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Original Research Article

EFFECT OF SOME FEED-ADDITIVES ON THE GROWTH PERFORMANCE, PHYSIOLOGICAL RESPONSE AND HISTOPATHOLOGICAL CHANGES OF RABBITS SUBJECTED TO OCHRATOXIN-A FEED CONTAMINATION

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Abstract: This experiment aimed to evaluate the toxic influence of ochratoxin- A (OTA) feed contamination and the effectiveness of some feed-additives (Humic acids, Bio-Plus 2B, Bio-Mos) in detoxifying ochratoxicosis. Thirty five day old weaned Animal Production Research Institute (APRI) rabbits were selected and were allocated to four groups. Group 1 (control, OTA group): rabbits were fed basal diet (ochratoxin- A level: 19 µg /kg diet). Group 2: rabbits were fed a basal diet containing 0.2 % humic acid. Group 3: rabbits were fed a basal diet containing 0.4 mg/kg diet *Bacillus subtilis* and *Bacillus licheniformis* (Bio-Plus 2B). Group 4: rabbits were fed a basal diet containing 0.1% Manna oligosaccharides (Bio-Mos). The control group showed significantly reduced feed intake, daily weight gain and growth performance index. Furthermore, there was a significant increase in AST, ALT, malondialdehyde, creatinine, and urea. Moreover, Bio-Mos group revealed significantly increased serum Hemoglobin, PCV, RBCs, MCV and RBCs/ lymphocyte ratio, and significantly decreased serum SOD and CAT activities. Additionally, internal organs (liver, kidney and intestine) of groups received feed additives revealed less deteriorative change in histopathological investigations in comparison with the control group. In conclusion, supplementation of contaminated feed with ochratoxin-A by feed-additives improves feed intake and final body weight and reduces mortality rate of rabbits. In addition, it improves liver and kidney functions and reduces its pathological changes, and restores antioxidant to its normal level.

Key words: rabbits; ochratoxin-A; growth performance; anti-oxidant

Introduction

Animal feed and feed ingredients are commonly contaminated with different types of mycotoxins which produced by some fungal species (1). One of the most dangerous mycotoxins types is ochratoxin-A. It created by some species of *Aspergillus* and *Penicillium*. Presence of ochratoxin-A (OTA) in animal feeds raises concerns in poultry, rabbits and livestock industry due to subclinical intoxications and poor growth in animals (2). Contamination of animal

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feed with OTA is very come and its toxic consequences are focused on the internal organs in form of hypertrophy, increase weight and sever destruction in active cells (3). In case of rabbits and some other poultry, the most affected organ is kidney (39). However, the other organs (liver, intestine, muscle, bone, bone marrow, testes, ovaries and lymphatic organs) may also be influenced by OTA toxicity (4). (3) found that rabbits suffering from OTA toxicity showed lower consumption of feed, reduced weight gain, higher feed conversion ratio, lower profit gain and consequently reduced overall production of rabbit (5).

Due to the severity of OTA toxicity, many trials were done to overcome its bad effects. Using of feed additives is a novel method used for reducing the destructive effects of OTA by reducing its absorption from animal intestine. (6). Humate is a substance originated from the decayed animal and pants in soil and contains many active ingredients such as humic acid, humus, fulvic acid, and some micro-minerals that have strong binding and absorption activity (7). Therefore, humic acid was used as therapeutic agents in the field of veterinary and human medicine for example as a coating agent in diarrhea treatment, antibacterial agent and immune stimulator (8).

Some *Bacillus species* have a strong powerful capability in toxins removal especially in the field of food production (9). (10) found that most of toxins produced by sever pathogenic fungi are biologically controlled by using *Bacillus subtilis*. Moreover, the growth of *Aspergillus species* could be inhibited under effect of *Bacillus subtilis* (11) and the aflatoxin produced by *A. flavus* and *A. parasiticus* and could be prevented under the effect of *Bacillus stearothermophilus* (12, 13). Another example for feed additives is manna oligosaccharides that produced by yeast *(Saccharomyces cerevisiae*) cell wall. It characterized by its strong binding capacity with microbes, chemical agents and toxins preventing its accumulation and absorption from intestine (14).

The aim of this experiment was to evaluate the influence of OTA and the antitoxic efficacy of some feed-additives on feed utilization, performance, hemato-biochemical parameters and histopathological changes of APPRI growing rabbits.

Materials and methods

Chemical analysis of OTA in rabbit feed

The feed was prepared to supply experimental animals with their basic requirements for the growth (Table 1) according to (15). Analysis of feed ochratoxin-A was performed using florometer- antibody column method in Vet. Med. Laboratory, Kafrelsheikh University, Egypt and the value of OTA in basal diet of rabbit was 19 μg/kg diet.

Experimental design

The experiment was conducted in a Sakha Station rabbits farm), Animal Production Research Institute, Agriculture Research Center, Egypt. Animals used in the experiment were weaned and 35-day old APRI-line rabbits. Each animal was housed in a separate box of a larger cage. Animals were arbitrarily allocated to four groups (20 rabbit each), two replicate each. For acclimatization, all rabbits were fed the same feed for a week before starting the experiment. At 35 days of age, each experimental group received its specific feed as follow: Group 1 (control): rabbits were fed only on basal diet (ochratoxin- A level: 19 μg /kg diet). Group 2 (Humic acids): rabbits were fed a basal diet contain 0.2 % humic acids. Group 3 (PLUS): rabbits were fed a basal diet contain 0.4 mg/kg diet *Bacillus subtilis* and *Bacillus licheniformis* (Bio-Plus 2B). Group 4 (MOS): rabbits were fed a basal diet contain 0.1% Manna oligosaccharides (Bio-Mos) (8 - 11). Feed additives were mixed with pelleted feed daily by spraying of feed with molasses mixed with water as a binder for additives and then additives mixed carefully with feed. In case of group 1 (control, OTA group), feed was mixed only with molasses mixed with water. All groups received continuous supply of feed and water. Rabbits feed consumption was recorded on daily bases. Whereas, body weight (BW), weight gain (WG) and feed conversion ratio (FCR) were

measured weekly. Mortality rate (%) was recorded along the experimental period. Economic efficiency (16), relative growth rate [(W2 - W1) x100]/ [1/2 (W2+W1)] and performance index [(final body weight (kg)/ feed conversion ratio) x 100] were calculated for each group (17).Whereas: W1is the initial weight, and W2 is the final body weight.

Blood was aspirated from the marginal ear vein at the end of the experiment. Blood (3 ml) was aspirated and mixed gently in heparinized tubes. Whole blood was used for hematological assay. Plasma was obtained by centrifugation of 2 ml whole blood at 3000 rpm for 15 min. Then, plasma was stored at -20 °C until used for biochemical assay.

At the end of experiment (91 day), six rabbits were randomly selected from group 1, 2, 3 and 4and were slaughtered using sharp knife to obtain internal organs (liver, kidney and intestine) for histopathological examination.

The Ethical Committee, Faculty of Veterinary Medicine, University of Kafrelsheikh and the Agricultural Administrative Authority approved the conduct of this experiment.

Hematological examination

Heparinized whole blood was analyzed after collection by 2 hours for estimation of red blood cells (RBCs) count, packed cell volume (PCV), haemoglobin concentration, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), white blood cells (WBCs) according to (18). On two clean microscope slides, thin blood smears were prepared from each blood sample. Slides were left to dry in room temperature. Slides were stained with a modified Wright's stain, and covered. One hundred cells were counted under × 100 lense and the number of neutrophils count, lymphocytes count, monocyte, eosinophil and basophil were calculated.

Biochemical examination

Plasma samples were used for calorimetric estimation of total protein (TP), globulin, albumin (ALB), triglyceride (TG), total cholesterol (TC) , HDL-C, LDL-C, VLDL-C, cholesterol/ HDL ratio, LDL/ HDL ratio, glucose, urea, creatinine (CREA), alanine aminotransferase (ALT), aspartate aminotransferase (AST) by using commercial kits (Bio-Diagnosis Co., Cairo, Egypt).

Antioxidant parameters and lipid peroxidation biomarker

Lipid peroxidation biomarker used in this study was malondialdehyde (MDA). The antioxidant indicators used in this study were superoxide dismutase (SOD) and catalase activity that were estimated as previously described (19), (20) and (21).

Histopathological changes

Kidneys, liver and intestine tissues specimens were fixed in 10% formalin immediately after slaughtering. The fixed tissues were paraffin embedded, sectioned (3 μm) and stained by hematoxylin and eosin (H&E) (22).

Statistical analysis

Data was tested for distribution normality. Data were analyzed using SAS® statistical system, Package v9.2, version 2002. Data were reported as means and SEM, compared by one-way ANOVA and the Duncan's multiple range tests was used as a post hoc test (23) except for mortality rate that was analyzed using Chi-square test. The level of significance was at (P<0.05).

Results

Feed consumption and rabbits performance

Feeding rabbits on diet contaminated with OTA (control group) led to a significant (P<0.05) decrease in feed consumption, WG, final BW and performance index as compared to the other groups (Table 2). On the other hand, administration of MOS, plus and humic groups reduced the negative influence of OTA and significantly (P<0.05) enhanced feed consumption, final BW, relative growth rate, and performance index in comparison with group 1 (control). Rabbits in the control group showed higher FCR and mortality (30 %) rate than other groups (Table 2).

Biochemical parameters

Although TP, ALB and globulin concentration showed no significant differences (Table 3), glucose concentration reached highest level significantly ($P<0.05$) in MOS and plus groups. While, the TG and TC recorded the lowest significant levels ($P<0.05$) in MOS group matched to other groups (Table 3). The LDL and VLDL show lower significant ($P<0.05$) differences in MOS group, while HDL shown higher significant different in Bio-Plus 2B (PLUS) group. The MOS and plus groups revealed a significant reduction ($P<0.05$) in blood serum creatinine and urea compared to humic and control groups. Meanwhile, AST and ALT activities significantly ($P<0.05$) increased in control group compared to other groups (Table 3).

Hematological parameters

Supplementation of MOS and Bio-Plus 2B improved ($P<0.05$) blood hemoglobin, PCV, RBCs, MCV and RBCs/ lymphocyte ratio (Table 4). However, the MCH, WBCs, neutrophils and lymphocyte percentages were significantly ($P<0.05$) higher in the control group (Table 4). While, MCHC, monocyte and eosinophil revealed absence of significant effect of all groups.

Lipid peroxidation and antioxidants enzymes activity

Plasma MDA level was significantly higher in rabbits supplied with basal diet (control group) and was lowered following supplementation with MOS, PLUS and humic acid (Table 5). Plasma CAT and SOD activities were increased significantly ($P<0.05$) with MOS and PLUS supplementation compared to the control group.

Histopathological changes

Histopathological findings of the control group showed marked swelling of hepatocytes with granular vacuolated cytoplasm (Fig. 1a), tubular degeneration and necrosis of kidney (Fig. 1b) and intestinal sub-epithelial lymphocytic infiltration (Fig. 1c). While, liver of the humic (Fig. 2a) fed rabbits showed slightly swollen hepatocytes with granular vacuolated cytoplasm, mild to moderate degree of renal tubular epithelium degeneration of kidney (Fig. 2b) and mild degree of intestinal villi atrophy associated with sub-epithelial infiltration of lymphocytes (Fig. 2c). Yet, in group 3 the liver showed normal hepatocytes (Fig. 3a), only mild degree of renal tubular degeneration of the kidney (Fig. 3b) and slight degree of mononuclear inflammatory cell within the lamina propria and hyperplasia of the covering epithelium of intestine (Fig. 3c). Meanwhile, liver (Fig. 4a), kidneys and intestine (Fig. 4b, c), sections of the MOS group 4 showed nearly normal morphological appearances.

Table 1: Composition and chemical analysis of basal diet

Ingredients	%	Chemical analysis (% as DM):	%
Berseem hay	30.05	Dry matter (DM)	85.81
Barley grain	24.60	Crude protein (CP)	17.36
Wheat brain	21.50	Organic matter (OM)	91.42
Soybean meal (44% CP)	17.50	Crude fiber (CF)	12.37
Molasses	3.00	Ether extract (EE)	2.230
Limestone	0.95	Digestable energy(DE, kcal/kg)(2)	2412
Di-calcium phosphate	1.60	Calcium(2)	1.243
Sodium chloride	0.30	Phosphorus(2)	0.808
Mineral-vitamin premix(1)	0.30	Methionine(2)	0.454
DL-Methionine	0.20	Lysine(2)	0.862
Total	100		

(1) One kilogram of mineral–vitamin premix provided: Vitamin A, 150,000 UI; Vitamin E, 100 mg; Vitamin K3, 21mg; Vitamin B1, 10 mg; VitaminB2, 40mg; Vitamin B6, 15mg; Pantothenic acid, 100 mg; Vitamin B12, 0.1mg; Niacin, 200 mg; Folic acid, 10mg; Biotin, 0.5mg; Choline chloride, 5000 mg; Fe, 0.3mg; Mn, 600 mg; Cu, 50 mg; Co, 2 mg; Se, 1mg; and Zn, 450mg

Table 2: Effect of experimental diets on growth performance of growing APRI-line rabbits from 5 to 13 weeks of age

Parameters	Control	Humic acids	PLUS	MOS	SEM	*P*-value
Initial body weight (g)	674.6	671.7	672.9	676.2	11.53	0.9953
Final body weight (g)	2052.5^{d}	2134.0^{c}	2201.0^{b}	2332.0^{a}	20.75	0.0001
Daily weight gain (g)	24.6^{c}	26.1^{b}	27.3^{b}	29.6^{a}	0.421	0.0001
Feed intake (g/d)	83.9^{c}	85.8^{bc}	87.2^{ab}	89.2^{a}	0.824	0.0007
Feed conversion ratio	3.421^{a}	3.295^{ab}	3.199^{b}	3.025^{c}	0.057	0.0005
Relative growth rate	101.1^{c}	104.3^{bc}	106.3^{ab}	110.1^{a}	1.396	0.0018
Performance index (%)	60.3^{c}	65.1^{bc}	69.0^{b}	77.4^{a}	1.703	0.0001
Mortality rate (%)	30^{a}	20^{b}	10^{c}	10^{c}	-	-

SEM = Standard error of means

a, b, c, d, Means in the same row with different superscripts are significantly different ($P<0.05$)

Table 3: Effect of experimental diets on some blood parameters of APRI-line rabbits

Items	Control	Humic acids	PLUS	MOS	SEM	*P*-value
Total protein (g /dl)	5.19	5.57	5.71	5.88	0.143	0.1921
Albumin (g /dl)	3.50	3.72	3.75	3.85	0.127	0.4375
Globulin (g /dl)	1.69	1.85	1.95	2.03	0.113	0.2178
Glucose (mg/ dl)	89.3^{b}	93.0^{b}	102.3^{ab}	114.7^{a}	4.631	0.0259
Triglycerides (mg/ dl)	97.3^{a}	86.7^{a}	86.0^{a}	70.0^{b}	3.844	0.0048
Cholesterol (mg /dl)	67.0^{a}	53.0^{b}	47.0^{bc}	38.0^{c}	3.055	0.0007
HDL (mg /dl)	13.9^{b}	13.0^{b}	18.0^{a}	14.5^{b}	0.888	0.0089
LDL (mg /dl)	26.3^{a}	18.3^{b}	17.2^{b}	9.47^{c}	2.210	0.0019
VLDL (mg /dl)	27.7^{a}	21.3^{b}	18.7^{bc}	14.0^{c}	2.517	0.0063
Kidney function:						
Creatinine (mg/ dl)	1.37^{a}	1.27^{a}	1.20^{ab}	1.08^{b}	0.061	0.0346
Urea (mg/ dl)	33.7^{a}	31.3^{a}	29.6^{ab}	26.7^{b}	1.167	0.0213
Liver function:						
AST (U/L)	125.3^{a}	101.3^{b}	83.3^{b}	86.3^{b}	6.960	0.0076
ALT (U/L)	71.0^{a}	69.7^{a}	62.3^{ab}	51.3^{b}	3.464	0.0138

SEM = Standard error of means, a, b, e, Means in the same row with different superscripts are significantly different ($P<0.05$)

Table 4: Effect of experimental diets on blood hematological values of APRI-line rabbits

Items	Control	Humic acids	PLUS	MOS	SEM	*P*-value
Hemoglobin (g/ dl)	10.5^{c}	11.7^{b}	12.1^{ab}	12.4^{a}	0.200	0.0005
PCV [(1)] (%)	32.7^{c}	35.7^{bc}	37.1^{b}	38.8^{a}	0.623	0.0007
RBCs ($x10^{6}$/ µl)	5.28^{b}	5.49^{ab}	5.60^{ab}	6.20^{a}	0.240	0.0748
MCV[(2)] (fl)	60.1^{c}	61.3^{bc}	63.8^{ab}	66.1^{a}	0.800	0.0030
MCH[(3)] (pg)	20.4^{a}	19.6^{ab}	19.3^{bc}	18.5^{c}	0.300	0.0152
MCHC[(4)] (g/ l)	32.3	32.0	32.1	31.5	0.384	0.4456
WBCs ($x10^{3}$/ µl)	9.23^{a}	9.03^{a}	7.67^{b}	6.30^{c}	0.351	0.0011
Neutrophils (%)	52.0^{a}	51.7^{a}	32.0^{b}	35.7^{b}	3.283	0.0016
Lymphocyte (%)	53.7^{a}	44.3^{b}	39.3^{bc}	34.3^{c}	2.333	0.0012
RBCs/ Lymphocyte ratio	0.10^{c}	0.13^{bc}	0.14^{b}	0.18^{a}	0.012	0.0041
Monocyte (%)	9.00	8.67	9.67	8.33	1.155	0.8640
Eosinophil (%)	5.33	5.33	4.67	3.67	0.333	0.1617
Basophil (%)	0	0	0	0	-	-

SEM = Standard error of means, a, b, e, means in the same row with different superscripts are significantly different ($P<0.05$).[1] Packed cell volume, [2]Mean corpuscular volume, [3]Mean Corpuscular Hemoglobin, [4]Mean Corpuscular Hemoglobin Concentrations.

Table 5: Effect of experimental diets on anti-oxidant parameters of APRI-line rabbits

Items	Control	Humic acids	PLUS	MOS	SEM	*P*-value
MDA (N/ mol)	2.45^{a}	2.05^{ab}	1.90^{ab}	1.65^{b}	0.250	0.0903
CAT (U/ l)	1.75^{b}	1.87^{ab}	2.05^{a}	2.09^{a}	0.087	0.0585
SOD (U/ ml)	17.5^{c}	19.5^{bc}	21.6^{ab}	22.4^{a}	0.722	0.0034

SEM = Standard error of means, a, b,….e, Means in the same row with different superscripts are significantly different (P<0.05)

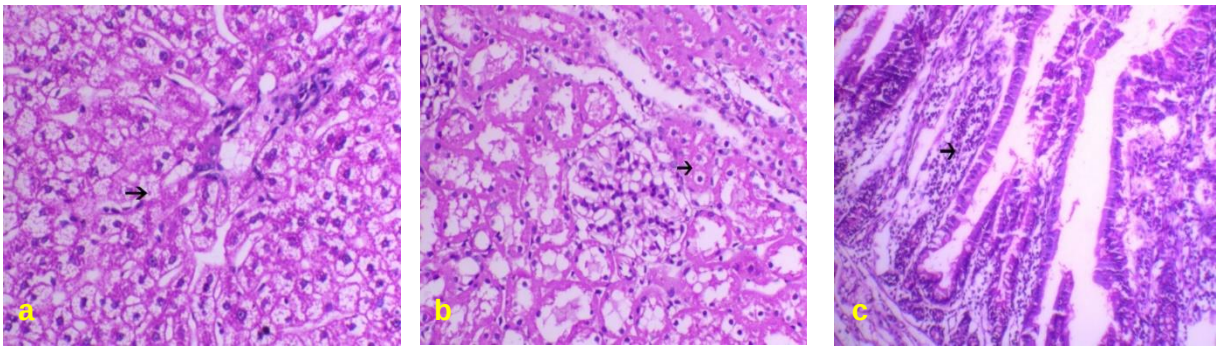

Figure 1: Control group 1 showing (a) liver showing marked swelling of hepatocytes with granular vacuolated cytoplasm (arrow), (b) the kidney showing tubular degeneration and necrosis (arrow), and (c) the Intestine showing marked sub-epithelial lymphocytic infiltration. (H&E, X 200)

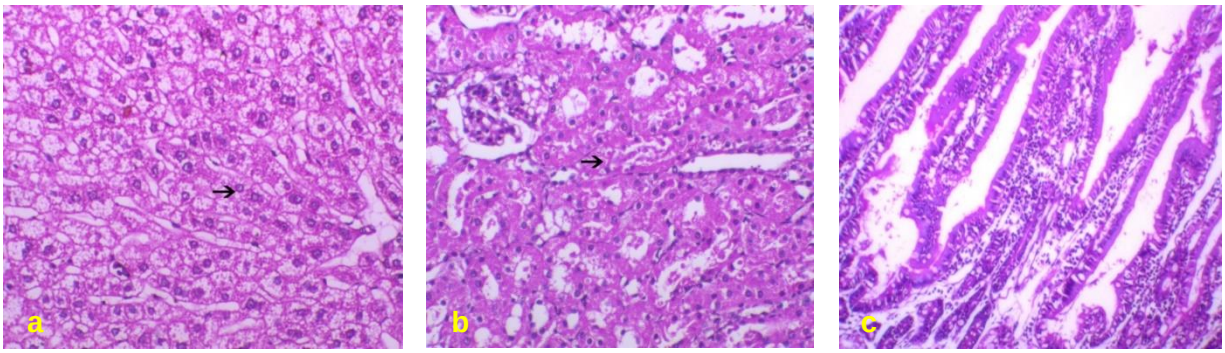

Figure 2: Humic group 2 showing (a) liver showing swelling of hepatocytes and with granular vacuolated cytoplasm (arrow), (b) the kidney showing mild to moderate degree of renal tubular epithelium degeneration (arrow), and (c) the Intestine showing mild degree of intestinal villi atrophy associated with sub-epithelial infiltration of lymphocytes. (H&E, X 200)

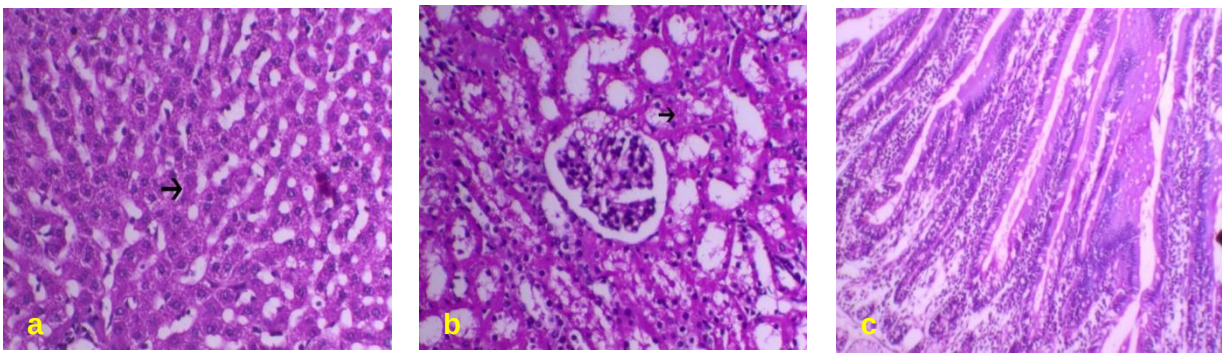

Figure 3: Plus, group 3 showing (a) liver showing normal hepatocytes (arrow), (b) the kidney showing mild degree of renal tubular degeneration (arrow), and (c) the Intestine showing slight degree of mononuclear inflammatory cell within the lamina propria and hyperplasia of the covering epithelium. (H&E, X 200)

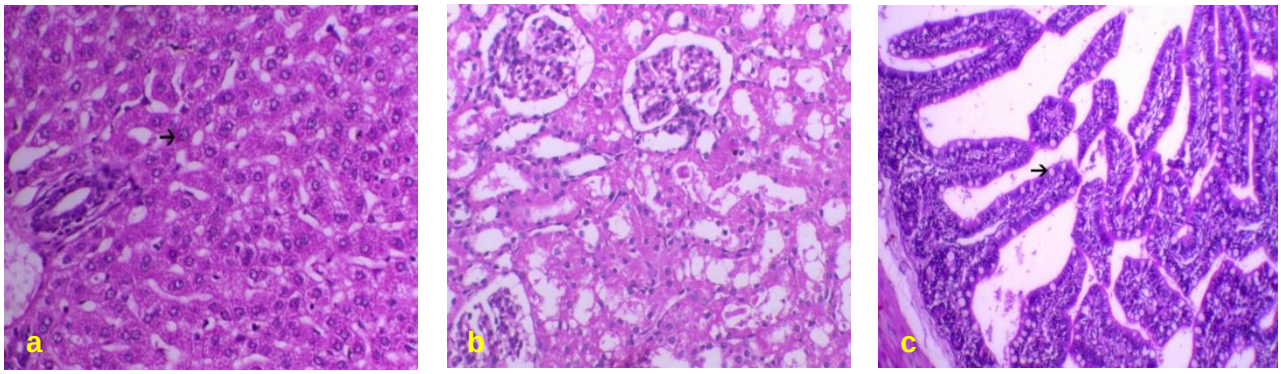

Figure 4: MOS group 4 (a) normal hepatocytes arranged in cords around the portal area (b) the kidney showing normal renal glomeruli and tubules, and (c) the Intestine showing normal branched intestinal villi with normal pseudo stratified epithelium with goblet cells. (H&E, X 200)

Discussion

To Feeding rabbits on feed contain OTA reduced final BW, WG and daily feed intake and performance index observed in the control group. These findings are in agreement with earlier studies recording the adverse effects of OTA on feed utilization and performance (3). These adverse effects might be attributable to phenylalanine moiety that present in OTA, which inhibit synthesis of phenylalanine-t-RNA and consequently reduce protein synthesis (24). Furthermore, OTA reduce carbohydrate metabolism, mainly glucogenolysis through inhibition of DNA, RNA and protein synthesis. While, feed additives especially MOS improve feed consumption, WG, final BW and decrease FCR. These results agreed with (25) who observed that using of MOS increase the height of intestinal villi. Accordingly, the surface area of intestinal villi increased and the absorption capacity improved that may improve feed consumption and performance.

Rabbits in control group showed higher levels ALT and AST. These findings are in concordance with the previous studies conducted by (26, 27). Higher level of ALT indicates changes in the hepatocyte membrane permeability due to the vascular congestion induced by OTA toxicity and finally hepatic destruction (28). While, higher level of AST secretion implies skeletal muscle destruction (29).

In present study, the control group showed an increase in the concentration of creatinine and urea that may give an indication about kidney damage due to OTA toxicity. These findings are similar to that stated in rabbits and some domestic animals (26, 28). (30) reported that toxicity by OTA is a direct cause of nephrotoxicity. Furthermore, due to nephrotoxicity urea and creatinine are accumulated in destructed nephrons resulting in further damage and kidney failure.

Supplementation of feed additives especially MOS and PLUS improved the reduced blood hemoglobin, PCV, RBCs, MCV and RBCs/ lymphocyte ratio those were elevated in the control group. These results are in consistence with (31) who reported internal bleeding, hemorrhages or bruising, stomach ulcers in ochratoxicosis rabbits that resulted in lower hemoglobin and RBCs. Control group appeared in exhausted condition due to the signs of anemia that may be due to increased anaerobic metabolism and reduced aerobic one. (32) reported that during OTA toxicity, rabbits showed lower RBCs count, haemoglobin content and PCV which might lead to an increase in anaerobic metabolism and decrease in oxygen supply to tissue and cells. Furthermore, RBCs cytotoxicity that may resulted from destruction of RBCs and may be occur in case of OTA toxicity (33).

Glucose concentration reached the lowest level in the control group. This result agreed with (34) who reported ochratoxicosis induced hypoglycemia; this finding may be due to intestinal villi destruction and subsequently poor absorption. In addition, it may be due to destruction in the membrane of hepatocytes. On the other hand, kidney failure and nephrotoxicity may lead to sever loss of glucose while using

of feed additives protects the intestinal villi, hepatocytes membrane and kidney nephrons from destruction (35, 26). In some cases of OTA toxicity, low glucose levels may be the cause of nervous manifestations observed in rabbits before death (36).

Triglycerides and cholesterol are significantly increased in control group compared to other groups.it may be due to destruction of hepatocytes and changes in metabolism of lipid which was prevented by using feed additives. (37) observed similar results in broiler subjected to ochratoxicosis.

Rabbits in the control group showed high oxidative destruction and changes in the plasma biochemical levels. While, rabbits supplied with feed additives showed high resistance to the OTA induced oxidative damage. Toxicity with OTA causes destruction in liver, kidney, intestine and muscle cell membranes that may be due to the increased lipid peroxidation (38). Therefore, activities of antioxidant were investigated due to its protective action in form of superoxide anion production, which protect cell membranes from OTA induced destruction that may be due to the effect of feed additives (38).

Kidney is the primary target organ for ochratoxin-A (39). The control group showed kidney tubular degeneration and necrosis while, Kidney histopathological lesions included mild to moderate degree of renal tubular epithelium degeneration of plus and humic groups. Liver histopathological lesions showed marked swelling of hepatocytes with granular vacuolated cytoplasm of control group. Meanwhile, intestine showing slight degree of mononuclear inflammatory cell within the lamina propria and hyperplasia of the covering epithelium, and mild degree of intestinal villi atrophy associated with sub-epithelial infiltration of lymphocytes while control group marked sub-epithelial lymphocytic infiltration. These indicate the feed additives improve liver, kidney and intestinal histology and function. The results of histopathological examination in kidney, intestine and liver confirmed by biochemical profile of examined rabbits were consistent with those reported by (40).

Toxicity with OTA resulted in impaired feed utilization and performance of rabbits. In addition, it caused high mortality rate. Consequently, the outcomes of this study represented by higher relative growth rate and performance index in grouped received feed additives may be reflected on the rabbit's economic production. Therefore, using feed additives especially MOS might improve feed utilization, WG, FCR and performance resulting in high economic revenue.

Conclusions

Supplementation of contaminated feed with ochratoxin-A by feed-additives especially MOS improves feed intake and final body weight and reduces mortality rate of rabbits. In addition, it improves liver and kidney functions and reduces its pathological changes, and the antioxidant biomarkers.

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Conflict of interest

The authors declare that there is no conflict of interest.

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