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Research Article

Effect of the Bacteriocin, Nisin, and Gingerol on Microbial Status of Chicken Carcasses

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Abstract | Chicken meat represents a major source of animal-derived protein, essential amino acids, vitamins, and minerals. This study was taken to investigate the hygienic status of chicken meat including breast and thigh collected from rural and urban localities in Egypt. Evaluation of the hygienic status of chicken meat was done via estimation of total bacterial count (TBC), most probable number (MPN) of coliforms, total staphylococcus count (TSC), and total mold count (TMC). An experimental trial for the improvement of the hygienic status of chicken meat (breast) was done using the bacteriocin, nisin, and gingerol at two concentrations (1%, and 2%). The achieved results indicated an unsatisfactory hygienic status of the retailed chicken meat in the study area, in terms of high microbial counts. Chicken breast collected from rural areas had significantly ($p< 0.05$) the highest counts. A significant reduction of the microbial load of chicken breast was achieved after treatment with nisin, and gingerol, particularly at 2%. For instances, TBC was significantly ($p< 0.05$) reduced by 26.98%, 31.94%, 32.84%, and 38.83% after treatment with nisin 1.0%, nisin 2.0%, gingerol 1.0%, and gingerol 2.0%, respectively. In conclusion, it is highly recommended to use nisin, and gingerol in the chicken meat industry for the purpose of improving the microbiological quality of the final products.

Keywords | Chicken meat, Microbial load, Bacteriocin, Nisin, Gingerol

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INTRODUCTION

Chicken meat is regarded as a major source of animal-derived protein, minerals, and vitamins. It supplies humans with a major part of their needs from essential amino acids, and polyunsaturated fatty acids. The chicken meat industry is developed worldwide to fill the gap in the shortage of the red meat (El Bayomi et al., 2018). Furthermore, this industry is the most developed one around the globe, as it plays important roles in the economy of many countries, particularly Egypt (Darwish et al., 2018; Muaz et al., 2018).

Microbial spoilage of chicken carcasses is controlled by the hygienic practices followed during slaughtering, scalding, defeathering, plucking, and further processing. Moreover, the initial microbial count is a critical determinant for the sanitary status of chicken carcasses and their shelf life (Aberle et al., 2001). Furthermore, the hygienic status of chicken meat is affected by the method and the place of slaughter, either rural or urban, and the level of the hygienic measures adopted during the processing of the chicken carcasses (Darwish et al., 2015). Therefore, there is a large need to confirm the sanitary status of the retailed chicken meat in Egypt.

Food antimicrobials are either chemical compounds or natural substances that might delay or cease the microbial growth in a food matrix. Natural antimicrobials or spices represent promising tools for reducing the microbial load

in chicken meat and meat products (Jessica Elizabeth et al., 2017).

The bacteriocin, nisin, is a polypeptide produced by certain species of lactic acid bacterium, *Lactococcus lactis*. Nisin was approved as a natural food preservative as it has antimicrobial activities against a vast array of microorganisms, particularly Gram's positive bacteria (Davies et al., 1997; Tang et al., 2020).

Gingerol is a major component of ginger with several documented biological activities. The antimicrobial effects of gingerol were reported before against several bacterial species (Park et al., 2008; Tang et al., 2020). However. the use of gingerol to improve the hygienic status and to reduce the microbial counts of chicken meat had received less attention.

In sight of the previous facts, the objectives of the current study were firstly to investigate the hygienic status of the retailed chicken meat (breast and thigh) collected from rural and urban places in Egypt. Evaluation of the hygienic status of chicken meat was done via estimation of total bacterial count (TBC), most probable number (MPN) of coliforms, total *Staphylococcus* count (TSC), and total mold count (TMC). Secondly, a trial for improvement of the sanitary status of the chicken breast was conducted using bacteriocin, nisin, and gingerol at two concentrations (1%, and 2%).

MATERIAL AND METHODS

Collection of Samples

A total of two hundred samples were collected randomly and equally from chicken meat (breast, and thigh, one hundred each) retailed in urban and rural areas in Dakahliya Governorate, Egypt. Each sample weighs 100 g. The collected samples were transferred cooled directly without delay to the laboratory for microbiological examination.

Organoleptical examinations

Organoleptical examination for the examined samples was conducted using the method of Varnam and Sutherland (1995). Samples with blue-whitish color, fresh odor and firm consistency were considered normal.

Microbiological examinations

The recommended protocol of APHA (2001) for sample preparation was followed. In brief, twenty-five grams from each sample were weighed and homogenized under aseptic conditions with 225 ml of sterile buffered peptone water 0.1% (LAB104, LAB M, UK) for two minutes at 2000 rpm using sterile meat homogenizer, making a homogenate of 10^{-1} dilution, and further ten-fold decimal serial dilutions were prepared.

Determination of total bacterial count (TBC): Total bacterial count was done according to the pour platting method recommended by APHA (2001). In short, one ml from each dilution was pipetted to a clean and sterile Petri dish. Then, 12-15 ml of plate count agar (Difco, Detroit, Michigan, USA) cooled to 45 ± 1 °C were poured on each Petri dish. After thorough mixing, plates were left to solidify and then incubated at inverted position for 48 h at 35 ± 2 °C. Plates with 25-250 pinpoint size colonies were recorded. TBC was calculated from the following formula:
TBC/g = average No. of colonies × reciprocal of the dilution
Counted colonies were expressed as log 10 cfu/g.

Determination of the most probable number (MPN) of Coliforms: The three tubes method recommended by APHA (2001) for determination of the most probable number (MPN) of coliforms was applied. Briefly, 1 ml of each prepared dilution was used to inoculate distinctly into three test tubes containing MacConkey broth with inverted Durham's tubes. The inoculated tubes were kept at 37 °C for 24-48 hrs. Acid (yellow color) and gas producing tubes were regarded as positive and recorded. The MPN of coliforms was calculated according to the recommended tables.

Determination of total Staphylococcus aureus count (TSC): *Staphylococcus aureus* (*S. aureus*) count was done according to the method of Quinn et al. (2011) using Baird Parker agar (Biolife, Italy) added with egg yolk-tellurite emulsion (Himedia, India). Cultures were incubated at 37°C for 48 h. Typical colonies (shiny, convex, black, 1–1.5 mm in diameter, and surrounded by a clear halo zone) and/or atypical colonies (black with no zones) were counted and noted. TSC was calculated according to the following formula:
TSC/g = average No. of colonies × reciprocal of the dilution
Counted colonies were expressed as log 10 cfu/g.

Determination of total mold count (TMC): Total mold counts were determined according to the protocol of APHA (2001) using the pour plate technique. The used culture medium was Sabouraud's dextrose agar media (Oxoid, Basingstoke, UK) supplemented with chloramphenicol 100 mg/L. After culture, plates were incubated in dark at 25°C for 5-7 days. During the incubation time, the plates were observed daily for mold growth. Estimation of TMC was obtained by direct counting of the cultured plates.
TMC/g = average No. of colonies × reciprocal of the dilution
Counted colonies were expressed as log 10 cfu/g.

Table 1: Improvement of the sanitary status of the chicken breast meat using nisin, and gingerol.

	TBC		MPN		TSC		TMC	
	Mean ± SE	Reduction %	Mean ± SE	Reduction %	Mean ± SE	Reduction %	Mean ± SE	Reduction %
Control	6.16 ± 0.33^{a}	0	3.08 ± 0.14^{a}	0	3.74 ± 0.05^{a}	0	3.68 ± 0.05^{a}	0
Nisin 1.0%	4.50 ± 0.15^{b}	26.98	2.77 ± 0.05^{ab}	10.09	3.08 ± 0.14^{b}	17.67	3.19 ± 0.21^{b}	13.08
Nisin 2.0%	4.19 ± 0.12^{bc}	31.94	2.49 ± 0.06^{b}	19.17	2.12 ± 0.07^{b}	27.78	3.10 ± 0.22^{bc}	15.40
Gingerol 1.0%	4.14 ± 0.08^{bc}	32.84	2.54 ± 0.05^{ab}	17.22	2.68 ± 0.14^{b}	28.38	3.17 ± 0.06^{bc}	13.51
Gingerol 2.0%	3.77 ± 0.10^{c}	38.83	2.25 ± 0.09^{b}	27.96	2.36 ± 0.17^{b}	36.95	2.97 ± 0.07^{c}	18.96

Values within the same column carrying different superscript letters are significantly different at p <0.05.
TBC refers to total bacterial count, MPN refers to most probable number of coliforms, TSC refers to total staphylococcus count, and TMC refers to total mold count

Improvement of the microbial status of the chicken breast

In a trial for reduction of the microbial load of the chicken breast, food grades of bacteriocin, nisin, (SIDLEY chemical, Linyi city, China), and 6-gingerol (Biopurify Phytochemicals, Chengdu, China) at 1.0, and 2.0% concentrations were used. Five of the collected minced chicken breast meat samples (250 g/each) were sub-divided into five pieces (5 pieces from each sample, 50 g/each). The pieces were grouped into 5 groups, namely, group 1 which was immersed in corn oil and served as a control; group 2 which was immersed in nisin 1.0%; group 3 which was immersed in nisin 2.0%; group 4 which was immersed in 6-gingerol 1.0%; group 5 which was immersed in 6-gingerol 2.0%. All treatments were lasted for 30 min at room temperature. Microbiological examination was conducted as mentioned before.

Statistical analysis

All values are expressed as means SE, and all observations were carried out in duplicates. Microbial counts were converted into base logarithm 10 of colony forming units per g (log 10 cfu/g). Statistical significance was estimated using one way analysis of variance (ANOVA), followed by the Tukey–Kramer HSD post hock test. In all analyses, p < 0.05 was taken to indicate statistical significance.

RESULTS

All examined samples from different localities in Egypt had normal sensory properties (blue-whitish color, fresh odor, and firm in consistency) (data are not shown). Microbiological examination of the examined samples in the present study indicated that the average values ± SE of TBC were 5.49 ± 0.26, and 4.53 ± 0.16-log 10 cfu/g in breast and thigh samples collected from rural localities, and 4.77 ± 0.21, and 4.59 ± 0.19-log 10 cfu/g in breast and thigh samples collected from urban localities, respectively (Figure 1).

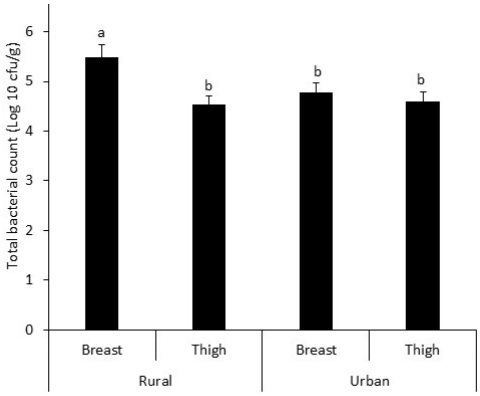

Figure 1: Total bacterial counts among breast and thigh samples of chicken collected from rural and urban localities in Egypt
Total bacterial counts of chicken breast and thigh samples collected from rural and urban localities in Egypt. Values represent means SE (log 10 cfu/g) of fifty samples from each group. Columns carrying different letters differ significantly at p < 0.05.

Most probable number of coliforms was further calculated among the examined samples, the obtained results revealed that the mean values of MPN of coliforms were 2.90 ± 0.08, and 2.28 ± 0.04-log 10 cfu/g in breast and thigh samples collected from rural localities, and 2.48 ± 0.04, and 2.41 ± 0.04-log 10 cfu/g in breast and thigh samples collected from urban localities, respectively (Figure 2).

Total *S. aureus* was further examined in the collected chicken meat samples. The obtained results indicated that the mean TSC in the examined samples were 3.47 ± 0.09, and

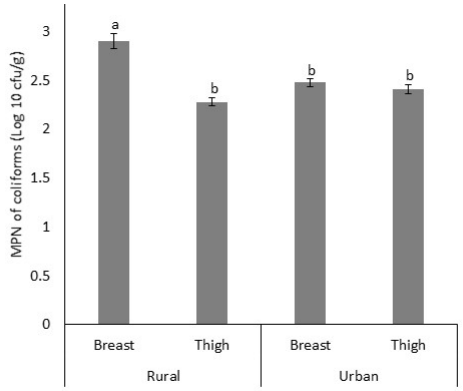

Figure 2: Most probable number of coliforms among breast and thigh samples of chicken collected from rural and urban localities in Egypt

MPN of coliforms in chicken breast and thigh samples collected from rural and urban localities in Egypt. Values represent means SE (log 10 cfu/g) of fifty samples from each group. Columns carrying different letters differ significantly at $p < 0.05$.

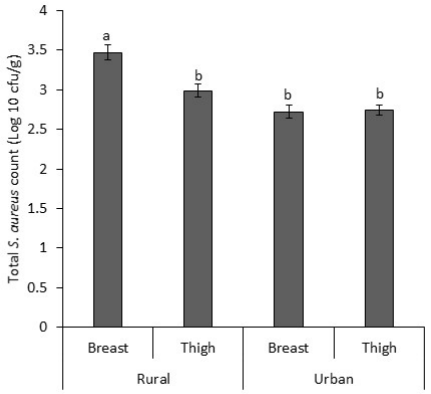

Figure 3: Total *Staphylococcus aureus* among breast and thigh samples of chicken collected from rural and urban localities in Egypt

Total *S. aureus* counts in chicken breast and thigh samples collected from rural and urban localities in Egypt. Values represent means SE (log 10 cfu/g) of fifty samples from each group. Columns carrying different letters differ significantly at $p < 0.05$.

2.99 ± 0.08-log 10 cfu/g in breast and thigh samples collected from rural localities, and 2.72 ± 0.08, and 2.74 ± 0.07-log 10 cfu/g in breast and thigh samples collected from urban localities, respectively (Figure 3).

Total mold count was examined among the collected samples. The recorded mean values of TMC were 3.66 ± 0.04, and 3.22 ± 0.09-log 10 cfu/g in breast and thigh samples collected from rural localities, and 3.16 ± 0.09, and 2.95 ± 0.10-log 10 cfu/g in breast and thigh samples collected from urban localities, respectively (Figure 4). In order to improve the microbiological status of chicken breast, an improvement a trial was conducted using nisin as a bacteriocin and gingerol as a natural food additive at two concentrations, 1% and 2%. The obtained results for this experimental trial were presented in Table 1. The achieved results indicated that TBC was significantly reduced by 26.98%, 31.94%, 32.84%, and 38.83% after treatment with nisin 1.0%, nisin 2.0%, gingerol 1.0%, and gingerol 2.0%, respectively. The reduction rates of the MPN of coliforms after same treatments were 10.09% (nisin 1.0%), 19.17% (nisin 2.0%), 17.22% (gingerol 1.0%), and 27.96% (gingerol 2.0%), respectively. These treatments improved TSC by 17.67%, 27.78%, 28.38%, and 36.95%, respectively. TMC was significantly reduced by 13.08%, 15.40%, 13.51%, and 18.96% after treatment with nisin 1.0%, nisin 2.0%, gingerol 1.0%, and gingerol 2.0%, respectively.

Figure 4: Total Mold counts among breast and thigh samples of chicken collected from rural and urban localities in Egypt

Total mold counts in chicken breast and thigh samples collected from rural and urban localities in Egypt. Values represent means SE (log 10 cfu/g) of fifty samples from each group. Columns carrying different letters differ significantly at $p < 0.05$.

DISCUSSION

Chicken meat represents a major source of the animal-derived protein worldwide. In addition, it represents a major sector in the national economy in many countries worldwide. Presence of spoilage microorganisms in the retailed meat affects both meat safety and quality. Furthermore, the initial bacterial load of chicken meat has a significant effect on its hygienic status (Darwish et al., 2018). A major task for the food safety sector is to ensure safety and wholesomeness of the meat available to the public. In sight of these facts, investigation of the microbial load in retailed chicken meat (breast and thigh) at both rural and urban localities was conducted. The results obtained in the present study revealed a high contamination level of the retailed chicken meat in terms of high TBC, MPN of coliforms,

TSC, and TMC. In particular, breast meat retailed in the rural localities had the highest contamination level. Sources of contamination of chicken meat may arise from poor hygienic measures adopted during slaughtering, dressing, and evisceration. Coliform bacteria are significant microbiological sanitary indicators, which highlights hygiene at all steps of preparation and handling of meat and meat products and their presence indicates fecal contamination (Darwish et al., 2015). The high numbers of TBC and MPN of coliforms indicate inadequacy of general hygiene in the meat-processing plant, or rupture of the intestinal tract during evisceration of the birds (ICMSF, 1996). The recorded TBC of chicken breast and thigh in the present study goes in agreement with Capita et al. (2002) who recorded mean TBC value of 5.19 ± 0.43-log10 cfu/g in chicken carcasses retailed in Spain. However, higher values were reported by Buzón-Durán et al. (2017) who recorded TBC value of 6.44 ± 1.16-log10 cfu/g in chicken meat products retailed in Spain. The recorded MPN of coliforms agrees with the values obtained from studies conducted in Spain on chicken carcasses (2.73 ± 0.29-log10 cfu/g by Capita et al. (2001), and chicken meat products (2.86 ± 0.76-log10 cfu/g by Buzón-Durán et al. (2017). Ingestion of *S. aureus*-contaminated food might lead to food-borne-intoxication, which is considered the third largest cause of food-related illnesses worldwide. It is characterized by its rapid onset, vomiting, abdominal cramps, and severe diarrhea with normal or sub-normal temperature (Darwish et al., 2018). The obtained values of TSC in the current investigation are comparable to that reported in chicken meat products retailed in Spain (Buzón-Durán et al., 2017), and chicken giblets retailed in Egypt (Darwish et al., 2018). Mold contamination of chicken meat might lead to their spoilage and production of mycotoxins with potential health hazards to humans due to their carcinogenic effects, liver diseases, and organ damage (Darwish et al., 2014). Mold contamination of chicken breast and thigh in the present study goes in agreement with reports from Italy (Iacumin et al., 2009), Spain (Martin-Sanchez et al., 2011), and Egypt (Darwish et al., 2016). Contamination of the retailed chicken meat with molds indicates inadequate hygienic practices adopted during slaughtering, defeathering, evisceration, storage, and distribution. Sanitary conditions and facilities of the slaughterhouses, butchery shops, freezing rooms, and stores are critical factors for the mold contamination (Mizakova et al., 2002).

In an improvement trial for the microbial status of the retailed chicken breast meat, nisin and gingerol were used as natural preservatives. Interestingly, both used preservatives could significantly ($p< 0.05$) reduce the spoilage parameters at variable rates and in a concentration-dependent manner. In particular gingerol 2.0% had the highest antimicrobial activities. In agreement with the obtained results, nisin had clear *in vitro* anti-listerial activities (Avery and Buncic, 1997). Besides, nisin had significant preservative effects against Gram-positive organisms (He et al., 2017). Furthermore, Tang et al. (2020) reported similar antimicrobial effects of nisin and gingerol, particularly against spoilage and indicator organisms. Thus, it is highly recommended to use either nisin, gingerol or their combination to improve chicken meat's microbial quality.

CONCLUSION

The obtained results of the present study demonstrated that strict hygienic precautions should be adopted during handling, processing, transportation, and distribution of chicken meat, particularly at the rural localities. In addition, treatment of the chicken meat with nisin, gingerol or their combination is of value for improving their microbial quality.

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CONFLICT OF INTEREST

There is no conflict of interest.

NOVELTY STATEMENT

This study describes for the first time the possible use of ginger, and nisin or their combination for the improvement of the chicken meat quality in Egypt.

AUTHORS CONTRIBUTION

Both authors contributed equally to the manuscript

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