Does the dietary graded levels of herbal mixture powder impact growth, carcass traits, blood indices and meat quality of the broilers?

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ABSTRACT

The present study was conducted to investigate the impacts of dietary supplementation of graded levels of herbal mixture powder (HMP) on growth performance, carcass traits, blood indices and meat quality of broilers. The herbal mixture consisted of (300 g [Capsicum annuum] Hot red pepper + 300 g Thymus vulgaris + 300 g Salvia rosmarinus + 150 g Pimpinella anisum + 150 g Mentha spicata + 300 g Nigella sativa + 300 g garlic [Allium sativum]). A total of 360 chicks of Hubbard broiler one-day-old were randomly distributed into six treatment groups. The chicks fed a basal diet plus 5 g HMP/kg diet had the highest (p < .05) values of live body weight (LBW) and body weight gain (BWG) at five weeks of age. Serum total protein, globulin, liver enzymes and kidney parameter values were reduced in groups treated with different levels of HMP compared with the control. At the same time, values of albumin, A/G ratio, IgM and lysozymes were increased in chicks treated with HMP. The quality of stored meat and antioxidative parameters were improved in groups fed HMP. Thus, it is concluded that HMP could be used as a natural additive to the broiler diet to enhance the growth rate and improve blood parameters and meat quality. Also, the oxidative rancidity was less in the meat of treated chicks during the storage period as compared to control.

HIGHLIGHTS

• Phytogenic additives are used to enhance growth and productive performance.
• Chicks fed 5 g herbal mixture/kg diet had the best growth performance.
• The quality of stored meat and antioxidative parameters were improved in groups fed the herbal mixture.

Introduction

There are a lot of dietary factors that have a major function in animal existence by affecting health, antioxidants and productive performance. Phytogenic additives are used in poultry production as natural feed additives to enhance the growth rate and productive performance. Beneficial impacts of herbs in poultry feeding such as improvement feed intake, appetite, energising secretion of digestive enzyme, stimulation immune response and antioxidant, antimicrobial, antihelminthic, antiviral properties and anti-heat stress modulators (Shewita and Taha 2018; Alagawany et al. 2019, 2020; Gado et al. 2019; Khafaga et al. 2019; Hafez and Attia 2020; Abd El-Hack, Alagawany et al. 2020; Abd El-Hack, Abdelnour et al. 2020; Ashour, Bin-Jumah et al. 2020; Ashour, El-Kholy et al. 2020; Battha et al. 2020). Several studies reported useful impacts of phytogenic additives on growth performance, nutrient retention, gut health, intestinal microflora, reduced the susceptibility to diseases, enhanced immunity function and improved carcass traits in broiler chickens (Ashour et al. 2014; Abd...
El-Hack and Alagawany 2015; Alagawany, Farag et al. 2015; Alagawany, Abd El-Hack et al. 2015; Taha et al. 2019; Abo Ghanima et al. 2020; Ashour, Bin-Jumah et al. 2020).

The manufacturing of poultry breeding and enhancement feed efficiency have quickened the use of feed additives in the broiler diet. The feed is a major input for the broiler rearing and contributes about 70–80% of production cost, hence plays a vital role in the broilers economy (Singh et al. 2018). The essential purpose of scientists is to enhance production by the preservation of broiler health. To resistance these problems, herbs and medicinal plant extracts are investigated to be growth promoters in the poultry diet (Allouii et al. 2013). Various researchers tried many medicinal plants; some of them proved the ability of bioactive compounds of plants (phytobiotics) to prevent disease and promote the growth of broilers at the same time. The advantage of phytogenic feed additives over synthetic growth promoters is mainly due to the natural synergistic effect of all agents within the plants. Some traditional medicinal herbs/plants like hot red pepper, Thyme, Al-Ruzmari, anise, mint and *Nigella sativa* seeds are alone or, in combination, proved for their novel property of phytophobiotics (Attia and Al-Harthi 2015; Attia, Al-Harthi et al. 2017; Attia, Bakhashwain et al. 2017; Attia et al. 2018; Arif et al. 2019; Attia et al. 2019; AL-Sagan et al. 2020). There is no previous reports studied the effect of a herbal mixture containing *Capsicum annuum*, *Thymus vulgaris*, *Salvia rosmarinus*, *Pimpinella anisum*, *Mentha spicata*, *Nigella sativa* and garlic with graded levels on broiler performance. Thus, the present experiment was planned to investigate the effective range of condensations of the current preparation which, consist of mixing natural herbs as phytogenic feed additives of broiler chickens and a natural antioxidant in growing broiler chick diets on the growth, carcass, serum biochemicals and quality of meat in Hubbard chickens.

**Materials and methods**

**Plant materials**

*Thymus vulgaris* (T.V), *Pimpinella anisum* (P.A), *Capsicum annuum* (C.A), *Mentha spicata* (M.S), *Salvia rosmarinus* (S.R), *Allium sativum* (A.S), and *Nigella sativa* (N.S) were bought from the botanicals, spices and herbs market (Zagazig City, Sharqia Governorate, Egypt).

**Chemical characterisation**

Folin and Ciocalteu’s phenol reagent, (DPPH) 2, 2-Diphenyl-1-picrylhydrazyl, gallic acid, quercetin, methanol, ethanol and aluminium chloride were obtained from Merck (Merck KGaA, Darmstadt, Germany).

**Sample preparation**

Ten grams of *Thymus vulgaris* (T.V), *Pimpinella anisum* (P.A), *Capsicum annuum* (C.A), *Mentha spicata* (M.S), *Salvia rosmarinus* (S.R), *Allium sativum* (A.S), *Nigella sativa* (N.S) and its flour were mixed individually with 70% methanol (200 mL), then was stirred for 3 h and then purified through filter paper Whatman No.2. Methanol was removed under vacuum from an extract in a BüCHL- water bath -B-480 evaporator at 45°C followed by lyophilisation using a freeze-Dryer (Thermo-electron Corporation–Heto power dry LL 300 Freeze dryer). The resulting extract was kept at −20°C until used for the following analyses (Abd El-Hack et al. 2018; Ashour, Bin-Jumah et al. 2020).

**Total phenolic compounds (TPCs) estimation**

The TPCs of the methanolic extract (1000 μg/mL) gained from each sample were evaluated by the Folin–Ciocalteu checked as observed by. Gallic acid was a standard phenolic compound at different concentrations (10–1000 μg/mL) for the preparation of the standard curve \( y = 0.001x + 0.0563 \) \( R^2 = 0.9792 \), where \( y \) and \( x \) are the gallic acid absorbance and concentration in μg/ml, respectively. One millilitre from each sample or standard gallic acid plus 3 ml from sodium carbonate 7.5% was mixed and kept in the darkness for 0.5 h at 25°C. Finally, the blend absorbance was recorded at 760 nm by using a spectrophotometer (JENWAY, 6405 UV/Vis, U.K.) (Abdel-Shafi et al. 2019).

**Total flavonoids (TFs) estimation**

Total TFs of the extract (1000 μg/mL) as recommended by (Ordonez et al. 2006), as explained in (Abdel-Shafi et al. 2019). Quercetin was used as a standard phenolic compound at different concentrations (10–1000 μg/mL) for the preparation of the standard curve \( y = 0.0012x + 0.008 \) \( R^2 = 0.944 \), where \( y \) is absorbance and \( x \) is concentration of quercetin in μg/mL, respectively. One millilitre from quercetin solution or extract plus One millilitre aliquot of 20 g/L AlCl3 ethanol was mixed. The spectrophotometer has recorded the absorbance of colour at 420 nm.
Antioxidant activity estimation

Antioxidant efficacy of the methanolic extractor of Thymus vulgaris (T.V), Pimpinella anisum (P.A), Capsicum annuum (C.A), Mentha spicata (M.S), Salvia rosmarinus (S.R), Allium sativum (A.S), Nigella sativa (N.S) and its blend flour was determined by their ability to scavenge DPPH-assay (Ramadan et al. 2008; Osman et al. 2014). One millilitre from each sample was blended individually with three millilitres methanolic- DPPH solution and recorded the absorbance at 520 nm (DPPH-assay) after incubation for 30 min using a spectrophotometer. The extract condensation that scavenges 50% of the ABTS and DPPH radicals (SC50) was estimated as described by Abdel-Hamid et al. (2017).

Experimental design and diets

This investigation was conducted at the Department of Poultry, Faculty of Agriculture, Zagazig University, Egypt. Animal auspices and protection were achieved in conformity to guidelines of the Research Ethics Committee. The institutional committee approved this study. A total number 360 unsexed Hubbard chickens one day old at average 44.89±0.47 g initial body weight were randomly divided into six treatments; 60 chicks in six replicates (6 × 6 × 10). All birds were reared adopting uniform management conditions; chicks were vaccinated against most common viral diseases in Egypt, such as Newcastle disease, Infectious bronchitis, Gambaro and Avian influenza virus according to the recommended veterinarian program. Feed was produced to all experimental groups in mash form. The dietary treatments consisted of the basal diet as a control group and A powdered herbal mixture (HMP) additives groups (2.0, 3.0, 4.0, 5.0 and 6.0 g HMP/kg diet) in mash form and recorded the absorbance at 520 nm (DPPH-assay) after incubation for 30 min using a spectrophotometer. The extract condensation that scavenges 50% of the ABTS and DPPH radicals (SC50) was estimated as described by Abdel-Hamid et al. (2017).

Table 1. Composition and chemical analysis of the basal diets as fed.

| 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 | 17.00 | 15.00 |
| Di calcium phosphate | 12.40 | 11.50 |
| Limestone | 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**: | | |
| 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 |
| Phosphorous (Available) % | 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 1,20,00,000 IU; Vit D3, 20,00,000 IU; 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.

At the end of the experiment, six birds were randomly selected from each group (3 males and 3 females); they fasted overnight, weighted and then slaughtered. After that, birds were scalded and de-feathered. The heart, gizzard, liver, thigh, and breast were weighted and expressed as g/kg of slaughter weight (SW). The characteristics studied of the carcass were a carcass, giblets (liver, heart and gizzard) and dressing (dressed percentage – carcass weight inclusion giblets weight/live weight × 100).

Serum biochemical analysis

At the end of the experiment, six birds per group were chosen randomly slaughtered; blood samples were collected into clean tubes without coagulate. Serum samples were separated from blood samples (Sitohy et al. 2013). By using commercial diagnostic kits provided by Bio Diagnostic Co. (Giza, Egypt),
determined urea, creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST), total protein and albumin. Globulin was calculated from the following equation (Abdel-Hamid et al. 2020):

\[
\text{Globulin} = \text{Total protein} - \text{Albumin}
\]

IgM estimated as described by (Amer et al. 2020). Lysozyme is estimated according to (Ellis 1990).

**Meat quality samples**

**The TBA test**

Lipid oxidation is estimated, according to Fernandez-Lopez et al. (2005), with a little modulation. Meat samples 10 g was dissolved with 100 mL distilled water each group for 2 min. The sample pH regulates to 1.5 by supplementing a few drops of 4 N HCl and then transported to a distillation tube. 50 mL of the distilled mixture was gathered. 5 mL of 0.02 M 2-thiobarbituric acid in 90% acetic acid and added (TBA reagent) to 5 mL of the distillate in a flask then well mixed. To develop the chromogen, the flask was covered and put for 30 min in a boiling water bath. After that, samples were refrigerated to the temperature room. At 538 nm, absorbance was estimated, prepared the blank against 5 mL TBA-reagent and 5 mL distilled water, using a Spectrophotometer. Numbers of TBA were calculated as malondialdehyde/kg sample according to the following equation:

\[
\text{TBA number (kg)} = \text{Absorbance at 538nm} \times 7.8
\]

Measured pH values of beef burger and minced meat samples Fernandez-Lopez et al. (2005) Measurement colour of poultry meat was performed to Rao et al. (2011).

**Statistical analysis**

By using SPSS (2008) of GLM procedures, statically analysed the present data according to a completely randomised design with the following model:

\[
Y_{ij} = \mu + T_i + e_{ij}
\]

where \(Y_{ij}\) observed value; \(\mu\), overall mean; \(T_i\), treatment effect (C: control; MIX1, MIX2, MIX3, MIX4 and MIX5: basal diet + 2, 3, 4, 5 and 6 g herbal mixture/kg diet, respectively); \(e_{ij}\) random error. The test of student Newman keuls was determination the differences between means. Significant is depending on \(p < .05\).

**Results**

**Chemical characterisation**

Total phenolic (TP; mg GAE g\(^{-1}\) extract) and total flavonoid (TF; mgQE g\(^{-1}\) extract) contents and DPPH activity (\(SC_{50}\); \(\mu\)g mL\(^{-1}\)) of the methanolic extract acquired from *Thymus vulgaris* (T.V), *Pimpinella anisium* (P.A), *Capsicum annuum* (C.A), *Mentha spicata* (M.S), *Salvia rosmarinus* (S.R), *Allium sativum* (A.S), *Nigella sativa* (N.S) and its blend.

| Samples | TP (mg GAE g\(^{-1}\) extract) | TF (mg QE g\(^{-1}\) extract) | DPPH activity (\(SC_{50}\); \(\mu\)g mL\(^{-1}\)) |
|---------|-------------------------------|-----------------------------|---------------------------------|
| T.V.    | 9.7                           | 2.4                         | 150                             |
| P.A.    | 12.15                         | 4.9                         | 100                             |
| C.A.    | 4.9                           | 0.9                         | 270                             |
| M.S.    | 10.23                         | 3.9                         | 118                             |
| S.R.    | 5.2                           | 1.8                         | 230                             |
| A.S.    | 6.6                           | 2.4                         | 200                             |
| N.S.    | 4.5                           | 1.3                         | 300                             |
| Blend   | 25                            | 16                          | 13                              |

**Table 2.**

**Table 2.** Total phenolic (TP; mg GAE g\(^{-1}\) extract) and total flavonoid (TF; mg QE g\(^{-1}\) extract) contents and 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) activity (\(SC_{50}\); \(\mu\)g mL\(^{-1}\)) of the methanolic extract acquired from *Thymus vulgaris* (T.V), *Pimpinella anisium* (P.A), *Capsicum annuum* (C.A), *Mentha spicata* (M.S), *Salvia rosmarinus* (S.R), *Allium sativum* (A.S), *Nigella sativa* (N.S) and its blend.

**Growth performance**

The growth performance, as impacted by herbs mixture powder (HMP), is clarified in Table 3. LBW and BWG of broilers fed a diet enriched with HMP was influenced (\(p < .05\)) during the studied period. Whereas chicks received 5.0 and 6.0 g HMP/kg had the highest BW and BWG values throughout 3 and 5 weeks of age for body weight or (0–3, 3–5 and 1–5 weeks of age) for BWG compared to control and another trail groups. Data on feed intake in Table 3 cleared insignificant impacts of added HMP on broiler FI among most treatment periods except the first term.
Table 3. Growth performance parameters of broiler chicks as affected by graded levels of dietary herbal mixture supplementation.

| Items   | LBW (g) | BWG (g) | DFC (g/day) | FCR (g feed/ g gain) |
|---------|---------|---------|-------------|----------------------|
|         | 0 wk    | 3 wk    | 5 wk        | 0–3 wk               | 3–5 wk               | 0–5 wk               | 0–3 wk   | 3–5 wk | 0–5 wk |
| C       | 45.33   | 948.67  | 1938.46     | 43.02b               | 70.70a               | 56.86a               | 58.99bc  | 148.93 | 103.71 |
| MIX1    | 46.15   | 927.00  | 1893.07     | 41.95bc              | 69.01b               | 55.47b               | 55.67a   | 140.03 | 99.36  |
| MIX2    | 42.20   | 873.50  | 1742.39     | 39.59c               | 62.06bc              | 50.83b               | 56.87bc  | 142.46 | 99.67  |
| MIX3    | 44.00   | 944.25  | 1882.02     | 42.87b               | 66.99bc              | 54.93bc              | 58.24bc  | 141.17 | 99.71  |
| MIX4    | 46.40   | 1014.0a | 2011.75     | 46.08a               | 71.27a               | 58.67a               | 61.27a   | 151.44 | 106.35 |
| MIX5    | 45.30   | 954.17  | 1963.57     | 43.28b               | 72.10a               | 57.69a               | 57.78bc  | 152.07 | 104.92 |
| SEM     | 0.47    | 11.84   | 25.29       | 0.56                  | 1.20                 | 0.79                 | 0.51     | 1.69   | 0.98   |
| P value | 0.550   | 0.116   | 0.35        | 0.006                | 0.020                | 0.027                | 0.007    | 0.236  | 0.115  |

C: control; MIX1, MIX2, MIX3, MIX4 and MIX5: basal diet + 2, 3, 4, 5 and 6 g herbal mixture/kg diet, respectively. LBW: live body weight; BWG: body weight gain; DFC: daily feed consumption; FCR: feed conversion ratio.

Table 4. Carcass characteristics of broiler chicks as affected by graded levels of dietary herbal mixture supplementation.

| Items   | Carcass traits (relative to pre-slaughter weight %) |
|---------|--------------------------------------------------|
|         | Carcass | Dressing | Breast | Thigh | Liver | Heart | Gizzard |
| C       | 72.32   | 76.28    | 44.43  | 27.80 | 2.24  | 0.50a  | 1.21ab  |
| MIX1    | 72.71   | 76.72    | 44.36  | 28.29 | 2.05  | 0.38ab | 1.58a   |
| MIX2    | 73.39   | 77.36    | 46.45  | 26.20 | 2.35  | 0.48a  | 1.15b   |
| MIX3    | 73.40   | 77.19    | 46.22  | 27.07 | 2.35  | 0.48a  | 0.95b   |
| MIX4    | 72.87   | 76.77    | 43.95  | 28.70 | 2.19  | 0.40b  | 1.31ab  |
| MIX5    | 73.18   | 76.99    | 45.37  | 27.92 | 2.22  | 0.45b  | 1.14b   |
| SEM     | 0.17    | 0.16     | 0.35   | 0.29  | 0.07  | 0.01   | 0.06    |
| P value | 0.441   | 0.526    | 0.160  | 0.132 | 0.039 | 0.013  | 0.045   |

C: control; MIX1, MIX2, MIX3, MIX4 and MIX5: basal diet + 2, 3, 4, 5 and 6 g herbal mixture/kg diet, respectively. SEM: standard error mean.

Blood parameters

The effects of dietary plus herbal mixture powder on serum metabolites of broiler chicks are stated in Table 5. Concentrations of serum ALT, AST, urea, creatinine, total protein and globulin reduced (p < 0.05) in all chick groups trail with HMP whereas, the highest concentrations of ALT, AST, urea, creatinine, total protein and globulin (10.81 IU/L, 123.67 IU/L, 44.89 mg/dL, 6.62 g/dL and 2.90 g/dL respectively) presents chicks fed the basal diet. Data for creatinine show that there was no significant effect (p > 0.05) of the treatments (MIX2 and MIX4) compared to control. Albumin concentration and A/G ratio values enhanced (p < 0.05) in the serum of trail broilers by HMP. In the present work, IgM and lysozyme enhanced (p < 0.05) with increasing levels of treatments.

Meat quality samples

pH values

pH values of chicken meat through cold storage (4 ± 1°C for 14 days) were given in Table 6. All samples showed increases in pH values during cold storage. This increase in pH values throughout meat storage was lead to the growth micro-organisms on the meat surface, stimulating the decomposition of different meat elements such as amino acids and fat (Zhao et al. 2019).

Thiobarbituric acid reactive substances (TBARS)

The effect of herpes mixture powder addition to broiler diet on the oxidative constancy of stored meat chicken in the refrigerator clarified in Table 7. Throughout the storage period, TBARS was increased in all studied samples. However, rancidity oxidative was lowered in storage meat chicken of broiler fed...
Table 5. Biochemical parameters of broiler chicks supplemented with different concentrations of herbal mixture.

| Biochemical parameters | C          | MIX1     | MIX2     | MIX3     | MIX4     | MIX5     |
|------------------------|------------|----------|----------|----------|----------|----------|
| ALT (IU/L)              | 10.81a     | 10.58b   | 10.28c   | 10.13cd  | 9.98cd   | 9.80d    |
| AST (IU/L)              | 123.67a    | 121.50b  | 119.50c  | 118.50c  | 116.50d  | 115.50d  |
| Urea (mg/dL)            | 44.89a     | 35.50d   | 39.50b   | 36.75c   | 35.15d   | 33.50a   |
| Creatinine (mg/dL)      | 0.54a      | 0.43b    | 0.56c    | 0.48ab   | 0.53c    | 0.42c    |
| Total protein (g/dL)    | 6.62a      | 6.45b    | 6.38bc   | 6.40bc   | 6.31cd   | 6.25d    |
| Globulin (g/dL)         | 3.23bc     | 3.16c    | 3.33a    | 3.28ab   | 3.33a    | 3.34a    |

C: control; MIX1, MIX2, MIX3, MIX4 and MIX5: basal diet + 2, 3, 4, 5 and 6 g herbal mixture/kg diet, respectively.

Table 6. Effect of herpes mixture on PH of broilers samples during cold storage (±4°C).

| Items | 0 days | 7 days | 15 days |
|-------|--------|--------|---------|
| C     | 5.83a  | 5.99a  | 6.24a   |
| MIX1  | 5.75b  | 5.90c  | 6.12b   |
| MIX2  | 5.83b  | 5.98b  | 6.16b   |
| MIX3  | 5.76b  | 5.93b  | 6.15b   |
| MIX4  | 5.62b  | 5.86a  | 6.09b   |
| MIX5  | 5.62b  | 5.82a  | 6.03b   |
| SEM   | 0.02   | 0.06   | 0.02    |
| P value | <0.001 | <0.001 | <0.000  |

C: control; MIX1, MIX2, MIX3, MIX4 and MIX5: basal diet + 2, 3, 4, and 6 g herbal mixture/kg diet, respectively.

Table 7. Effect of herpes mixture on TBA (mg MD/Kg) of broilers samples during cold storage (±4°C).

| Items | 0 days | 7 days | 15 days |
|-------|--------|--------|---------|
| C     | 0.34a  | 0.49a  | 0.85a   |
| MIX1  | 0.24b  | 0.41b  | 0.70b   |
| MIX2  | 0.26bc | 0.32c  | 0.52d   |
| MIX3  | 0.33a  | 0.43b  | 0.69b   |
| MIX4  | 0.16c  | 0.28c  | 0.41i   |
| MIX5  | 0.27b  | 0.34a  | 0.51i   |
| SEM   | 0.01   | 0.02   | 0.04    |
| P value | <0.000 | <0.000 | <0.000  |

C: control; MIX1, MIX2, MIX3, MIX4 and MIX5: basal diet + 2, 3, 4, 5 and 6 g herbal mixture/kg diet, respectively.

Discussion

Chemical characterisation

The total phenol amount was 9.7, 12.15, 4.9, 10.23, 5.2, 6.6, 4.5, and 25 mg GAE g⁻¹ extract for TV, PA, CA, MS, SR, AS, NS and its blend, respectively. While, the total flavonoid amount was 2.4, 4.9, 0.9, 3.9, 1.8, 2.4, 1.3 and 16 mg QE g⁻¹ extract for TV, PA, CA, MS, SR, AS, NS and its blend, respectively. Low values of SC₅₀ mean antioxidant activity higher (Osman et al. 2019). In DPPH-radical scavenging activity assay, antioxidant activity (SC₅₀) was 150, 100, 270, 118, 230, 200, 300 and 13 mg L⁻¹ for TV, PA, CA, MS, SR, AS, NS and its blend, respectively. The antioxidant ability of different foods inclusive coffee is backed to polyphenol aggregation (Acidri et al. 2020).

Growth performance

This noticeable improvement in LBW at the whole experimental period (1–5 wks of age) might be due to improved digestion and absorption of diet nutrients by some components of the phytotherapeutic additives (Tables 2 and 3). However, that may be contributed to enhancing the utilisation of feed consequence, enhancing the growth rate. Prohibition of used antibiotics in poultry production due to a widely used of herbs and plant medicines as feed additives to improve growth condition, its induced saliva secretion and improve digestion processes (Suganya et al. 2016). Phytogenic additives may also reduce the environmental problems dangerous that are produced by using antibiotics as feed additives such as bacterial resistance (Perić et al. 2009). Fotina et al. (2013) reported that to preserve broiler growth rate, quality of meat and immune responses, its necessary added optimal antioxidant level to diets.

Meat colour evaluation

The changes in meat colour values of chickens treated with different levels of HMP throughout storage cold were illustrated in Table 8. The L* and a* grade values lowered in all samples slowly through cold storage. In control, values of L* and a* grads of samples were lower than treatment groups.
On the other hand, Marzoni et al. (2014) cleared that the growth rate did not impact adversely by adding a mix of natural antioxidants in the broiler diet. Results also showed no statistical differences in FI due to the dietary supplementation of HMP except the term of age 1-3 wk. In agreement, Cho et al. (2014) claimed that feed intake and FCR of broiler chickens did not affect by the addition of phytogenic additives to broiler diet compared to the control. In contrast, Perić et al. (2009) and Scheuermann et al. (2009) stated that additives of phytogenic could improve the consuming and transformation of nutrients, but the action mechanism is not yet clear. The positive effect of these additives leads to BWG improvement, as presented in Table 3. However, this improvement may be due to the synergetic effect of chemical ingredients present in HMP (Table 2). Kohlert et al. (2000) reported that phytogenic additives theoretically enhance digestion and absorption by hyperactivation of intestinal villi and fast body metabolism. It stimulates modification in membrane permeability and penetration characteristics, such as proteins synthesis linked with the function of cytoskeletal, consequence enhance absorption in small intestine surface (Khajuria et al. 2002).

Carcass characteristics

Positive impacts were observed on gizzard (p > .01) and heart (p < .05) yields, while carcass, dressing, breast and thighs yields were adversely influenced (p > .05 and .01) by dietary HMP supplementation. In a partial agreement with our findings, Marzoni et al. (2014) cleared that broiler carcass yields did not impact by adding a natural antioxidant mixer to chick’s diet. In disagreement with our results, Cabuk et al. (2006) concluded that a herbs mixture were not had significant impacts on body weight or internal organs of the broilers at the marketing age.

Blood parameters

Blood biochemical estimations are usually expressed to bird health conditions. These estimations are a good index of nutritional, physiological and pathological conditions of the bird and have the possibility of starting used to illustrate the influence of feed ingredients and additives dietary provided. This present finding is positively confirmed by Swain and Johri (2000) and Biswas et al. (2010). Our results are agreeing with Suganya et al. (2016), who showed that the herbs, flavonoids, carotenoids and vitamin C were useful for the immune system. Also, Lavinia et al. (2009) revealed that dietary additives of essential oils of medicinal plants enhance duodenum mucosa and immune response due to improving animal performance.

Meat quality samples

pH values

The glycolysis rate after slaughter causes meat acidity that responsible for the primary variety of meat quality. The pH values increasing in storage meat responsible for the growth micro-organisms on storage meat surface, stimulating fat and amino acid decomposition, Shengming Zhao et al. (2019). The lower pH in chicken meat treatment with herpes mix may be responsible for inhibition of the integration of growth microorganism deterioration.

Thiobarbituric acid reactive substances (TBARS)

Food oxidative rancidity is estimated by using the methods of thiobarbituric acid reactive substances. The changes of treated samples or control were not significant at zero time, which indicates that oxidative deterioration of broilers lipid occurred during storage time (Table 7). During the storage period, TBARS values tended to significantly (p < .05) increases, and all samples recorded values lower than the critical limit.

| Table 8. Effect of herpes mixture on meat colour of broiler samples during cold storage (±4 °C). |
|---------------------------------------------------------------|
| **Meat colour**                                                |
|                  | 0 days | 7 days | 15 days |
|                  | L⁰     | a⁰    | b⁰     | L⁰     | a⁰    | b⁰     | L⁰     | a⁰    | b⁰     |
| C                | 53.35ᵃ  | 5.27ᵃ  | 13.56ᵃ | 52.52ᵃ  | 5.05ᵈ  | 13.31ᶜ | 52.46ᵇ  | 6.70ᵈ  | 12.55ᶜ |
| MIX1             | 53.72ᵃ  | 8.74ᵃ  | 12.57ᵇ | 51.52ᵇ  | 6.57ᵇ  | 12.34ᵃ | 49.86ᵇ  | 8.13ᵃ  | 14.15ᵇ |
| MIX2             | 41.79ᵇ  | 8.43ᵇ  | 13.91ᵇ | 47.69ᵇ  | 6.57ᵇ  | 13.88ᵇ | 55.29ᵇ  | 7.38ᵇ  | 11.80ᵇ |
| MIX3             | 51.56ᵇ  | 6.41ᵇ  | 12.68ᵇ | 50.64ᵇ  | 5.34ᵇ  | 12.28ᵇ | 48.32ᵇ  | 4.61ᵇ  | 13.17ᵇ |
| MIX4             | 48.41ᶜ  | 7.14ᶜ  | 12.63ᵇ | 48.09ᵈ  | 5.44ᶜ  | 13.65ᵇ | 45.70ᵇ  | 5.93ᵃ  | 10.37ᵇ |
| MIX5             | 47.19ᵈ  | 8.11ᵇ  | 13.62ᵇ | 46.82ᵈ  | 7.55ᵃ  | 13.13ᵈ | 47.40ᵇ  | 7.51ᵇ  | 12.40ᵈ |
| SEM              | 1.00    | <0.000 | <0.000 | <0.000 | <0.000 | <0.000 | <0.000 | <0.000 | <0.000 |

C: control; MIX1, MIX2, MIX3, MIX4 and MIX5: basal diet + 2, 3, 4, 5 and 6 g herbal mixture/kg diet, respectively. SEM: standard error mean; L: lightness; a: redness; b: yellowness. Different letters within one column are significantly different (p < .05).
(0.9 mg malonaldehyde/kg sample), as reported by Greene and Cumuze (1982). The inhibitory effect of herpes mix of lipid oxidation may be linked by the contents of biochemical constituents and phenolic components that mostly participate in the activity of antioxidant (Zhang et al. 2010 and Jia et al. 2012). Also, the expression of antioxidant activity as SC50 (µg mL⁻¹) of the extract. Low SC50 values lead to high antioxidant activity (Osman et al. 2019). The blend material contains 13 DPPH activity (SC50; µg mL⁻¹) as reported in Table 2 that means that it has a strong antioxidant activity.

**Meat colour evaluation**

Cold chicken marketing during refrigerated storage period depends on chicken surface colour, which indicates to its freshness and commercial acceptability. Reducing water retention leads to less surface light reflecting which, responsible for L* value decreasing (Hughes et al. 2014). Metmyoglobin (MetMb) cumulating on the storage meat surface participate in the discoulouration of storage meat (Bekhit et al. 2007), which clears the differences in a* value. The formation of MetMb and high lipid oxidation are the major elements responsible for the differences in b* value (Xiong, 2000).

**Conclusions**

It is concluded that HMP could be used as a natural additive to the broiler diet to enhance the growth rate and improve blood parameters and meat quality. Chicks fed 5 g herbal mixture/kg diet had the best growth performance. Also, the oxidative rancidity was less in the meat of treated chicks during the storage period as compared to control samples.

**Ethical approval**

All procedures of the current study were followed according to the Directive 2010/63/EU of the European Parliament and the Council of September 22, 2010, on the safety of animals used for scientific purposes. The experimental protocol regarding the care and handling of animals had been approved by the Ethics Committee of Department of Poultry, Faculty of Agriculture, Zagazig University, Egypt.

**Disclosure statement**

The authors declare that there is no conflict of interest associated with the paper. The authors alone are responsible for the content and writing of this article.

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**References**

Abd El-Hack ME, Alagawany M, Shaheen H, Samak D, Othman SI, Allam AA, Taha AE, Khafaga AF, Arif M, Osman A, et al. 2020. Ginger and its derivatives as promising alternatives to antibiotics in poultry feed. Animals. 10(3): 452.

Abd El-Hack ME, Abdelnour SA, Taha AE, Khafaga AF, Arif M, Aysan T, Swelum AA, Abukhalil MH, Alkahtani S, Aleya L, et al. 2020. Herbs as thermoregulatory agents in poultry: An overview. Sci Total Environ. 703(703):134399

Abd El-Hack ME, Alagawany M. 2015. Performance, egg quality, blood profile, immune function, and antioxidant enzyme activities in laying hens fed diets with thyme powder. J Anim Feed Sci. 24(2):127–133.

Abd El-Hack ME, Ashour EA, Elaraby GM, Osman AO, Arif M. 2018. Influences of dietary supplementation of peanut skin powder (Arachis Hypogaea) on growth performance, carcass traits, blood chemistry, antioxidant activity and meat quality of broilers. Anim Prod Sci. 58(5):965–972.

Abdel-Hamid M, Osman A, El-Hadary A, Romeih E, Sitohy M, Li L. 2020. Hepatoprotective action of papain-hydrolyzed buffalo milk protein on carbon tetrachloride oxidative stressed albino rats. J Dairy Sci. 103(2):1884–1893.

Abdel-Hamid M, Otte J, De Gobba C, Osman A, Hamad E. 2017. Angiotensin I-converting enzyme inhibitory activity and antioxidant capacity of bioactive peptides derived from enzymatic hydrolysis of buffalo milk proteins. Int Dairy J. 66:91–98.

Abdel-Shafi S, Al-Mohammadi AR, Sitohy M, Mosa B, Ismaiel A, Enan G, Osman A. 2019. Antimicrobial Activity and Chemical Constitution of the Crude, Phenolic-Rich Extracts of Hibiscus sabdariffa, Brassica oleracea and Beta vulgaris. Molecules. 24(23):4280.

Abo Ghanima MM, Elsadek MF, Taha AE, Abd El-Hack ME, Alagawany M, Ahmed BM, Elshafie MM, El-Sabrouk K. 2020. Effect of housing system and rosemary and cinnamon essential oils on layers performance, egg quality, haematological traits, blood chemistry, immunity, and antioxidant. Animals. 10(2):245.

Acdrí R, Sawai Y, Sugimoto Y, Handa T, Sasagawa D, Masunaga T, Yamamoto S, Nishihara E. 2020. Phytochemical profile and antioxidant capacity of coffee plant organs compared to green and roasted coffee beans. Antioxidants. 9(2):93.

Alagawany MM, Farag MR, Dhama K, Abd El-Hack ME, Tiwari R, Alam GM. 2015. Mechanisms and beneficial applications
of resveratrol as feed additive in animal and poultry nutrition: a review. Int J Pharmacol. 11(3):213–221.

Alagawany M, Abd El-Hack ME, Farag MR, Tiwari R, Dhama K. 2015. Biological effects and modes of action of carvacrol in animal and poultry production and health – a review. Adv Anim Vet Sci. 3(25):73–84.

Alagawany M, Attia Y, Farag MR, ElNesr SS, Nagadi SA, Shafi ME, Khafaga AF, Ohran H, Aalaqil AA, Abd El-Hack ME. 2020. The strategy of boosting the immune system of food-producing under CoViD-19 pandemic. Front Vet Sci. 7:398.

Alagawany M, Elnesr SS, Farag MR, Abd El-Hack ME, Khafaga AF, Taha AE, Tiwari R, Yatoq Ml, Bhatt P, Marappan G, et al. 2019. Use of licorice (Glycyrrhiza glabra) herb as a feed additive in poultry: Current knowledge and prospects. Animals. 9(8):536.

Alloui MN, Szczurek W, Swiątkiewicz S. 2013. The usefulness of prebiotics and probiotics in modern poultry nutrition. Annals Anim Sci. 13(1):17–32.

Al-Sagan AA, Khalil S, Hussein OE, Attia YA. 2020. Effects of fennel seed powder supplementation on growth performance, carcass characteristics, meat quality, and economic efficiency of broilers under theroneutral and chronic heat stress conditions. Animals. 10(2):206.

Amer SA, Ahmed SAA, Ibrahim RE, Al-Gabri NA, Osman A, Sitoby M. 2020. Impact of partial substitution of fish meal by methylated soy protein isolates on the nutritional, immunological, and health aspects of Nile tilapia, Oreochromis niloticus fingerlings. Aquaculture. 518:734871.

Arif M, Hayat Z, Abd El-Hack ME, Saeed M, Imran HM, Alowaimer AN, Saadeldin IM, Taha AE, Swelum AA. 2019. Impacts of supplementing broiler diets with a powder mixture of black cumin, Moringa and chicory seeds. SA J Anim Sci. 49(3):564–572.

Ashour EA, Alagawany M, Reda FM, Abd El-Hack ME. 2014. Effect of supplementation of yucca schidigera extract to growing rabbit diets on growth performance, carcass characteristics, serum biochemistry and liver oxidative status. Asian J Anim Vet Adv. 9:732–742.

Ashour EA, Bin-Jumah M, Abou Sayed-Ahmed ET, Osman AO, Taha AE, Momenah MA, Allam AA, Suelum AA, Abd El-Hack ME. 2020. Effects of dried okra fruit (Abelmoschus esculentus) powder on growth, carcass characteristics, blood indices, and meat quality of stored broiler meat. Poult Sci. 99(6):3060–3069.

Ashour EA, El-Kholy MS, Alagawany M, Abd El-Hack ME, Mohamed LA, Taha AE, El Sheikh Al, Laudadio V, Tufarelli V. 2020. Effect of dietary supplementation with Moringa oleifera leaves and/or seeds powder on production, egg characteristics, hatchability and blood chemistry of laying Japanese quails. Sustainability. 12(6):2463. 2463.

Attia YA, Al-Harthi MA, Hassan SS. 2017. Turmeric (Curcuma longa Linn.) as a phytogetic growth promoter alternative for antibiotic and comparable to mannan oligosaccharides for broiler chicks. Rev Mex Cienc Pecu. 8 (1):11–21.

Attia YA, Bakhashwain AA, Bertu NK. 2017. Thyme oil (Thyme vulgaris L.) as a natural growth promoter for broiler chickens reared under hot climate. Italian J Anim Sci. 16(2):275–282.

Attia YA, Al-Harthi MA. 2015. Nigella seed oil as an alternative to antibiotic growth promoters for broiler Chickens. Europ Poult Sci. 79.

Attia YA, Bakhashwain AA, Bertu NK. 2018. Utilisation of thyme powder (Thyme vulgaris L.) as a growth promoter alternative to antibiotics for broiler chickens raised in a hot climate. Europ Poult Sci. 82.

Attia YA, Rawia SH, Bovera F, Mohammed AA, Abd El-Hamid EA, LE, Hossam AS. 2019. Milk thistle seeds and rosemary leaves as rabbit growth promoters. Anim Sci Papers. Rep. 37:277–295.

Bathiya EG, Beshbishy MA, Wasef GL, Elewa YH, Al-Sagan AA, Abd El-Hack ME, Taha AE, Abd-Elhakim MY, Prasad DH. 2020. Chemical constituents and pharmacological activities of garlic (Allium sativum L.): A review. Nutrients. 12(3):872.

Bekhit AED, Cassidy L, Hurst RD, Farouk MM. 2007. Post-mortem metmyoglobin reduction in fresh venison. Meat Sci. 75(1):53–60.

Biswas A, Ahmed M, Bhati VK, Singh SB. 2010. Effect of antioxidants on physio-biochemical and hematological parameters in broiler chicken at high altitude. Asian Australas J Anim Sci. 24(2):246–249.

Cabuk M, Bozkurt M, Alicek A, Akbas Y, Kucukyilmaz K. 2006. Effect of a herbal essential oil mixture on growth and internal organ weight of broilers from young and old breeder flocks. S Afr J Anim Sci. 36:135–141.

Cho JH, Kim HJ, Kim IH. 2014. Effects of phytogenic feed additive on growth performance, digestibility, blood metabolites, intestinal microbiota, meat color and relative organ weight after oral challenge with Clostridium perfringens in broilers. Livestock Sci. 160:82–88.

Ellis AE. 1990. Lysozyme assays. Tech Fish Immunol 1, 101–103.

Fernandez-Lopez J, Zhi N, Aleson-Carbonell L, Perez-Alvarez JA, Kuri V. 2005. Antioxidant and antibacterial activities of natural extracts: application in beef meatballs. Meat Sci. 69(3):371–380.

Fotina AA, Fisinin VI, Surai PF. 2013. Recent developments in usage of natural antioxidants to improve chicken meat production and quality. Bulgarian J Agri Sci. 19:889–896.

Gado AR, Ellakany HF, Elbestawy AR, Abd El-Hack ME, Khafaga AF, Taha AE, Arif M, Mahgoub SA. 2019. Herbal medicine additives as powerful agents to control and prevent avian influenza virus in poultry–a review. Ann Anim Sci. 241. (ahead-of-print).

Greene BE, Cumuze TH. 1982. Relationship between TBA numbers and in experienced panelist assessment of oxidized flavor in cooked beef. J Food Sci. 47(1):52–58.

Hafez MH, Attia YA. 2020. Challenges to the poultry industry: Current perspectives and strategic future after the COVID-19 outbreak. Front Vet Sci.

Hughes JM, Oiseth SK, Purslow PP, Warner RD. 2014. A structural approach to understanding the interactions between colour, water-holding capacity and tenderness. Meat Sci. 98(3):520–532.

Jia N, Kong B, Liu Q, Diao X, Xia X. 2012. Antioxidant activity of black currant (Ribes nigrum L.) extract and its inhibitory effect on lipid and protein oxidation of pork patties during chilled storage. Meat Sci. 91(4):533–539.

Khafta AF, Abd El-Hack ME, Taha AE, ElNesr SS, Alagawany M. 2019. The potential modulatory role of herbal additives...
against Cd toxicity in human, animal, and poultry: A review. Env Sci Poll Res. 2026(5):4588–4604.

Khajuria A, Thusu N, Zutshi U. 2002. Piperine modulates permeability characteristics of intestine by inducing alterations in membrane dynamics: influence on brush border membrane fluidity, ultrastructure and enzyme kinetics. Phytomedicine. 9(3):224–231.

Kohler C, van Rensen I, Marz R, Schindler G, Greife EU, Veit M. 2000. Bioavailability and pharmacokinetics of natural volatile terpenes in animals and humans. Planta Med. 66(6):495–505.

Lavinia S, Gabi D, Drinceanu D, Stef D, Daniela M, Julean C, Ramona T, Corcionivoschi N. 2009. The effect of medicinal plants and plant extracted oils on broiler duodenum morphology and immunological profile. Romanian Biotechnol Lett. 14:4606–4614.

Marzoni M, Chiarini R, Castillo A, Romboli I, Marco M de, Schiavone A. 2014. Effects of dietary natural antioxidant supplementation on broiler chicken and Muscovy duck meat quality. Anim Sci Papers Rep. 32:359–368.

NRC, National Research Council. 1994. Nutrient Requirements of Poultry, National Academy of Sciences, Washington, D.C., USA.

Ordonez A, Gomez J, Vattuone M, Lsla M. 2006. Antioxidant activities of Sechium edule (Jacq.) Swartz extracts. Food Chem. 97(3):452–458.

Osman A, Abd-Elaziz S, Salama A, Eita AA, Sitohy M. 2019. Health protective actions of phycocyanin obtained from an Egyptian isolate of Spirulina platensis on albino rats. EurAsian J BioSci. 13:105–112.

Osman A, Mahgoub S, Sitohy M. 2014. Hindering milk quality storage deterioration by mild thermization combined with methylated chickpea protein. Int Food Res J. 21 (2): 693–701.

Perić L, Žikić D, Lukić M. 2009. Application of alternative growth promoters in broiler production. Biotechnol Anim Husb. 25:387–397.

Ramadan MF, Osman AOM, El-Akad HM. 2008. Food ingredients total antioxidant potential of juices and beverages screening by DPPH in vitro assay. Deutsche Lebensmittel-Rundschau. 104:235–239.

Rao L, Hayat K, Lv Y, Karangwa E, Xia S, Jia C, Zhong F, Zhang X. 2011. Effect of ultrafiltration and fining adsorbents on the clarification of green tea. J Food Eng. 102(4):321–326.

Scheuermann GN, Cunha Junior A, Cypriano L, Gabbi AM. 2009. Phyrogenic additive as an alternative to growth promoters in broiler chickens. Cienc Rural. 39(2):522–527.

Shewita RS, Taha AE. 2018. Influence of dietary supplementation of ginger powder at different levels on growth performance, haematological profiles, slaughter traits and gut morphology of broiler chickens. S Afr J Anim Sci. 48(6):997–1008.

Singh VB, Singh VK, Tiwari D, Gautam S, Ruma D, Singh SP, Chaturvedi S, Singh P. 2018. Effect of a phyrogenic feed additive supplemented diet on economic efficiency and cost of production of broiler chickens. Int J Curr Microbiol App Sci. 7:2831–2837.

Sitohy M, Osman A, Gharib A, Chobert JM, Haertlé T. 2013. Preliminary assessment of potential toxicity of methylated soybean protein and methylated β-lactoglobulin in male Wistar rats. Food Chem Toxicol. 59:618–625.

SPSS 2008. Statistical package for the social sciences, Release. SPSS INC, Chicago, USA. 16

Swain BK, Johri TS. 2000. Effects of supplementation of combinations of different levels of selenium and vitamin E on relative weight of some organs and serum enzymes level in broilers. Indian J Poult Sci. 35:66–69.

Taha AE, Hassan SS, Shewita RS, El-Seidy AA, Abd El-Hack ME, Hussein ES, Saadeldin IM, Swelum AA, El-Edel MA. 2019. Effects of supplementing broiler diets with coriander seed powder on growth performance, blood haematology, ileum microflora and economic efficiency. J Anim Physiol Anim Nutr. 103(5):1474–1483.

Xiong YL. 2000. Protein oxidation and implications for muscle food quality. In: Decker E, Faustman C, Lopez-Bote CJ editors. Antioxidants in muscle foods: Nutritional strategies to improve quality. New York: John Wiley and Sons. p. 85–111.

Zhang Y, Li X, Wang Z. 2010. Antioxidant activities of leaf extract of Salvia miltiorrhiza Bunge and related phenolic constituents. Food Chem Toxicol. 48(10):2656–2662.

Zhao S, Ningning L, Zhao L, Hongju H, Yanan Z, Mingming Z, Zhengrong W, Zhuangli K, Hanjun M. 2019. Shelf life of fresh chilled pork as affected by antimicrobial intervention with nisin, tea polyphenols, chitosan, and their combination. Int J Food Prop. 22(1):1047–1063.