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Shelf life of chilled broiler which fed on mycotoxins contaminated ration

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ABSTRACT

The aim of this study is to find out the relation between broiler feed contaminated with mycotoxins and shelf life of chilled broiler meat. A total of 50 samples of broiler feed and their meat collected (from the same farm) and analyzed for incidence of total aflatoxins and ochratoxin A and total fungal count. Meat samples examined for aerobic plate count, thiobarbituric acid values "TBA" and total volatile basic nitrogen values "TVN" on day of sample collection (zero day) to assess broiler meat quality. Such examinations were repeated on the chilled broiler meat at constant time interval (every three days) until meat spoilage. The results shown that 100% of samples contaminated with various levels of both mycotoxins. In broiler feed samples, mean value of total aflatoxins was 4.67±1.20 ppb, five samples (10%) exceeded FAO permissible limit; while, mean value of ochratoxin A was 6.73±0.73 ppb, twenty-six samples (52%) exceeded the permissible limit (5ppb). In broiler meat samples, mean value of total aflatoxins was 0.80 ±0.09 ppb; While, mean value of ochratoxin A was 1.80±0.47 ppb. All tested meat samples were within FAO permissible limits. The mean count of fungi of broiler feed was 3.45±0.19 (log10) cfu/g, while in meat samples were 2.12±0.08, 2.75±0.16 and 3.13±0.31 (log10) cfu/g on zero day, 3rd day and 6th day, respectively. Moreover, mean values of total aerobic plate count were 4.30±0.12, 4.58±0.11 and 5.50±0.16 (log10) cfu/g on zero day, 3rd day and 6th day, respectively. Results showed that 34% of samples became unacceptable on the 6th day of chilling. TVN

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mean values of broiler meat were 14.35±0.72, 16.23±0.33 and 19.41±0.44 mg % on zero day, 3rd day and 6th day, respectively. Resulting in 40% unacceptable samples on the 6th day of chilling. TBA mean values were 0.25±0.05, 0.38±0.04 and 0.70±0.18 mg malondialdehyde/kg on zero day, 3rd day and 6th day, respectively. Resulting in 30% unacceptable samples on the 6th day of chilling. All samples were totally spoiled on 9th day of chilling.

It could be concluded that broiler ration is the main source of mycotoxin and fungal contamination of broiler meat. The more mycotoxin and fungal contamination that broiler ration contain, the less broiler meat shelf life will be.

INTRODUCTION

Consumption of chicken meat increased worldwide due to the high content of essential amino acids, and their competitive price as they considered as good and low cost source of protein. Chicken meat is a significant source of protein in Egypt due to the lack of red meat production (**Hussein et al. 2018),** in addition to, poultry meat is nutritious, good flavor and simply digestible. Poultry meat has about 20 to 23% protein **(Smith, 2001).** On the other hand, poultry production continues to face a diverse range of challenges, including feed cost issues, bacterial and parasitic infection, heat stress, mycotoxins, etc., which can result in altered body composition, increased food safety concerns, and reduced meat yield and quality **(Choi and Kim, 2020).**

There is an association between the incidence of outbreaks of foodborne illness and consumption of the poultry meat. In most of the countries, poultry and poultry products ranked top foods to be associated with the diseases **(Lunden et al. 2003).**

It is suggested that yeast and molds play an important role in meat spoilage. Fungal contaminations in food is very useful indicator to evaluate the quality of food **(Taniwaki et al. 2001);** Fungi commonly contaminate meat and its products by causing spoilage with producing mycotoxins which further damages liver, cause liver cancer and food poisoning in humans **(Mossel, 1982).**

Mycotoxins, the secondary metabolites of fungi, such as the genera Fusarium, Aspergillus, etc., are frequently found in poultry feed **(Ochieng et al. 2021).** There are more than 400 mycotoxins with different absorption rates and effects on broiler chickens. For example aflatoxin causes mild negative effects on the gastrointestinal tract induced oxidative stress, damaged liver, damaged RNA and DNA and suppressed immune system **(Umaya et al. 2021 and Yunus et al. 2011).** Ochratoxin causes damaged RNA and DNA, induced oxidative stress, lipid peroxidation, cell apoptosis and inhibited protein synthesis and finally suppressed immune system **(Abdelrahman et al. 2022)**

Fungal and mycotoxins contamination of rations are unavoidable problem, aflatoxins and ochratoxin A are mycotoxins of greatest public health and agro economic significance. Mycotoxins remain a worldwide problem and account for millions of dollars lost annually in terms of human health, animal health and condemned agricultural products **(Zain, 2011).**

Consumption of aflatoxin and/or ochratoxin contaminated diet is potentially a health hazards for both humans and animals through induction of acute and chronic effects that have teratogenic, carcinogenic or immunosuppressive impact **(Felizardo and Câmara, 2013).**

Aflatoxins are implicated in human hepatocellular carcinoma **(Felizardo and Câmara, 2013),** while ochratoxin A is highly nephrotoxic, causing both acute and chronic lesions of the kidneys, and is implicated in urinary tract tumors **(IARC, 1993).**

Mould contamination of chicken meat indicates bad sanitary and hygienic conditions adopted during the production cycle. The growth of mould on chicken meat surfaces results in high economic losses and human health hazard. Mould commonly produces extracellular proteases and lipases resulting in degradation of protein and fats (**Sabotič and Kos, 2012 and Ghaly et al. 2010).**

Short period of shelf life of poultry meat at refrigerator temperature can not only be associated with its composition, but also with spoilage microorganisms present during poultry rearing and primary production. Such microorganisms can multiply at a relatively low temperature, and the result of their metabolic activity is manifested as product spoilage and consequently, they are the most important factors of chicken meat shelf life. The shelf life of poultry meat depends on the initial number of microorganisms, which emphasises the importance of hygienic conditions and control during various stages of the production process **(Yashoda et al. 2001).** The aerobic plate count considered as an index of quality, which gives an idea about the hygienic measures and help in assessing meat keeping quality **(Aberle et al. 2001).**

Decomposition processes are manifested by a change in specific sensoric and chemical properties of meat. In a majority of cases, the changes and the degree of contamination with microorganisms, and their biochemical activity, are in correlation with the meat ammonia content **(Bilgili, 2001 and Baeza, 2004).**

Thiobarbituric acid (TBA) and total volatile nitrogen (TVN) are reliable guideline tests for the quality of meat, meat products and various foodstuffs, where thiobarbituric acid (TBA) test is widely used to measure oxidative rancidity in fat containing food. It is a sensitive test to determine the decomposition of highly unsaturated fatty acid products (**Melton, 1983).**

Total Volatile Nitrogen (TVN) values increase in meat products with increasing storage period as the growth of microbes and proteolytic enzymes break down protein (ammonia**) (Alina and Ovidiu, 2007).**

Degradation of chicken meat due to chemical and/or physical factors can occur depending on the microbiological conditions of poultry carcasses **(Balamatsia et al. 2006).**

MATERIALS and METHODS

Sampling:

2.1.1 Samples collection

Fifty samples of broiler feed and meat (of the same farm) collected in the period from October 2022 to June 2023, from different farms in Beni-Suef Governorate, Egypt.

Samples preparation

Collected samples transferred to Animal Health Research Institute, Beni-Suef Branch to be examined. Broiler were slaughtered, meat samples were taken under good hygienic condition from broiler breast and thigh. Part of meat samples were examined fresh (zero day) and the other parts were chilled for examination later at constant time interval (each three days) until meat spoilage.

2.2. Microbiological indices:

2.2.1 Bacteriological examination: total aerobic plate count of broiler muscle samples was done according to (APHA, 2001) on days 0, 3, 6 and 9 of chilling.

2.2.2 Fungal examination: broiler feed and broiler muscle samples were prepared and examined for total fungal count according to the technique recommended by ISO (217-1-2) (ISO, 2008) broiler muscle samples were examined on days 0, 3, 6 and 9 of chilling.

2.3. Chemical indices:

2.3.1 Determination of total aflatoxins and ochratoxin A in broiler feed and broiler muscle samples using VICAM Series-4EX fluorometer according to the manufacturer's instructions (**AOAC, 1991).**

2.3.2 Determination of total volatile nitrogen (TVN), TVN was determined in broiler muscle samples on days (0, 3, 6 and 9) of chilling according to **(AOAC, 1992).**

2.3.3 Determination of thiobarbituric acid (TBA), TBA was determined in broiler muscle samples on days (0, 3, 6 and 9) of chilling according to **(Kirk and Sawyers, 1991).**

2.4. Statistical analysis:

Microbial counts were translated to cfu/g using base10 logarithms and collected data were statistically analyzed for maximum, minimum, mean and standard error using t-test, One-way ANOVA **(Graph Pad InStat, 2017).**

RESULTS

Results of total aflatoxins and ochratoxin A incidence in broiler feed and broiler meat are summarized in table (1), while table (2) contains fungal evaluation of broiler ration and meat and table (3) contains bacteriological evaluation of broiler meat. Finally, chemical evaluation of broiler meat is summarized in table (4).

Table 1. Incidence of total aflatoxins and ochratoxin A in some broiler feed and broiler meat (ppb).

Item	Broiler feed		Broiler meat	
	Total aflatoxins	Ochratoxin A	Total aflatoxins	Ochratoxin A
Minimum (ppb)	0.10	0.50	0.50	0.10
Maximum (ppb)	32	29.40	1.10	3
Mean±SE	4.67±1.20	6.73±0.73	0.80±0.09	1.80±0.47
No. of +ve samples	50	50	50	50
% of +ve samples	100%	100%	100%	100%
No. of samples exceeded MPL of FAO	5	26	-----	-----
% of samples exceeded MPL of FAO	10%	52%	-----	-----

ppb: part per billion.
MPL: Mean Permissible Limit

Table 2. Fungal evaluation of broiler ration and meat (values in log 10) cfu/g.

Item	Total fungal count of ration	Total fungal count of broiler meat		
		On zero day	On 3rd day	On 6th day
Minimum cfu /g	3.18	1.70	2.30	2.23
Maximum cfu /g	4	2.40	3.30	4.30
Mean ±SE	3.45±0.19	2.12±0.08	2.75±0.16	3.13±0.31

N=50 cfu /g : colony formatting unit per gram

Table 3. Bacteriological evaluation of broiler meat (values in log 10) cfu/g.

Item	Total aerobic plate count of broiler meat		
	On zero day	On 3rd day	On 6th day
Minimum cfu /g	4	4	5
Maximum cfu /g	4.77	5	6
Mean ±SE	4.30±0.12	4.58±0.11	5.50±0.16
* No. of acceptable samples	50	50	33
% of acceptable samples	100%	100%	66%
No. of unacceptable samples	-----	-----	17
% of unacceptable samples	-----	-----	34%

N=50 cfu /g : colony formatting unit per gram
* according to **Egyptian standards No. 1651 (2005)** as total aerobic plate count up to 10^5 cfu/gram.

Table 4. Chemical evaluation of broiler meat.

Item	T.V.N. of broiler meat mg %			T.B.A. of broiler meat mg/Kg		
	On zero day	On 3rd day	On 6th day	On zero day	On 3rd day	On 6th day
Minimum	12.60	15.40	18.20	0.17	0.26	0.44
Maximum	16.10	16.80	20.10	0.39	0.45	1
Mean±SE	14.35±0.72	16.23±0.33	19.41±0.44	0.25±0.05	0.38±0.04	0.70±0.18
*No. of acceptable samples	50	50	30	50	50	35
% of acceptable samples	100%	100%	60%	100%	100%	70%
No. of unacceptable samples	-----	-----	20	-----	-----	15
% of unacceptable samples	-----	-----	40%	-----	-----	30%

N=50
* The permissible limit of TVN is 20 mg / 100 gm and for TBA is 0.9 mg malondialdehyde/ kg **(Egyptian standards No. 1651, 2005)**

DISCUSSION

Results show that 100% of samples contaminated with various levels of both mycotoxins (Table 1). Total aflatoxins levels in broiler feed samples ranged from 0.10 to 32 ppb with mean of 4.67±1.20, five samples (10%) exceeded FAO permissible limit; while, ochratoxin A levels ranged from 0.50 to 29.40 ppb with mean of 6.73±0.73, twenty six samples (52%) exceeded the permissible limit (5ppb).

FAO (2004) has set 20 ppb as a maximum permissible limit for total aflatoxins and 5 ppb as a maximum permissible limit for ochratoxin A in cereals and cereal products.

Total aflatoxins levels in broiler meat samples ranged from 0.50 to 1.10 ppb with mean of 0.80±0.09; while, ochratoxin A levels ranged from 0.10 to 3.00 ppb with mean of 1.80±0.47. All tested muscle samples are within maximum permissible limits regulated by **FDA (2000)** and **FAO (2004).**

Food and Drug Administration **(FDA, 2000)** set 20 ppb as the maximum permissible limit of total aflatoxins and Food and Agriculture Organization **(FAO, 2004)** set 4ppb as the maximum permissible limit of total aflatoxins and 5ppb for ochratoxin in meat and meat products

High incidence of total aflatoxins and ochratoxin A in broiler ration samples agree with **Torky et al. (2003), Hassan et al. (2012) and El-Alfy and Abdein (2016)** who recorded nearly similar result in broiler ration in Assiut, Ismailia and Dakahalia Governorates, respectively.

The most important factors that influence growth and mycotoxin production are environmental temperature, substrate water activity, relative humidity, gas composition, substrate composition, inoculum concentrations, microbial interactions and mechanical or insect damage **(Guynot et al. 2003 and Giorni et al. 2007).**

In particular, the interaction between some or all of these factors that determines the increscent of fungal contamination and mycotoxins production. Interactions between available water and temperature are fundamental because fungi may be able to germinate, grow and actively compete for the allocation of the available resources (**Samapundo et al. 2005 and Marín et al. 2012).**

Higher levels of total aflatoxins and ochratoxin A residues in broiler liver samples recorded by **Ali and Hassan (2023)** in Beni-Suef Governorate as aflatoxins levels ranged from 0.51 to 6.52 ppb with mean of 2.15 ±0.72; while, ochratoxin A levels ranged from 1.90 to 5.40 ppb with mean of 2.55 ± 0.32.

Residues of mycotoxins in broiler muscles are less than in their liver, such trend agree with previous observation by **Hassan et al. (2012) and Darwish et al. (2016).**

Results of fungal evaluation of broiler feed and meat were recorded in table (2). Fungal examination of broiler feed revealed that, total mycological count ranged from 3.18 to 4 (log10) cfu/g with mean of 3.45±0.19. On the other hand, microbiological examination of broiler meat showed increase in both total fungal count and total aerobic plate count during chilling. Total fungal count ranged from 1.70 to 2.40 (mean value of 2.12±0.08), 2.30 to 3.30 (mean value of 2.75±0.16) and 2.23 to 4.30 (mean value of 3.13±0.31) (log10) cfu/g on zero day, 3rd day and 6th day, respectively. Moreover, total aerobic plate count ranged from 4 to 4.77 (mean value of 4.30±0.12), 4 to 5 (mean value of 4.58±0.11) and 5 to 6 (mean value of 5.50±0.16) (log10) cfu/g on zero day, 3rd day and 6th day, respectively. Results cleared that 34% of samples became unacceptable on the 6th day of chilling.

Results of fungal evaluation of broiler meat agree with the results recorded by **Shaltout et al. (2022) and Shaltout et al. (2016)** who examined broiler meat samples which had total fungal count of 2.85 and 2.83 (log 10) cfu/g, respectively. On the other hand, different counts were recorded by **Ogu et al. (2017)** who examined fresh and frozen broiler meat for total fungal counts, which ranged from 4.04 to 4.34 (log 10) cfu/g for fresh samples and from 2.11 to 2.6 (log 10) cfu/g for frozen samples.

Results of bacteriological evaluation of broiler meat recorded in table (3). Total aerobic plate count ranged from 4 to 4.77 (mean value of 4.30±0.12), 4 to 5 (mean value of 4.58±0.11) and 5 to 6 (mean value of 5.50±0.16) (log10) cfu/g on zero day, 3rd day and 6th day, respectively. Results cleared that 34% of samples became unacceptable on the 6th day of chilling. Similar total aerobic plate count was recorded by **Maharjan et al. (2019)** which was 4.45 (log 10) cfu/g, while higher counts were recorded **by Yassen et al. (2019) and Zakki et al. (2017)** who recorded counts of 8.23 and 7.9 (log 10) cfu/g. On the other hand, lower counts were recorded by **Enver et al. (2021**) revealing counts of 3.68 (log 10) cfu/g.

Total fungal and total aerobic plate counts increased during chilling, which agrees with **Bailey et al. (2000)** who examined broiler meat to determine the effect of different refrigeration and freezer temperatures on the microbiological profile, where total aerobic plate counts were about log 4.6 on day 0, increased by 2 log after 7 days. Moreover, inadequate cold preservation of broiler meat, giblets, and products may results in mould growth, especially, if the initial microbial load is high **(Morshedy and Sallam, 2009).**

Less qualified and hygienic handling of carcasses on preparation after slaughtering beside improper evisceration lead to highly bacterial count. In addition to bad chilling, proliferate mould contamination so food spoilage occur that enhance toxins production as aflatoxins (**Lacumin et al. 2009 and Martín-Sánchez et al. 2011).**

Results of chemical evaluation of broiler meat recorded in table (4). Chemical examination of chicken muscles showed the expected increase in TVN and TBA during chilling. TVN ranged from 12.6 to 16.1(mean value of 14.35±0.72), 15.40 to 16.80 (mean value of 16.23±0.33) and 18.20 to 20.10 (mean value of 19.41±0.44) mg % on zero day, 3rd day and 6th day, respectively, resulting in 40% unacceptable samples on the 6th day of chilling. TBA ranged from 0.17 to 0.39 (mean value of 0.25±0.05), 0.26 to 0.45 (mean value of 0.38±0.04) and 0.44 to 1 (mean value of 0.70±0.18) mg malondialdehyde/ kg on zero day, 3rd day and 6th day, respectively, resulting in 30% unacceptable samples on the 6th day of chilling.

All samples were totally spoiled on 9th day of chilling.

TVN results are relatively agree to Afifi (2000) who recorded TVN values of 13.87±0.18 mg% for fresh broiler breast and 12.57 ±0.22 mg% for fresh broiler thigh samples. While **Hasanine and Hassan (2003)** recorded higher results of TVN values 30.76±1.07 mg % for fresh chicken thigh samples.

Accordingly, Total Volatile Nitrogen (TVN) can be considered as a reliable indicative measure for the quality of various food articles specially meat and meat products. In general, TVN may be increased as the days of storage increased where protein break down (ammonia) may occur due to microbial growth and its proteolytic enzymes **(Alina and Ovidiu, 2007)**

TBA results are relatively agree to Bhawana et al. (2023) who recorded TBA values ranged from 0.20 to 0.34 mg/kg for fresh broiler thigh samples. In addition, **Moawad (1995)** recorded TBA values of 0.31 mg/Kg for fresh broiler breast. While, Shams El-Din and Ibrahim (1990) recorded higher results of TBA values 0.58±0.12 and 0.81± 0.15 mg/Kg for fresh chicken breast and thigh samples, respectively.

The oxidative rancidity in fresh, frozen and cooked broiler breast and thigh meat was evaluated by measuring malonaldehyde in fat meat with an improved thiobarbituric acid (TBA) assay with antioxidant protection (**Abd El-Kader, 1996).** Once oxidation has run its course, the oxidized food article will have essentially changed, chemically different from its original form and potentially toxic, which is why it is considered rancid and unusable **(Amato et al. 1989).**

CONCLUSION

Mycotoxins have negative effect on carcass quality and shelf life as a reason of high microbial contamination causing serious economic loses. Total fungal count of broiler meat considers an important factor in meat spoilage and increase their mycotoxins content. Fungal and mycotoxin contamination of broiler ration directly proportion to broiler meat spoilage and inversely proportion to broiler meat shelf life. Further researches are of importance to eliminate or reduce mycotoxins and their effect on chicken meat.

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