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Microbiological and mycotoxin risk assessment in ruminant feeding in Sulaimani governorate

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Abstract

Background: Feed contamination and poisoning by microbial and fungal genera pose obvious health threat to animals. Method: Vesa, ZAB, MIK, Deern, Zano and Zizar types of ruminant feed used in Sulaimani Governorate were analyzed for their bacterial and fungal quality. Identification of fungal isolates with determination of aflatoxin, ochratoxin and T-2 toxin for all trademarks and local feed and the prime target of this research would be background foreshadow for some danger of ruminant feedstuff in Sulaimani governorate and taking these aspects in consideration to assess microbiological quality and mycotoxins in ruminant feed. Result: The genera of bacteria and fungi isolated and their occurrence were *Salmonella sp.*, total plate count and total fungal count. Based on standard method identification of fungal species in brands ruminant feed and local ruminant feed, the all commonly isolated genera were *Aspergillus spp*., *Penicilliumspp* and *Fusarium spp*. Finally, detected concentration of each aflatoxin, ochratoxin and T-2 toxin in brands ruminant feed and local ruminant feed. Most types of ruminant feed under study were lower than the maximal standard limits of aflatoxin while all types of brands ruminant feed and local ruminant feed under study were lower than the maximal tolerance limits of ochratoxin and T-2 toxin.

Introduction

Feed stuff have significant role in the animal production sector and health such as it reduces the coast of the production and reducing the competition between human and animal for food by forming non-traditional feed ingredients for animal feeding [6]. Domesticated animals products are important part of raising national economy and providing high quality food for human beings. The role of livestock sector in development of agricultural productivity can contribute to raise poverty in rural areas [1]. Therefore, food safety has a crucial role to draw the attention of food manufacturers, consumers and producers. Thereby, the term food-borne illness that means ingestion of contaminated food with presence of bacteria or other microbes [4]. Thus, Assessment of microbiological profiling for animal feeding stuffs, trade and production are an important element in quality assurance system [31; 16 and 20]. Feed is at a high risk of contamination from presence and activities of insects and microbes. Microbes such as viruses, bacteria, fungi and yeast can invade the feed stuff and forming toxic substance inside or outside of the cell and mycotoxins [1]. The microbial population of the ruminant is diverse and live throughout the gastrointestinal tract of mammals like the *E. coli* [9]. Prevention at the first part of the food chain which is contaminated food is an essential step to prevent illness of consumers and better control from zoonotic pathogens such as *Campylobacter* and *Salmonella*. In human *salmonellosis* and campylobacteriosis took big portion of

causing gastroenteritis in the developed country around the world [18]. Animal feed ingredients and rations, as a result of their high rich nutrients and moisture, its provide an optimum media to grow of moulds at all stages in the food chain, through production to harvesting, handling, processing and storage. The toxic metabolites that produced by mold are varied and some of these are well known as mycotoxins. Aflatoxin, Ochratoxin and T-2 toxin are secondary metabolites of *Aspergillus flavus- Aspergillus parasiticus*, *Aspergillus ochraceus* and *Fusarium sporotrichoides* respectively, are often encountered in animal feeds stuffs [36].The secondary metabolites toxes (mycotoxins) produced by several fungal species is considered a toxic to humans, animals and plants. Their ingestion, inhalation or dermal absorption may cause different diseases and even death.

The prime target of this research would be to draw background foreshadow some of our most pressing current issues of public policy in: As one of ways to keep safety of ruminant feed stuffs in Sulaimanigovernorate and taking these aspects into consideration, the study was undertaken to assess microbiological quality and mycotoxins and Identification of fungal of ruminant feed produced and used in Sulaimanigovernorate.

Material and methods

A. Analysis and identification of mycotoxins in feeds

This study was conducted in the laboratories of the Faculty Agricultural sciences/ University of Sulaimani/ Kurdistan Region/ Iraq and veterinary laboratories directorate, for the period of 1st October 2014 till 1st February 2015. The inspections of feed ruminant included three (3) imported trademarks were collected from the market, which were Vesa, ZAB and MIK feed trademark, as well as three types of local feed ruminant most commercially used in Sulaymaniah governorate, which were Deern, Zano and Zizarlocal feed ruminant. Thirty-sixth samples of ruminant feed were examined for bacteria, fungi and mycotoxin, for each trademark used sixth samples. All treatments were examined for the presence of *Salmonella sp.*, *Enterobacteriaceae*, total plate count, total fungal count and fungal with determination of aflatoxin, ochratoxin and T-2 toxin for all trademarks and local feed were conducted in this study. Based on International methods dealing with quality assurance for microbiology in feed analysis laboratories, the detection of *Salmonella sp.*, *Enterobacteriaceae*, total plate count, number total fungal count and determination of aflatoxin, ochratoxin as well as T2 toxin were conducted in this study. The more details regarding procedures for laboratory examination were as following:

B. The Salmonella detection and enumeration of Enterobacteriaceaewas done by following the FAO, 2013 ***[12]*** dealing with quality assurance for microbiology in feed analysis laboratories.

C.The enumeration of total plate countwas done according to method used by International Standard ISO 6579: dealing with microbiology of food and animal feeding stuffs.

D. Isolation of ruminant feed fungi:Dilute plate technique was used for isolation of fungi from the samples. According to Pitt (1987) ***[28].***

E. Mycotoxins analyses:Feed parts intended for mycotoxin estimation were subjected to three standard kits for aflatoxin, ochratoxin and T-2 toxin using Neogen mycotoxin extraction kits (Neogen Corporation).

F. Statistical analysis:All data were subject to one-way analysis of variance (ANOVA) using XLSTAT 2015.1 program for Windows. Differences between the means were tested by Fisher's Least Significant Difference (LSD) test. The significance between means was done according to Addinsoft***[2]***.

Results and discussion

There were significant differences ($p<0.05$) in the total aerobic count of bacterial and fungal in ruminant feeds (table 1). Deern feed has the highest total aerobic count (11.667 cfu/gm), while Zizar feed contained low percentage of total bacteria (1 cfu/gm), but there were not any colonies Vesa feed, ZAB feed and Zano feed. *Salmonella sp.* identification in four types of ruminant feed (Vesa, Deern, Zano and Zizar), which present in table 1. Vesa feed was the highest count of *Salmonella sp.*, while Zizar feed showed the lowest of

the *Salmonella sp.*. Differences was appeared among Vesa, ZAB, MIK, Deern, Zano and Zizar in *Salmonella sp*. (10, 0, 0, 6, 6, 2 cfu/gm, respectively). As for the number of *Enterobacteriaceae* in feeding stuffs, it was not detected, and the total fungal count in the majority of the examined feeding stuff samples ranged from 2 to16 cfu/gm. The most often stated levels of fungi in MIK feed sample was 16 cfu/gm, while Vesa feed, ZAB feed and Zano showed lower total fungal count (2, 2 and 2 mg cfu/gm feed respectively).

Table-1: Levels of Occurrence of Bacterial and Fungal Isolates in the Brands and local of ruminant Feeds (cfu/gm).

Feed type	Total aerobicbacteria Count 10^{-7}	*Salmonella sp.* 10^{-2}	Total fungal Count 10-4	Number of positive samples/ fungi
Vesa feed	0.000 ^{c} ±0.000	10.000 ^{a} ±1.155	2.00 ^{b} ±0.000	1
ZAB feed	0.000 ^{c} ±0.000	0.000 ^{c} ±0.000	2.00 ^{b} ±1.619	1
MIK feed	5.333 ^{b} ±2.603	0.000 ^{c} ±0.000	16.00 ^{a} ±4.064	2
Deern feed	11.667 ^{a} ±0.882	6.000 ^{b} ±0.577	3.33 ^{b} ±1.453	1
Zano feed	0.000 ^{c} ±0.000	6.000 ^{b} ±0.000	2.00 ^{b} ±0.000	1
Zizar feed	1.000 ^{c} ±0.577	2.000 ^{c} ±1.000	4.33 ^{b} ±0.667	1
$LSD_{0.05}$	2.889	1.679	3.404	

Least significant differences ($LSD_{(0.05)}$) are based on a Fisher test. Means with different letters in the same column are differ significantly ($P < 0.05$).

High numbers of fungal species were isolated from the few types of ruminant feed as observed in table 2. Based on standard method of identification for ruminant feed, three genera were isolated which were *Aspergillus spp.*, *Penicilliumspp* and *Fusarium spp.* Whereas, based on morphological characteristics, the first fungal of *Aspergillus spp* groups, can be described as a colonies of a velvety, hairy, milky- creamy color, but later converted to black colony of fungi with yellowish color at the another side, and septate with unbranched conidophores, Then radiate head was formed as a result of double sterigmate cover for whole vesicles. The isolates of*Penicillium* spp. produced branched septate hyphae with flask shaped sterigmata, the conidia is unbranched with a penicillate or blue color appearance, white-creamy powdery surface but later turned blue-green with whitish reverse side and edges in the agar. The *Fusarium* spp. had a hairy growth with creamy color, and then turned to pink with a yellowish at the opposite side and septate, branched conidiophore with rectangle conidia.

Table-2: Identification characteristics of fungal of Brands ruminant feed and local ruminant feed.

Serial No of Isolates	Macroscopic characteristics and texture	Microscopic Characteristic	Organism
1	Velvety, hairy, milky- creamy color, but later converted to black colony of fungi with yellowish color at the another side	Septate with unbranched conidiophores, then radiate head was formed as a result of double sterigmate cover for whole vesicles	*Aspergillus spp*
2	White-creamy powdery surface but later turned blue-green with whitish reverse side and edges in the agar.	Branched septate hyphae with flask shaped sterigmata, the conidia is unbranched with a penicillate or blue color appearance.	*Penicilliumspp*
3	Hairy growth with creamy color that later turned to pink with a yellowish at the opposite side	Septate with branched conidiophore and rectangle conidia.	*Fusarium spp*

The mean concentration of each aflatoxin, ochratoxin and T-2 toxin in imported and local ruminant were illustrated in table 3. The results showed aflatoxin was found in all samples with significance differences ($p< 0.05$) in aflatoxin contents for all feed types; the highest aflatoxin content was in Vesa feed, while the lowest was in Zano feed. Ochratoxin were ranged from 0 – 3 ppb of ruminant feed, there was non-significant difference between five feed types except for Zano feed, which showed difference among them. T-2 toxin level was also ranged from 17.43 – 30.16 ppb, the highest T-2 toxin levels were occurred as 30.16 ppb in the Zano feed and the lowest level was 17.43ppb in MIK feed. Figure 1 shows the aflatoxin concentrations (ppb) in the six feed types as compared maximum limit. While, figure 2 shows ochratoxin concentration in six types ruminant feed compared tolerance limit, and the T-2 toxin concentration is shown in the figure 3.

Table-3: Mycotoxin levels in brands ruminant feed and local ruminant feed.

Feed type	Aflatoxin ppb	Ochratoxin ppb	T-2 toxin ppb
Vesa feed	28.333 ^{a} ±0.882	2.600^{a}±0.231	22.000 ^{c} ±0.577
ZAB feed	18.867 ^{cd} ±0.088	2.867 ^{a} ±0.088	19.700 ^{d} ±0.173
MIK feed	24.000 ^{ab} ±0.577	2.567 ^{a} ±0.233	17.433 ^{e} ±0.296
Deern feed	16.667 ^{d} ±3.333	3.000 ^{a} ±0.231	24.000 ^{b} ±0.577
Zano feed	2.033 ^{e} ±0.033	0.000 ^{b} ±0.000	30.167 ^{a} ±0.120
Zizar feed	21.567 ^{bc} ±0.296	2.667 ^{a} ±0.176	20.000 ^{d} ±0.577
LSD0.05	3.610	0.460	1.0946

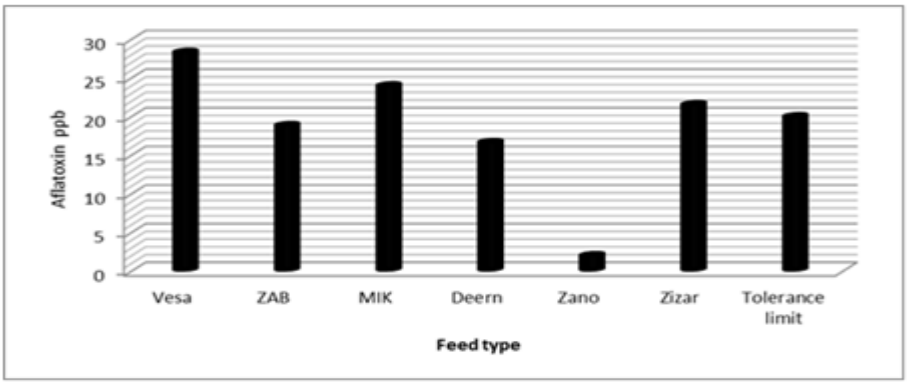

Figure-1: Mean Aflatoxin concentration in six type ruminant feed compared tolerance limit.

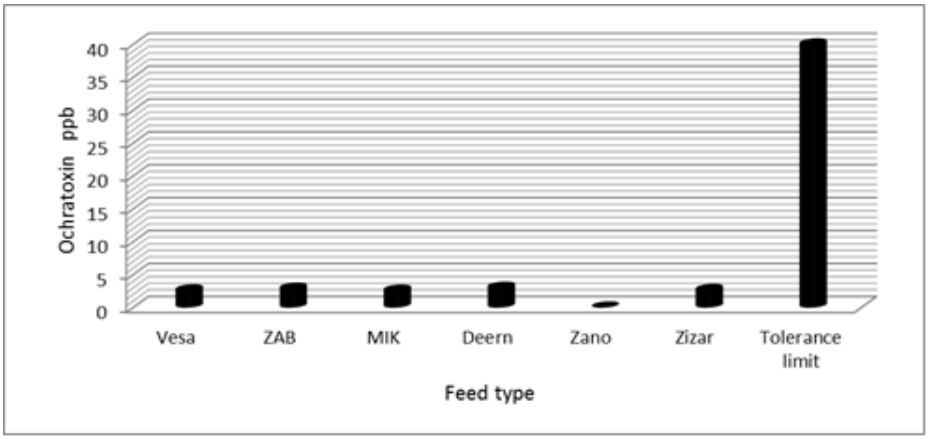

Figure-2: Mean Ochratoxin concentration in six type ruminant feed compared tolerance limit.

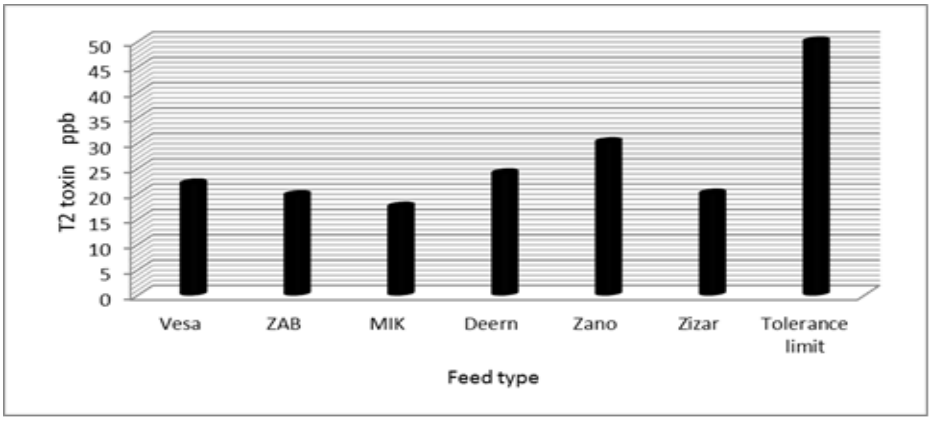

Figure- 3: Mean T-2 toxin concentration in six type ruminant feed compared tolerance limit

The obtained results indicate that some (the majority) of examined ruminant feeding stuff samples had a proper microbiological quality and achieve the established criteria. There was small percentage of samples which did not achieve the established criteria and some of ruminant feed contained total aerobic bacteria, *Salmonella sp.* and fungal isolates(faungi). However, *Enterobacteriaceae*was not observed in all examined ruminant feed. The results indicated that aerobic bacterial colony counts in ruminant feed samples was ranged between 1.00 to 11.66 cfu/gm.Wojdat et al., ***[34]*** found that total plate count of bacteria in feeding stuff ranged from 10^3 to 10^5cfu/gm, Level of contamination by aerobic bacteria in the examined animal feeding stuffs was diversified and sometimes relatively high, the reason might be related to feed compositions (the level of carbohydrate, fat and protein), storage condition, production technology and microbiological quality of feed materials. The study state that the main contribution to infection by pathogenic bacteria in feed stuff is the high levels of contamination that occurrence by microrganisms.

The datain this study found that *Salmonella sp*. showed significant differences for each kind of ruminant feed, in which the highest counts was 6 cfu/gm, and the lowest count was 2 cfu/g. However, *Salmonella* in feed can generally be killed or reduced by pelleting; Pelleted feed process might have a great contribution to reduce the possibility of feed infection with Salmonella in both time of animal life and after slaughter, particularly in a contaminated environment***[19].****Salmonella*through ingestion or dealing with contaminated food such as animals' organ meat can cause a serious health implication to human***[37].*** In addition, the emerge of *Salmonella*'s resistance strain against the antibiotic raise more attention and concern worldwide, especially in meat industry sector . Therefore, the emphases of controlling *Salmonella* in meat production field become an interested target of many studies in animal science product and it have been considered as a main source of posing a direct threat to human health ***[11]*** and ***[35]***. The data do not show any *Enterobacteriaceae* in ruminant feed for all trademarks.Wojdat et al.,***[34]***reported a 10 cfu of *Enterobacteriaceae*/gm in feeding stuffs for fattening animals. *Escherichia coli* in the rumen of cattle are not often cultured in high numbers, commonly less than 10^6 cells/ml in population of more than 10^{10} cells/ml ***[21]*** and ***[23]***.

According to the obtained data, higher fungal counts were indicated in the MIK brand of ruminant feed, and the lower were gained in the Vesa, ZAB and Zano feed. Wojdat et al., ***[34]*** recorded that yeast and molds are present in feeding stuff with average level between 10^1 - 10^4 cfu/gm. The present of fungal and bacterial species can be passed to the meat through feed staff and then cause many heath implications, especially through their toxins ***[13]***. Fungal species can be actively transported from the field, then during handling and other post-harvest process more contamination ***[29]***.

The data identified three fungal species in brands ruminant feed and local ruminant feed as shown in table 2. Including members of the *Aspergillus, Penicillum,* and *Fusarium* genera, which may contaminate high numbers of agricultural feed samples such as soybean, corn, barley, wheat and others commodities. Molds'

mycotoxin gains high focus because of their deleterious effect ***[8].*** The pathogenic property of these fungi might also be a reason for their highly presence in feeding stuff, which can affect both human and farmed animal ***[29]***.

In this study, the aflatoxin concentration in six types of ruminant feed is shown in table 3. Three types of ruminant feed studied were lower than the maximal standard limits of aflatoxin but the other three types feed were higher than maximal aflatoxin limitation. Pacin et al., ***[27]*** reported that each of the *aspergillus* (including Eurotium), *fusarium* and*penicillium*are common fungal that inhabiting the mixture of feed materials, and the main mycotoxins that recovered in feed mixture are ochratoxin A, fumonisin and aflatoxin. In additional, these fungi are very well known for their ability their ability to form high numbers of harmful secondary metabolize mycotoxins ***[30]*** and ***[14]*** As classified by the (IARC, 1993)***[17]***,these fungi are considered group one of human's carcinogen and exposure to aflatoxin for long term animals is associated with liver tumors and lesions in animal [33] and other implication with animal health production sector and products as well ***[5]*** and ***[7].***

As shown in table 3, all the studied six brands of ruminant feed and local ruminant feed were lower than maximal tolerance limits of ochratoxin. Some toxic substances are known to be either immunosuppressive or carcinogenic. The immunosuppressive are caused by aflatoxin B1, ochratoxin A and T-2 toxin, where carcinogenic can be caused by aflatoxin B1, ochratoxin A in additional to fumonisin B. Other side effect include neurotoxic by fumonisin B1, dermatotoxic via trichothecenes and nephrotoxic by ochratoxin A ***[22].***Ochratoxin A is commonly found in cereals, peanuts and beans and often localize in a hot location such as grain store. The animal organ that is primary targeted for ochratoxinA toxicity is the kidney and its contamination has been associated with to nephropathy in poultry and pigs, immunosuppression, reduction of growth rate and increasing mortality rate ***[10]***. The T-2 toxin concentration of six type ruminant feed show in table 3, with significant difference between T-2 toxin ($P<0.05$) in ruminant feed and all types of studied feed were lower than the maximal standard limits for T-2 toxin concentration. Aflatoxin, fumonisins and two cancer promoting mycotoxins, ochratoxin and T-2 toxin can have great negative influence on animal's health and productivity ***[15]***.

Conclusion

In conclusion, the types of bacteria and fungi isolated and their occurrence were *Salmonella sp.,* total plate count and total fungal count andperceived concentration of each aflatoxin, ochratoxin and T-2 toxin in imported ruminant feed and local ruminant feed then isolated genera were *Aspergillus spp.,Penicilliumspp* and *Fusarium spp*., it has to be emphasized that the most results obtained will be beneficial for establishing microbiological and mycotoxin standards for most ruminant feed types in Sulaimani governorate.

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