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EFFECT OF CLAY SOURCES ON REPRODUCTIVE PERFORMANCE OF DOE RABBITS FED AFLATOXICATED DIET

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ABSTRACT

A total of 60 female Bouscat rabbits at 10-12 months of age with an average body weight 3.48±0.11 kg were divided into three homogeneous groups (n=20). The 1st group was fed on a formulated pelleted diet as a control. The 2nd and 3rd groups were fed on the same diet replaced with 30 and 40% of diet naturally contaminated with 450 ppb aflatoxins (B_1+G_1, AF-diet), respectively. Each group was divided into two equal sub groups. The 1st and 2nd sub groups were fed on diet supplemented with 4 g of either tafla or bentonite clay/kg diet, respectively. Doe in each group was naturally mated with proven fertile buck after two weeks from start of treatments and continued for three consecutive parities.

Progesterone concentration at mid gestation period, litter size and litter weight at birth and at weaning, total milk yield from birth to 21 days of lactation and litter weight gain were reduced (P<0.001 or 0.01 or 0.05) with increasing AF-diet intake and feeding duration time (from 1st till 3rd parities), while number of services per conception, abortion percentage and pre-weaning mortality rate tented to increase significantly. At 21st day of pregnancy through 3rd parity, body weight, ovaries weight, total embryos recovered, and maternal placenta weight decreased (P<0.01 or 0.05) with increasing aflatoxin level in the diet. Moreover, lost embryos through pre-implantation and abnormal embryos percentages tended to increase significantly. Inclusion bentonite clay to the AF-diet reduced the harmful effect of aflatoxicosis and improved markedly reproductive performance, litter traits, milk production and embryo quality compared to tafla supplementation, especially with increasing feeding duration. Genitalia organs were adversely affected by aflatoxins consumed, especially with elevating AF-diet. Does fed 30% AF-diet+bentonite showing normal genitalia appearance than that fed the same diet+tafla. Liver and kidneys of doe rabbits fed 40% AF-diet+tafla showed more deterioration than that fed 30% AF-diet+tafla or 30 and 40% AF-diet+bentonite. Generally, inclusion 4 g bentonite clay/kg diet protect mature doe rabbits fed diet contaminated with 135 ppb aflatoxins+ without retardation in the reproductive performance.

Key words: Aflatoxins, bentonite, tafla clay, doe rabbits, reproductive performance.

INTRODUCTION

Pollution of air, feed and water represent great problem for each of human and animals. Pollution of feed animal with aflatoxins represents great economical losses resulting from decreasing animal performance (Bueno & Oliver, 2004 and Groopman & Kensler, 2005) and death induced from ingestion of toxic material (Harvey *et al.,* 1993). Aflatoxins are fungal metabolites produced by different Aspergillus species, which B1 is the most frequently found in contaminated feed samples. Aflatoxins are considered carcinogenic. Both the potency and carcinogenicity are dependent on the species, dose and duration of intake, age of animal and nutritional state (Diekman and Green, 1992).

Controlling the effect of mycotoxin contaminated feeds may represent a great hope for decreasing toxicity and minimizing hazards effect. Nowar *et al*. (2001), Abd El-Baki *et al*. (2002), Schatzmayr *et al*. (2006) and Meshreky *et al.* (2007) succeeded in using clay minerals (bentonites, tafla and zeolites) for detoxification and controlling toxicity of aflatoxins contaminated feeds, suggesting that detoxification natural clays would adsorb toxic products of digestion and decrease accumulation of toxic substance in tissues (Harvey *et al.*, 1993 and Elmore, 2003).

The aim of the present study is to define the effects of aflatoxine levels in the rabbit diets (AF-diet) and source of natural clay as a detoxifying agent in the prevention of toxic effects of aflatoxins on reproductive performance of doe Bouscat rabbits during three consecutive parities.

MATERIALS AND METHODS

The present study was carried out in the Rabbitry of EL-Gemmeza Research Station, EL-Gharbiya Governorate, Animal Production Research Institute, Agricultural Research Center, Ministry of Agriculture, Egypt.

A total of 60 female Bouscat rabbits at 10-12 months of age with an average body weight 3.48±0.11 Kg were used. Animals were divided into three equal homogeneous groups (n=20). The 1st group was fed on a formulated pelleted diet as a control (25.0% yellow corn, 25.0% soya bean meal, 22% wheat bran, 15.0% berseem hay, 8.4% berseem straw, 0.3% common salt, 0.3% premix, 3% molasses and 1.0% limestone) containing 18.13 % crude protein, 2.69 % fat and 12.7 % fibers according to NRC (1984). The 2nd and 3rd groups were fed on the same diet replaced with 30 and 40% of diet naturally contaminated with 450 ppb aflatoxins (B_1+G_1, AF-diet), respectively. Each group was divided into two equal subgroups. The 1st and 2nd subgroups were fed the diet supplemented with 4 g tafla or bentonite clay/kg diet, respectively. Such natural clay (Manganes Sinai Company, Kasser El-Nile Street, Cairo) contained: 52.0% SiO_2, 22% Al_2O_3, 3.5% Fe_2O_3, 2.3%Na_2O, 3.0% CaO, 1.6% MgO, 1.7% K_2O, 10.0% moisture content (110°C), Surface area (m^2/g) of raw ore for tafla clay and 50-55% SiO_2, 18-22% Al_2O_3, 3-5% Fe_2O_3, 2.0-3.2%Na_2O, 0.1-0.3 CaO, 0.5-1.6% MgO, 1.2-2.2 k_2O, 8-14 L.O.I, 10.0 max% moisture content (110°C), Surface area (m^2/g) of raw ore for bentonite clay.

Rabbits were individually housed in galvanized wire cages. Stainless steel drinking nipples and feeders were supplied for each cage. Feed and water were offered *ad libitum*. Animals were kept under similar managerial conditions. Each doe was naturally mated with proven fertile buck after two weeks of treatments and continued for three consecutive parities. On the twelve day after mating, each mated doe was abdominally palpated to detect pregnancy. After 5-7 days of kindling, does were mated. At 3-4 days before the expected day of parturition nest box was placed in the cage and for 28 days following parturition. Number of services per conception (NSC), abortion percentage (Ab), gestation length (GL), litter size and litter weight (g) at birth and at weaning (28 days), bunny weight at birth and at weaning, litter weight gain and pre-weaning mortality percentage, were recorded.

Milk yield (g) of each doe was recorded at the seventh, 14^{th} and 21^{st} days after kindling, using the weight-suckle-weight technique described by McNitt and Lukefahr (1990). Total milk yield (TMY) of the first 21 days of the suckling period was considered as seven times of the sum of milk yield at 7^{th}, 14^{th} and 21^{st}. Daily milk intake of young (DMI) in grams was recorded. Milk to litter conversion ratio (MC) during the first 21 days of lactation period was calculated (total milk yield /litter weight gain from birth to 21 days).

Five blood samples were collected from marginal ear vein of rabbits using heparinized tubes at mid day of gestation (day 15) of each treatment in each parity, centrifuged at 3000 rpm for 15 minutes. Plasma was separated and stored at -20 °C till analysis. Progesterone (P_4) concentration was determined by using RIA kit (Diagnostic Systems Laboratories, Inc., USA) according to the manufacturer information.

On day 21 of pregnancy in the 3^{rd} parity, three rabbits from each treatment were chosen and slaughtered to study some phenomenal embryos and histopathological parameters of the genitalia, liver and kidneys. Tissue samples from such organs were taken and fixed in Boun's solution, then processed by standard technique and stained with haematexylin and Eosin (Bancroft and Cook, 1994).

Data were analyzed using GLM procedure in SAS® (1996) program and the differences among means were tested using Duncan's New Multiple Range Test (Duncan, 1955). Data estimated in percentage were transformed with the arcsine square-root procedure to normalize variance before analysis and were retransformed again to the original scale before presentation.

RESULTS AND DISCUSSION

1. Progesterone hormone (P_4) level:

Progesterone concentration (P_4) at mid gestation of Bouscat rabbits was reduced ($P<0.01$ or 0.05) with increasing AF-diet consumed and feeding duration time (Figure 1). These may be due to aflatoxicosis effects, indirect effect of aflatoxins that leads to fluctuations in LH level, which has a primary stimulating effect on P_4 secretion in domestic animals, and/or a reduction rate of embryo survival (Parr *et al.,* 1982). Mills and Stopper (1989) stated that plasma progesterone concentrations

during pregnancy are related to the number of corpora lutea, their synthesis and secretion**.** Inclusion of bentonite clay to the AF-diet was more effective in reduction of the harmful effect of aflatoxicosis compared with tafla supplementation (Figure 1). Progesterone level was higher ($P<0.05$) in does fed basal diet without aflatoxins with bentonite clay than in that fed the same diet supplemented with tafla clay.

2. Reproductive and litter traits:

Number of services per conception (NSC), abortion (Ab) percentage and pre-weaning mortality rate increased ($P<0.001$, 0.01 or 0.05) by increasing AF-diet consumed and feeding duration, while litter size and litter weight at birth and at weaning, and litter weight gain tented to decreased (Table 1). The same finding was observed by Meshreky *et al.* (2007) who found that aflatoxins reduced significantly oestrogen level, receptivity and subsequently conception rate of female rabbits. In addition, Cheeke and Shull (1985) reported that the mechanism by which aflatoxin reduce growth rate is probably related to disturbances in protein, charbohydrate and lipid metabolism. The increase stillborn and pre-weaning mortality rate with increasing AF-diet and advancement of parity (Table1) might be due to the penetrated aflatoxin through the alimentary system and blood for milk synthesis in alveolar cells of the mammary gland (Veldman *et al.*, 1992) and the direct effect of aflatoxicosis on the sensitive young offspring during suckling and with start eating from feeder (18 day old). Ferrufino *et al.* (2000) found a linear relationship between the ochratoxin A concentrations in milk of rabbit does fed a naturally-contaminated feed and in the plasma of the sucklings, indicating an effective transfer of the toxin to the sucklings. Moreover, Fernandez *et al*. (1997) stated that aflatoxin caused a failure in the acquired immunity system by decreasing antibody production and reduced serum protein, which may be due to changes in hormonal balance.

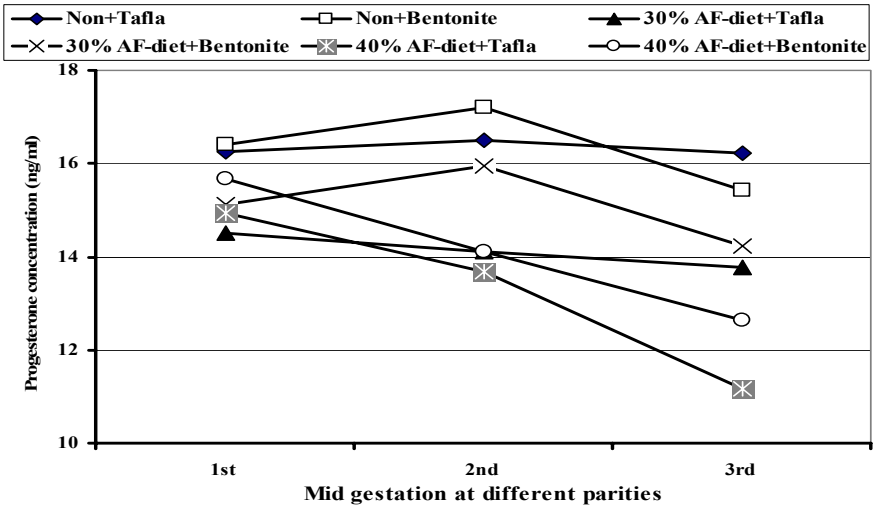

Figure 1. Progesterone levels (ng/ml) at mid gestation period as affected by dietary aflatoxins contaminated diet (AF-diet) and type of detoxifying agents.

Table 1. Reproductive traits of female Bouscat rabbits through three parities as affected with aflatoxin contaminated and detoxified rations.

Items	No AF-diet (Basal diets)		30% AF-diet (135 ppb aflatoxin/kg diet)		40% AF-diet (180 ppb aflatoxin/kg diet)		Sig
	Tafla	Bentonite	Tafla	Bentonite	Tafla	Bentonite	
			1st Parity				
NSC	1.2±0.11	1.2±0.11	1.3±0.12	1.3±0.13	1.4±0.11	1.3±0.13	NS
Abortion (%)	0.0	0.0	0.0	0.0	0.0	0.0	NS
LSB (total)	7.8±0.29	8.1±0.26	8.1±0.25	7.9±0.27	7.9±0.28	8.2±0.26	NS
LSB (alive)	7.6±0.31	7.8±0.27	7.6±0.32	7.6±0.31	7.3±0.35	7.8±0.27	NS
LS-21 days	7.4^{ab}±0.25	7.7^{a}±0.21	7.2^{bc}±0.27	7.3^{abc}±0.26	6.8^{c}±0.26	7.4^{ab}±0.24	*
LS-28 days	7.4^{ab}±0.27	7.5^{a}±0.26	6.8^{c}±0.32	6.9^{bc}±0.30	6.1^{d}±0.34	7.0^{bc}±0.29	*
LWB (g)	420±18	415±15	401±15	402±17	396±18	407±14	NS
LW 21 d(g)	1901^{b}±43	2012^{a}±38	1795^{c}±40	1840^{bc}±40	1689^{d}±45	1786^{c}±39	*
LW 28 d(g)	3028^{ab}±117	3230^{a}±112	2710^{c}±139	2850^{bc}±136	2358^{d}±148	2737^{c}±131	*
LWG B-21d	1481^{b}±39	1597^{a}±35	1394^{c}±43	1438^{bc}±41	1294^{d}±49	1374^{c}±41	*
LWG 21-28d	1126^{b}±25	1221^{a}±24	912^{d}±30	1009^{c}±27	669^{e}±32	951^{d}±28	**
Stillborn (%)	2.56	3.70	6.17	3.80	7.59	4.88	NS
MB-21d (%)	2.63	1.28	5.26	3.95	6.85	5.13	NS
M21-28d(%)	0.00^{a}	2.60^{a}	5.56^{a}		10.29^{b}	5.41^{a}	*
			2nd Parity				
NSC	1.4^{a}±0.13	1.4^{a}±0.13	1.8^{b}±0.17	1.6^{ab}±0.16	2.1^{c}±0.19	1.7^{b}±0.16	**
Abortion (%)	0.0	0.0	0.0	0.0	10.0	10.0	*
LSB (total)	7.2^{ab}±0.21	7.5^{a}±0.17	7.3^{a}±0.20	7.3^{a}±0.19	6.8^{b}±0.21	7.2^{ab}±0.26	*
LSB (alive)	7.0^{a}±0.20	7.3^{a}±0.19	6.9^{a}±0.23	7.0^{a}±0.21	6.2^{b}±0.23	6.9^{a}±0.20	*
LS-21 days	6.7^{ab}±0.24	7.0^{a}±0.21	6.3^{b}±0.25	6.7^{ab}±0.21	5.7^{c}±0.27	6.6^{b}±0.22	*
LS-28 days	6.6^{b}±0.19	7.0^{a}±0.16	5.6^{d}±0.23	6.3^{bc}±0.20	5.1^{e}±0.25	6.1^{c}±0.21	**
LWB (g)	369^{a}±26	386^{a}±23	390^{a}±27	365^{a}±25	306^{b}±27	358^{ab}±25	*
LW 21 d(g)	1696^{b}±57	1877^{a}±53	1533^{c}±59	1678^{b}±51	1313^{d}±62	1553^{c}±58	**
LW 28 d(g)	2812^{b}±89	3028^{a}±87	2215^{c}±97	2726^{b}±91	1827^{d}±106	2364^{c}±95	**
LWG B-21d	1327^{b}±46	1492^{a}±43	1143^{c}±50	1313^{b}±44	1007^{d}±53	1194^{c}±46	**
LWG 21-28d	1116^{ab}±48	1151^{a}±43	682^{d}±47	1048^{b}±46	513^{e}±52	810^{c}±49	***
Stillborn (%)	2.78^{a}	2.67^{a}	5.48^{ab}	4.11^{a}	8.20^{b}	4.62^{ab}	*
MB-21d (%)	4.29	4.11	8.70	4.29	8.93	4.84	NS
M21-28d(%)	1.49^{a}	0.00^{a}	11.11^{d}	5.97^{ab}	9.80^{cd}	6.78^{bc}	*
			3rd Parity				
NSC	1.7^{ab}±0.21	1.6^{a}±0.21	2.4^{cd}±0.24	1.9^{ab}±0.23	2.7^{d}±0.26	2.1^{bc}±0.22	***
Abortion (%)	0.0	0.0	0.0	0.0	30.0	10.0	**
LSB (total)	7.86^{ab}±0.19	8.14^{a}±0.18	7.14^{c}±0.21	7.57^{b}±0.19	6.25^{d}±0.21	6.83^{c}±0.26	**
LSB (alive)	7.71^{a}±0.20	7.86^{a}±0.19	6.43^{c}±0.23	7.29^{b}±0.21	5.25^{d}±0.24	6.33^{c}±0.20	**
LS-21 days	7.43^{a}±0.24	7.57^{a}±0.21	6.00^{c}±0.25	6.71^{b}±0.21	4.25^{d}±0.25	5.83^{c}±0.22	**
LS-28 days	7.29^{a}±0.19	7.43^{a}±0.16	5.14^{c}±0.23	6.43^{b}±0.20	3.25^{d}±0.25	5.17^{c}±0.21	***
LWB (g)	420^{a}±31	441^{a}±32	318^{c}±32	380^{b}±31	281^{d}±37	328^{c}±34	**
LW 21 d(g)	1928^{a}±65	1924^{a}±64	1520^{c}±67	1660^{b}±61	1030^{d}±68	1457^{c}±63	***
LW 28 d(g)	2990^{a}±102	3097^{a}±105	1993^{c}±111	2586^{b}±106	1236^{d}±116	2019^{c}±112	***
LWG B-21d	1508^{a}±67	1482^{a}±64	1203^{bc}±68	1280^{b}±64	749^{d}±74	1129^{c}±70	***
LWG 21-28d	1062^{b}±51	1174^{a}±49	472^{d}±51	927^{c}±50	205^{e}±56	562^{d}±53	***
Stillborn (%)	1.82^{a}	3.51^{ab}	10.00^{c}	3.77^{ab}	16.00^{d}	7.32^{bc}	*
MB-21d (%)	3.70^{a}	3.64^{a}	6.67^{a}	7.84^{a}	19.05^{b}	7.89^{a}	**
M21-28 d(%)	1.92^{a}	1.89^{a}	14.29^{c}	4.26^{a}	23.53^{d}	11.43^{b}	***

NSC=Number of service per conception, GL= Gestation length, LSB=Litter size at birth, LWB=Litter weight at birth, MB-21d (%) =Mortality rate from birth to 21-days and MB-28 d(%) =Mortality rate from birth to 28-days (weaning). *** P<0.001, ** P<0.01, * P<0.05 and NS= No significant. ^{a,b,c} Values with different superscripts in the same row differ significantly (P<0.05).

The highest values of litter size and weight and the lowest NSC and pre-weaning mortality were estimated in does rabbits fed basal diet without aflatoxins supplemented with bentonite or tafla clay groups compared with other groups (Table 1). Addition of bentonite clay to the AF-diet reduced the harmful effect of aflatoxicosis more than tafla supplementation, especially with increasing feeding duration. Similar to that reported by Harvey *et al.* (1993) who found that aluminosilicate compounds (natural clay) are able to protect chickens, swine, and lambs from the deleterious toxic effects of AF and to reduce AF residues in milk of dairy cows and goats. In this respect, Veldman *et al.* (1992) revealed that AF content in milk was significantly reduced with bentonite supplementation. Ramos and Fernandez (1993) demonstrated that bentonite at 1% adsorbed nearly 100% of the existing 5 ppm AFB_1 in a simulated intestinal fluid of cows.

3. Milk yield, milk intake and milk conversion ratio:

Total milk yield from birth to 21 days of lactation decreased ($P<0.001$, 0.01 or 0.05) in does fed AF-diet (Table 2). The differences among treatments in milk yield and subsequently daily milk intake of their young may be related to differences in number of young's at birth (Zerrouki *et al.,* 2002), as well as, differences in amount of aflatoxins intake and long period of aflatoxins hazard. Amin *et al.* (1991) observed increased fibrosis and collagen deposition with thick-walled blood vessels in mammary gland of Bouscat rabbits received aflatoxin B1 (50 µg/kg body weight) during pregnancy and lactation. These changes may be the cause of decreased secretion of milk inside the alveoli. Similarly, in ewes (Hassan, 2003) and in lambs (Soliman *et al.,* 2005) found AF in diet decreases the milk yield. In addition, Applebaum *et al.* (1981) showed that 8% decrease in milk production of cows fed diet containing 1 ppm AF after six weeks; moreover cows fed a diet containing 4 ppm AF given the same decrease in production, but only after two weeks on the diet. Addition of bentonite clay to the basal diet or AF-diet increased significantly milk yield compared with those fed the same diet with tafla (Table 2). Inclusion bentonite clay to the AF-diet enhanced significantly milk conversion ratio than tafla clay during 3rd parity (Table 2).

4. Body weight and some ovarian and embryos measurements:

Body weight (Kg), ovaries weights (g), total embryos recovered and placental weight (g) decreased ($P<0.01$ or 0.05) with increasing aflatoxin in the diet, whereas, lost embryos during pre-implantation and abnormal embryos percentages tended to increase significantly (Table 3 and Pate 1). These results are in harmony with results obtained by Diekman and Green (1992) who mentioned that the potency of aflatoxins is dependent on the dose of intake, duration of intake, age of animal and nutritional state. Also, Badawy (1997) reported that the presence of implantation sites in the uterine horns of rabbits fed aflatoxin diet after mating or during mid-pregnancy indicated early fetal resorption. Results in Table (3) and Plate (1) revealed that addition bentonite to 30 or 40% AF-diet reducing the detrimental effects of aflatoxin more than that fed the same diet supplemented with tafla.

Table 2. Total milk yield (TMY) of doe rabbit (g), daily milk intake of young (DMI, g) and milk conversion ratio (MC) as affected by dietary aflatoxins contaminated diet (AF-diet) and type detoxifying agents.

Items	No AF-diet (Basal diet)		30% AF-diet (135 ppb aflatoxin/kg diet)		40% AF-diet (180 ppb aflatoxin/kg diet)		Sig.
	Tafla	Bentonite	Tafla	Bentonite	Tafla	Bentonite	
			1st Parity				
TMY	2595.9^{ab}±93	2772.8^{a}±93	2593.3^{ab}±93	2600.4^{ab}±93	2387.1^{b}±93	2615.5^{ab}±93	*
DMI	16.83±0.59	17.38±0.59	17.30±0.59	17.46±0.59	16.77±0.59	16.91±0.59	NS
MC	1.75^{a}±0.031	1.74^{a}±0.031	1.86^{b}±0.031	1.81^{ab}±0.031	1.84^{ab}±0.031	1.91^{b}±0.031	**
			2nd Parity				
TMY	2444.9^{b}±97	2758.2^{a}±97	2107.8^{c}±97	2427.9^{b}±97	2012.5^{c}±108	2199.6^{bc}±108	**
DMI	17.25±0.92	18.90±0.92	15.82±0.92	17.44±0.92	16.74±1.03	16.57±1.03	NS
MC	1.84±0.039	1.85±0.039	1.84±0.039	185±0.039	1.95±0.043	1.83±0.043	NS
			3rd Parity				
TMY	2801.9^{a}±107	2838.7^{a}±107	2242.3^{b}±107	2335.4^{b}±107	1637.2^{c}±139	2114.7^{b}±121	***
DMI	18.50±0.83	17.97±0.83	17.88±0.83	16.44±0.83	16.92±1.07	17.77±0.93	NS
MC	1.86^{a}±0.043	1.92^{ab}±0.043	1.86^{a}±0.043	1.82^{a}±0.043	2.01^{b}±0.056	1.87^{a}±0.048	*

^{a,b,c} Values with different superscripts in the same row differ significantly (P<0.05).
*** P<0.001, ** P<0.01, * P<0.05 and NS= Not significant.

Table 3. Body weight (Kg) and some ovarian and embryo measurements of female Bouscat rabbits at day-21 of pregnancy through the 3rd parity as affected with aflatoxin contaminated and detoxified rations.

Items	No AF-diet (Basal diets)		30% AF-diet (135 ppb aflatoxin/kg diet		40% AF-diet (180 ppb aflatoxin/kg diet)		Sig
	Tafla	Bentonite	Tafla	Bentonite	Tafla	Bentonite	
Body weight	3.97^{ab}±.05	4.06^{a}±0.05	3.68^{c}±0.05	3.88^{b}±0.05	3.46^{d}±0.05	3.63^{cd}±.05	**
Ovaries weight(g)	1.18^{ab}±.06	1.25^{a}±0.06	1.08^{bc}±.06	1.11^{ab}±.06	0.85^{c}±0.06	0.99^{bc}±.06	**
No. CL	8.33± 0.7	8.67±0.7	7.67±0.7	8.67±0.7	7.33±0.7	7.33±0.7	NS
Total embryos	8.00^{a}±0.6	8.33^{a}±0.6	6.67^{b}±0.6	8.00^{a}±0.6	6.00^{b}±0.6	6.33^{b}±0.6	*
LEPI (%)	3.70^{a}	4.17^{a}	13.23^{b}c	7.04^{ab}	17.86^{c}	12.17^{bc}	*
Ab. Emb. (%)	4.17^{a}	3.03^{a}	8.33^{ab}	7.41^{a}	25.40^{c}	15.08^{b}	*
Embryo weight	14.04^{a}±.26	13.74^{a}±.24	13.80^{a}±.31	13.62^{a}±.26	9.15^{c}±.34	11.40^{b}±.33	**
PW (g)	3.26^{ab}±.12	3.05^{ab}±.11	3.47^{a}±.15	3.16^{ab}±11	2.28^{c}±.17	2.72^{b}±.16	*

No. CL= Number of corpora lutea, LEPI= Lost embryos pre-implantation percentage, Ab. Emb.= Abnormal embryos percentage and PW= Placental weight.
^{a,b,c} Values with different superscripts in the same column differ significantly (P<0.05).
*** P<0.001, ** P<0.01, * P<0.05 and NS= Not significant.

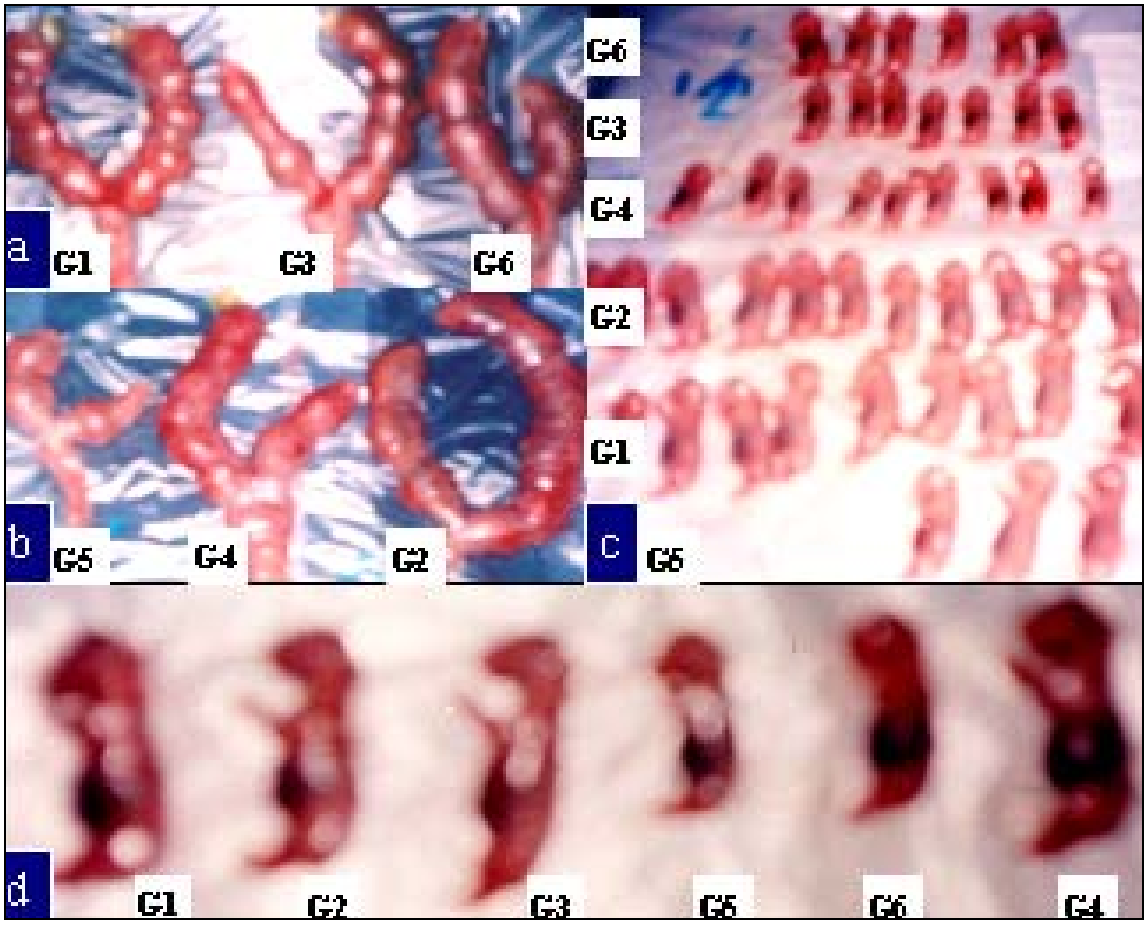

Plate 1. (a & b) Showing uterus of doe rabbits with resorption sites in 5th (fed diet with 180ppb aflatoxin+tafla) and 6th (fed diet with 180ppb aflatoxin+bentonite) groups, whereas in the 1st (fed diet without aflatoxin +tafla), 2nd (fed diet without aflatoxin +bentonite), 3rd (fed diet with 135ppb aflatoxin+tafla) and 4th (fed diet with 135ppb aflatoxin+bentonite) groups showing normal uterus without resorption sites. (c) Embryo recovered from different treated groups: showing retardation of growth in rabbit fetuses recovered from dam fed diets with different levels of aflatoxin+tafla or bentonite clay (G3, G4, G5 and G6) compared with that fed diet without aflatoxin+tafla or bentonite clay (G1 and G2). (d) Showing abnormality and retardation growth in rabbit fetuses recovered from dam in G3, G5 and G6.

5. Histopathological examination:

1. Genitalia organs:

Ovarian of rabbits fed free diet+bentonite or tafla, showed large numbers of Graafian follicles and their uterus showed more number and activity of uterine glands, these numbers were more pronounced than that on ovaries of does fed diet with aflatoxin (Plate 2). Ovaries of rabbits fed 40% AF-diet+tafla showed presence of few numbers of mature follicles with secondary follicles than that of rabbits fed 30% AF-diet+tafla, which showed several follicles in different stages of maturity and few

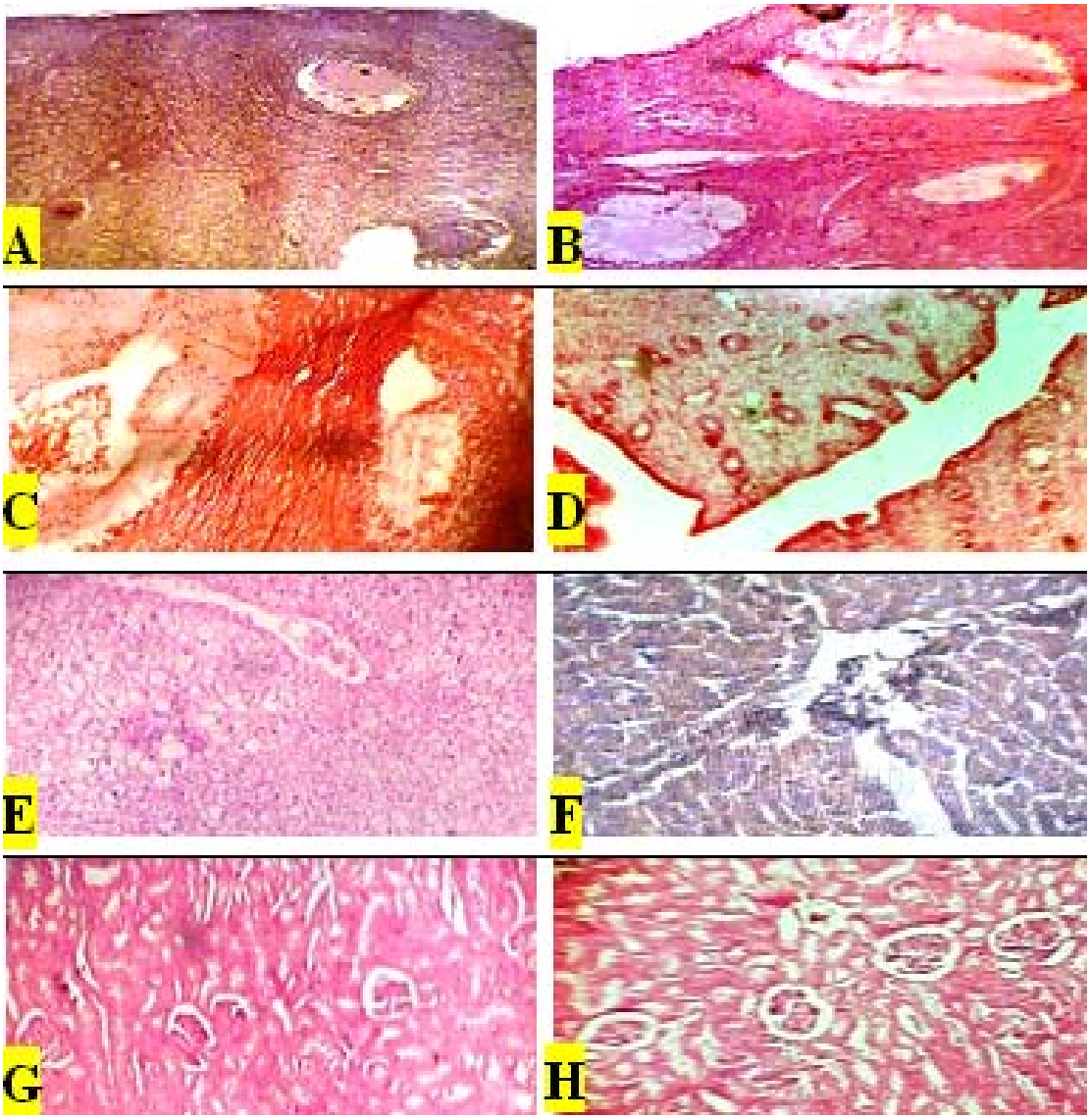

Plate 2. Cross sections in (A) Ovary of doe rabbits fed 40%AF-diet+tafla, showing higher interstitial tissue and low number of mature follicles (H & E x33). (B) Ovary of does fed 30%AF-diet+bentonite, showing increase in number of mature follicle (H & E x33). (C) Ovary of doe rabbits fed free diet+tafla, showing presence of high number of mature follicles in the cortex (H & E x33). (D) Uterus of does fed 30%AF-diet+tafla, showing few numbers in the uterine glands (H & E x66). (E) Liver of doe rabbit fed the 30%AF diet+bentonite showing normal architecture of the hepatic lobules, central vein and hepatocytes as well as the portal lobules (x100, H&E stains). (F) Liver of doe rabbit fed 40%AF diet+tafla showing abnormal hepatocytes and high amount of red blood cells in the central vein (x150, H&E stains). (G) Kidneys of doe rabbit fed 30%AF diet+tafla showing apparently normal architecture of the renal cortex with neoplastic signs and periglomerular and peritubular cell infiltration (x150, H&E stains). (H) Kidneys of doe rabbits fed free diet+bentonite showing normal architecture of the renal cortex and intact structure of both glomerulosa and renal tubules (x150, H&E stains).

numbers of follicles degenerated. However, ovary of does fed 30% AF-diet+bentonite showed mature follicles in addition to presence of atretic follicles than that fed 30 or 40% AF-diet+tafla. Uterus of does fed 30%AF-diet+tafla, proved few numbers of uterine glands, meanwhile, uterus of does fed 40%AF-diet+bentonit, had more number and great activity of uterine glands. These results agreed with those observed by Badawy (1997) who found severe pathological changes in the ovaries and uterus of rabbits fed AF-diet compared with control group.

2. Liver and kidneys:

Liver and kidneys of doe rabbits fed 40% AF-diet+tafla were clearly changed in compared with that fed diet without aflatoxin (Plate 2). These finding agreed with that observed by Soliman *et al.* (2001) who found particularly alteration in liver and kidneys of rabbits fed AF-contaminated diet (833 µg of aflatoxins/kg). Winston and Hagler (1988) also found that AFB_1 caused noticeable damage in liver. Rrabbits fed 40% AF-diet+tafla showed deterioration in liver and kidneys structures more than that fed 30% AF-diet+tafla. Addition bentonite to rabbits fed diet with 30 or 40% AF-diet reducing the detrimental effects of aflatoxin more than that fed the same diet supplemented with tafla.

CONCLUSION

1. Elevating aflatoxins ratio in the diet and increasing its consumption period adversely affected progesterone and genitalia organs and consequently declined the reproductive performance, litter traits and milk production of Bouscat doe rabbits
2. Bentonite clay in the rabbit diet would relive the harmful effects of aflatoxicosis and consequently improved their reproductive performance markedly than tafla clay.

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تأثير مصادر الطين المعدني على الأداء التناسلي لإناث الأرانب المغذاة على علائق ملوثة بالأفلاتوكسين

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أ ستخدم ٦٠ أنثى أرنب بوسكات عمر ١٠-١٢ شهر بمتوسط وزن جسم ٣.٤٨±١١.٠كجم. قسمت الأرانب إلي ثلاث مجموعات متساوية متجانسة (٢٠ أنثى لكل منها). غذيت المجموعة الأولي علي عليقه مشكلة محببة كمجموعة ضابطة. بينما غذيت المجموعة الثانية و الثالثة علي نفس العليقه مع استبدال ٣٠ أو ٤٠% منها بعليقة ملوثة طبيعيا ب ٤٥٠ جزء في البليون (أفلاتوكسينB_1+G_1) على التوالي. قسمت كل مجموعة إلى مجموعتين فرعيتين متساويتين. غذيت المجموعة الفرعية الأولى و الثانية علي عليقة مضافا إليها ٤ جم طفلة أو بنتونيت/ كحم عليقه ، علي التوالي. لقحت كل أنثى طبيعيا مع ذكر عالي الخصوبة بعد أسبوعين من بداية المعاملة و استمرت التجربة لثلاث بطون متتالية.

أظهرت النتائج انخفاض تركيز هرمون البروجستيرون عند منتصف فترة الحمل، حجم خلفة البطن، وزن خلفة البطن عند الميلاد و الفطام، محصول اللبن الكلى من الميلاد إلى ٢١ يوم من الرضاعة، و الزيادة في خلفة البطن للأرانب البوسكات معنويا (على مستوى ١ أو ٥%) مع زيادة المأكول من العليقة الملوثة بالافلاتوكسين و طول فترة التغذية، بينما عدد التلقيحات اللازمة للحمل، نسبة الإجهاض و معدل النافق قبل الفطام أتجة إلى الزيادة المعنوية. عند اليوم ال٢١ من الحمل أثناء البطن الثالثة، سببت زيادة الافلاتوكسين في العليقة انخفاض وزن الجسم، وزن المبيض و عدد الأجنة المسترجعة و وزن المشيمة معنويا (على مستوى ١ أو ٥%)، بينما نسبة الأجنة المفقودة قبل الانغراس و الاجنة الغير طبيعية أتجة الى الزيادة المعنوية. أدت إضافة البنتونيت إلى العلائق الملوثة بالافلاتوكسين الى أنخفاض معنوي للتأثيرات الضارة للافلاتوكسين و والى تحسن الأداء التناسلي و صفات خلفة البطن و أنتاج اللبن و جودة الأجنة أكثر من إضافة الطفلة، خاصة مع زيادة طول فترة التغذية. تأثرت الأعضاء التناسلية تأثيرا ضارا بالتغذية على علائق ملوثة بالافلاتوكسين خاصة مع زيادة نسبة الافلاتوكسين فى العليقة. أظهرت الإناث التي غذيت على عليقه مستبدلة ب ٣٠% عليقه ملوثة+البنتونيت مظهرا شبه طبيعي للأعضاء التناسلية مقارنة بالحيوانات التي غذيت على نفس العليقة و مضافا لها الطفلة. كبد و كلى الأرانب المغذاة على ٤٠% عليقه ملوثة+طفلة أظهرت تدهور أكثر من التي غذيت على ٣٠% عليقة ملوثة+طفلة أو ٣٠ و ٤٠% عليقه ملوثة+بنتونيت. عموما، إضافة ٤ جم بنتونيت/كجم عليقة يحمى إناث الأرانب الناضجة عند التغذية على عليقة ملوثة ب ١٣٥ جزء في البليون أفلاتوكسين بدون أعاقة في النشاط و الأداء التناسلي.