infiltration (H and E stain X 400)

Figure.(14): Kidneys of chicken from group 7 showing necrobiotic changes of renal tubular epithelium, congestion of intertubular blood capillaries associated with focal renal hemorrhage (H and E stain X 200)

Figure.(15): Kidneys of chicken from group 8 showing necrobiotic changes of renal tubular epithelium,



- Figure.(16): Brain of chicken from group 3 showing pyknosis of neurons (H and E stain X 200)
- Figure.(17): Brain of chicken from group 5 showing necrosis of neurons (H and E stain X 400)
- Figure.(18): Brain of chicken from group 6 showing neuronophagia of necrotic neurons (H and E stain X 200)
- Figure.(19): Brain of chicken from group 7 showing necrosis of purkinje cells of the cerebellum necrosis of purkinje cells of the cerebellum (H and E stain X 200)

Metabolism of AFT in liver cells by cytoplasmic reductases and microsomal oxygenases resulted in different other forms of AFT. One of them is AFB₁-exo-8,9-epoxide form (Mclean and Dutton, 1995). This epoxide form is too active to form ducts with different component of the cell such as DNA, RNA, amino acids, proteins, enzymes...etc. (Chou and Chen, 1997; Shebl, 1999; Wang *et al.*, 2002; Smela *et al.*, 2002; Dash *et al.*, 2007; and Polychronak, 2007). All the alterations in cell biochemistry disturb cell vital activities and lead to water and ionic shifts, which finally cause cell damage and death (Butler *et al.*, 1981). That speculation can explain all the histopathological changes which recorded herein .

II- Aflatoxin residue :

The residues of aflatoxin in different tissues are illustrated in Tables (1-2) and Figures (20-21). No AFT residue was detected in any type of tissues of groups 1:4 which fed on AFT - free ration, as well as in breast muscle of all the tested groups. On the other hand , the levels of AFT residue in the remaining groups, AFT- treated groups (5- 8) ,were varied from group to group as well as from tissue to tissue.

Table (1) and Figure (20) represented the amount of AFT residue in thigh muscle of different tested groups.

As it is evident from the recorded data, significant levels of AFT were detected in thigh muscles of all AFT- treated groups(5-8). The highest levels were in groups 6 and 8 ,which fed on AFT- contaminated diet with HSCAS singly or in combinations with YCW. The levels were 2.097 and 3.23 µg/kg respectively.

Table (2) and Figure (21) represented the amount of AFT residue in liver tissues of different tested groups.

Data recorded revealed that significant levels of AFT were detected in liver tissues of all AFT- treated groups. The highest (12.26 µg/kg) was in group 6 which fed on AFT- contaminated diet with HSCAS.

Most of the previous finding are in parallel with those recorded by Trucksess *et al.*(1983) , Sova *et al.*(1984) , El-samra *et al.*(1991) and Denli *et al.*(2009), where they found that chickens fed AFT-contaminated diet accumulate residues of AFT in their meat and liver , and the levels in liver were many folded than in meat. Hassan(2006), detected high levels of AFB₁-residues in tissues and liver of growing local chickens treated with 1000 µg AFB₁/kg feed. He cited that the addition of HSCAS or Bio- Mos (yeast cell wall) to the contaminated diet decreased AFB₁ residue in meat and liver at the end of the treatment. He also noticed that ,the concentration of AFB₁ residue in liver tissues was higher 7-10 times than in meat. The last observation confirm our result, where in the present study, the level of AFT -residue in liver tissues is higher 5-10 times than in thigh.

Also, the previous findings with our finding are in parallel with those obtained in rabbits and sheep fed on AFT-contaminated diet (Abdelhamid *et al.*, 1990 and Allam *et al.* ,1999 and 2002). They estimated AFT-residues in muscles, liver , heart and kidneys of AFT- treated animals. They also added that, the addition of HSCAS to the contaminated ration can effectively provide a highly protection against the toxic effects of aflatoxin on the growing animals.

Hydrated Aluminosilicates of alkali and alkaline earth cations, having infinite , three -dimensional structure . they are further characterized by an ability to lose and gain water reversibly and to exchange constituent cations (Mumpton and Fishman , 1977) . Bonding between AFT and Aluminosilicates appears to be in the furan rings. Other possible bonding is with the two oxygen in the coumarin ring of AFT and interlayer cations or their associated water molecules. Evidence of octahedral Fe in smectite and amorphous silica in the clays both indicate greater AFT adsorption potential. Other smectites with spectral absorption indicating predominantly Al in the octahedral positions adsorbed less AFB₁(Tenorio Arvide *et al.* , 2008). The binding ability mainly appear to be pH -dependant (Ledoux *et al.*, 1999)

On the other hand, *Saccharomyces cerevisiae* , yeast cell wall (YCW) components have been used in animal feeding since the last decades (Hooge, 2004 and Rosen,2007). Their inclusion in broiler diets has resulted in improvements of animal productivity, which was attributed to physiological effects on intestinal digestive mucosa (Santin *et al.*, 2001; Zhang *et al.*, 2005 and Baurhoo *et al.*, 2007). However, the mode of action of YCW products in

broiler chicken diets is not well understood and the characteristics of YCW products have been poorly defined. Typically, commercial YCW are composed of 30 to 60% polysaccharides (15 to 30% of β -1, 3/1, 6-glucan and 15 to 30% of mannan sugar polymers), 15 to 30% proteins, 5 to 20% lipids, and no more than 5% of chitin (Aguilar-Uscanga and François, 2003 and Eurasyp, 2007). Most of the protein is linked to the mannanoligosaccharides (MOS) and is referred to as the mannoprotein complex (MP). In the digestive tract of animals, MOS present in YCW could act as high-affinity ligands, with the potential benefit of offering a competitive binding site for pathogenic bacteria mannose-specific type-1 fimbriae (Spring *et al.*, 2000). In lactating cows, Battacone *et al.* (2009) cited that the addition of a yeast that was not specifically manufactured as a mycotoxin-sequestering agent did not reduce the transfer of AFM1 from feed into milk. The previous speculation can explain the variation in AFT- residue which recorded herein in different tested groups and tissues.

III- Micro- and macro-elements:

The results of the determination of Al, As, Cd, Pb, Se, Cu, Fe, Mn, Zn, K, Na and Mg in meat and liver of different tested groups are illustrated in Tables (3-5) and Figures (22-24).

In both meat [breast muscle (Table 3 and Figure 22), and thigh muscle (Table 4 and Figure 23)] and liver tissues (Table 5 and Figure 24), there were a significant change between the groups, but control groups (2,3,4) showing, somewhat, closely related levels. During aflatoxicosis (groups 5,6,7,8), drastic elevation in AS, Pb and Zn was recorded. The most noticeable record was the drastic elevation in meat level of Al in the groups which treated with HSCAS (groups 6, 8). The same observation was recorded also in liver, but in less degree. Data recorded revealed also significant elevation in As and Zn in thigh muscle. Mn level was affected vigorously by the addition of HSCAS to the diet. Its levels were significantly reduced in thigh muscle, as well as in liver tissue.

Data recorded revealed that, breast tissue is more richer than thigh in Se and K, whereas thigh is more richer in Cu and Fe. Liver is more richer than both, nearly in all types of mineral (micro- and macro-elements).

There is a lack of information available about the effect of AFT or the detoxification materials such as HSCAS or YCW on the pattern of metal in meat and liver tissues. In the present study, the average mineral composition of different tissues are in parallel with Demirbas (1999) and Surtipanti *et al.* (2001) where they determined different levels of As, Cd, Ca, Co, Fe, Pb, Mg, Mn, Hg, K, Na, Zn, Ni, Sb and Se in chicken meat, intestine, liver and eggs.

Metals which present in both meat and liver are mainly originated from feed (Reilly, 1980). These metals play a variety of roles. They may be structural components of control mechanisms (e.g. in nerves and muscles), and above all enzyme activator, or component of redox systems. Some metals are essential (Co, Cr, Cu, Fe, Ni, Zn), and others are non-essential (As, Cd, Pb, Hg). A deficiency in essential elements results in impairment of biological function, but when present in excess, essential elements become toxic. Non-essential elements when enter into the body, will cause toxic effects.

T1

The major route of entry of most elements into the body is through the diet (Surtipanti *et al.*, 2001).

In rats treated with $AlCl_3$ and/or AFT, Shebl *et al.*, (2004b) found that Al residue in hepatic cystol was 10 folds in contrasted with the control group, that elevation was concomitant with marked decrease in hepatic cystol Se level. They cited that, the toxicity of Al- AFT mixture take the pattern of Al-toxicity. This speculation confirm the elevation in liver Al, which recorded herein concomitant with reduction in Se levels.

In the shadow of the previous speculation with the fact that HSCAS have ability to lose and gain water, and to exchange constituent cations (Mumpton and Fishman, 1977), the disturbance in the levels of different metals in different type of tissues can be explained .

Data didn't show clear improvements of the recommended dose of HSCAS and YCW singly or in combination during aflatoxicosis on the tested parameter , and more specific research is needed to if their have a possible beneficial effects on broiler or not .

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التسمم الأفلاتوكسيني في الدجاج:

2 - فعالية سليكات الألومنيوم وجدر خلايا الخميرة في الحد من التأثيرات السمية للأفلاتوكسينات في أنسجة دجاج التسمين

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سليكات الألومنيوم وجدر خلايا الخميرة قيمتا لمعرفة مقدرتهما على الحد من التأثيرات الضارة للأفلاتوكسين في علائق التسمين. أدخلنا منفردين أو مجتمعين إلى عليقة ملوثة ب 211.88 ميكروجرام أفلاتوكسين / كجم عليقة. أوضح الفحص النسيجي لكبد مجموعة الأفلاتوكسين وجود تحرضات سيتوبلازمية في خلايا الكبد، زيادة في سمك جدار القناة المرارية مصحوباً بارتشاح في المنطقة البابية بالخلايا البيضاء. أما النسيج الكلوي فقد لوحظ تغيرات تنكزية في الخلايا المبطننة للأنابيب الكلوية وضمور في أنويتها مع زيادة خلوية في الحويصلة الكلوية. الملح به تتركز في الخلايا العصبية مع التهام للخلايا العصبية الميتة و تتركز في خلايا بركنجي في المخيخ. مستويات متبقيات الأفلاتوكسين في أنسجة المجموعات التي تعاطت الأفلاتوكسين تفاوتت في النوع والكم من مجموعة إلى مجموعة ومن نسيج إلى نسيج. أحدثت الأفلاتوكسين زيادة في مستويات عناصر الأرسنك والرصاص والزنك في الأنسجة المحتبرة. تحسن خلوى سجل للأنسجة السابقة بعد إضافة سليكات الألومنيوم وجدر خلايا الخميرة للعلائق الملوثة. من الملاحظات الجديرة بالذكر وجود ارتفاع هائل في مستوى عنصر الألومنيوم في أنسجة المجموعات التي أضيفت السليكات إلى علائقها، نفس المجموعات عكست ارتفاع مستوى متبقيات الأفلاتوكسين في اللحم والكبد. إضافة جدر خلايا الخميرة إلى العلائق الملوثة لم يؤدي إلى تحسن ملحوظ. خلصت هذه الدراسة إلى أن إضافة سليكات الألومنيوم وجدر خلايا الخميرة بالجرعة المقررة مجتمعين أو منفردين إلى العلائق الملوثة لم يؤدي إلى تحسن ملحوظ في العناصر المختبرة، وأن استخدام تلك المواد يحتاج لمزيد من الدراسات المتخصصة لبيان تأثيراتها الغير معروفة الغير مرغوب فيها مع دجاج التسمين.

قام بتحكيم البحث

أ. د/ عبد الحميد محمد عبد الحميد

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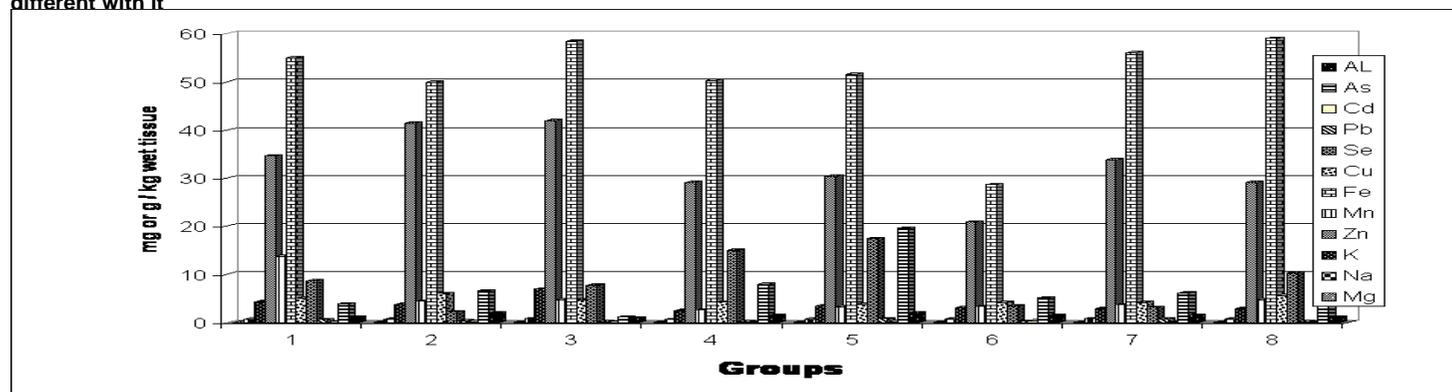
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Table(5) & Fig(24): Residue of some elements in liver tissues of broiler of different tested groups

Groups	Type and unit of elements											
	Micro-elements(mg / kg wet tissues)								Macro-elements(g/kg wet tissues)			
	Al	As	Cd	Pb	Se	Cu	Fe	Mn	Zn	K	Na	Mg
1	1.102 ^{2b} ±0.201	3.851 ^{4,5b} ±1.283	0.00 ^{2,4,5b,7,8} ±0.00	0.612 ^{2,3,4,5b,6} ±0.077	8.65 ^{2,4,5b,7} ±1.585	5.090 ±1.079	55.007 ^o ±9.857	13.757 ^{2,3,4,5b,7,8} ±2.987	34.633 ^{1b} ±4.07	4.327 ^{3,4,5,7,8} ±0.852	0.649 ±0.153	0.031 ² ±0.01
2	1.911 ^{1,3a} ±0.35	6.588 ^{3,5a} ±1.332	0.087 ^{1,3a,7} ±0.017	0.261 ^{1,3,4,5,7} ±0.037	2.209 ^{1,3,4,5a} ±0.803	6.013 ^{4,5a,7} ±1.04	49.9 ^o ±5.11	4.417 ¹ ±1.002	41.333 ^{4,5a,7,8} ±6.028	3.706 ³ ±0.939	0.733 ±0.217	0.064 ^{1,3} ±0.02
3	0.808 ^{2,3,7} ±0.131	1.139 ^{2,4,5b,7,8} ±0.205	0.02 ^{2,4,5b} ±0.008	0.115 ^{1,2,3,7} ±0.034	7.779 ^{2,4,5b,7} ±1.148	4.727 ±1.074	58.393 ^o ±11.819	4.67 ¹ ±1.501	41.99 ^{1,4,5b,7,8} ±4.687	6.914 ^{1,2,4,5b,7,8} ±0.88	0.761 ±0.155	0.036 ¹ ±0.007
4	1.359 ±0.452	7.859 ^{1,3a} ±1.715	0.06 ^{1,3a,5} ±0.016	0.111 ^{1,2,3,7} ±0.027	14.983 ^{1,2,3,4,5,7,8} ±2.245	4.2 ¹ ±0.855	50.315 ^o ±5.531	2.609 ¹ ±0.617	29.053 ^{3,5a} ±3.872	2.479 ^{1,3} ±0.934	0.578 ±0.189	0.044 ±0.013
5	1.937 ^{1,3a} ±0.379	19.599 ^{1,2,3,4,5,7,8} ±4.623	0.117 ^{1,3,4,5,7,8} ±0.031	0.795 ^{1,2,3,4,5a} ±0.13	14.43 ^{1,2,3,4,5,7,8} 2.498	3.816 ^{2,3} ±0.73	51.6 ^o ±6.089	3.199 ¹ ±0.528	30.3 ^{3,5a} ±5.027	3.358 ³ ±0.298	0.680 ±0.097	0.043 ±0.019
6	1.375 ±0.424	5.137 ^{3,5a} ±1.219	0.273 ^{1,2,3,4,5,7,8} ±0.05	0.234 ^{1,2,3,7} ±0.054	3.436 ^{1,2,3,4,5a} ±0.241	4.104 ² ±0.584	28.631 ^{1,2,3,4,5,7,8} ±5.543	3.325 ¹ ±0.527	20.863 ^{1,2,3,4,5,7,8} ±2.208	2.972 ^{1,3} ±0.466	0.645 ±0.152	0.046 ±0.012
7	1.508 ³ ±0.512	6.062 ^{3,5a} ±1.129	0.043 ^{1,2,3a} ±0.009	0.685 ^{2,3,4,5a} ±0.114	3.118 ^{1,3,4,5a} ±0.159	4.106 ² ±0.6	56.066 ^o ±8.923	3.687 ¹ ±0.518	33.767 ^{2,3a} ±3.356	2.803 ^{1,3} ±0.626	0.758 ±0.152	0.041 ±0.013
8	1.113 ^{2,3} ±0.336	10.615 ^{1,2,3,4,5,7,8} ±2.033	0.056 ^{2,4,5a,7} ±0.011	0.048 ^{1,2,3,7} ±0.048	10.28 ^{2,4,5a,7} ±1.489	5.505 ³ ±0.891	59.04 ^o ±6.493	4.682 ¹ ±0.54	29.09 ^{2,3a} ±3.456	2.867 ^{1,3} ±0.611	0.717 ±0.205	0.047 ±0.01

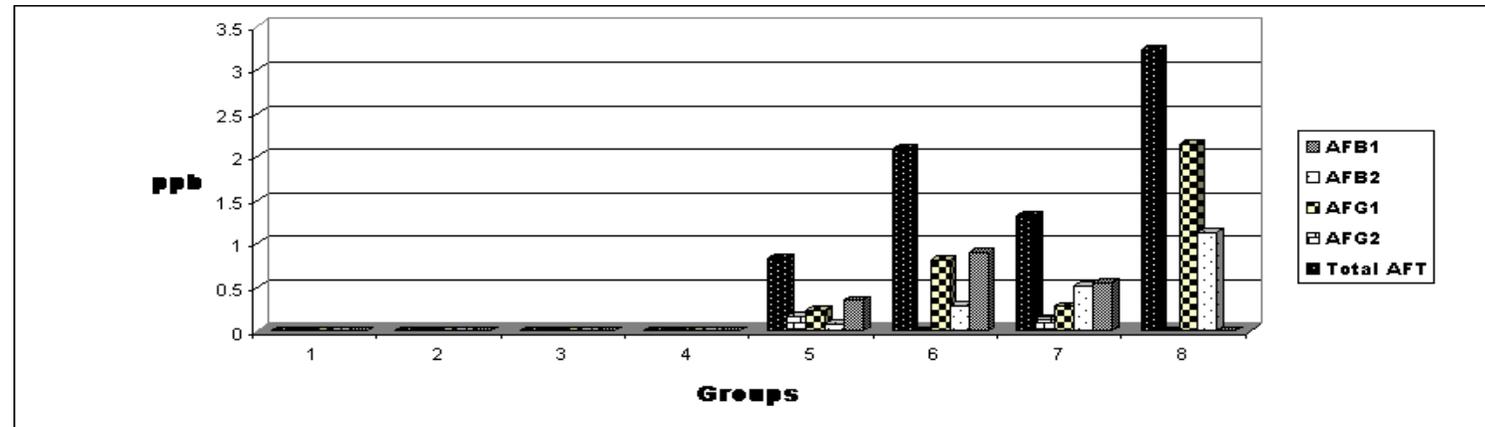
Data expressed as mean±SD , means within the same column are labeled (superscript no.) with the group(s) no. which they significantly (P<.05) different with it



Table(1) & Fig(20) : Residue of aflatoxin in thigh muscle (µg/kg wet tissue) of broiler of different tested groups

Group	Type of Aflatoxin				Total AFT
	AFB ₁	AFB ₂	AFG ₁	AFG ₂	
1	0 ^{5,6,7}	0 ^{5,6,7,8}	0 ^{5,6,7,8}	0 ^{5,7}	0 ^{5,6,7,8}
2	0 ^{5,6,7}	0 ^{5,6,7,8}	0 ^{5,6,7,8}	0 ^{5,7}	0 ^{5,6,7,8}
3	0 ^{5,6,7}	0 ^{5,6,7,8}	0 ^{5,6,7,8}	0 ^{5,7}	0 ^{5,6,7,8}
4	0 ^{5,6,7}	0 ^{5,6,7,8}	0 ^{5,6,7,8}	0 ^{5,7}	0 ^{5,6,7,8}
5	0.35 ± 0.03 ^{1,2,3,4,6,7,8}	0.079 ± 0.014 ^{1,2,3,4,6,7,8}	0.237 ± 0.04 ^{6,8}	0.163 ± 0.025 ^{1,2,3,4,6,8}	0.824 ± 0.028 ^{1,2,3,4,6,7,8}
6	0.897 ± 0.055 ^{1,2,3,4,5,7,8}	0.29 ± 0.02 ^{1,2,3,4,5,7,8}	0.807 ± 0.031 ^{1,2,3,4,5,7,8}	0 ^{5,7}	2.097 ± 0.27 ^{1,2,3,4,5,7,8}
7	0.547 ± 0.061 ^{5,6}	0.51 ± 0.036 ^{1,2,3,4,5,6,8}	0.28 ± 0.05 ^{1,2,3,4,6,8}	0.127 ± 0.025 ^{1,2,3,4,6,8}	1.32 ± 0.163 ^{1,2,3,4,5,6,8}
8	0 ^{5,6,7}	1.13 ± 0.113 ^{1,2,3,4,5,6,7}	2.147 ± 0.4 ^{1,2,3,4,5,6,7}	0 ^{5,7}	3.23 ± 0.456 ^{1,2,3,4,5,6,7}

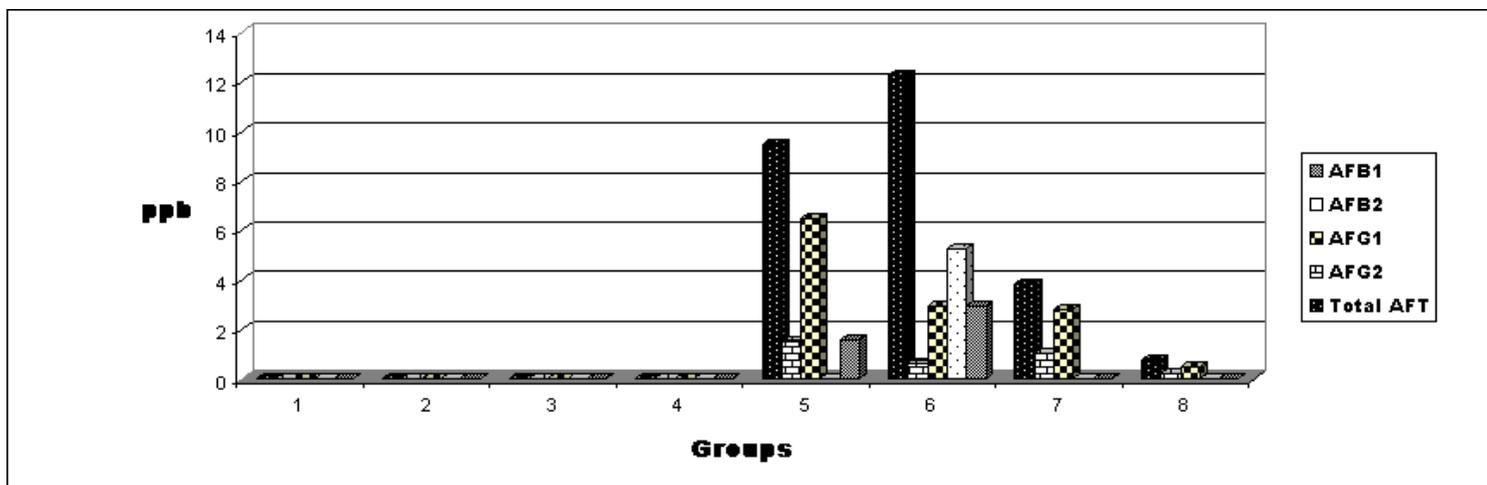
Data expressed as mean±SD , means within the same column are labeled (superscript no.) with the group(s) no. which they significantly (P<.05) different with it



Table(2) & Fig(21) : Residue of aflatoxin in liver tissues (µg/kg wet tissue) of broiler of different tested groups

Group	Type of Aflatoxin				Total AFT
	AFB ₁	AFB ₂	AFG ₁	AFG ₂	
1	0 ^{5,6}	0 ⁶	0 ^{5,6,7,8}	0 ^{6,8}	0 ^{5,6,7,8}
2	0 ^{5,6}	0 ⁶	0 ^{5,6,7,8}	0 ^{6,8}	0 ^{5,6,7,8}
3	0 ^{5,6}	0 ⁶	0 ^{5,6,7,8}	0 ^{6,8}	0 ^{5,6,7,8}
4	0 ^{5,6}	0 ⁶	0 ^{5,6,7,8}	0 ^{6,8}	0 ^{5,6,7,8}
5	1.593 ± 0.179 ^{1,2,3,4,6,7,8}	0 ⁶	6.51 ± 0.285 ^{1,2,3,4,6,7,8}	1.507 ± 0.333 ^{1,2,3,4,6,7,8}	9.493 ± 0.272 ^{1,2,3,4,6,7,8}
6	2.967 ± 0.351 ^{1,2,3,4,5,7,8}	5.267 ± 0.301 ^{1,2,3,4,5,7,8}	2.9667 ± 0.351 ^{1,2,3,4,5,8}	0.647 ± 0.015 ^{1,2,3,4,5,7,8}	12.263 ± 0.707 ^{1,2,3,4,5,7,8}
7	0 ^{5,6}	0 ⁶	2.827 ± 0.181 ^{1,2,3,4,5,8}	1.017 ± 0.097 ^{1,2,3,4,5,6,8}	3.81 ± 0.306 ^{1,2,3,4,5,6,8}
8	0 ^{5,6}	0 ⁶	0.51 ± 0.036 ^{1,2,3,4,5,6,7}	0.25 ± 0.01 ^{1,2,3,4,5,6,7}	0.75 ± 0.05 ^{1,2,3,4,5,6,7}

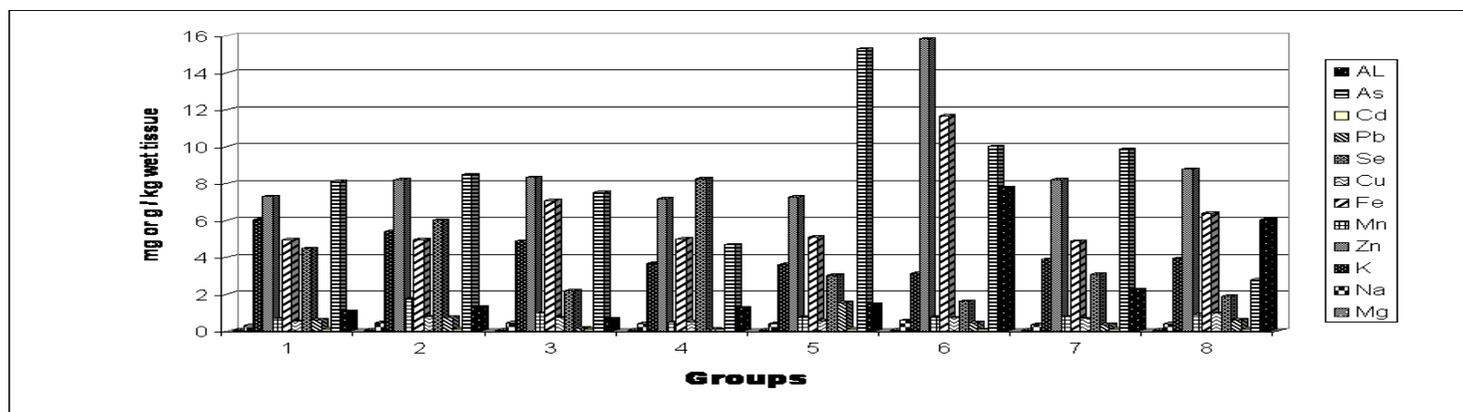
Data expressed as mean±SD , means within the same column are labeled (superscript no.) with the group(s) no. which they significantly (P<.05) different with it



Table(3) & Fig(22): Residue of some elements in breast muscle of broiler of different tested groups

Groups	Type and unit of elements											
	Micro-elements(mg / kg wet tissues)									Macro-elements(g/kg wet tissues)		
	Al	As	Cd	Pb	Se	Cu	Fe	Mn	Zn	K	Na	Mg
1	1.039 ^{0,7,0} ± 0.211	8.100 ^{4,5,0} ± 1.202	0.099 ⁴ ± 0.01	0.59 ^{3,4,0} ± 0.137	4.463 ^{2,3,4,5,0,7,0} ± 0.792	0.523 ^{1,2,0,0} ± 0.068	4.93 ^{3,0} ± 1.138	0.663 ^{2,3,0} ± 0.14	7.29 ⁰ ± 1.066	6.016 ^{4,5,0,1,0} ± 1.120	0.289 ⁰ ± 0.062	0.047 ± 0.011
2	1.269 ^{3,0,7,0} ± 0.2	8.467 ^{4,5,0} ± 1.35	0.090 ± 0.017	0.75 ^{3,4,0,7} ± 0.19	5.997 ^{1,3,4,5,0,7,0} ± 0.305	0.767 ^{1,3,0,0} ± 0.049	4.936 ^{3,0} ± 0.675	1.734 ^{1,3,4,5,0,7,0} ± 0.166	8.183 ⁰ ± 1.87	5.396 ^{4,5,0} ± 1.323	0.445 ± 0.07	0.046 ± 0.007
3	0.652 ^{2,4,5,0,7,0} ± 0.124	7.504 ^{4,5,0,7,0} ± 1.045	0.085 ± 0.014	0.151 ^{2,0,0,0} ± 0.03	2.163 ^{1,2,4} ± 0.404	0.718 ^{1,4,0,0} ± 0.102	7.051 ^{1,2,4,5,0,7} ± 0.809	0.956 ^{1,2,4,5,0} ± 0.155	8.33 ⁰ ± 0.83	4.855 ⁰ ± 0.852	0.442 ± 0.111	0.033 ^{0,0} ± 0.007
4	1.235 ^{3,0,7,0} ± 0.248	4.659 ^{1,2,3,5,0,7} ± 0.789	0.0667 ^{1,0,0} ± 0.021	0.107 ^{1,2,0,0,0} ± 0.022	8.256 ^{1,2,3,5,0,7,0} ± 1.088	0.486 ^{2,3,0,7,0} ± 0.122	5.002 ^{3,0} ± 0.8	0.465 ^{2,3,5,0,7,0} ± 0.073	7.174 ⁰ ± 1.044	3.654 ^{1,2} ± 0.974	0.408 ± 0.108	0.035 ^{0,0} ± 0.011
5	1.427 ^{3,0,7,0} ± 0.24	15.283 ^{1,2,3,4,0,7,0} ± 2.023	0.103 ⁴ ± 0.016	1.529 ^{2,3,4,5,0,7,0} ± 0.406	3.000 ^{1,2,4,0,0} ± 0.5	0.534 ^{2,3,0,0} ± 0.068	5.1 ^{3,0} ± 0.656	0.729 ^{2,3,4} ± 0.128	7.26 ⁰ ± 0.865	3.604 ^{1,2} ± 1.392	0.385 ⁰ ± 0.084	0.051 ^{3,4} ± 0.01
6	7.779 ^{0,1,2,3,4,5,0,7,0} ± 0.674	9.993 ^{3,4,5,0} ± 1.135	0.078 ± 0.014	0.45 ^{2,3,4,0} ± 0.088	1.609 ^{1,2,4,0,7} ± 0.319	0.718 ^{1,4,0,0} ± 0.074	11.67 ^{1,2,3,4,5,0,7,0} ± 1.529	0.739 ^{2,3,4} ± 0.06	15.837 ^{1,2,3,4,5,0,7,0} ± 2.244	3.117 ^{1,2} ± 0.432	0.569 ^{0,1,0} ± 0.094	0.045 ± 0.007
7	2.233 ^{1,2,3,4,5,0,0} ± 0.208	9.853 ^{3,4,5,0} ± 1.785	0.080 ± 0.01	0.357 ^{2,0} ± 0.053	3.069 ^{1,2,4,0,0} ± 0.495	0.663 ^{4,0} ± 0.066	4.865 ^{1,2,3,4,5,0,0} ± 0.68	0.763 ^{2,4} ± 0.066	8.183 ⁰ ± 1.026	3.874 ¹ ± 0.707	0.332 ⁰ ± 0.088	0.036 ⁰ ± 0.01
8	6.036 ^{1,2,3,4,5,0,7} ± 0.197	2.774 ^{1,2,3,5,0,7} ± 0.44	0.102 ⁴ ± 0.014	0.582 ^{3,4,0} ± 0.068	1.864 ^{1,2,4,0,7} ± 0.282	0.963 ^{1,2,3,4,5,0,7} ± 0.154	6.362 ⁰ ± 0.913	0.889 ^{2,4} ± 0.1	8.770 ⁰ ± 1.968	3.931 ¹ ± 0.636	0.38 ⁰ ± 0.118	0.055 ^{3,4,7} ± 0.009

Data expressed as mean±SD , means within the same column are labeled (superscript no.) with the group(s) no. which they significantly (P<.05) different with it



Table(4) & Fig(23): Residue of some elements in thigh muscle of broiler of different tested groups

Groups	Type and unit of elements											
	Micro-elements(mg / kg wet tissues)									Macro-elements(g/kg wet tissues)		
	Al	As	Cd	Pb	Se	Cu	Fe	Mn	Zn	K	Na	Mg
1	0.236 ^{2,3,4,5,6} ± 0.033	1.523 ^{2,3,4,5,6,7,8} ± 0.546	0.149 ^{4,5,7,8} ± 0.045	0.275 ^{2,3} ± 0.043	0.825 ^{4,5,6} ± 0.118	4.51 ^{1,6,7,8} ± 1.305	8.46 ^{2,3,4,5,6,7} ± .924	1.872 ^{2,3,4,5,6,7} ± 0.313	9.469 ^{2,6,7,8} ± 1.377	1.925 ^{2,3,4,5,6,7,8} ± 0.427	0.391 ^{3,7,8} ± 0.136	0.056 ± 0.009
2	0.85 ^{1,4,5,7} ± 0.131	5.082 ^{1,4,5,7} ± 0.976	0.102 ⁶ ± 0.010	0.579 ^{1,3,4,6} ± 0.173	1.399 ^{4,5,6} ± 0.306	0.811 ^{1,6,7,8} ± 0.625	5.573 ^{1,3,6} ± 0.41	0.495 ^{1,6} ± 0.111	13.702 ^{1,6,7,8} ± 2.403	3.414 ^{1,6} ± 0.336	0.397 ^{3,7,8} ± 0.165	0.043 ± 0.006
3	0.853 ^{1,4,5,7} ± 0.152	3.983 ^{1,4,5,7} ± 0.529	0.125 ^{4,7,8} ± 0.031	1.670 ^{1,2,4,5,6,7,8} ± 0.44	1.521 ^{4,5,6} ± 0.312	0.4 ^{1,6,7,8} ± 0.054	2.84 ^{1,2,6,7,8} ± 0.511	0.296 ^{1,6} ± 0.061	10.808 ^{6,7,8} ± 2.452	4.085 ^{1,4,5} ± 0.310	0.764 ^{1,2,6} ± 0.121	0.041 ± 0.006
4	0.540 ^{1,2,3,6,8} ± 0.067	9.977 ^{1,2,3,5,6,8} 1.065	0.056 ^{1,3,6,8} ± 0.006	0.244 ^{2,3} ± 0.05	5.703 ^{1,2,3,5,6,7,8} ± 1.085	0.569 ^{1,6,7,8} ± 0.098	3.896 ^{1,7,8} ± 0.727	0.328 ^{1,6} ± 0.022	14.5 ^{6,7,8} ± 2.291	3.001 ^{1,6} ± 0.709	0.49 ^{7,8} ± 0.118	0.045 ± 0.013
5	0.717 ^{1,6,7} 0.088	17.327 ^{1,2,3,4,6,7,8} ± 2.511	0.079 ^{1,6} ± 0.011	0.32 ³ ± 0.079	9.067 ^{1,2,3,4,6,7,8} ± 1.160	0.632 ^{1,6,7,8} ± 0.098	3.637 ^{1,7,8} ± 0.818	0.398 ^{1,6} ± 0.056	10.843 ^{6,7,8} ± 1.729	2.525 ^{3,6,7,8} ± 0.404	0.47 ^{3,7,8} ± 0.118	0.045 ± 0.013
6	0.302 ^{2,3,4,5,6} ± 0.052	4.088 ^{1,4,5,7} ± 0.152	0.111 ^{4,6} ± 0.017	0.534 ^{2,3,6} ± 0.071	0.443 ^{4,5,6} ± 0.081	2.767 ^{1,2,3,4,5,7} ± 0.679	4.675 ^{1,6} ± 0.641	0.698 ^{1,6} ± 0.114	19.667 ^{1,2,3,4,5,7} ± 3.698	3.93 ^{1,6} ± 0.234	0.631 ⁷ ± 0.069	0.043 ± 0.006
7	0.395 ^{2,3,5,6} ± 0.092	9.233 ^{1,2,3,5,6,8} ± 0.874	0.084 ^{1,3,6} ± 0.012	0.418 ³ ± 0.066	0.814 ^{4,5,6} ± 0.117	6.654 ^{1,2,3,4,5,6,8} ± 1.333	6.403 ^{1,3,4,5} ± 0.81	0.582 ^{1,6} ± 0.105	16.669 ^{1,2,3,4,5} ± 2.889	3.691 ^{1,6} ± 0.538	1.051 ^{1,2,4,5,6} ± 0.356	0.043 ± 0.007
8	0.846 ^{1,4,5,7} ± 0.163	4.431 ^{1,4,5,7} ± 0.41	0.327 ^{1,2,3,4,5,6,7} ± 0.055	0.165 ^{2,3,6} ± 0.054	3.415 ^{1,2,3,4,5,6,7} ± 0.918	3.346 ^{2,3,4,5,7} ± .792	7.586 ^{1,3,4,5,6} ± 0.803	1.936 ^{2,3,4,5,6,7} ± 0.562	18.091 ^{1,2,3,4,5} ± 2.916	4.628 ^{1,2,4,5} ± 1.002	0.796 ^{1,2,4,5} ± 0.114	0.043 ± 0.007

Data expressed as mean±SD , means within the same column are labeled (superscript no.) with the group(s) no. which they significantly (P<.05) different with it

