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Ameliorating effects of organic and inorganic mycotoxin binders on the performance of Ossimi sheep

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ABSTRACT

This investigation is an endeavor to prevent or limit the negative effects resulted of ingesting diets contaminated with a combination of aflatoxin B1 (AFB1) and ochratoxin A (OTA). This experiment aimed to evaluate the efficacy of supplementing diets with organic, inorganic and/or mixed toxin binders, throughout a feeding and digestibility trials, to relieve mycotoxins' negative effect towards maintaining the productive and reproductive performance of Ossimi ewes and their offspring. One hundred Ossimi ewes averaged 38.97 ± 0.55 kg body weight and aged 3 to 4 years were chosen, 30 days before the expected lambing date and divided randomly into five equal groups (20 each). The first group received an uncontaminated diet and served as a negative control (NC). The second group fed a diet contaminated with AFB1 mixed with OTA and served as a positive control (PC), while the third group fed contaminated diets and supplemented with organic toxin binder (OB). The fourth group fed contaminated diet supplemented with inorganic toxin binder (IOB) and the fifth group fed contaminated diet and supplemented with a mixture of OB and IOB (OB+IOB).

The results showed that PC group had decrease in levels of digestibility coefficients, feeding values, milk production, feed intake, serum total protein, albumin, glucose and cholesterol while had increase of ALT, AST and urea concentrations. The decrease of ewes' body weight was associated with increased age at 1st estrous post-lambing versus decreasing the fertility parameters of the PC group. Besides, there was a decrease in thyroid hormones and antioxidant activities versus the increase in malondialdehyde concentrations. In the same group, changes in ewes' body weight decreased, while the feed conversion ratio increased with the PC group compared to the NC control group. Whereas, all treatments tested in the current study could reverse the mycotoxin-induced effects significantly and restore the normal levels of animals. In conclusion, IOB alone, or the mixture of OB+IOB, can be added to ewes' diets for the relief of symptoms induced by mycotoxin.

Keywords: Sheep, mycotoxin, binders, productive, reproductive

INTRODUCTION

Mycotoxins (MYCs) usually cause great economic losses for livestock production. The consumption of feeds contaminated with MYCs poses a potential hazard for animal health thus food safety due to the transfer of toxin through the food chain to humans.

Aflatoxin B1 (AFB1) and ochratoxin A (OTA) are perceived as main MYCs, and they present simultaneously in animal diets **(Solfrizzo et al., 2014)**. **Kourousekos et al. (2012)** illustrated that 50 µg/kg aflatoxin-B1 reduced goat's milk yield**.** However, **Battacone et al. (2003)** reported that 128 µg/kg AFB1 significantly raised serum ALT level and diminished the ALP level in sheep. Symptoms of the toxic influences of AFB1 and OTA mixture were reported on rabbits **(Prabu et al., 2013),** and dairy goats **(Huang et al., 2018)**.

An important method for avoiding mycotoxicosis in animal is supplementation of non-dietary adsorbents in the ration, which bind with MYCs in the digestive tract, thus can decrease their bioavailability. These binding factors don't subject to any alterations in the

digestive tract. They also bind to AFB1 fragments and decrease their toxic impacts **(Bhatti et al., 2017)**.

Mycotoxin binders (MYCB) contain substances with a high absorption capacity, including polysaccharides, peptidoglycan, cellulose, aluminosilicate activated carbon, and synthetic polymers like polyvinylpyrrolidone, cholestyramine, and their derivatives **(Avantaggiato et al., 2005)**. **Boudergue et al. (2009)** classified the MYCB to silica-based inorganic polymers or carbon-based organic compounds**.**

Inorganic binders generally incorporate aluminosilicate minerals, which are the biggest class of MYCB, and many studies have focused on the mycotoxicosis mitigation by the utilization of adsorbents on these clays **(Santos et al., 2011).** The organic binders are complicated carbohydrates that are not digestible (polysaccharides in the cellular yeast walls, cellulose, and bacteria like peptidoglycans, glucomannans, and others), and synthetic polymers like cholestyramine can adsorb MYCs **(Oguz, 2016).** The beneficial impacts of yeast have been due to the mannan in the yeast cell wall. Through utilizing just cell walls of yeast (consisted of mannan oligosaccharides and beta-glucans) rather than the entire cell, MYCs binding can be improved **(Karaman et al., 2005).**

European Food Safety Authority **(EFSA, 2010)** confirmed that besides testing the effectiveness of MYCB; their protection must be verified since the additions of binders to the diets are believed to produce unspecified bindings. Therefore, we investigated the ameliorative effect of organic and inorganic toxic binders supplemented to diets to relieve the toxic effects of AFB1 and OTA mixture on milk production, feed intake, metabolic blood measurements, antioxidant status and the performance of Ossimi ewes and their suckling lambs.

MATERIALS AND METHODS

The current study was performed in the Fac. of Agri. Exp. Sta., El-Fayoum Univ., in cooperation with APRI, ARC, Ministry of Agri., Egypt. One hundred pregnant Ossimi ewes averaged 38.97±0.55 kg BW and aged 3 to 4 years old were selected, 30 days prior expected lambing date, and divided randomly into five equal groups (20 each). The 1st group received the uncontaminated diet, composed of concentrate feed mixture, corn silage, and rice straw and served as a negative control (NC). The 2nd group fed the basal diet contaminated with 50 µg aflatoxin B1/kg DM and 100 µg ochratoxin A / kg DM and served as positive control (PC), while the 3rd group fed the same contaminated diet supplemented with 500 gm/ton organic toxin binder (OB) contains cell wall of *cerevisia* yeast, mannan-oligosaccharide, β-glucan, organic acid, plant extracts, and some enzymes stimulate the enzymatic transformation of mycotoxins such as epoxidase and esterase). The 4th group fed the contaminated diet supplemented with 250 g / ton inorganic toxin binder (IOB) contains choline chloride 70% (contains 21g choline), Cynara (artichoke dry powder), Ca and Al silicates, bentonite, clinoptilolite, clenobentonite, sepiolite and calcium carbonate (carrier). The 5th group fed the contaminated diet supplemented with a mixture of OB and IOB (OB+IOB).

All MYCs were given in the purified form, and dosages were picked in accordance with the national standards for MYCs, which set the upper limits of feeding at 100 µg/kg for OTA, and 50 µg/kg for AFB1 **(National Health, 2011).**

Dietary requirements for tested ewes determined by **NRC (2007)** tables utilizing total mixed ration (TMR) during the experimental period. The chemical analysis of ration's ingredients and TMR were performed according to **AOAC (2003)** and are presented in Table 1. Nutrient digestion coefficients and nutrient values were determined by acid insoluble ash method, according to **Van Keulen and Young (1977).** Body weight change was recorded biweekly before morning feeding. The ewes were fed two times per day, and feed

Table (1): Chemical composition of feedstuffs and total mixed ration, DM basis (%).

Items	DM	Chemical composition, as DM basis					
		OM	CP	EE	CF	NFE	Ash
CFM	89.71	87.12	13.86	2.81	23.25	47.20	12.88
RS	91.13	85.55	2.65	1.20	38.33	43.37	14.45
CS	30.22	92.65	10.75	2.60	24.30	55.00	7.35
TMR	75.19	88.11	10.28	2.36	27.28	48.19	11.89

CFM= Concentrate feed mixture, consisted of 24 % cotton seed meal; 40% wheat bran; 30% yellow corn 1.5% lime stone; 1% sodium chloride, 0.5% vitamins and mineral mixture and 3% molasses; **RS=** Rice straw; **CS**= Corn silage; **TMR=** Total mixed ration (50% CFM + 25% RS + 25% CS).

consumption was recorded daily. Fresh-water was accessible all the time. The hand milking technique was utilized to stimulate and determine milk yield weekly. Besides, milk samples were obtained biweekly post-lambing for 75 days. Milk samples were protected directly post milking by adding 3 drops of $K_2Cr_2O_7$ (5 ppm). Milk composition was analyzed by Milko-Scan® analyzer (USA). **Economides and Louca (1981)** equation was used to correct sheep milk yield to 6% fat, as follows: Fat corrected milk (FCM) = DMY × (0.428 + 0.095 × fat %). The feed conversion ratio (FCR) was determined and represented in terms of dry matter (g) and total digestible nutrients (g), needed to produce 1 gram of milk.

Fifteen days post-lambing, blood samples were drawn monthly from 3 fasting ewes per group. Sera samples were isolated by centrifugation at 1,800 × *g* for 20 min and afterwards frozen at -20 °C until analyzed.

Serum protein profile, aspartate aminotransferase (AST), alanine aminotransferase (ALT), cholesterol, glucose and urea were measured according to **Young (2000)** method by biosystems automated reagent kits purchased from Costa Brava 30, Chemical Company, Barcelona (Spain). Also, total T_3 and T_4 were measured by radioimmunoassay procedures, in accordance with **Chopra et al. (1971)**, and **Irvin and Standeven (1968**), respectively. Serum total antioxidant capacity (TAC), glutathione peroxidase (GPX), superoxide dismutase (SOD), catalase (CAT), and malondialdehyde (MDA) were measured in accordance with the manufacturer's guidance of assay kits (Biodiognostic Company, Dokki, Giza, Egypt).

Estrous behaviour for ewes post-lambing was recorded. Days and body weight at first estrous post-lambing were determined for each ewe. When the females were ready for the mating number of services/conception and fertility parameters were recorded as follow:
Fertility rate (%) = (No. pregnant ewes / No. mated ewes) × 100; Pregnancy rate (%) = (No. pregnant ewes / No. ewes present to rams) × 100; Lambing rate (%) = (No. of lambed ewes / No. of mated ewes) × 100; Litter size = (No. lambs / No. lambed ewes); Stillbirth (%) = (No. died births / No. lambs born) × 100; Twining rate (%) = (No. of twins / No. lambed ewes) × 100; Fecundity rate (%) = (No. live births / No. lambed ewes) × 100.

Data were statistically analyzed using SPSS statistical program to perform ANOVA analysis of variance on current data. When significant variations by ANOVA analysis were found, the variations between the means of the control and treated groups were tested by using Dennett's t-test. Fertility parameters and survival rate results were analyzed by the Chi-square test.

RESULTS

Effect of binding MYCs on digestibility coefficients and nutrient values:

The effect of binding MYCs by two different binders on ewes' digestibility coefficients and nutrient values are presented in Fig. 1 (A, B, C & D). MYCs induced significant ($P < 0.001$) decrease in all nutrient digestibility coefficients (Fig. 1 A, B & C) and nutritive values (DCP and TDN) (Fig. 1 D) *vs.* control (NC). In contrary, all treated groups restored the digestibility coefficients and feeding values levels ($P < 0.05$ for OB or IOB and $P < 0.001$ for OB+IOB) to normal *vs.* PC and NC groups.

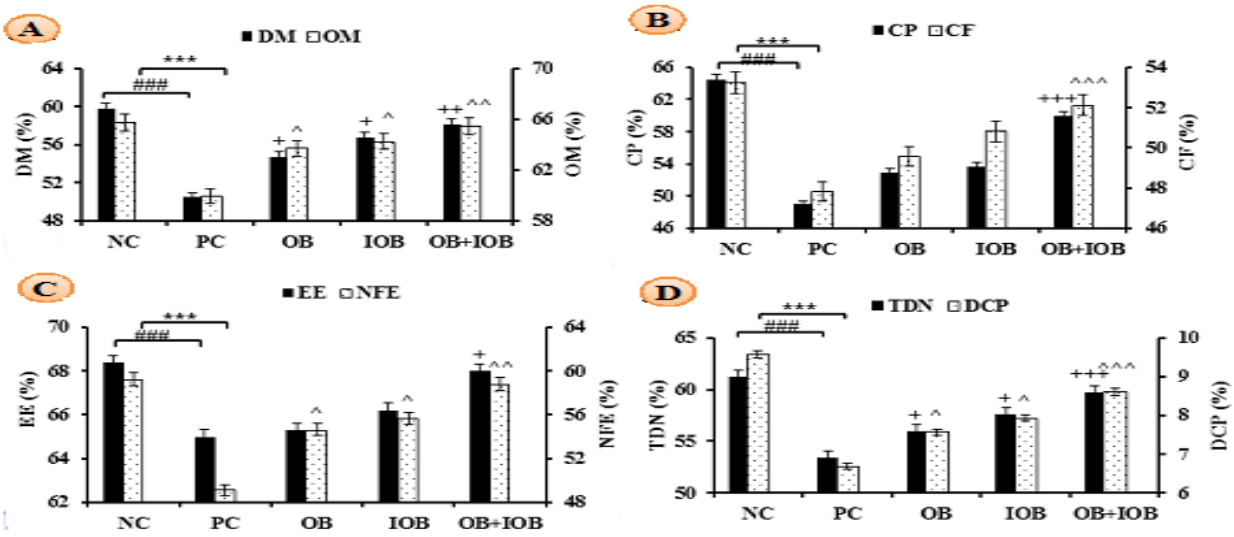

Fig. 1. Effect of organic and inorganic mycotoxin binders on digestibility coefficients such as DM & OM (a), CP & CF (b) and EE & NFE (c) and feeding values such as TDN & DCP (d) of Ossimi ewes fed experimental rations.

NC= Negative control (uncontaminated diet); **PC**= positive control (contaminated diet by aflatoxin B1 mixed with ochratocin A); **OB**= organic binders; **IOB**= inorganic binders. Data are expressed as mean±SE. # & * represent the degree of significant difference between PC & NC groups, while + & ^ represent the difference between treatments and PC groups.

Effect of binding MYCs on ewes' performance:

Milk production:

Milk yield, FCM, and milk composition are summarized in Fig. 2 (A & B) and Table 2. PC group had the greatest adverse effects on milk production in comparison with the NC group ($P < 0.001$), while the OB group was almost similar to the PC group ($P > 0.05$). However, the IOB and OB+IOB mixture groups had an ameliorative effect ($P < 0.05$ & $P < 0.001$, respectively) and restored yields to normal levels compared with PC and NC groups (Fig. 2 A).

Concerning FCM and milk composition, PC group induced significant ($P < 0.001$) decrease in FCM level (percentage change = -38.81 %) (Fig. 2 B) and significant ($P < 0.001$) decrease in milk fat, protein and total solids content (percentage change = -15.08, -7.83, & -13.69 %, respectively) (Table 2) compared to negative control (NC).

Therefore, treated ewes fed contaminated diets with different mycotoxin binders specially IOB or OB+IOB showed significant increment ($P < 0.001$) in yield of FCM (percentage change = 31.71 & 56.10 %, respectively), while milk contents of fat, protein and total solids showed light increase (percentage change = 13.55, 13.16, & 17.95 % for IOB and 14.64, 15.92, & 17.83 % for OB+IOB, respectively) relative to positive control (PC) which is expected because contamination had light effect on milk contents (15.08, 7.8, 13.7 % decrease in PC compared to NC for fat, protein and ash, respectively).

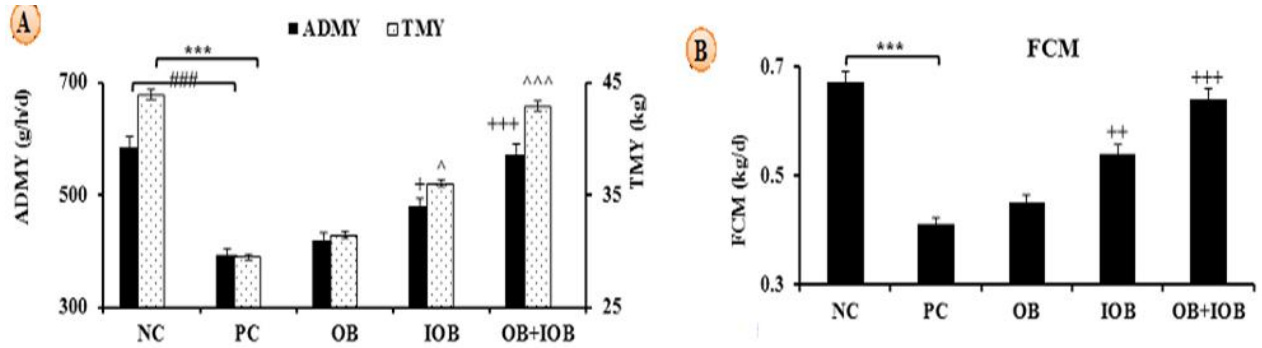

Fig. 2. Effect of organic and inorganic mycotoxin binders on milk yield (a) and fat corrected milk (FCM) (b) of Ossimi ewes fed experimental rations.

NC= Negative control (uncontaminated diet); **PC**= positive control (contaminated diet by aflatoxin B1 mixed with ochratocin); **OB**= organic binders; **IOB**= inorganic binders. **ADMY**= average daily milk yield; **TMY**= total milk yield

Data are expressed as mean±SEM. # & * represent the degree of significant difference between PC & NC groups, while + & ^ represent the difference between treatments and PC groups.

Milk yield was corrected to 6% equation: **FCM** = daily milk yield × (0.428+0.095× fat %).

Table (2): Effect of organic and inorganic mycotoxin binders on milk composition (%) of Ossimi ewes fed experimental rations.

Traits (%)	Treatments				
	NC	PC	OB	IOB	OB+IOB
Fat	7.56±0.13	6.42±0.10###	6.90±0.16	7.29±0.11**	7.36±0.13**
Protein	5.11±0.18	4.71±0.11#	5.20±0.10*	5.33±0.16*	5.46±0.05**
Lactose	4.91±0.11	4.08±0.17#	4.85±0.20	5.24±0.14*	5.07±0.11*
Solid not fat	10.76±0.27	9.41±0.30#	10.80±0.23*	11.36±0.28***	11.27±0.18***
Total solids	18.33±0.28	15.82±0.36###	17.70±0.35*	18.66±0.37***	18.64±0.25***
Ash	0.74±0.02	0.62±0.02###	0.75±0.02***	0.79±0.02***	0.74±0.03***

Degree of statistical difference between PC and NC groups ($P < 0.05$), ### ($P < 0.001$).

*Degree of statistical difference among treated and PC groups ($P < 0.05$), ** ($P < 0.01$), *** ($P < 0.001$).

NC= Negative control (uncontaminated diet); **PC**= positive control (contaminated diet by aflatoxin B1 mixed with ochratocin); **OB**= organic binders; **IOB**= inorganic binders.

Feed intake and feed conversion ratio:

Total dry matter intake (TDMI) and daily TDN intake were significantly lower ($P < 0.001$) in the PC group, but PC group showed an increase in feed conversion ratio for TDMI and TDN ($P < 0.001$) versus the NC group (Fig. 3 A & B). However, there were no significant variations ($P > 0.05$) in the OB group compared with PC group. However, the IOB and OB+IOB groups had an ameliorating effect ($P < 0.001$) on feed intake and FCR, and they had the nearest values to the control group (NC). Moreover, anti-mycotoxins addition (OB, IOB, or OB+IOB) enhance FCR for TDMI by reducing it by 8.99, 17.01 and 26.92, respectively, compare with those fed contaminated diets (PC).

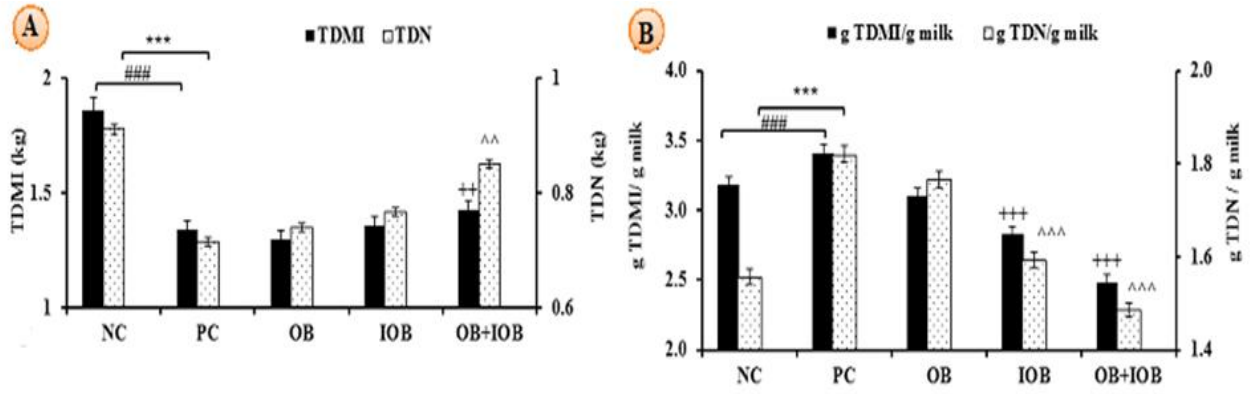

Fig. 3. Effect of organic and inorganic mycotoxin binders on feed intake (a) and feed conversion ratio (b) of Ossimi ewes fed experimental rations.

NC= Negative control (uncontaminated diet); **PC**= positive control (contaminated diet by aflatoxin B1 mixed with ochratocin); **OB**= organic binders; **IOB**= inorganic binders; **TDMI**= Total dry mater intake; **TDN**= Total digestible nutrients.

Data are expressed as mean±SEM. # & * represent the degree of significant difference between PC & NC groups, while + & ^ represent the difference between treatments and PC groups.

Body weight changes of ewes

Feeding mycotoxins (PC) induced significant loss ($P < 0.001$) for the body weight at weaning compared to the negative control (NC). However, treated ewes that fed a contaminated diet with OB, IOB, or a mixture of them (OB+IOB) revealed significant ($P < 0.01$) recovery of the body weight loss compared to PC (Fig. 4 A).

Post-lambing Days and weight at first estrous

Treated ewes fed contaminated diet plus OB, IOB or OB+IOB reached the 1st estrous post-lambing early with heavier body weights compared to the PC group (Fig. 4 B).

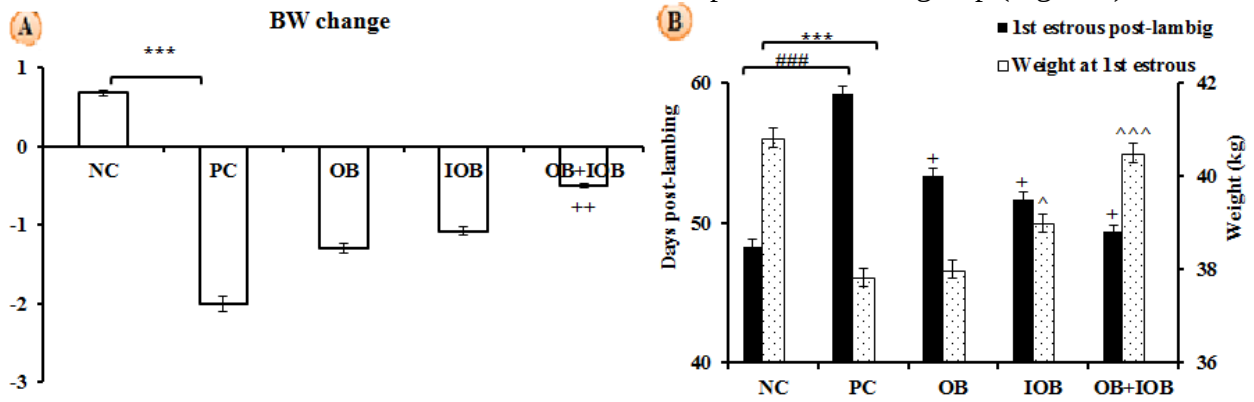

Fig. 4. Effect of organic and inorganic mycotoxin binders on body weight changes (a) and post-lambing days & weight at first estrous (b) of Ossimi ewes fed experimental rations.

NC= Negative control (uncontaminated diet); **PC**= positive control (contaminated diet by aflatoxin B1 mixed with ochratocin); **OB**= organic binders; **IOB**= inorganic binders; **BW**= Body weight.

Data are expressed as mean±SEM. # & * represent the degree of significant difference between PC & NC groups, while + & ^ represent the difference between treatments and PC groups.

Table (3): Effect of organic and inorganic mycotoxin binders on suckling lambs' performance of different experimental groups.

Traits	Treatments				
	NC	PC	OB	IOB	OB+IOB
Birth weight, kg	3.46±0.16	2.53±0.18#	2.89±0.15	2.95±0.10	3.26±0.11*
Weaning weight, kg	17.12±0.15	13.40±0.50#	14.14±0.17	15.63±0.28*	16.53±0.23*
Total gain, kg	13.66±0.18	10.87±0.66#	11.25±0.31	12.68±0.31*	13.27±0.26*
Average daily gain, g	182.13±2.42	144.93±8.82#	150.00±4.17	169.07±4.10*	176.93±3.45*
Survival rate (%)	100.00±0.00	50.00±18.90	83.33±14.09	83.33±14.09	83.33±14.09

Degree of statistical difference between PC and NC groups ($P < 0.05$).
*Degree of statistical difference among treated and PC groups ($P < 0.05$).
NC= Negative control (uncontaminated diet); **PC**= positive control (contaminated diet by aflatoxin B1 mixed with ochratocin); **OB**= organic binders; **IOB**= inorganic binders.

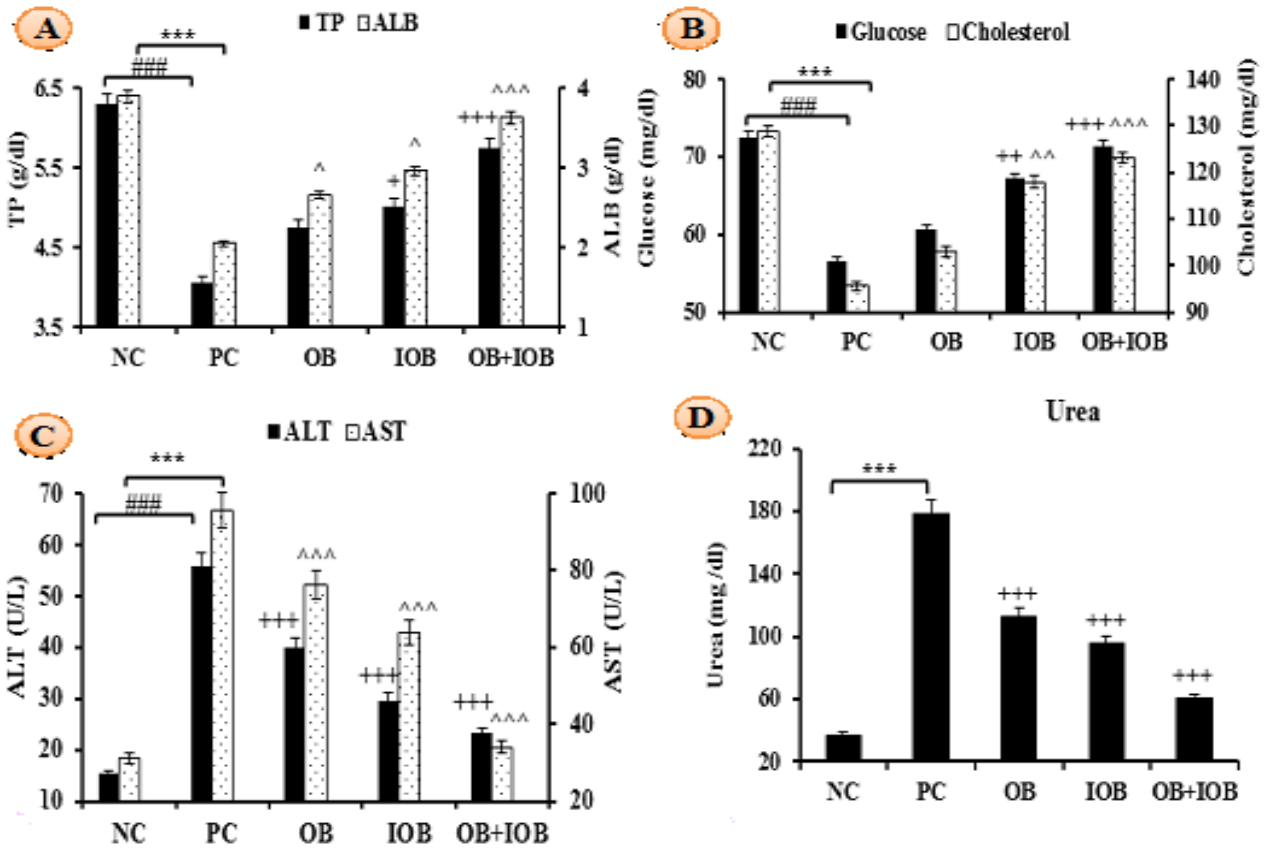

Fig. 5. Effect of organic and inorganic mycotoxin binders on some blood parameters such as TP & ALB (a), glucose & cholesterol (b), ALT & AST (c) and urea (d) of Ossimi ewes fed experimental rations.

NC= Negative control (uncontaminated diet); **PC**= positive control (contaminated diet by aflatoxin B1 mixed with ochratocin); **OB**= organic binders; **IOB**= inorganic binders; **TP**= Total protein; **ALB**= Albumin; **ALT**= Alanine aminotransferase; **AST**= Aspartate aminotransferase**.**
Data are expressed as mean±SEM. # & * represent the degree of significant difference between PC & NC groups, while + & ^ represent the difference between treatments and PC groups.

Effects of binding MYCs on suckling lambs performance:

BW gain, ADG and survival rate of suckling lambs (Table, 3) from birth to weaning were significantly ($P < 0.05$) decreased in PC group, that their mother fed MYCs contaminated diet. However, those from mothers treated groups had significant ($P < 0.05$) improve these parameters, especially with IOB or OB+IOB treatments.

Effects of binding MYCs on some blood parameters:

Serum protein, albumin, glucose, and total cholesterol levels were significantly ($P < 0.001$) decreased by dietary mycotoxins (PC) compared to negative control (Fig. 5 A & B), although these levels in ewes received IOB or OB+IOB were greater ($P < 0.001$) as compared with those receiving contaminated diets (PC). However, AST, ALT, and urea concentrations were significantly ($P < 0.001$) increased in PC group compared to NC group, while, they significantly ($P < 0.001$) decreased in treated groups OB, IOB, and OB+IOB, compared with PC group (Fig. 5 C, D).

Effects of binding MYCs on the thyroid hormones (T_3 & T_4):

Serum T_3 and T_4 concentrations increased ($P < 0.05$ & 0.001), with ewes, fed IOB or OB+IOB, respectively, approximately 1 or 2 times higher than ewes fed OB. However, T_3 and T_4 concentrations of positive control ewes (PC) remained the lowest ($P < 0.05$ & 0.001) compare to those of negative control and treated ewes (Fig. 6).

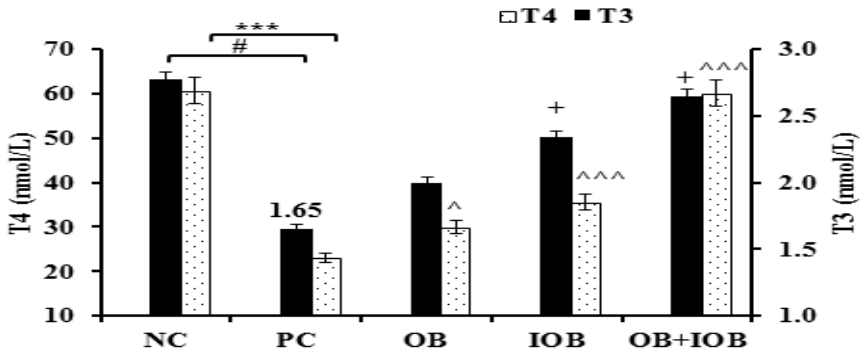

Fig. 6. Effect of organic and inorganic mycotoxin binders on thyroid hormones (T_3 & T_4) of Ossimi ewes fed experimental rations.

NC= Negative control (uncontaminated diet); **PC**= positive control (contaminated diet by aflatoxin B1 mixed with ochratocin); **OB**= organic binders; **IOB**= inorganic binders.

Data are expressed as mean±SEM. # & * represent the degree of significant difference between PC & NC groups, while + & ^ represent the difference between treatments and PC groups.

Effects of binding MYCs on antioxidant statues:

MYCs contaminated diets (PC) induced significant ($P < 0.001$) increase in the level of MDA (percentage change = 80.53) (Fig. 7 E) and a significant ($P < 0.001$) decrease in serum TAC content (Fig. 7 A) and the activity of CAT, GPX, and SOD (percentage change = -62.21, -37.08, -35.60, & -27.32, respectively) (Fig. 7 B, C & D) compared to negative controls (NC). However, the ewes fed contaminated diets plus different mycotoxin binders specially IOB or OB+IOB groups caused significant increase ($P < 0.001$) in the level of TAC (percentage change = 96.46 & 147.79 %, respectively) and in the activities of CAT, GPX, and SOD (percentage change = 40.83, 28.69 & 26.72 % for IOB and 57.37, 45.55, & 32.40 % for OB+IOB, respectively), while caused significant decrease ($P < 0.001$) in MDA concentration (percentage change = -28.16 & -36.85 %, respectively) relative to positive control (PC).

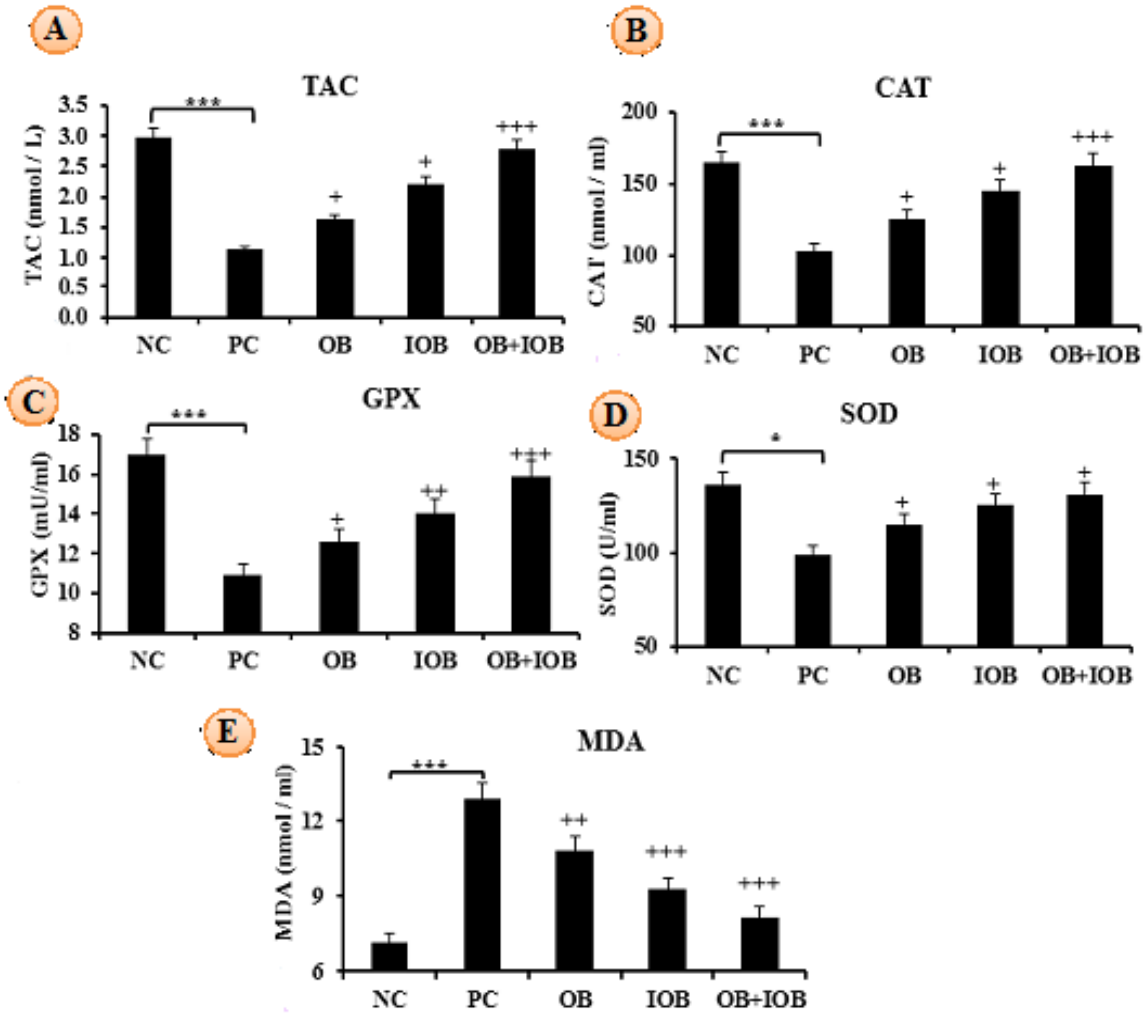

Fig. 7. Effect of organic and inorganic mycotoxin binders on antioxidant statutes such as TAC (a), CAT (b), GPX (c), SOD (d) and MDA (e) of Ossimi ewes fed experimental rations.

NC= Negative control (uncontaminated diet); **PC**= Positive control (contaminated diet by aflatoxin B1 mixed with ochratocin); **OB**= Organic binders; **IOB**= Inorganic binders; **TAC**= Total antioxidant capacity; **CAT**= Catalase; **GPX**= Glutathione peroxidase; **SOD**= Superoxide dismutase; **MDA**= Malondialdehyde.

Data are expressed as mean±SEM. * represent the degree of significant difference between PC & NC groups, while + represent the difference between treatments and PC group.

Effects of binding MYCs on reproductive performance of ewes:

Results in Table (4) showed that ewes fed contaminated diets and treated by mycotoxins binders (OB, IOB, or OB+IOB) insignificantly enhanced their fertility and reproductive traits (percentage change = 22.35, 43.15 & 51.58 % for fertility rate; 30.00, 70.00 & 71.01% for pregnancy rate; 41.18, 57.90 & 78.94 % for lambing rate; 39.68, 58.73 & 84.13 % for litter size; 33.33, 51.11 & 56.87 % for fecundity rate, respectively) relative to the positive control group (PC). However, the IOB and OB+IOB groups had an ameliorative economical effects ($P < 0.01$) on stillbirth and twining rate compared to PC group (percentage change = -73.68 & -77.28 % for stillbirth; 113.36 & 135.28 % for twining rate, respectively). The PC group showed negative effect on reproductive traits (percentage change = -37.50 % for fertility rate; -44.60% for pregnancy rate; -44.12% for lambing rate; -47.93% for litter size; -39.29% for fecundity rate; 359.77% for

stillbirth; -64.58 % for twining rate, respectively) compared to the NC group.

Table (4): Effect of organic and inorganic mycotoxin binders on the reproductive performance of Ossimi ewes fed experimental rations

Traits	Treatments				
	NC	PC	OB	IOB	OB+IOB
Total No. of ewes	20	20	20	20	20
No. of ewes presented to rams	20	19	19	19	20
No. of mated ewes	19	16	17	19	19
No. of pregnant ewes	19	10	13	17	18
No. of lambing ewes	17	8	12	15	17
No. of lambs born	23	10	15	19	22
No. of live births	21	6	12	17	20
No. of twins	6	1	2	4	5
Estrous duration (h)	20.10±1.59	24.60±1.22	23.40±1.44	22.10±1.76	19.80±1.69
No of services / conception	1.25±0.10	1.56±0.12	1.45±0.11	1.39±0.11	1.30±0.11
Fertility rate (%)	100.00±0.00	62.50±8.06	76.47±7.62	89.47±8.60	94.74±5.26
Pregnancy rate (%)	95.00±5.00	52.63±9.61	68.42±8.60	89..47±8.60	90.00±6.88
Gestation period (day)	151.21±0.38	151.65±0.38	151.31±0.42	151.94±0.42	151.38±0.41
Lambing rate (%)	89.47±7.23	50.00±11.39	70.59±10.08	78.95±10.38	89.47±8.60
Litter size (prolificacy)	1.21±0.12	0.63±0.11#	0.88±0.11	1.00±0.11	1.16±0.12*
Fecundity rate (%)	123.53±15.35	75.00±18.27	100.00±16.91	113.33±16.91	117.65±15.82
Stillbirth (%)	8.70±7.87	40.00±10.08###	20.00±9.15	10.53±9.15**	9.09±8.23***
Twining rate (%)	35.29±11.95	12.50±11.24##	16.67±11.38	26.67±11.38**	29.41±11.97**

Degree of statistical difference between PC and NC groups ($P < 0.05$), ## ($P < 0.01$), ### ($P < 0.001$).
*Degree of statistical difference among treated and PC groups ($P < 0.05$), ** ($P < 0.01$), *** ($P < 0.001$).
NC= Negative control (uncontaminated diet); **PC**= positive control (contaminated diet by aflatoxin B1 mixed with ochratocin); **OB**= organic binders; **IOB**= inorganic binders.

DISCUSSION

MYCs are toxic metabolites generated by numerous types of fungi species, with the fumonisins, aflatoxins, zearalenone, ochratoxin A and trichothecenes being the most prominent found in diets. The economic impact of MYCs stimulated the study of detoxification methodologies to decrease its bioavailability by enterosorption. Although there are numerous varieties of adsorbents, absorption ability depends on the chemical and physical properties of both adsorbents and MYCs. MYCs are the explanation of a wide scope of metabolic harms and lesions in the liver, reduction of digestion enzymes, immune repression and animal performance **(Zhao et al., 2010; Barati et al., 2018)**.

The effects of MYCB on the digestion coefficient of the nutrient may be affected by existing MYCs. We found that the mixture of organic and inorganic toxin binders (TBs) improved digestibility in ewes fed treated diets (OB, IOB or OB+IOB). Currently, TBs include special plant extracts intended to make harmful conditions in the gastrointestinal tract produced by MYCs **(Pietri et al., 2009)**. Meanwhile, diatomaceous earth and bentonites can adsorb polar MYCs **(Kiyothong et al., 2012).** As a result of the numerous constituents of TBs, it was impractical to identify the components responsible for enhancing the digestion of nutrients. Accordingly, TBs meet the essential requirement of MYCB because it didn't affect the absorption of nutrients **(Avantaggiato et al., 2005).** MYCB have been developed basically to adsorb MYCs and inhibit their absorption in the gastrointestinal tract **(Binder, 2007).**

In the current study, ewes fed a contaminated diet (PC) had reduced most parameters of ewes' performance and their offspring. However, groups treated by TBs

(OB, IOB or OB+IOB) have been consistently unaffected by mycotoxins, especially the IOB and OB+IOB groups. The study identified the potential of organic, inorganic or mixture binders on MYCs binding and improving ewes' performance. IBO and mixture groups showed better effectiveness on the binding of MYCs among the toxin binders utilized. These outcomes are in agreement with **Yalcin et al. (2018)**. IOB utilized in this investigation mainly contain Al silicates and Ca, clinoptilolite, sepiolite, and bentonite. IOB has been reported to have a greater binding effect on MYCs in other studies. Likewise, another IOB utilized as nutrient additive was hydrated sodium calcium aluminosilicate **(Neeff et al., 2013)**, clinoptilolite **(Ortatatli and Oguz, 2001)** and montmorillonite **(Shi et al., 2009)** which were successful to adsorb aflatoxin B1 *in vitro* and *in vivo* conditions. **Diaz et al. (2002)** reported that the binding efficacy of aflatoxin B1 by Na and Ca bentonite were 98.4% and 98.5%, respectively, in an *in vitro* study. There are other ordinarily binding factors used like activated charcoal, aluminosilicates, mannan oligosaccharide, bentonite, etc. They have been observed to have diverse effects on ochratoxicosis binding **(Goryacheva et al., 2007),** and aflatoxin **(Gowda et al., 2008)**.

The superiority of the IOB or OB+IOB groups in favour of milk yield may be attributed to the raise of nutrient digestibility and nutrient value of these groups. Nutrient digestibility and the feeding values of TBs groups (OB, IOB & OB+IOB) positively reflected on the 6% FCM produced via ewes fed those diets, which assessed to be more by 9.76, 31.71 and 56.10%, respectively than that of the PC group. FCR that calculated as DM and TDN g per g of milk yield were significantly improved with TBs supplementation, especially IOB and OB+IOB compared with the PC group. The IOB or OB+IOB groups demonstrated the best feed efficiency among the experimental treatments. However, the consumption of contaminated diet (PC) alone led to a decrease in body weight and poorer FCR when compared to the NC group and other groups. Also, either organic or inorganic TBs supplementation to the contaminated diet improved lambs' survival rate and body weight gain. This may be attributed to the role of mannan-oligosaccharides (MOS) which have been demonstrated concerning their value on immune modulation **(O'Quinn et al., 2001),** and also, on the decline of intestinal pathogen colonization **(Line et al., 1998)** and enhancement of growth performance of young pigs **(Pettigrew, 2000)**.

Variations in serum parameters of ewes fed contaminated diets are indicators of liver harm and disturbance in the pathways of metabolites **(Kececi et al., 1998).** It has been illustrated that the low serum protein, glucose, and cholesterol levels and the increase of ALT and AST concentrations accompanied MYCs condition are indicators of liver damage by the toxin **(Zhao et al., 2010).** AFB1 intake significantly reduced glucose, total protein, albumin, and globulin levels and elevated liver enzymes. Also, the serum level of protein, albumin, glucose, cholesterol, thyroid hormones and antioxidant parameters were decreased, while ALT, AST, urea, and MDA levels were increased in the PC group, which was opposite to the other treated groups receiving MYCs binders. The lowering of serum glucose and total protein, resulted by MYCs is compatible with previous investigations **(Bovo et al., 2015; Barati et al., 2018).** However, Aluminosilicates, probiotic bacteria and yeast cell wall cause diminish of MYCs influence on glucose level **(Bagherzadeh et al., 2012; Bovo et al., 2015).**

Also, thyroid hormones are associated with milk production and components that are higher with high yielders (TBs ewes) and lower with low yielders (PC group). These outcomes are in accordance with that reported by **Gueorguiev (1999),** who found that T_3 and T_4 concentrations increased in the high producing cattle. **Collier et al. (1984)** revealed that pivot is a major physiological factor controls milk secretion and metabolic procedures. T_3 and T_4 hormones interact with different hormones to develop the mammary gland and improve and maintain lactation **(Neville, 1990).** Hydrated sodium/calcium aluminosilicate can absorb MYCs by its surface or by its inner spaces.

Adsorption of MYCs is performed by binding or replacing positive charges inside these surfaces. Thus, MYCs can be adsorbed via the pores and caught by electrostatic charges **(Boudergue et al., 2009).** Supplemented yeast to rations improved its useful impact, which caused by many reasons, such as the occurrence of minerals, vitamins, and proteins in the cells of yeast **(Amata, 2013)** and MOS and 1,1-6, D-glucan that binde some MYCs, involving aflatoxin, which reflected on enhancing the growth rate due to enhancement of intestinal mucosa; besides, it raises the height of villus, number of cellular anaerobic bacteria that promote the use of lactate and modifies the intestine pH, thus improve the digestion of nutrients and growth rate **(Abdel-Azeem, 2002).** Binding MYCs to glucomannan in the yeast wall is assessed by hydrogen bonds and van der Waals forces, and this binding will stay constant during the gastrointestinal tract **(Jouany, 2007).** It has been demonstrated that some cell walls of bacteria can adsorb different types of toxins **(Devreese et al., 2013).** Cell wall proteins and carbohydrates of *Bacilli* can bind and adsorb AFB1. This potential has been proved *in vitro* and *in vivo* **(Huwig et al., 2001).** The most significant reduction due to mycotoxicosis are made in the antioxidant markers like TAC, SOD, GPX, and CAT, besides an increase of MDA in the PC group was observed.

Serum MDA activity is a pointer of oxidative stress. Earlier work indicated that the diet containing a mixture of selenium-yeast and Deoxynivalenol (DON) caused a significant lower in MDA tissue **(Placha et al., 2009)**. Thus, the current results confirmed the rising pattern with elaborated serum MDA level, suggesting that multiple MYCs in a contaminated diet can lead to worse harm than pure MYCs.

GPX, CAT, and SOD activities are the major criteria for anti-oxidative stress. Supplementing the contaminated diet with AFB1 and OTA led to reducing the antioxidant enzymes. The present results illustrate that antioxidant activity, especially GPX of the TBs ewes (groups, 3 & 4), had higher values contrasted with the PC ewes (group, 2), indicating that animals could increase GPX generation to dispose H_2O_2 resulting from O_2 disintegration following the administration of MDA **(Hou et al., 2013)**.

Superoxide dismutase, is an essential intracellular antioxidant enzyme for detoxifying superoxide anion, hence protecting cells against the oxidative stress of diets contaminated by MYCs compared to catalase and glutathione peroxidase **(Yuan et al., 2010).** However, catalase has dual enzymatic roles, not just cracking H_2O_2 to H_2O and O_2, but stimulating the electronic donors' oxidation like phenols or ethanol in the existence of low H_2O_2 level. In the current study, the serum SOD activity was diminished, followed by the lower CAT activity of the PC group. The potential explanation for this result is that the MYCs-metabolism happens in the kidneys and liver, and these three antioxidant enzymes contribute as follows: SOD stimulates the breakdown of O_2^- to H_2O_2, preserving the tissues from O_2- harm. GPX stimulates the lowering of H_2O_2 in water. CAT, another essential antioxidant metalloenzyme, is also involved in the transformation of H_2O_2 in water.

The reproductive and fertility parameters in this study showed an insignificant decrease with the PC group compared with the NC group. In the current study, although the binders were effective in enhancing fertility in general, the IOB or OB+IOB groups showed highly positive effects in enhancing fertility. Additionally, ameliorative economic effects were measured due to decreasing stillbirth and raising the twinning rate. The improvement in fertility values, stillbirth and twinning rate with IOB or OB+IOB groups could attribute to the binding of AFB1 and OTA and the hepatic protection from damage. **Afzali (1998)** guessed that the glucomannan pattern of modified–MOS preparations trap MYCs irreversibly. The mode of action of bentonite is assumed to be through binding the MYCs molecule and discharging it in the faeces.

Adverse effects of dietary MYCs on fertility have been notified by **Manafi (2011)** in broiler breeder hens. On the contrary, **Muthiah (1996)** observed no influence of dietary AF on fertility percentage in breeder hens.

CONCLUSION

As conclusion, MYCs contaminated rations induced a significant decrease in milk yield, daily feed intake, TDN, and body weight. Additionally, serum constituents and antioxidant measurements indicated impaired liver function and digestive disturbances in ewes fed MYCs. The addition of inorganic binder alone or a combination of it with organic binder was able to modify the productive and reproductive performance by enhancing digestibility and feeding values and changing antioxidant statues. It could approximately normalize the adverse effects of MYCs, perhaps due to its effect on blood metabolites and improving digestibility and animal performance. From the economic point of view, it can be satisfied to use inorganic binders alone, because their effects on the productive and reproductive performance of sheep converge with that of the mixture of them with organic binders.

Recommendation

The exact status of the antitoxic effect may require further explanation and further *in vivo* studies using the preceding materials, which could give a beneficial tool for improving the nutrient utilization efficiency in the rumen and thus can be recommended as a supplement to sheep diet for its potentiality with MYCs contamination.

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التأثيرات المحسنه لمضادات السموم الفطرية العضوية وغير العضوية على أداء الأغنام الأوسيمى

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تعتبر هذه الدراسة محاولة لمنع أو الحد من الآثار السلبية الناجمة عن تناول العلائق الملوثة بمزيج من الأفلاتوكسين والأوكراتوكسين. هدفت هذه الدراسة إلى تقييم فاعلية اضافة مضادات السموم العضوية وغير العضوية أو المزيج بينهما عند استخدامهم كمكملات للعلائق وذلك لتخفيف التأثير السلبي للسموم الفطرية للحفاظ على الأداء الإنتاجي والتناسلى للنعاج الأوسيمي ونتاجها. تم اختيار مائة نعجة أوسيمي بمتوسط وزن جسم ٣٨,٩٧ ± ٠,٥٥ كجم وعمر ٣ إلى ٤ سنوات ، قبل ٣٠ يومًا من تاريخ الولادة المتوقع وتقسيمها عشوائيًا إلى خمس مجموعات متساوية (٢٠ نعجه/ لكل مجموعة). تغذت المجموعة الأولى على عليقة غير ملوثه وأعتبرت كمجموعة ضابطة سلبية. تغذت المجموعة الثانية على عليقة غذائيه ملوثه بواسطة الأفلاتوكسين المخلوط مع الأوكراتوكسين وأعتبرت كمجموعة ضابطة إيجابية ، في حين أنه تغذت المجموعة الثالثة على علائق ملوثه مضاف اليها مضادات سموم عضوية. تغذت المجموعة الرابعة على علائق ملوثه مضاف اليها مضادات سموم غير عضوية والمجموعة الخامسة تغذت على علائق ملوثه مضاف اليها بمزيج من مضادات سموم العضوية وغير العضوية معاً .

أظهرت النتائج انخفاض مستويات معاملات الهضم ، والقيم الغذائية ، وإنتاج اللبن ، والغذاء المأكول ، والبروتين الكلي ، والألبيومين ، والجلوكوز ، والكوليسترول فى مصل الدم ، ولكن زادت تركيزات إنزيمات الكبد (ALT & AST) واليوريا في المجموعة الضابطة الإيجابيه. ارتبط انخفاض وزن جسم النعاج بزيادة العمر عند دورة الشبق الأولى بعد الولادة نتيجة قياسات الخصوبة المنخفضة للمجموعة الضابطة الإيجابيه. إلى جانب ذلك ، انخفضت هرمونات الغدة الدرقية وأنشطة مضادات الأكسدة مقابل الزيادات في تركيزات MDA. وفي نفس المجموعة، انخفضت معدلات وزن جسم النعاج ، بينما زادت نسبة تحويل الغذاء فيما يتعلق بالمجموعة الضابطة الإيجابيه مقارنة بالمجموعة الضابطة السلبيه. ,وفقا لذلك فإن جميع المعاملات المستخدمة في الدراسة الحالية يمكن أن تحد من التأثيرات التي يسببها السم الفطري بشكل كبير واستيعادتها للمستويات الطبيعية. في الختام ، يمكن إضافة مضادات السموم غير العضوي منفردة ، أو مختلطة مع العضوية إلى علائق النعاج لتخفيف الأعراض السلبية التي يسببها السم الفطري.