**Impact of Dietary Garlic Inclusion on Microbial population in the Intestinal content in Broiler**

**ABSTRACT**

The present study aimed to assess the impact of garlic (*Allium sativum*) powder as a dietary supplement on the microbial population in broiler chickens. A total of 120 unsexed day-old broiler chicks (Cobb 500) were randomly assigned to four treatment groups (n=30), with each group further divided into three replicates, following a completely randomized design (CRD) over a 42-day period.

The control group (C) received a basal diet without additives or antibiotics, while experimental groups (T1, T2, and T3) were supplemented with garlic powder at inclusion rates of 0.2%, 0.4%, and 0.6%, respectively, starting from the second week of feeding. Feed intake was monitored throughout the study.

At the end of the experiment, birds were slaughtered, and microbial counts were recorded. Statistical analysis revealed a highly significant (P ≤ 0.001) positive effect of garlic powder supplementation, leading to the complete absence of Salmonella spp. in the ileo-cecal digesta.

**Keywords: Garlic, Broiler, Escherichia coli, Salmon**e**lla**

Introduction

The poultry industry plays a pivotal role in ensuring food security for the rapidly growing global population. Broiler production, characterized by its short cycle, high feed efficiency, and substantial biomass yield per unit of agricultural land, is particularly advantageous in meeting the rising demand for animal protein. However, the success of broiler production is largely dependent on the availability of high-quality feed and effective disease management strategies.

Historically, the use of antibiotics and chemical additives in poultry feed has been instrumental in controlling infectious diseases and enhancing feed efficiency (Engberg et al., 2000). These compounds, produced by lower fungi and certain bacteria, are widely utilized to prevent bacterial proliferation and mitigate health risks in both humans and animals. However, extensive scientific research indicates that the indiscriminate use of antibiotics has contributed to the emergence of antibiotic-resistant bacterial strains (Diarra et al., 2007; Furtula et al., 2010). Furthermore, antibiotic residues in feed and the environment pose significant health risks (Carvalho & Santos, 2016; González Ronquillo et al., 2017), compromising overall human and animal welfare (Diarra et al., 2010).

Given the mounting concerns surrounding antibiotic resistance and the potential carcinogenic and immunosuppressive effects of chemical additives, regulatory bodies such as the European Union have imposed strict bans on their use in animal feed. This has necessitated the exploration of alternative feed additives that are both effective and safe. One promising alternative is garlic powder (*Allium sativum)*, a natural phytogenic feed additive renowned for its antimicrobial and immunomodulatory properties.

Phytogenic feed additives (PFAs), derived from plants, herbs, and spices, have gained prominence in poultry nutrition due to their ability to enhance growth performance, bolster immune function, and mitigate stress responses (Sun et al., 2005). Among these, garlic (Allium sativum), a widely used medicinal plant belonging to the family Liliaceae and the genus Allium (Ebesunun et al., 2007), has exhibited considerable potential as a natural growth promoter and antibiotic alternative (Demir et al., 2003). Garlic has long been recognized for its antibacterial, antifungal, antiparasitic, antiviral, antioxidant, anti-cholesteremic, anticancer, and vasodilatory properties (Khan et al., 2007; Hanieh et al., 2010). Moreover, some studies suggest that commercial garlic products—including garlic oil, garlic powder, and extracts—may possess hypocholesterolemic effects (Aporn et al., 2008).

Beyond its antimicrobial benefits, garlic has been demonstrated to enhance feed palatability and subsequently improve feed intake in poultry (Newall et al., 1996). Given these attributes, garlic powder serves as a viable natural alternative to conventional antibiotics in broiler diets. This study aims to evaluate the efficacy of garlic powder supplementation in broiler feed and its impact on the microbial population in intestinal content, contributing to the development of sustainable and antibiotic-free poultry production systems.*.*

**2. Materials and Methods**

**2.1 Experimental Site and Duration**

This study was conducted at the poultry farm of Merowe University of Technology (Abdulatif Alhamad) in Sudan from December 29, 2019, to February 8, 2020.

**2.2 Experimental Housing**

The chicks in each replicate were housed in pens measuring 1 square meter within an open-sided deep litter system. The house temperature was systematically controlled during the fattening period, starting at 33°C on the first day and gradually reduced by 2°C per week. Continuous lighting was maintained throughout the experimental period. Each –pen was equipped with feeding troughs, and water was provided ad libitum through a self-feed pump.

**2.3 Experimental Birds**

A total of 120 unsexed, one-day-old broiler chicks (Cobb 500) were obtained from the Sudanese Nile Poultry Company, Northern Sudan. The birds were maintained under strict hygienic conditions throughout the study. They were randomly selected and weighed to ensure uniform initial body weight, without significant variation. The chicks were then allocated into four treatment groups (C, T1, T2, and T3), with 30 birds per group, further divided into three replicates each, following a completely randomized design (CRD) over 42 days.

**2.4 Experimental Diets**

**2.4.1 Garlic Preparation**

Garlic bulbs (*Allium sativum*) were sourced from the local market, sun-dried separately, finely ground into powder, and stored until use**.**

**2.4.2 Diet Formulation**

Two basal diets (Starter and Finisher) were formulated to meet the nutritional requirements of the Cobb 500 strain during the starter and finisher phases. The experimental birds were divided into four groups:

Control group (C): Fed a basal diet without additives. Treatment groups (T1, T2, and T3): Received basal diets supplemented with garlic powder at inclusion rates of 0.2%, 0.4%, and 0.6%, respectively, starting from the second week.

All birds were provided the starter feed from Day 1 to Day 21, followed by the finisher feed from Day 22 to Day 42. Nutritional analysis was conducted in accordance with the methods outlined by AOAC (1998). Details of ingredients, calculated chemical composition, and determined chemical analysis for the basal diets are presented in Tables 1 while the chemical composition of the super concentrate used in the basal diets is shown in Table (2, 3).

**Table 1. Calculated analysis of the experimental diet**

|  |  |  |
| --- | --- | --- |
| Item | Starter (from 1st to 21st day) | Grower(22nd to 42nd day) |
| Metabolizable energy (Kcal/kg) | 2991 | 3089 |
| Crude fat, % | 4.43 | 5.17 |
| Crude protein,% | 2143. | 18.7 |
| Lysine, % | 1.15 | 1.14 |
| Methionine,% | 0.53 | 0,54 |
| Cystine, % | 0.29 | 0.26 |
| Methionine + cystine,% | 0.82 | 0.80 |
| Calcium,% | .0.98 | 0.87 |
| Available phosphorus,% | 0.77 | 0.68 |
| Caloric-protein ratio | 140 | 165 |

**Table)2( Chemical composition of the super concentrate**

**(Hendrix starter broiler concentrate)**

|  |  |
| --- | --- |
| Metabolizable energy | 2150 (Kcal/kg) |
| Crude protein | **35%** |
| Lysine | **12.65%** |
| Methionine | **5.8%** |
| Methionine + cysteine | **6.1%** |
| Calcium | **6.8%** |
| Available phosphorus | **7.9%** |

**Table )3( Chemical composition of the super concentrate**

**(Champrix broiler concentrate)**

|  |  |
| --- | --- |
| Metabolizable energy | 2000 (Kcal/kg) |
| Crude protein | **35%** |
| Lysine | **14%** |
| Methionine | **5.6%** |
| Methionine + cysteine | **6.8%** |
| Calcium | **6.1%** |
| Available phosphorus | **6.5%** |

**2.5 Microbial Population Analysis**

On day 42, three chickens were randomly selected from each replicate group and humanely sacrificed. The ileal content was immediately collected for microbial culture and transported to the Microbiology Laboratory of Merowe Medical City using appropriate transport media for microbial identification and enumeration.

For analysis, 1 g of digesta from each sample was suspended in 9 ml of cooked meat broth and mixed for 1 minute. A tenfold serial dilution was then performed by transferring 1 ml from each mixture into fresh broth volumes. Subsequently, 1 ml from the 1/1000 dilution test tube of each sample was inoculated onto solid culture media prepared in Petri dishes on the previous day.

Microbial dispersion was carried out using a sterile spreader, which was sterilized over a Bunsen flame after each step to prevent contamination. Yeasts and mold fungi were cultured on Sabouraud agar medium, supplemented with 250 mg of chloramphenicol to inhibit bacterial growth, and incubated at room temperature for up to two weeks to allow proper identification.

For bacterial enumeration, selective culture media were utilized: Salmonella/Shigella agar medium for Salmonella and Shigella species MacConkey agar for Escherichia coli and other enterobacteria. This methodological approach ensured precise microbial assessment and identification, contributing to the study's evaluation of garlic powder as a natural feed additive in broiler diets.

**2.6 Statistical Analysis**

The experimental data were analyzed using the General Linear Model (GLM) in SPSS Statistics 17.0 (Statistical Package for the Social Sciences, released on August 23, 2008). Significant differences among treatments were evaluated using One-Way Analysis of Variance (ANOVA), followed by Duncan’s Multiple Range Test (DMRT) to assess pairwise comparisons. A significance threshold of (P ≤ 0.05) was applied, as outlined by Duncan (1955).

**3. RESULTS AND DISCUSSION**

The results presented in Table (4) indicate that there were no significant differences (P ≥ 0.05) among the treatment groups in terms of *Escherichia coli* colony counts. However, the total colony-forming unit (CFU) count for Salmonella bacteria exhibited a highly significant effect (P ≤ 0.001), as Salmonella was completely absent in all treatment groups compared to the control.

**Table. (4).** Effects of garlic on microbial population of broilers chicken

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Treatment | C | T1 | T2 | T3 | P level |
|  | **control** | **0.2%garlic** | **0.4%garlic** | **0.6%garlic** |  |
| *Escherichia coli* | 8.8±1.6 | 8±1.9 | 11.4±1.7 | 7.5±2.7 | N.S |
| *Salmonella.SPP* | 8.5±7.4a | 0000b | 0000b | 0000b | \*\*\* |

Means on the same raw with the same superscripts are not significantly different (P≥0.05). Means with different superscripts within the same row are significantly different (P<0.05). N.S = Not significant. \* = (P≤ 0. 05). \*\* = (P ≤ 0. 01). \*\*\* = (P≤0. 001).

The findings of the present study are consistent with those of Ismoyowati et al. (2015), who reported that the concentration of *Escherichia coli* in the caecum was not significantly affected by dietary supplementation with aqueous garlic extract. Similarly, Rafeeq et al. (2017) demonstrated that garlic, when used as an antimicrobial feed additive, had a profound impact on microbial populations, leading to a reduction in various bacterial species.

Conversely, some studies have reported differing outcomes. Damanik et al. (2015) found that the administration of garlic extract significantly (P ≤ 0.05) reduced *Escherichia coli* counts in broiler chicken feces, suggesting that garlic extracts could serve as an effective means of controlling *E. coli* proliferation in poultry. Likewise, Dieumou et al. (2009) observed a significant (P ≤ 0.001) decrease in colony-forming units (CFU) of *Escherichia coli* and other enterobacteria in the ileo-cecal digesta compared to the control group. Their study further noted a P ≤ 0.05 reduction in Salmonella and Shigella populations as garlic essential oil dosage increased.

The antimicrobial properties of garlic have been shown to positively influence gut microbiota, reducing bacterial loads and contributing to overall health maintenance. These findings reinforce the potential of garlic as a natural feed additive with broad-spectrum antimicrobial benefits. Given its efficacy in reducing pathogenic bacteria such as E. coli and Salmonella, garlic supplementation in poultry diets has been linked to improved bird performance and enhanced production potential.

Moreover, garlic has demonstrated immunomodulatory effects, benefiting consumers' health by promoting stronger immune responses. Given these attributes, garlic could serve as a viable replacement for conventional antibiotic growth promoters in poultry nutrition, supporting sustainable and antibiotic-free poultry production systems.

**4.2. Recommendations.**

From the present study we would like recommend

1. Uses garlic at different three level in broilers diet as an effective alternative of antibiotic growth promoters.
2. Induce garlic in broiler diets to reduce the number of pathogenic bacteria like salmonella spp.
3. When use garlic in wider commercial applications there is a test their effects at lower inclusion levels and other preparation as well as administration methods for economic point of view.
4. Future Research Directions: Additional studies are required to establish standardized guidelines for garlic usage in poultry feed, ensuring its efficacy, safety, and economic feasibility.

**DISCLAIMER (ARTIFICIAL INTELLIGENCE)**

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (Chat GPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

**REFRENCE**

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