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Efficacy of Rumen Fluid from Various Types of Egyptian Sheep and Goats on The Biodegradation of Aflatoxin B1 *In-vitro.*

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ABSTRACT

In this study eighteen of Egyptian native sheep and goats were tested for their ability to degrade and metabolize aflatoxin B1 (AFB1) in other less toxic metabolites aflatoxin B2a (AFB2a), aflatoxin M1 (AFM1), and aflatoxicol (AFRo) *in three in vitro* trial sets. One set trial evaluated the aflatoxin B1 degradation ability of different rumen fluid donors (sheep vs. goats) by incubating whole rumen fluid (WRF) with three different AFB1 concentrations 5, 10 and 20µg/ ml WRF, for 12h. Another set examined AFB1 degradation by collecting WRF at five different times (0, 2, 4, 6 and 12 h) after feeding and incubated for 12h with AFB1 5µg/ ml WRF. For the third set AFB1 at 5µg/ ml WRF, was incubated for 12h with intact rumen fluid (WRF) or fractions of rumen protozoa (RP) and bacteria (RB) from sheep and goats. AFB1and their metabolites were determined by HPLC. All animals under investigation were fed a 70% concentrated diet and 30% roughage with free access to water. Results showed that rumen fluid from the Egyptian native goats demonstrated higher ($p < 0.05$) AFB1 degradability than Egyptian native sheep (AFB1 content decreased by an average of 48.5% in case for baldy goats). However, differences in sheep types had no significant influence on degradability. The capacity of rumen fluid to degrade aflatoxin B1 decreased 3 h after feeding, but this activity was gradually increased till 12 h feeding time. Also, protozoa were more active than bacteria. In addition, AFB1 was cleaved into none or less toxic metabolites by rumen contents from all tested animals. It was concluded that aflatoxin degradation was depending upon aflatoxin concentration, rumen fluid source and collection time after feeding, as significant differences were observed.

Keywords: aflatoxin B1, degradation, rumen. Egyptian goats, Egyptian sheep.

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INTRODUCTION

In comparison to non-ruminant species ruminant animals are generally considered to be less susceptible to the adverse effects caused by contamination of feeds with mycotoxins, this is mainly based on the assumption that the rumen microflora has the ability of biotransformation of mycotoxins to less toxic or non toxic metabolites (e.g., aflatoxicol), thus protecting the animal [1,2].

Rumen is considered an important factor in reducing the severity of harmful effect of mycotoxins toxins through broken into other compounds less harmful or almost non-existent damage. In ruminal fluid, a diverse ecosystem of microflora and microfauna is present. It consists of more than 50 genera of bacteria (10^{10}-10^{11} cells/mL), 25 genera of ciliate protozoa (104-106/mL), five genera of anaerobic fungi (103-105 zoospores/mL) and 10^{8}-10^{9}/ml of bacteriophages [3].

Ruminal microbes cause metabolism of ingested material which is considered to be a first line of defense system against toxic material present in the diet. However, ruminal degradation might be a disadvantage as toxic substances may be converted into much toxic compounds [4]. Protozoa are found to be more active than bacteria when mycotoxins are present in the rumen [4].

In regard to the ruminal degradation of aflatoxins, differences in the findings of *in vitro* studies are based upon the tested aflatoxins (crystalline pure AfB1 or extracted from natural contaminated feeds), the dilution factor (AfB1 concentration, w/v), the AfB1: SA ratio (w/w), the pH conditions and the media in which adsorption test was conducted [5,6].

There is no doubt that the type of animal and conditions of the rumen environment of diversity in the microflora as well as the difference in the degree of degrading ability of Aflatoxins. Aflatoxin B1 (AFB1) is converted to aflatoxicol in rumen [2, 7], but the percentage of its formation is still unknown as it is readily converted back to parent AFB1 [8]. Diets containing 40-60% of concentrates stimulate highest density of protozoa [9].

Due to no attempt was made to identify any AFB1 metabolites in rumen of sheep and goats under the Egyptian environmental conditions. So, this study aimed to in vitro investigate the relationship between the ruminal microflora components (bacteria and protozoa) on the AFB1 degradability, the degree to which the sampling time after feeding as well as aflatoxin concentration affect biodegradation of aflatoxin B1under the Egyptian environmental conditions. Our intent is to use the findings for the future selection of potential ruminant species containing bacteria and protozoa having aflatoxin degradation ability.

MATERIAL AND METHODS

Experimental design:

The present study was conducted to evaluate aflatoxin (AFB1) degradability to other species using rumen fluid collected from 18 healthy Egyptian native sheep and goats. The ability of rumen fluid to degrade AFB1 was studied in the presence of different concentrations of AFB1. The effect of rumen collection time after feeding on the removal of AFB1 was studied along with the effect of microbial population on the degradability of the same toxin. So, three sets of experiment trials were conducted in this study as follow:

Trial A: To study the AFB1 degradation ability of rumen fluid of native sheep and goat versus AFB1 concentration. In this experiment we employed a 6 × 3 factorial arrangement consisting of the rumen fluid donors (sheep *vs.* goats) versus three different levels of AFB1 (5, 10 and 20 µg/ml WRF).

Trial B: To study the effect on AFB1 degradation of rumen fluid collected at different times after feeding (0 h, 2 h, 4 h, 6h and 12 h) after feeding.

Trial c: To study AFB1 degradation based on types of the rumen microbial population, three types of rumen microbial population were obtained by the method of Orpin [10].

Due to there is a somewhat variation between rumen fluids, microflora profiles of sheep and goats kept in the same environment. Further, all tested animals in the present study were maintained with the same feed and under the same conditions.

Animals and diet:

Two patterns of eighteen apparently healthy Egyptian male sheep and goats were selected from a local private farm of Giza province, Egypt, and used for this study.

The first pattern was consisted of nine male sheep with a mean body weight of 28 - 30 ± 1.65 kg, and age from 6 to 8 months; in equal three types groups. These three types are: Balady, Rahmany and Saidy. The second pattern was also consisted of nine male goats with a mean body weight of 23 - 26 ± 1.43 kg, and age from 6 to 7 months; in equal three types groups. These types are: Balady, Saidy, and Zariby.

Animals under study are checked for safety research and especially making sure not to contain any rumen liquor or diet contaminated with mycotoxins.

Before withdrawal period of rumen fluid samples, there was a pre-sampling stage which extended for two weeks to make the animal will be adapted for the tested diet, and to avoid any differences in both type and number of ruminal microflora before treatment. During this stage, all experimental animals were fed sound rations (without aflatoxins), checked and observed for any abnormalities. The daily ration fed to the animals had contained 70 % concentrated diet (60 % wheat, 9 % soy meal and 1 % mineral/vitamin mixture) and 30 % hay as roughage. Animals had free access to water**.** The whole range of pH of freshly collected rumen fluid was measured and varied from 6.6 to 6.9.

Aflatoxins standard and chemicals.

Standard aflatoxins in the present study were aflatoxin B1 (AFB1), aflatoxicol (AFRo), aflatoxin B2a (AFB2a) and aflatoxin M1 (AFM1). All standards of aflatoxins were purchased from Sigma Chem., Co., USA. All Chemicals and solvents used were of ACS grade. Thin layer TLC aluminum plates recoated with 0.25 mm silica gel 60 (Merk).

Preparation of aflatoxin B1 (AFB1) test solution

Pure extract of AFB1 (2 mg powder) was procured from Sigma-Aldrich (USA) and was dissolved in 2 ml methanol HPLC, then diluted with sterilized deionized water to make the working standard solution at a concentration of 20 µg/ml. The working standard solution was further diluted as required for the HPLC analysis.

Sampling of rumen fluid and microbial treatments.

To investigate AFB1 degradation based on source of rumen fluid and / or rumen microbial fractions versus AFB1 concentration (Trials A &C), approximately 125 ml of rumen fluid samples were immediately collected before the morning feeding, using a rubber stomach tube, subjected to oxygen-free CO_2 using a gassing apparatus, homogenized for 1 min, then strained through 4-layer cheese cloth to eliminate large feed particles. After filtration, the fluid was used directly (WRF) or separated into bacterial (B fraction) and protozoal (P fraction) fractions. To obtain the fractions, in brief, rumen fluid was centrifuged at 200 x g for 10 min. The pellet, which contained the protozoa and some bacteria, was diluted with buffer to the same volume as the supernatant, which contained the bulk of the bacteria. The rumen fluid was defaunated of protozoa by adding dioctyl sodium sulfosuccinate $(OT)^{-1}$ mg of rumen fluid per ml, by the method of Orpin [10]. To prepare rumen fluid samples supplemented with AFB1 in concentration of 5, 10 and 20 µg/ml, known weights of powdered AFB1 were dissolved in less volume of methanol and diluted to the required volume with different fluids samples under investigation. 1 mL of the fluid contents was taken in to glass tubes and transferred to the laboratory in pre- warmed thermo flasks.

To investigate AFB1 degradation based on sampling time after feeding (Trial B), rumen fluid was collected at 0, 2, 4, 6 and 12 h after feeding in 15 ml sterilized falcon tubes in triplicates and immediately inoculated into sterilized Hungate tubes containing AFB1 to give a final concentration of 5 µg/ml.

For a control in each experiment, rumen fluid was autoclaved, supplemented with aflatoxin and incubated under the same conditions and incubation was done for 12 h at 39°C. After incubation time, the reaction was stopped by adding 1 mL methanol.

Aflatoxin extraction and analysis

The extraction of residual AFB1 was performed using AOAC methods [11]. Samples of rumen fluid one ml each were extracted and cleaned-up with sep-pak florisil cartridge and quantitatively analyzed by HPLC Technique [11]. Residues of AFB1 and their metabolites AFB2a, AFM1, and AFRO; were determined by HPLC with reverse phase at a flow rate 1 ml/min, 40 ^{o}C column temperature, and fluorescence detector. Analysis was performed using an HPLC instrument consisting of a LC-200 pump (Perkin Elmer, Norwalk, CT, USA) an AS-2055 sampling system, a FP-1520 fluorescence detector (Jasco Corporation, Tokyo, Japan), and a UV derivatizer (UVE TM derivatizer, LC tech, Dorfen, Germany); the instrument was controlled by Borwin 1.5 software (Jasco). A Superspher RP-18 column (4 µm particle size, 125x4 mm i.d., Merck) was used at room temperature with a mobile phase of water: methanol: acetonitrile (64:23:13, v/v/v) at 1 mL/min.

Statistical analysis

The *in vitro* data were examined and analyzed of each variable in triplicate, using F- Test through the analysis of variance (ANOVA) according to Snedecor and Cochran [12]. The differences among variables were tested using Duncan's multiple range tests [13]. All data were represented by means ± standard error (SE), n = 3. All differences were considered statistically significant at ($P < 0.05$).

RESULTS AND DISCUSSION

In the present study, *in vitro* methods were used to evaluate the AFB1 degradability by rumen fluid from the Egyptian native sheep and goats. The *in vitro* incubation method is one of the best ways to investigate the fate of aflatoxin metabolism when exposed to a broad range of concentrations. This is because the *in vitro* tests provide an effective approximation of *in vivo* tests and have the advantage that reproducibility is good, as it is possible to control conditions better than in vivo tests [14].

Trial A: the AFB1 degradation ability of rumen fluid of native sheep and goat versus AFB1 concentration (5, 10 and 20 µg/ml).

In the first trial whole rumen fluid (WRF) samples with AFB1 at three different concentrations (5, 10 and 20 µg/ml WRF) were incubated for 12 h. The degradation of AFB1 was measured at above three different concentrations, the degraded content of AFB1 (µg/ml WRF**),** degradation percentages and relative degradation percentages are shown in Table 1.

The results obtained in Table 1 clearly indicate very mixed effects when different concentrations of aflatoxin B1 are exposed to the rumen microorganisms where the activity of WRF was found to vary between 5, 10 and 20 µg/ml WRF and the rumen fluid supplemented with AFB1 had higher ($p < 0.05$) degradability when derived from native goats than from native sheep.

With regard to WRF source, the highest degradation ratio was observed with Balady goats group, showing a maximum removal of 48.5% ($P < 0.05$) at a concentration of 5µg/ml WRF, which indicates the most resistant AFB1 among the six selected groups (Figure 1). We could say that AFB1 relative degradation % ; which was 100% for Balady goats group at 5µg/ml WRF ; decreased to 53.40% and 38.35 % when WRF of the same group treated with AFB1 at concentration of 10 and 20 µg/ml ,respectively (Figure 1). Thus, the degradation of AFB1 was shown to be dose dependent, with the highest percentage of degradation observed at low concentration (5 µg/ml WRF). On the other hand, no significant differences were noticed between the three sheep groups although numerical differences and a slight decrease in the degraded amount of aflatoxin B1 occurred. However, the lowest AFB1degradation was observed with Rahmani sheep where, the maximum degradation was recorded as 24.5% at lower concentration (Figure 1). The degradation was fall down to 5.35% and 1.2% when AFB1 concentration was increased to 10 and 20 µg/ml WRF, respectively (Figure 1). Relative degradation percentages of Rahmani sheep group were 50.51, 22.06 and 9.89 when their WRF treated with AFB1 at concentration of 5, 10 and 20 µg/ml, respectively.

In our study we used a crystalline pure AFB1 and this may illustrate the differences in findings of our *in vitro* study than those reported in previous studies. In this respect, some researchers [5, 6]**,** mentioned that the pH conditions and the tested aflatoxins (crystalline pure AfB1 or extracted from natural contaminated feeds were affected in the AFB1 degradability rates.

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Data represented in Table 1 clearly indicate the higher degree of AFB1 degradation than those observed by Upadhaya *et al.* [15], who confirmed that AFB1 added at levels of 0.08–0.1 µg/ml can be degraded in rumen fluid, and the degradation was influenced by the species of animal and type of forage fed to the animals. Similarly, Westlake *et al.* [16] noted that 0.10 µg/ml of AFB1 was degraded after 12 h incubation at 39 °C when AFB1 was added at levels of 1.0 or 10 µg/ml AFB1 in ovine rumen fluid. On the other hand, results of the present study are in contrast with those observed by Keisling *et al.* [4], who found that Aflatoxin B1 and deoxynivalenol (DON) were not degraded by rumen microorganisms when incubated with rumen fluid for 3 h. Differences in results may be due to difference in experimental conditions (Feeding and animal type, rumen collection time after feeding and examined concentration of AFB1).

From the results of Table 1, it could be concluded that aflatoxin B1 degradation was both rumen microorganisms' source and AFB1-dose dependent. The obtained data can provide useful information for those interested and those in charge of the sheep and goat rearing in Egypt.

Table 1: Effect of rumen fluid source on AFB1 degradation versus three different concentrations (5, 10 and 20 µg/mL) for 12 h

Source of rumen fluid donor	*AFB1 concentration (µg/ml WRF)								
	5			10			20		
	AFB1 µg	D%	RD%	AFB1 µg	D%	RD%	AFB1 µg	D%	RD%
Sheep									
Rahmany	1.225^{Ca}± 0.036	24.5	49.95	0.535^{Cb}±0.003	5.35	22.06	0.240^{Bc}±0.005	1.20	9.89
Balady	1.465^{Ca}±0.011	29.3	60.43	0.925^{Bb}±0.005	9.25	38.14	0.375^{Bc}±0.016	1.87	15.46
Saidy	1.325^{Ca}±0.018	26.5	54.63	0.620^{Cb}±0.01	6.20	25.56	0.285^{Bc}±0.013	1.42	11.75
Goats									
Zariby	1.985^{Ba}±0.013	39.7	81.85	0.925^{Bb}±0.029	9.25	38.14	0.675^{Bc}±0.041	3.37	27.83
Balady	2.425^{Aa}±0.017	**48.5**	100.0	1.295^{Ab}±0.018	12.95	53.40	0.930^{Ac}±0.018	4.65	38.35
Saidy	1.810^{Ba}±0.010	36.2	74.63	0.905^{Bb}±0.027	9.05	37.31	0.515^{Bc}±0.023	2.57	21.23
LSD	0.255								

D% = Degradation percent,

$$\text{Relative degrade percent (RD\%)} = \frac{\text{Concentration of degraded AFB1}}{\text{Hightest concentration of degraded AFB1}} \times 100$$

a, b, c, d = means with same letter are not significantly different.

A, B, C = Means in the same column having different superscripts are significantly different at ($p<0.05$)

* AFB1 concentration is expressed as mean values ± standard deviation.

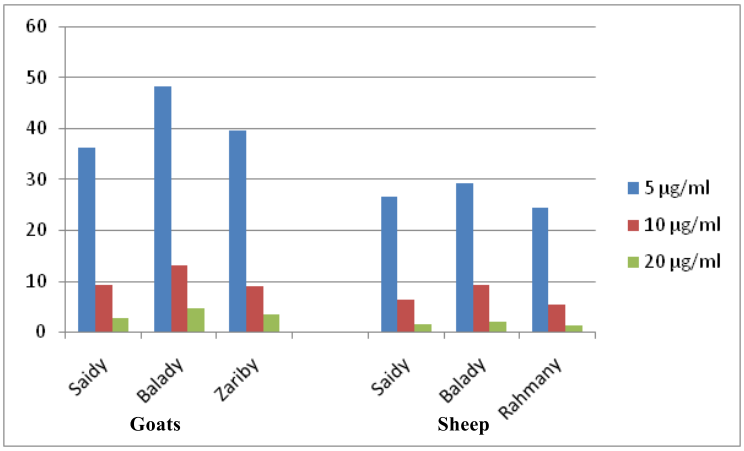

Figure 1: % Degradability under three concentrations of AFB1 by different rumen fluid source within 12 h of incubation.

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Trial B: the AFB1 degradation ability of rumen fluid as affected by collection time after feeding (0 h, 2 h, 4 h, 6 h and 12 h).

In relation to sampling time after feeding, results in Table 2 show that the capacity of WRF to degrade AFB1 varied according to the interval between feeding and sampling. We observed that overall AFB1 degradability tended to be higher at sampling time just before feeding (0h), then seemed to decrease immediately after feeding and gradually increased with the increase in time after feeding to reach maximum at 12 h of feeding (Table 2 and Figure 2). Goats have been shown the higher AFB1 degradabilities than sheep. In the microbial source from goats, Saidy and Zariby goats had significantly lower ($p < 0.05$) degradation than Balady goats, but in the microbial source from sheep and except for Balady sheep, there was much individual difference in AFB1 degrading ability in different sampling times (Table 2 and Figure 2).

In our study, degradation of AFB1 could be seen after incubation of 2 h or more, in the all tested animal species. In fact, the microbial population as well as the metabolic activity of the microbes increases at specific times after feeding, leading to higher AFB1 degradation. These results are in agreement with those observed by Michalowski and Muszyniski [17], who found that the capacity of rumen fluid to degrade aflatoxin B1 varied according to the interval between feeding and sampling, and whether this is an effect of feed inhibition or a variation in the population of protozoa; since a similar variation in the number of protozoa in rumen fluid has been observed.

The above degradabilities of AFB1 shown in Table 2 and Figure 2 were much higher than values reported by Upadhaya *et al.* [15]. The differences may be due to the effect of type of feed fed to donor animals or the donor animal species, on the rumen microbial ecosystem compared to much shorter incubation times in earlier studies by Jiang *et al.* [18], the long incubation time used in the present study would be expected to enable microbes to degrade AFB1 to a larger extent.

Table 2: Effect of rumen fluid collection time on AFB1 degradability

Source of rumen fluid donor	Sampling time after feeding				
	0h	2h	4h	6h	12h
Sheep					
Rahmany	1.225^{Ca}±0.036	0.56^{Cb}±0.002	0.735^{Cb}±0.06	0.94^{Ca}±0.016	1.085^{Ca}±0.021
Balady	1.465^{Ca}±0.011	0.825^{Db}±0.09	0.99^{Cb}±0.011	1.12^{Ca}±0.023	1.265^{Ca}±0.008
Saidy	1.325^{Ca}±0.018	0.585^{Cc}±0.008	1.225^{Ba}±0.011	0.99^{CD}±0.004	1.175^{Ca}±0.014
Goats					
Zariby	1.985^{Ba}±0.013	1.23^{Bb}±0.013.	1.315^{Bb}±0.012	1.575^{Ba}±0.014	1.76^{Ba}±0.031
Balady	2.425^{Aa}±0.017	1.53^{Ad}±0.019	1.760^{Ac}±0.033	1.825^{Ac}±0.022	2.09^{Ab}±0.022
Saidy	1.810^{Ba}±0.010	1.225^{Bb}±0.036	1.37^{Bb}±0.025	1.650^{Ba}±0.015	1.725^{Ba}±0.09
LSD	0.248				

a, b, c, d = means with same letter are not significantly different.

A, B, C = Means in the same column having different superscripts are significantly different at ($p<0.05$)

* AFB1 concentration is expressed as mean values ± standard deviation.

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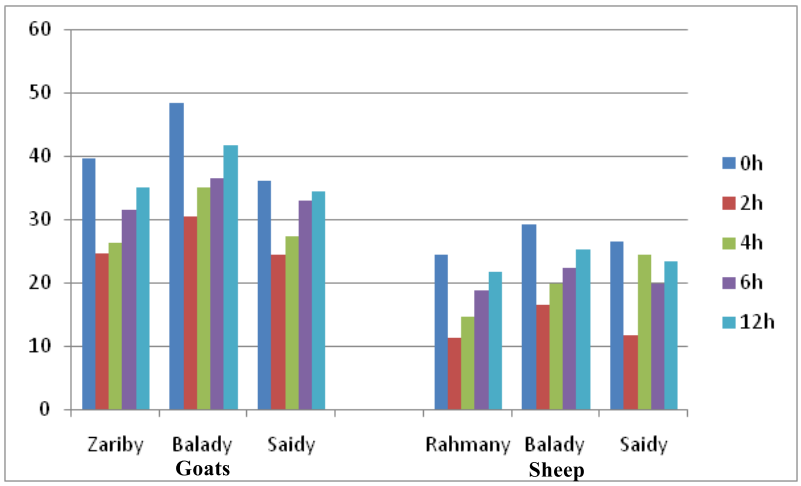

Figure 2: Sampling time after feeding dependent degradation of AFB1 by WRF within 12 h of incubation and initial concentration of AFB1 was 5 µg/ml WRF.

Trial c: AFB1 degradation based on types of the rumen microbial population, three types of rumen microbial population.

This trial experiment was to find out whether AFB1 degradation was performed by protozoa or bacteria. So, rumen fluid was fractionated to different microorganisms rich fractions. The results mentioned in Table 3 and Figure 3 showed that AFB1 was degraded by rumen microorganisms, but the protozoa are significantly more active than the bacteria. The non-significant differences between PF and WRF in the degradation of AFB1 as shown in table 3 proved the highly metabolic role of protozoa against AFB1. Our findings agreed to a large extent with those obtained by Kiessling et al. and Abdel-Fattah et al. [2, 4], who reported that protozoa were more active than bacteria in the degradation of mycotoxins. Teunisssion and Robertson [19], mentioned that Cells of the protozoon *Tetrahymena pyriformis* W have the ability to degrade pure AFB1 to another bright-blue fluorescent product and decrease the AFB1 concentration to 25% over 30 hours. It was concluded by Robertson et al. [20] that *T. pyriformis* W reduced the carbonyl in the cyclopentane ring of AFB1 to a hydroxyl group. The reduced aflatoxin appears to be identical to AFRo.

Only minor differences were observed in the rate of aflatoxin metabolism between BF from sheep and goats. In contract, the ability of goats PFs to metabolize AFB1 was significantly higher than those obtained by sheep. In this respect, Westlake et al. [16], mentioned that the faunal composition of protozoa in the rumen can vary with the dietary composition.

Table 3: Effect of rumen fluid fractions on aflatoxin B1 (AFB1) degradation

Source of rumen fluid donor	AFB1		
	WRF	BF	PF
Sheep			
Rahmany	1.225^{Ca}±0.036	0.435^{Ab}±0.027	1.265^{Ca}±0.032
Balady	1.465^{Ca}±0.011	0.56^{Ab}±0.018	1.525^{Ca}±0.011
Saidy	1.325^{Ca}±0.018	0.67^{Ab}±0.024	1.405^{Ca}±0.014
Goats			
Zariby	1.985^{Ba}±0.013	0.675^{Ab}±0.021	1.995^{Ba}±0.022
Balady	2.425^{Aa}±0.017	0.61^{Ab}±0.016	2.585^{Aa}±0.019
Saidy	1.810^{Ba}±0.010	0.84^{Ab}±0.017	1.875^{Ba}±0.024
LSD	0.315		

a, b, c, d = means with same letter are not significantly different.

A, B, C = Means in the same column having different superscripts are significantly different at ($p<0.05$)

* AFB1 concentration is expressed as mean values ± standard deviation.

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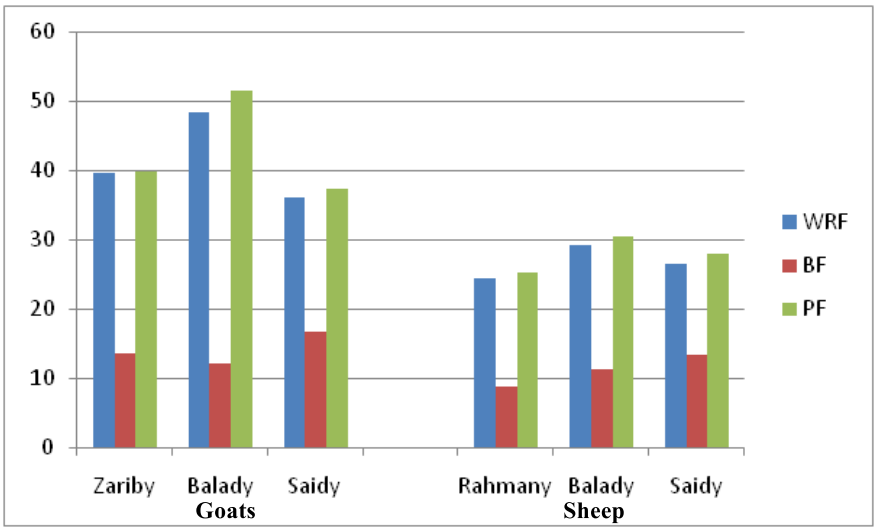

Figure 3: % Degradability of AFB1 by Whole rumen fluid (WRF), Bacterial fraction (RB) and Protozoa fraction (PR) within 12 h of incubation and initial concentration of AFB1 was 5 µg/ml WRF.

Table 4: Residues of AFB1 and their metabolites in WRF, incubating time 12h and AFB1 5µg/ml WRF

Source of rumen fluid donor	Mean values of AFB1 and their metabolites (ng/ml WRF)				
	AFM1	AFB2a	AFRO	Total identified metabolites	% Identified metabolites
Sheep					
Rahmany	133.5^{Bb}±12	105.4^{Ab}±10	474.1^{Da}±19	713.00	58.20
Balady	197.8^{Bb}±6	165.5^{Ab}±11	681.2^{Ca}±23	1044.5	71. 29
Saidy	164.3^{Bb}±8	141.8^{Ab}±6	443.9^{Da}±13	740.00	55.84
Goats					
Zariby	204.5^{Bb}±12	209.5^{Ab}±13	628.4^{Ca}±9	1042.4	52.51
Balady	390.4^{Ab}±16	235.2^{Ab}±10	1547.2^{Ab}±14	2172.8	89.60
Saidy	231.7^{Bb}±9	130.3^{Ab}±17	870.6^{Ba}±11	1232.6	68.09
LSD	157				

a, b, c, d = means with same letter are not significantly different.

A, B, C = Means in the same column having different superscripts are significantly different at ($p<0.05$)

* AFB1 concentration is expressed as mean values ± standard deviation.

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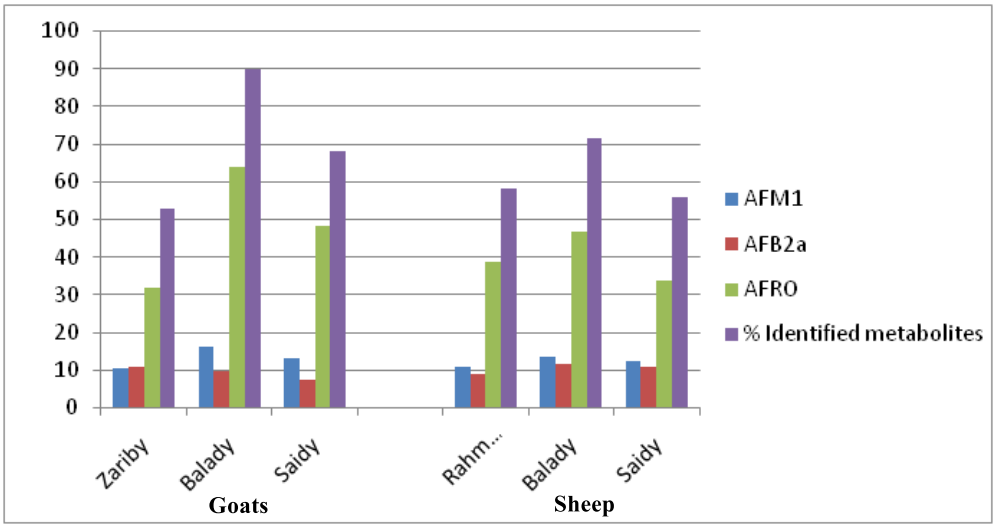

Figure 4: %, degradability of less toxic metabolites of the degraded amounts of AFB1, by WRF within 12 h of incubation.

Our results (Table 4 and Figure 4) showed that rumen microbes were capable to convert AFB1 into other less toxic metabolites, besides a different efficiency in degradation between sheep and goats. When WRF was treated with AFB1 at a concentration of 5 µg/ml, the percentages of metabolites were as follow: AFRo (33.5-63.8%), AFM1 (10.3-16.1%) and AFB2a (7.2-11.3%); of the degraded amounts of AFB1. As usual the highest percentages of these metabolites were found with goats, especially baldy goats, than sheep (Figure 4).

In similar manner total identified metabolites by HPLC technique ranged from 52.51-89.6% of the degraded amounts of AFB1 with the superior of Balady goats (table 4).

The higher activities of Balady goats shown in the present study may be referred to a higher number of microbes in WRF. Moreover, a higher number of microbes will often increase metabolic activity, leading to higher degradation of aflatoxin. However, individual differences in aflatoxin degradation existed among the three sheep groups. This may be because individual animals have unique physical abilities, organ sizes, functions, sensory abilities and microbial populations**.** Results obtained by Upadhaya *et al.* [15], may support these hypothis.

In previous studies many investigators [1,2,15,16,18,21] described the degradation of AFB1 by ruminants and mentioned that although it appears that some microorganisms in the rumen may be disturbed by aflatoxin, other rumen microorganisms may be able to degrade and transform aflatoxin to less toxic metabolites e.g., aflatoxicol.

The aflatoxicol is a significant member of the aflatoxin family which is produced by reduction of AFB1 by an NADPH-dependent cytoplasmic enzyme present in the soluble fraction of the liver [22]. Other significant members of the AFB1 family are AFM1 and AFB2a. AFM1 is oxidative form of AFB1 modified in the digestive tract of some animals and isolated from milk, urine and feces as a result of hydroxylation reaction of AFB1 [23]. The hydration process of AFB1 results in a metabolite AFB2a [24]. The aflatoxicol can also be metabolized to AFM1 and AFH1 [22].

Santin [25], mentioned that rumen microorganisms can degrade up to 42% of aflatoxin B1 but they are also capable of producing aflatoxicol. Auerbach *et al.* [7], reported that AFB1 is converted to aflatoxicol in rumen, this may support our results. But the percentage of its formation is still unknown as it is readily converted back to parent AFB1 [8, 26]. Fedele et al [27] reported that the aflatoxins B1, B2, M1, M2 and aflatoxicol (AFRo) were found both in the degraded maize and in the rumen fluid.

Jiang *et al.* [18], reported that AFB1 degradability was about 0.50 µg/ml after 12 h incubation in rumen fluid from the goats fed alfalfa hay. This may be support our results. Kiessling *et al.* [4], has previously

suggested that mycotoxins are not completely degraded and furthermore, the extent of degradation tends to vary between different species, age, sex and breed. This could be attributable to the types of microbes inhabiting the rumen. Some reports indicate mycotoxin effects were moderated by different environmental factors, stress, animal's physiological ability and their preference for food [28]. The influence feed could have on the number and types of microbes residing in the rumen ecosystem [15].

CONCLUSION

Our experimental findings show rumen microbes from Egyptian native goats demonstrated higher AFB1 degradability compared to Native sheep. In contrast to the opinions in many publications, the rumen bacteria of the Egyptian native sheep and goats played an important role in AFB1 degradation. Also, the protozoa are invariably more active than the bacteria. We observed AFB1 degradation in rumen fluid was influenced by animal species and time after feeding as well as AFB1 concentration. The findings from this study further our research in selecting species as potential rumen fluid donors for the isolation of bacteria and protozoa having aflatoxin degrading ability.

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