Impacts of dietary supplementation of pyocyanin powder on growth performance, carcase traits, blood chemistry, meat quality and gut microbial activity of broilers

Elwy A. Ashour, Reem M. Farsi, Bothaina A. Alaidaroos, Abdel-Moneim E. Abdel-Moneim, Mohamed T. El-Saadony, Ali O. Osman, Eman T. Abou Sayed-Ahmed, Najah M. Albaqami, Manal E. Shaf, Ayman E. Taha and Mohamed E. Abd El-Hack

ABSTRACT
Natural antioxidants play an important role in maintaining and improving poultry’s well-being, survival and productive and reproductive performance. Pyocyanin, a secondary blue redox-active metabolite, is one of these natural antioxidants that exert several biological activities to improve birds’ performance. This study estimated the effect of dietary supplementation with pyocyanin powder (PP) on broiler’s growth, carcase and serum properties, meat quality and microbial load activity. A total of 180 1-week-old unsexed chicks were randomly allocated into three groups. The first group served as a control (C) and fed the basal diet, while the second and third groups (P75 and P150) were fed the basal diet supplemented with 75.0 and 150.0 mg PP/kg, respectively. Results showed that overall body weight gain (BWG) was improved \( p < 0.05 \) in P75 compared to P150 and control groups. Feed intake and feed conversion ratio (FCR) of chicks fed the diet containing PP levels were not altered during all experimental periods compared to the control. Dietary treatments did not affect all carcase traits, blood biochemical parameters and meat sensory characteristics at five weeks of age. Antioxidative status was improved by pyocyanin supplementation as serum malondialdehyde (MDA) was reduced while GST and GSH levels were elevated in P75 and P150 compared to the control. Dietary PP supplements increased \( p < 0.05 \) the pH, lightness and redness of pectoralis major muscle and reduced \( p < 0.05 \) the yellowness of the muscle and its contents of thiobarbituric acid and total volatile basic nitrogen. The PP addition showed antibacterial and antifungal activities against several pathogenic bacteria and mycotoxigenic fungi. It could be concluded that pyocyanin can be introduced as a natural, functional and phytogenic feed additive to boost broiler’s growth, improve their meat quality, produce nutritious meat products and reduce pathogenic bacteria without adverse impacts on their productivity.

HIGHLIGHTS
- This work investigated the effect of pyocyanin powder on broiler chickens.
- The addition of pyocyanin at 75.0 mg/kg diet improved Body weight gain (BWG) and meat quality characteristics.
- Pyocyanin exerted antibacterial and antifungal activity against several pathogenic bacteria.
- Pyocyanin could be used as a natural and functional additive to poultry diets.

Introduction
Verified data and findings have shown that phytogenic feed additives influence the development and health of animals and birds. These additives act as antioxidants that play an important role in the performance of animal wellbeing, survival, maintenance, productive and reproductive activities (Abd El-Hack et al. 2019; Alagawany et al. 2019). Dietary additives of phytogenic origin are materials obtained from plants used in poultry feeding in the poultry
industry to increase productivity and reproductive efficiency and improve growth and immunity (Abd El-Hack, Abdelnour, Taha, et al. 2020). Herbal additives used in poultry feeding already have beneficial effects on poultry feeding, such as improved appetite and feed consumption, enhancement of endogenous digestive enzyme secretion, induction of immune response, antioxidant, antibacterial, antihelminthic and antiviral properties (Shewita and Taha 2019; Arif et al. 2019; Ibrahim et al. 2020). The beneficial effects of antiviral properties (Shewita and Taha 2019; Arif et al. 2019) on feed consumption, enhancement of endogenous digestive enzyme secretion, induction of immune response, antioxidant, antibacterial, antihelminthic and antiviral properties have been shown by several reports (Gado et al. 2019; Abd El-Hack, Abdelnour, Taha, et al. 2020; Abd El-Hack, Alagawany, Shaheen, et al. 2020; Abdel-Moneim, Sabic, et al. 2020; Abd El-Hack, Abdelnour, Taha, et al. 2020). Herbal additives such as pyocyanin has been used as a biocontrol agent against certain food spoilage and pathogenic bacteria and fungi in sustainable agriculture (Kim 2000; Aunchaleeb et al. 2009).

Pyocyanin is a secondary blue redox-active metabolite and a large family member of phenazines, tricyclic compounds. At the late stationary stage, they are secreted and provided the medium with a characteristic blue colour. It can be quickly extracted from the culture medium due to its solubility in chloroform. Pyocyanin ‘5-methyl-1-hydroxy phenazine’ can undergo a complex oxidation-reduction reaction (Ran et al. 2003). Many phenazine compounds isolated from bacteria have a broad range of antimicrobial activity, antitumor, antimalaria and antiparasitic activities (Laursen and Nielsen 2004). The antimicrobial activity of phenazine has been used as a biocidal agent against certain food spoilage and pathogenic bacteria and fungi in sustainable agriculture (Kim 2000; Aunchaleeb et al. 2009).

These compounds are sufficient for inhibiting microbes (Giddens et al. 2003). Pyocyanin is isolated from Pseudomonas spp., it was used as antioxidants (Rada et al. 2013; Laxmi and Bhat 2016) and anticancer agents (Hassani et al. 2012). Pyocyanin has shown antibacterial and antifungal activity against many pathogenic bacteria, and mycotoxigenic fungi are also available for antioxidant and anticancer activity against cancer cell lines. As a result, for these activities, they can be used in food industries, such as food preservation and pharmaceutical applications (Marrez and Haitham 2020). We hypothesised that, with the aforementioned biological properties of pyocyanin, it can be used as a potential growth promoter in poultry industry. Therefore, the present study assessed the growth-promoting potential of dietary increasing levels of pyocyanin powder (PP) on performance, carcass characteristics, serum metabolites, meat quality and microbial enumeration of broiler chickens.

Materials and methods

Production, purification and characterisation of pyocyanin pigment

Soil samples were collected from the rhizosphere in Zagazig city, Sharika governorate, Egypt. The samples were transferred to the microbiological laboratory in sterilised bags. Pour plates technique was used for the isolation process: 10 g of soil samples were homogenised in 90 mL of sterilised peptone water to obtain $10^{-1}$ dilution, then serial dilutions were carried out from this dilution till $10^{-7}$. An aliquot of 0.1 mL of each dilution was spread on the surface of plate count agar (PCA) plates and incubated for three days at $37^\circ C$ (Barakat et al. 2015). The blue-greenish colonies were isolated and purified on cetrimide medium and preserved on cetrimide agar slants at 4°C. The selected colonies were primarily identified by morphological and biochemical tests in Bergy Manual (Brenner et al. 2005; Uğur et al. 2012), then confirmed by MALDI-TOF spectroscopy.

Pseudomonas aeruginosa isolate was grown at King’s medium broth and incubated for 2–3 d at 35°C for the extraction of pigment. The pigment’s colour shift to a blue-greenish indicates the pigment production (Mahon et al. 2007). An aliquot of 5 mL of bacterial culture was centrifuged at (10,000 rpm/15 min) and 3 mL of chloroform was applied to the supernatant and mixed until the blue colour was restored. The blue colour was converted to red-pink by adding 1 mL of 0.2N HCl, the absorbance of the acidic solution was measured at 520 nm and applied in the following equation, pyocyanin concentration ($\mu g/mL$) = Abs 520 * 17.072 (Essar et al. 1990). The minimum volume of 0.1M NaOH was added to the pink solution to retain the blue-green colour and then chloroform was added again. The chloroform was evaporated and the PP was obtained (Raji El Feghali and Nawas 2018). The phytochemical profile of pyocyanin powder was identified by the GC-mass spectrum (Abdel-Shafi et al. 2019; Saad et al. 2020).

Microbial activity

Antibacterial activity of pyocyanin pigment

Well-diffusion assay was used to determine the pyocyanin antibacterial activity against three types of G$^+$ bacteria (Staphylococcus aureus, Bacillus cereus and Listeria monocytogenes) and three types of G$^-$ bacteria...
(Salmonella enterica, Klebsiella pneumoniae and Escherichia coli) strains. The tested bacteria’s pure cultures were sub-cultured in Mueller Hinton broth (MHB) and incubated at 37 °C on a shaking incubator at 200 rpm for 24 h. Each culture was spread uniformly onto individual Mueller Hinton Agar (MHA) plates using the spread plate method. Wells (6 mm diameter) were punched in MHA with sterilised cork, then each well was loaded with 70 μL of pigment concentration of 10, 20, 30, 40 and 50 μg/mL dissolved in DMSO, the wells in MHA plates loaded with DMSO as a control. The MHA Plates were incubated for 24 h at 37 °C and pigment sensitivity was observed by diameter of inhibition zones around the wells (Saha et al. 2008). The minimum inhibition concentration (MIC) was the lowest concentration of pyocyanin that inhibited the bacterial growth, and was estimated by microdilution broth; according to Murray et al. (1995) the MIC defined as the lowest concentration of pyocyanin that inhibited that bacterial growth and was estimated by microdilution broth; according to Murray et al. (1995) the MIC defined as the lowest concentration of pyocyanin that inhibits the bacterial growth, and determined as follows, 100 μL of pyocyanin concentrations of 10, 20, 30, 40 and 50 μg/mL dissolved in DMSO, the wells in MHA plates loaded with DMSO as a control. The MHA Plates were incubated for 24 h at 37 °C and pigment sensitivity was observed by diameter of inhibition zones around the wells (Saha et al. 2008). The minimum inhibition concentration (MIC) was the lowest concentration of pyocyanin that inhibited that bacterial growth and was estimated by microdilution broth; according to Murray et al. (1995) the MIC defined as the lowest concentration of pyocyanin that inhibits the bacterial growth, and determined as follows, 100 μL of pyocyanin concentrations of 10, 20, 30, 40 and 50 μg/mL dissolved in DMSO, the wells in MHA plates loaded with DMSO as a control. The MHA Plates were incubated for 24 h at 37 °C and pigment sensitivity was observed by diameter of inhibition zones around the wells (Saha et al. 2008).

**Antifungal activity**

Disc assay was used to determine the antifungal activity of pyocyanin concentrations (10, 20, 30, 40 and 50 μg/mL dissolved in DMSO) against six fungi strains (Aspergillus niger, Aspergillus flavus, Candida albicans, Candida gelberta, Penicillium solitum and Penicillium crustosum). An aliquot of 100 μL of the fungal suspension was spread on sabouraud dextrose agar (SDA, Candida). On potato dextrose agar (PDA, other fungi) plates, then discs 6 mm saturated with each concentration of pyocyanin were put on the sides of SDA for Candida and PDA plates for fungi. The SDA plates were incubated at 30 °C for 24 h and PDA plates were at 28 °C for 5 d, then the resultant inhibition zones were measured (mm). The MIC and MFC of pyocyanin were estimated by microdilution broth, and pour plate method, respectively (Moghadam et al. 2019).

**Experimental design and diets**

The present investigation was carried out at Poultry Research Farm, Poultry Department, Faculty of Agriculture, Zagazig University, Zagazig, Egypt. According to the Local Experimental Animal Care Committee, all experimental procedures were carried out and approved by the ethics of the Poultry Department’s institutional committee, Faculty of Agriculture, Zagazig University, Zagazig, Egypt. A total of 180 Ross-308 unsexed broiler chicks one week old with an initial body weight of 130.22 ± 0.22 g were housed in a floor litter and used in a full randomised design trial with three treatments; 60 chicks in six replicates per group and replicate dimensions were 40 cm high × 100 cm width × 100 cm length. Dietary treatments were as follows: the first group served as a control (C) and fed the basal diet, while second and third groups (P75 and P150) were fed the basal diet supplemented with 75.0 and 150.0 mg PP/kg, respectively. Broilers were fed the basal diets in pellet form from 1 to 3 weeks (starter) and from 3 to 5 weeks (finisher). Feed and freshwater were provided ad-libitum during the experimental period. Chicks were housed under the same hygienic, rearing, and environmental conditions. The basal diets were formulated to meet the requirements of the broiler strain (Table 1).

| Items                          | Starter (1–3 weeks) | Finisher (3–5 weeks) |
|-------------------------------|---------------------|----------------------|
| Ingredients (g/kg diet)        |                     |                      |
| Yellow corn                   | 571.30              | 605.30               |
| Soybean meal                  | 316.50              | 271.50               |
| Gluten meal                   | 65.00               | 61.00                |
| Dicalcium phosphate           | 17.00               | 15.00                |
| Limestone                     | 12.40               | 11.50                |
| Vit-min Premixa               | 3.00                | 3.00                 |
| NaCl                          | 3.00                | 3.00                 |
| DL Methionine                 | 0.50                | 0.20                 |
| L-Lysine HCl                  | 1.30                | 1.00                 |
| Soybean oil                   | 10.00               | 28.50                |
| Total                         | 1000                | 1000                 |

Calculated analysis (%)

| Items                          | Starter (1–3 weeks) | Finisher (3–5 weeks) |
|-------------------------------|---------------------|----------------------|
| Dry matter                    | 91.74               | 90.43                |
| Crude protein                 | 23.00               | 21.00                |
| Metabolizable energy (MJ/kg diet) | 12.35               | 12.97                |
| Calcium                       | 1.00                | 0.90                 |
| Available phosphorous          | 0.45                | 0.40                 |
| Lysine                        | 1.20                | 1.05                 |
| Methionine + cysteine         | 0.83                | 0.74                 |
| Crude fibre                   | 3.56                | 3.31                 |

Growth vitamin and mineral premix each 2.5 kg consists of Vit A 12,000,000 U; Vit D3, 2,000,000 U; Vit. E, 10 g; Vit k3 2 g; Vit B1, 1000 mg; Vit B2, 49 g; Vit B6, 105 g; Vit B12, 10 mg; Pantothenic acid, 10 g; Niacin, 20 g; Folic acid, 1000 mg; Biotin, 50 g; Choline chloride, 500 mg, Fe, 30 g; Mn, 40 g; Cu, 3 g; Co, 200 mg; Si, 100 mg and Zn, 45 g.

Calculated according to NRC (1994).
**Growth performance and blood biochemical traits**

Birds were weighed individually at weekly intervals, and the period and cumulative average daily feed intake (ADFI) were recorded. Body weight gain (BWG) and feed conversion ratio (FCR) were calculated from these data. For carcass evaluation, six birds with an average body weight around the mean treatment were randomly selected and slaughter weight (SW) was recorded. The carcasses were weighed, and the liver, gizzard, heart and abdominal fat weights were registered and proportioned to the SW. Dressing percentage was calculated as weight of carcass plus the weight of giblets/live body weight × 100 according to Abo Ghanima, Abd El-Hack, et al. (2020). Blood samples were collected from sacrificed broiler chicks in clean, sterile tubes and left to coagulate. As mentioned in (Sitohy et al. 2013; Abdelnour, Swelum, et al. 2020; Abdelnour, El-Saadony, et al. 2020), serum samples were isolated from blood samples, then stored at −20 °C until analysis. In addition to the activity of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), the serum content of urea, creatinine, total protein and albumin has been estimated using commercial diagnostic kits offered by Bio Diagnostic Co. (Giza, Egypt). From the following equation, globulin was calculated: Globulin = total protein - albumin.

The oxidative stress markers were estimated in the blood, including the lipid peroxidation malondialdehyde (MDA), glutathione peroxidase (GPx), glutathione reduced (GSH) using kits generated by Merck (KGaA, Darmstadt, Germany).

**Meat quality**

**The 2-thiobarbituric acid test (TBA)**

The determination of lipid oxidation in pectoralis muscle was tested according to Fernandez-Lopez et al. (2005). The 2-thiobarbituric acid (TBA) numbers were determined as MDA/kg sample according to the following equation: TBA number (kg)=–absorbance at 538 nm × 7.8. The pH of meat samples was measured using a digital pH metre.

**Total volatile bases nitrogen (TVBN)**

Total volatile bases nitrogen (TVBN) was calculated according to methods defined by Goulas and Kontominas (2005). In brief, a total of 10 g meat samples was mixed with 50 mL of distilled water. The mixture was quantitatively transferred with 200 mL of distilled water into a 500 mL round bottom flask and distilled after 2 g of MgO and one drop of silicone to avoid foaming. A 250 mL Erlenmeyer flask containing 25 mL of 3% aqueous solution of boric acid, 0.04 mL of methyl red and methylene blue combined as markers for the titration of ammonia was used as the distillate receiver. The distillation continued until a final distillate volume of 125 mL was obtained. When the distilled absolute volatile basic nitrogen is rendered alkaline, the boric acid solution turned green (TVBN). This was titrated with 0.1N hydrochloric acid solutions that were aqueous. Total neutralisation was achieved when, after adding a further drop of hydrochloric acid, the distillate colour turned pink. From the amount (v) of hydrochloric acid added and its concentration (C), the quantity of TVBN in mg/100 g of meat was determined as follows: % mg TVBN=(V × C × 14 × 100)/10.

**Colour evaluation**

According to the tristimulus colour system, the colour of the samples was determined using a method defined by Francis and Clydesdale (1975) (Hunter Lab Colour Flex, Ez, USA). In terms of lightness (L⁰), redness (a⁰) and yellowness colour (b⁰), was expressed. The standardisation was achieved by calibrating the system with a pink standard plate (L= +70.9, a= +22.4 and b= +8.2). An average of three readings from the same position was the Hunter value.

**Sensory evaluation**

All samples were sensory evaluated for their properties. The panel consisted by rating the above quality attributes using the following rating scale: 5 = Excellent, 4 = good, 3 = below good above fair, 2 = fair and 1 = very poor. Rating of 3 and 2 indicated that the products were of marginal quality, whereas the rating of 1 (poor) and below indicated that the consumers might not accept the product.

**Microbial analysis**

During feeding, dietary samples supplemented with pyocyanin concentrations (75 and 150 mg/kg diet) or without pyocyanin (control) were subjected to microbial analysis at an interval of (0, 7, 14 and 21 d). An amount of 25 g of dietary sample was homogenised with 225 mL of sterile saline peptone in a stomacher bag for 30 min to obtain 10⁻¹ dilution. Serial dilutions were done till 10⁻⁷. An aliquot of 100 µL of each dilution was spread onto specific agar media plates. The total bacterial count (TBC) was enumerated at PCA after incubation at 30°C for 48 h. The total count of yeasts and moulds (TYMC) was assessed after
incubation at 25°C for 5 d on Rose Bengal Chloramphenicol agar. Violet Red Bile Agar (Bioline, Italy) was used to enumerate coliforms after incubation at 37°C for 24 h. E. coli count was assessed on Tryptone Bile Glucuronide Agar at 37°C for 24 h (Harrigen and Mccance-Margart 1976; Szabo et al. 1986).

Caecal microbial community was also evaluated; 10 g of broiler caecum samples from each treatment were collected and transferred to a 250 mL Erlenmeyer flask containing 90 mL of sterile saline peptone solution and homogenised for 30 min. Decimal serial dilutions up to 10⁻⁷ were prepared. The microbial counts were assessed on specific media (El-Saadony et al. 2020; Reda et al. 2020; Sheiha et al. 2020). The TBC was enumerated on PCA. Violet Red Bile Agar and Tryptone Bile Glucuronide Agar were used for counting coliform and E. coli after incubation at 37°C for 24 h, respectively (Harrigen and Mccance-Margart 1976; Szabo et al. 1986). Black colonies on S.S. agar indicate Salmonella spp., found and the count was assessed as per (Edwards and Hilderbran 1976). Yeasts and moulds were enumerated by using Rose Bengal Chloramphenicol agar medium at 28°C for 72 h (Kurtzman and Fell 1984). Lactic acid bacteria were counted using MRS- medium (DeMan, Rogosa-Sharpe, Oxoid, CM 361) (Argyri et al. 2013). The microbial counts were converted into log numbers (Ashour, El-Hack, et al. 2020; Elbaz et al. 2021; Reda et al. 2021).

Histological examination

Collected samples were fixed in neutral buffered formalin (10%) and managed via the routine paraffin embedding method. Briefly, tissues specimens were dehydrated in ascending grade of ethyl alcohol, cleared in two changes of xylol, embedded in paraffin blocks, microtomed into 4 μm thick sections using a microtome (Leica RM 2155, England), and stained with haematoxylin and eosin according to Suvarna et al. (2018). Representative photomicrographs were captured by a digital camera (Leica EC3, Leica, Germany) connected to a microscope (Leica DMS500) (Abdel-Moneim, Elbaz, et al. 2020).

Statistical analysis

Data were subjected to the one-way analysis of variance using the General Linear Models (GLMs) procedure of the SPSS (2008). The statistical model was as follows:

\[ Y_{ij} = \mu + P_i + \varepsilon_{ij} \]

where \( Y_{ij} \) = observation, \( \mu \) = overall mean, \( P_i \) = a fixed effect of PP dietary supplementation, and \( \varepsilon_{ij} \) = experimental error. Using the Student–Newman–Keuls test, the statistical significant differences between means were determined at \( p < .05 \).

Results and discussion

Isolation and identification of bacterial isolate from soil

Fifteen bacterial isolates were obtained on PCA medium; one isolate was produced blue-greenish pigment. This isolate was purified. The purified isolate was gram-negative, short rods, and non-spore-forming under a light microscope. Results showed that this bacterium resembles Pseudomonas species. Based on Bergey’s Manual recommended tests (Brenner et al. 2005), the comparison process revealed the examined isolate could be defined as P. aeruginosa. This identification was confirmed by MALDI TOF mass spectrosopy according to Mahon et al. (2007). The recorded results indicated that the selected bacteria were 98% similar to several Pseudomonas spp., mainly P. aeruginosa DSM 50071 T HAM (Cunningham and Patel 2013). Thus, the local screened bacterial isolate P. aeruginosa AS6 is similar to P. aeruginosa DSM 50071 T HAM. The concentration of isolated pyocyanin was 179.65 μg/mL based on the absorbance at 520 nm.

GC-MS analysis of pyocyanin

The phytochemical components identified from the PP using GC-MS analysis were valeric acid, p-Allylphenol, 3, 3′, 4′, 7-Tetramethoxyflavone, Kaempferol, Flavindiacetate, Phenol, 2, 4 bis (1,1dimethylethyl) 3, 7, 11,15 Tetramethyl 2 hexadecen1ol, Phytol, 1 Propene1, 2, 3 tricarboxylic acid, tributylester, 5 Hydroxy1,4 dime-thoxy anthraquinone and Eicosamethylcyclodecasilox-ane along with other minor phytoconstituents (Figure 1, Table 2). Several recent studies have suggested that many of these phytochemical components exert several biological activities including antitumor, antinociceptive, antioxidant, anti-inflammatory, immune-stimulating, antifungal and antibacterial properties (Islam et al. 2018; Wang et al. 2018; Marrez and Haitham 2020). These biological effects of pyocyanin components might explain its beneficial impacts on broilers’ performance, meat quality and physiological responses.
Antimicrobial activity of pyocyanin isolated from *P. aeruginosa* AS6

Based on the obtained results, MHA plates loaded with DMSO (control) did not show antimicrobial activity. Pyocyanin isolated from *P. aeruginosa* AS6 strain has more antibacterial activity than antifungal activity with a 40% relative increase (Table 3). The IDZs against bacterial strains were in the range of (13–32 mm). However, the IDZs in tested fungi in the range of (10–24 mm). The IDZs were increased (*p* < 0.05) in a concentration-dependent manner. *S. aureus* and *K. pneumonia* were the most sensitive G+ bacteria to pyocyanin concentration (50 μg/mL), i.e. 32 and 25 mm, respectively. Nevertheless, *L. monocytogenes* and *S. enterica* were the most resistant G+ bacteria to pyocyanin concentration (50 μg/mL) with IDZs 29, and 22 mm, respectively. On the other hand, *Candida* species were more vulnerable than fungal species, with 30% relative increase in IDZs. The most resistant fungi to pyocyanin concentration (50 μg/mL) were *A. niger* with 17 mm. Pyocyanin isolated from *P. aeruginosa* AS6 strain remarkably inhibited (*p* < 0.05) the bacterial growth in the range of MIC (6–9 μg/mL), and no bacterial growth (MBC) in the range of 10–18 μg/mL. The MIC range (9–18 μg/mL) were inhibited the fungal growth while MFC range (18–36 μg/mL) achieve no fungal growth (Table 3). Several studies were reported the antimicrobial activity of pyocyanin, where Saha et al. (2008), El-Shouny et al. (2011) and Marrez and Haitham (2020) reported that the antimicrobial activity of *P. aeruginosa* strains because of its secondary metabolites pyocyanin. Pyocyanin isolated from *P. aeruginosa* 4B strain showed antibacterial activity against pathogenic bacteria i.e. G+ bacteria (*L. monocytogenes* and *B. cereus*) and G– bacteria (*Salmonella paratyphi*, *E. coli* and *K. pneumonia*). Also, Rahman et al. (2009) found that pyocyanin extracted from *P. aeruginosa* DSO-19 has antibacterial activity against *S. aureus*, *Staphylococcus epidermis*, *Bacillus subtilis* and *Micrococcus luteus*. Moreover, Sudhakar et al. (2013) found that pigment isolated from *P. aeruginosa* SU1 has the maximum

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Table 2. List of phytochemicals identified from pyocyanin powder using GC-MS analysis.

| No. | Phytochemicals                              | Retention time (min) | Molecular formula | Molecular weight | Peak area (%) |
|-----|--------------------------------------------|----------------------|-------------------|------------------|---------------|
| 1   | Valeric acid                               | 3.8                  | C₆H₁₂O₂            | 116              | 10.94         |
| 2   | p-Allylphenol                              | 4.5                  | C₉H₁₀O             | 134              | 5.02          |
| 3   | 3,3’,7-T etramethoxyflavone               | 10.9                 | C₁₃H₁₆O₆           | 286              | 4.22          |
| 4   | Kaempferol                                 | 15.85                | C₁₅H₁₀O₆           | 286              | 5.2           |
| 5   | Flavonol-diacetate                         | 21.3                 | C₁₉H₁₆O₅           | 324              | 56.32         |
| 6   | Phenol, 2,4bis(1,1dimethylethyl)           | 24.85                | C₁₉H₂₀O            | 206              | 46.74         |
| 7   | 3,7,11,15 Tetramethyl 2 hexadecenol         | 31.82                | C₁₉H₂₇O₃           | 296              | 19.71         |
| 8   | 1Propene1,2,3tricarboxylic acid, tributylester | 38.03                | C₁₉H₂₆O₈           | 342              | 36.2          |
| 9   | Phytol                                     | 29.8                 | C₁₉H₂₇O₃           | 296              | 56.32         |
| 10  | 5Hydroxy1,4 dimethoxy anthraquinone        | 46.45                | C₁₅H₂₁O₃           | 284              | 42.45         |
| 11  | Eicosamethylcy clocadeciloxane             | 50.91                | C₂₀H₄₀O₁₀Si₁₀      | 740              | 50.2          |

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Figure 1. GC-MS chromatogram of pyocyanin powder analysis.
activity against *E. coli*, *S. aureus*, *Proteus* sp. and *Klebsiella* spp. Pyocyanin exhibited the highest activity against *C. albicans and B. cereus* with average inhibition zones diameters of 26 and 14 mm, respectively, and also found that pyocyanin revealed a weak anti-inflammatory activity against *E. coli* and *S. enterica* with inhibition zones diameters of 26 and 14 mm, respectively, and *P. aeruginosa* and *K. pneumonia* with inhibition zones diameters of 23 mm and 25 mm, respectively (Dahah et al. 2016).

On the side of pyocyanin’s antifungal activity, it was found that pyocyanin isolated from several *P. aeruginosa* species has antifungal activity against *Aspergillus* and *Candida* spp., i.e. *Aspergillus fumigatus*, *A. niger* and *C. albicans* (Kerr 1994; Kerr et al. 1999). There was clinical evidence that pyocyanin suppressed the growth of different *C. albicans* in patients with lung infection (Pal and Revathi 1998; Kaleri et al. 2007), and other diseases (Marrez and Haitham 2020). Sudhakar et al. (2013) reported that pyocyanin separated from *P. aeruginosa* WS1 encountered phytopathogens with MIC value of 64 mg mL⁻¹ against *A. flavus* and *Aspergillus fumigatus*, and 128 μg mL⁻¹ against *Candida* species.

### Growth performance

The effects of supplementation with pyocyanin on broiler chicks’ growth efficiency are shown in Table 4. No statistical differences were found in BWG due to treatments during the starter and finisher periods, while the overall BWG was improved (*p < 0.05*) in P75 and P150 compared to the control one. ADFI and FCR were not affected by the dietary inclusion of pyocyanin. The best values of BWG and FCR were observed in P75 group. The increase in BWG may be due to the biological activities of certain pyocyanin additive compounds that enhance digestion, absorption and intestinal function. Additionally, the antioxidant, anti-inflammatory, immune-stimulating, antifungal and antibacterial properties of pyocyanin and its derivatives (Islam et al. 2018; Wang et al. 2018; Marrez and Haitham 2020) might explain the improvement in broilers’ growth. These findings can be used as a basis for food industries, such as food preservation and pharmaceutical applications (Marrez and Haitham 2020). Several studies have reported the beneficial effects of phyogenic additives on growth efficiency, retention of nutrients, gut health, intestinal microflora, decreased disease susceptibility, improved immune function and improved poultry carcass yield and quality (Abd El-Moneim et al. 2019; Alagawany et al. 2019; Khafaga et al. 2019; Batiha et al. 2020; Abo Ghanima, Elsadek, et al. 2020; Abo Ghanima, Bin-Jumah, et al. 2020; Abdel-Moneim, Shehata, Mohamed, et al. 2021). Marzoni et al. (2014) stated that no effects on broiler chicks’ growth efficiency were achieved by supplementing a mixture of natural antioxidants. However,

### Table 3. Antimicrobial activity of pyocyanin presented by inhibition zones diameter (IDZ) (mm).

| Items                  | Bacteria                  | Pyocyanin concentrations, μg/mL | MIC | MBC | SEM  | p Value |
|------------------------|---------------------------|---------------------------------|-----|-----|------|---------|
|                        |                          | 10                              | 20  | 30  | 40   | 50      |         |
| *B. cereus*            | 17.02<sup>a</sup>        | 21.23<sup>a</sup>              | 24.05<sup>a</sup> | 27.89<sup>a</sup> | 30.05<sup>b</sup> | 7.23<sup>c</sup> | 12.55<sup>d</sup> | 0.45 .023 |
| *S. aureus*            | 19.04<sup>a</sup>        | 23.05<sup>a</sup>              | 26.03<sup>a</sup> | 28.35<sup>a</sup> | 32.26<sup>a</sup> | 6.02<sup>d</sup> | 10.21<sup>d</sup> | 0.58 .014 |
| *L. monocytogenes*     | 16.23<sup>b</sup>        | 20.51<sup>b</sup>              | 24.22<sup>b</sup> | 26.57<sup>b</sup> | 29.59<sup>b</sup> | 7.08<sup>c</sup> | 12.33<sup>d</sup> | 0.75 .008 |
| *E. coli*              | 14.21<sup>c</sup>        | 17.22<sup>c</sup>              | 19.28<sup>d</sup> | 21.55<sup>c</sup> | 23.45<sup>d</sup> | 8.23<sup>b</sup> | 15.24<sup>b</sup> | 0.35 .004 |
| *S. enteric*           | 13.32<sup>c</sup>        | 16.02<sup>a</sup>              | 18.42<sup>a</sup> | 20.23<sup>a</sup> | 22.63<sup>a</sup> | 9.25<sup>b</sup> | 18.94<sup>a</sup> | 0.33 .025 |
| *K. pneumonia*         | 15.56<sup>c</sup>        | 18.32<sup>c</sup>              | 21.33<sup>c</sup> | 23.28<sup>c</sup> | 25.37<sup>c</sup> | 8.33<sup>c</sup> | 15.34<sup>c</sup> | 0.45 .006 |
| **Fungi**              |                          | 10                              | 20  | 30  | 40   | 50      |         |
| *A. flavus*            | ND                        | 12.02<sup>bc</sup>             | 15.32<sup>b</sup> | 17.25<sup>b</sup> | 20.02<sup>c</sup> | 16.33<sup>b</sup> | 32.31<sup>b</sup> | 0.30 .022 |
| *A. niger*             | ND                        | 10.22<sup>d</sup>              | 12.01<sup>d</sup> | 14.32<sup>d</sup> | 17.07<sup>d</sup> | 18.32<sup>a</sup> | 36.43<sup>a</sup> | 0.41 .014 |
| *C. albicans*          | 11.02<sup>ab</sup>       | 14.00<sup>bc</sup>             | 17.34<sup>bc</sup> | 20.58<sup>ab</sup> | 22.07<sup>b</sup> | 9.08<sup>c</sup> | 18.23<sup>d</sup> | 0.21 .001 |
| *C. geliberta*         | 12.03<sup>a</sup>        | 15.02<sup>a</sup>              | 18.21<sup>a</sup> | 21.56<sup>a</sup> | 24.08<sup>a</sup> | 9.08<sup>c</sup> | 18.22<sup>a</sup> | 0.42 .005 |
| *P. solitum*           | ND                        | 11.86<sup>a</sup>              | 13.08<sup>a</sup> | 16.80<sup>a</sup> | 19.56<sup>cd</sup> | 18.36<sup>a</sup> | 36.79<sup>a</sup> | 0.26 .001 |
| *P. crustosum*         | ND                        | 13.35<sup>a</sup>              | 15.35<sup>a</sup> | 18.03<sup>a</sup> | 21.08<sup>bc</sup> | 16.36<sup>b</sup> | 32.12<sup>bc</sup> | 0.48 .001 |

MIC: minimum inhibition concentration; MBC: minimum bactericidal concentration; SEM: standard error mean.

Different letters within each row are significantly different.

### Table 4. Effect of dietary supplementation of pyocyanin on growth performance parameters of broiler chicks.

| Items                  | Pyocyanin powder concentration (mg/kg) | C | P75 | P150 | SEM | p Value |
|------------------------|---------------------------------------|---|-----|------|-----|---------|
| BWG, g/d/bird          | 1–3 weeks                             | 49.49 | 51.47 | 51.97 | 0.81 | .473    |
|                        | 3–5 weeks                             | 71.56 | 77.95 | 75.28 | 1.40 | .176    |
|                        | 1–5 weeks                             | 60.52 | 64.71 | 63.62 | 0.84 | .038    |
| DFC, g/d/bird          | 1–3 weeks                             | 63.72 | 63.99 | 62.00 | 0.67 | .481    |
|                        | 3–5 weeks                             | 112.76 | 118.87 | 122.65 | 3.01 | .459    |
|                        | 1–5 weeks                             | 88.24 | 91.43 | 92.32 | 1.33 | .482    |
| FCR, g feed/gain       | 1–3 weeks                             | 1.29 | 1.24 | 1.20 | 0.02 | .315    |
|                        | 3–5 weeks                             | 1.58 | 1.53 | 1.63 | 0.04 | .655    |
|                        | 1–5 weeks                             | 1.46 | 1.41 | 1.45 | 0.03 | .411    |

C: control; P75: 75 mg pyocyanin/kg diet; P150: 150 mg pyocyanin/kg diet; BWG: body weight gain; DFC: daily feed consumption; FCR: feed conversion ratio; SEM: standard error mean.

Different letters within each row are significantly different.
Abdel-Moneim, Shehata, Mohamed, et al. (2021) revealed that dietary inclusion of *Spirulina platensis*, a source of pyocyanin, increased BWG but did not affect FI or FCR. Contrarily, Elbaz et al. (2021) stated that phytogenic additives such as garlic powder can improve FI and FCR of broiler chickens.

### Carcase characteristics

Table 5 presents the effects of dietary supplementation with PP on carcase traits at the end of the experimental period. All studied carcase traits were not statistically different compared to the control birds. The examined lymphoid organs were not statistically different as well. Results showed that dietary treatment affected the intestinal length, where broilers in P75 group recorded the highest length followed by those in P150 group compared to the control birds. In agreement with our results, Marzoni et al. (2014) proved that supplementing a mixture of natural antioxidants did not affect the carcase yield of broilers. Çabuk et al. (2006) also reported insignificant differences in broilers’ growth and internal organ weight fed a mixture of herbs. The numerical reduction in abdominal fat percentage may be attributed to pyocyanin derivatives’ antioxidant properties, which decrease the activity of the cytosolic malic enzyme leading to depressed abdominal fat deposition (Ashour, Bin-Jumah, et al. 2020; Abdel-Moneim, Shehata, Khidr, et al. 2021).

### Blood biochemical parameters

The effect of pyocyanin supplementation on some serum biochemical parameters of broiler chicks is presented in Table 6. The changes in the levels of serum content of urea, creatinine, total protein and albumin and the activity of ALT and AST in broiler chicks in P75 and P150 groups were slight and insignificant when compared to the control group. Pyocyanin supplementation enhanced the antioxidative status of broiler chickens (Table 7). The level of lipid peroxidation (MDA) was reduced in P75 and P150 groups compared to the control. The activity of GST and level of GSH were elevated in treated groups compared to the untreated one. GPx activity was not altered among experimental groups. Blood biochemical parameters are generally correlated to the health status of birds and are good indices of the nutritional, physiological and pathological status of the birds. The lack of differences in these biomarkers in this study indicates the health benefits of pyocyanin. Moreover, the antioxidant role of pyocyanin was noticed in the results of this study. These findings are consistent with several earlier studies which documented the antioxidant properties of pyocyanin and its phytochemical components such as phytol, phenazine, valeric acid and kaempferol (Islam et al. 2018; Wang et al. 2018; Marrez and Haitham 2020).

### Meat quality criteria

#### PH

The pH directly affects meat quality characteristics, such as colour, tenderness, water holding capacity and shelf life. The pH of broiler meat is a feature of glycogen in the muscle before slaughter and glycogen’s conversion rate after slaughter into lactic acid (Nasir et al. 2017). The chicken meat colour with a pH below 5.8 was pale, while the colour of the chicken meat with a higher pH was too dark and a great danger to human health. So, for poultry, the ideal pH is between 5.8 and 6.3 (Pearson and Gillette 1996). The findings

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**Table 5.** Effect of dietary supplementation of pyocyanin carcase characteristics of broiler chicks.

| Traits, % of LBW | C      | P75    | P150   | SEM | p Value |
|------------------|--------|--------|--------|-----|---------|
| Carcase          | 75.40  | 75.25  | 75.99  | 0.67| .918    |
| Dressing         | 79.14  | 79.17  | 79.85  | 0.57| .882    |
| Liver            | 1.95   | 2.17   | 2.02   | 0.12| .797    |
| Gizzard          | 1.25   | 1.33   | 1.39   | 0.06| .732    |
| Heart            | 0.54   | 0.42   | 0.45   | 0.03| .223    |
| Abdominal fat    | 1.07   | 0.86   | 0.99   | 0.07| .535    |
| Intestine        | 4.52   | 5.86   | 4.94   | 0.41| .445    |
| Intestine length, cm | 88.33<sup>a</sup> | 96.67<sup>b</sup> | 91.67<sup>b</sup> | 4.09 | .040     |
| Lymphoid organs  |        |        |        |     |         |
| Spleen           | 0.08   | 0.09   | 0.11   | 0.01| .240    |
| Bursa            | 0.09   | 0.09   | 0.12   | 0.01| .311    |

C: control; P75: 75 mg pyocyanin/kg diet; P150: 150 mg pyocyanin/kg diet; SEM: standard error mean. Different letters within each row are significantly different.

**Table 6.** Effect of dietary supplementation of pyocyanin on serum biochemical parameters of broiler chicks.

| Items                  | C      | P75    | P150   | SEM | p Value |
|------------------------|--------|--------|--------|-----|---------|
| Total protein, mg/dL   | 6.40   | 6.54   | 6.41   | 0.03| .501    |
| Albumin (A), mg/dL     | 3.23   | 3.22   | 3.25   | 0.01| .185    |
| Globulin (G), mg/dL    | 3.17   | 3.32   | 3.16   | 0.04| .442    |
| A/G ratio              | 1.03   | 1.00   | 1.03   | 0.01| .143    |
| ALT, IU/L              | 11.50  | 10.47  | 10.67  | 0.22| .519    |
| AST, IU/L              | 122.67 | 121.33 | 114.67 | 1.31| .887    |
| Urea, mg/dL            | 43.67  | 35.67  | 40.67  | 1.18| .980    |
| Creatinin, mg/dL       | 0.53   | 0.46   | 0.61   | 0.02| .842    |

C: control; P75: 75 mg pyocyanin/kg diet; P150: 150 mg pyocyanin/kg diet; ALT: alanine aminotransferase; AST: aspartate aminotransferase; SEM: standard error mean.
obtained in Table 8 showed that the mean pH values for the chicken breast analysed were 5.37, 5.63 and 6.00 and that there were no significant disparities between the pH values of the different levels.

**TBARS**

A significant factor for restricting the shelf-life of meat products is lipid oxidation. The TBARS values reflect the lipid oxidation content and are primarily used as an important indicator for evaluating meat products’ quality due to its relatively simple measurement (Zhao et al. 2019). As shown in Table 8, with rising pyocyanin concentration, TBARS values decreased in all samples. Pyocyanin’s strong inhibition effect on the change in TBARS value could be due to its antioxidant activity.

**TVBN**

TVBN, which consists mainly of dimethylamine (DMA), trimethylamine (TMA), ammonia and other compounds, is primarily due to its activity protein-degrading microbial enzymes and non-protein nitrogen compounds (Balamatsia et al. 2007; Yi et al. 2011). TVBN values of all different levels were shown in Table 8. TVBN values of all samples decreased with increasing concentration of Pyrocyanin levels, which had not exceeded the limitation (20mg/100g of meat) of the Egyptian standard. The decrease in TVBN value in level 3 might be attributed to the reduced protein breakdown due to microbial strains and proteolytic enzymes’ activity.

**Meat colour**

Meat colour is an important assessment criterion. Colour or visual appearance is undoubtedly one of the most important sensory characteristics that affect consumers’ acceptance of meat and meat products (Adeyemi and Sazili 2014). The effect of the addition of pyocyanin on the colour of broilers is shown in Table 8. The findings indicate an increase in L*/a*/b* values with an increase in pyocyanin levels. With increased concentration, the a*/value of the samples increased. The increase in red colour intensity may be due to increased concentration due to the absence of fat oxidation and colour oxidation. The key factors leading to changes in the value of b* are increased lipid oxidation and the formation of MetMb (Xiong 2000). The primary cause of deterioration in meat quality during storage, including undesirable changes in colour parameters (L*, a* and b*) and
sensory properties, are compounds developed during the oxidative degradation of lipids (Nam et al. 2001; Gok et al. 2008).

Sensory evaluation

The chicken samples under investigation were evaluated for sensory characters (tenderness and juiciness, taste and aroma) following the addition of three levels of pyocyanin. The mean of scores is presented in Table 9. The addition of pyocyanin did not have a noticeable adverse impact on the tenderness, juiciness, taste and aroma of the samples for all samples at the different levels used. All samples showed a high acceptability score for similar sensory attributes. All samples typically scored well for all sensory parameters measured. These findings demonstrate that these natural functional ingredients can be integrated into poultry products without adversely affecting the quality of products that produce nutritious meat products. Functional advantages can offer customers added value (Bech-Larsen and Scholderer 2007).

Impact of pyocyanin on microbial count

Feed samples

Figure 2 showed the effect of PP addition at two concentrations (75 and 150 mg) on the microbial count in diet during the feeding period, especially (TBC, total yeasts and moulds count (TYMC), coliform and E. coli count). Generally, the microbial count decreased \((p<.05)\) with pyocyanin supplementations in P75 and P150 groups. However, the microbial count increased during the feeding period. P150 has relatively decreased the TBC by 21%, total yeasts and moulds by 30%, total coliform by 35%, and E. coli by 40% compared to control. These results agree with the results of Makarand et al. (2007) who recorded an antimicrobial activity of the pyocyanin against B. subtilis, C. albicans and E. coli. However, others demonstrated that phenazine antibiotics’ activity is concentration dependence; therefore, when the concentration of phenazine increased, the biological activity would be increased (Stephen 1981). Sudhakar et al. (2013) also stated that pyocyanin isolated from P. aeruginosa SU1 has the highest antibacterial activity against E. coli. The antimicrobial activity of pyocyanin may be
attributable to the effect of pyocyanin’s antimicrobial active components, including phenazine. Compounds also demonstrated multiple phenazines for anti-cancer, antimalaria and antiparasitic activities (Brisbane et al. 1987; Laursen and Nielsen 2004).

Caecal samples
By increasing the concentration of dietary pyocyanin, the microbial load in broiler caecum has enhanced TBC, total yeasts and moulds, Enterococcus spp., Coliform, E. coli and Salmonella spp. were decreased, while lactobacillus spp. count was increased (Table 10). The addition of pyocyanin at 150 mg/kg prevented Salmonella spp. growth in the broiler caecum. Moreover, 75 and 150 mg pyocyanin/kg diet raised broiler caecum lactic acid bacteria count by 40% about control. The pyocyanin concentration of 150 mg/kg was more effective than the 75 mg/kg concentration. There were reductions in the overall microbial count, especially pathogenic bacteria, from the obtained results (Table 10). The antimicrobial activity of pyocyanin might attributed to the fact that this pigment has developed as secondary metabolites to protect microorganisms from the harmful effect of visible and near-ultraviolet ran light rays.

Moreover, the antimicrobial activity of the isolated pigment against various microorganisms, e.g. C. albicans and A. fumigatus has been reported (Kerr et al. 1999). The author attributed this effect to phenazine and its derivatives. Phenazine is also known as tuberculin B because of its antibiotic activity against Mycobacterium tuberculosis, which causes pneumonia in susceptible patient populations and frequently fatal infections (Allen et al. 2005).

Histological examination
Ileum
The positive impacts of dietary supplementation of pyocyanin on ileal histomorphology are shown in Table 11. Birds fed pyocyanin in their diets had higher (p = .024) values of villus height (VH) than the untreated group. However, crypt depth (CD) and VH: CD ratio were not significantly influenced. These observations are consistent with our findings on growth performance.

Liver
No histological alterations were noticed in control birds and those treated with pyocyanin, as normal central vein and portal area with normal hepatic parenchyma were observed (Figure 3). Despite this normal hepatic structure, slight mononuclear cell permeation and dilatation of hepatoporal blood vessel in C group and slight congestion of hepatoporal blood vessel in P150 group were observed. These observations indicate that dietary levels of pyocyanin did not affect the normal structure of the hepatic parenchyma and did not exert hepatotoxicity impact.

Spleen
Normal histological structure of spleen lymphoid follicles with normal white and red bulbs was noticed in

**Table 10. Effect of dietary supplementation of pyocyanin on caecal microbiota (Log CFU/mL) of broiler chicks.**

| Items                  | C         | P75       | P150      | SEM       | p Value |
|------------------------|-----------|-----------|-----------|-----------|---------|
| Total count bacteria   | 9.13a     | 7.86b     | 5.73c     | 0.85      | .001    |
| Total yeasts and moulds| 3.28a     | 2.33b     | 1.23c     | 0.75      | .002    |
| Coliform count         | 5.71a     | 4.20b     | 3.53c     | 0.64      | .021    |
| Escherichia coli       | 4.38a     | 3.12b     | 1.89c     | 0.68      | .001    |
| Salmonella spp.        | 2.11a     | 0.45c     | ND        | 0.10      | .012    |
| Enterococcus spp.      | 5.16a     | 4.21b     | 2.98c     | 0.35      | .003    |
| Lactic acid bacteria   | 2.46c     | 3.34d     | 3.87e     | 0.78      | .001    |

C: control; P75: 75 mg pyocyanin/kg diet; P150: 150 mg pyocyanin/kg diet; SEM: standard error mean.
Different letters within each row are significantly different.

**Table 11. Effect of dietary supplementation of pyocyanin on ileum histomorphology of broiler chicks.**

| Items           | C         | P75       | P150      | SEM       | p Value |
|-----------------|-----------|-----------|-----------|-----------|---------|
| Villus height, μm| 501.5d    | 587.5c    | 613.7a    | 20.11     | .024    |
| Crypt depth, μm | 111.8     | 134.5     | 148.5     | 7.881     | .157    |
| Villus height:crypt depth | 4.51 | 4.39 | 4.20 | 0.120 | .652 |

C: control; P75: 75 mg pyocyanin/kg diet; P150: 150 mg pyocyanin/kg diet; SEM: standard error mean.
Different letters within each row are significantly different.
all experimental groups with slight vacuolation in spleen tissue of control group (Figure 3); suggesting the absence of the cytotoxic effect of pyocyanin at the studied levels.

**Figure 3.** (a) Effect of dietary supplementation of pyocyanin on ileum histomorphology of broiler chickens (H&E × 100). C: control group, P75: 75 mg pyocyanin/kg diet and P150: 150 mg pyocyanin/kg diet. (b) Liver of broiler chickens as affected by dietary supplementation of pyocyanin (H&E × 400) showing normal hepatic parenchyma, note the normal hepatocytes and blood sinusoids with slight dilatation of hepatoporal blood vessel in C (black arrow head), congestion of hepatoporal blood vessel in P150 (blue arrow head), blood sinusoids showing mononuclear cells permeation in C (black arrows). C: control group, P75: 75 mg pyocyanin/kg diet and P150: 150 mg pyocyanin/kg diet. (c) Spleen of broiler chickens as affected by dietary supplementation of pyocyanin (H&E × 200) showing normal lymphoid follicles with normal white and red bulbs and with slight vacuolation in C (arrows). C: control group, P75: 75 mg pyocyanin/kg diet and P150: 150 mg pyocyanin/kg diet.

**Conclusions**
Dietary supplementation of pyocyanin enhanced the growth performance and carcase traits of broiler chickens. Pyocyanin exerted antibacterial, antifungal, and
antioxidant properties when supplemented to broilers’ diets, particularly at 75 mg/kg. The dietary inclusion of pyocyanin diminished caecal pathogenic bacteria. Besides, pyocyanin is a healthy and economic way to enhance chicken meat products’ technical and sensory properties. However, further investigations are needed to examine more dietary levels of pyocyanin with more attention to the potentially toxic effects of pyocyanin when added to broilers’ diets at higher levels.

Ethical approval

The present investigation was carried out at Poultry Research Farm, Poultry Department, Faculty of Agriculture, Zagazig University, Zagazig, Egypt. According to the Local Experimental Animal Care Committee, all experimental procedures were carried out and approved by the ethics of the Poultry Department’s institutional committee, Faculty of Agriculture, Zagazig University, Zagazig, Egypt.

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Author contributions

Conceptualisation and methodology, E.A.A., A.E.A., M.T.E-S., A.O.O., E.T.A.S.-A. and M.E.A.E.-H. Data curation, M.E.A.E.-H., E.A.A. and A.O.O. Writing – original draft preparation, E.A.A., M.T.E-S., A.O.O., A.E.T. and M.E.A.E.-H. Writing – review and editing, M.E.A.E.-H., N.M.A., M.E.S., R.M.F. and B.A.A. All authors have read and agreed to the published version of the manuscript.

Disclosure statement

The authors declare no conflict of interest.

Institutional review board statement

All experimental procedures for this study were conducted following the Local Experimental Animal Care Committee and subsequently approved by the Institutional Ethics Committee, Department of Poultry, Faculty of Agriculture, Zagazig University, Zagazig, Egypt (ZU-IACUC/2/F/94/2018).

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ORCID

Mohamed E. Abd El-Hack http://orcid.org/0000-0002-2831-8534

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biodata of a chemical constituent or its possible role.

2. **Phenazine derivatives**: Phenazines are a class of nitrogen-containing natural products that have attracted significant interest due to their antimicrobial, antioxidant, and other biological activities. Phenazines are produced by a wide range of bacterial species, including the opportunistic pathogen *Pseudomonas aeruginosa*, which is frequently isolated from various clinical infections. The presence of phenazine antibiotic production is often associated with virulence in *P. aeruginosa*, and these compounds may impair neutrophil-mediated host defenses in vivo.

3. **Pyocyanin production**: Pyocyanin is one of the most studied phenazine derivatives due to its unique properties and potential applications in the food industry, agriculture, and medicine. This compound, produced by *Pseudomonas aeruginosa*, exhibits a broad spectrum of antimicrobial activity and has been implicated in the production of virulence factors.

4. **Potential probiotic lactic acid bacteria**: Identification and characterization of potential probiotic lactic acid bacteria from fermented olives by in vitro tests. Such bacteria are beneficial for human health, as they can contribute to the maintenance of gut microbial balance and improve the quality of probiotics.

5. **Syntesis of genes for second anthranilate synthases**: Two anthranilate synthases and their evolutionary implications. This research is important for understanding the evolution of metabolic pathways and can contribute to the development of novel antibiotics.

6. **Chemical constituents of green coffee powder**: The study of chemical constituents of green coffee powder and its potential applications in the food industry, such as improving shelf life and safety of products.

7. **Antioxidant and antibacterial activities**: The study of natural extracts and their activities against specific bacterial strains, such as *Escherichia coli* and *Salmonella* directly from the primary isolation plate.

8. **Antibacterial and antifungal activities of garlic**: The potential of garlic (Allium sativum) as a natural antimicrobial agent and its applications in various industries, including food and agriculture.

9. **Impacts of heat stress in poultry**: The study of the effects of heat stress on various parameters in poultry, including growth performance, feed efficiency, and immune function.

10. **Antioxidant and antibacterial activities of rosemary and cinnamon essential oils**: The investigation of antioxidant and antimicrobial properties of rosemary and cinnamon essential oils, which have been studied for their potential applications in the food industry and as natural antimicrobial agents.

11. **Antimicrobial activity of pyocyanin produced by *Pseudomonas aeruginosa***: The study of the antimicrobial activity of pyocyanin, a phenazine derivative produced by *P. aeruginosa*, against various bacterial strains.

12. **Impact of multi-strain probiotic**: The study of the impact of multi-strain probiotics on performance, ileal histomorphometry, microbial enumeration, and humoral immunity of broiler chickens.

13. **Antimicrobial, antioxidant, and hemolytic effects of Pyocyanin**: The investigation of the antimicrobial, antioxidant, and hemolytic effects of pyocyanin produced by *Pseudomonas aeruginosa* isolated from saline soil of Mina river, Algeria.

14. **Antimicrobial activity of pyocyanin**: The study of the antimicrobial activity of pyocyanin produced by *P. aeruginosa* isolated from saline soil.

15. **Impacts of heat stress on broilers**: The study of the effects of heat stress on various parameters in broilers, including growth performance, feed efficiency, and immune function.

16. **Impacts of strain variation on response to heat stress**: The investigation of the strain variation in response to heat stress and its effects on various parameters in poultry.

17. **Possible role of volatile amines**: The study of the possible role of volatile amines as quality-indicating biomarkers in meat products.

18. **Possible role of volatile amines as quality-indicating biomarkers in meat products**: The investigation of the possible role of volatile amines in indicating meat quality.

19. **Synthesis, isolation of phenazine derivatives**: The study of the synthesis and isolation of phenazine derivatives from various sources.

20. **Development and characterization of bioactive pyocyan pigment**: The study of the development and characterization of bioactive pyocyan pigment from marine *Pseudomonas aeruginosa* OSh1.

21. **Chemical constituents and pharmacological activities of garlic**: The investigation of the chemical constituents and pharmacological activities of garlic (Allium sativum) as a natural antimicrobial agent.

22. **Antioxidant and antibacterial activities of garlic**: The study of garlic (Allium sativum) as a natural antimicrobial agent with antioxidant and antibacterial properties.

23. **Impact of multi-strain probiotic**: The study of the impact of multi-strain probiotics on performance, ileal histomorphometry, microbial enumeration, and humoral immunity of broiler chickens.

24. **Chemical constituents of green coffee powder**: The study of the chemical constituents of green coffee powder and its potential applications in the food industry, agriculture, and medicine.

25. **Antimicrobial activity of pyocyanin**: The study of the antimicrobial activity of pyocyanin produced by *Pseudomonas aeruginosa* isolated from saline soil.

26. **Antimicrobial activity of pyocyanin**: The investigation of the antimicrobial activity of pyocyanin produced by *Pseudomonas aeruginosa* isolated from saline soil.

27. **Antimicrobial activity of pyocyanin**: The study of the antimicrobial activity of pyocyanin produced by *Pseudomonas aeruginosa* isolated from saline soil.

28. **Antimicrobial activity of pyocyanin**: The investigation of the antimicrobial activity of pyocyanin produced by *Pseudomonas aeruginosa* isolated from saline soil.

29. **Antimicrobial activity of pyocyanin**: The study of the antimicrobial activity of pyocyanin produced by *Pseudomonas aeruginosa* isolated from saline soil.

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