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INCIDENCE OF MOLD AND AFLATOXIN IN POULTRY MEAT PRODUCTS AND THE ROLE OF ORGANIC ACIDS AND PROBIOTICS IN THEIR CONTROL

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ABSTRACT

The present study aimed to assess mold contamination and aflatoxin levels in some local chicken meat products, as well as evaluate the impact of organic acids and probiotics on reducing these contaminants. In this context, a total of 120 samples of chicken meat products (fillet, sausage, burger, nuggets, and luncheon) were collected from various markets in the Sharkia governorate, Egypt. The samples were subjected to mold contamination assessment using culture techniques and aflatoxin levels measurement using competitive direct ELISA. Treatments with organic acids and probiotics were applied as well. *Aspergillus* species were the most common mold detected, with *Aspergillus flavus* being dominant in 73.5% of the samples. Aflatoxin was found in 58.3% of chicken sausage and burger samples and in 41.7% of chicken nugget samples, however, none exceeded the permissible limits except five luncheon samples. Applying organic acids to chicken fillet samples significantly reduced the mold count to 47% and 49% for the concentration of 2% of citric acid and acetic acid, respectively. Concurrently, probiotics in minced meat achieved a reduction in aflatoxin levels up to 75% and 84% for *Lactobacillus acidophilus* and *Bifidobacterium lactis*, respectively, after 6 days. The findings recommend using organic acids and probiotics as effective interventions for reducing mold contamination and aflatoxin levels in poultry products for enhancing food safety in Egypt.

Key words: Mold, Aflatoxins, Poultry products, ELISA, Probiotics, Organic Acids.

INTRODUCTION

Molds, the microscopic fungi that grow on plant or animal matter, producing their toxic metabolites (mycotoxins), are considered a potential hazard for food consumers at different ages and circumstances worldwide. As a ubiquitous microorganism, molds can contaminate a wide variety of foodstuffs, constituting an economic and public health burden. On the large list of foodstuffs, poultry meat and its products constitute main sources of protein, vitamins, and essential polyunsaturated

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fatty acids. Moreover, its availability and low cost made it the first-choice meat source in many developing countries. On the other side, contamination of poultry meat during different preparation stages can happen, being a crucial public health and economic hazard, especially mold contamination. Most cereals used in poultry feed, including maize, groundnuts, and wheat, exposed to be contaminated with mycotoxins, particularly aflatoxins (Ráduly *et al.,* 2020). In chicken feeds, aflatoxins have been found anywhere between 64% and 100% of the time (Aboagye-Nuamah *et al.,* 2021).

Mycotoxins greatly affect the health of humans and animals, as well as lead to economic impact by affecting public health, trade, and the marketing of food products (Meneely *et al.,* 2022). The Food and Agriculture Organization, FAO, reported that about twenty-five percent of annual crops are affected by various mycotoxins all over the world, with subsequent losses of about one billion tons of food and food products each year (Smith *et al.,* 2016). Furthermore, billions of people are exposed to mycotoxins via crops, spices, meat, coffee, and dairy products (Marc, 2022).

Aspergillus species is considered one of the most common fungi isolated from poultry meat (Ghanem *et al.,* 2022). The greatest danger behind *Aspergillus* contamination lies in the carcinogenic mycotoxin secretion .Aflatoxins, a family of mycotoxins produced by *Aspergillus flavus* and *Aspergillus parasiticus,* are associated with a great threat of liver cancer.

Despite considerable research on aflatoxin contamination in the literature, there is still low aflatoxin awareness. This gap of awareness is further exacerbated by food insecurity and lack of regulatory limits, which contribute profoundly to the high levels of aflatoxin exposure in developing populations. In this respect, raising awareness as well as finding effective control methods to reduce mold growth and aflatoxin residues in food became an urgent challenge. Numerous studies have suggested several physical, chemical, and biological methods for the elimination of these hazards. For example, altering the favorable environmental conditions for molds by changing humidity and O2 exposure, using irradiation, applying adsorbents in food that bind and remove aflatoxins, and intervening with chemical agents such as acids, enzymes, and gases were discussed (Sipos *et al.,* 2021). U.S. Department of Agriculture (2008) stated that acetic and citric acids are generally recognized as safe (GRAS) in food processing. Commonly used on meat surfaces, inexpensive, simple, fast, and proved obvious antimicrobial efficacy (Hinton and Corry, 1999). And so, probiotics work as biological absorbents of aflatoxins and decrease their transmission to the human or animal gut (Ahlberg *et al.,* 2015). From this standpoint, our current study aimed to assess the prevalence of mold and aflatoxin contamination in select local chicken products, highlight the associated health hazards, and identify safe and effective methods, such as organic acids and probiotics, to reduce their levels in food.

MATERIALS AND METHODS

1. Sample collection and preparation

120 random samples of poultry products (chicken fillet, chicken sausage, chicken burger, chicken nuggets, and chicken luncheon (24 of each) were collected from different markets in Sharkia governorate, Egypt. Aseptically transferred to the Zagazig laboratory, where mycological examinations were done. From each sample, 25 g were blended in 225 mL of sterile buffered peptone water 0.1% with subsequent decimal dilution ISO 6887-3 (2017).

2. Determination of the total mold count (BAM, FDA, 2001)

Aseptically pipet 0.1 ml of dilutions on pre-poured, solidified Dichloran rose bengal chloramphenicol (DRBC) agar plates and spread inoculum with a sterile bent glass rod. Incubate plates in the dark at 25°C for 5 days**,** then count the plates. If there is no growth, an additional 48 h of incubation was required. The identification of mold colonies by macroscopic and microscopic observation.

3. Evaluation of proteolytic and lipolytic activity of isolated mold

Colonies were cultured on casein hydrolysis medium supplemented with skim milk at 30°C for seven days for detection of proteolytic activity, which appeared as a clear zone around the colony (Paterson and Bridge, 1994). The lipolytic activity of mold was carried out on Tween 80 for 7 days at 30°C, according to Ullman and Blasins (1974). Positive lipolytic activity was seen as an opaque zone around the colony due to the liberation of oleic acid by mold enzyme that interacts with calcium salt crystals.

4. Estimation of total aflatoxins

Total aflatoxins were estimated via a competitive direct enzyme-linked immunosorbent assay (CD-ELISA) method (Lesczcynska *et al.,* 2018).

5. Experimental reduction of mold and aflatoxin

5.1. Effect of organic acids on total mold count

Organic acids used:

Acetic acid glacial 99-100% (that was prepared with sterile distilled water to reach (1% & 2%).

Citric acid anhydrous oral that was prepared with sterile distilled water to reach (1% & 2%).

Acetic acid 1%- citric acid 1% mixture

Chicken fillet samples with a known mold count (3.05 $\log_{10}$cfu) were dipped for 15 min in acetic acid 1% and 2%, citric acid 1% and 2%, and a mixture of both acids 1% separately; the treated groups were mycologically examined. The experiment was conducted in triplicate.

5.2. Effect of probiotics on aflatoxin content in chicken fillet (Ibrahim *et al.*, 2018)

Lactobacillus acidophilus, and *Bifidobacterium lactis* were refreshed by culturing on De Man Regosa & Sharp medium (MRS) broth and agar at 37°C for 24 hrs for 3 sequential times. The inoculated broth was centrifuged at 1.700 X g for 15 minutes. The supernatant was discarded, and the bacterial pellet was washed with phosphate buffered saline (PBS), and then their concentration was adjust to obtain the inoculum levels 10^7cfu/ml (Eid *et al*., 2015).

A chicken fillet sample that contains a known amount of aflatoxin (3.88 ppb) that was estimated using ELISA was divided into two groups, one group inoculated with *Lactobacillus acidophilus* and another with *Bifidobacteriuml actis* in a concentration of 10^7cfu/g. Then examine the samples for total aflatoxin at zero time, then day after day, ELISA.

6. Statistical analysis

The mycological counts were converted to the base-10 logarithms of the number of colony-forming units per g of examined samples (logCFU/g). Mean ± standard errors (SE), minimum, and maximum were calculated. Differences between log cfu/g of control and treated samples were calculated as log reduction of treatments was compared by analysis of variance (ANOVA) tests by the general linear models of SPSS 14.0 for Windows.

RESULTS

Table 1: Frequency and count of mold (log_{10} CFU/g) of examined chicken meat products (n = 24 each).

Samples	Positive samples		Min.	Max.	Mean counts ± SE
	No.	%			
Chicken fillet	5	20.8	1.70	3.08	**2.16 ±0.24**
Chicken sausage	13	54	1.95	2.60	**2.34 ± 0.04**
Chicken burger	20	83	1.95	2.48	**2.19 ± 0.05**
Chicken nuggets	12	50	1.85	2.48	**2.18 ± 0.05**
Chicken luncheon	3	12.5	2.00	2.18	**2.11 ± 0.06**

Table 2: Frequency of mold genera in examined chicken meat products.

Samples	***Aspergillus***		***Penic-illium***	***Alter-naria***	***Curv-ularia***	***Clado-sporum***	***Sporo-tricum***	***Mucor***
	A. flavus	***A. niger***						
Chicken fillet **(5)**	1 (20%)	4 (80%)	3 (60%)	-	3 (60%)	-	-	2 (40%)
Chicken sausage **(13)**	7 (53%)	-	3 (23%)	2 (15.4%)	2 (15.4%)	1 (7.6%)	-	1 (7.6%)
Chicken burger **(20)**	18 (90%)	4 (20%)	3 (15%)	3 (15%)		5 (25%)	-	2 (10%)
Chicken nuggets **(12)**	10 (83%)	3 (25%)	5 (41.6%)	5 (41.6)	-	1 (8.3%)	1 (8.3%)	-
Chicken luncheon **(3)**	3 (100%)	-	2 (66.6)	-	-	-	-	-
Total	39	11	16	10	5	7	1	5

Table 3: Lipolytic and proteolytic activities of the isolated molds.

		Proteolytic				**Lipolytic**			
	No	**+ve**	**High**	**Moderate**	**Weak**	**+ve**	**High**	**Moderate**	**Weak**
Aspergillus flavus	**39**	**36**	**30**	**6**	**-**	**35**	**35**	**-**	**-**
Aspergillus niger	**11**	**11**	**10**	**-**	**1**	**10**	**10**	**-**	**-**
Penicillium	**16**	**16**	**14**	**2**	**-**	**16**	**10**	**3**	**3**
Alternaria	**10**	**10**	**7**	**-**	**3**	**9**	**-**	**3**	**6**
Curvularia	**5**	**-**				**-**			
Cladosporum	**7**	**6**	**6**	**-**	**-**	**7**	**-**	**3**	**4**
Sporotricum	**1**	**-**				**1**			**1**
Mucor	**5**	**5**		**2**	**3**	**4**			**4**

High activity (H): more than 11 mm; moderate (M): 6-10 mm; weak (W): less than 5 mm.

Table 4: Aflatoxin residues (µg/ kg) of examined chicken meat products (N=24 for each)

Samples	Positive samples		Min.	Max.	Mean counts ± SE	Samples exceed PL(20*, 0**)	
	No.	%				No.	%
Chicken fillet	4	16.7	0.16	3.88	1.62 ±0.83	0	0
Chicken sausage	14	58.3	0.30	6.02	1.91 ± 0.52	0	0
Chicken burger	14	58.3	0.15	5.25	2.09 ± 0.43	0	0
Chicken nuggets	10	41.7	0.12	1.02	0.47 ± 0.09	0	0
Chicken luncheon	5	20.8	0.17	1.88	0.92 ± 0.21	5	20.8

***:** permissible limits of aflatoxins in food according to FAO 2004,
****:** permissible limits of aflatoxin in poultry luncheon according to ES 1696 (2005)

Table 5: The effect of organic acid on total mold count in chicken fillet sampls

Treated groups Durations	Control	Acetic acid 1%	Acetic acid 2%	Citric acid 1%	Citric acid 2%	Acetic-citric acid 1% mixture
Mean ± SE	3.05± 0.012^{a}	1.87± 0.017^{b}	1.56± 0.04^{d}	1.83± 0.02^{bc}	1.63± 0.03^{d}	1.75± 0.03^{c}
Log reduction (Reduction %)	--	1.18 (39%)	1.49 (49%)	1.22 (40%)	1.42 (47%)	1.3 (43%)

Table 6: The effect of probiotics on percentage of total aflatoxins in contaminated chicken fillet sample*s*

Probiotic strain	Zero day		2nd day		4th day		6th day	
	AF conc.	R %	AF conc.	R %	AF conc.	R %	AF conc.	R %
Lactobacillus acidophilus	3.88	-	2.89	26	1.45	63	0.97	75
Bifidobacterium lactis	3.88	--	2.54	35	1.03	73	0.62	84

AF conc.**:** Aflatoxin concentration R %**:** Reduction percent

DISCUSSION

As mentioned in the result section, particularly Table 1, the information clarified the variation of mold count and prevalence among different tested products, which reflects an enormous difference in their exposure to contamination.

For mold count, the mean count among all products ranged from 2.11 to 2.34 log10 CFU/g; almost equivalent results were recorded by Shaltout *et al.* (2014), where the mean mold count among chicken meat products ranged from 2.2 to 2.5 log10 CFU/g, and while slightly higher results were mentioned by Hussein (2021), where the mean count among all examined samples ranged from 1.9 to 3.4 log10 CFU/g.

Regarding the prevalence of mold contamination among the tested chicken meat products, the chicken burger had the highest rate of 83%. This high prevalence rate may return to the potential contaminated additives added to the burger and storage conditions. On the other hand, chicken luncheon recorded the lowest prevalence rate of 12.5%, which may return to the exposure of luncheon to heat during manufacturing process steps. In

contrast, higher results (60%) were recorded before (Shaltout *et al.*, 2014).

Concerning chicken sausage, it recorded a lower prevalence rate than burger, where the highest mean mold count among tested products was 2.34 ± 0.04 log10 CFU/g. This could indicate that sausage hold higher levels of mold due to their nature and processing steps that may be a favorable environment for mold proliferation.

It is noteworthy that chicken fillets, the least processed samples among the tested products, exhibited a significant mold count (2.16 ± 0.24 log10 CFU/g), which may reflect the power of cross-contamination during handling rather than processing.

Data in Table (2) highlighted the prevalence of mold genera in examined chicken meat products. Evidently, there was a predominance of certain species, such as *Aspergillus* spp., *Alternaria,* and *Cladosporium*, with the assertion of the intense prevalence of *Aspergillus* species, particularly *Aspergillus flavus* and *Aspergillus niger.* In accord, Darwish *et al.* (2016) isolated four mold genera, namely, *Aspergillus* spp., *Penicillium* spp., *Cladosporium* spp., and *Alternaria* spp., from frozen chicken giblets and meat cuts. Approximately, comparable results were recorded by Abdallah *et al.* (2021), where *Aspergillus* spp. (*flavus* and *niger*) was positive in about 55% of examined samples. Moreover, Abuzaid *et al.* (2020) also detected *A. flavus* and *A. niger* in 40% and 80% of the examined samples of chicken origin, respectively.

It is worth mentioning that *A. Flavus* is a major producer of aflatoxin, which is a crucial carcinogen (Klich, 2007). On the other hand, despite not being a key producer of aflatoxin, *A. niger* is still a potential hazard that can cause food spoilage and allergic reactions. Regarding the prevalence of such genera, it may return to their ability to grow over a wide range of temperatures. Furthermore, they are spore-forming microorganisms and can stand very low oxygen environments (Plahar *et al.*, 1991).

The lipolytic and proteolytic activities of the isolated molds are illustrated in Table 3. Foremost among the molds, *Aspergillus flavus* showed high proteolytic activity in 30 out of 39 isolates, with moderate activity in 6 isolates. Additionally, its lipolytic activity was recorded as high in 35 isolates. Furthermore, *Aspergillus niger* showed high proteolytic and lipolytic activities in 10 isolates, with only one isolate detected as having weak proteolytic activity. *Penicillium* species exhibited high proteolytic activity in 14 isolates, with 2 demonstrating moderate activity. Lipolytic activity recorded high levels in 10 *Penicillium* isolates and moderate in 3. Pertaining to *Alternaria* species, high proteolytic activity was recorded in 7 isolates and moderate activity in 3, with high and moderate lipolytic activity in 3 and 6 isolates, respectively. Comparable results were detected among the other remaining molds, where *Cladosporium* species caused high proteolytic activity in 6 isolates, while lipolytic activity was moderate in 3 isolates and weak in 4 isolates. In addition, 2 isolates and 3 isolates were of moderate and weak proteolytic activities, respectively. Besides, 4 isolates were of weak lipolytic activity in *Mucor* species. *Sporotrichum* and *Curvurlaria* species showed no proteolytic activity, yet *Sporotricum* exhibited only one isolate with weak lipolytic activities.

According to Table (3), which provided the lipolytic and proteolytic activities of the molds isolated from chicken meat. *Aspergillus flavus* showed the highest enzymatic activities with 30 isolates, and 35 isolates exhibited high proteolytic and lipolytic activities, respectively, succeeded by *Aspergillus niger* and *Penicillium* with notable high enzymatic activities. Meanwhile, there were moderate to weak

activities among some genera. Nearly similar results were recorded by Ouf *et al.* (2010) and Shaltout *et al.* (2014).

The incidence of molds of high proteolytic and lipolytic activities in food is a crucial concern for food quality and safety. High proteolytic activity suggests that mold can significantly degrade the proteins, accelerating the spoilage and deterioration of food. On the other hand, high lipolytic activity is related to off-flavor and rancidity due to the breakdown of fats.

The presence of aflatoxin residues in different chicken meat products was assessed in Table (4). The chicken burger samples recorded the highest mean aflatoxin residue concentrations of 2.09 ± 0.43 µg/kg, followed by chicken sausage samples of 1.91 ± 0.43 µg/kg. Remarkably, none of the samples exceeded the permissible limit of 20 µg/kg according to FAO (2004), except for contaminated luncheon samples, 5 of 24 (20.8); chicken luncheon should be free from fungal growth and their toxins according to ESO 1695 (2005). There are no available established norms for other chicken meat products in Egypt.

The highest incidence of aflatoxin is in burger and sausage, followed by nuggets, luncheon, and fillet. This may be due to the frequent unhygienic handling and processing of meat, especially when additives of low quality, such as flavorings especially spices, were used. Darwish *et al.* (2016) noted that the liver and muscle tissues of chickens contain the most aflatoxin residues, as opposed to any other organs. In the study of El Asuoty *et al.* (2023), they found that chicken muscles and livers were exposed to different degrees of contamination during meat processing. Concerning data in tables 1 and 4, processed chicken products, in particular those with higher mold contamination, are more inclined to harbor high aflatoxin residue levels.

Table (5) declared the effect of organic acids on total mold count in chicken fillet samples. Groups treated with 1% acetic acid and 1% citric acid showed a significant reduction in mold count, compared to the control group, with no significant difference between the two treatments. However, the combination of 1% acetic and 1% citric acids exhibited a significant reduction in mold count, compared to the control and 1% acetic acid groups, yet no significant difference was recorded, compared to the 1% citric acid group. A substantial, significant difference was observed between the control group and both the 2% acetic and 2% citric acid groups. Meanwhile, no significant difference was detected between the two treatments themselves. In this framework, Hassan *et al.* (2012) reported that 10% acetic acid and 10% citric acid achieved a reduction of *Aspergillus flavus* by 45.42% and 17.71% and *penicillium purpurogenum* by 40.92% and 20.16%, respectively. Obtained results indicated that both acetic and citric acid were effective in reducing mold growth, with acetic acid demonstrating slightly higher efficacy. This came in alignment with the findings of El-shemy *et al.* (2016), who reported that the antimicrobial activity of acetic acid is higher than citric acid. In addition, Dalie *et al.* (2010) reported that acetic acid was more effective than lactic acid and was the best inhibitor for fungal growth.

Data in Table (6) highlighted the impact of *Lactobacillus acidophilus* and *Bifidobacterium lactis* on aflatoxins in contaminated chicken fillet samples over 6 days. The samples were examined for total aflatoxin levels at zero-time, 2, 4, and 6 days of treatment. After 6 days, the reduction percentages were 75% for samples treated with *Lactobacillus acidophilus* and 84% for those treated with *Bifidobacterium lactis.* These findings align roughly with results reported by Ibrahim *et al.* (2018), who recorded slightly higher reduction percentages of 88%

and 98.2% for *Lactobacillus acidophilus* and *Bifidobacterium lactis*, respectively, in similar treatment groups. In this respect, El-Nezami *et al.* (1998) found that probiotics had binding ability with aflatoxins and removed them. Moreover, Al-Ruwaili (2018) tested the effect of LAB in yogurt whey added at 5% to drinking water of broiler chickens that fed AFB1-contaminated diets for 6 weeks. The result showed a significant reduction in aflatoxin concentrations of 55.46% and 59.35% in leg and breast, respectively, at the end of week 6 in the group that received whey in drinking water, emphasizing the profound effect of probiotics in reducing aflatoxin levels in chicken meat.

CONCLUSION

The results of the present study emphasize the potential use of organic acids and probiotics as safe and effective methods for controlling mold contamination and reducing aflatoxin levels in food, particularly in regions with low food safety regulations and low awareness levels of aflatoxin dangers.

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تقييم تواجد الفطريات و الأفلاتوكسين فى منتجات لحوم الدواجن ودور الأحماض العضوية والبروبيتيك فى التحكم فيها

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هدفت دراستنا إلى تقييم مستويات التلوث بالفطريات والأفلاتوكسين في بعض منتجات لحوم الدجاج المحلية وكذلك تقييم تأثير الأحماض العضوية والبروبيوتيك في الحد من هذه الملوثات. وفي هذا الإطار تم جمع ١٢٠ عينة من منتجات لحوم الدجاج المتمثلة في (فيليه، سجق، برجر، ناجتس، لانشون) من أسواق مختلفة بمحافظة الشرقية بمصر, حيث تم فحص العينات لتقييم التلوث بالعفن وقياس مستويات الأفلاتوكسين باستخدام الاليزا التنافسي المباشر. وكذلك المعالجة بالأحماض العضوية والبروبيوتيك أيضًا. وقد كان فطر الاسبريجلس هو الأكثر تواجدا، وبالتحديد فطر أسبريجلس فلافس بنسبة ٧٣,٥٪ من العينات. أما الأفلاتوكسين وجد بنسبة ٥٨,٣٪ من عينات نقانق الدجاج والبرغر وفي ٤١,٧٪ في عينات ناجتس الدجاج. ومع ذلك، لم يتجاوز أي منها الحدود المسموح بها فيما عدا عينات اللانشون الملوثة .وقد أدى تطبيق الأحماض العضوية على عينات شرائح الدجاج إلى تقليل عدد الفطريات بشكل ملحوظ إلى ٤٧٪ و٤٩٪ لتركيز ٢٪ من حمض الستريك وحمض الأسيتيك على التوالي. كما حقق استخدام البروبيوتيك انخفاضًا في مستويات الأفلاتوكسين بعد ٦ أيام بنسبة تصل إلى ٧٥٪ و٨٤٪ لكل من *Lactobacillus acidophilus* و *Bifidobacterium lactis* على التوالي. وبذلك توصي النتائج باستخدام الأحماض العضوية والبروبيوتيك كمعالجات فعالة للحد من تلوث منتجات الدواجن بالفطريات و الأفلاتوكسين .