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# *IN VITRO* EVALUATION OF ANTIBACTERIAL ACTIVITY OF GARLIC *ALLIUM SATIVUM* AGAINST POULTRY PATHOGENS AND EFFECT OF GARLIC SUPPLEMENTATION ON DUCKLING GROWTH PERFORMANCE

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**Abstract.** Poultry production provides the source of protein and contributes an important income for Vietnamese farmers. Among the poultry in Viet Nam, ducks account for 27.3 % of head of poultry and even 55.7 % in Mekong Delta region. Along with the development of rearing ducks, bacterial, viral and fungal diseases emerging in the last two decades caused bad economic effect for poultry producer. *Escherichia coli* and *Salmonella enterica* act as major pathogenic bacteria in duck. The aims of this study were to investigate the antibacterial activity of garlic *Allium sativum* against *E. coli*, *Staphylococcus aureus*, *Salmonella* Typhimurium and to evaluate the effect of garlic on growth performance of duck from 1-28 days old. The results indicated that fresh garlic and dried garlic powder showed inhibitory effect against pathogenic tested strains at the concentration of at least 2 % and 4 % w/v, respectively. The inhibition zones and the minimal inhibitory concentration (MIC) values of garlic extract ranged from 11.3 - 28.3 mm and 0.02 - 0.2 g/ml, respectively. After 28 days of diet with garlic supplemented, the diet containing 2 % of fresh garlic showed significantly different in weight gain, feed conversion ratio, protein efficiency ratio and average daily weight gain; whereas, the diet of adding of garlic powder in basal diet only possessed a difference significant in feed consumption compared to the control. The obtained results demonstrated the potential of garlic application in poultry production.

*Keywords:* garlic, duck, antibacterial activity, growth performance, poultry production.

*Classification numbers:* 1.2.1, 1.3.4

## 1. INTRODUCTION

Poultry production provides essential protein source and contributes important revenue for Vietnamese farmers. The production of poultry, especially chickens and ducks, has been increasing during the last two decades (broiler meat rising from 226 thousand tons in 1997 to 550 thousand tons in 2018). Among the poultry, ducks occupy 27.3 % of head of poultry in Viet Nam and even 55.7 % in Mekong Delta region. At present, the consumption of duck egg and meat is increasing in Viet Nam thanks to the low price and typical flavor [1]. Along with the

raising of rearing ducks, bacterial, viral and fungal diseases occurring in the last two decades have been caused severe damage to the poultry producer. *Escherichia coli* and *Salmonella* spp. act as major bacterial pathogens in duck. Nowadays, antibiotics are used to prevent and treat bacterial infections in poultry industry. However, the overuse of antibiotic in livestock and poultry production has led to the antimicrobial resistance which is one of the most serious threats to global health [2]. Therefore, the Ministry of Agriculture and Rural Development of Viet Nam is planning to impose a ban on all kinds of antibiotics in animal feed by 2020. Alternative methods including the use of herbal products in poultry production are being evaluated [3]. Plant extracts have been reported to various activities like anti-stress, growth promotion, appetite stimulation, and enhancement of immune responses, anti-pathogen properties in animal due to active compositions such as alkaloids, terpenoids, tannins, saponins, glycosides, flavonoids, phenolics, steroids or essential oils [3]. A number of researchers over the years have investigated the varying influences of different garlic forms in poultry production [4].

Garlic (*Allium sativum*), which belongs to the *Alliaceae* family, is one of important spices in Viet Nam and throughout the world. Garlic contains many bioactive compounds such as sulfur compounds (e.g. allicin) and polyphenols. Garlics extracts have been shown antimicrobial, antioxidant and immunomodulatory effects [4, 5]. In Viet Nam, farmers have been used garlic in poultry feed to attain the aforementioned results.

The aims of this study were to investigate the antibacterial activity of garlic *A. sativum* against pathogenic bacteria in duck including *Escherichia coli, Staphylococcus aureus, Salmonella* Typhimurium, and to evaluate the effect of garlic extract on growth performance of duck from 1 to 28 days old.

## 2. MATERIALS AND METHODS

### 2.1. Materials

***Garlic preparation:*** Fresh garlic *A. sativum* was purchased from Kinh Mon, Hai Duong. Fresh garlic (moisture content ≈ 65 %) was dried at 50 $^{o}$C for 2 days to moisture content of ≈ 30%, then milled to obtain dry garlic powder. The fresh garlic extract was prepared by milling the fresh garlic in sterilized water (ratio 1:3), whereas the dry garlic powder was extracted in water (ratio 1:10). Both extractions were filtered using a 0.2 μm pore size membrane to remove all suspended solids (Minisart-Plus filters, Merck, Germany). After filtration, the extracts were diluted in water and were used for antibacterial experiment.

***Pathogenic bacteria:*** Three American Type Culture Collection (ATCC) strains were used in this study including *E. coli* ATCC 25922, *S.* Typhimurium ATCC 14028 and *S. aureus* ATCC 25923.

***Commercial feed*** was provided by Tan Phat animal feed, JSC.

### 2.2. Disc diffusion method

The antibacterial activities of garlic extract against *E. coli* ATCC 25922, *S. aureus* ATCC 25923, *S.* Typhimurium ATCC 14028 were investigated using the well diffusion agar method described by Balouiri *et al*. [6]. Inhibition activity was determined by clear zone surrounding each agar well. The test strains were inoculated into 10 ml of sterile nutrient broth (peptone 10 g/l, beef extract 10 g/l, sodium chloride 5 g/l), and incubated at 37 °C for 8 h. The cultures were swabbed on the surface of sterile nutrient agar (peptone 10 g/l, beef extract 10 g/l, sodium

chloride 5 g/l, agar 15 g/l) plates using a sterile cotton swab. Agar wells were prepared with the help of sterilized cork borer of 8 mm diameter. Using a micropipette, 100 µl of different concentrations of garlic extracts (100 %, 75 %, 50 %, 25 % and 10 %) were added to the wells. The plates were incubated in an upright position at 37 ℃ for 24 h. The assays were performed in triplicate. The inhibition zones including the diameter of the well was measured in mm with a ruler and the results were recorded. The inhibition zones with diameter more than 8 mm were considered as having antibacterial activity.

**2.3. Antibacterial activity test**

The MICs (Minimum Inhibitory Concentration) and MBC (Minimum Bactericidal Concentration) of garlic extracts were determined using micro-broth dilution assay in 96-well microplates as described previously with slightly modification [6]. The bacterial suspension of $10^6$ CFU/ml was prepared from an overnight culture in NB medium by using previously established Optical Density (OD) at 600 nm versus concentration standard curves. Each well contained 20 µl of garlic extracts at different concentration and 180 µl of bacterial suspension. Then, the plates were incubated at 37 $^oC$ for 24 h. MIC was determined as the lowest concentration showing no visible growth. The MBC was determined by spreading 100 µl of the cultures on NA plates and then incubated for 24 h at 37 $^oC$. MBC was identified as the lowest concentration showing no bacterial growth on agar plates. The assays were carried out in triplicate. A positive control containing only the culture of bacterial and a negative control containing the medium NB and garlic extract were performed under the same conditions.

**2.4. Experimental diets**

The experiment was carried out at the poultry farm at Son Tay, Hanoi. A total of 90 ducklings (vịt bầu cánh trắng) (1 day old, 45.0 ± 0.3 g) obtained from a hatchery (Ha Noi) and then ducks were randomly allocated to nine cage (10 ducks per pen; three treatments and three replicates). Three treatments including:

Diet 1 (D1): basal diet with no herbal plant (control), tap water,

Diet 2 (D2): basal diet supplemented with 2 % of garlic powder, tap water,

Diet 3 (D3): basal diet with no herbal plant added, drink water supplemented with 2 % of fresh garlic.

The ingredients were blended thoroughly in a mixer and pelleted using a pellet-maker. The feeding program involved in suppling starter diet (1-28 days old). The ducks were fed two times per day by the prepared feed at rate of 30-35 % of the estimated duckling biomass per day. Feeding rates were adjusted weekly based on estimates of biomass.

**2.5. Determination of growth performance**

The growth performance parameters were calculated according to the following formula:

Weight Gain (WG) = Final weight (g) – Initial weight (g);

Feed Conversion Rate (FCR) = Total feed (g)/Weight gain (g);

Average daily weight gain (ADG) = (Final weight – Initial weight)/Days;

Protein Efficiency Ratio (PER) = Weight gain (g)/Protein intake (g);

Feed Consumption (FC, % w/day) = (Feed given – Feed uneaten/W) × 100 %;

Feed cost for 1 kg WG (VND/kg) = FCR × price of feed.

### 2.6. Statistical analysis

All data obtained were analyzed by ANOVA using EXSTAT software. Differences between means were determined and compared by Fisher's test at the threshold of $p < 0.05$.

## 3. RESULTS AND DISCUSSION

### 3.1. Antibacterial activities of garlic extract

The antibacterial activities of garlic extract against pathogenic bacteria were presented in Table 1 and Table 2.

*Table 1*. Inhibition zones (mm) of garlic against pathogenic strains.

| | **Concentration (% w/v)** | *E. coli* | *S.* Typhimurium | *S. aureus* |
|---|---|---|---|---|
| **Fresh garlic extract** | 10 | 28.3 ± 1.5 | 25.7 ± 1.5 | 18.6 ± 1.5 |
| | 5 | 20.0 ± 3.0 | 18.3 ± 2.0 | 14.3 ± 1.5 |
| | 4 | 15.0 ± 1.0 | 13.7 ± 0.5 | 12.3 ± 0.5 |
| | 2 | - | - | - |
| **Dry garlic powder extract** | 10 | 22.0 ± 1.7 | 16.3 ± 1.5 | 13.0 ± 1.7 |
| | 5 | 15.7 ± 2.5 | 12.7 ± 0.5 | 11.6 ± 1.5 |
| | 4 | - | - | - |
| | 2 | - | - | - |

(-): Non-inhibitory effect.

Fresh garlic extract showed higher antibacterial activity than dry garlic powder extract against test bacteria (Table 1 and Table 2). All test strains were inhibited by fresh garlic extract and dry garlic powder extract up to concentration of 2 % and 4% w/v, respectively. The inhibition zone was varied from 12.3 - 28.3 mm for fresh garlic extract and from 11.6 - 22.0 mm for dry garlic powder extract. *S. aureus* was the most sensitive strain among three tested strains. In addition, the higher MIC values were obtained in the case of dry garlic powder (0.2 g/ml) compared to those of fresh garlic (0.02 - 0.04 g/ml) (Table 2). In all cases, garlic extract showed bactericidal effect against test bacteria (MBC/MIC < 4).

*Table 2.* MIC and MBC (g dry matter/ml) values of garlic against pathogenic strains.

| Strains | **Fresh garlic extract** | | **Dry garlic powder** | |
|---|---|---|---|---|
| | MIC | MBC | MIC | MBC |
| *E. coli* | 0.02 ± 0 | 0.02 ± 0 | 0.2 ± 0 | 0.2 ± 0 |
| *S.* Typhimurium | 0.04 ± 0 | 0.04 ± 0 | 0.2 ± 0 | 0.2 ± 0 |
| *S. aureus* | 0.02 ± 0 | 0.02 ± 0 | 0.2 ± 0 | 0.2 ± 0 |

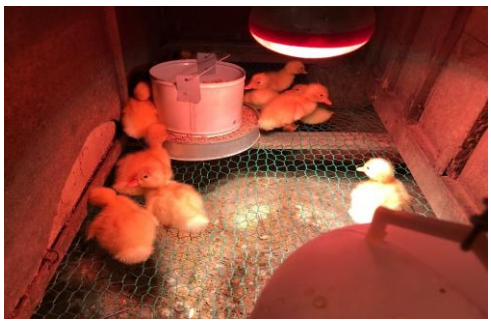
The results obtained were in accordance with those of previous studies. Garlic and its extracts have been shown to inhibit numerous microorganisms, including bacteria (*Bacillus, Escherichia, Mycobacterium, Pseudomonas, Staphylococcus and Streptococcus*), fungi, and yeasts. Durairaj *et al*. reported that aqueous garlic extract at different pH value (5.8 - 9.0)

possessed an inhibition zones ranged from 12.0 - 33.0 mm, 15.0 - 44.0 mm and 0 - 33.0 mm against *E. coli*, *S.* Typhimurium and *S. aureus*, respectively [7]. In addition, garlic extract at 0.04 - 0.10 mg/ml concentration inhibited the growth of *S. aureus* with the inhibition zones ranged from 15 - 23 mm [8]. However, Gull *et al*. reported higher inhibitory effect of garlic extract in Lahore, Pakistan (MIC = 0.1 mg/ml) than our study in the case of *E. coli* [9]. This may be due to the garlic preparation, different concentrations of the active compounds present in the extract and their interactions in the culture media. Several mechanisms have been proposed for the antibiotic activity of garlic, including modulation of sulfhydryl enzymes, inhibition of RNA synthesis, and partial inhibition of DNA and protein synthesis [10]. Recently, Booyens *et al*. (2014) investigated the antibacterial mechanism of crude garlic clove extract by using electron microscopy. The micrographs obtained showed the change of morphology such as cell elongation, distorted cells with bulbous ends and the condensation of cytoplasmic material, disintegration of membranes, loss of structural integrity of garlic clove extract-treated cells and leading to the cell death [11].

The antibacterial activity of garlic is widely attributed to allicin. Previous studies demonstrated that allicin blocked the action of bacterial enzymes by reacting with thiols, inhibited RNA synthesis and thereby inhibited the growth of the micro-organism [11, 12]. However, this constituent is photo- and thermo-sensitive, and easily metabolized into various compounds. Therefore, dry garlic powder possessed lower antimicrobial activity than fresh garlic.

### 3.2. Effect of garlic extract on growth parameters of duckling

As the antibacterial activity of garlic extract against poultry pathogen bacteria was demonstrated, our study continued to evaluate the effect of their extract on the growth of duckling. The previous study concluded that the incorporation of phytogenic substances in feed could improve the growth performance and thus decrease mortality rate of birds [13].



1 day old duckling

28 days old duckling

*Figure 1.* Duckling experimental diets.

The growth performance parameters of duckling fed experimental diets were presented in Figure 1 and Table 3. No adverse effect was observed for duckling during 28 days-feeding period. At the end of the experiment, significant differences were observed in the Weight Gain (WG), Feed Conversion Rate (FCR), Protein Efficiency Ratio (PER) and Average daily weight gain (ADG) duck fed D3 and D1 ($p > 0.05$; Table 3). The result of the WG indicated that D3 gained the highest weight (1012.9 ± 31.8 g), followed by D2 (956.0 ± 21.3 g) and D1 (894.6 ± 42.5 g). FCR of D3 groups (2.80 ± 0.13), on the other hand, showed lowest value whereas the

D1 exhibited the highest value (2.47 ± 0.06). The D2 showed highest FC value, following by D1 and D2, no significant difference was observed between D1 and D3 (Table 3).

*Table 3.* Growth parameters of duckling after 28 days of feeding with garlic supplemented.

| Treatment | WG (g) | FCR (g/g) | PER (g/g protein) | ADG (g/bird/day) | FC (g/bird) |
|---|---|---|---|---|---|
| D1 | $894.6^{a} \pm 42.5$ | $2.80^{a} \pm 0.13$ | $2.03^{a} \pm 0.10$ | $31.95^{a} \pm 1.52$ | $2520.0^{a} \pm 5.8$ |
| D2 | $956.0^{ab} \pm 21.3$ | $2.73^{a} \pm 0.09$ | $2.10^{a} \pm 0.07$ | $34.14^{ab} \pm 0.76$ | $2607.6^{b} \pm 23.9$ |
| D3 | $1012.9^{b} \pm 31.8$ | $2.47^{b} \pm 0.06$ | $2.31^{b} \pm 0.06$ | $36.17^{b} \pm 1.14$ | $2503.9^{a} \pm 28.0$ |

WG: Weight Gain, FCR: Feed Conversion Ratio, PER: Protein Efficiency Ratio, ADG: Average Daily Weight gain, FC: Feed Consumption; Values with different letters (a-b) within the same columns differ significantly ($p < 0.05$) by Fisher's test.

The results of the present study showed that 2 % garlic powder supplementation in diet had no significant effects whereas supplementation of 2 % fresh garlic in water led to significant effects on the growth performance of duck. As reported in literature, phytogenic substances are supposed to increase performance of birds by stimulating secretion of digestive enzymes, leading to enhanced digestion and absorption. Furthermore, the presence of active ingredients and phenolic compounds can reduce numbers of intestinal pathogens, thus minimizing nutrient loss and improving performance. Elagib *et al.* concluded that the incorporation of garlic at 3 % in feed significantly enhanced growth and performance of broiler chicks without any side effects, and therefore decreased mortality rate [13]. Using 0.3 % of garlic to the basal experimental diet on the broiler chicks resulted in a significant positive effect on birds' growth performance, mortality rate and immune response [14]. Additionally, garlic is rich in calcium, phosphorus, carbohydrates, contains many valuable compounds and vitamins and has a high nutritive value. In addition, garlic and its main active component, allicin, were demonstrated to improve the immunological parameter of poultry such as reduce low density lipoprotein, triglyceride and cholesterol in serum; decrease serum and liver cholesterol levels; reduce oxidative stress [4].

*Table 4.* Feed cost of garlic supplemented diets.

| Parameters/pens | D1 | D2 | D3 |
|---|---|---|---|
| Price of basal diet | 10.000 | 10.000 | 10.000 |
| Price of garlic* | 0 | 1440 | 600 |
| Price of diet | 10.000 | 11.440 | 10.600 |
| **Feed cost** | **28.000** | **31.231** | **26.182** |

Unit: VND; (*) Price of garlic = Amount of fresh garlic used in 1kg of feed * 10.000 VND/kg

The feed cost per kilogram body WG in D1, D2 and D3 were 28.000, 31.231 and 26.182 VND, respectively (Table 4). The feed cost per kilogram body WG of control group D1 was 6.49 % higher than the treatment D3 group and was 10.35 % lower than the treatment D2 group. Feed is the major component of total costs of duck rearing. The change of feed cost was due to

the difference of the FCR. In the current study, the FCR of two treatment groups were lower compared to the control (Table 3). However, due to the higher cost of garlic, rate feed cost is different between groups. The results indicated that fresh garlic showed positive effect in the cost of production.

## 4. CONCLUSIONS

In conclusion, the current study demonstrated the inhibitory effect of the garlic extract on pathogenic bacteria. The fresh garlic exhibited higher antibacterial activity than dry garlic powder. The zone of inhibition and the MIC values ranged from 11.6 – 28.3 mm and 0.02 – 0.2 g/ml, respectively. Dietary of fresh garlic supplemented in water had significant effects on the growth performance of duckling including weight gain, feed conversion ratio, protein efficiency ratio, and average daily weight gain. Due to wide distribution of garlic, their extract could be proposed to apply at industrial scale in order to strengthen the sustainable development in poultry production.

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