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Influence of Dietary Supplementation of Ginger, Garlic and their Combination on Mycological Assessment of Quail Carcasses

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ABSTRACT

Objective: This work was under taken to evaluate the influence of dietary supplementation of ginger (*Zingiber officinale*), garlic (*Allium sativum*) and their combination on quail meat through assessment of its contamination with fungi and its toxins and its effect on human beings.

Design: Randomized-controlled experimental study.

Samples: We obtained 110, Japanese quail chicks of one day old were included in this study.

Procedures: Quail chicks were further subdivided into four groups (n = 25/group): Control group was free from any dietary additions and the other three treated groups with ginger, mixed and garlic supplements, respectively for 45 days. After scarifying, skin swab specimens were assembled from quail breast and thigh muscles.

Results: Revealed that breast and thigh muscles in garlic treated group have the lowest counts of total mold and yeast than those of the others. Notably, control group represented the highest incidence in mold and yeast contamination as compared to that of the others. The isolated mold species were *Aspergillus* spp. (22, 15, 18& 9), *Mucor* (11, 8, 9& 7), *Penicillium* spp. (26, 19, 19& 10), *Fusarium* spp. (7, 3, 4& 2), *Rhizopus* (1, 1, 1& 0), *Alternaria* (1, 1, 2& 0) and *Cladosporium* spp. (1, 0, 0& 0) from control, ginger, mixed and garlic treated groups, successively. The isolated *Aspergillus* strains were *A. flavus* (10, 7, 7& 3), *A. fumigatus* (5, 4, 5& 3) and *A. niger* (7, 4, 6& 3) from control, ginger, mixed and garlic treated groups, successively.

Conclusions and Clinical relevance: It could be concluded that this research established the significance of the dietary addition of ginger, garlic and their combination as antifungal agents in the quail meal whereas, the garlic was the superior supplement for an obvious and considerable decreasing of yeast and mold loads in quails meat.

Keywords: Garlic, Ginger, Quails, Carcass, *Aspergillus*.

1. INTRODUCTION

Quails are considered an essential protein source that is characterized by good quality and cheap price. Quails meat is rich in essential amino acids (oleic, linoleic, palmitic and stearic), moreover, ratio of poly unsaturated fatty acids: saturated ones is 0.073 *Genchev et al.* [1]. Besides, meat of quails is a white meat has a little amount of fat and cholesterol, but it is rich in iron content *Jaturasitha et al.* [2]. Contamination of quail with fungus can exist in any stage from its production stages *Wagner et al.* [3].

Products of fungal growth which include mycotoxins and its metabolites carry a certain health risk for human through consumption of affected quails *Darwish et al.* [4]. Garlic and ginger are considered natural growth activators and can be effective substitutes for dominant artificial ones as antibiotics *Demir et al.* [5].

In-vitro, scientists indicated that the ginger extracts might limit free radicals quantity and lipids peroxidation *Al-Amin et al.* [6] and have anti-diabetic characteristics *Morakinyo et al.* [7]. During cutting of garlic and under the action of allinase enzyme, cysteine sulfoxide in garlic can be changed into thiosulfates which have powerful antibacterial and antifungal properties *Hanieh et al.* [8]. In native medicine, garlic is consumed as a spice for several

years, because it has possessed a powerful activity against bacteria, fungi, parasites, viruses, oxidation, cholesterol, cancers and vasoconstriction *Al-Shuwaili et al.* [9]. Gingerol, gingerdiol and gingerdione are considered the most important components in ginger that have the eligibility to activate digestive enzymes, influence the microbial activity acting as antioxidants *Dieumou et al.* [10]. In this research we studied the effective antifungal activity of ginger, garlic and their combination and also, we evaluated the quality of meat in quail of supplemented groups in comparison with control one.

2. MATERIALS AND METHODS

Guide for the Care and Use of Laboratory Animals' announced by the Ethics Committee of Veterinary Medicine Faculty, Mansoura University (Permit numbers 20-17) was applied in animal rearing for all experiments. This experiment was performed from August-November, 2019, in Veterinary Teaching Hospital, Veterinary Medicine Faculty, Mansoura University at Dakahlia Governorate, Egypt

2.1 Birds and experimental design:

One hundred and ten, Japanese quail chicks had one day old were obtained from (Dakahlia Poultry Company, Egypt).

All quails were reared in wire boxes, held in a well closed room 23 h under illumination and one hour of dim.

Birds were distributed into four groups (n = 25 per group): Control one was birds without any dietary supplements while the other three treated ones were ginger, mixed and garlic supplements, respectively. Quails in control group were supplied with a mercantile starter, constant feed devoid of any medicaments. Quails in supplemented groups had the same feed in addition to 14 g/Kg commercial dried, ginger, garlic and their combination powders, respectively (El- Gomhoria Company, Egypt) as feed additive. The food and water were supplied without stoppage for each group of quails throughout the course of the

Experiment (45 days). The house temperature was kept at about 35°C during the first 3 days, then gradually decreased by 2°C weekly until reached 24°C and kept constant until the end of the experiment.

2.2 Samples collection

Total number of birds (n = 100) were unfed for 5 h and slaughtered at the end of the experiment (45 days). Skin swab specimens were obtained by aseptic technique (10 cm²) from each quail carcass at 2 separate places (breast and thigh muscles). The samples were assembled in 9 ml test tube that

contains sterile buffered peptone water. After samples collection, from each quail, we obtained breast and thigh muscles independently and put in air-proof polyethylene boxes in relation to each dietary treatment and were chilled at 5±1°C for 2 days. All skin swab samples were taken under sterile conditions for mycological analysis.

2.3. Mycological analysis

The total mold and yeast counts were performed according to, **APHA** [11]. The assembled skin swab samples were mixed in 90 ml of sterile buffered peptone water to give 10⁻¹ dilution and were incubated for half an hour/25°C and then tenfold dilutions were setted. Two parts of 0.1 ml from every dilution were cultured in the same time on the surface of malt extract agar medium and Czapek-Dox agar with 5% NaCl and incubated aerobically away from light at 25°C/one week.

The mold and yeast counts were estimated by direct counting of the cultured agar plates. Mold counts were converted into base logarithms of colony forming units per gram of meat product samples (log cfu/g). Based on micro morphological characteristics, the predominant fungi in each specimen was distinguished to class level according to **Raper et al. [12]**, **Barnett et al. [13]** and **Samson et al. [14]**.

Table 1. Total mold count cm⁻² in the examined surface of quail specimen: (n = 25 each).

GRPUP	BREAST			THIGH		
	Min.	Max.	M±SE*	Min.	Max.	M±SE*
CONTROL	1×10 ²	1×10 ³	3.9×10 ² ±0.88×10 ²	0.95×10 ²	3×10 ³	2×10 ² ±1.15×10 ²
GINGER	0.5×10 ²	0.98×10 ²	0.75×10 ² ±0.72×10 ²	0.5×10 ²	2.5×10 ²	1.3×10 ² ±1.12×10 ²
MIXED ⁺	0.8×10 ²	3.2×10 ²	2.35×10 ² ±1.07×10 ²	0.56×10 ²	2.8×10 ²	1.5×10 ² ±0.81×10 ²
GARLIC	0.42×10 ²	0.79×10 ²	0.74×10 ² ±0.86×10 ²	0.22×10 ²	1.9×10 ²	1×10 ² ±0.79×10 ²

*M±SE, MEAN COUNT±STANDARD ERROR, +SIGNIFICANT DIFFERENCES (P<0.05)

Table 2. Total yeast count cm⁻² in the examined surface of quail specimen: (n = 25 each).

GRPUP	BREAST			THIGH		
	Min.	Max.	M±SE*	Min.	Max.	M±SE*
CONTROL	3.1×10 ²	4.9×10 ³	2.5×10 ³ ±0.68×10 ³	1.9×10 ²	9.6×10 ²	4×10 ² ±0.54×10 ²
GINGER	2.6×10 ²	2.7×10 ²	1×10 ² ±0.37×10 ²	0.98×10 ²	1.89×10 ²	1.14×10 ² ±0.22×10 ²
MIXED++	2.8×10 ²	3.4×10 ²	2.3×10 ² ±0.93×10 ²	1.8×10 ²	2.42×10 ²	2.2×10 ² ±0.22×10 ²
GARLIC	1.7×10 ²	2×10 ²	1.05×10 ² ±0.35×10 ²	0.7×10 ²	1.88×10 ²	0.77×10 ² ±0.13×10 ²

++Means high significant differences (p<0.01)

Table 3. Incidence of molds and yeasts colonies identified from the examined surface of quail specimen: (n=25 each).

GROUPS	MOLD		YEAST	
	Breast	Thigh	Breast	Thigh
CONTROL	8 (32)	10 (40)	8 (32)	11 (44)
TOTAL	8 (32)	10 (40)	8 (32)	11 (44)
GINGER	6 (24)	8 (32)	7 (28)	9 (36)
MIXED	7 (28)	9 (36)	6 (24)	10 (40)
GARLIC	5 (20)	4 (16)	5 (20)	8 (32)
TOTAL	18(24)	21 (28)	18 (24)	27 (36)

2.4. Statistical analysis

All values are expressed as means + SD, and all measurements were carried out in duplicates. Mold and yeast counts were converted into base logarithms of colony forming units per gram (log cfu/g). Statistical significance was evaluated using Tukey-Kramer HSD test. In all analyses, $p < 0.05$ was taken to indicate statistical significance using non-parametric ANOVA test (SPSS version 20) JMP statistical package, SAS Institute Inc., Cary, NC.

3. RESULTS

3.1. Dedication of mold and yeast number counts in quail carcasses

Quails looked well and rate of death did not represent any value throughout the experiment. Table 1 and 2 showed the influence of supplementation with 1.4% ginger, garlic and their mixed powders on mold and yeast counts during the research which had an important difference compared to control. Breast and thigh muscles in garlic treated group have the least numbers in total mold and yeast count compared to those of the others. Also, a considerable ($p < 0.05$) higher number of mold and yeast in breast and thigh specimens of both control and mixed supplemented groups as compared to those which were collected from both ginger and garlic supplemented ones, respectively.

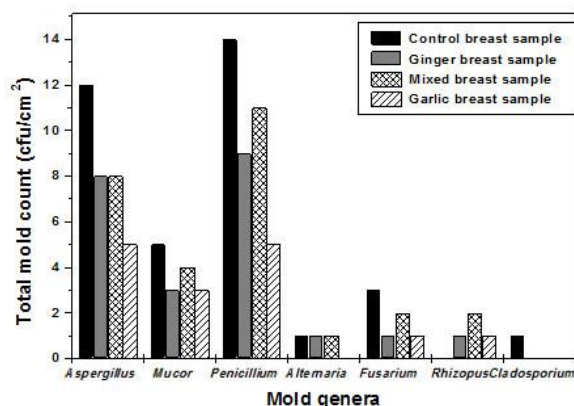


Figure 1. Incidence of the mold colonies identified from the examined breast specimen in control, ginger, mixed and garlic supplemented groups, respectively (n=25 each).

3.2. Prevalence of molds and yeasts in quail carcasses

Results in Table 3 revealed that feeding of the birds on feeds treated with garlic, mixed and ginger powders, respectively resulted in reducing molds in their carcasses comparing to those fed on the ordinary control one. All control groups represented the highest incidence of molds with a percentage of 32 and 40%, respectively in comparison with the other three treated ones which represented percentage 24 and 28%, respectively. Also, All treated samples represented a lower yeast incidence (24% for breast and 36% for thigh) compared to the control group (32% for muscle of the breast and 44% for muscle of thigh). Through this work, in control group the isolated molds were 26

Penicillium isolates, 22 *Aspergillus* isolates, 11 *Mucor* isolates, 7 *Fusarium* isolates, 1 isolate for *Rhizopus*, *Cladsporium* and *Alternaria*, (Table 4) (figures 1&2). In contrast, the isolated molds in ginger, mixed and garlic treated ones, respectively were 15, 18 & 9 *Aspergillus* isolates, 8, 9 & 7 *Mucor* isolates, 19, 19 & 10 *Penicillium* isolates, 3, 4 & 2 *Fusarium* isolates, 1, 1 & 0 *Rhizopus* isolates and 1, 2 & 0 *Alternaria* isolates and absence of *Cladosporium*.

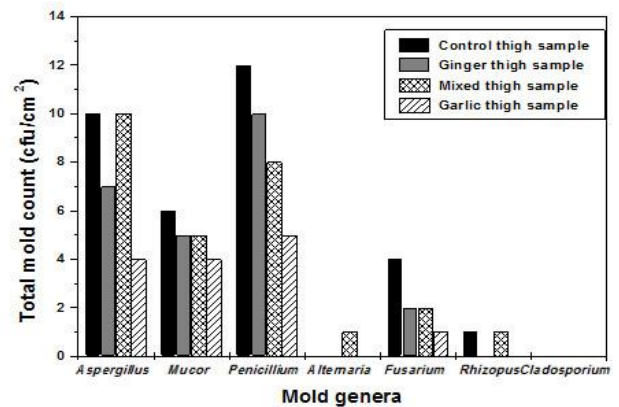


Figure 2. Incidence of mold colonies from the examined quail thigh specimens in control, ginger, mixed and garlic supplemented groups, respectively (n=25 each).

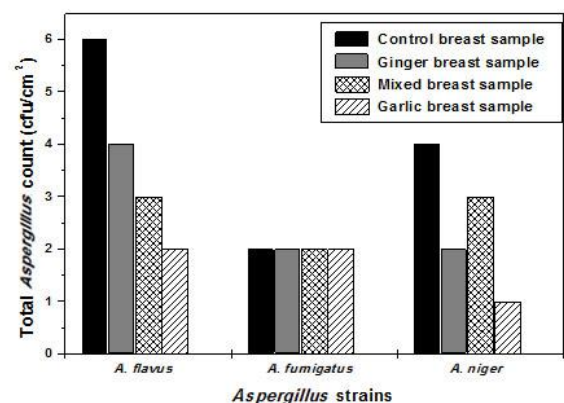


Figure 3. incidence of identified *Aspergillus* strains from the examined quails breast samples in control, ginger, mixed and garlic supplemented groups, respectively (n=25 each)

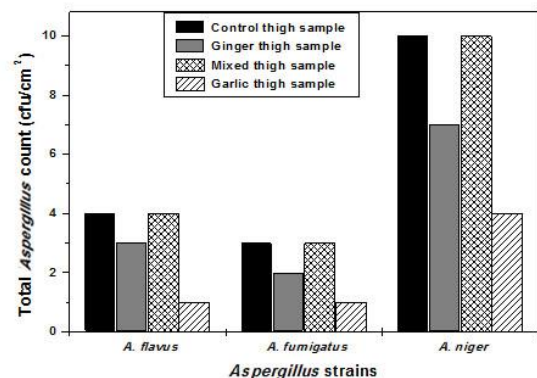


Figure 4. incidence of identified *Aspergillus* strains from the examined quails thigh samples in control, ginger, mixed and garlic supplemented groups, respectively (n=25 each)

3.3. Incidence of isolated *Aspergillus* strains

Twenty-two (44%) *Aspergillus* isolates in control group which were divided into: 10 (20%) *A. flavus*, 5 (10%) *A.*

fumigatus and 7 (14%) *A. niger* (Table 5) (figures 3&4). In contrast, 42(28%) of *Aspergillus* isolates in ginger, mixed and garlic treated ones were obtained as following: 17 (11.33%) *A. flavus*, 12 (8%) *A. fumigatus* and 13 (8.66%) *A. niger*.

4. Table 4. Incidence of the mold colonies identified from the examined quail specimen: (n = 25 each).

MOLD GENERA	CONTROL GROUP		TOTAL (N=50)	TREATED GROUPS (%)						TOTAL (N = 150)
	(%)			Ginger		Mixed		Garlic		
				Breast	Thigh	Breast	Thigh	Breast	Thigh	
ASPERGILLUS	12 (48)	10 (40)	22(44)	8 (32)	7 (28)	8 (32)	10 (40)	5 (20)	4 (16)	42(28)
MUCOR	5 (20)	6 (24)	11(22)	3 (12)	5 (20)	4 (16)	5 (20)	3 (12)	4 (16)	24 (16)
PENICILLIUM	14 (56)	12 (48)	26(52)	9(36)	10(40)	11(44)	8 (32)	5 (20)	5 (20)	48 (32)
ALTERNARIA	1 (4)	0	1(2)	1 (4)	0	1 (4)	1 (4)	0	0	3 (0.5)
FUSARIUM	3 (12)	4 (16)	7(14)	1 (4)	2 (8)	2 (8)	2 (8)	1 (4)	1 (4)	9 (1.5)
RHIZOPUS	0	1 (4)	1(2)	1 (4)	0	0	1 (4)	0	0	2 (0.33)
CLADOSPORIUM	1 (4)	0	1(2)	0	0	0	0	0	0	0

Table 5. Incidence of isolated *Aspergillus* strains from the examined quail specimens: (n = 25 each).

ASPERGILLUS STRAINS	CONTROL GROUP (%)		TOTAL (N=50)	TREATED GROUPS (%)						TOTAL (N = 150)
	Breast	Thigh		Ginger		Mixed		Garlic		
				Breast	Thigh	Breast	Thigh	Breast	Thigh	
A. FLAVUS	6 (24)	4 (16)	10 (20)	4 (16)	3 (12)	3 (12)	4 (16)	2 (8)	1 (4)	17(11.33)
A. FUMIGATUS	2 (8)	3 (12)	5 (10)	2 (8)	2 (8)	2 (8)	3 (12)	2 (8)	1 (4)	12(8)
A. NIGER	4 (16)	3 (12)	7 (14)	2 (8)	2 (8)	3 (12)	3 (12)	1 (4)	2 (8)	13(8.66)
TOTAL	12 (48)	10(40)	22 (44)	8 (32)	7 (28)	8 (32)	10(40)	5 (20)	4 (16)	42 28)

4. DISCUSSION

Fungal contamination of quail muscles has a certain public health importance in the field of food control and hygiene because it results in food putrefaction which leads to mycotoxins production as recorded by **Bryden [15]**. The present research showed the occurrence of fungal contamination in carcasses which were taken from control and the other three treated groups. The previous results assured the theory that garlic has a powerful activity against fungi. Several studies have reported that the garlic decreases fungi contamination by reducing the oxygen uptake **Szymona [16]** decreasing of the pathogen growth, prevention of the lipids, proteins and nucleic acids synthesis **Adetumbi et al. [17]** and membranes damage **Ghannoum [18]**. Control group was highly contaminated with molds more than the other three treated groups, and thus resulting in a hazardous effect for human health.

These results showed obviously the decreasing of mold colonies number either in breast or thigh muscles which were taken from the three treated groups than those of control one. **Ali et al. [19]** documented that garlic addition in meals enhanced chicken meat quality under conditions of refrigeration and freezing.

Moreover, **Fayed et al. [20]** reported that garlic addition in meals reduced aerobic plate count and coliforms. Also, an earlier work showed that garlic supplementation to the feeds prevented the growth of aflatoxin producing fungi **Meraj [21]**. So, these results revealed the highest incidence of mold count of all groups in fresh quail carcasses because the conditions of wetness and warmth are suitable for fungal growth. Similarly, **Mohsen [22]** found that the total mold count/g showed the mean, minimum and maximum values which were $2.5 \times 10^2 \pm 2 \times 10^2$, $<10^2$ and 1×10^4 for fresh samples, but $1.2 \times 10^2 \pm 1.0 \times 10^2$, <10 and 2×10^2 for frozen ones, respectively. Besides, **Mostafa [23]**, mentioned that the mean values of total mold count for fresh and frozen quail carcasses were 5.7×10^4 and 6×10^4 respectively. Also, **El-Abbassy [24]** studied the mean values of total mold count for 80 quail carcasses inspected by surface technique after removal of skin which varied from 1×10^2 to 3×10^3 with a mean value of $4.65 \times 10^2 \pm 1.87 \times 10^2 / \text{cm}^2$. Concerning to yeasts, the present work revealed a highly considerable lower count of yeasts in the breast and thigh specimens from group treated with garlic in comparison with that of the other three ones (control, ginger and mixed treated ones). This result was in consonance with **Fayed et al. [20]** who mentioned that yeast

contamination was reduced in broiler chickens when fed on garlic. So, this work revealed the greater number of yeasts in the control and mixed groups than that in ginger and garlic treated ones with great high remarkable differences ($p < 0.01$). In an earlier study, the total yeast number was (37×10^2 and 42×10^2 /g) in chilled poultry for markets I and II, **El-Shora [25]**. Another work, it was found that 9.4×10^4 (count/ g) of yeasts between specimens which were gathered from 45 sparrows after picking up of feathers in Egypt **Yassien et al. [26]**. Also, **Rodríguez-Calleja et al. [27]** reported that the preliminary level of yeasts on chilled stored rabbit carcasses was 3.46 ± 0.32 which grew quicker than the rest of micro-organisms and became abundant at the terminal of rack life. In our work, carcasses of control group had a high count of mold genera than that in the other three treated ones including; *Aspergillus* species (*A. flavus* was the most predominant molds, *A. fumigatus* and *A. niger*), *Mucor*, *Penicillium* spp., *Alternaria*, *Fusarium* spp., *Rhizopus* spp. and *Cladosporium*. **Hechelmann [28]** isolated the most significant mold genera from meat, *Aspergillus*, *Penicillium*, *Cladosporium*, *Alternaria*, *Fusarium*, *Mucor* and *Rhizopus* in gradually decreased percentages. The former researches declared that the garlic has a powerful activity against *Aspergillus* species as mycotoxin producing molds **Dankert et al. [29]** and **Tekeli et al. [30]**, *Fusarium* spp. and *Rhizopus* spp. **Kutawa et al. [31]**.

Our results were in an agreement with **Gad et al. [32]** who studied the effect of dietary garlic addition on mycological evaluation of fresh and frozen quail carcasses and they found that garlic treated group was lower in total mold and yeast counts either breast or thigh muscles than those of control one, concerning that, the fungal contamination was higher in fresh quail carcasses than that of frozen ones and they isolated the following mold species: *Aspergillus* species (*A. flavus* as the most predominant molds, *A. fumigatus* and *A. niger*), *Mucor*, *Penicillium* spp., *Alternaria*, *Fusarium* spp., *Rhizopus* spp. and *Absidia* from both groups. The antifungal effect of garlic is as a result of the presence of allicin or ajoene which has powerful antimicrobial and antifungal activities which hinders the activity of some enzymes that are essential for fungi. Thus, the decreasing of fungal contamination was noticed in this study. **Oleforuh-Okoleh et al. [33]** indicated that the ground ginger and garlic significantly ($p < 0.05$) influenced the carcass parts of the broilers. Though, contrary to **Dieumou et al. [10]** and **Pourali et al. [34]** who found that carcass parts were not affected by ginger or garlic, it is however in consonance with **Raeesi et al. [35]** who detected an essential effect on the carcass parts of broiler which fed on garlic. It has been reported that the diets containing ginger might stimulate lactic acid bacteria and reduce pathogenic ones like mesophilic aerobic, coliform and *E. coli* and mycotoxins producing molds and thus enhance the absorption of nutrients resulting in a better weight gain of the birds **Tagoe et al. [36]**. Our results were in an agreement with **Karangiya et al. [37]** who reported that garlic supplementation was the superior supplement in comparison to ginger and mixture of garlic and ginger. Also, **Zewell et al. [38]** concluded that bee

propolis, ginger or their mixture could effectively be used as a growth promoter and natural antioxidant in growing Japanese quail diets for improving feed conversion ratio, humeral immunity and to optimize lipid profile in blood serum and enhancing anti-oxidative status without negative effects on bird viability leading to unfavorable media for fungal growth.

Conclusion

It could be concluded that the garlic addition was the superior supplement in quails feeding which could enhance their carcass quality through its decreasing the mycological hazard and contamination.

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Authors Contributions

Tamer M. Gad: Revision of the whole article and shared as a corresponding author with the journal. Dalia E. El-Atriby: Collection of the samples, application of the practical study and scientific writing of the article. Amany M. Abd El-Moaty: Revision of the article and examining of the English language.

Conflict of interest statement

There is no any conflict of interest in the current research work.

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