The Effects of Dietary Supplementations with Barley Seeds and Hay on Ochratoxin A- Toxicity using Lactating Egyptian Goats.

Abdel- Fattah, Sh. M.[*1]; Yehia .H. A[1]; EL-Maaz[2], A.A. and El-Nemr, S. A.[2]

[1] Department of food toxins and contaminants National research centre, [2] Biochemistry department, Giza provincial laboratory, Animal health research institute, Cairo, Egypt.
[*]shaabanmostafa@yahoo.com

Abstract: The effects of dietary supplementation with barley and hay at levels of 45% of the composition of daily rations, on the performance and toxicity of Egyptian lactating gaots received ochratoxin A (10mg/kg ration, of dry matter basis), were successively examined for four weeks. Sixteen native apparently healthy female goats with average weight of 47.6 ± 1.26 kg., were put under observation for two weeks, then they were divided into four equal groups according to their live body weight for performing feeding trials. The 1st., group used as a negative control which fed ochratoxin A-free diets consisting of 70% concentrates and 30% hay (dry matter basis, energy to supply 1.1 times the requirement for maintenance), the 2nd., group used as a positive control which fed ochratoxin A-contaminated diets (70% concentrates and 30% hay), the 3rd., group fed the same diet used for the 2nd., group except the hay level which elevated to 45%, and the 4th., group which fed the same diet used for the 3rd., group except the hay portion which supplemented with 45% barley seeds. Dry matter intake (DM), apparent nutrient digestibilities, nutritive values, serum chemistry profiles and ochratoxin A (OA) concentrationa in feed intake, orts, serum, feces, urine and milk; were evaluated. The obtained results indicated that goats fed ochratoxin A-contaminated diets without barley or hay supplementation, showed reduction in feed intake, nutritive value, body weight gain, and immunoglobulin production as well as in some serum constituents. After 1,.2,.3, and 4 weeks of the trial, significant concentrations of ochratoxin A were detected in serum, feces and urine samples of all treated animals. Ochratoxin A was found in significant concentrations, because of escaping from the gastrointestinal tract. Addition of barley or hay can in part, able to counteract the toxic effects of ochratoxin A as well as the immunoglobulin dysfunction. The antitoxic effects of hay were generally better than barley seeds to minimize or to avoid the immunosuppressive effect of OA, the mycotoxin status of goats diet must be controlled if good goat performance is to be targeted. The current experiment demonstrates that OA hydrolysis in the gastrointestinal tract of Egyptian goats is substantially less than previously described, especially if OA is ingested in combination with concentrate-rich diets. The results suggest that OA is hydrolyzed much faster in the rumen of goats fed hay than in goats fed grains, presumably because of the different ruminal microbial population, which in turn influenced the rate of hydrolysis of OA. This experiment established that very good conversion of OA to ochratoxin alpha (Oα) occurs systematically; 3.5 to 5.15% of the OA was recovered as unaltered OA in the urine. Most of the great amount of Oα recovered was also in the urine. [Journal of American Science 2010; 6(9):504-514]. (ISSN: 1545-1003).

Key Words: Ochratoxins, Ruminants, Diets, Hydrolysis, Bioavailability

1. Introduction

Ochratoxins, of which ochratoxin A (OA) is the most prevalent, are highly toxic secondary fungal metabolites of some toxigenic species of Aspergillus and Penicillium (Krogh, 1987; Frisvad and Samson, 1991). The natural occurrence of OA in food and feedstuffs is widespread (Dwivedi and Burns, 1986), especially in temperate areas (Jorgensen *et al.,* 1996). The presence of ochratoxins in dairy products reflects the contamination of feedstuffs. Following ingestion of contaminated feeds, OA is largely transformed by rumen microorganisms into the less toxic metabolite Oα (Kiessling *et al.,* 1984). Ochratoxin A and Oα are mainly eliminated in the urine and feces, but they can also be found in milk. In dairy sheep, the carryover is less than 1% (Boudra *et al.,* 2005) among the various feedstuffs susceptible to ochratoxin contamination, cereals and cereal by-products are the major source of ochratoxin A.

Degradation of ochratoxin A to its alpha metabolite (Oα) by microorganisms within the gastrointestinal is known to be the principal means of detoxification of OA (Chu *et al.,* 1972; Chu, 1974). Ruminants seem to be resistant to the acutely toxic effects of OA (Ribelin *et al.,* 1978), and they should be able to degrade up to 12 mg of OA/kg of contaminated feed (Hult *et al.,* 1976).

Little was known, about the importance of the rumen in the detoxification of OA until Sreemannarayana *et al*. (1988), who reported that the bioavailability of OA in ruminant calves was only 27 to 36% of that in preruminant calves, and that the functional rumen may greatly reduce the toxidity of OA. Some studies on goats suggested that the type of diet is important, the rate of in vitro hydrolysis of ochratoxin A was more rapid with ruminal digesta from sheep fed hay than with ruminal digesta from those fed grain (Xiao *et al.*, 1991). In addition, the numbers and types of microorganisms (include the protozoa) in the rumen of sheep are affected by the composition of the diet (Nakamura and Kanegasaki, 1969; Eadie and Mann, 1970) and the ruminal protozoa are able to hydrolyze OA more efficiently than ruminal bacteria. The bioavailability of OA (i.e., the amount of OA reaching systemic circulation, (Shargel and Yu, 1985) and its toxic effects should therefore be lower in sheep fed hay than in sheep fed grain. There for, the present study aimed to study the influence of diet on the hydrolysis of OA in the rumen of goats and its subsequent bioavailability and to determine whether dietary doses of hay and barley seeds could be minimize the adverse effects of 10 mg of OA/kg of feed, concentrations that have on nonruminants, tolerated by a ruminant such as goats.

2. Materials and Methods:

1- Treatment groups and diets.

A total of 16 Egyptian dairy goats with a mean body weight of 47.6 ± 1.26 kg, were selected from a local private farm Giza province were used in the this experiment. All animals were apparently healthy and were checked for any abnormalities for two weeks observation before the experiment and then, they were fed daily diets consisting of (DM basis) 70% concentrates (60% wheat, 36% wheat bran, 4% soy oil, minerals, trace elements, and vitamins) and 30% berseem hay for three weeks pretreatment period. The concentrates were offered twice daily at 0700 a.m. and 1900 p.m., but hay offered once daily at 1200. At the experiment period, animals were divided into four equal groups and each of which was subjected to one of four diets (as shown in table 1), for 30 days. The OA-contaminated material was mixed with the basal diet every week, and the mixed diet was stored under dry conditions at ambient temperature. Feed and orts were weighed daily and sampled (10%) to estimate OA intake during the feeding period based on TLC analysis of feed and orts.

To ensure that each goat received its allotment of barely grains or berseem hay, the barely or hay supplements were added individually to each animal's diet. The amount of feed fed to each animal was adjusted twice weekly to minimize feed refusal (5 to 10% of the daily ration) without restricting consumption. The energy supply was 1.1 times the requirement for maintenance (AFRC, 1993), and animals had ad libitum access to tap water. Body weight was monitored weekly (experimental days 0, 6, 13, 20, and 27).

Digestibility trail: During the digestibility trail (at the third week of the treatment stage), samples of rations, refusal, and feces were taken daily to measure digestibility over a week following the growth trial. Refusals were collected daily at 07.30h, weighed, sampled, and then stored. Total daily fecal output for each animal was also collected, weighed, homogenised and 10% of feces samples were dried at 70º C for 24 hr, then blended and kept for fecal analysis. Nutrient consumed during digesitibility evaluation trail were calculated according to feeds consumed and their chemical composition, as follows:

TDN % = % DCP + % DCF + % DNFE + 2, 25 * % DCEE

With DCP: digestible crude protein
DCF: digestible crude fibre
DNFE: digestible nitrogen-free energy
DCEE: digestible crude ether extract

D% = DMI – DMO/DMI * 100%

With DMI: Dry Matter Intake (DM eaten)
DMO: Dry Matter Output (DM in faeces)

2- Preparation of OA- artificially contaminated ingedient.

OA which used in this study was produced by a culture of Aspergillus ochraceous NRRL 3174 (obtained from the Mycotoxin Lab., N.R.C., Dokki, Giza, Egypt.) on wheat which was used as a basal material (Alex, 1972). Each 500 gm quantity of wheat was potted in sealed 2.8 L-Fernbach flask with sufficient tap water to give 30% water by weight, and then autoclaved at 121 °C for 15 min. Autoclaved samples were cooled at room temperature, and inoculated with 10 ml suspension of Aspergillus ochraceous spores scraped from a 14 day-old potato dextrose agar (PDA) slant. The infected medium was incubated for 17 days at 28 °C. Qualitative and quantitative assay has been caried out using HPLC (Agilent 1100) as recommended by AOAC (1990). The determined OA was 29.4 mg of OA per kilogram of wheat dry matter. OA-contaminated material was incorporated into the concentrated diet in ratio 23.89 % of the daily ration, to form a final concentration of 10 mg OA/Kg ration.

Table (1): Ingredient and Chemical composition of the experimental diets (% D.M.) offered to goats during the study.

	Groups			
	Negative Control (group 1)	Treated groups		
		Positive control (group 2)	Hay (group 3)	Barley (group 4)
aIngredients				
Berseem hay	30	30	45	0.0
Wheat	60	36.11	21.11	21.11
Wheat bran	7.2	7.2	7.2	7.2
Barley supplement	0.0	0.0	0.0	45
bOther ingridients	2.8	2.8	2.8	2.8
Ochratoxin A-cont.wheat	0.0	23.89	23.89	23.89
Chemical composition				
DM (g/100 g sample)	89.14	87.65	87.63	88.42
Nutrient composition (g/100 g DM):				
Organic matter (OM)	89.94	87.25	88.94	88.38
Crude protein (CP)	13.83	13.06	14.05	13.35
Crude fiber (CF)	24.03	23.34	21.19	23.03
Ether extract (EE)	2.41	2.35	2.18	2.25
Nitrogen free extract (NFE)	49.67	48.50	51.52	49.75

[a] All ingredients except Ochratoxin A-cont.wheat were OA- free.

[b] Other ingredients: 1.2% limestone, .24% calcium phosphate, .38% KCl, .4% Co-I salt, .5% trace mineral mix, and 20% vitamin mix. The trace mineral mix was formulated to provide 22 mg of &SO,, .ll mg of Se, 28 mg of ZnO, 28.5 mg of MnO, 750 mg of MgO, 2.0 g of KC1, and 1.6 g of Co-I NaCl per kilogram of mixed diet. The vitamin mix was formulated to provide 7,000 IU of vitamin A, 3,000 IU of vitamin D3, and 6 IU of vitamin E per kilogram of mixed diet.

3- Mycotoxins standard:

Ochratoxin A (OA) and ochratoxin alpha (Oα) were from Sigma, St. Louis, Mo.

4- TLC plates

TLC plates precoated with 0.25 mm of silica gel containing gypsum and a fluorescent UV (254 nm) indicator (Macherey-Nagel Co., Duren, Germany) and high-performance TLC-plates precoated with Lichrosorb RP-18 (E. Merck AG, Darmstadt, Germany) were used. The solvent systems were (vol/vol): benzene-methanol- acetic acid (90:5:5)

5- Sampling

Blood samples were taken weekly from the jugular vein prior to the morning feeding, at the following times: 0, 6, 13, 20 and 27 days of experimental period with the volume or weight being determined. Blood samples were placed on ice, allowed to clot and after centrifugation; serum was separated and frozen at -20 °C until it was analyzed for OA and Oα.

During the 3rd wk of the experiment, total feces and urine of the animals were collected twice daily over a 7-days period. Urine was collected from an indwelling cyatic catheter, which was also placed 1-day before the toxin administration. Feces were collected in fecal bags (Frohlich *et al.*, 1987). Following the collection period, total samples of urine and feces from each animal were homogenized, and aliquot samples were stored at -20°C until further analysis.

Milk samples were preserved by adding 1.5 µg/mL of Na NO3, kept on ice during transport from the farm to the laboratory, and stored at –20°C until analysis.

6- Analytical Methods.

6.1. Analysis of OA and Oa involved their extraction from the matrix followed by TLC analysis.

6.1.1. Extraction, isolation, and purification of OA and Oa from the samples of serum, feces, urine and milk.

Serum samples were extracted following the procedure of Hult *et al.*, (1979). Feces were also extracted using the procedure of Hult *et al.*, (1979), except they were first freezedried and then ground to

pass through a 1-mm sieve, and 1 g was added into a centrifuge tube (35 mL).

Urine was freeze-dried, and an amount of urine equivalent to 5 mL was reconstituted in 5 mL of .1 M sodium bicarbonate and sonicated. The pH of the sample was adjusted to 2.5 with H3PO4, and it was extracted for 20 min with 10 mL of CHCl3. Four milliliters of CHCl3 layer was transferred into opticlear glass vials (8 mL) and the CHCl3 layer was evaporated in a fume hood under a stream of nitrogen. Extracted samples were reconstituted with 1 mL of methanol, sonicated, centrifuged at 900xg for 10 min, and analyzed using TLC procedure. This procedure was similar to that reported by Xiao *et al.*, (1996) and Li *et al.*, (1997).

Milk samples were extracted following the procedure of Hana and Michael (1996). A 50-ml portion of milk was mixed with 15 ml of methanol and acidified to pH 1.6-2.0 with 2 M hydrochloric acid. After addition of 40 ml of chloroform, the flask was shaken automatically for 30 min. The mixture was centrifuged for 25 min in a refrigerated centrifuge at 4 °C and 2000g. A 30-ml aliquot of the chloroform phase was washed once with 10 ml of bidistilled water and dried over 1.5g anhydrous Na2 SO4. The chloroform extract was run through a silica gel cartridge, prewetted with chloroform. The column was washed with 10 ml of chloroform, and OA was eluted with 15 ml of chloroform- formic acid (100:2, v/v). The elute was washed with 5 ml of bidistilled water to remove formic acid and chloroform was removed by evaporation on a rotary evaporator.The last traces were blown under a gentle stream of nitrogen and dissolved in HPLC-grade methanol.

6.1.2. Visualization and quantitation.

The methanol fraction containing OA, and its fungal metabolite Oα was applied to a flexible preparatory thin-layer chromatography (TLC) plate (20 by 20 cm, silica; Whatman, Clifton, N.J. with the maximum loading being 5 mg per plate. The plate was developed with a solvent mixture of ethyl acetate and acetic acid (95:5). Ochratoxin A and ochratoxin alpha were located by UV exposure. The plates placed at the filter surface of a transilluminator with an intensity of 6,000 LW/cm2 at a wave length of 365 nm. For the quantitative determination of the compounds, the silica plates were developed in solvent system benzene-methanol- acetic acid (90:5:5) and scanned in a Vitatron LTD 100 densitometer equipped with a mercury lamp (excitation at 366 nm and emission at 460 mm). The recorded areas of the spots were compared with standards of the respective compounds. All samples and standard were analyzed in duplicate. The minimum detectable level for both OA and Oα was 5 ng/plate, and recoveries were 94%. Recoveries were estimated on the basis of recovery of OA from spiked samples of serum, urine, feces, and milk.

6.2. Analysis of several metabolic variables

The profiles of several metabolic variables, including urea (Patton and Cruch; 1977), glutamate-oxalacetate-transaminase (GOT; Reitman and Frankel, 1957), glutamate-pyruvate-transaminase (GPT; Reitman and Frankel; 1957), lactate dehydrogenase (LDH; Kachman and Moss; 1976), creatine kinase (CK) and alkaline phosphatase (AP), were determined in the serum samples of each animal using a commercial kits (Stanbio Laboratory, North Main, Boerne, TX USA). The immunoglobulins fractions were determined using ELISA technique by Erhard *et al.*, (1992).

Feed and feces were analyzed for crude nutrients and fiber components. Feces were mixed, and DM was estimated by freeze-drying and subsequent oven-drying at 105°C overnight. Concentrates, hay, Barley and feces were successively ground in mills with 3 and 1 mm screens and, for starch analysis, with a 0.2 mm screen. Nitrogen was determined using the standard Kjeldahl procedure with K2SO4 and CuSO4 as catalysts. The OM was determined by ashing at 550°C overnight. The NDF (cell wall), ADF, and 72% H2SO4 lignin were analyzed as described by Goering and Van Soest (1970), except that NaSO3 was not used in the NDF preparation.

7. Statistical Analysis.

Data are presented as means ± SD. The obtained data were statistically analyzed by T-student test and the F-test through Anaysis of Varience (ANOVA) according to Snedecor and Cochran, 1969).

3. Results and Discussion:

1. Nutrients intake, nutritive values and body weight

Nutrient consumed during digestibility evaluation trial were calculated according to feeds consumed and their chemical composition. The composed diets showed comparable nutrients as shown in Table (1). Dry matter intake, body weight gains and nutritive values of all experimental groups are presented in Table (2). Except the positive control group, the amount of feed offered was always consumed completely, the OA content of 10 mg/kg of concentrate feed had no effect on the goats's intake of the feed, and no overt illness or disturbance was observed. During the 4th wk of the trial, the goats gained an average of 2.59 kg of BW (47.56 ± 1.26 to 50.15 ± 1.6 kg). Compared to the negative control group, hay and barley supplemental gruops, had

apparently no significant (P>0.05) effect on the daily body gain. The differences in the body weight gain between the positive control group and the other three groups, were significant (P>0.05), these differences were in aliner trend for the daily DM intake, and this result is supported by Hohler *et al.,* (1999) and Abdel- Fattah *et al.,* (2004). The total DM intake kg/head/day appeared to higher with animals in group (negative control diet) than those of the other groups. Consequently, the daily body weight gain was higher in animals fed the same diet when expressed as body weight gained, gm/day, and this was the apparent reason for such observation.

Table 2: Nutrients intake, nutritive values and body weight gained during the study.

Parameters	Expermental groups (Means ±SE)				LSD (P<0.05)
	Neg. control (group 1)	Pos. control (group 2)	Hay group (group 3)	barley group (group 4)	
Daily DM intake,gm	1680 ± 17.2 ^{A}	1516 ± 26.5 ^{B}	1604 ±13.0 ^{A}	1665 ± 19.7 ^{A}	58.5
Body weight					
Initial B.W.	47.25±0.47 ^{A}	48.60 ±1.17 ^{A}	46.89 ±0.68 ^{A}	47.51 ±0.97 ^{A}	1.73
Final B.W.	50.02 ±0.86 ^{A}	49.76±0.89 ^{A}	49.58 ±1.03 ^{A}	50.24 ±1.17 ^{A}	1.42
Total B.W. gained	2.77 ±0.23 ^{A}	1.16 ±0.34 ^{B}	2.69 ±0.15 ^{A}	2.73 ±0.68 ^{A}	0.58
B.W. gain (gm/day)	92.33 ±2.84 ^{A}	38.67 ±3.60 ^{B}	89.67 ±4.13 ^{A}	91.00 ±2.85 ^{A}	21.69
Nutritive values:					
TDN intake (gm/day)	875.78 ±11.42 ^{A}	741.38±17.8 ^{B}	853.22 ±23.12 ^{A}	861.69 ±15.63 ^{A}	28.54
DCP intake (gm/day)	174.25 ±4.71 ^{A}	137.38 ±9.16^{B}	169.84 ±3.38 ^{A}	172.62 ±7.60 ^{A}	11.43
TDN%	56.14	48.02	54.17	55.06	
DCP%	11.17	9.52	10.74	11.03	

The same capital litters in columns denotes no significant difference between treatments in the same raw at ($p \leq 0.05$) and vise versa.

In regard to nutritive values,.the nutritive valueas TDN for the four diets showed comparable results and ranged between 56.14 and 48.02 %. It is clear that the nutritive values for the hay, barley supplemental groups and the negative control group, were not significant (P<0.05), but they were significantly higher than that for positive control group. The low digestibility of CP (9.52 %) for the positive control diet tended to be significant (P<0.05) the lowest digestible crude protein (DCP), being 9.52 % while those of negative control, hay and barley supplemental groups were 11.7, 10.74, and 11.03 % respectively. The lower nutrient digestibilities of positive control group resulted in lower TDN% than that of other three groups and this may be due to a tendency for reduced digestibility of nutrients. Also, positive control group showed significantly (P<0.05) lower OM, CP, CF, EE and NFE digestibility coefficients than those negative control, hay and barley supplemented groups. The results for TDN and DCP of the hay and barley groups are very close to those reported by Abdel- Fattah et al., (2004).

2- Effect of Hay and barley fed on the level of ochratoxin A (OA) and its metabolite ochratoxin alpha (Oa) in the blood serum of lactating goats after feeding 10 mg of OA per kilogram of feed (DM bases).

Prior to initial exposure to ochratoxin (on day 0 of the experiment), there was no detectable OA in the serum of the goats. After 6, 13, 20, and 27 of exposure, OA/mL of serum was observed in all ochratoxin -A treated groups (Table 3). This indicates that some of the OA-mycotoxin was not degraded by the ruminal microflora, and it was absorbed and distributed in the animal tissues. The corresponding OA values for the animals that received 10 mg of OA/kg at the end of experiment were much higher, in positive control group (301±16.3 ng/mL) than the hay (145.7 ±7.2 ng/mL) or barley (197.6 ± 23.5ng/mL) supplemental groups. In both treatment groups, considerable amounts of the metabolite Oa were detected in the serum samples during the experiment. The concentrations of Oa ranged from 22.5 ± 8.2 to 51.2 ± 6.8, 75.3 ± 8.5 to 237.5± 16 and 47.1 ± 6.5 to 165± 22.5 ng/mL serum, for positive control, the hay-fed and barley-fed groups, respectively. As determined for the serum data, the bioavailability of OA was much greater in goats fed a hay diet than in

goats fed barley diet. Our results were in agreement with those obtained by Xiao *et al.,* (1991), who indicated the bioavailability of OA, was much greater in goats fed a hay diet than in goats fed barley diet. This implies that the amount of OA in the rumen greatly affected the amount of OA in the blood in our study. Also, these results demonstrated that the rate of disappeaxance of OA from the rumen was much higher in goats fed hay than in goats fed grain, and the relative bioavailability of OA for goats fed grain was much greater than for goats fed hay. It was also shown that the rate of formation of the OA metabolite (Oa), was much greater in the rumen of goats fed hay than in the rumen of those fed grain, this may be due to that diet influences the pH and probably the type of microflora that developed in the rumen, and as a result, the rate of hydrolysis of OA was reduced in goats fed grain compared with those fed hay (Eadie and Mann,1970; Kiessling et al.,1984; Jouany et al., 1988 and Xiao *et al.,* 1991).

The present results and those of a previous study by Xiao *et al..,* (1991) indicate that diet may influences the pH and probably the type of microflora that developed in the rumen. Diets that promote rapid fermentation in the rumen result in rapid production of VFA, which is usually associated not only with a reduction in pH of the rumen, but also with a change in the microbial population, particularly the ruminal protozoa (Jouany *et al.,* 1988). Presumably, diet affected the population of protozoa and as a result, the rate of hydrolysis of OA was reduced in goats fed barley grain compared with those fed hay (Eadie and Mann, 1970 and Kiessling *et al.,* 1984).

Table (3). Concentration of ochratoxin A (OA) and its metabolite ochratoxin alpha (Oa) in the blood serum of lactating goats after feeding 10 mg of OA per kilogram of concentrate feed (four goats/treatment group).

Treatment	Day of treatment			
	6	13	20	27
	OA, ng/mL serum			
Negative control	0±0	0±0	0±0	0±0
Positive control	133.9±12.5^{A}	247.4±18^{A}	220.6±17.1^{A}	301±16.3^{A}
Hay group	104.5±8.17^{B}	123.4±21.3^{B}	122.5±6.2^{B}	145.7±7.2^{B}
Barley group	106.2±13.6^{B}	142.1±11.4^{C}	167±11.6^{C}	197.6±23.5^{C}
LSD at P (0.05)	15.9	11.2	19.1	31.8
	Oa, ng/mL serum			
Negative control	0±0	0±0	0±0	0±0
Positive control	27.8±5.11^{A}	33.5±3.5^{A}	22.5±8.2^{A}	51.2±6.8^{A}
Hay group	75.3±8.5^{B}	77.2±10.7^{B}	182.5±14.6^{B}	237.5±16^{B}
Barley group	47.1±6.5^{C}	55.3±16.3^{C}	122.5±11^{C}	165±22.5^{C}
LSD at P (0.05)	13.7	10.5	27.4	23.3

The same capital litters in columns denotes no significant difference between treatments in the same raw at ($p \leq 0.05$) and vise versa.

3- Proportional urinary, fecal and milk excretion of ochratoxin A, and its corresponding metabolite (Oα), of lactating goats.

As noted from the serum analysis, the concentrations of OA and Oα in feces, urine and milk varied according to the intake of OA and dietary treatment. The proportional excretion of OA via feces, urine and milk is shown Table (4). The cumulative excretion of Oα is expressed in terms of OA equivalents. Data represented in Table (4) indicate that in both treatment groups, most of the excreted OA was found as the metabolite Oα in the urine (43.3 to 76.6%), whereas approximately 5.2 to 8.7% was found as Oα in the feces. Relatively small amounts of intact OA were excreted in the urine (3.5 to 5.15% of intake) and the feces (0.6 to 1.8% of intake). Fecal excretion of Oα was significantly reduced by the hay and barley-supplementations, and this might be explained by a more pronounced renal elimination, which in turn might result in lower biliary secretion of Oα. In the hay and barley-supplemental groups, mainly Oa and only very small amounts of OA were excreted with the feces, and the percentage of OA and Oα excreted was relatively high, being greater than 79% of the amount of OA that was ingested.. Our reslts were in the same trend with those observed by (Hagelberg *et al.,* (1989), who found that the lower toxicities of Oa and OH-OA in mammals are mainly as a result of a faster rate of clearance via urine and feces compared with that of OA. The half-life of ochratoxins in the blood is directly related, in part, to the ability of plasma proteins to bind ochratoxins. In studies with rats, Li *et al.,* (1997) calculated half-lives of OA, Oα, and OH-OA of 103, 9.6, and 6.0 h, respectively, indicating that the OA metabolites are cleared at a much faster rate than OA. Hence, the rate of OA

biotransformation represents the main mechanism through which detoxification occurs. Therefore, processes that enhance the conversion of OA to Oα or to OH-OA will tend to reduce the general toxicity of OA in the animal itself or the toxic potential of OA-contaminated feed (e.g., when feed containing OA is administered to ruminants rather than to non ruminants).

From the 128 milk samples that were analyzed, 28 samples (25%) contained traces of OA (less than 6 ng/ml), whereas its metabolite, Oα, was not detected at any of the analyzed samples, probably because of the low binding of this metabolite to the IAC used (Boudra and Morgavi, 2006). The low level of OA contamination we observed in milk, does not possess any particular risk for milk consumers. Kiessling *et al.*, (1984) reported that Oα is excreted in milk and could be used as a marker of exposure. It is of interest to mention that in the absence of a reliable immunoaffinity binder for OA, it was not possible to attribute the observed low level of contamination to the efficiency of rumen biodegradation or just to the low level of toxin in feeds consumed by the animals.

Analysis of the feces, urine and milk samples reflect the findings of OA and Oα in the serum, because both compounds were found in significant concentrations, indicating substantial escape of OA from gastro intestinal tract (Table 4). According to Marquardt and Frohlich (1992), OA is absorbed in a passive manner in the nonionized form, which occurs at low pH values but not at neutral pH values. Under such conditions, the absorption of OA from the rumen would have been minimal. These observations, therefore, suggest that the OA was present systematically not only escaped hydrolysis in the rumen but was probably absorbed in the stomach.

The type of diet used in the present work could have affected the metabolism of OA in the rumen as well as absorption of intact OA from the rumen. Based on the data of Kiessling et al. (1984) who postulated that ruminal protozoa are especially effective at degrading OA to Oα, and the results of Xiao *et al*., (1991) who found that Protozoa are abundant in the rumen of hay-fed, but not of grain-fed, ruminants, our results reflect these findings, because disappearance of OA was much lower in grain-fed than in hay-fed goats.

As noted above, ruminants are thought to have a high capacity for the intraruminal degradation of OA and, thus, should be resistant to the toxic effects of OA. Based on the data of Hult *et al.*, (1976) and Kiessling *et al.*, (1984), ruminants should be able to degrade OA in amounts of up to 5 to 12 mg/kg of feed. Muller (1995) concluded on the basis of in vitro studies that the capacity of a dairy cow to degrade OA in the rumen is 33 to 72 mg/d, and that sheep should be able to degrade 3 to 7 mg of OA/day. In part agreement, the current data support these findings, because a concentration of 10 mg OA/kg feed was not appear any toxic lesions to lactating goats fed hay or barley supplemental diets.

Table (4) Proportional urinary and fecal excretions of ochratoxin A, and its corresponding metabolite (Oα), of lactating goats fed a diet containing OA or a control diet (OA-free).

Item		Groups								LSD (p≤0.05)
		Group 1		Group 2		Group 3		Group 4		
		M+SD	%	M+SD	%	M+SD	%	M+SD	%	
Feces	OA	0 ±0^{A}	0 ±0^{A}	50.23 ±7.4^{C}	0.6 ± .5^{B}	69.9± 12.06^{C}	0.9 ± 0.1^{C}	106.7 ± 17.5^{C}	1.8 ±2.15^{D}	11.8
	Oα	0 ±0^{A}	0 ±0^{A}	518 ±23.6^{B}	8.7 ±2.1^{B}	1737.5±28.7^{C}	5.3 ±1.2^{C}	1945 ± 36.2^{D}	5.2 ± 1.78^{C}	45.4
Urine	OA	0 ±0^{A}	0 ±0^{A}	8.55 ±2.4^{B}	3.5 ±1.5+^{B}	32 ± 6.5^{C}	4.8 ±.1.4^{C}	37.1 ± 9.3^{D}	5.15 ± 1.03^{C}	4.6
	Oα	0 ±0^{A}	0 ±0^{A}	149.82 ±17.5^{B}	43.3 ±8.1^{B}	753.69±15.4^{C}	76.6± 3.9^{C}	851 ± 18.4^{D}	67.82 ± 2.17^{C}	28.3
Milk	OA	0 ±0^{A}	5.8±0.13^{B}	2.37 ±0.19^{A}	0±0^{A}	0 ± 0^{A}	0 ±0^{A}	0 ± 0^{A}	0 ±0 A	0.0
	Oα	0 ±0^{A}	0 ±0^{A}	5.8 ± .13	0±0^{A}	0± 0^{A}	0±0^{A}	0±0^{A}	0 ±0^{A}	0.0
Total recovery, %		0 ±0^{A}		58.47_± 7.2^{B}		87.60__± 4.5^{C}		79.97_± 5.3^{C}		

N.B: 1- The same capital litters in columns denotes no significant difference between treatments in the same period at ($p \leq 0.05$) and vise versa.

2- Excretion of Oα expressed in terms of OA equivalents as calculated from the molecular weight of OA/molecular weight of Oα (403/256) x mg of Oα.

4- Some biochemical analysis of goats serum (liver and kidney function tests).

Metabolic indicators did not show a consistent pattern as influenced by the mycotoxin or the two dietary supplemental groups. Table (5) depicts the changes in the serum biochemical variables, recorded from the beginning and the end of the experiment. Except the positive control group, there were no significant ($p \leq 0.05$) differences amang other experimental groups concerning the activities of SGOT and SGPT enzymes, or the concentrations of serum urea. This may lead us to suggest that there is no significant liver dysfunction or necroosis in in these groups, i.e. barley and hay could protect liver of female goats from deleterious effects of ochratoxin pollution. It's of interest to mention that an increase in serum GOT is indicative of altered hepatocyte membrane integrity with the leakage of the enzyme or hepatic necrosis, but GPT level increase essentially with hepatic disease (Katchman and moss, 1976; Duncan and Prasse, 1986). Increased GOT activity due to OA has previously been observed in rats and sheep (Atroshi *et al.*, 2000; Abdel-Fattah *et al.*, 2004). Also, non significant changes in serum urea could detected by barley group than negative control group, and this may explained the protective effect of barley on kidney as compared with the two other OA-feeding groups (Abdel-Fattah *et al.*, 2001). The serum urea levels may not change significantly until 50% of renal function is impared as mentioned by (Hayes, 1989). So, we could suggest that the barley seeds could protect goats from hepato- or nephro-toxicities which induced by OA-contaminated diet when used as a sole diet for goats.

Table (5): Some biochemical analysis of goats serum (M ± SD)

Items / groups	Urea (mg/dL)		GOT (U/L)		GPT (U/L)		AP (U/L)		LDH (U/L)		CK (U/L)	
	Day (0)	Day (27)	Day (0)	Day (27)	Day (0)	Day (27)	Day (0)	Day (27)	Day (0)	Day (27)	Day (0)	Day (27)
Negative control	29 ± 5.02^{A}	27± 3.9^{A}	40 ± 12.3^{A}	39 ± 7.2^{A}	13± 1.4^{A}	11± 1.8^{A}	351± 7.2^{A}	245± 26.2^{B}	612± 14^{A}	569± 33.4^{A}	27 ± 7.3^{A}	24± 3.5^{A}
Positive control	31 ± 8.5^{A}	59± 7.3^{B}	36 ± 6.9^{A}	63 ± 13.4^{B}	9 ± 3.5^{A}	27± 1.5^{B}	287± 23^{B}	194± 21.1^{A}	585± 34.2^{A}	686± 18.7^{C}	27 ± 5.7^{A}	26± 6.2^{A}
Hay group	32± 8.3^{A}	35± 6.8^{A}	41 ± 13.5^{A}	40 ± 11.3^{A}	7± 2.8^{A}	9 ± 2.1^{A}	310± 28.7^{B}	263± 17.5^{B}	490± 16.3^{B}	458± 19.2^{B}	32 ± 10.7^{A}	28± 3.8^{A}
Barley group	35 ± 8.4^{A}	26± 4.6^{A}	36 ± 17.1^{A}	38 ± 8.8^{A}	12± 4.6^{A}	11± 2.6^{A}	305± 25.5^{B}	313± 25.5^{C}	519± 22.3^{B}	483± 28.4^{B}	28 ± 6.5^{A}	25± 4.2^{A}
LSD p≤ 0.05	9.4		7.1		6.3		28.4		47.5		11.9	

N.B.: 1- The same capital litters in columns denotes no significant difference between treatments in the same period at ($p \leq 0.05$) and vise versa.
2- a GOT = glutamate-oxalacetate-transaminase; GPT = glutamate-pyruvate transaminase; AP = alkaline phosphatase; LDH = lactate dehydrogenase; and CK = creatine kinase.
Day (0): Beginning of the experiment Day (27): End of the experiment

Except the positive control group, ther were no significant ($p \leq 0.05$) changes occurred in the serum LDH activities, withen 27days, compared to other experimental groups. The relative increase in serum LDH activities was significantly higher in the positive control group than those of the other groups. Increased serum LDH indicates myocardial infraction, skeletal muscle disease, liver damage and some anemia (Murray *et al.*, 1990).

Alkaline phosphatase (AP) activity exhibited a different pattern of change. The enzyme activity

reduced significantly ($p \leq 0.05$) in both the negative and positive control groups, however, a significant increase ($p \leq 0.05$) was found in the hay supplemental group. For the barley supplemental group, there was a slight none significant increase in AP levels, these were in the normal range for goats. In contrast, data obtained by (Hoheler *et al.*, 1999), on sheep fed OA-contaminated diet at level up to 5mg/kg diet, for 28 days, showed no significant changes in serum constituents either in GOT, LDH or urea levels. These differences may attributed to the high dose of the OA used in our study, feeding system or the animal itself. Creatine kinase (CK) activities were not changed by treatments guring this studym and there is no apparent reason for this observation.

5- Immunosuppressive action of OA.

The results obtained in Table (6) regarding OA as an immunosuppressive agent in goats. Immunoglobulin production reduced significantly ($p \leq 0.05$) as a result of OA feeding. In addition, significant differences between immunoglobulin fractions had been occurred. Values of IgG and IgM were reduced significantly in the positive control group rather than other groups. On the other hand, there were no significant differences in the IgA values between the all experimental proups. Previous studies by (Luster *et al.*, 1985 and Ueno, 1991), have reported that immunosuppressive effects associated with OA feeding in domestic animals included lymphocytpenia, reduced T cell-mediated immunity and reduced immunoglobulin production. Hay and barley supplementations were able to counteract the immunosuppressive effect of OA, and this was in the same trend with our previous results on sheep (Abdel-Fattah *et al.*, 2004). IgG and IgM values were significantly ($p \leq 0.05$) elevated in both the hay and barley-supplemental groups, to reach nearly normal values in the negative control group. On the other hand, no significant differences in IgG or IgM values were found between negative control group and the hay or barley-groups. IgA values were not affected by feeding treatments. The persent data on the immunosuppressive effect of OA make it obvious that the mycotoxin status of goats diet must be controlled if good goats performance is to be synthesized in response to antigenic stimulus, and also it is the second of the greatest concentration, after IgG, in the serum (Tizard, 1987) with the large molecular weight, so that it is intravascular antibody (Kaneko, 1989). The IgG is the second antibody appeared in the serum (after IgM) in response to antigenic stimulus, but the first of the great concentration in the serum and can distribute throughout the extra vasular tissue fluid for various protection mechnisms in various tissues and blood, such as: bacterial opsonization, complement fixation and neutralization of various microbial antigens and toxins (Kaneko, 1989). The IgA is important antibody secreted in the intestinal tract and lung through neutralization of virus and prevent adhesion of bacterial pathogens (Tizard, 1987).

Table (6). Blood serum immunoglobulins (mg/ml) in goats at the beginning and the end of experiment (M ± SD).

Groups / Items	Negative control		Positive control		Hay group		Barley group		LSD p≤ 0.05
	Day (0)	Day (27)	Day (0)	Day (27)	Day (0)	Day (27)	Day (0)	Day (27)	
IgG	16.7 ± 0.9^{A}	16.4 ± 0.9^{A}	17.2 ± 1.66^{A}	11.1 ± 1.66^{B}	16.4 ± 1.28^{A}	16.4 ± 1.28^{A}	17.0 ± 1.95^{A}	17.0 ± 1.95^{A}	2.29
IgM	1.6 ± 0.2^{A}	1.6 ± 0.2^{A}	1.6 ± 0.11^{A}	0.6 ± 0.11^{B}	1.4 ± 0.18^{B}	1.2 ± 0.18^{A}	1.6 ± 0.15^{A}	1.6 ± 0.15^{A}	0.16
IgA	0.4 ± 0.11^{A}	0.4 ± 0.11^{A}	0.4 ± 0.08^{A}	0.3 ± 0.08^{A}	0.4 ± 0.08^{A}	0.4 ± 0.08^{A}	0.4 ± 0.07^{A}	0.4 ± 0.07^{A}	0.06

N.B. Different litters in rows denote significant varience between means (p=0.05) and vise versa.
Day (0): Beginning of the experiment Day (27): End of the experiment

4. Conclusion

This study demonstrates that the rumen contents of Egyptian lactating goats are able to hydrolyze efficiently OA up to 10mg/kg contaminated feed and that the diet is an important factor affecting this hydrolysis. From a practical view, OA-contaminated grain should be fed to animals consuming a diet containing a high proportion of hay rather than grain. Under these conditions, OA would be efficiently hydrolyzed in the rumen and, therefore, its toxic effects should be greatly reduced. Research however, needs to be carried out to establish relationships between percentage of OA-content in the diet, relative rates of hydrolysis of OA, and corresponding toxic effects of OA to the animal, including OA-level in milk. The type of diet used in the present work could have affected the metabolism of OA in the rumen as well as absorption of intact OA from the rumen.

Corresponding author
Abdel- Fattah, Sh. M.
Department of food toxins and contaminants National research centre, Cairo, Egypt.
shaabanmostafa@yahoo.com

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