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# Bacterial Community Changes and Immune Response in Broiler Chickens as Affected with Various Feed Additives

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**Abstract**

This study aimed to compare the effectiveness of commercially available probiotic growth promoters (Micro-BACLA) and the performance of the antibiotic growth promoter zinc bacitracin (55g/ton) with the control group (without any additives) on growth performance and meat quality. A total of 120 day-old Ross chicks were allotted into three groups (n=40) with four repetitions per treatment. The current study examines a considerable rise in the probiotic group's (G3) features like total Live Body Weight (LBW), which was 2127.00 g/bird, compared to the control (G1) and antibiotic group (G2) values of 1957.28 and 1998.80 g/bird, respectively. Additionally, the G3 group had a much higher carcass weight (CW), at 1628.28 g/bird as opposed to 1442.66 and 1546.28 g/bird in the G1 and G2 groups. Moreover, compared to the G1 and G2 groups, the G3 group had the best feed intake and feed conversion ratio (FCR). Additionally, meat from the G3 group had a larger effective intramuscular fat (IMF) percentage than meat from the G1 and G2 groups, as well as a middle value for crude protein between the G2 and G1 groups and no significant difference in collagen between the three groups. Also In the current study, the ratio of unsaturated fatty acids (linoleic and oleic acids) to saturated fatty acids (myristic and palmitic acids) was high. Furthermore, G3 meat had a considerably higher lightness (L*) value than G1 and G2 meats. The current study suggests that using (Micro-BACLA) additives instead of antibiotics can improve the meat quality and productive performance of Ross broiler chickens.

**Keywords:** Poultry, probiotics, growth performance, meat quality, intramuscular fat, fatty acids.

## Introduction

The development of antibiotics led to improvements in feed efficiency and the management of infectious diseases **(Engberg *et al.*, 2000).** They are frequently employed to treat and stop infections in people and animals. However, scientific data indicates that the widespread usage of these substances has contributed to an increased issue of antibiotic resistance **(Diarra *et al.*, 2007).** Moreover antibiotic residues in feed and the environment **(Carvalho and Santos, 2016).** The main benefit of a probiotic is that it does not cause antibiotic resistance through eating or leave behind any residues in animal production. To close this gap, probiotics are being studied, and some farmers currently choose to use them over antibiotics. As a result, several researchers now use probiotics in place of some antibiotics to treat illnesses and spur development **(Jha *et al.*, 2020).** Previous research has demonstrated the pathogen-inhibiting and immunity-modifying properties of Lactobacillus acidophilus (L. acidophilus) **(Li *et al.*, 2018).** Furthermore, Due to their ability to form spores, which produce resistance against high temperatures, pH, bile, and enzymes encountered in the gastrointestinal tract (GIT), withstand harsh conditions, and provide health benefits to the host, bacillus-based probiotics are widely used as antibiotic alternatives in animal and chicken feed **(Haque *et al.*, 2017, Xu *et al.*, 2017).** The research studies conducted to date in both challenged and non-challenged situations illustrate the opportunity for successfully using xylanase, amylase, protease, and bacillus strains in combination due to their complementary modes of action. **Momtazan *et al.* (2011)** found that a combination of enzyme complex and probiotics can improve the health condition of poultry and their production**.**

This study aimed to shed light on the effect of bacterial probiotics plus an enzyme mixture (Micro-BACLA) compared to antibiotic (zinc bacitracin) on growth and productive performances and meat quality in poultry (Ross broilers chicken).

## Materials and Methods

### 1.1. Experimental location and time

This study was carried out in a private poultry farm at Met Fadala, Aga, Dakahlia Governorate, Egypt (30°52'43.2"N; 31°20'58.3" E) during the period from November 20th, 2022 to December 25th, 2022.

### 1.2. Birds and feed additives

A total of 120 un-sexed birds (one day old), Ross chicks' strain, was obtained from a commercial hatchery in Egypt. Two commercial products were applied in this experiment as feed additives, the first one is the antibiotic called zinc bacitracin (15%) used at the rate of 55g/ton, while the second one is a pre/probiotic called Micro-BACLA® and applied at the rate of 500g/ton feed. Both antibiotic and Micro-BACLA® were obtained from PROPYN International Inc., (https://www.probyn.com/microbacla.html).

### 1.3. Experimental design and housing

Totally 120 birds were allotted into three groups (n=40) with four repetitions per treatment. The experimental groups were as follows:

Group 1 (G1): Birds fed on feeder without any additives were kept in control,

Group 2 (G2): Birds fed on feeder supplemented with zinc bacitracin.

Group 3 (G3): Birds fed on feeder supplemented with Micro-BACLA®.

In a spotless, well-ventilated space that had previously undergone burning, Virkon-S, and TH4 disinfection, broiler chicks were kept. The space was divided into nine sections, each measuring 1.0 $m^2$ for the first 10 days and 3.0 $m^2$ thereafter. Five centimeters of fresh, clean wheat straw bedding material were provided for each partition. With the help of a gas heater and a 200-watt electric bulb, chicks were brooded at a beginning temperature of 33°C during the first week, which subsequently decreased to 25°C by the end of the fifth week. Continuous natural and artificial light programs were used on the birds.

### 1.4. Feeding and medical care

Birds were fed on a starter mash ration for the first three weeks from Apex Feed Company in Egypt (https://www.apexcairo.net), followed by a grower diet containing 21.5% protein and 3150 kcal/kg of metabolized energy until the experiment's termination in the fifth week.

Four fortifications were used to immunize the birds against New Castle disease namely Hitchner B1 live virus intra-ocular at day 7, ND plus AI killed virus S/C on day 9, Gumboro E228 live virus in drinking water on day 14, and ND Colon 79 live virus in drinking water at day 19. Both the grower feeder and fortifications were from Dakahlia for Poultry Company in Egypt (https://dakahliapoultry.com).

### 1.5. Growth and productive performances

Body weight was calculated weekly, and weight increase was calculated as the difference between two weights taken successively. Feed intake was calculated by consistently giving a known amount of feed at eight o'clock in the morning, and the residual portion was weighed at the end of the week. An estimate was made of the typical daily feed intake. According to **Wagner et al. (1983)**, feed conversion is calculated by the following equation:

$$\text{Feed conversion (FCR)} = \frac{\text{feed intake(g)/bird (Total FI)}}{\text{weight gain(g)/bird (BWG)}}$$

### 1.6. Samples collection

Random samples from each group were used to butcher the birds after the trial (35 days of age) and evaluate the carcass's characteristics. After each bird was weighed separately before slaughter, the remaining corpse was weighed separately after the legs and viscera were removed. The raw breast samples were kept at -20°C until the fatty acid profile, meat color, and chemical makeup were evaluated.

All analyses were conducted at Cairo University Research Park (CURP) and the Faculty of Agriculture, Cairo University, Egypt.

### 1.7. Meat color

The manufacturer-calibrated Chroma meter (Konica Minolta, model CR 410, Japan) was used to measure the meat color of a chicken breast muscle segment using a white plate and light trap. The CIE Commission International de l'E clairage, 1976 L, a, and b color schemes were used to express the color. Each sample had three separate spectral readings recorded at various points on the LD muscle:

- Redness (a*) values range from reddish to greenish,
- Lightness (L*) values range from dark to light,
- Yellowness (b*) values (yellowish to bluish).

### 1.8. Chemical composition

Samples of chicken breast muscle were examined for chemical composition according to **Kelrich (1990)** by collecting 50.0 g of each meat sample and mixing for no longer than 60 seconds to get a homogenous mixture. Five samples from each group were chemically analyzed using a Food ScanTM Pro meat analyzer (Foss Analytical A/S, Model 78810, Denmark) according to the manufacturer's instructions. After the samples were placed in a petri dish. Each sample's moisture, fat, protein, and collagen content were estimated.

#### 1.8.1. Moisture content

Samples of chicken breast muscle were examined for moisture content as weight loss after the samples were dried in an oven at 105˚C for 16 h.

#### 1.8.2. Protein content

Protein content was determined by using micro Kjeldahl and was calculated as follows:

$$\text{Protein \%} = \text{Nitrogen} \times 6.25$$

#### 1.8.3. Fat contents

Total lipids from breast muscle samples were quantitatively extracted, according to the method of **(Folch *et al.*, 1957)**. To produce FAME, 60.0 mg of fat was mixed with 1.0 mL of hexane, 500 L of sodium methoxide, and 500 mg of hexane. The GC model 7890B from Agilent Technologies, which was equipped with a flame ionization detector, was used to detect fatty acid methyl esters (FAMEs). Separation was accomplished using a Zebron ZB-FAME column (60 m x 0.25 mm internal diameter x 0.25 m film thickness). The following temperature program was used during the analysis, with hydrogen serving as the carrier gas at a flow rate of 1.8 ml/min in split-1:50 mode and an injection volume of 1 l: 3 minutes at 100 °C, followed by 10 minutes at 240 °C with a 2.5 °C/min increase. The injector and detector (FID) were maintained at respective temperatures of 250 °C and 285 °C.

### 1.9. Statistical analysis

The data were analyzed using the General Linear Models (GLM) method of the SPSS software, version 25. All data were analyzed using one-way ANOVA. Group differences were evaluated using Duncan's multiple comparison tests **(Duncan, 1955)** when the P-value was less than 0.05. Results were given as means and standard deviations (± SD).

## Results and discussion

### 1.10. Growth performance

The impact of probiotic and antibiotic addition to the broiler chicks feeding compared to control (no additives) on their growth performance was estimated as live body weight, carcass weight, body weight gain, feed intake, and feed conversion ratio **(Tables 1-4).** For live body, data presented in **Table (1)** and graphically illustrated by **Fig. (1a)** indicated that the antibiotic-fed group recorded the highest significant live body weight (310.73 g/bird) over the first ten days compared to the other groups. Also, no significance was observed between the control and probiotic-fed groups. This might be due to the direct and rapid effect of antibiotics on pathogenic microbes that may attack the bird at the beginning of its life, so antibiotics have been regularly used as growth promoters in the chicken industry for over 60 years **(El-Faham *et al.*, 2022).**

On the other hand, the probiotics added to the bird's feed need time to grow and multiply then show their activity and influence on the bird's growth performance, which led to at the age of 20 days, the probiotics-fed group recording slightly higher values of live body weight than the antibiotic-fed group but with no significance. Additionally, after 30 days, the probiotic-fed group outperformed the other two groups and recorded significantly higher live weights (1744.59 g/bird) with a difference of 112.59 and 134.45 g from the control and antibiotics-fed groups, respectively. Additionally, the LBW reached 2127.0 g/bird at the age of slaughter (35 days) in the probiotics-fed group compared to 1998.8 and 1957.28 g/bird in the antibiotic-fed and control groups, respectively **(Table 1)**.

Regarding the carcass weight, results in **Table (1)** and **Fig. (1b)** showed that the probiotic-fed group had a significant carcass weight higher than other groups with (1628.28 g/bird) vs (1546.28 and 1442.66) for the antibiotic-fed and control groups, respectively. Similar results have been previously confirmed by **Abd El-Hack *et al.* (2020)** that the probiotics alter the intestinal ecosystem by delivering digestive enzymes, lowering pH, and influencing intestinal bacteria.

**Table 1.** The mean of live body weight (LBW) and carcass weight (CW)/bird during the experimental period.

| Experimental period (day) | | Experimental groups | | |
|---|---|---|---|---|
| | | G1 (Control) | G2 (Antibiotic) | G3 (Probiotic) |
| LBW (g) | 1st | 38.8 ± 0.0 | 38.8 ± 0.0 | 38.8 ± 0.0 |
| | 10th | 287.64±27.27$^{b}$ | 310.73±18.79$^{a}$ | 292.21±20.78$^{b}$ |
| | 20th | 758.09±103.78$^{b}$ | 815.57±82.88$^{a}$ | 821.71±85.33$^{a}$ |
| | 30th | 1610.14±104.14$^{b}$ | 1632.00±111.47$^{b}$ | 1744.59±94.60$^{a}$ |
| | 35th | 1957.28±187.95$^{b}$ | 1998.80±183.59$^{b}$ | 2127.00±158.13$^{a}$ |
| CW(g)/3rd day | | 1442.66±139.70$^{c}$ | 1546.28±130.24$^{b}$ | 1628.28±115.70$^{a}$ |

**G1: Control group (fodder without any additives).**
**G2: The antibiotics-feeding group.**
**G3: The probiotics-feeding group.**
**a,b, and c values in the same row with different letters showed significant results (P ≤ 0.05).**

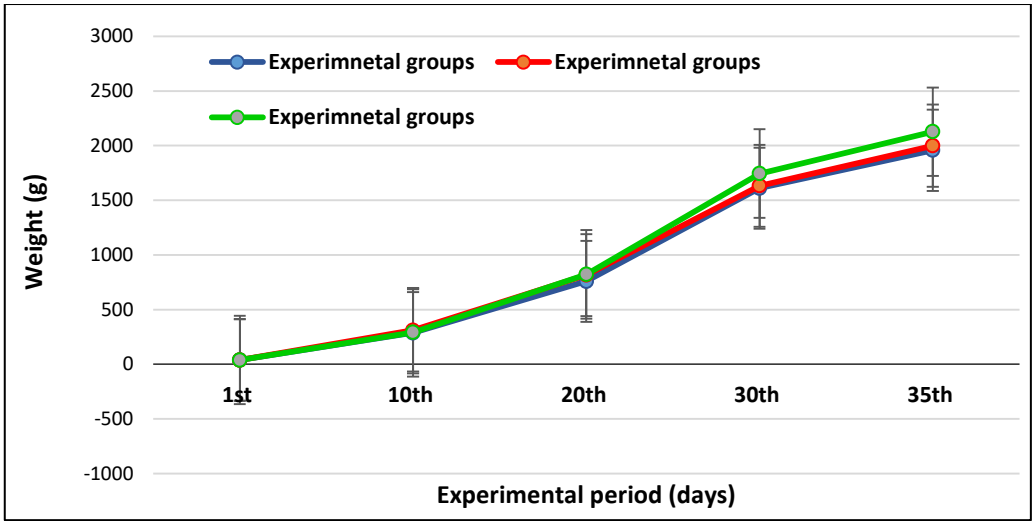


**Figure 1a.** Mean of live body weight (LBW)/bird during the experimental period.

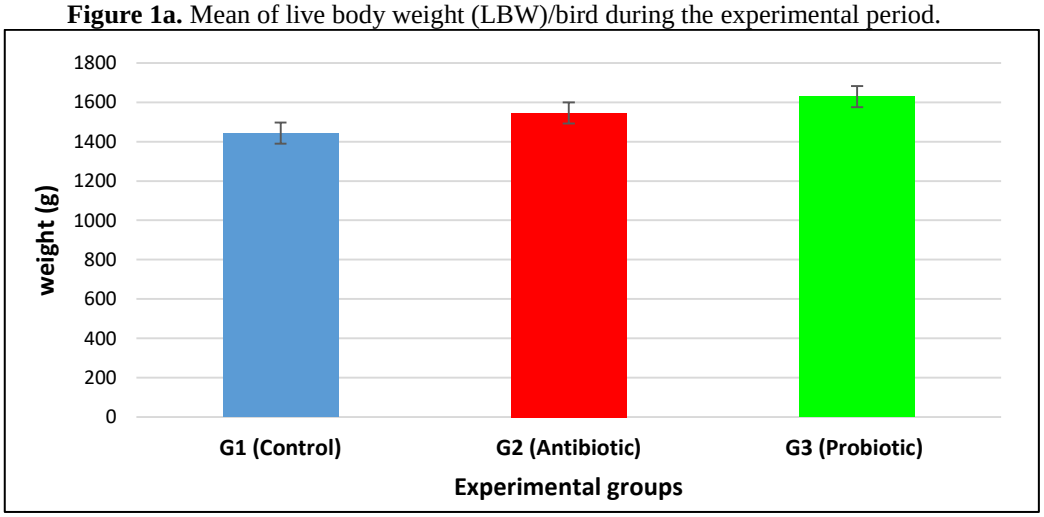


**Figure 1b.** Mean carcass weight (CW)/bird during the experimental period.

Generally, the antibiotic-feeding and probiotic-feeding groups recorded higher values of both live body and carcass weights. This trend of results was true during all experimental periods. As **Soomro *et al.* (2019)** reported supplementing with probiotics improved carcass yield, live weight, immunological response, and the appearance of prominent cut-up meat pieces.

Concerning the body weight gain (BWG), results showed significant changes among all groups. Compared to the control and antibiotic groups, the BWG of the probiotic group was highest (2088.4 g/bird vs. 1960.2 and 1918.68 g/bird, respectively **(Table 2 and Figs. 2 a&b)**.

**Table 2.** The mean of body weight gain (BWG) during the experimental periods.

| Experimental period (day) | | Experimental groups | | |
|---|---|---|---|---|
| | | **G1 (Control)** | **G2 (Antibiotic)** | **G3 (Probiotic)** |
| BWG (g) | **1st** | 38.8 ±0.0 | 38.8 ±0.0 | 38.8 ± 0.0 |
| | **10th** | 249.04±27.27$^{b}$ | 272.13 ±18.79$^{a}$ | 253.61±20.78$^{b}$ |
| | **20th** | 470.45±110.21 | 504.83±94.39 | 529.50±78.57 |
| | **30th** | 852.04±164.71$^{ab}$ | 816.42±115.11$^{b}$ | 922.88±135.39$^{a}$ |
| | **35th** | 347.14±133.39 | 366.80±110.61 | 382.40±100.09 |
| **Mean** | **0- 35th** | 1918.68±187.95$^{b}$ | 1960.20±183.59$^{b}$ | 2088.40±158.13$^{a}$ |

**G1: Control group (fodder without any additives).**
**G2: The antibiotics-feeding group.**
**G3: The probiotics-feeding group.**
**a,b, and c values in the same row with different letters showed significant results (P ≤ 0.05).**

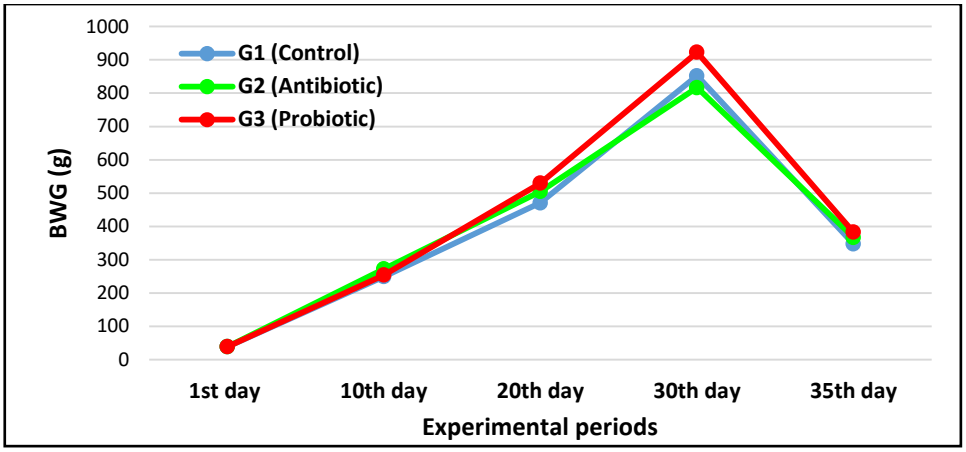


**Figure 2a.** Changes in body weight gain (BWG) during the experimental period.

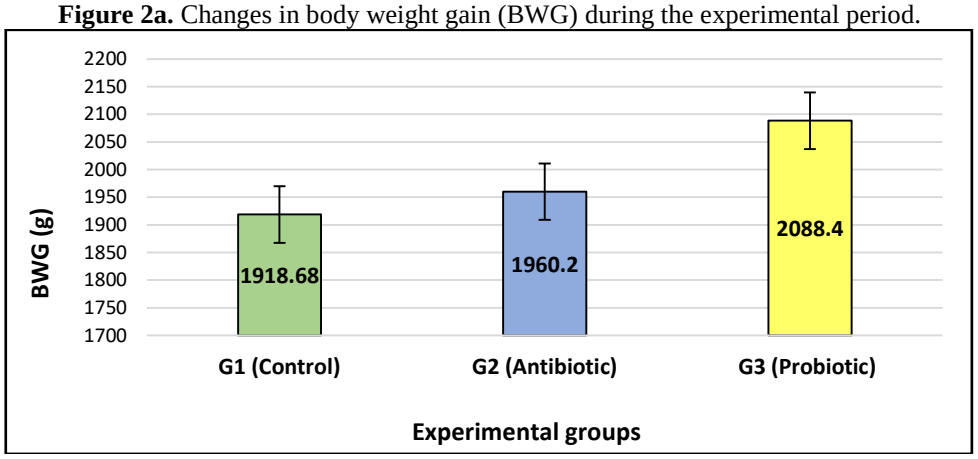


**Figure 2b.** Mean of body weight gain (BWG) during the experimental period.

A similar trend of results were observed by many researchers that probiotics in broiler diets have been proven to boost growth performance when used instead of antibiotic growth promoters in several studies **(Shim *et al.*, 2010; Wang and Gu 2010; Zakeri and Kashefi 2011)**. In addition, **Manafi *et al.* (2018)** found that probiotics improve growth performance and humoral immune response and leave no residues in meat that could be harmful to consumers' health.

And about the feed intake (FI), it was observed that during the first 20$^{th}$ days, the FI was significantly lower in the antibiotic group compared to the other groups. The FI in all experimental groups gradually increased from the first 10$^{th}$ days and reached its maximum value after 30$^{th}$ days, then decreased after **(Table 3** and **Fig. 3a)**.

**Table 3.** The mean of feed intake (FI) during the experimental periods.

| Experimental periods (day) | | Experimental groups | | |
|---|---|---|---|---|
| | | G1 (Control) | G2 (Antibiotic) | G3 (Probiotic) |
| FI | 1-10$^{th}$ days | 241.2±0.0$^{a}$ | 232.5±0.0$^{c}$ | 240.5±0.0$^{b}$ |
| | 10-20$^{th}$ days | 798.0±0.0$^{a}$ | 747.7±0.0$^{c}$ | 787.3±0.0$^{b}$ |
| | 30$^{th}$ | 1683.1±0.0$^{b}$ | 1706.6±0.0$^{a}$ | 1597.3±0.0$^{c}$ |
| | 35$^{th}$ | 611.6±0.0$^{b}$ | 571.1±0.0$^{c}$ | 619.2±0.0$^{a}$ |
| Mean | 0- 35$^{th}$ | 3333.9±0.0$^{a}$ | 3257.9±0.0 | 3244.3±0.0$^{c}$ |

**G1: Control group (fodder without any additives).**
**G2: The antibiotics-feeding group.**
**G3: The probiotics-feeding group.**

**a,b, and c values in the same row with different letters showed significant results (P ≤ 0.05).**

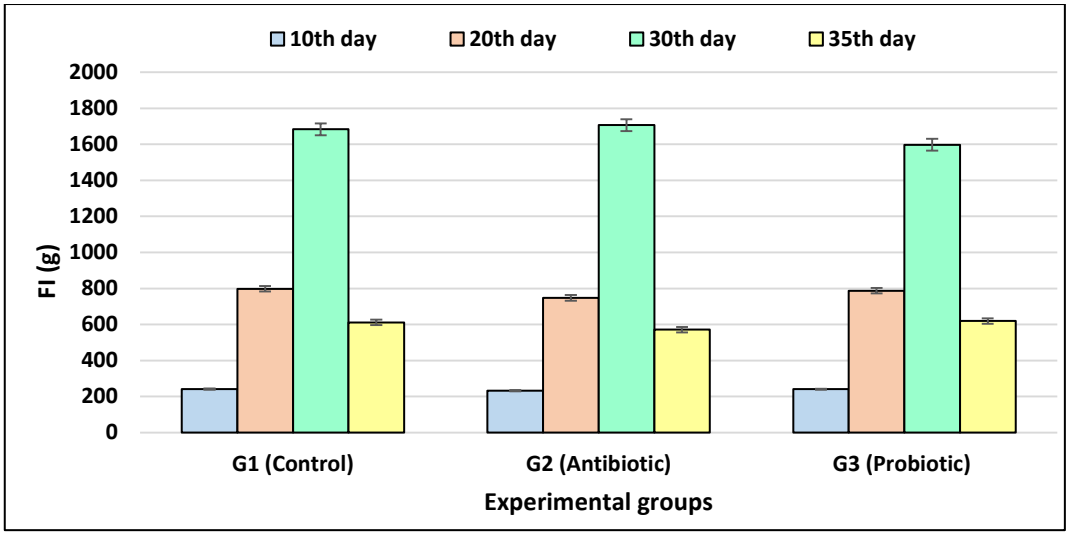


**Figure 3a.** Changes in feed intake (FI) during the experimental period.

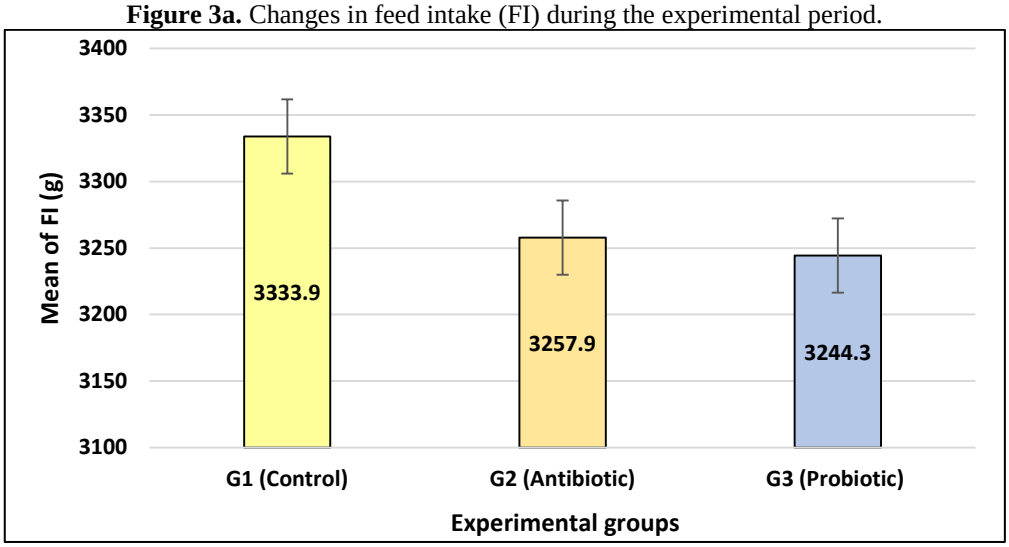


**Figure 3b.** Mean of feed intake (FI) during the experimental period.

Generally, the mean of FI (0- 35$^{th}$ day) was significantly lower in the probiotic group during the experimental period, with 3244.3 g/bird vs. 3333.9 g/bird and 3257.9 g/bird in the control and antibiotic groups, respectively **(Table 3** and **Fig. 3b)**.

Regarding the the feed conversion ratio (FCR), results presented in **Table (4)** and graphically illustrated by **Fig. (4a)** indicated that the FCR recorded the lowest values during the first 10$^{th}$ days and gradually increased in the following days and reached their maximum values at the 30$^{th}$ days, then decreased. This trend of results means that the birds had higher efficient FCR during the first 10$^{th}$ days than in other periods. Generally, the mean of FCR was lower in the probiotic group with a significant value of 1.56 which indicates higher efficiency in this group compared with 1.75 and 1.67 in the control and antibiotic groups, respectively **(Table 4** and **Fig. 4b)**.

**Table 4.** The mean of feed conversion ratio (FCR) during the experimental periods.

| Experimental periods | | Experimental groups | | |
|---|---|---|---|---|
| | | G1 (Control) | G2 (Antibiotic) | G3 (Probiotic) |
| FCR | 1-10$^{th}$ days1-1 day | 0.97±0.11$^{a}$ | 0.85±0.06$^{b}$ | 0.95±0.08$^{a}$ |
| | 20$^{th}$ day | 1.80±0.53$^{a}$ | 1.54±0.38$^{b}$ | 1.51±0.21$^{b}$ |
| | 30$^{th}$ day | 2.04±0.39$^{a}$ | 2.14±0.39$^{a}$ | 1.76±0.26$^{b}$ |
| | 35$^{th}$ day | 1.99±0.70$^{a}$ | 1.68±0.48$^{c}$ | 1.72±0.44$^{b}$ |
| Mean | 0-35$^{th}$ day | 1.75±0.16$^{a}$ | 1.67±0.15$^{a}$ | 1.56±0.10$^{b}$ |

**G1: Control group (fodder without any additives).**
**G2: The antibiotics-feeding group.**
**G3: The probiotics-feeding group.**

a,b, and c values in the same row with different letters showed significant results (P ≤ 0.05).

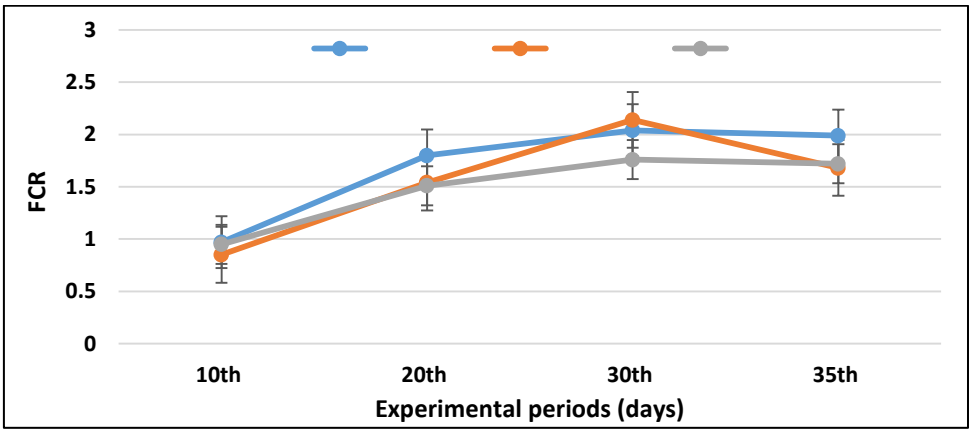


**Figure 4a.** Changes in feed conversion ratio (FCR) during the experimental period.

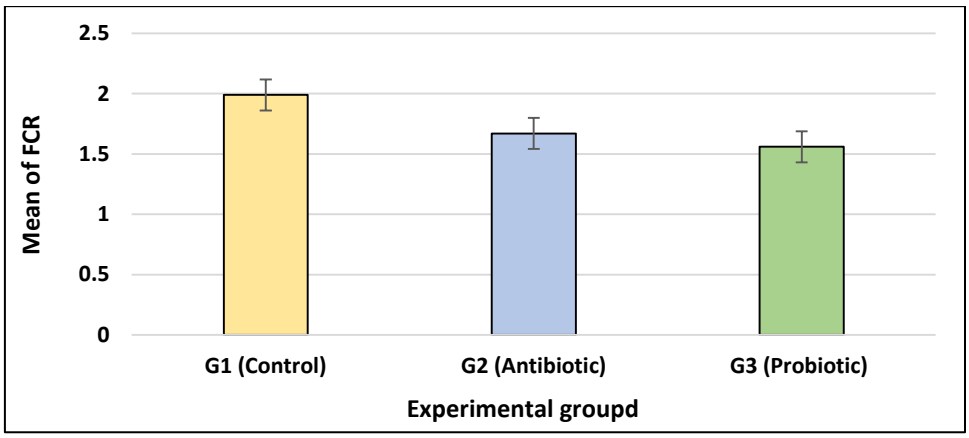


**Figure 4b.** Mean of feed conversion ratio (FCR) during the experimental period.

Finally, the current study showed that the addition of bacterial probiotic mixed with some enzymes (Micro-BACLA®) (*Lactobacillus acidophilus*, *Bacillus subtilis*, *Aspergillus oryzae*, xylanase, hemicellulase, β-glucanase, α-Amylase, protease, cellulase) as alternative feed additives increased broilers' body weight and also reduced feed intake and feed conversion ratio.

In line with this research, **Mohamed *et al.* (2014)** reported that Micro-BACLA® feed additives improved total body weight, total weight gain, and total feed conversion compared to the control group. Research studies conducted to date in both challenged and non-challenged situations illustrated the opportunity for successfully using xylanase, amylase, protease, and bacillus strains in combination due to their complementary modes of action. **Momtazan *et al.* (2011)** found that a combination of enzyme complexes and probiotics can improve the health condition of poultry and thereby their production. Moreover, supplementing feed with probiotics and symbiotics enhances growth, feed effectiveness, and gut health **(Ghasemi *et al.*, 2014; Giannenas *et al.*, 2012).**

Also, proteins, phytates, and glucans can all be broken down with the help of enzymes. For instance, endo-b-1-4-xylanases and b-1,3,4-glucanases have been added to wheat and barley broiler diets to enhance their digestion ***(Cowieson et al.*, 2006).** Moreover, **Toghyani *et al.* (2011)** discovered that utilizing probiotics at a dose of 15 mg/kg can dramatically improve live body weight (LBW), feed conversion ratio (FCR), and feed intake (FI) compared to the control group. **Pourakbari *et al.* (2016)** discovered that adding probiotics to broiler diets up to 0.02 percent increased DBWG and improved FCR, but probiotics did not affect FI.

Similarly, **Machado *et al.* (2020)** found that supplementing broiler diets with probiotics improved LBW and increased FI, while there was no effect on FCR. Probiotics supplementation in broiler feed, on the other hand, did not influence broiler performance **(Rehman *et al.*, 2020)** and the microorganisms in the small intestine **(Abd El-Hack *et al.*, 2020)**. Several studies have reported a significant reduction in the feed conversion ratio of broilers fed diets supplemented with probiotics **(Pourakbari et al. 2016; Sarangi et al. 2016).**

### 1.11. Physical and chemical characteristics of chicken breast muscle

The chemical composition of chicken breast meat including intramuscular fat, protein, collagen,

and moisture is presented in **Table (5)**. Meat from the probiotic group had a greater effective intramuscular fat content of 1.96% than meat from the control and antibiotic groups measuring 1.43 % and 1.64%, respectively **(Fig 5a)**.

On the contrary, several studies recorded a decrease in fat percentage in broiler meats supplemented with probiotics and reported that might be due to the presence of enzymes like lipase and esterase which break ester bonds that link glycerol to fatty acids, preventing the formation and/or decreased the absorption of triglycerides into plasma, thereby reducing the fat content of meat **(Suryadi *et al.* 2019** and **Bhogoju *et al.*, 2021).** Although **Rehman *et al.* (2020)** and **Sarangi *et al.* (2016)** reported that *Lactobacillus* culture in probiotics could reduce fat content in Caracas, the current study observed that the addition of *Lactobacillus acidophilus* mixed with other microorganisms like *Bacillus subtilis* and *Aspergillus oryzae* caused an increase in fat content due to the synergistic action among them **(Table 5; Fig 5a).**

The amount of intramuscular fat (IMF), total muscle fat, and the composition of the meat's fatty acids are the key factors that determine meat quality characteristics like juiciness, flavor, water-holding capacity, and tenderness **(Cui *et al.*, 2012, Hocquette *et al.*, 2010).** Additionally, intramuscular fat improves meat quality by reducing cooking and drip loss **(Gerbens *et al.*, 2001).** In this context, the current study showed that adding probiotic products as an alternative to feed additives significantly increased intramuscular fat (IMF%) compared to the control and antibiotic groups.

On the other hand, crud protein was significantly higher in the antibiotic group with 23.22% compared to 21.09 and 22.82 % in the control and probiotic groups respectively **(Fig 5b).** Similarly, in the control group, collagen recorded the lowest value across the three groups, and probiotic was the best without any significant differences between the three experimental groups **(Fig 5c)**. Otherwise, moisture recorded the highest significant value in the control group with 75.95 % compared to 74.33 and 74.07 in the antibiotic and probiotic groups, respectively **(Fig 5d).**

**Table 5.** Chemical composition and physical analysis of chicken breast muscle.

| **Parameters** | | **Experimental groups** | | |
|---|---|---|---|---|
| | | **G1 (Control)** | **G2 (Antibiotic)** | **G3 (Probiotic)** |
| **Fat*** | (%) | 1.43±.01$^{c}$ | 1.64±.07$^{b}$ | 1.96±.05$^{a}$ |
| **Protein** | | 21.09±.03$^{c}$ | 23.22±.12$^{a}$ | 22.82±.04$^{b}$ |
| **Collagen** | | 1.080±.27 | 1.208±.04 | 1.244±.21 |
| **Moisture** | | 75.95±.16$^{a}$ | 74.33±.15$^{b}$ | 74.07±.18$^{b}$ |
| **Col** | Lightness (L*) | 50.63 ± 0.28$^{c}$ | 53.96 ± 0.53$^{b}$ | 56.27±0.27$^{a}$ |
| | Redness (a*) | 13.09 ± 0.83$^{a}$ | 11.62 ± 0.52$^{b}$ | 10.27±0.20$^{c}$ |
| | Yellowness (b*) | 5.99 ± 0.27$^{b}$ | 8.18 ± 0.44$^{a}$ | 7.86±0.29$^{a}$ |

**a,b, and c values in the same row with different letters showed significant results (P ≤ 0.05).**
**Lightness (L*) (dark to light), Redness (a*) values (reddish to greenish), Yellowness (b*) values (yellowish to bluish).**
**G1: Control group (fodder without any additives).**
**G2: The antibiotics-feeding group.**
**G3: The probiotics-feeding group.**

The high protein content of broilers supplemented with probiotics was potentially due to the presence of lactic acid bacteria which survive in the digestive tract and bind to the intestinal walls, producing digestive enzymes such as proteases to break down chemical bonds in nutrients resulting in macromolecules which easier to absorb (**Suryadi et al. 2019)**. Additionally, a study by **Wang et al. (2017)** stated that probiotics increase the beneficial microbial community in the intestines which increased the chicken meat quality.

In the current study, results indicated that the supplementation of broiler feeds with the commercial probiotics (Micro-BACLA)® caused a significant increase in all estimated parameters which might be due to the presence of three microorganisms (*Lactobacillus acidophilus, Bacillus subtilis, Aspergillus oryzae*), mixed with some commercial enzymes namely xylanase, hemicellulase, β-glucanase, α-amylase, protease, cellulase, these digestive enzymes could accelerate nutrient decomposition **(Fooks and Gibson, 2002)**. The availability of protein in small forms increased meat protein synthesis which manifested in increased meat protein content.

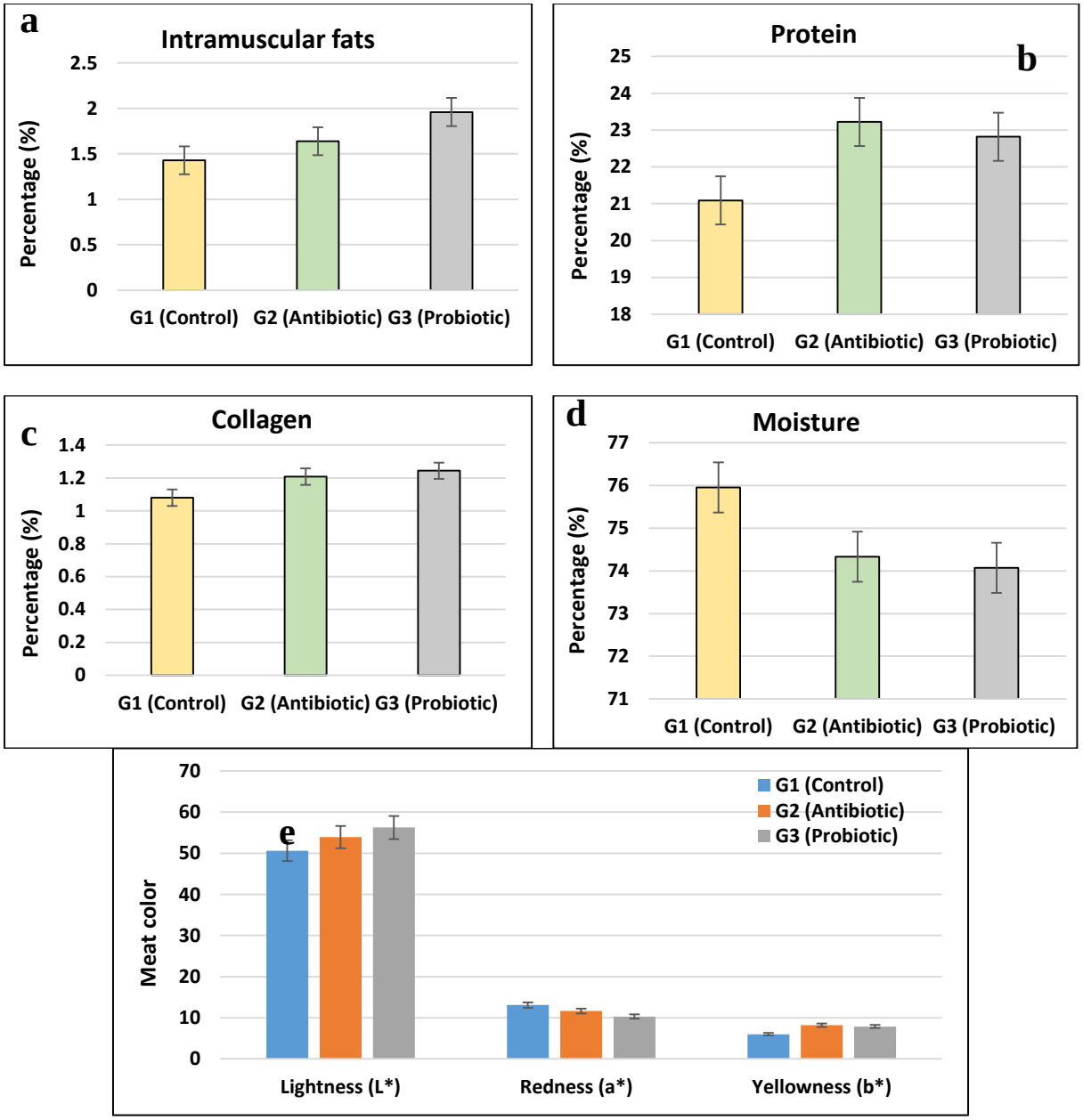


**Figure 5.** Chemical composition and physical analysis of chicken breast muscle, **a)** Intramuscular fats (%); **b)** protein (%); **c)** collagen, **d)** moisture content (%), and **e)** flesh color.

In terms of flesh color, probiotic meat had considerably higher lightness (L*) values than control and Antibiotic meat ($P \leq 0.05$), with a score of 56.27 compared to 50.63 and 53.96 for control and antibiotic, respectively **(Table 5).** On the other hand, meat from the control and antibiotic groups had considerably higher reddish (a*) values than meat from the probiotic group. The Antibiotic group's meat had the highest yellowness (b*) values, at 8.18, compared to the probiotic group's meat at 7.86, with no statistical significance **(Fig. 5e).**

Chicken breast muscle was categorized into three groups by **Qiao et al. (2001)** based on the color of the muscle: "lighter than normal" (L* > 53), "normal" (48 L* >53), and "darker than normal" (L*< 48). However, the breast meat L* values in the Probiotic meat group in the current investigation were higher than usual in the earlier study. According to another study, the lightness of the (L*) values is dark (L*< 50), standard (50 L* 56), or pale (L* > 56) **(Petracci et al., 2004).** In general, pale red meat is the preferred color for meat, while dark meat typically has a low consumer appeal score **(Jeremiah et al., 1972)**.

### 1.12. Fatty acid profile of chicken breast muscle

The fatty acid profile containing four fatty acids was estimated for each group. Among the three groups, Myristic acid (C14:0) was the highest in the probiotic group (G3) with 0.2% compared to 0.02%

in both the antibiotic (G2) and control (G1) groups. Also, Oleic acid (18:1 ) was the greatest in the probiotic group (G3) with 2.13% compared to 0.32% and 1.96% in the (G1) and (G2) groups, respectively **(Table 6)**. On the other hand palmitic acid was the highest in the control group (G1) with 34.87%, probiotic (G3) was the next with 31.96%, and (G2) was the lowest with 25.58%. In contrast, Linoleic acid (C18:2) recorded the highest value in the Antibiotic group (G2) with 72.71% vs 65.71 in (G3) and 65.79 in control **Fig (6).**

**Table 6.** Fatty acids profile of chicken breast muscle.

| **Fatty acids** | | **Experimental groups** | | |
|---|---|---|---|---|
| | | **G1 (Control)** | **G2 (Antibiotic)** | **G3 (Probiotic)** |
| **Myristic (C14:0)** | (%) | 0.02 | 0.02 | 0.2 |
| **Palmitic (C16:0)** | | 34.87 | 25.58 | 31.96 |
| **Oleic (18:1 )** | | 0.32 | 1.96 | 2.13 |
| **Linoleic (C18:2)** | | 64.79 | 72.71 | 65.71 |

**G1: Control group (fodder without any additives).**
**G2: The antibiotics-feeding group.**
**G3: The probiotics-feeding group.**
**a,b, and c values in the same row with different letters showed significant results (P ≤ 0.05).**

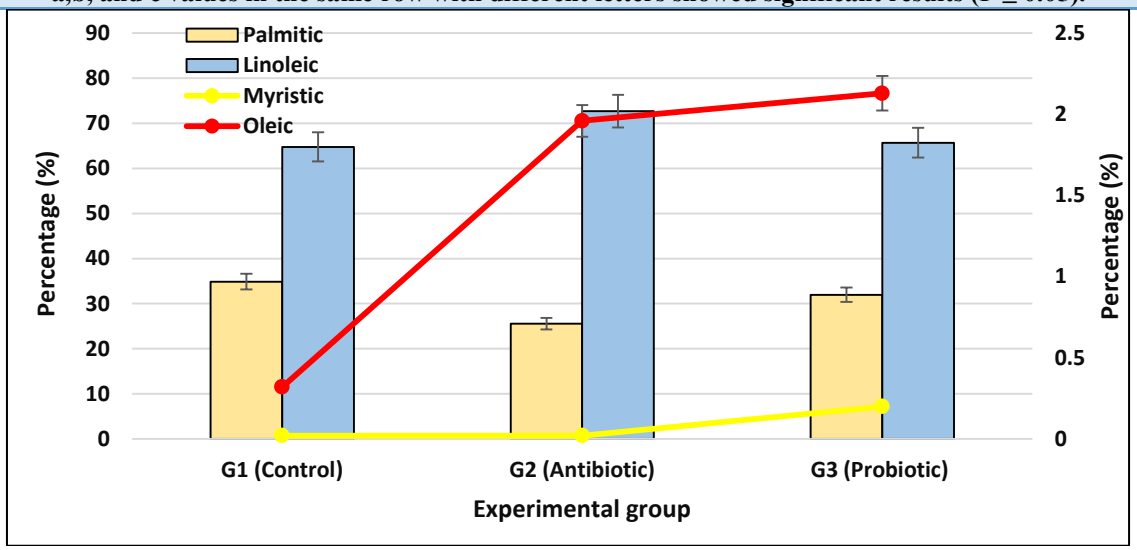


**Figure 6. Fatty acids profile of chicken breast muscle.**

The urge to produce healthier meat with a higher ratio of polyunsaturated (PUFA) to saturated fatty acids and a more favorable balance between n-6 and n-3 PUFA is the main driver of interest in meat fatty acid composition **(Wood et al., 2004).** The ratio of unsaturated fatty acids, such as linoleic and oleic acids, in the present study, was higher than that of other saturated fatty acids, such as Myristic and palmitic acids. In addition, linoleic acid is the PUFA that humans consume the most of in their diets. When consumed, linoleic acid has four main outcomes. It has the same potential for usage as an energy source as all fatty acids. It can be esterified to create polar and neutral lipids such as cholesterol esters. triacylglycerols, and phospholipids. Linoleic acid serves as a structural element of membrane phospholipids and helps to maintain a specific amount of membrane fluidity of the transdermal water barrier of the epidermis. Additionally, it can be enzymatically oxidized to a range of derivatives important in cell signaling when freed from membrane phospholipids **(Whelan and Fritsche, 2013)**

## Conclusion

This study concluded that the use of commercially available probiotic growth promoters (Micro-BACLA) instead of antibiotics can improve the meat quality and productive performance of Ross broiler chickens. Our results recorded a considerable rise in the probiotic group's features like total Live Body Weight (LBW), carcass weight (CW), feed intake (FI) and feed conversion ratio (FCR) as well as intramuscular fat (IMF) percentage than antibiotic and control groups. Moreover, a middle value for crude protein between the antibiotic and control groups was recorded and no significant difference in collagen was observed between the three groups. Also In the current study, the ratio of unsaturated fatty acids (linoleic and oleic acids) to saturated fatty acids (myristic and palmitic acids) was high. Furthermore, the meat color in probiotic group had a

considerably higher lightness (L*) value than other groups.

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