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Biochemical Effects of Aflatoxin on Rabbit Serum and Meat Chemical Constituents.

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Abstract
In the current study, Biochemical examination of aflatoxin intoxicated rabbits serum revealed significant increase in aspartate aminotransferase (AST), alanine aminotransferase (ALT) and γ-glutamy ltransferase (γGT) activities, malondialdhyde (MDA), triglycerides, very low density lipoprotein (VLDL), atherogenic index (LDL/HDL%) and VLDL/HDL%, creatinine, urea and uric acid concentrations. On the other hand, serum superoxide dismutase (SOD activity), reduced glutathione (GSH), cholesterol, high density lipoprotein (HDL) and low density lipoprotein (LDL) concentrations were significantly decreased in serum of rabbits fed aflatoxin contaminated ration. Chemical analysis of meat showed that SOD activity, GSH content, total protein%, fat%, ash%, triglycerides, cholesterol, calcium (Ca), inorganic phosphorus (P), sodium (Na) and potassium (K) concentrations in the meat of aflatoxin intoxicated rabbits were significantly decreased comparing with control. However, MDA concentration and moisture % were significantly higher than control rabbits. Additionally, chemical analysis of intoxicated rabbit's meat samples revealed presence of aflatoxin residues (3.2 ppb) in such meat.

Keywords: *Aflatoxin, oxidative stress, liver& kidney functions, lipid profile, meat constituents, aflatoxin residues and rabbit.*

Introduction

Rabbits farming have been taken up in the world for meat, fur, and biomedical purposes **(Lakkawar *et al.*, 2004)**. The importance of the domestic rabbit as a supplier of meat for human consumption is widely recognized throughout the world. **(Murshed *et al.*, 2014).** Breeding of rabbit in developing countries constitutes an extended activity among their populations. It aims to improve the financial situation and provide a high protein diet for these populations **(Verspecht *et al.*, 2011).** However, production of rabbit meat in Africa remains marginal **(Bléyéré *et al.*, 2013).**

Rabbits are prolific breeders. The animal breeds at least four times a year with a litter of five to eight rabbits. That means a female rabbit can give 90-105 young ones during its reproductive life that extends for 3 to 4 years. **(Para *et al.*, 2015)** In an efficient breeding, rabbits convert up to 20% of the consumed protein into meat, which is more than that of pigs (15-18%) and cattles (9-12%) **(Suttle, 2010).**

Global rabbit meat production approached 1.8 million tones per year in 2010, being predominantly concentrated in Asia (48.8%), Europe (28.4%), and the Americas (18.1%). The largest producer was China (669,000 tons) followed by Italy (255,000 tons), South Korea (133,000 tons) and Egypt (70,000 tons), **(FAOSTAT 2010 & 2012).** Rabbit meat repre-

sents a typical food for many Mediterranean countries like Algeria, Cyprus, Egypt, France, Italy, Spain, and some other European countries such as Belgium, Czech Republic, Luxembourg and Portugal **(Dalle Zotte** and **Szendrő, 2011).**

Worldwide high-value animal protein demand is in a continuous increase. The global per capita consumption of meat was reported to be 36.4 kg/year in 1999 and, more recently, per capita meat consumption is expected to increase to be 45.3 % by 2030 **(Bruinsma, 2003; Fiala, 2008; Schönfeldt,** and **Gibson, 2008)** due to the increasing world population, rising incomes and urbanization **(Steinfeld *et al.*, 2006).**

Meat is a major source of many nutrients, some of which are exclusive of meat or have higher bioavailability compared to other sources, thus playing a crucial role in maintaining human health **(Arihara, 2006).** Consequently, meat can be considered a functional food to the extent that it naturally contains many nutritive elements essential for humans, such as protein, fats, vitamins, essential amino acids, and minerals **(Zhang *et al.*, 2010).**

Rabbit meat is an interesting white meat ideal for modern consumers who are increasingly aware of the link between diet and health and see it as a valuable way to improve the quality of their lives **(Olmedilla-Alonso *et al.*, 2013).** Rabbit meat is appreciated for its vitamin B-complex content Particularly B12, being considered the highest among common animal meat species such as pork, beef, veal and chicken, for its low sodium, and low cholesterol **(Dalle Zotte and Szendrő, 2011).**

Nutritionally, rabbit meat is flavorful and easily digested, with high nutritional and dietetic properties: this meat contains 20–21% proteins of a high biological value; high levels of essential amino acids **(Dalle Zotte, 2004)**, high levels of unsaturated fatty acids (oleic and linoleic; 60% of all fatty acids), potassium, phosphorus, and magnesium. It has low concentrations of fat, cholesterol, and sodium **(Bielanski *et al.*, 2000** and **Hermida *et al.*, 2006).** So, rabbit meat is better than other kinds of meat (beef, lamb, or pork) **(Enser *et al.*, 1996)** and is recommended for persons with cardiovascular illnesses **(Hu and Willett, 2002).** Moreover, the energy value of rabbit meat (427–849 kJ/100 g of fresh meat) is comparable to various commonly consumed sorts of red meat **(Dalle Zotte, 2002).** Also, rabbit meat does not contain uric acid and has a low content of purines **(Hernández, 2007).**

Rabbit meat contains higher amounts of threonine, histidine, lysine, serine, glutamic acid, and glycine than ostrich meat and also higher amounts of threonine, lysine, glutamic acid, and glycine as compared to chicken meat **(Sales and Hayes, 1996** and **Strakova *et al.*, 2006).**

The amount of cholesterol in rabbit meat is about 59 mg/100 g in muscle**(Combes, 2004)**, lower than those of other species (61 mg in pork, 70 mg in beef, 81 mg in chicken) **(Dalle Zotte, 2002).**

Rabbit meat offers excellent nutritive and dietetic properties because it is a lean meat; rich in protein, contains a high level of polyunsaturated fatty acids (PUFAs) and a low cholesterol content **(Hernandez and Dalle Zotte 2010).**

Rabbit meat is also an important source of minerals P, K, Zn and Se and it is favorably low in Na (37-47 mg /100 g edible fraction) **(Dalle Zotte & Szendrő, 2011).** The mineral fraction of rabbit meat is characterized by its low contents in sodium (49 and 37 mg/100 g for hind leg and loin, respectively) and iron (1.3 and 1.1 mg/100 g for hind leg and loin, respectively), while the phosphorus level is high (230 and 222 mg/100 g for hind leg and loin, respectively **(Combes, 2004)**.

On the other hand, consumers have started associating meat and meat products with a more negative image. This is mainly due to their contents of fat, saturated fatty acids, cholesterol, sodium and nitrite, which have been associated with an increased risk to develop chronic diseases like obesity, cardiovascular disease, diabetes mellitus and some types of

cancer **(Arihara, 2006).** Foods of animal origin play an important role in determining the exposure of human beings to contaminants either of biological or chemical origin **(Hernández, 2008).** In the last decades, meat started to be seen as a food containing harmful compounds which could increase the risk to develop diseases **(Larsen, 2003).**

One of the food contaminants of greatest concern in food safety is aflatoxins **(Sabran *et al.*, 2012).** Aflatoxins are secondary toxic fungal metabolites produced by toxigenic strains of *Aspergillus* species of fungi, mainly *Aspergillus flavus* and *Aspergillus parasiticus*, *Aspergillus fumigatus* and *A. nomius*. They are toxic to a wide variety of animals **(Benneti and Klish, 2003).** The main route of exposure is through the diet, and this toxin is ubiquitously found in a variety of food commodities such as nuts, cereals, spices and herbs **(Mohd-Redzwan *et al.*, 2013)**. Aflatoxin B1 (AFB1) is classified by the International Agency for Research on Cancer **(IARC, 2002)** as a Group 1 carcinogen to humans**,** which is linked to the development of hepatocellular carcinoma (HCC) **(Strosnider *et al.*, 2006).** Moreover, it estimated that more than five billion people in developing countries face uncontrolled exposure to this food contaminant, especially in certain Asian and African countries where aflatoxin contamination is prevalent**. (Adilah and Redzwan**, **2017)**.

Rabbits are one of the most sensitive animals for aflatoxins (AF) **(Guerre *et al.*, 1996)**. Aflatoxicosis caused by AFB1 represents one of the most serious diseases of rabbits. Ingestion of AFs by rabbits showed many effects including, reduction of feed intake, poor efficiency of feed conversion and feed efficiency, poor growth, mal-absorption of various nutrients, decreased tissues integrity, increased susceptibility to infection, vaccine and drug failure and increased sensitivity to high temperature**,** great pathological changes, organs dysfunction, genetic damage and decreased productive and reproductive performance **(Marai and Asker, 2008)**.

Food safety is an important and essential aspect for consumers, especially on the meat sector since numerous crises have recently affected meat production **(Hernández, 2008).** Aflatoxin persists as a threat to the health of animals and humans in spite of the extensive research on preventive measures (**Dixon, *et al.*, 2008)**. Aflatoxin residues had reported in the meat of broiler chickens fed with aflatoxin-contaminated feed **(Hussain *et al.*, 2010).**

Since AFB1 has been implicated in the etiology of hepatic cancer, the presence of aflatoxin residues in milk, eggs and meat that represents a potential human health hazard, most countries have restricted the marketing of aflatoxin-contaminated grains **(Van Egmond, 1989).** The US Food and Drug Administration has set the maximum residue levels (MRLs) for aflatoxins in most food and feed ingredients at 20 μg kg^{-1}. The European Commission has set the MRLs for AFB1 and total aflatoxins in nuts and cereals for human consumption at 2 and 4 μg kg^{-1} respectively**(Brinda *et al.*, 2013)**.

The aim of the present work was to throw light on the harmful effects of aflatoxin B_1 presented in ration on rabbit's antioxidant status, liver & kidney functions and lipid profile, concentrations and to study the effect of aflatoxin on the oxidative stability, nutritional quality (some chemical constituents) and aflatoxin residues in rabbit meat to indicate the economic loss in rabbit's farms due to ration aflatoxicosis.

Materials and Methods

Ration analysis:

Ration samples were analyzed for the detection of aflatoxins according to the procedures described by **Truckess *et al.*, (1991),** using Vicam™ Fluorometer.

Aflatoxin B1 (AFB1) Production and quantification:

Aflatoxin was produced using a reference toxi-

genic strain of *Aspergillus parasiticus* obtained from mycology department, faculty of Veterinary. Medicine, Cairo university as the method of **Shotwell *et al.*, (1966)**.The fermented cultures were harvested (6^{th} day), autoclaved, oven dried, pulverized and AF was extracted **(Romer, 1975)** and quantified by the procedures of **Truckess *et al.*, (1991)**.

The culture material was mixed with the experimental crushed pellets to contain total AF concentration of 60.0 µg/Kg of feed. **(Karakilcik *et al.*, 2004)**

Animals and experimental design:

Twenty five male, healthy, New Zealand rabbits, aged between 5 and 6weeks, weighing between 1 and 1.2 kg were used. The rabbits were caged in flat deck wire cages, fitted with nipple drinkers and feeders. Rabbits were randomly assigned to treatments, five per cage. Animals had free access to feed and water. The animals were divided into 2 groups. Group 1 (10 rabbits) was fed a crushed pellets diet for 4 successive weeks and designed as **control group.** Rabbits in the Group 2 (15 rabbits) were fed a crushed pellets diet containing Aflatoxin B1, with concentration of 60.0 µg / Kg for 4 successive weeks **(affected group)**.

All the care and experimental procedures involving animals were followed according to the guidelines stated in the Guide for the Care and Use of Laboratory Animals.

Blood samples:

At the end of the experiment all rabbits were slaughtered and blood samples were collected from each rabbit of both groups in clean dry tubes. Serum was then separated by centrifugation of blood at 3000 rpm for 20 minutes and used for biochemical analysis.

Biochemical analysis:

Serum malondialdehyde (MDA) and reduced glutathione (GSH) concentrations were performed according to **Albro *et al.*, (1986) and Chanarin, (1989)** respectively Superoxide dismutase (SOD) activity assayed after **Masayasu and Hiroshi, (1979).**

Serum samples were subjected to determination of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities according to **Reitman and Frankel, (1957)**, γ-glutamyltransferase (γ GT) activity according to **Persijn, and Vander, (1976),**

Serum creatinine concentration was measured by the method adopted by **Bowers and Wong, (1980)**. Serum urea level was estimated according to **Fawcett and Scott, (1960)**. Determining serum uric acid concentration was performed by the assay of **Fossati et al. (1980)**.

Triglycerides concentration was measured according to the method described by **Fassati and Prencipe, (1982)**, Cholesterol level was estimated according to **Allain *et al.*, (1974)**, high density lipoprotein (HDL), and low density lipoprotein **(**LDL) were determined according to **Friedewald *et al.*, (1972)**. Further, atherogenic index (AI) was determined by using a method explained by **Bahramikia and Yazdanparast, (2008)** as AI = LDL/HDL

Rabbit meat samples (a part of the hind leg) were dissected from the fat surface, and the lean part was then finely minced and subjected to chemical analysis for moisture, protein, fat, ash, calcium, phosphorus, sodium, potassium and lipid profile (cholesterol, HDL& LDL).

Meat portions were homogenized according to **Combs, (1987).** The tissue homogenates were used for determination of MDA, GSH concentration and (SOD) activity as serum**.** The protein content was measured in tissue homogenate according to **Lowry *et al.* (1951).**

Proximate composition of rabbit meat was determined according to the procedures of **AOAC, (2005).** Moisture content of meat samples was determined by oven drying 2 g of meat at 105°C for 4 hours until a constant weight results. Crude protein was determined by using kjedahl method including, digestion distillation, and titration of the distillate. Crude protein value was obtained by converting nitrogen (N %) content obtained with a constant (6.25), thus crude protein was obtained as (6.25 x N %). Fat from meat samples was determined with Soxhlet extraction method using petroleum ether. Ash content was obtained by igniting 2 g of meat samples in a muffle fur-

nace set at 550°C for 4-6 h.

Meat lipids were extracted according protocol of **Folch *et al.*, (1957)** then, meat lipid profile (cholesterol, HDL& LDL) carried out as serum.

Meat Content of calcium (Ca), phosphorus (P) sodium (Na), and potassium (K), in thigh muscle were studied after dry mineralization. Calcium concentration was determined according to **Barnett (1965)**. Inorganic phosphorus concentration was determined according to **Daly and Ertingshausen (1972)**. Sodium concentration was measured according to **Henry *et al.*, (1974)**. Potassium concentration was measured as described by **Hillman *et al.*, (1967)**.

Statistical analysis:

Data were presented as mean ± standard error (SE) and the significance of differences was evaluated by one way analysis of variance (ANOVA) using The Statistical Package for the Social Sciences PC statistical program (**SPSS 14, 2006)**.

Results

Serum biochemical changes:

As shown in tables (**1, 2& 3**), AST, ALT and γ GT, activities, creatinine, urea, uric acid, MDA, triglycerides, VLDL, AI (LDL/HDL%) and VLDL/HDL% concentrations in serum of rabbits fed ration contained aflatoxin B_1 were significantly higher than control. On the other hand, serum SOD activity, GSH, cholesterol, HDL and LDL concentrations in the rabbits fed ration containing aflatoxin B_1 were significantly decreased compared with control.

Meat chemical constituents:

Chemical analysis of meat showed that SOD activity, GSH content, total protein%, fat%, ash%, triglycerides, cholesterol, Ca, P, Na, and K concentrations in the meat of affected rabbits were significantly decreased comparing with that of control, While MDA, concentration and moisture%, were significantly higher than that of control (**tables 1, 4& 5**).

Aflatoxin B_1 in rabbit Meat:

Table (**6**), represents that level aflatoxin B_1 residues in meat of affected rabbits was 3.2 ppb. No detectable levels of aflatoxin B_1 in control rabbits were recorded.

Table (1). Serum and Meat antioxidant profile in control and affected (fed aflatoxin B_1 contaminated ration) rabbits, (Mean ± SE).

Tissue	Serum (nmol/ml) [@], (µmol/ml) [$], (U/ml) [#]		Meat (nmol/mg protein) [@], (µmol/mg protein) [$], (U/mg protein) [#]	
Group / Parameter	Control rabbits	Affected rabbits	Control rabbits	Affected rabbits
MDA[@]	1.3 ± 0.06	3.42 ± 0.16*	1.24 ± 0.08	4.52 ± 0.22*
GSH [$]	17.84 ± 0.47	12.9 ± 0.51*	16.6 ± 0.25	12.2 ± 0.8*
SOD [#]	133.0 ± 0.95	92.6 ± 3.63*	143.2 ± 1.53	88.0 ± 3.03*

* Significant difference against control at $p \leq 0.05$.

Table (2). Serum biochemical parameters in control and affected (fed aflatoxin B_1 contaminated ration) rabbits, (Mean ± SE).

Group / Parameter	Control rabbits	Affected rabbits
AST (U/L)	19.2 ± 0.8	68.4 ± 2.15*
ALT (U/L)	12.6 ± 0.98	27.4 ± 0.87*
γ GT (U/L)	10.4 ± 1.17	20.0 ± 0.63*
Creatinine (mg/dl)	0.98 ± 0.09	1.52 ± 0.06*
Urea (mg/dl)	18.94 ± 1.17	44.0 ± 0.71*
Uric acid (mg/dl)	2.44 ± 0.26	3.86 ± 0.18*

* Significant difference against control at $p \leq 0.05$.

Table (3). Serum lipid profiles in control and affected (fed aflatoxin B_1 contaminated ration) rabbits, (Mean ± SE).

Group / Parameter	Control rabbits	Affected rabbits
Triglycerides (mg/dl)	91.0 ± 1.87	101.8 ± 1.2*
Cholesterol (mg/dl)	125.2 ± 1.43	92.0 ± 0.95*
HDL (mg/dl)	47.0 ± 0.63	26.6 ± 1.07*
LDL (mg/dl)	60.0 ± 1.14	45.0 ± 0.84*
VLDL (mg/dl)	18.2 ± 0.49	20.4 ± 0.68*
AI (LDL / HDL %)	1.28 ±0.021	1.7 ±0.082*
VLDL / HDL%	0.387 ± 0.01	0.775 ± 0.05*

* Significant difference against control at $p \leq 0.05$.

Table (4). Meat chemical constituents in control and affected (fed aflatoxin B_1 contaminated ration) rabbits, (Mean ± SE).

Group / Parameter	Control rabbits	Affected rabbits
Total protein %	22.52 ± 0.26	20.9 ± 0.28*
Total fat %	3.6 ± 0.15	2.74 ± 0.14*
Moisture %	71.9 ± 0.43	74.34 ± 0.46*
Ash %	1.18 ± 0.037	0.92 ± 0.026*
Triglycerides (mg/100g)	81.2 ± 0.80	72.6 ± 1.08 *
Cholesterol (mg/100g)	56.72 ± 1.51	37.68 ± 0.85*

* Significant difference against control at $p \leq 0.05$.

Table (5). Meat mineral profiles concentrations in control and affected (fed aflatoxin B_1 contaminated ration) rabbits, (Mean ± SE).

Group / Parameter	Control rabbits	Affected rabbits
Ash (mg/100g)	1180.0 ± 37.42	920.0 ± 25.5*
Calcium (mg/100g)	18.78 ± 0.3	16.41 ± 0.42*
Phosphorus (mg/100g)	314.6 ± 7.43	262.6 ± 3.9 *
Sodium (mg/100g)	60.3 ± 0.41	55.4 ± 0.8*
Potassium (mg/100g)	398.4 ± 9.26	366.4 ± 6.88*
Total Ca, P, Na& K(mg/100g)	792.08 ± 8.42	700.81 ± 9.43*
Total Ca, P, Na& K/Ash %	67.32 ± 1.5	76.3 ± 1.24*
Other (trace) Elements %	32.68 ± 1.5	23.7 ± 1.24*

* Significant difference against control at $p \leq 0.05$.

Table (6). Meat Aflatoxin B_1 residues in the control and affected (fed aflatoxin B_1 contaminated ration) rabbit, (Mean ± SE).

Group / Parameter	Control rabbits	Affected rabbits
Aflatoxin B_1 (ppb).	Not detected	3.2 ± 0.4

Discussion

Aflatoxins are secondary toxic fungal metabolites produced by toxigenic strains of *Aspergillus species*. They are toxic to a wide variety of animals **(Benneti and Klish, 2003).** Rabbits are one of the most sensitive animals for AF **(Guerre *et al.*, 1996)**.

The observed Clinical signs in AF-fed rabbits were decreased food and water consumption, dullness, dehydration, emaciation, and finally death in agree with that reported by **Çam *et al.*, (2008)**.

Effects of aflatoxin toxicity on rabbits serum biochemical parameters:

Nutrition, environment and toxins are involved in the occurrence of oxidative damage **(Shehata and Yosef, 2010).**

In the present study there was a significant increase in lipid peroxidation product; malondialdehyde (MDA) in the serum of AF intoxicated rabbits. While, glutathione (GSH) concentration, and superoxide dismutase (SOD) activity showed a significant reduction in AF fed group compared with control **(table 1)**. Such results are consistent with the findings of **Zhang *et al.*, (2017)** and **Al-Afifi *et al.*, (2018)** in broilers. Also, **Sharmila *et al.*, (2009)**, reported a marked increase in lipid peroxide levels and a concomitant decrease in enzymatic (SOD, CAT, GPx, glutathione reductase and glutathione-S-transferase) and non -enzymatic (reduced glutathione, vitamin C and vitamin E) antioxidants in the hepatic tissue of AFB1-administered rats. Additionally, Feeding rats a diet containing 40 μgkg−1 AFB1 significantly increased thiobarbituric acid-reactive substance and decreased glutathione (GSH) concentration, superoxide dismutase, glutathione peroxidase and glutathione *S*-transferase activities **(Rotimi *et al.*, 2016).**

Ingested AFB1 is metabolized by liver cytochrome P450 (CYP450) enzymes into AFB1-8, 9-epoxide intermediate **(Kensler *et al.*, 2011).** It had been reported that the toxic effect of AFB1 could be related to the generation of re-

active oxygen species (ROS) **(Costa *et al.*, 2007);** mainly superoxide (O2-), hydrogen peroxide (H_2O_2), and hydroxyl ($OH^{\cdot}$)**(Mehrzad *et al.*, 2011).**

The antioxidative enzymes, SOD, CAT and glutathione peroxidase (GPx) constitute the endogenous enzymatic line of defense against ROS **(Laguerre *et al.*, 2007).** GSH protects tissues from AFB1 oxidative damage or acts as a cofactor for enzymatic detoxification of aflatoxin **(Rotimi *et al.*, 2016).**

Production of free radicals has been referred to as a possible contributor to AFB1-induced hepatotoxicity **(Costa *et al.*, 2007).**

In AF group, serum liver enzymes activities (ALT, AST& γGT), kidney function markers (creatinine, urea & uric acid concentration) were significantly elevated than control levels **(table 2)**. The aflatoxin effects on biochemical alterations in this study are in consistent with **Çam *et al.*, (2008)**; They recorded a significant increase in serum AST, ALT and γGT activities and urea concentration in rabbits given AF at a dose of 0.4 mg/kg. Furthermore, **Brinda *et al.*, (2013)** found that intraperitoneal administration of AFB1 (1.5 mg kg−1 body weight) to rats significantly elevated levels of AST, ALT enzymes and urea level compared with control group, indicating hepatic damage**.**

The Increased serum AST, ALT and γGT activities are due to hepatocellular destruction and cholestasis that are frequently observed in aflatoxicosis in rabbits **(Soliman *et al.*, 2001** and **Yoesef *et al.*, 2003).**

Renal dysfunction is one of the pronounced effects of aflatoxin, which is known to be associated with increased levels of urea and creatinine. The increased urea level might be attributed to either the impaired glomerular filtration due to damage of renal tissues **(Tessari *et al.*, 2006)** or to dehydration **(Çam *et al.*, 2008).** The increase of uric acid levels in aflatoxin B_1 affected rabbits could be resulted from liver damage induced by aflatoxin (**Carlson *et al.*, 2001**).

The obtained results in table **(3)** revealed that serum cholesterol, HDL and LDL concentrations, in the rabbits fed ration containing aflatoxin B_1 were significantly decreased compared with control but, triglycerides, VLDL concentrations, AI(LDL/HDL%) and VLDL/ HDL% were significantly higher than control. The decreased levels of serum triglycerides and cholesterol concentrations in affected rabbits is concomitant with that recorded by **Çam *et al.*, (2008)**; They recorded A significant decrease in total cholesterol levels in rabbits given AF at a dose of 0.4 mg/kg. Also, **Brinda *et al.*, (2013),** reported that intraperitoneal administration of AFB1 (1.5 mg kg−1 body weight) to rats significantly increased the levels of VLDL and LDL compared with control group**.** The effects of AF on serum cholesterol and triglyceride levels suggest that lipid metabolism and transport were disrupted due to hepatic damage induced by AF being the main organ affected by aflatoxin **(Adilah and Redzwan, 2017)**.

Elevated levels of LDL (low density lipoprotein) and declined levels in HDL (high density lipoprotein) are important risk factors for coronary heart disease (CHD). An increase in HDL is associated with decrease in coronary risk (**Asdaq, 2015).** LDL is attributed to deposition of cholesterol in the arteries and aorta culminating in coronary heart diseases **(De Graaf *et al.*, 2002). Ulbricht and Southgate (1991)** suggest that the atherogenic index (AI) is a more suitable measure of the atherogenicity of foods than the ratio of polyunsaturated fatty acids (PUFA) to saturated fatty acids (P/S ratio).

<u>Effects of aflatoxin toxicity on rabbits meat biochemical parameters and chemical constituents:</u>

The main components of meat, excluding water, are proteins and lipids. Rabbit meat is a lean meat rich in proteins of a high biological value as it is characterized by high levels of essential aminoacids **(Dalle Zotte, 2004),** with low cholesterol and low lipids content that rich with unsaturated fatty acids(noticeable quantities of linolenic fatty acid; C18:3 ω3). Also, it

displays a low content of sodium and a high content of calcium and phosphorus than other meats **(Hernández and Gondret, 2006** and **Williams, 2007).**

In the present study, meat SOD activity, GSH content, total protein%, fat%, ash%, triglycerides and cholesterol concentrations were significantly decreased in AF fed rabbits than control levels. While MDA concentration and moisture%, were significantly higher than that of control (**tables 1, 4 & 5**).

Lipid oxidation represents one of the most important causes of deterioration of meat and meat products and it affects unsaturated fatty acids, particularly PUFA in membrane phospholipids, and cholesterol; mainly LDL cholesterol. The end-products of this process deteriorate color, aroma, flavor, texture of meat and meat products consequently, reduce their nutritive value **(Gray *et al.,* 1996).**

Additionally, lipid oxidation in foods generates end-products which may be harmful to human health. Compounds such as malondialdehyde and cholesterol oxidation products are reported to have cytotoxic and genotoxic potential and have been linked to the promotion of atherosclerosis, cardiovascular disease, and cancer **(Kanner, 2007; Muselík *et al.,* 2007** and **Soyer *et al.,* 2010).**

Meat contains several natural antioxidants such as superoxide dismutase, glutathione peroxidase (GSH-Px) and catalase, **(Pradhan *et al.,* 2000).** These endogenous antioxidant enzymes could potentially delay the onset of oxidative rancidity in meat **(Hernández *et al.,* 2002)** through, detoxification of ROS **(Laguerre *et al.,* 2007).**

The decreased total protein level in this study might be due to impairment of protein synthesis by the inhibitory effect of AF on DNA and RNA synthesis (**Rosa *et al.,* 2001** and **Bennett and Klich, 2003)**.

Juiciness depends much on the fat content of the carcass; the fatter the carcass the lower its water content **(Hoffman *et al.,* 2004).** Consequently, meat of aflatoxin affected rabbits was less Juicy due to its low fat and high moisture contents.

Data presented in table **(5)** demonstrated that individual Ca, P, Na, & K, total Ca, P, Na& K and other (trace) elements % concentrations in the meat of affected rabbits were significantly decreased comparing with that of control but, ratio of the total Ca, P, Na& K/Ash % was significantly higher than control. Reduction of calcium, and phosphorus levels were observed in broilers fed AF-contaminated diet **(Chen *et al.,* 2014a** and **Al-Afifi *et al.,* 2018).** The hypocalcaemia and hypophosphatemia observed in the aflatoxin B_1 fed rabbits may be due to defective intestinal absorption or impaired renal function **(Gill *et al.,* 1988** and **Chen *et al.,* 2014b)**. The decreased Ca and P concentration in our study may be also attributed to the direct or indirect effects of aflatoxin on Ca and phosphorous metabolisms, and may be related to altered vitamin D and parathyroid hormone (PTH) metabolism **(Glahn *et al.,* 1991** and **Kececi *et al.,* 1998)**. The hypokalaemia in the affected rabbits might result from impaired active reabsorption of potassium in renal tubules **(Gill *et al.,* l989).**

Residues of aflatoxins and their metabolites may be present in meat, dairy products and eggs and other products of animals fed rations contaminated with aflatoxins **(Hussain *et al.,* 2010)** and potentially result in health problems in human **(Pandey and Chauhan, 2007** and **Denli *et al.,* 2009).** In many countries, the maximum tolerance level of AFB1 in human food products is 2µg/kg **(Zhang *et al.,* 2017)**.

As shown in table **(6)**, AF residues were detected in meat of AF group (3.2 ppb). These results appear to go parallel with that obtained by **Al-Afifi *et al.,* (2018)** they detected AF residues in both liver and muscles tissues in AF fed broilers (250ppb/kg diet) for 6 weeks with higher concentrations in liver (3.4 ppb) than muscles (2.6 ppb).

From the above mentioned results, it is obvious that aflatoxin contaminated ration not only affect on rabbit meat quality and its nutritional value but also represents a potential human health hazard due to dissemination of aflatoxin residues in the produced meat.

Conclusion

In the present study, Aflatoxin B_1 showed adverse impacts on organs functions, serum parameters and meat chemical constituents of rabbits. Aflatoxin B_1 residues appeared in the meat of rabbits fed aflatoxin B_1 contaminated rations. So, it is advisable to apply suitable measures to control the aflatoxins contamination in feeding stuffs of rabbit ration and supplying the rabbits with aflatoxin free ration to avoid their hazard effects on rabbit's performance and human health and to obtain healthy rabbit meat of high quality and nutritive value.

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