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Research Article
Influence of Dietary Garlic Supplementation on Mycological Assessment of Fresh and Frozen Quail Carcasses

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Abstract

Background and Objective: Fungi and their spores are everywhere in the environments and most of them are mycotoxigenic. These micro-organisms can possibly contaminate the meat during processing chain such as slaughtering, feather plucking, evisceration and storage. Thus, this study was conducted to assess the effect of dietary garlic (*Alliumsativum*) supplementation on the meat quality of quail carcasses through evaluation of mycological contamination that of public health concern. **Materials and Methods:** A total of 101 day old Japanese quail chicks were divided into two groups (n = 50/group): Control group without garlic supplement and treated group with garlic supplement for 42 days. After slaughter, surface samples were collected from breast and thigh muscles of fresh and frozen quail carcasses. **Results:** The results showed that total mold and yeast counts in garlic supplemented group were lower in breast and thigh muscles than control group. Moreover, a lower incidence of molds and yeasts were detected in garlic supplemented group (either breast or thigh muscles) as compared to control group. In particular, the mold and yeast contamination was higher in fresh quail carcasses than frozen ones. The isolated mold species were *Aspergillus* spp. (37, 22) *Mucor* (19, 11), *Penicillium* spp. (45, 29), *Fusarium* spp. (10, 5), *Rhizopus* (1, 1), *Alternaria* (2, 0) and *Absidia* spp. (1, 0) from control and garlic supplemented groups, respectively. The isolated *Aspergillus* strains were *A. flavus* (17, 9), *A. fumigatus* (9, 6) and *A. niger* (11, 7) from control and garlic supplemented groups, respectively. **Conclusion:** This study confirmed the importance of garlic supplementation as antifungal agent in the quail diet for a clear and significant reduction of mold and yeast loads in quail carcasses.

Key words: Garlic, fungi, *Aspergillus*, quail, carcass

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The world as a whole begins to search for new resources as protein. That's why, quails seem as an important source for protein which characterized by fine consistency and low cost. Quails meat is a very rich source in essential amino acids (oleic, linoleic, palmitic and stearic), ratio of poly unsaturated fatty acids: Saturated fatty acids[1] is 0.073. Furthermore, quails meat is a white meat contains low fat and cholesterol contents, but high iron content[2]. Dietary factors play a key role in the development of various human diseases.

Generally, the fungal contamination can occur at any stage of quail production[3]. Fungal growth may introduce a meat product to consumers containing aflatoxins, ochratoxins and other mold metabolites like antibiotics and allergens which represent a potential health hazard to consumers too[4].

Garlic supplements in birds have been recognized for their strong stimulating effect on the immune system and their very rich aromatic oils which enhance digestion in birds[5]. The key active ingredient in garlic is a powerful plant chemical called *allicin* which rapidly decomposes to several volatile organosulfur compounds with bioactivities[6,7]. Garlic contains sulfur-containing compounds such as cysteine sulfoxide that when chopped is converted by alliinase enzyme into thiosulfinates, that possess a well-documented antibacterial and antifungal activities[8]. Moreover, garlic exhibit antiatherosclerotic, hypolipdemic, antithrombotic, antidiabetic effects[9], antioxidant, anticarcinogenic, anti-inflammatory effects[10], chemopreventive and chemotherapeutic effects[11].

Therefore, the present study was performed to confirm the potential antifungal effect of garlic as well as to assess the meat quality characteristics of fresh and frozen quail carcasses of garlic treated group in comparing with control one.

MATERIALS AND METHODS

Ethics approval and consent to participate: All animal experiments were reared under 'Guide for the Care and Use of Laboratory Animals' approved by the Ethics Committee of the Faculty of Veterinary Medicine, Mansoura University (Permit numbers 20-17). This experiment was performed form February-July, 2018, in Veterinary Teaching Hospital, Faculty of Veterinary Medicine, Mansoura University at Dakahlia Province, Egypt.

Birds and experimental design: One hundred and one day old Japanese quail chicks were purchased from commercial farms (Mansoura Poultry Company, Egypt). All birds were reared in cages, kept in strictly isolated room under 23 h light and 1 h dark. Quails were divided into two groups (n = 50 per group): Control quails with no garlic supplement group and garlic supplemented group. Experimental birds in control group were provided with a commercial starter, balanced ration free from any medications. Birds in treated group were fed on the same ration supplemented with 0.5 g/100 g commercial dried garlic powder (El-Shrouk Company, Egypt) as feed additive. The feed and water were offered *ad libitum* to all the birds throughout the experimental period (42 days).

Sampling: All birds (n = 100) were starved for 4 h and sacrificed at the end of experiment (42 days). Surface samples were aseptically taken (10 cm^2) of each quail carcass at 4 different areas (right and left breast and right and left thigh muscles). The samples were collected in 9 mL tube containing sterile buffered peptone water. After sampling, breast and thigh muscles from each quail carcass were separately taken and packed into sealable polyethylene bags relative to each dietary treatment. One side form each muscle sample was frozen for 3 months immediately at -18°C, while the other side was chilled at 5+1°C for 2 days prior to frozen storage for 3 months. During freezing, the same areas of quail carcass were sampled at regular interval under chilling conditions until control of spoilage. All surface samples were obtained under aseptic conditions and subjected for mycological analysis.

Mycological analysis: The total mold and yeast counts were occurred according to APHA[12]. Briefly, the collected surface samples were incubated for 30 min/25°C and then10-fold serial dilutions were prepared. Two portions of 0.1 mL from each dilution were spread in parallel on the surface of malt extract agar medium and Czapek-Dox agar with 5% NaCl (Oxoid, Basingstoke, UK) and incubated aerobically in the dark at 25°C/5-7 days. The fungal count was expressed as colony forming unit per cm^2. Based on macroscopic and microscopic features, the dominating fungi in each sample was identified to genus level according to Raper and Fennell[13], Barnett and Hunter[14] and Samson *et al.*[15].

Statistical analysis: The obtained data were statistically analyzed using Pearson's chi-square exact test using the SPSS Statistics 17.0 software program. Variability in the data is expressed as standard error (SE) and a probability level of $p<0.05$ was considered as statistically significant.

RESULTS

Determination of total mold and yeast counts in quail carcasses: All quail birds were apparently healthy and mortality was not significant in the whole course of the experiment. According to Table 1 and 2, the effect of treatment with 0.5% garlic powder on mold and yeast count during the experiment showed significant difference compared to control. The results for total mold and yeast counts in garlic supplemented group were lower in breast and thigh muscles than control group. Moreover, a significant ($p<0.05$) greater number of mold and yeast in the fresh breast and thigh samples as compared to the frozen samples either collected from control or treated group.

Prevalence of molds and yeasts in quail carcasses: Data in Table 3 showed that quails when fed diets supplemented with garlic led to decreasing the incidence of molds in their carcasses than those fed control diets. The highest incidence of molds were detected in all control samples (either breast or thigh) with percentage 10 and 16%, respectively as compared to treated group. Moreover, a lower yeast incidence was demonstrated in all treated samples (11% for breast and 16% for thigh) in comparison to control group. Generally, the molds and yeasts contamination were extremely illustrated in fresh samples more than frozen ones either from control or treated group.

During this experiment, the isolated mold genera from quail carcasses in control group were 37 isolates of *Aspergillus* species, 19 isolates of *Mucor*, 45 isolates of *Penicillium* spp., 2 isolates of *Alternaria*, 10 isolates of *Fusarium* spp., one isolate of *Rhizopus* and *Absidia*, each (Table 4). On the other hand, the isolated mold genera from quail carcasses in garlic treated group were 22 isolates of *Aspergillus* species, 11 isolates of *Mucor*, 29 isolates of *Penicillium*, 5 isolates of *Fusarium* spp., one isolate of *Rhizopus* and absence of *Alternaria* and *Absidia*.

Occurrence of identified *Aspergillus* strains: A total of 37 (18.5%) *Aspergillus* isolates in control group were obtained which included: 17 (8.5%) *A. flavus*, 9 (4.5%) *A. fumigatus* and 11 (5.5%) *A. niger* (Table 5). Whereas, a total of 22 (11%) *Aspergillus* isolates in treated group were obtained as following: 9 (4.5%) *A. flavus*, 6 (3%) *A. fumigatus* and 7 (3.5%) *A. niger*.

Table 1: Statistical analytical results of total mold count cm^{-2} on the surface of quail carcass samples in control and treated groups (n = 50 each)

		Breast			Thigh		
Groups	Samples	Min.	Max.	M±SE*	Min.	Max.	M±SE*
Control	Fresh+	1×10^2	1×10^3	$3.9\times10^2\pm0.89\times10^2$	0.9×10^2	3×10^3	$2.1\times10^2\pm1.2\times10^2$
	Frozen	0.5×10^2	3×10^2	$2.6\times10^2\pm1.09\times10^2$	0.4×10^2	5×10^2	$1.4\times10^2\pm1.08\times10^2$
Treated	Fresh+	0.5×10^2	1×10^2	$0.8\times10^2\pm0.7\times10^2$	0.6×10^2	4×10^2	$1.1\times10^2\pm0.91\times10^2$
	Frozen	0.35×10^2	0.75×10^2	$0.7\times10^2\pm0.89\times10^2$	0.2×10^2	2×10^2	$1\times10^2\pm0.78\times10^2$

*M±SE, Mean count±standard error, +Significant differences ($p<0.05$)

Table 2: Statistical analytical results of total yeast count cm^{-2} on the surface of quail carcass samples in control and treated groups (n = 50 each)

		Breast			Thigh		
Groups	Samples	Min.	Max.	M±SE*	Min.	Max.	M±SE*
Control	Fresh++	3×10^2	5×10^3	$2.5\times10^3\pm0.7\times10^3$	2×10^2	9.5×10^2	$4.1\times10^2\pm0.55\times10^2$
	Frozen	2×10^2	2×10^2	$1.1\times10^2\pm0.35\times10^2$	1×10^2	1.9×10^2	$1.19\times10^2\pm0.23\times10^2$
Treated	Fresh++	2.7×10^2	3.5×10^2	$2.9\times10^2\pm0.95\times10^2$	1.8×10^2	2.5×10^2	$2.2\times10^2\pm0.23\times10^2$
	Frozen	1.5×10^2	2.1×10^2	$1.05\times10^2\pm0.33\times10^2$	0.68×10^2	1.75×10^2	$0.75\times10^2\pm0.11\times10^2$

++Means high significant differences ($p<0.01$)

Table 3: Incidence of molds and yeasts isolated from the surface of quail carcass samples in control and treated groups (n = 50 each)

		Molds		Yeast	
Groups	Samples	Breast	Thigh	Breast	Thigh
Control	Fresh	6 (12)	10 (20)	8 (16)	11 (22)
	Frozen	4 (8)	6 (12)	7 (14)	8 (16)
	Total	10 (10)	16 (16)	15 (15)	19 (19)
Treated	Fresh	5 (10)	8 (16)	6 (12)	9 (18)
	Frozen	3 (6)	5 (10)	5 (10)	7 (14)
	Total	8 (8)	13 (13)	11 (11)	16 (16)

Table 4: Incidence of the isolated mold genera from the examined quail carcasses in control and treated groups (n = 50 each)

	Control group (%)					Treated group (%)				
	Fresh		Frozen			Fresh		Frozen		
Mold genera	Breast	Thigh	Breast	Thigh	Total (n = 200)	Breast	Thigh	Breast	Thigh	Total (n = 200)
Aspergillus	12 (24)	10 (20)	8 (16)	7 (14)	37 (18.5)	8 (16)	7 (14)	4 (8)	3 (6)	22 (11.0)
Mucor	5 (10)	6 (12)	3 (6)	5 (10)	19 (9.5)	2 (4)	4 (8)	2 (4)	3 (6)	11 (5.5)
Penicillium	14 (28)	12 (24)	9 (18)	10 (20)	45 (22.5)	11 (22)	8 (16)	5 (10)	5 (10)	29 (14.5)
Alternaria	1 (2)	0	1 (2)	0	2 (1.0)	0	0	0	0	0
Fusarium	3 (6)	4 (8)	1 (2)	2 (4)	10 (5.0)	2 (4)	2 (4)	0	1 (2)	5 (2.5)
Rhizopus	0	1 (2)	0	0	1 (0.5)	0	1 (2)	0	0	1 (0.5)
Absidia	1 (2)	0	0	0	1 (0.5)	0	0	0	0	0

Table 5: Incidence of identified *Aspergillus* strains from the examined quail carcasses in control and treated group (n = 50 each)

	Control group (%)					Treated group (%)				
	Fresh		Frozen			Fresh		Frozen		
Aspergillus strains	Breast	Thigh	Breast	Thigh	Total (n = 200)	Breast	Thigh	Breast	Thigh	Total (n = 200)
A. flavus	6 (12)	4 (8)	4 (8)	3 (6)	17 (8.5)	3 (6)	3 (6)	2 (4)	1 (2)	9 (4.5)
A. fumigatus	2 (4)	3 (6)	2 (4)	2 (4)	9 (4.5)	2 (4)	2 (4)	1 (2)	1 (2)	6 (3.0)
A. niger	4 (8)	3 (6)	2 (4)	2 (4)	11 (5.5)	3 (6)	2 (4)	1 (2)	1 (2)	7 (3.5)
Total	12 (24)	10 (20)	8 (16)	7 (14)	37 (18.5)	8 (16)	7 (14)	4 (8)	3 (6)	22 (11.0)

DISCUSSION

Fungal contamination of fresh and frozen quail carcasses has a particular public health significance in the field of food safety due to its related food spoilage and production of mycotoxins[16]. However, garlic has antifungal activity that might reduce mold growth, especially, in the frozen carcasses. Accordingly, considering the fungal contamination in fresh and frozen quail carcasses is a matter of importance for public health. The current study described the incidence of fungal contamination of fresh and frozen quail carcasses obtained from control and garlic supplemented quail groups. The above results clearly confirmed the fact that garlic has antifungal properties. Numerous documents have shown that the garlic reduces the contamination with fungi through decreasing the oxygen uptake[17], reduction of the pathogen growth, inhibiting the synthesis of lipids, proteins and nucleic acids[18] and destruction of membranes[19]. On the other hand, there has been only limited study evaluating garlic supplementation on fungal growth in quails.

The contamination in the control group was comparable to garlic supplemented group as this kind of contamination might poses a health risk for humans. The present results significantly revealed the decreasing of total mold counts either in breast or thigh muscles got from garlic supplemented group than control group. Ali and Zahran[20] reported that garlic supplementation in diets improved chicken meat quality during refrigerated and frozen storage. Also, Fayed *et al.*[21] demonstrated that garlic supplementation in diets decreased aerobic plate count and coliforms. Additionally, a previous study detected that the addition of garlic to the diets inhibited the growth of aflatoxin producing fungi[22].

Furthermore, such results declared that the fresh quail carcasses had the highest mold count of all groups as a wet and warm climate is suitable for fungal growth. Thus, frozen samples were the least contaminated at the preserving stages. Similarly, *Mohsen*[23] reported that the total mold count/g revealed the mean, minimum and maximum values were $2.5\times10^2\pm2\times10^2$, $<10^2$ and 1×10^4 for fresh quail samples, while $1.2\times10^2\pm1.0\times10^2$, <10 and 2×10^2 for frozen quail samples, respectively. In addition, *Mostafa*[24], who studied the total mold count for frozen and fresh quail carcasses and found that the mean values for total mold count were 5.7×10^4 and 6×10^4, respectively. As well, El-Abbasy[25] found that the mean values of total mold count for 80 quail carcasses examined by swab method after skin excision ranged from 1×10^2 to 3×10^3 with a mean value of $4.65\times10^2\pm1.87\times10^2$/cm^2.

Regarding yeasts, the present study showed a significant lower number of yeasts in the breast and thigh samples from garlic supplemented group as compared to the control group either fresh or frozen quail caracess. This result is compatible with Fayed *et al.*[21], who found that garlic supplementation in diets decreased yeast contamination in broiler chickens in both groups B and C (fed 500 mg and 1000 mg kg^{-1} diet, respectively).

Furthermore, this study showed the higher count of yeasts in the fresh quail carcasses than in frozen ones with high significant differences ($p<0.01$). A previous investigation established the total yeast count (37×10^2 and 42×10^2/g) in poultry for cool stores I and II, respectively[26]. Another study detected 9.4×10^4 (count g^{-1}) of yeasts among samples collected from 45 defeathered sparrows in Egypt[27]. Also, Rodriguez-Calleja *et al.*[28] found that the initial level of yeasts on chilled stored rabbit carcasses was 3.46 ± 0.32 which grew faster than the remaining micro-organisms and became predominant at the end of the shelf life.

In this study, a high number of mold genera were isolated from quail carcasses in control group than in garlic supplemented group involving; *Aspergillus* species (*A. flavus* as the most abundant molds, *A. fumigatus* and *A. niger*), *Mucor*, *Penicillium* spp., *Alternaria*, *Fusarium* spp., *Rhizopus* spp. and *Absidia*. *Hechelman*[29] isolated the most important mold genera from meat, *Aspergillus*, *Penicillium*, *Cladosporium*, *Alternaria*, *Fusarium*, *Mucor* and *Rhizopus* in descending percentages. The previous studies exhibited that the garlic has antifungal activity against *Aspergillus* species as mycotoxin producing molds[30,31], *Fusarium* spp. and *Rhizopus* spp.[32]. The antifungal action of garlic is due to the compound allicin. It has strong antimicrobial and antifungal activities. Thus, the reduction of fungal contamination observed in this study might be related to allicin or ajoene which curbs the performance of some enzymes that are important to fungi.

CONCLUSION

It could be concluded from this study that the garlic powder supplementation in quails nutrition could improve their carcass quality either in fresh or frozen carcass through its reduction of the mycological risk and contamination. It is suggested, however, that further evaluation of this additive is warranted.

SIGNIFICANCE STATEMENT

Information on mycological quality of quail meat is scanty. This study investigated the potential antifungal activity of garlic (*Allium sativum*) in quail carcasses. The presence of fungi in edible food is of great public health importance. Thus, this study will help the researchers to uncover the critical areas of medicinal plant supplementation such as garlic to bird diets. Considering the importance of quail meat assessment for fungal contamination and public health safety that many researchers were not able to explore.

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