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# Microbial contamination of imported soybean meal which used as animal feed in Iraq

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**Abstract**
Soybean meal is most important components in diets for animals; therefore, the objective of this study was to evaluate the microbial contamination rate and types of pathogens in imported Soybean meal to Iraq because of this contamination causing animal diseases that often causing death and great economic losses. A total of 100 samples were collected from Iraqi borders points, cultured, bacteriological and mycological analysis were down.
Bacteriological analysis results identified Gram-negative bacteria in (78) samples at (78%) such as *E.coli* in (34) samples at (34%) then *Salmonella* spp*.* in (20) samples at (20%), *Klebsiella* spp in (15) samples at (15%), *Enterobacter* (11) isolates at (11%) and *Proteus* spp. in (9) samples at (9%).
Mycological analysis results shows presence of mycotoxogenic fungi: *Aspergillus flavus* (25) isolates, *Fusarium* (35) isolates, *Penecillium* (13) isolates and *Alternaria* (10) isolates, when we doing total count of fungi, all samples were acceptable to Iraqi standard specification which limited down ($10^5$) is required for soybean used as animal feed except (8) samples were unacceptable because its value ($2.2\times10^6$).
Mycotoxin analysis results show presence of Aflatoxin in (66) samples, T2/HT2 in (78) samples, Ochratoxin in (45) samples.
This study concluded presence of microbial contamination (bacteriological and mycological contamination) in most samples and the severity when presence of bacteriological, mycological contamination and mycotoxin in same sample which increase multiple risk for pathogens and its mycotoxin which lead to animal diseases and death causing a loss economic, therefore, it must be assessed the microbial quality of imported Soybean meal by manufacturers and health authorities to ensure its safety because it may harbor potential animal and human pathogens*.*

**Keywords:** Mycotoxogenic fungi, Mycotoxin, Pathogens

## 1. Introduction
Soybean (*Glycine max* (L.) Merr.) is an important oilseed and protein crop but Soybean meal is the product remaining after extraction of oil from whole seeds followed by grinding of solid residue (FAO, 2004) [7] and it is vegetal origin feedstuff widely used in diets for animals because of its high nutrient value: a high content of crude protein (Vojtech Rada, *et al*., 2017) [22] which have a high biological value, a high energy and a high content of fat and unsaturated fatty acids (with about 50% linoleic acid) (Vojtech Rada, 2017) [22], also Soybean is a rich in iron, calcium, magnesium, and B-vitamins (Prestamo and Fontecha, 2007) [17].
Because of its characteristics: high protein, high water content, and neutral pH (Asharaf *et al*., 1999) [3], it is easily spoiled by microbial growth in environmental conditions, such as moisture, temperature, pH and light in the fields, upon harvesting, during storage and even during processing leading to the loss of nutrients and to the development of mycotoxins (Pitt, 2000, Yaling *et al*., 2008) [15, 18], Thus, its shelf life is short and storage / distribution is limited.
Type of processing and storage conditions are the factors that have an effect on contamination levels and pathogens type. (Muhsin L., *et al*, 2016) [13]
The objective of this study was to evaluate the microbial contamination rate and types of pathogens in imported Soybean meal to Iraq because of this contamination causing animal diseases that often causing death and great economic losses.
We conclude it must be assessed the microbial quality of imported Soybean meal by manufacturers and health authorities to ensure its safety because it may harbor potential animal and human pathogens.

## 2. Materials and Methods
### 2.1 Samples Collection
This study had conducted in the Directorate of Animal Resources / Department of Quality Control on Feed in Baghdad, Iraq. (100) samples of imported Soybean meal collected from border points about (3) Kg as representative sample

### 2.2 Preparation of media used
The media were prepared depending on the manufacturer's instructions on the labels of the media and autoclaved at 121°C for 15 min *.*

### 2.3 Isolation of bacteria
For Salmonella isolation, (25) g of soya bean meal sample added to (225) ml peptone water, mix and incubated, (1) ml from pervious mixture was transferred to (9) ml of Selenite Cystine Broth and incubated at 37°C for 24 hours. Three differential media used Xylose Desoxycholate agar, Hiktone Enteric agar were streaked and incubated at 37°C for 24 hours. Salmonella suspect colonies were picked up for biochemical tests (triple sugar iron (TSI) and urease) [Holt JG, 1994] [10], agglutination test (O&H antiserum for salmonella) and isolates stained with gram stain to know Microscopical Characteristics of isolates [Holt JG, *et al*., 1994] [10]*.*

For isolation of Escherichia coli and other bacteria uses Eosine Methlyen Blue agar (EMB agar), Nutrient agar and macconkey agar**.**
Identification of isolates by API kit (remel one rapid system)

### 2.4 Mycological analysis

#### 2.4.1 Total fungi count

We used dilution technique to determinate total fungal count (Pitt, J.I. and Hocking, A.D 2009) [9]. 10 g of soybean meal samples were added into 90 ml peptone water. Four dilutions of each sample were made and 1 ml of the dilutions ($10^{-3}$and $10^{-4}$) was pour plated on potato dextrose agar (PDA). All plates were incubated for 5- 7 days at 28°C. All colonies were counted and expressed in colony forming unit per gram (CFU/g) of the sample using the formula**:**

$$\frac{\text{No. of colonies x dilution factor}}{\text{Volume used}}$$

#### 2.4.2 Fungi identification

Sub-cultured were made on potato dextrose agar (PDA) then incubated at 28°C for 7 days then identification of fungal species was based on the macroscopic and microscopic characteristics of pure cultures of obtained isolates from analyzed samples**.**
Fungi were identified by slide cultures preparing for microscopic examination then the isolation frequency (Fr) and relative density (RD) of species were calculated according to (González, *et al*., 1995) [9] as followin

$$\text{Fr (\%)} = \frac{\text{No. of samples with a species or genus}}{\text{Total number of samples}} \times 100$$

$$\text{RD (\%)} = \frac{\text{No. of isolates of a species or genus}}{\text{Total number of fungi isolated}} \times 100$$

#### 2.4.3 A rapid identification method for aflatoxin-producing strains of *Aspergillus flavus* by ammonia vapor:

Sub-cultured of obtained isolates of *Aspergillus flavus* were made on potato dextrose agar (PDA), exposed to ammonia vapor and incubation at 25°C for 7 days, these cultures were turned pink pigmentation after incubation. [Saito M. and Machida, S., 1999] [19].

#### 2.4.4 Quantification of mycotoxins using ELISA

To quantitative analysis by using competitive direct enzyme-linked immunosorbent Assay (ELISA) [Neogen corporation, 2013] [14]. The procedure was conducted according to manufacturer's instructions (Neogen. USA). In brief, 5g of ground sample was extracted with 25 ml of 70% methanol for aflatoxin and T2/HT2 toxin and 10g of ground samples were extracted with 40 ml of 50% methanol for ochratoxin. Afterwards, samples were shaken for 3 minutes and the extracts filtered through Whatman No.1paper. Then, 100$\mu$L of the diluted filtrate per well was used for testing for aflatoxin, ochratoxin and T2/HT2 toxin by using Agri-Screen kit. Then, ELISA reader, which it provided from BioTek Company used to obtain mycotoxin concentration.

### 3. Statistical analysis

It carried by Microsoft Excel (2010) to find average, minimum, maximum of concentration (ppb) and chart for each mycotoxin.

### 4. Results and Discussion

Soybean meal is used as a protein source in animal diets, including chicken, cattle, and fish feed. In this study two principal aspects can be analyzed in order to assess the possibility of microbial contamination in imported Soybean meal, the first aspect is related to bacterial pathogens, second aspect is related to total fungi count, presence of toxigenic fungi species and mycotoxin levels observed in imported soybean meal.
A total of 100 samples were collected and analyzed for Bacteriological and mycological tests and the results show contamination with Bacterial pathogenic isolates, which is Gram negative bacteria belonging to Enterobacteriaceae family at (78%) in (78) samples of total samples and the isolates were identified according to their microscopic, cultural and biochemical properties, the contamination rate of these bacteria is summarized in table (1), among all them; *E.coli* was the most frequently isolated bacterial species, it was (34) isolates at (34%) due to presence of feces which indicate to unsuitable storage conditions then *Salmonella* spp. was (20) isolates at (20%), as it known is pathogen for human and animal, while *Klebsiella* spp. was (15) isolates at (15%) then *Enterobacter* spp. (11) isolates at (11%), Finally least frequently isolated bacteria was *Proteus* spp. (9) isolates at (9%). These results meaning occurrence of contamination with enteric bacteria, as indicated by the presence of *E. coli* and *Salmonella* spp. which occurring in storage, transport or handling for processing due to this material may be serve as carriers for wide variety of pathogens.
On contrast, pervious study in Iraq by F. H. Dhaher *et al*.(2011) reported a presence of *Salmonella* spp. in (3) samples of (11) imported soybean meal samples to Iraq, another study by Aleksandra (2011) reported *Proteus* spp. in (1) samples and *E. coli* in (2) samples of soybean meal, addition to Wierup M. and Häggblom P. (2010) [21] reported in Sweden (14.6%) of (795) imported soybean meal samples were found to be contaminated by *Salmonella* during 2004–2005, also AEJ Okwori, *et al*. (2010) [1] reported *Klebsiella* spp. in (10)samples at (6.3%) of soya bean flour samples.

**Table 1:** Bacterial isolates in imported soybean meal samples

| Bacterial isolates | No. of positive samples for Bacteria | Percentage of total Isolation (1) |
|---|---|---|
| *E. coli* | 34 | 34% |
| *Salmonella spp.* | 20 | 20% |
| *Klebsiella spp.* | 15 | 15% |
| *Enterobacter* | 11 | 11% |
| *Proteus spp.* | 9 | 9% |
| Total | 78 | 78% |

(1) Total No. of samples (100)

Because Total fungi count in soybean meal is an important indicator of its quality, results in table (2) indicated high fungi contamination (100%) was observed in all samples, the highest level of contamination was in (82) samples where the number of Total fungi count ranged from ($1.2\times10^4$ - $6.7\times10^4$ CFU/g) while the lowest level of contamination was in (10) samples with Total fungi count ranged from ($7.5\times10^3$ - $9.8\times10^3$ CFU/g), both these two groups samples were acceptable according to Iraqi standard specification which limited down ($10^5$ CFU/g) as safe limit

is required for soybean meal used as animal feed, except the third group (8) samples were unacceptable because of its value ($2.2\times10^6$ CFU/g) which is more than safe limit ($10^5$ CFU/g), in pervious study by Al-Seeni (2012) [12] reported the results of (20) soybean meal samples show total counts of fungi were ranged from ($3.9x10^4$ to $10^5$) CFU/g.

**Table 2:** Total fungi count in imported soybean meal samples

| | Total fungi counts (CFU/g) | No. of sample |
|---|---|---|
| 1 | $7.5\times10^3$ - $9.8\times10^3$ | 10 |
| 2 | $1.2\times10^4$ - $6.7\times10^4$ | 82 |
| 3 | $2.2\times10^6$ | 8 |
| | Total No. of samples | 100 |

Results of mycological tests in table (3) shows presence of mycotoxigenic fungi: the most frequently isolates were *Fusarium* spp. (35) isolates with Fr. (35%) but RD. (42%), then *Aspergillus flavus* (25) isolates with Fr. (25%) and RD. (30%) which they are all producing for aflatoxin when there are tested a rapid identification method by ammonia vapor, then *Penicillium* spp. (13) isolates with Fr. (13%) and RD. (16%). Finally *Alternaria* spp. were (10) isolates with Fr. (10%) but RD. (12%) which it's occurrence consider as an indicator of recently harvested soybean which means high quality of the examined soybean samples as it reported by Christensen (1987) [5]. Another study by Seyed Soheil (2016) [20] was isolated and identified: (26) *Aspergillus* isolates, (30.5) *Fusarium* isolates and (4.3) *Penicillium* isolates in (22) soybean meal samples.

**Table 3:** mycotoxogenic fungi in imported soybean meal samples

| fungal species | No. of fungi isolates | Fr (%) | RD (%) |
|---|---|---|---|
| *Aspergillus flavus* | 25 | 25 | 30 |
| *Fusarium* spp. | 35 | 35 | 42 |
| *Penecillium* spp. | 13 | 13 | 16 |
| *Alternaria* spp. | 10 | 10 | 12 |
| Total No. isolates | 83 | | |

Because of growth of mycotoxigenic fungi, we observed Mycotoxin incidence and results in table (4) show total Aflatoxin presence in (66) samples, it's quantities at average (3.075 ppb), minimum (1.0 ppb) for 33 samples and maximum (5.0 ppb) for 10 samples, distributed between them, (2.5 ppb) for 8 samples and (3.7 ppb) for 15 samples as it shown in fig (1) while we noted non-presence of total Aflatoxin in (34) samples, all these samples were acceptable according to Iraqi standard specification which limited (< 20 ppb) for Aflatoxin as a safe limit as we known, it's production may occur in the field, processing, storage and depending on the level and duration of exposure this toxin become carcinogenic and hepatotoxic. On the other hand, T2/ HT2 toxin presence was much higher than other mycotoxins in (78) samples, it's quantities at average (57.0 ppb), minimum (6.5 ppb) for 32 samples and maximum (103.5 ppb) for 20 samples, distributed between them, (40.0 ppb) for 15 samples, (55.0 ppb) for 8 samples and (80.0 ppb) for 3 samples as it shown in fig (2) while we noted non-presence of T2/ HT2 toxin in (22) samples.

Finally ochratoxin presence was in (45) samples, it's quantities at average (3.7 ppb), minimum (1.9 ppb) for 9 samples and maximum (6.5 ppb) for 20 samples, distributed between them, (2.8 ppb) for 5 samples and (3.6 ppb) for 11 samples as it shown in fig (3) while we noted non-presence of ochratoxin in (55) samples, Although the tested soybean meal samples were not contaminated with highest level of mycotoxins which exceeded the regulatory limit but we observed more than one mycotoxin being present in tested soybean meal samples because as it is known that the same fungus species can synthesize many mycotoxins, also fungi that produce different mycotoxins which lead to exposure the animal to a mixture of several mycotoxins by feed consumption, as it has been reported by Binder E.M (2007) [4], and these multiple concentration of different mycotoxins when accumulated in the body causing diseases such as cancer and this may be one of reasons of spreading of cancer in Iraq,

There are study by H. Hüseyin ORUC, *et al*. (2007) [11] was reported T2/HT2 toxin quantities ranged from (11.90 to 16.50 µg/kg) in 6 soybean meal samples, addition to Ghulam Fareed, *et al*, (2014) [6] was reported for all 12 soybean meal samples tested, concentration of aflatoxin was maximum level 56.9 µg/kg.

**Table 4:** mycotoxins in imported soybean meal samples

| Mycotoxin type | No. of undetected samples* | detected samples | | | |
|---|---|---|---|---|---|
| | | No. of samples | Average ppb | Minimum ppb | Maximum ppb |
| Total Aflatoxin (ppb) | 34 | 66 | 3.075 | 1.0 | 5.0 |
| T2/HT2 toxin (ppb) | 22 | 78 | 57.0 | 6.5 | 103.5 |
| Ochratoxin (ppb) | 55 | 45 | 3.7 | 1.9 | 6.5 |

* Undetected samples: which concentration is (0 ppb)

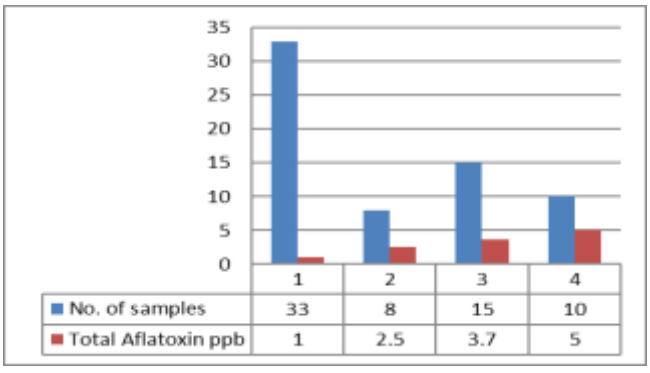


**Fig 1:** Total Aflatoxin ppb in imported soybean samples

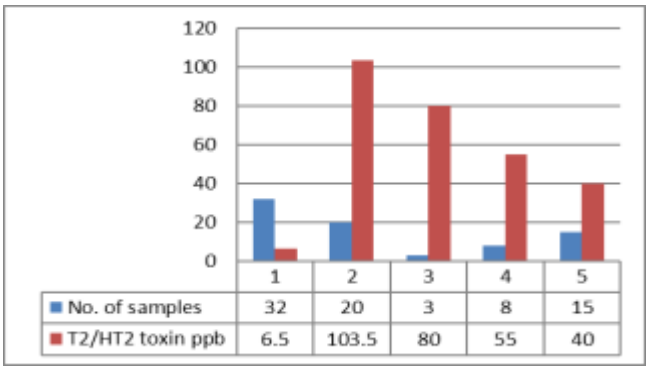


**Fig 2:** T2/HT2 ppb in imported soybean samples

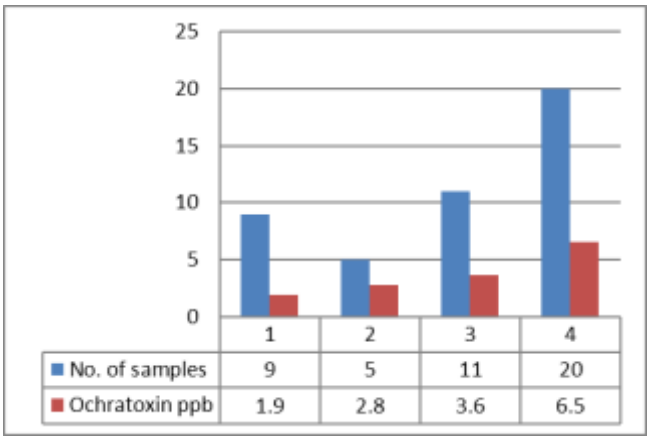


**Fig 3:** Ochratoxin ppb in imported soybean samples

it is clear from all results of this study, these soybean meal samples because its characteristics: high protein, high water content, and neutral pH, it appeared to be a rich substrate for pathogens effecting health which are five bacterial species, four mycotoxogenic fungi and three mycotoxin together in same sample which lead to a big severity due to increase multiple risk for pathogens and its mycotoxin, this case resulting animal diseases and sometime death causing a loss economic in addition to human diseases by contaminated animal products consumption. Therefore it is important to soybean meal are tested before giving to animal.

## Conclusion

the results show microbial contamination in most samples and to avoid the severity, its necessary to ensure animal and human health against mycotoxins, governmental agencies need to inform both importers and dairy companies about the severity of mycotoxins to force them to apply a control measure and suitable storage condition because some business operators are disregard a good practices of production and manufacturing.

## References


1. AEJ Okwori, Enokela MO, Nwankiti OO, Shidali NN, Bacteriological, Contamination of Soya Bean Flour Sold in Makurdi Metropolis, Benue State Nigeria, Journal of Medical Laboratory Science. 2010; 19(2):32-36.
2. Aleksandra Sergije Bočarov, Stančić Aleksandra Sergije Bočarov, Stančić Natasa Salma Vladimir Pantic, Microbiological and mycotoxicological correctness of protein feed ingredients in Vojvodina, Zbornik Matice srpske za prirodne nauke. 2011; 120:213-220.
3. Asharaf HR, White M, Klubek B. Microbiology survey of tofu sold in a rural Illinois county. J. Food Protect. 1999; 62:1050-1053.
4. Binder EM, Tan LM, Chin LJ, Handl J, Richard J, Worldwide occurrence of mycotoxins in commodities, feeds and feed ingredients. Anim. Feed Sci. Technol. 2007; 137:265-282.
5. Christensen CM. Field and storage fungi, In: Beuchat L R. (eds) Food and beverage mycology, Van Nostrand Reinhold, New York, 1987, pp. 211-232.
6. Ghulam Fareed, Sohail Hassan Khan. Muhammad Ashraf Anjum, Naveed Ahmed, Determination of Aflatoxin and Ochratoxin in poultry feed ingredients and finished feed in humid semi-tropical environment, J. Adv. Vet. Anim. Res. 2014; 1(4):201-207.
7. FAO (Food and Agriculture Organization of the United Nations). Animal production and health, protein sources for the animal feed industry, ISBN 92-5- 105012-0, Rome, Italy, 2004.
8. Dhaher FH, Awni MDH, Mahmood NR, Jamil MM, Rasheed HS. Isolation and Diagnosis of Salmonella in Animal Origin Food, Import feed in Baghdad Local Markets and Local Poultry Farms, Iraqi journal for market researches and consumer protection. 2011; 3(5):1-19.
9. González HHL, Resnik SL, Boca RT, Marasas WFO. Mycoflora of Argentinean corn harvested in the main production area in 1990. Mycopathologia. 1995; 130:29-36.
10. Holt JG, Krieg NR, Sneath PHA, Staley JT, Williams ST. Bergy's manual of determinative bacteriology. 9th ed. Williams and Wilkins, Baltimore, USA, 1994.
11. Hüseyin ORUC H, Murat Cengiz, İlknur Uzunoğlu. Occurrence of Aflatoxin B1 and T-2 Toxin in Feed and Raw Ingredients Used for Animal Feeding Stuffs, Uludag Univ. J. Fac. Vet. Med. 2007; 26:1-2, 1-5.
12. Madeha Al-Seeni N. Natural occurrence of heavy metal, fungi and mycotoxins in soybean meal samples used in animal feeding in Saudi Arabia, African Journal of Biotechnology. 2012; 11(38):9288-9294.
13. Muhsin Al-Musawi L, Siham Hussein B, Marwa Gatoof J, Noor Alhuda S, Hanash Baraa Z, Abdulkareem, Bacterial contamination of imported poultry feed in Iraq, Pharmaceutical and Biological Evaluations. 2016; 3(issue 5):495-499.
14. Neogen Corporation. Mycotoxin handbook, third edition, 2013. www.neogen.com.
15. Pitt JI. Toxigenic fungi and mycotoxins. Br. Med. Bull. 2000; 56(1):184-192.
16. Pitt JI, Hocking AD. Fungi and Food Spoilage, Springer, Berlin, Germany, 3rd edition, 2009.
17. Prestamo G, Fontecha J. High pressure treatment on the tofu fatty acids and acylglycerols content. Innovat. Food Sci. Emerg. Tech. 2007; 8:188-191.
18. Yaling W, Tongjie C, Guozhong L, Chunsan Q, Huiyong D, Meiling Y, *et al*. Simultaneous detection of airborne aflatoxin, ochratoxin and zearlaenone in poultry house by immunoaffinity column and high performance liquid chromatography. Environ. Res. 2008; 107:139-144.
19. Saito M, machida S. Arapid identification method for aflatoxin- producing strains of Aspergillusflavus and A. parasiticus by ammonia vapor. mycoscience. 1999; 40:205-208.
20. Seyed Soheil Ghaemmaghami, Mehrdad Modirsaneii, Ali Reza Khosravi, Mehdi Razzaghi-Abyaneh. Study on mycoflora of poultry feed ingredients and finished feed in Iran, Iran J Microbiol. 2016; 8(1):47-54.
21. Wierup M, Häggblom P. An assessment of soybeans and other vegetable proteins as source of Salmonella contamination in pig production.Acta Vet Scand. 2010; 52:15. [PMC free article] [PubMed].
22. Vojtech Rada, Martina Lichovnikova, Ivo Safarik. The effect of soybean meal replacement with raw full-fat soybean in diets for broiler chickens, JOURNAL OF Applied Animal Research. 2017; 45(1):112-117.