

## Growth Performance and Microbial Population of Broiler Chicken Fed Different Levels of Grape Pomace Meal

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### ABSTRACT

This study evaluated the effect of adding different levels of grape pomace meal on growth performance and microbial population of broilers dietary. A total of 275 one-day-old ROSS 308 chicks were randomly allotted into five treatments. Each treatment had five replicates, with 11 birds per replicate. The control birds were fed a diet consisting primarily of corn and soybean meal. The four treatments were fed a similar diet with the corn meal partially substituted by 2, 4, 6, and 8% grape pomace meal. Results showed no significant effect of grape pomace meal was observed on body weight and weight gain at days 24 and 35. The birds fed an 8% grape pomace meal diet reported higher ( $P<0.01$ ) feed intake compared with other treatments. The feed conversion ratio increased ( $P<0.05$ ) by 6% and 8% grape pomace meal diets. The population of *E. coli* and *total bacteria* declined ( $P<0.01$ ), whereas the number of *Lactobacillus spp* inclined ( $P<0.01$ ) with increasing the amount of grape pomace meal in the diets. In conclusion, the study indicated that feeding grape pomace meal up to 6% showed no deleterious effects on the growth performance, and the number of *Lactobacillus spp* was increased.

**Key words:** Feed alternatives, Intestinal health, Poultry diet.



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### INTRODUCTION

The feed cost represents about 70% of the total cost of the poultry production process, whether for meat or egg production (Borkar et al., 2021; De Oliveira et al., 2021; Thirumalaisamy et al., 2016). Alternative feedstuffs are materials used as a partial or complete alternative to traditional feed, intending to improve animal production efficiency, reduce costs, and enhance environmental sustainability (Hamodi, 2023; Matoo et al., 2001; Van den Abbeele et al., 2020). These alternatives include multiple sources, such as agricultural wastes, byproducts of the food industry, and non-traditional feeds such as insects and algae (Correddu et al., 2023; Galanakis, 2012; Vilela et al., 2022). One substitution is grape pomace, a solid byproduct extracted from processing processes (Bennato et al., 2020; Moncalvo et al., 2016; Muhlack et al., 2018). Grape pomace

constitutes approximately 20% of the grapes produced (Dwyer et al., 2014; Kalli et al., 2018; Kumanda et al., 2019; Muhlack et al., 2018; Yu & Ahmedna, 2013). Using food factory waste in feed is an important step to improve environmental efficiency and reduce production costs (Alhotan, 2021; De Oliveira et al., 2021). Grape pomace consists of proteins, lipids, and carbohydrates (de Carvalho Tavares et al., 2019; Taşeri et al., 2018), as well as vitamins, microelements, and phenolic compounds with antioxidants, antimicrobial, and anti-inflammatory activity which makes it a good addition to animals' diets. However, the content of these components may vary depending on the grape variety, cultivation conditions, climate, and maturity stage (Chikwanha et al., 2018; Peixoto et al., 2018). Phenolic compounds are essential components of grape pomace (Taşeri et al., 2018). It also contains important amino

acids such as lysine, phenylalanine, tyrosine, arginine, and histidine, in addition to biogenic amines such as putrescine and cadaverine, which are important indicators of food quality, especially in processed products (Gowman et al., 2019; Sánchez-Pérez et al., 2018). Grape pomace seeds are also rich in oils and a rich source of phenolic compounds (Beres et al., 2017). Due to important biological properties of grape pomace and its many positive functions, it may represent a promising option as a partial substitution for maize in poultry feed. However, studies related to its effect on the productive performance of broilers and the balance of beneficial and harmful microorganisms in the small intestine are still insufficient and require further research (Alhotan, 2021). The main objective of this study is to investigate the potential of using grape pomace as a partial alternative to conventional feed in broiler nutrition, with the aim of improving production efficiency and reducing costs. Additionally, the study aims to assess the impact of grape pomace on productive performance and its effect on the balance of beneficial and harmful microorganisms in the small intestine of broilers.

## MATERIALS AND METHODS

**Diets and management:** The experiment was conducted in the Department of Animal Production poultry field at the College of Agricultural Engineering Sciences - University of Baghdad (Abu Ghraib) from 26/12/2023 to 30/1/2024 for 35 days. Two hundred seventy-five broiler chicks (ROSS 308) were used in the study, sexed at one day old and with an initial weight of 43.2 g/chicken, distributed randomly into five treatments, with five replicates for each treatment. Each replicate contained 11 chicks. As follows: the first treatment (T1) is a control treatment (without addition), T2 treatment is a 2% substituting of grape pomace, and other treatments (T3, T4 and T5) are a substituting of grape pomace powder with a percentage of 4, 6, and 8%, respectively. Feed and water were provided

freely during the experimental period. The chicks were fed rations to meet the nutritional requirements according to the requirements (**National Research Council [NRC], 1994**) (Table 1, 2 and 3). Artificial light was used alongside normal daylight for a photoperiod of 23 hours/day and one hour of darkness. The hall temperature was controlled using a gas incubator and electric heaters to achieve a temperature of 34°C during the first three days of the chicks' lives, after which it was reduced to 32-33°C until the end of the first week. Then, it was gradually reduced to the end of the trial period to settle to a temperature of 20-22°C. The experiment lasted for 35 days, and production characteristics were measured for each week of the experiment, which included body weight, weight gain, feed consumption, Feed conversion ratio (FCR), and the number of microorganisms in the small intestine of the birds.

**Plant material:** Grape pomace was sourced from juice production outlets, where black raisins were soaked in water for 24 hours prior to juice extraction. The chemical composition and detection of phytochemical substances were determined by high performance liquid chromatography (HPLC) technique (Table 4 and 5).

**Bacterial Culture Media:** *Lactobacillus* grows in media containing nutrients rich in carbohydrates, especially sugars, and in low pH environments such as MRS agar. The culture medium for Total Count is Nutrient Agar and the culture medium for *Escherichia coli* is MacConkey Agar.

**Statistical analysis:** A completely randomized design was used to compare replacing different levels of dietary grape pomace powder in broiler diets and their effect on productive performance and the number of microorganisms. The significance of the means was compared with **Duncan's (1955)** multiple range test, and the **SAS** statistical program (**SAS Institute, 2012**) was used in the statistical analysis.

**Table 1.** Ingredient composition (%) of the experiment diets used from days 1 to 10

| Ingredients                                           | Control diet<br>Without any<br>addition | Treatment T <sub>2</sub><br>2% grapes | Starter diets<br>1 – 10 days             |                                          | Treatment T <sub>5</sub><br>8% grapes |
|-------------------------------------------------------|-----------------------------------------|---------------------------------------|------------------------------------------|------------------------------------------|---------------------------------------|
|                                                       |                                         |                                       | Treatment<br>T <sub>3</sub><br>4% grapes | Treatment<br>T <sub>4</sub><br>6% grapes |                                       |
| Crushed maize                                         | 47                                      | 45                                    | 43                                       | 41                                       | 39                                    |
| Ground wheat<br>(local)                               | 12                                      | 12                                    | 12                                       | 12                                       | 12                                    |
| Soybean meal<br>48%                                   | 32                                      | 32                                    | 32                                       | 32                                       | 32                                    |
| Protein<br>concentrate (1)                            | 5                                       | 5                                     | 5                                        | 5                                        | 5                                     |
| Sunflower oil                                         | 2                                       | 2                                     | 2                                        | 2                                        | 2                                     |
| limestone                                             | 1                                       | 1                                     | 1                                        | 1                                        | 1                                     |
| Dicalcium<br>phosphate DCP                            | 0.5                                     | 0.5                                   | 0.5                                      | 0.5                                      | 0.5                                   |
| salt                                                  | 0.3                                     | 0.3                                   | 0.3                                      | 0.3                                      | 0.3                                   |
| Vitamins and<br>minerals<br>mixture (2)               | 0.2                                     | 0.2                                   | 0.2                                      | 0.2                                      | 0.2                                   |
| Grape pomace<br>powder                                | 0                                       | 2                                     | 4                                        | 6                                        | 8                                     |
| Total                                                 | 100                                     | 100                                   | 100                                      | 100                                      | 100                                   |
| <u>Chemical analysis calculated based on NRC (29)</u> |                                         |                                       |                                          |                                          |                                       |
| Crude protein<br>(%)                                  | 22.74                                   | 22.76                                 | 22.78                                    | 22.80                                    | 22.82                                 |
| Dietary energy<br>(kcal/kg feed)                      | 3029.2                                  | 3027.08                               | 3024.96                                  | 3022.84                                  | 3020.72                               |
| Methionine +<br>Cysteine (%)                          | 0.92                                    | 0.92                                  | 0.91                                     | 0.90                                     | 0.89                                  |
| Lysine (%)                                            | 1.33                                    | 1.33                                  | 1.32                                     | 1.32                                     | 1.31                                  |
| Calcium (%)                                           | 0.78                                    | 0.78                                  | 0.78                                     | 0.78                                     | 0.78                                  |
| Available<br>phosphorus (%)                           | 0.49                                    | 0.49                                  | 0.49                                     | 0.48                                     | 0.48                                  |
| Crude fiber%                                          | 2.8                                     | 3.6                                   | 4.4                                      | 5.3                                      | 6.1                                   |

(1) Protein concentrate type BROCON – 5 SPECIAL W is of Dutch origin. Each kg contains 40% crude protein, 2.40% fat, 4.40% fiber, 3.60% calcium, 5.5% phosphorus, 2.50% sodium, 3.88% chloride, 3.85% lysine, 4.60% methionine, 5.10% methionine + cysteine, 3290 kcal/kg dietary energy, 20,000 IU Vitamin A, 80,000 IU Vitamin D3, 600 mg Vitamin E, 50 mg Vitamin K3, 50 mg Vitamin B1, 140 mg Vitamin B2, 80 mg Vitamin B6, 700 µg B12, 20 mg folic acid, 5 mg citric acid, 2 mg Biotin, 800 mg niacin, 1 mg iron, 200 mg copper, 1,600 mg manganese, 1,200 mg zinc, 20 mg iodine, 5 mg selenium, 6 mg cobalt, 33.50 mg antioxidant (BHT).

(2) A mixture of vitamins and minerals, each kg of which contains: 720,000 IU Vitamin A, 168,000 IU D3, 800 mg E, 80 mg B1, 80 mg B2, 80 mg B6, 5,800 mg niacin, 500 µg folic acid, 5 mg diphosphate Calcium, 40 mg zinc, 100 mcg cobalt, 50 mg lysine

**Table 2.** Ingredient composition (%) of the experiment diets used from days 11 to 24

| Ingredients                                    | Control diet<br>Without any<br>addition | Grower diets<br>11 – 24 days          |                                       |                                       |                                       |
|------------------------------------------------|-----------------------------------------|---------------------------------------|---------------------------------------|---------------------------------------|---------------------------------------|
|                                                |                                         | Treatment T <sub>2</sub><br>2% grapes | Treatment T <sub>3</sub><br>4% grapes | Treatment T <sub>4</sub><br>6% grapes | Treatment T <sub>5</sub><br>8% grapes |
| Crushed maize                                  | 48                                      | 46                                    | 44                                    | 42                                    | 40                                    |
| Ground wheat (local)                           | 14.9                                    | 14.9                                  | 14.9                                  | 14.9                                  | 14.9                                  |
| Soybean meal 48%                               | 27                                      | 27                                    | 27                                    | 27                                    | 27                                    |
| Protein concentrate (1)                        | 5                                       | 5                                     | 5                                     | 5                                     | 5                                     |
| Sunflower oil                                  | 3                                       | 3                                     | 3                                     | 3                                     | 3                                     |
| limestone                                      | 1.3                                     | 1.3                                   | 1.3                                   | 1.3                                   | 1.3                                   |
| Dicalcium phosphate                            | 0.3                                     | 0.3                                   | 0.3                                   | 0.3                                   | 0.3                                   |
| DCP                                            |                                         |                                       |                                       |                                       |                                       |
| salt                                           | 0.3                                     | 0.3                                   | 0.3                                   | 0.3                                   | 0.3                                   |
| Vitamins and minerals<br>mixture (2)           | 0.2                                     | 0.2                                   | 0.2                                   | 0.2                                   | 0.2                                   |
| Grape pomace powder                            | 0                                       | 2                                     | 4                                     | 6                                     | 8                                     |
| Total                                          | 100                                     | 100                                   | 100                                   | 100                                   | 100                                   |
| Chemical analysis calculated based on NRC (29) |                                         |                                       |                                       |                                       |                                       |
| Crude protein (%)                              | 20.75                                   | 20.77                                 | 20.79                                 | 20.81                                 | 20.83                                 |
| Dietary energy (kcal/kg<br>feed)               | 3121.5                                  | 3119.06                               | 3116.94                               | 3114.82                               | 3112.70                               |
| Methionine + Cysteine<br>(%)                   | 0.87                                    | 0.86                                  | 0.85                                  | 0.85                                  | 0.84                                  |
| Lysine (%)                                     | 1.2                                     | 1.19                                  | 1.18                                  | 1.18                                  | 1.17                                  |
| Calcium (%)                                    | 0.84                                    | 0.84                                  | 0.84                                  | 0.83                                  | 0.83                                  |
| Available phosphorus<br>(%)                    | 0.45                                    | 0.44                                  | 0.44                                  | 0.44                                  | 0.44                                  |
| Crude fiber%                                   | 2.7                                     | 3.0                                   | 4.3                                   | 5.2                                   | 6.0                                   |

(1) Protein concentrate type BROCON – 5 SPECIAL W is of Dutch origin. Each kg contains 40% crude protein, 2.40% fat, 4.40% fiber, 3.60% calcium, 5.5% phosphorus, 2.50% sodium, 3.88% chloride, 3.85% lysine, 4.60% methionine, 5.10% methionine + cysteine, 3290 kcal/kg dietary energy. 20,000 IU Vitamin A, 80,000 IU Vitamin D3, 600 mg Vitamin E, 50 mg Vitamin K3, 50 mg Vitamin B1, 140 mg Vitamin B2, 80 mg Vitamin B6, 700 µg B12, 20 mg folic acid, 5 mg citric acid, 2 mg Biotin, 800 mg niacin, 1 mg iron, 200 mg copper, 1,600 mg manganese, 1,200 mg zinc, 20 mg iodine, 5 mg selenium, 6 mg cobalt, 33.50 mg antioxidant (BHT). (2) A mixture of vitamins and minerals, each kg of which contains: 720,000 IU Vitamin A, 168,000 IU D3, 800 mg E, 80 mg B1, 80 mg B2, 80 mg B6, 5,800 mg niacin, 500 µg folic acid, 5 mg diphosphate Calcium, 40 mg zinc, 100 mcg cobalt, 50 mg lysine

**Table 3.** Ingredient composition (%) of the experiment diets used from days 25 to 35

| Ingredients                                    | Control diet<br>Without any<br>addition | Finisher diets<br>25 – 35 days        |                                       |                                       |                                       |
|------------------------------------------------|-----------------------------------------|---------------------------------------|---------------------------------------|---------------------------------------|---------------------------------------|
|                                                |                                         | Treatment T <sub>2</sub><br>2% grapes | Treatment T <sub>3</sub><br>4% grapes | Treatment T <sub>4</sub><br>6% grapes | Treatment T <sub>5</sub><br>8% grapes |
| Crushed maize                                  | 50                                      | 48                                    | 46                                    | 44                                    | 42                                    |
| Ground wheat (local)                           | 14.9                                    | 14.9                                  | 14.9                                  | 14.9                                  | 14.9                                  |
| Soybean meal 48%                               | 24                                      | 24                                    | 24                                    | 24                                    | 24                                    |
| Protein concentrate (1)                        | 5                                       | 5                                     | 5                                     | 5                                     | 5                                     |
| Sunflower oil                                  | 4                                       | 4                                     | 4                                     | 4                                     | 4                                     |
| limestone                                      | 1.3                                     | 1.3                                   | 1.3                                   | 1.3                                   | 1.3                                   |
| Dicalcium phosphate                            | 0.3                                     | 0.3                                   | 0.3                                   | 0.3                                   | 0.3                                   |
| DCP                                            |                                         |                                       |                                       |                                       |                                       |
| salt                                           | 0.3                                     | 0.3                                   | 0.3                                   | 0.3                                   | 0.3                                   |
| Vitamins and minerals<br>mixture (2)           | 0.2                                     | 0.2                                   | 0.2                                   | 0.2                                   | 0.2                                   |
| Grape pomace powder                            | 0                                       | 2                                     | 4                                     | 6                                     | 8                                     |
| Total                                          | 100                                     | 100                                   | 100                                   | 100                                   | 100                                   |
| Chemical analysis calculated based on NRC (29) |                                         |                                       |                                       |                                       |                                       |
| Crude protein (%)                              | 19.4                                    | 19.5                                  | 19.52                                 | 19.54                                 | 19.56                                 |
| Dietary energy (kcal/kg<br>feed)               | 3204                                    | 3202                                  | 3200                                  | 3198                                  | 3196                                  |
| Methionine + Cysteine<br>(%)                   | 0.83                                    | 0.83                                  | 0.82                                  | 0.81                                  | 0.80                                  |
| Lysine (%)                                     | 1.1                                     | 1.1                                   | 1.1                                   | 1.1                                   | 1.0                                   |
| Calcium (%)                                    | 0.83                                    | 0.83                                  | 0.83                                  | 0.83                                  | 0.83                                  |
| Available phosphorus (%)                       | 0.44                                    | 0.44                                  | 0.44                                  | 0.44                                  | 0.43                                  |
| Crude fiber%                                   | 2.6                                     | 3.4                                   | 4.3                                   | 5.1                                   | 5.9                                   |

(1) Protein concentrate type BROCON – 5 SPECIAL W is of Dutch origin. Each kg contains 40% crude protein, 2.40% fat, 4.40% fiber, 3.60% calcium, 5.5% phosphorus, 2.50% sodium, 3.88% chloride, 3.85% lysine, 4.60% methionine, 5.10% methionine + cysteine, 3290 kcal/kg dietary energy. 20,000 IU Vitamin A, 80,000 IU Vitamin D3, 600 mg Vitamin E, 50 mg Vitamin K3, 50 mg Vitamin B1, 140 mg Vitamin B2, 80 mg Vitamin B6, 700 µg B12, 20 mg folic acid, 5 mg citric acid, 2 mg Biotin, 800 mg niacin, 1 mg iron, 200 mg copper, 1,600 mg manganese, 1,200 mg zinc, 20 mg iodine, 5 mg selenium, 6 mg cobalt, 33.50 mg antioxidant (BHT). (2) A mixture of vitamins and minerals, each kg of which contains: 720,000 IU Vitamin A, 168,000 IU D3, 800 mg E, 80 mg B1, 80 mg B2, 80 mg B6, 5,800 mg niacin, 500 µg folic acid, 5 mg diphosphate Calcium, 40 mg zinc, 100 mcg cobalt, 50 mg lysine

**Table 4.** Chemical analysis of grape pomace powder

|                | Analysis         | Value |
|----------------|------------------|-------|
| Components (%) | Protein          | 9.5   |
|                | Fat              | 3.65  |
|                | Fibers           | 44.3  |
|                | Moisture         | 7.14  |
|                | Ash              | 3     |
|                | Energy (kcal/kg) | 3244  |

Reference: This analysis was conducted in the Laboratories of the Quality Control Department/Livestock Department/Ministry of Agriculture

**Table 5.** Chemical analysis of grape pomace powder for amino acids and minerals constituents

| No | Name                | Con  |
|----|---------------------|------|
| 1  | Ca (µg / g)         | 24.5 |
| 2  | P (µg / g)          | 19.8 |
| 3  | A (IU)              | 20.1 |
| 4  | E (µg / g)          | 24.6 |
| 5  | C (µg / g)          | 29.0 |
| 6  | Methionine (µg / g) | 17.5 |
| 7  | Lysine (µg / g)     | 14.9 |
| 8  | Cysteine (µg / g)   | 21.9 |
| 9  | Carotene (µg / g)   | 1.6  |

## RESULTS AND DISCUSSION

**Live body weight:** The results of Table (6) show the average live weight of the body to which grape pomace powder was added, showing a significant increase ( $P>0.05$ ) during the first period (1-10 days) in favor of treatment T4 compared to treatments T2 and

T3. No significant differences were shown among treatments T5 and the control group. No significant differences were observed in the average live body weight in the second (10-24 days) and the third (24-35 days) periods.

**Table 6.** Live body weight of broilers fed different levels of dietary grape pomace powder (Mean±SE)

| treatments         | average live body weight (g/bird) |                   |               |               |
|--------------------|-----------------------------------|-------------------|---------------|---------------|
|                    | 0 day                             | 10 day            | 24 day        | 35 day        |
| T1                 | 46.00                             | 252.13±2.73<br>ab | 1030.00±21.52 | 2060.75±49.73 |
| T2                 | 46.33                             | 247.18±3.09<br>b  | 980.36±14.87  | 1943.55±46.67 |
| T3                 | 47.18                             | 248.78±5.61<br>b  | 1005.45±21.15 | 1974.16±39.47 |
| T4                 | 46.36                             | 262.09±4.98<br>a  | 996.45±14.06  | 2067.36±50.11 |
| T5                 | 46.78                             | 250.13±1.28<br>ab | 969.45±23.90  | 1948.89±40.76 |
| significance level | -                                 | $P\leq 0.05$      | NS            | NS            |

T<sub>1</sub>: control treatment (without addition); T<sub>2</sub>, T<sub>3</sub>, T<sub>4</sub>, and T<sub>5</sub> with Grape pomace powder at 2, 4, 6, and 8 g/kg, respectively.

<sup>a, b</sup> Means in the same column with different superscripts indicate significant differences among treatments ( $p\leq 0.05$ ). Means within the same column with no superscripts are not significantly different

**Weight gain:** Table (7) show the weight gain rates of broiler birds to which grape pomace powder was added in different proportions for the period from (0-10) days showed a significant increase in favor of the T<sub>4</sub> treatment compared to T<sub>2</sub>, T<sub>3</sub>, and T<sub>5</sub>. At the same time, it did not differ from the control group. However, the increase favored the

control treatment for the period from (11-24) days, which did not differ from treatments T<sub>2</sub>, T<sub>3</sub>, and T<sub>4</sub>. Also, it was superior ( $P\leq 0.05$ ) to the T<sub>5</sub> treatment, and no significant differences in the rate of weight gain were noticed for the third period (24-35) days of the experiment.

**Table7.** The weight gain of broilers fed different levels of dietary grape pomace powder (Mean±SE)

| treatments         | The average weight gain |                    |               |               |
|--------------------|-------------------------|--------------------|---------------|---------------|
|                    | 0 day                   | 10 day             | 24 day        | 0-35 day      |
| T1                 | 206.13±2.73<br>ab       | 777.87±19.06<br>a  | 1030.75±38.07 | 2014.75±49.85 |
| T2                 | 200.85±3.02<br>b        | 733.18±13.93<br>ab | 963.18±38.92  | 1897.22±46.61 |
| T3                 | 201.60±5.46<br>b        | 756.67±20.32<br>ab | 968.71±31.34  | 1926.98±39.15 |
| T4                 | 215.73±4.58<br>a        | 734.36±10.72<br>ab | 1070.91±40.97 | 2021.00±49.73 |
| T5                 | 203.35±1.30<br>b        | 719.33±22.92<br>b  | 979.44±23.64  | 1902.11±40.02 |
| significance level | P≤0.05                  | P≤0.05             | NS            | NS            |

T1: control treatment (without addition); T2, T3, T4, and T5 with Grape pomace powder at 2, 4, 6, and 8 g/kg, respectively

<sup>a, b</sup> Means in the same column with different superscripts indicate significant differences among treatments ( $p \leq 0.05$ ). Means within the same column with no superscripts are not significantly different

**Feed consumption:** It was observed from Table (8) of the rate of feed consumption with dietary grape pomace powder of broilers for the period from 0-35 days that there is a significant superiority ( $P \leq 0.05$ ) in the first period in favor of the T4 treatment compared to the control and T2, while it did not differ from T3 and T5 treatments for the period from 0-10 days, The increase was highly significant ( $P \leq 0.01$ ) in favor of the T4 treatment compared to the control treatment

and the rest of treatments for the period 11-24 days. Also, there was a significant increase ( $P > 0.05$ ) in favor of the T4 treatment compared to the control and the rest of the treatments, while the control treatment did not differ from T5 and T2 for the period of 25-35 days. Moreover, there is a presence of a highly significant superiority ( $P < 0.01$ ) for the cumulative increase (0-35 days) in favor of treatment T4 compared to the control and the rest of the experimental treatments.

**Table 8.** The feed intake of broilers fed different levels of dietary grape pomace powder (Mean±SE)

| treatments         | Average feed intake (g/bird) |                    |                     |                    |
|--------------------|------------------------------|--------------------|---------------------|--------------------|
|                    | 0-10 day                     | 11-24 day          | 25-35 day           | 0-35 day           |
| T1                 | 288.36±3.59<br>b             | 1082.77±25.20<br>b | 1464.43±54.04<br>bc | 2835.56±73.61<br>b |
| T2                 | 290.47±7.56<br>b             | 1064.67±10.08<br>b | 1461.43±45.16<br>bc | 2816.57±45.56<br>b |
| T3                 | 305.15±3.96<br>ab            | 1114.05±14.04<br>b | 1382.55±32.24<br>c  | 2801.75±44.81<br>b |
| T4                 | 315.13±4.83<br>a             | 1182.61±9.44<br>a  | 1644.45±44.86<br>a  | 3142.18±56.31<br>a |
| T5                 | 300.42±5.54<br>ab            | 1104.46±24.58<br>b | 1517.19±28.11<br>b  | 2922.07±33.61<br>b |
| significance level | ( $p \leq 0.05$ )            | ( $p \leq 0.01$ )  | ( $p \leq 0.05$ )   | ( $p \leq 0.01$ )  |

T1: control treatment (without addition); T2, T3, T4, and T5 with Grape pomace powder at 2, 4, 6, and 8 g/kg, respectively

<sup>a, b</sup> Means in the same column with different superscripts indicate significant differences among treatments ( $p \leq 0.05$ )

<sup>a, b</sup> Means in the same column with different superscripts indicate significant differences among treatments ( $p \leq 0.01$ )

**Feed conversion ratio:** The result Table (9), which deals with the effect of adding different levels of grape pomace powder to the diets of broilers during the period from 1 to 35 days, showed significant differences in the feed conversion. At the age of 10 days, a considerable ( $P < 0.05$ ) was observed in favor

of treatment which was better than T3 treatment. while no significant differences appeared compared to the rest of the treatments (T2, T4 and T5). During the period from 11 to 24 days, the control treatment T1, achieved a significant superiority in the feed conversion compared to the treatments T4 and

T<sub>5</sub>, while there was no significant difference with the T<sub>4</sub> and T<sub>5</sub> treatments T<sub>2</sub> and T<sub>3</sub>. In the last period (24-35 days), the experimental treatments did not show significant differences in the feed conversion rate. As for the cumulative increase during the entire period

(0-35 days), there was a considerable superiority ( $P < 0.05$ ) in favor of treatments T<sub>1</sub> and T<sub>2</sub> compared to treatments T<sub>4</sub> and T<sub>5</sub>, while there was no significant difference with treatment T<sub>3</sub>.

**Table 9.** The feed conversion ratio of broilers fed different levels of dietary grape pomace powder (Mean±SE)

| treatments         | feed conversion ratio g feed / g weight gain |                   |           |                   |
|--------------------|----------------------------------------------|-------------------|-----------|-------------------|
|                    | 0-10 day                                     | 11-24 day         | 25-35 day | 0-35 day          |
| T1                 | 1.40±0.01<br>b                               | 1.39±0.02<br>c    | 1.42±0.01 | 1.41±0.01<br>b    |
| T2                 | 1.45±1.45<br>ab                              | 1.45±0.02<br>bc   | 1.53±0.07 | 1.49±0.04<br>b    |
| T3                 | 1.52±0.03<br>a                               | 1.48±0.04<br>bc   | 1.43±0.01 | 1.45±0.01<br>ab   |
| T4                 | 1.46±0.02<br>ab                              | 1.61±0.02<br>a    | 1.54±0.04 | 1.56±0.03<br>a    |
| T5                 | 1.48±0.04<br>ab                              | 1.54±0.03<br>ab   | 1.55±0.04 | 1.54±0.02<br>a    |
| significance level | ( $p \leq 0.05$ )                            | ( $p \leq 0.05$ ) | NS        | ( $p \leq 0.05$ ) |

T<sub>1</sub>: control treatment (without addition); T<sub>2</sub>, T<sub>3</sub>, T<sub>4</sub>, and T<sub>5</sub> with Grape pomace powder at 2, 4, 6, and 8 g/kg, respectively

<sup>a, b</sup> Means in the same column with different superscripts indicate significant differences among treatments ( $p \leq 0.05$ ). Means within the same column with no superscripts are not significantly different

Although there are no significant differences between the addition and control treatments, these additions are considered positive. This is because the treatments were a partial substitute for maize, yet they provided the same productive level. This may be attributed to the use of grape pomace (GP), which can protect the intestine from possible oxidative damage during the digestion process because it contains polyphenol compounds with antioxidant properties (Gungor et al., 2021). Maintaining intestinal health is an important indicator of improved growth, enhanced nutrient absorption, and strengthened defense against pathogenic bacteria. Features of a healthy gut include longer villi, shallower crypts, and thicker muscle lamina, which promotes body growth (Chee et al., 2010). The observed superiority in body weight and weight gain rates for the experimental treatment (T<sub>4</sub>), which included the addition of grape pomace powder at a rate of 6%/kg, may be attributed to its content of important amino acids, as shown in the amino acid table. The most prominent of these amino acids is methionine, which is essential in forming proteins necessary to build body tissues and contributes to achieving a clear weight gain (Koshchayev et al., 2020). In addition,

resveratrol is considered one of the most important active compounds in grape pomace, as it acts as an antioxidant and contributes to weight gain by reducing the oxidative stress chickens may be exposed to. Heat stress is one of the most prominent forms of stress that lead to increased oxidative stress within the body, causing damage to the intestinal mucosa of chickens and affecting the oxidation and reduction balance in the body. This produces large amounts of reactive oxygen species (ROS) and reduces the body's ability to resist oxidative stress (Song et al., 2014; Wang et al., 2021; Zhang et al., 2018). In addition, grape pomace contains compounds of high biological value, such as polyphenols, which may contribute to reducing the degradation of vitamin E in the intestine (Chedea et al., 2018), which enhances the absorption of larger amounts of it and improves oxidative stability in many tissues. In addition, grape pomace is characterized by its ability to enhance the synthesis of vitamin E in the liver (Goñi et al., 2007), improving birds' productive performance. The microbial count in the small intestine of broilers at 35 days of age. The data in Table (10) shows the effect of adding different levels of grape pomace powder to broiler diets for (1–35) days on the numbers of

beneficial and harmful bacteria in the intestines of broilers. A highly significant superiority ( $P \leq 0.01$ ) was obtained in favor of treatment T4 and T5 in the numbers of *Lactobacillus* bacteria compared to control treatment T1, T2, and T3. The control treatment recorded the lowest number of *Lactobacillus* bacteria. As for the numbers of *E. coli* bacteria, it can be noticed a highly significant decrease ( $P \leq 0.01$ ) in favor of the T5 treatment compared to the control

treatment, which recorded the highest number of this type of bacteria compared to all addition treatments. At the same time, there was a highly significant superiority ( $P \leq 0.01$ ) in favor of the control treatment T1 in the complete count of beneficial and harmful bacteria in the intestines compared to the treatments T3, T4, and T5. In contrast, it did not differ from the control treatment T2 in the complete count of beneficial and harmful bacteria.=

**Table 10.** The microbial count of the intestines in broilers fed dietary grape pomace powder (Mean $\pm$ SE)

| Treatments         | E. Coli          | Lactobacillus     | Total count       |
|--------------------|------------------|-------------------|-------------------|
| T <sub>1</sub>     | 0.03 $\pm$ 7.89a | 0.11 $\pm$ 7.34d  | 0.19 $\pm$ 8.86a  |
| T <sub>2</sub>     | 0.11 $\pm$ 7.29b | 0.16 $\pm$ 8.22c  | 0.03 $\pm$ 8.56ab |
| T <sub>3</sub>     | 0.21 $\pm$ 5.89c | 0.14 $\pm$ 9.46b  | 0.14 $\pm$ 8.28b  |
| T <sub>4</sub>     | 0.03 $\pm$ 5.58c | 0.12 $\pm$ 10.52a | 0.04 $\pm$ 7.77c  |
| T <sub>5</sub>     | 0.18 $\pm$ 4.77d | 0.38 $\pm$ 10.84a | 0.06 $\pm$ 6.71d  |
| Significance level | $p \leq 0.01$    | $p \leq 0.01$     | $p \leq 0.01$     |

T<sub>1</sub>: control treatment (without addition); T<sub>2</sub>, T<sub>3</sub>, T<sub>4</sub>, and T<sub>5</sub> with Grape pomace powder at 2, 4, 6, and 8 g/kg, respectively

<sup>a,b</sup> Means in the same column with different superscripts indicate significant differences among treatments ( $p \leq 0.01$ )

The increased numbers of *Lactobacillus* bacteria in the ileal region of the intestines of broilers in treatments T4 and T5 can be explained by the fact that grape pomace contains fiber and polysaccharide carbohydrates, most notably inulin. Inulin is a type of carbohydrate that belongs to non-digestible dietary fiber and acts as a prebiotic. It promotes the growth of beneficial bacteria in the gastrointestinal tract, such as *Lactobacillus*, as it consists mainly of fructose units with a natural glucose end, and these units are linked by a  $\beta$ -(2-1) glycosidic linkage (Zeaiter et al., 2019). These glycosidic bonds make inulin resistant to digestive enzymes and non-degradable in the stomach, allowing it to reach the large intestine to be fermented by beneficial bacteria such as *Lactobacillus*. The fermentation process produces short-chain fatty acids which reduce the pH in the intestine (Hernández-González et al., 2021; Van den Abbeele et al., 2020), creating an acidic environment that hinders the growth of harmful bacteria such as *E. coli* (Royce et al., 2013; Yang et al., 2010). In addition, *Lactobacillus* contributes to limiting the proliferation of harmful bacteria through competitive exclusion by adhering to the intestinal mucous membranes, competing for nutrients, reducing the numbers of *E. coli*, and

inhibiting their growth. Furthermore, the production of natural antibiotics (bacteriocins) inhibits the growth of *E. coli*. These results confirm the positive role of adding grape pomace in improving the microbial environment of the intestine and promoting digestive health (Hernández-González et al., 2021; Lagha et al., 2017; Patterson & Burkholder, 2003). The addition of grape pomace at levels up to 8% in broiler diets improved productive performance and digestive health without negatively affecting production. This highlights the potential of grape pomace as an effective and environmentally friendly alternative to yellow corn in poultry feed, enhancing both environmental and health benefits.

## CONCLUSION

In conclusion, this study demonstrates that grape pomace meal can serve as a viable and sustainable alternative to yellow corn in broiler (ROSS 308) diets at an optimal inclusion level of up to 6% without any adverse effects on body weight or weight gain at 24 and 35 days of age. Although higher levels (6% and 8%) increased the feed conversion ratio and feed intake, grape pomace exerted a highly beneficial effect on intestinal health by significantly stimulating the proliferation of beneficial *Lactobacillus* spp. and reducing

pathogenic *E. coli* and total bacterial counts. Therefore, it is recommended to incorporate grape pomace meal at a maximum level of 6% to capitalize on its gut-health advantages and lower feed costs without compromising the growth performance of the birds.

### CONFLICT OF INTEREST

The authors declare no conflict of interest

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## اداء النمو والاحياء المجهرية لفروج اللحم المغذى على مستويات مختلفة من مسحوق ثفل العنب

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### المستخلص

أُجريت هذه الدراسة لتقييم تأثير إضافة مستويات مختلفة من مسحوق ثفل العنب في العلائق على أداء النمو و العد الميكروبي لفروج اللحم. وزعت طيور التجربة (275 فروج لحم) المتماثلة بالوزن بعمر يوم واحد عشوائيًا إلى خمس مجموعات تجريبية. كان لكل مجموعة تجريبية خمسة مكررات (11 طائرًا /مكرر). تم تغذية طيور مجموعة السيطرة على نظام غذائي يتكون أساسًا من الذرة الصفراء وكسبة فول الصويا. أما المجموعات الأربع الأخرى فقد تم تغذيتها بنظام غذائي مشابه مع استبدال جزئي لوجبة الذرة الصفراء بـ 2، 4، 6، و8% من مسحوق ثفل العنب. أظهرت النتائج أنعدام التأثير معنوي لمعاملات مسحوق ثفل العنب على وزن الجسم وزيادة الوزنية عند الايام 24 و 35. كما أظهرت الطيور التي تغذت على نظام غذائي يحتوي على 8% من مسحوق ثفل العنب زيادة (P<0.01) في استهلاك العلف مقارنةً بالمجموعات الأخرى. كما زادت قيمة نسبة التحويل الغذائي (P<0.05) في معاملات الغذائية التي تحتوي على 6% و8% من مسحوق ثفل العنب. كما انخفضت اعداد بكتريا *E. coli* والبكتيريا الكلية (P<0.01)، في حين ارتفعت اعداد البكتريا اللبنية من جنس *Lactobacillus spp* (P<0.01) مع زيادة كمية مسحوق ثفل العنب في العلائق. ومنها هذه الدراسة نستنتج أن تغذية فروج اللحم بعلائق تحتوي على مسحوق ثفل العنب حتى 8% لم يُظهر أي تأثيرات ضارة على أداء النمو، كما زاد عدد من انواع البكتريا من جنس *Lactobacillus spp*.

**الكلمات المفتاحية:** بدائل علفية، صحة الامعاء، تغذية الطيور.