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Antimicrobial effect of *Allium sativum* (garlic) against *Escherichia coli* and *Salmonella typhimurium* generated by poultry activities

Efecto antimicrobiano de *Allium sativum* (ajo) frente a *Escherichia coli* y *Salmonella typhimurium* generadas en actividades avícolas

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Abstract

The poultry sector plays a fundamental role on an industrial scale and in rural areas; one of the problems it faces today, through farming, meat and eggs production, preservation and sale to the consumer, is the presence of pathogenic microorganisms such as *Escherichia coli* and *Salmonella typhimurium*, which represent a latent risk to the health of the population. With the aim of contributing to the knowledge of an alternative for the reduction/elimination of these bacteria, this work proposes a treatment based on a 30% w-v garlic-aqueous solution, applied to a water sample obtained as a result of activities related to chicken farming. The evaluation of the effect was carried out at 0, 5, 10, 20, 30, 45, 60 and 90 minutes, using the plate count technique. At the end of the treatment, it was determined that the garlic-aqueous solution has an antimicrobial effect, which allows a reduction in concentrations of 1.6-$\log_{10}$ for *Escherichia coli* and 2.2-$\log_{10}$ for *Salmonella typhimurium* in the established treatment time. It is suggested to extend the knowledge in the use of this alternative, in addition to the existing technologies to contribute to sustainable development.

Garlic, Bacteria, Poultry-activities

Resumen

El sector avícola desempeña un papel fundamental a escala industrial y en zonas rurales; una de las problemáticas a las que se enfrenta hoy en día, a través de la crianza, la producción de carne y huevos, conservación y venta al consumidor, es la presencia de microorganismos patógenos tales como *Escherichia coli* y *Salmonella typhimurium*, las cuales representan un riesgo latente para la salud de la población. Con el propósito de contribuir al conocimiento sobre una alternativa para la reducción/eliminación de dichas bacterias, en este trabajo se propone un tratamiento con base en una solución acuosa de ajo al 30% p-v, aplicado a una muestra de agua obtenida como resultado de actividades vinculadas con la crianza de pollos. La evaluación del efecto se realizó a 0, 5, 10, 20, 30, 45, 60 y 90 minutos, empleando la técnica de recuento en placa. Al finalizar el tratamiento, se determino que la solución acuosa-ajo posee un efecto antimicrobiano, que permite en el tiempo de tratamiento establecido, una reducción en concentración de 1.6-$\log_{10}$ para *Escherichia coli* y de 2.2-$\log_{10}$ para *Salmonella typhimurium*. Se sugiere extender el conocimiento en la utilización de esta alternativa, en complemento a las tecnologías ya existentes para contribuir a un desarrollo sustentable.

Ajo, Bacteria, Actividades avícolas

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Introduction

Worldwide, the growth of poultry industry is accelerated due to food requirements generated as a result of demographic expansion. In 2019, meat production by the poultry sector accounted for about 39% in livestock sector at global scale and, in the period of 1961-2019, meat and eggs production reached increments of 14-fold and 6-fold, respectively (FAO, 2021). Technological advances have been reflected in reproduction, feeding, slaughter and safety methods, that are feasible to implement by large-scale producers; however, FAO (2021) highlights that, an important proportion of poultry production is developed on a small scale, in rural areas with family dependence and whose function, is important for the preservation of life in these environments, since it provides them the opportunity to generate income and a source of nutritional consumption (FAO, 2021; Farrell, 2013).

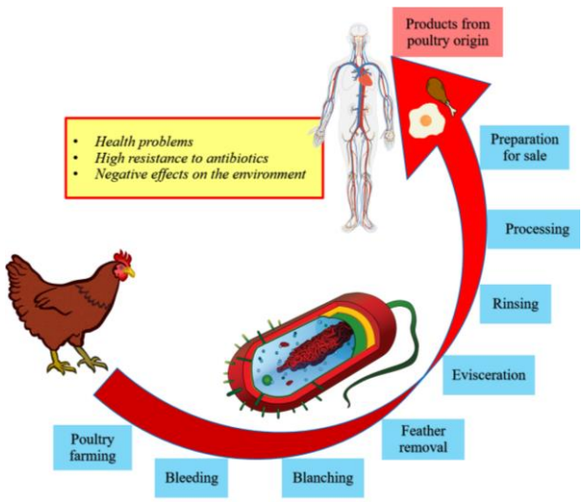

Figure 1 Stages for obtaining products in poultry industry Source: (*Own elaboration based on information by Ventura da Silva, 2013; Logue and Nde, 2007*)

Operation of poultry sector in both scales share one essential vulnerability: the existence of pathogenic microorganisms in the different rearing stages, as well as in the production process of meat, eggs and derivatives from them (Figure 1).

As a result, it is anticipated that those producers who do not have appropriate safety control systems in place to perform their functions within the poultry industry will face serious economic consequences. In addition, there is a latent health risk for the population, which would require the identification and solution of a problem that could compromise the life of people.

In this respect, López, Burgos, Díaz, Mejía and Quinteros (2018) conducted a research work in El Salvador due to cases associated to health problems such as: foodborne diseases, diarrhea and gastroenteritis. As a result, they identified a significant contamination in chicken meat due to the presence of *Salmonella spp*, *E. coli* and *S. aureus* in a supermarket product. They attributed this to failures generated during the production process, handling and even the transfer for sale to the final consumer. On the other hand, EFSA and ECDPC (2019), in 2018 reported that, diseases caused by *Salmonella* represented 30.7% of all cases of foodborne origin in the European Union, percentage that experimented an increase of 20.6% respect to 2017.

Some alternatives have been generated to reduce problems derived by pathogen microorganisms during different stages of poultry activities: using of formaldehyde for disinfection of eggs (Baylan, Akpinar, Canogullari and Ayasan, 2018); Facon *et al.* (2017) highlights the importance of vaccination for the control of colibacillosis in chickens, an infectious disease derived from *E. coli* and contracted because the bird is in contact with contaminated feces, water and dust. However, this infection, like others, continues to be treated with antibiotics (Selecciones avícolas, 2018). At the moment, it could be a benefit to farmers in the effectiveness production because it is an extensive productive activity; however, problems associated with water contamination from poultry effluents and bacterial resistance strains (Agyare, Boamah, Zumbi and Osei, 2018) and their dissemination in the environment, constitute a serious threat.

Bagust (2013) emphasizes the high probability that new pathogens may emerge from poultry farming in the coming years affecting not only this sector, but also to human being.

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In the last years, alternatives such as the use of herbal plants in the control of pathogen agents have been proposed. In this case, garlic, has been used due to its chemical components that allow it to act against diseases. Some research works have been concluded that garlic has a potential bactericidal action and, it is recommended for activities related in the different stages of meat poultry, eggs production, farming activities until the product is purchased by the final consumer (Nayab, 2022; Baylan *et al*., 2018; Fouad, Abdel-Hafez and El-Halim, 2018). Additionally, Ogbuewu, Okoro, Mbajiorgu and Mbajiorgu (2018) suggest the garlic, as an element to contribute significantly to animal nutrition, able to create beneficial effects associated with growth, health of livestock and a reliable alternative to reduce the use of antibiotic and chemical substances.

Based on the above, the aim of this work is determining the effect of garlic-aqueous solution, in relation to time, on the reduction in concentration of pathogen bacteria: *Escherichia coli* and *Salmonella typhymurium*, contained in an effluent of water from poultry activities. With this, it is expected to contribute to the knowledge on the behaviour of pathogenic microorganisms using a sustainable, safe and viable alternative to apply, for both large productive sectors and rural areas to satisfy needs of consumers and for those whose livelihood depends on poultry farming.

Methodology

Preparation of garlic-aqueous solution

Aqueous solution of *Allium sativum* (garlic) was prepared in 30% w/v concentration, according to the following equation:

$$\%\frac{w}{v} = \frac{mass\ of\ solute\ [g]}{volume\ of\ solution\ [mL]} * 100 \quad (1)$$

The solute, in this case garlic, was separated from its coating to be disinfected with ethanol. Subsequently, 15 g of garlic was placed in a mortar to be ground and introduced into a volumetric flask to be put in contact with water in order to complete 50 mL volume solution. Then, both components were incorporated and kept below room temperature until they were brought into contact with bacteria.

Sample of bacteria from poultry activities

Water is an important resource used in different stages of poultry activities, therefore, in this work it was contemplated as the bacteria resource (Figure 2). Subsequently, 100 ml volume of water from poultry activities was collected according to NOM-109-SSA1-1994. Then, material of significant size was removed to homogenize the sample for applying the microbiological analysis and later, the proposed treatment.

Figure 2 Identification of bacteria in poultry activities. *Source: Own elaboration*

Implementation of treatment: bacteria-garlic

The treatment was applied by triplicate. In it, 1 mL of water sample containing bacteria was poured into a test tube and subsequently, 9 mL of 30% w-v garlic-aqueous solution was introduced in it to be homogenized. At this time, the first sample (time = 0 s), with 0.1 mL volume, is taken to evaluate the effect of the garlic-aqueous solution on the inactivation of *Escherichia coli* and *Salmonella Typhimurium* through the application of microbiological analysis. Then, the solution was kept at rest and samples (volume = 0.1 mL) were taken at testing times of: 5, 10, 20, 30, 45, 60 and 90 minutes; before sampling, homogenization is important.

Microbiological analysis

Analysis to determine the effect of garlic-aqueous solution in *Escherichia coli* and *Salmonella typhimurium* bacteria was carried out extracting 0.1 ml volume of sample in the periods of time cited before. Subsequently, serial dilutions were carried out from each sample ($1{:}10^1$ to $1{:}10^5$); as inoculum was used the microbiological growth medium: Violet Red Bile Agar (VRB Agar, NEOGEN).

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For VRB Agar, 25 ml of microbiological medium at 318 K was poured in Petri dishes; once solidified, 0.1 ml of aliquot (sample taken in each testing time and dilutions) was distributed according to standard plate count (by duplicate). Later, Petri dishes were subjected to an incubation process at inverted position at 310 K during 18-24 h. Finally, microorganisms of interest were determined quantitatively by:

$$A\left[\frac{\text{CFU}}{\text{mL}}\right] = \frac{B \times C}{D\,[mL]} \quad (2)$$

where *A*, corresponds to bacteria concentration of the analyzed sample; *B* is the average of bacteria; *C*, dilution factor and *D*, represents the volume of inoculum.

Results

The results generated by the implementation of the experimental methodology proposed in this work are illustrated in Figure 3. Initial bacteria concentrations are showed graphically in testing time of 0 minutes; *Escherichia coli* with higher concentration (33×10^6 CFU/mL) than in the case of *Salmonella typhimurium* (16×10^5 CFU/mL). No immediate change is detected in concentrations bacteria when pathogen microorganisms are in contact with garlic-aqueous solution. Figure 4 shows the growth of both pathogen bacteria considered in microbiological medium VRB.

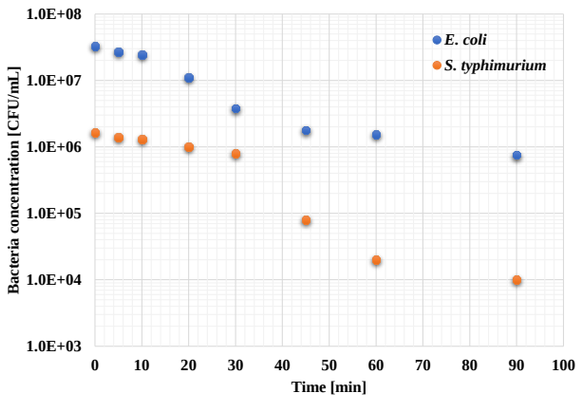

Figure 3 Bacteria reduction by the action of garlic-aqueous solution
Source: Own elaboration

In a period of 0-10 minutes of treatment, a linear trend in reduction (0.1-$\log_{10}$) is observed for both bacteria, obtaining concentrations of 24×10^6 CFU/mL and 13×10^5 CFU/mL for *Escherichia coli* and *Salmonella typhimurium* bacteria, respectively. Subsequently, in the specific case of *Escherichia coli* bacteria reduction increases (average: 0.3-$\log_{10}$) until the treatment finished (90 minutes). Mainly, at testing time 30 minutes, a greater effect of the garlic-aqueous solution on the inactivation of this pathogen microorganism is detected (0.5-$\log_{10}$). As a result of the treatment applied to *Escherichia coli* during 90 minutes, a total reduction of 1.6-$\log_{10}$ is achieved, which allows to obtain a final concentration of 76×10^4 CFU/mL.

Figure 4 Growth of *Escherichia coli* (red color) and *Salmonella typhimurium* (colorless) in VRB
Source: Own elaboration.

By the other hand, *Salmonella typhimurium* bacteria maintains a continuous reduction of 0.1-$\log_{10}$ before a testing time 45 minutes, in which garlic-aqueous solution reaches its maximum activity (0.9-$\log_{10}$ reduction). After, the inactivation effect starts to decrease until it reaches a concentration of 10×10^3 CFU/mL, thus achieving a reduction of 2.2-$\log_{10}$ respect to the initial concentration.

According to results, *Escherichia coli* and *Salmonella typhimurium* bacteria from poultry activities, experience vulnerability to the action of garlic-aqueous solution, as an agent to promote their reduction.

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Both bacteria, starting reduction with 0.1-log_{10} and ending the treatment with 0.3-log_{10}; however, during the evaluation time (90 s), *Salmonella typhimurium* shows greater reduction although a longer period of time is required to intensify bacterial decline in contrast to *Escherichia coli*.

These results could be attributed to substances such as allicin and diallyl trisulfide contained in garlic, which when it is crushed, interact with the water contained in the solution to reduce pathogen microorganisms.

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Conclusions

The garlic-aqueous solution in concentration 30% w-v, with treatment time 90 minutes proposed in this work, allows a simultaneous bacterial reduction for *Escherichia coli* (1.6-log_{10}) and *Salmonella typhimurium* (2.2-log_{10}). Due to the physical, chemical and microbiological properties of garlic, this kind of treatment can contribute to other methods or technologies to provide food with quality, reducing microbiological risks during the poultry farming and processes involved in reaching to the final consumer.

It is suggested to extend the knowledge by contemplating other concentrations of aqueous solutions of garlic due to its antimicrobial activity in the control of pathogen microorganisms, as well as to improve control over factors such as the physical, chemical and microbiological characteristics of the garlic to be used in the treatment.

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