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# Synergistic Effects of Selenium and Garlic-Based Synbiotic on Antioxidant Capacity and Meat Quality of Broilers Under High-Density Tropical Rearing

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**Abstract** | High-density rearing in broiler farming often causes stress, impairing growth, gut health, and meat quality, especially under tropical conditions. This study evaluated the combined effect of dietary selenium (Se) and a garlic-based synbiotic (*Lactobacillus casei* + garlic flour) on broilers reared at high density in a tropical climate. A total of 275 two-week-old broilers (448 ± 25 g) were arranged to four treatments in a completely randomized design: T0 (basal diet, 10 birds/$m^2$), T1 (basal diet, 15 birds/$m^2$), T2 (basal diet + 0.12 mg/kg Se, 15 birds/$m^2$), and T3 (basal diet + 1% garlic-based synbiotic, 15 birds/$m^2$). Growth performance, nutrient digestibility, intestinal morphology, microbiota, volatile fatty acids (VFA), antioxidant status, and meat quality were evaluated. The results show T3 significantly improved feed intake, body weight gain, and production ($P<0.05$), while reducing *coliform* counts and enhancing ileal growth. Although antioxidant markers (SOD and MDA) were not significantly affected, the trends indicated improved oxidative stability. Meat from T2 and T3 birds had higher protein and lower cholesterol levels ($P<0.05$). Conclusion: These findings highlight that dietary supplementation with selenium combined with a garlic-based synbiotic (*Lactobacillus casei* + garlic flour) effectively improved growth performance, intestinal health, and meat quality of broilers reared under high stocking density. Combined supplementation with selenium and a garlic-based synbiotic effectively mitigated the negative effects of high-density rearing.

**Keywords** | Antioxidant, High density, Selenium, Synbiotic, Tropical climate, Broiler production

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## INTRODUCTION

Efficient use of housing space is a key challenge in broiler production. Increasing stocking density is commonly practiced to maximize output per unit area, but it often compromises animal welfare, growth performance, and meat quality, particularly under tropical conditions where ambient temperatures are above the thermal comfort zone of broilers. High stocking density combined with heat stress increases oxidative stress, impairs gut barrier function, and reduces carcass quality (Abudabos *et al*., 2013; Goo *et al*., 2019; Son *et al*., 2022). These challenges highlight the urgent need for nutritional strategies that can mitigate stress-induced negative effects in intensive broiler systems.

Selenium (Se) is an essential trace mineral, which plays a central role in the antioxidant defense system, thereby reducing oxidative stress and protecting tissue integrity

(Sevim *et al.*, 2023; Wickramasuriya *et al.*, 2023). Meanwhile, synbiotics, which combine probiotics and prebiotics, have proven effective in enhancing gut microbial balance, improving intestinal morphology, and supporting nutrient absorption. Garlic is a natural prebiotic with antimicrobial and hypocholesterolemic properties. When combined with *Lactobacillus casei*, it has the potential to promote gut health and improve antioxidant capacity (Mangisah *et al.*, 2024). Several recent studies have shown that selenium supplementation in the diet of broiler chickens raised at high stocking densities and under heat stress has been shown to increase the activity of selenoenzymes such as glutathione peroxidase (GPx), protect intestinal epithelial cells from oxidative damage, and improve nutrient absorption (Bień *et al.*, 2023; Sevim *et al.*, 2023). Simultaneously, synbiotic supplementation can improve gut microbiota balance, enhance immunity, increase short-chain fatty acid (SCFA) production, and strengthen intestinal barrier function (Mangisah *et al.*, 2024; Khosravi *et al.*, 2025).

Therefore, combining Se with synbiotics is hypothesized to provide enhanced and complementary protection, with selenium acting at the cellular enzymatic level as an antioxidant, while the synbiotic supports gut microbial balance and nutrient digestion. This combined action offers dual protection against oxidative and physiological stress in broiler chickens raised at high stocking densities and tropical temperatures.

Previous research has mostly focused on either Se supplementation or synbiotic supplementation independently. Based on our observation, there is no study has evaluated the combined effect of selenium with a garlic-based synbiotic in broilers reared at high density under tropical climates. Therefore, this study aimed to evaluate the combined effects of selenium and a garlic-based synbiotic on growth performance, gut health, antioxidant capacity, and meat quality in broilers raised at high density in tropical conditions.

## MATERIALS AND METHODS

The experimental procedures in this study were approved by the Animal Research Ethics Committee, Faculty of Animal Husbandry and Agriculture, Universitas Diponegoro (Approval No. 57-01/A-4/KEP-FPP.2).

### Animal and experimental design

A total of 275 two-week-old broiler chickens (average body weight: 448 ± 25 g) were obtained from a commercial broiler farm. The study was arranged in a completely randomized design (CRD) consisting of four treatments with five replicates, resulting in 20 experimental units. The treatments were as follows:

T0: basal diet + normal-density (10 birds/m$^2$)
T1: basal diet + high-density (15 birds/m$^2$)
T2: basal diet + Se (0.12 mg/kg), high-density (15 birds/m$^2$)
T3: basal diet + Se (0.12 mg/kg) + garlic-based synbiotic (1%), high-density (15 birds/m$^2$)

Each pen measured 1 × 1 m$^2$, accommodating either 10 (normal-density) or 15 birds (high-density). All birds were managed under uniform lighting and husbandry conditions. Temperature and humidity were recorded daily. Water and feed were provided ad libitum. The basal diet was formulated based on NRC (1994) requirements, and its composition and nutrient content are presented in Table 1. Treatments were applied from day 21 to 35 of age.

**Table 1:** Composition and nutrient content of ration.

| Ingredients | Composition (%) | |
|---|---|---|
| | **Starter (1-21)** | **Finisher (22-35)** |
| Yellow corn | 56.10 | 63.40 |
| Soybean meal | 36.90 | 30.10 |
| Palm oil | 2.30 | 2.30 |
| Bentonite | 1.00 | 0.50 |
| Limestone | 1.33 | 1.33 |
| Mono calcium phosphate | 1.45 | 1.45 |
| Premix* | 0.57 | 0.57 |
| NaCl | 0.35 | 0.35 |
| Total | 100.00 | 100.00 |
| **Nutritional content** | | |
| ME (kcal/kg) | 2918 | 3003 |
| Dry matter | 86.35 | 87.15 |
| Crude protein (%) | 21.80 | 19.16 |
| Ether extract (%) | 2.61 | 2.82 |
| Crude fiber (%) | 3.32 | 3.21 |
| Calcium (%) | 0.85 | 0.87 |

### Preparation treatment

Selenium used in this study was commercial product “Introvit-ES-200 WS”. The synbiotic consisted of *Lactobacillus casei* (10$^8$ CFU/g) as a probiotic and 1.5% garlic powder as a prebiotic. Synbiotic preparation followed Mangisah *et al.* (2021), in which *L. casei* was inoculated into skim milk, incubated for 24 h at 37 °C, mixed with 1.5% garlic powder, and further incubated for 48 h. Synbiotic products (containing *L. casei* and 1.5% garlic powder) were mixed into the ration, amounting to 1% of the total ration given. Selenium and garlic-based synbiotic were thoroughly mixed into the diet during the treatment period.

The dietary treatments were applied from 21 to 35 days of age. The diets were provided according to the birds' nutritional requirements. Vaccination was carried out using the ND Lasota vaccine at 4 and 15 days of age.

### Parameter measurement

#### Nutrient digestibility

Nutrient digestibility was determined using the total collection method for 3 consecutive days after a 2-week adaptation to the experimental diets. Feed intake was recorded daily. Excreta were collected, dried, and analyzed for crude protein, dry matter, and ether extract following AOAC (2012) procedures.

#### Growth performance

Feed intake was calculated as the difference between feed offered and feed refusal. Body weight gain (BWG) was calculated from the difference between the final and the initial body weights. Production efficiency was calculated as the total live weight per experimental unit at 35 d.

#### Digestive organ morphometry

Lengths of the duodenum, jejunum, ileum, cecum, and colon were measured using a flexible measuring tape, and weights were recorded with an analytical balance.

#### Intestinal pH and microbiota

The pH of the proventriculus, gizzard, duodenum, jejunum, ileum, cecum, and colon were measured within 5 min post-mortem using a calibrated digital pH meter. Lactic acid bacteria (LAB), *coliform*, and total *Lactose negative enterobacteriaceae (LNE)* were quantified using the total plate count (TPC) method as described by Teymouri *et al.* (2021). Serial dilutions of intestinal content were spread on selective media (MRS agar for LAB and MacConkey agar for *coliforms*) and expressed as CFU/ml.

#### Intestinal villi morphology

Morphometry of villi was assessed following Jazi *et al.* (2018). Two-centimeter segments from the duodenum, jejunum, and ileum. These were fixed in buffered formalin, embedded in paraffin wax, sliced into 5 µm sections and stained with hematoxylin–eosin. Villus height (apex to crypt junction) and crypt depth were measured under light microscopy at 40× and 100× magnification. Villus height-crypt depth ratio (VH:CD) was calculated. Image-analysis software was used to analyzed at least 10 well-oriented villia and crypts per sample.

#### Volatile fatty acids (VFA)

Cecal contents were collected immediately post-mortem and analyzed for VFA concentrations using gas chromatography (GC), expressed as µmol/g digesta (Muhammad *et al.*, 2021).

#### Oxidative stress indicator

Blood samples (3 ml) were collected from the brachial vein into plain tubes, centrifuged at 3000 rpm for 10–15 min, and the serum analyzed for superoxide dismutase (SOD) activity and malondialdehyde (MDA) concentration using commercial assay kits. Absorbance was observed at 450 nm (SOD) and 532 nm (MDA) using a spectrophotometer (Hosseinzadeh *et al.*, 2023).

#### Blood biochemical profile

An additional 3 ml of blood was collected into EDTA tubes, centrifuged (3000 rpm, 10–15 min), and plasma analyzed for total cholesterol, HDL, and triglycerides by spectrophotometry. LDL was calculated using Friedewald's formula (Friedewald *et al.*, 1972).

#### Meat quality

Breast muscle samples (50–100 g, pectoralis major) were homogenized and analyzed for crude protein (Kjeldahl method), cholesterol (Lopes-Virella *et al.*, 1997), crude fat (Soxhlet extraction), and antioxidant capacity (commercial assay kit).

#### Statistical analysis

The data were analyzed using one-way ANOVA with SPSS software (version 22). Significant differences in the means of the treatments were indentified using Duncan's multiple range test at $p<0.05$ (Steel and Torrie, 1995).

## RESULTS

#### Nutrient digestibility

Crude fat digestibility showed a significant decrease ($P<0.05$) in T1 and T2 compared to T0 and T3, with the highest value observed in T3 (Table 2). Crude protein digestibility was not significantly affected ($P>0.05$) by treatments, although T3 presented the numerically highest value. Digesta passage rate also showed no significant differences ($P>0.05$) among treatments with or without Se and garlic-based synbiotic supplementation or under different stocking densities.

#### Performance

Feed intake and body weight gain (BWG) were significantly influenced ($P<0.05$) by the treatments. T0 and T3 had comparable values, both higher than T1 and T2 (Table 2). Production per unit area increased significantly ($P<0.05$) in T3 compared with the other treatments.

#### Digestive organ growth

The length and weight of digestive organs were generally not significantly affected ($P>0.05$) by Se and garlic-based synbiotic supplementation under normal or high stocking densities, except for the ileum (Table 3). Ileum weight was

**Table 2:** Effect of selenium (Se) and garlic-based synbiotic administration in normal and high density on the nutrient digestibility and performance of broiler chickens.

| Parameter | T0 | T1 | T2 | T3 | SEM | P value |
|---|---|---|---|---|---|---|
| Crude fat digestibility | 94.13 [a] | 91.13 [b] | 91.79 [b] | 94.56 [a] | 0.31 | 0.01 |
| Crude protein digestibility | 77.26 | 73.26 | 71.21 | 75.86 | 1.35 | 0.42 |
| Digestion rate (minutes) | 198.00 | 201.40 | 199.60 | 201.20 | 1.09 | 0.67 |
| Consumption (g/birds) | 2167.1[a] | 1883.22 [c] | 1840.14 [c] | 1935.82 [b] | 2.15 | 0.01 |
| BWG (g/birds) | 1422.80[a] | 1090.10 [c] | 1136.90 [c] | 1279.90 [b] | 11.55 | 0.01 |
| Production (kg/m$^2$) | 23.79 [d] | 30.09 [c] | 31.41 [b] | 33.54 [a] | 0.55 | 0.01 |

[a,b,c,d] Means with different superscripts in the same row are significantly different (P<0.05). T0: basal feed + density 10 birds/m$^2$, T1: basal feed + density 15 birds/m$^2$, T2: basal feed + density 15 birds/m$^2$ + selenium (Se) supplementation, T3: basal feed + density 15 birds/m$^2$ + selenium supplementation 0.12 mg/kg + 1% synbiotic.

**Table 3:** Effect of selenium (Se) and garlic-based synbiotic administration in normal and high density on digestive organ growth of broiler chickens.

| Parameter | T0 | T1 | T2 | T3 | SEM | P value |
|---|---|---|---|---|---|---|
| **Proventriculus** | | | | | | |
| pH | 4.46 | 4.62 | 4.66 | 4.42 | 0.05 | 0.31 |
| Weight (g) | 0.35 | 0.31 | 0.31 | 0.35 | 0.01 | 0.09 |
| **Ventriculus** | | | | | | |
| pH | 4.70 | 4.48 | 4.64 | 4.72 | 0.07 | 0.57 |
| Weight (g) | 1.31 | 1.33 | 1.35 | 1.35 | 0.01 | 0.61 |
| **Duodenum** | | | | | | |
| pH | 5.94 | 5.98 | 5.96 | 5.88 | 0.08 | 0.37 |
| Weight (g) | 0.51 | 0.46 | 0.46 | 0.51 | 0.01 | 0.11 |
| Length (cm) | 35.50 | 33.70 | 34.00 | 35.20 | 0.31 | 0.15 |
| **Jejunum** | | | | | | |
| pH | 5.62 | 5.76 | 5.88 | 5.96 | 0.04 | 0.14 |
| Weight (g) | 1.11 | 1.03 | 0.97 | 1.04 | 0.02 | 0.29 |
| Length (cm) | 87.00 | 83.00 | 83.70 | 86.40 | 0.61 | 0.44 |
| **Ileum** | | | | | | |
| pH | 5.68 | 5.56 | 5.62 | 5.52 | 0.06 | 0.06 |
| Weight (g) | 0.79 [b] | 0.85 [b] | 0.83 [b] | 0.9 [a] | 0.01 | 0.02 |
| Length (cm) | 86.7 [a] | 76.7 [c] | 78.3 [c] | 80.4 [b] | 0.86 | 0.00 |
| **Caeca** | | | | | | |
| pH | 7.18 [a] | 6.38 [b] | 6.40 [b] | 6.54 [b] | 0.09 | 0.04 |
| Weight (g) | 0.35 | 0.28 | 0.30 | 0.33 | 0.01 | 0.15 |
| Length (cm) | 19.00 | 18.96 | 19.24 | 19.54 | 0.48 | 0.97 |
| **Colon** | | | | | | |
| pH | 6.76 | 6.72 | 6.46 | 6.74 | 0.08 | 0.09 |
| Weight (g) | 0.12 | 0.11 | 0.11 | 0.12 | 0.01 | 0.08 |
| Length (cm) | 11.00 | 9.10 | 9.50 | 9.50 | 0.95 | 0.07 |

[a,b,c,d] Means with different superscripts in the same row are significantly different (P<0.05). T0: basal feed + density 10 birds/m$^2$, T1: basal feed + density 15 birds/m$^2$, T2: basal feed + density 15 birds/m$^2$ + selenium (Se) supplementation, T3: basal feed + density 15 birds/m$^2$ + selenium supplementation 0.12 mg/kg + 1% synbiotic.

significantly higher (P<0.05) in T3 compared to the other treatments, while ileum length was significantly shorter (P<0.05) in T1 and T2 than in T0. The addition of Se and garlic-based synbiotic did not significantly influence the pH of digestive organs (P>0.05), although a decreasing trend was observed in the caeca pH values of T1, T2, and T3.

## Intestinal microbes

The intestinal microbial population is presented in Table 4. *Coliform* counts were significantly reduced (P<0.05) in T3 compared with T1, but were similar to T0 and T2. Lactic acid bacteria (LAB) populations were not significantly different among treatments (P>0.05), although T1 showed a slightly higher count. *Lactose negative Enterobacteriaceae (LNE)* were significantly higher (P<0.05) in T1 compared with the other groups, while T2 and T3 did not differ from T0.

**Table 4:** Effect of selenium (Se) and garlic-based synbiotic administration in normal and high density on intestinal microbes of broiler chickens.

| Parameters (log CFU/ml) | T0 | T1 | T2 | T3 | SEM | P value |
|---|---|---|---|---|---|---|
| *Coliform* | 5.47 [c] | 8.37 [a] | 6.55 [c] | 7.13 [b] | 0.26 | 0.01 |
| Lactic acid bacteria | 11.59 | 11.63 | 11.55 | 11.34 | 0.05 | 0.26 |
| Lactose-negative enterobacteriaceae (LNE) | 7.48 [b] | 8.74 [a] | 7.32 [b] | 7.62 [b] | 0.17 | 0.04 |

[a,b,c,d] Means with different superscripts in the same row are significantly different (P<0.05). T0: basal feed + density 10 birds/m$^2$, T1: basal feed + density 15 birds/m$^2$, T2: basal feed + density 15 birds/m$^2$ + selenium (Se) supplementation, T3: basal feed + density 15 birds/m$^2$ + selenium supplementation 0.12 mg/kg + 1% synbiotic.

## Intestinal villi morphometry

The addition of Se and garlic-based synbiotic had no significant effect (P>0.05) on villus height and crypt depth of the duodenum and jejunum, except for jejunal crypt depth (Table 5). Jejunal crypt depth was significantly reduced (P<0.05) in T1, T2, and T3 compared with T0.

**Table 5:** Effect of selenium (Se) and garlic-based synbiotic administration in normal and high density on villi morphology of broiler chickens.

| Parameter | T0 | T1 | T2 | T3 | SEM | P value |
|---|---|---|---|---|---|---|
| **Duodenum (μm)** | | | | | | |
| Villi height/ VH | 1292.34 | 1016.23 | 1247.21 | 1372.38 | 54.04 | 0.15 |
| Crypth depth/ CD | 261.70 | 206.61 | 243.09 | 266.78 | 7.85 | 0.06 |
| VH/CD ratio | 4.94 | 4.92 | 5.20 | 5.47 | 0.32 | 0.92 |
| **Jejunum (μm)** | | | | | | |
| Villi height/ VH | 1858.64 | 1634.01 | 2070.83 | 1786.93 | 56.14 | 0.09 |
| Crypt depth/ CD | 301.79 $^{a}$ | 219.49 $^{b}$ | 308.72 $^{b}$ | 230.18 $^{b}$ | 5.40 | 0.01 |
| VH/CD ratio | 6.18 | 7.51 | 6.75 | 7.83 | 0.25 | 0.13 |

$^{a,b,c,d}$Means with different superscripts in the same row are significantly different (P<0.05). T0: basal feed + density 10 birds/m$^2$, T1: basal feed + density 15 birds/m$^2$, T2: basal feed + density 15 birds/m$^2$ + selenium (Se) supplementation, T3: basal feed + density 15 birds/m$^2$ + selenium supplementation 0.12 mg/kg + 1% synbiotic.

**Table 6:** Effect of selenium (Se) and garlic-based synbiotic administration in normal and high density on the production of Volatile Fatty Acid (VFA) of broiler caecum.

| Parameters (μmol/g) | T0 | T1 | T2 | T3 | SEM | P value |
|---|---|---|---|---|---|---|
| Acetate | 1.41 $^{c}$ | 9.82 $^{a}$ | 3.39 $^{b}$ | 2.29 $^{b}$ | 0.84 | 0.01 |
| Propionate | 0.37 $^{b}$ | 2.66 $^{a}$ | 0.95 $^{b}$ | 0.52 $^{b}$ | 0.23 | 0.01 |
| Butyrate | 0.26 $^{b}$ | 3.11 $^{a}$ | 0.65 $^{b}$ | 0.24 $^{b}$ | 0.26 | 0.01 |

$^{a,b,c,d}$Means with different superscripts in the same row are significantly different (P<0.05). T0: basal feed + density 10 birds/m$^2$, T1: basal feed + density 15 birds/m$^2$, T2: basal feed + density 15 birds/m$^2$ + selenium (Se) supplementation, T3: basal feed + density 15 birds/m$^2$ + selenium supplementation 0.12 mg/kg + 1% synbiotic.

**Table 7:** Effect of selenium (Se) and garlic-based synbiotic administration in normal and high density on the lipid profile and antioxidants of broiler chickens.

| Parameter | T0 | T1 | T2 | T3 | SEM | P value |
|---|---|---|---|---|---|---|
| Cholesterol (mg/dL) | 137.78 | 131.11 | 133.33 | 133.33 | 4.65 | 0.96 |
| HDL (mg/dL) | 27.08 | 20.87 | 23.98 | 24.42 | 1.28 | 0.42 |
| LDL (mg/dL) | 68.41 | 62.11 | 66.5 | 64.66 | 4.34 | 0.96 |
| Triglycerides (mg/dL) | 211.43 | 162.86 | 271.43 | 220.00 | 13.34 | 0.08 |
| Serum SOD (U/ml) | 21.37 | 21.15 | 21.46 | 29.24 | 2.00 | 0.43 |
| Serum MDA (nmol/ml) | 7.55 | 7.87 | 10.73 | 6.76 | 0.63 | 0.17 |

Means with no stats in the same row are not significantly (P>0.05). T0: basal feed + density 10 birds/m$^2$, T1: basal feed + density 15 birds/m$^2$, T2: basal feed + density 15 birds/m$^2$ + selenium (Se) supplementation, T3: basal feed + density 15 birds/m$^2$ + selenium supplementation 0.12 mg/kg + 1% synbiotic.

## Volatile fatty acids (VFA)

VFA concentrations in the caeca were significantly affected by treatments (P<0.01) (Table 6). Acetate production was highest in T1, followed by T2 and T3, while T0 showed the lowest values. Propionate and butyrate production followed a similar trend, with T1 significantly higher (P<0.05) than the other groups.

## Blood profile and serum antioxidants

Se and garlic-based synbiotic supplementation, under normal or high stocking densities, did not significantly influence (P>0.05) blood lipid profiles, including cholesterol, HDL, LDL, and triglycerides (Table 7). However, cholesterol and LDL levels tended to be lower in T1 compared to other groups. Serum antioxidant indicators (SOD and MDA) were also not significantly affected (P>0.05) by treatments.

## Meat quality

Meat quality parameters are presented in Table 8. Crude protein content of meat was significantly higher (P<0.05) in T2 and T3 compared with T0 and T1. Meat cholesterol content was significantly reduced (P<0.05) in T2 and T3 compared with T0, while T1 did not differ from the other treatments. No significant differences (P>0.05) were observed in crude fat or antioxidant activity among treatments.


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**Table 8:** Effect of selenium (Se) and garlic-based synbiotic administration in normal and high density on meat quality of broiler chickens.

| Parameter | T0 | T1 | T2 | T3 | SEM | P value |
|---|---|---|---|---|---|---|
| Crude protein (%) | 20.80[b] | 20.63[b] | 21.97[a] | 22.26[a] | 0.20 | 0.03 |
| Crude fat (%) | 0.79 | 1.09 | 0.74 | 0.87 | 0.04 | 0.06 |
| Cholesterol (mg/100g) | 52.27[b] | 52.31[b] | 40.26[a] | 41.93[a] | 0.18 | 0.01 |
| Antioxidants (μg/g) | 10.52 | 10.42 | 9.88 | 9.94 | 0.10 | 0.09 |

[a,b,c,d] Means with different superscripts in the same row are significantly different (P<0.05). T0: basal feed + density 10 birds/m$^2$, T1: basal feed + density 15 birds/m$^2$, T2: basal feed + density 15 birds/m$^2$ + selenium (Se) supplementation, T3: basal feed + density 15 birds/m$^2$ + selenium supplementation 0.12 mg/kg + 1% synbiotic.

## DISCUSSION

The present study demonstrated that the combined supplementation of selenium (Se) and a garlic-based synbiotic improved several key parameters of broiler performance, gut health, and meat quality under high-density tropical rearing conditions. Notably, improvements were observed in nutrient digestibility, intestinal morphology, microbial balance, and meat crude protein content, while reductions were evident in ileal length, cholesterol deposition, and pathogenic *coliform* counts. These findings highlight the combined of Se and garlic-based synbiotic in mitigating the adverse effects of stocking stress, which remains a critical challenge in tropical poultry production systems.

The significant enhancement in crude fat digestibility and improved ileal morphometry in the Se- garlic-based synbiotic group (T3) indicate that this supplementation enhanced nutrient absorption efficiency. Previous studies have shown that Se supports antioxidant enzyme activity, thereby maintaining intestinal epithelial integrity and improve nutrient digestibility (Surai *et al.*, 2019; Deng *et al.*, 2022).

The T1 treatment group (high-density control) had higher VFA (acetate, propionate, and butyrate) levels than the other treatment groups. This is thought to be related to changes in gut microbial balance due to stress, leading to increased intestinal fermentation and inflammation. Previous research (Sugiharto, 2022) reported that high-density stress can alter the composition of the gut microbiota, promoting the population of specific fermentative bacteria, which can influence VFA levels.

Higher VFA do not necessarily indicate a healthier gut environment. Stress-induced dysbiosis can lead to increased fermentative activity and impaired nutrient absorption. In this study, the supplemented groups (T2 and T3) likely maintained a more balanced microbial activity, resulting in similar VFA levels to T0 and better overall physiological performance.

The VFA in T2 and T3 are not due to decreased fermentative capacity, but rather to more stable gut microbial metabolism under conditions of reduced oxidative stress and inflammation. In parallel, garlic-derived prebiotics and synbiotics promote beneficial bacterial growth, which supports fermentation and short-chain fatty acid (SCFA) production (Liao *et al.*, 2020). The present results confirm these mechanisms, showing higher acetate and butyrate concentrations in supplemented groups, which are well-known to serve as energy substrates for enterocytes and to promote intestinal barrier function.

Gut health indicators, such as reduced *coliform* counts and increased ileal growth in T3, confirm the role of synbiotics in reshaping the intestinal microbiota and morphology. The reduction in coliform counts with Se and synbiotic supplementation is particularly relevant under high stocking densities, where pathogenic bacteria proliferation is common due to stress-induced immune suppression. The trend toward increased lactic acid bacteria in T1 and T3 supports previous reports that garlic-based prebiotics enhance the colonization of beneficial microbiota (Amad *et al.*, 2013; Kikusato and Toyomizu, 2023).

Higher *Lactose-negative enterobacteriaceae* (LNE) in T1 indicates that high density alone increases gut microbial imbalance, favoring the increase of potentially pathogenic bacteria. Supplementation with Se or a combination of Se and synbiotics (T2 and T3) effectively suppressed LNE bacteria, suggesting a protective effect against dysbiosis, under stressful conditions induced by high housing density at tropical temperatures. The antioxidant and antimicrobial activities of selenium and garlic-based bioactives helped restore microbial balance. Selenium supports host antioxidant defenses, thereby reducing oxidative stress, while prebiotics and phenolic compounds derived from garlic inhibit the growth of harmful bacteria and support beneficial lactic acid bacteria (Zhang and Lin, 2021; Daliri *et al.*, 2019).

Contrary to initial expectations, serum antioxidant indicators (SOD and MDA) were not significantly affected by the treatments. This suggests that Se and the garlic-based synbiotic may not have induced a strong systemic antioxidant response under the present experimental conditions. Alternatively, their effects may have been more localized at the intestinal or cellular level, as reported in earlier studies showing tissue-specific antioxidant responses to Se and plant bioactives (Zhang and Lin, 2021; Daliri *et al*., 2019). However, because tissue-level antioxidant parameters were not measured, this interpretation remains hypothetical. Future studies incorporating tissue assays or molecular markers of oxidative stress would help clarify this mechanism.

Overall, Se and synbiotic supplementation appeared to support gut health and nutrient utilization under stressful rearing conditions, while their antioxidant effects at the systemic level warrant further confirmation. Previous studies indicated that Se enhances glutathione peroxidase activity in tissues (Zhang and Lin, 2021), while garlic bioactives contribute phenolic antioxidants (Daliri *et al*., 2019). But this study, improvements in antioxidant status (SOD and MDA) were not statistically significant.

An important practical implication is the effect on meat quality. Birds in the Se only (T2) and Se plus garlic-based synbiotic groups (T3) produced breast meat with higher crude protein and lower cholesterol content, confirming the nutritional and functional benefits of this supplementation strategy. The increase in crude protein content and reduction in cholesterol concentration of broiler meat with Se- garlic-based synbiotic supplementation provides an important practical implication for consumer health. These results align with earlier findings that synbiotics improve nutrient partitioning toward muscle accretion while reducing lipogenesis (Pourabedin and Zhao, 2015). Moreover, the hypocholesterolemic effect observed in T2 and T3 groups underscores the functional potential of garlic-derived bioactives, consistent with human and animal studies (Alam *et al*., 2018). The novelty of this study lies in demonstrating that such benefits are retained under tropical high-density rearing conditions, a production scenario rarely explored in previous literature.

## CONCLUSION

This study indicates that dietary supplementation with selenium combined with a garlic-based synbiotic (*Lactobacillus casei* + garlic flour) effectively improved broiler performance, gut health, and meat quality under tropical high-density rearing conditions. Supplementation enhanced feed intake, body weight gain, and production efficiency, while reducing *coliform* populations and improving ileal growth. Although antioxidant markers showed no improvements, the overall trend supports the role of selenium in oxidative defense and synbiotics in microbial modulation. Further studies including individual (synbiotic or Se) and combined treatments are recommended to confirm possible synergistic effects.

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## NOVELTY STATEMENT

The novelty of this research is the use of selenium and garlic-based sybiotics on antioxidant capacity and meat quality of broilers rearing under high-density in tropical climate.

## AUTHOR'S CONTRIBUTION

Istna Mangisah: Arranged the research concept, methodology, collected data, analyzed data and compiled the original article.
Vitus Dwi Yunianto Budi Ismadi: Supervised this research and checked the research concept and results.
Mulyono Mulyono: Formal analysis and data validation.
Sugiharto Sugiharto: Conducted data curation, methodology and supervised this research.
Lisa Anjani: Writing and editing article.
Sri Sumarsih: Concepted research and methodology.

### Generative AI and AI-assisted technology statement

The authors declared that they have not used Generative AI and AI-assisted technology in the writing process before submission.

### Conflict of interest

The authors have declared there is no conflict of interest regarding the publication of this paper. All authors have read and approved the final version of the manuscript and agree with its submission to this journal.

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