INTRODUCTION

There has been a growing number of scientific papers regarding the use of plant extracts since a ban on antibiotics uses as growth promoters in animal feed were introduced in 2006. Therefore, plant extracts and their derivations are used as alternative supplements and have become of exceptional interest in poultry production (Abbas & Ahmed, 2010; Brzóska et al., 2015; Al-Shammari et al., 2017). Plant extracts (spices and herbs) and their phytobiotics effects (bioactive constituents present in essential oil) can be incorporated into poultry diets to boost the productivity, physiology, and even welfare of birds. In monogastric animals, plant extracts have a diversity of Phyto biotics which are reported to act as stimulating agents for appetite and enzymatic secretions, and with prebiotic, immunological, antimicrobial, and prophylactic activities (Wenk, 2003; Lee et al., 2004; Vidanarachchi et al., 2005). Also, it is well known that many dietary-plant extracts can adjust the intestinal microflora strongly, which in turn, can modify animals’ response to diet and homeostasis (Wenk, 2003). These plant-derived materials have their physiological roles in body cells and tissues because they are considered as less toxic and residue-free and have been proven to be the optimal feed supplements in comparison with synthetic and antibiotic compounds in the diets of farm animals (Vidanarachchi et al., 2005).

Garlic (Allium sativum L.) is a popular spice which has a pungent quality and has been used as a culinary spice in most of the traditional cultures since it contains a multitude of active components such as, allicin, s-allyl cysteine, ajoene, and diallyl sulphide (Rahmatnejad & Roshanfekr, 2009). These compounds have a variety of advantages, most of which have been confirmed scientifically, e.g., antithrombosis, anti-atherosclerosis, antimicrobial, hypolipidemic, anti-diabetes, anti-hypertension, and antimicrobial functions, etc. (Mansoub, 2011; Puvača et al., 2015). It was proven that garlic, as a natural feed additive, has improved growth, feed conversion ratio, carcass quality, and health status (Stanačev et al., 2011). It also lowers the number of triglycerides and low-density lipoprotein (Puvača et al., 2015) in broilers with hypocholesterolemia impact upon serum and the yolk of layer hens (Chowdhury, 2002). Additionally, garlic powder may inhibit ascites incidence because of its anti-hypertensive characteristics in the cardiac system without deteriorating broiler performance (Varmaghany et al., 2015).
Cinnamon (Cinnamomum verum) is one of the oldest familiar herbal medicines which contain more than 300 active substances such as trans-cinnamaldehyde and certain biologically active compounds such as cinnamyl acetate, cinnamyl alcohol, carvaerol, and eugenol present in its essential oils. Eugenol and cinnamaldehyde are two substantial terpenoids found in cinnamon (Qin et al., 2003, Tajodini et al., 2015). The powerful capacity of cinnamon as anti-allergenic, antioxidant, anti-inflammatory, and antiviral agents along with its blood purifying and digestion-aiding properties is attributed to these biological compounds (Tajodini et al., 2015).

Black cumin (Nigella sativa L.) is an aromatic plant. It has been applied in the treatment of several diseases over the course of 2000 years and has been exploited as a supplement in poultry feed because of its possibility to optimize performance via antimicrobial, antioxidant, and the other pharmacological properties found within (Kumar & Patra, 2017). Ramadan (2007) mentioned active compounds in black cumin present in essential oil consisting of carvone, an unsaturated ketone, terpene or limonene also called carvone, p-cymene, α-pinene, and nigelone. Thymol, thymoquinone, di-thymoquinone, and thymo-hydroquinone are pharmacologically effective constituents of the volatile oil (Ghosheh et al., 1999), while trans retinol, DL-γ-tocopherol, DL-α-tocopherol, and selenium are among important antioxidants (Al-Saleh et al., 2006).

The systematic approach with respect to the efficiency and safety of these plant-based substances and their active botanicals in the poultry industry has not been broadly researched. Further studies are required to improve the situation. Based upon the premise, the current study was carried out to assess the impact of garlic, cinnamon, and black cumin as phytogenic additives upon the performance characteristics, physiological, and immunological aspects of Ross 308 broilers fed the same levels of these dietary additives.

MATERIALS AND METHODS

Experimental Design and Birds Management

This experiment was conducted in the farm and laboratory facilities belonging to Al-Furat Al-Awset Technical University, Babylon, Iraq. The study ran for 42 d. These plant extracts (Hennahub India, Sojat, Rajasthan/India) were purchased from the local market for 42 d. These plant extracts (Hennahub India, Sojat, Rajasthan/India) were purchased from the local market as a fine powder and mixed freshly into diets every three days. Two hundred and forty broiler chickens Ross 308 were housed in (1x2 m) floor pens with 3 pens (replicates) per group. The experimental groups were divided into 4 groups, 60 chicks in each group. The first group was the control group (CO) (no dietary supplement), and 2nd, 3rd, and 4th groups’ diets were supplemented with 4 mg/kg garlic (GC), cinnamon (CN), and black cumin (BC), respectively. All birds were fed ad libitum (Table 1) on uniform isonitrogenous and isocaloric diet based on NRC recommendation (NRC, 1994) and maintaining all healthcare and preventive measures during the experiment.

### Characteristics Studied

The productive characteristics of birds involving live body weight and feed intake were recorded weekly and reported periodically to calculate weight gain and feed conversion ratio. At the end of the experiment, 9 birds per group (3 birds per pen) (n= 9) were bled. A blood sample was collected from the jugular vein and kept in non-heparinized plastic tubes to obtain blood serum. Blood samples were then centrifuged at 3000 RPM for 15 minutes to separate the serum. Subsequently, the serum was stored at -20°C until biochemical and immunological analyses were conducted. From fresh blood, packet cell volume (PCV) was determined using heparinized microhematocrit capillary tubes (Haen, 1995).

For serum biochemical evaluation, glucose, protein, cholesterol, and triglycerides levels were measured using commercial laboratory kits (Diagnostic Biosystems, Spain) and spectrophotometer VS721G according to methods coined by Burtis et al. (2005); Burris et al. (2005); Friedman & Young (2001) and Young (2000), respectively. Levels of thyroxine (T4), triiodothyronine (T3), and thyroid-stimulating hormone (TSH) in serum samples

### Table 1. Diet formula and chemical composition

| Ingredients (%) | Starter (1 day-3 weeks) | Finisher (4-6 weeks) |
|-----------------|-------------------------|----------------------|
| Yellow corn     | 40                      | 47                   |
| Wheat           | 20.5                    | 21.4                 |
| Soybean meal    | 26                      | 20                   |
| Protein concentrate       | 10                     | 8                    |
| Vegetable oil   | 2                      | 2                    |
| Salt            | 0.3                    | 0.3                  |
| Premix          | 0.2                    | 0.28                 |
| Limestone       | 1                      | 1                    |
| L-Lysine        | -                      | 0.02                 |
| Total           | 100                    | 100                  |
| Calculated analysis |             |                     |
| Crude protein (%) | 22.20                  | 19.30                |
| Metabolizable energy (kcal/kg) | 3006 | 3073                |
| Calcium (%)     | 1.27                    | 1.10                 |
| Available phosphorus (%) | 0.41 | 0.40                |
| Lysine (%)      | 1.16                    | 1.25                 |
| Methionine (%)  | 0.25                    | 0.40                 |

Note:

1. (Holde Mix, Jordan) provided following nutrients per kg of diet: 40% of crude protein; 1% of crude fiber; 3.5 of fat; 6% of calcium; 2100 of kcal metabolizable energy; 3% of phosphorus; 2.20% of salt; 2.5% of lysine; 3.50% of methionine; 3.90% of methionine + cystine; 40000 of IU of vitamin D3; 15 mg of vitamin B1; 3000 mg of vitamin B6; 50 mg of vitamin E; 200 of mg niacin; 1000 mg of iron; 6 mg of cobalt; 800 mg of zinc; 200000 IU of vitamin A; 15 mg of vitamin B12; 300 mg of vitamin B12; 30 mg of vitamin K; 100 mg of biotin; 100 mg of cupper; 1200 mg of manganese; 15 mg of iodine; 2 mg of selenium and 10 mg folic acid.

2. (Poultry Premix, Belgium) provided following nutrients per kg of diet: 9000 mg of methionine; 200 mg of vitamin D3; 250 mg of vitamin B1; 500 mg of vitamin B6; 5000 mg of vitamin E; 5000 mg of iron; 100 mg of cobalt; 125000 IU of vitamin A; 2 mg of vitamin B6; 200 mg of vitamin K; 2.5 mg of biotin; 800 mg of cupper; 100 mg of iodine; 10 mg of selenium; 150 mg of folic acid; 9.2 mg of magnesium; 5.2 mg of zinc and 4000 mg of choline.
were determined radio-immunologically by means of test kits (AccuBind® ELISA, USA). All samples were run in duplicate and kit calibrators, and controls were contained in each analysis. By using ELISA microplate reader (HumaReader HS, Germany), absorbance was measured at a specified value in the kit, with a reference wavelength of each analytical step (Braverman, 1996). To evaluate antibodies titer against Newcastle and Gumboro diseases, research kits (ProFLOK® NDV-T ELISA, USA, and ProFLOK® IBD-T ELISA, USA), were used respectively in compliance with manufacturers’ protocol and instruction by using an automated microplate reader (BMG LABTECH, Germany).

**Statistical Analysis**

The data were analyzed statistically by ANOVA with a completely randomized design (CRD) using SAS Software (SAS, 2001). To find significant differences (p<0.05) and (p<0.01) among the groups’ means, Duncan’s multiple tests were carried out.

**RESULTS**

The influence of three supplementary levels of plant extracts (GC, CN, and BC) on growth performances are shown in Table 2. The levels of GC, CN, and BC had high significant (p≤0.01) impacts on weeks 3 and 6 regarding body weight and weight gain from 1 day-3 weeks and from 1 day-6 weeks. From 1 day-3 weeks, there was no significant difference in feed intake between groups fed diets with all plant extracts and the CO group. However, from 1 day to 6 weeks, birds fed diets supplemented with GC had lower (p≤0.05) feed intake. Significant improvement (reduction) (p≤0.01) in feed conversion ratio was noticed in birds fed GC, CN, and BC than those in the CO group at both ages (1 day-3 weeks) and (1 day-6 weeks).

The birds fed diets containing experimental groups had high (p≤0.01) significant impact on PCV value and low (p≤0.01) significant levels of cholesterol and triglyceride values than birds fed the non-supplemented diet (Table 3). In the same table, there was a low level (p≤0.01) of glucose in GC and CN and a high level (p≤0.01) of protein in CN and BC compared with the CO group.

Table 4 revealed that GC and BC achieved high values (p≤0.01) and (p≤0.05) in T3 and TSH respectively, whereas all groups whose diet was supplemented with plant extracts recorded high values (p≤0.01) in T4 compared with the CO group. The means of Newcastle and Gumboro hemagglutination-inhibition titers in birds fed diets containing plant extracts were significantly higher (p≤0.01) than those fed the CO diet (Table 5).

**DISCUSSION**

**Growth Performance**

The accumulative increase in body weight and weight gain in the present data reflected the accumulatively positive reduction in feed conversion ratio (FCR) in birds fed diets based on GC, CN, and BC extracts. These results might be due to the availability of major natural bioactive elements found in essential oils of these extracts, e.g., allicin, sulfur-containing compounds in GC (Khan et al., 2012a; Puvača et al., 2015), trans-cinnamaldehyde in CN (Tajodini et al., 2015), quinine, carvone, carvene, α-pinene, p-cymene, and nigellone in BC (Abbas & Ahmed, 2010; Kumar & Patra, 2017). These compounds have been identified as having beneficial effects on poultry growth, health, and performance.

**Table 2. Productive traits of broilers fed on different sources of plant extracts (mean±standard error)**

| Groups | Body weight (g) | Weight gain (g) | Feed intake (g) | Feed conversion ratio |
|--------|-----------------|-----------------|-----------------|----------------------|
|        | 3 weeks**       | 6 weeks**       | 1 day-3 weeks*  | 1 day-6 weeks**      |
| CO     | 617.5±3.30[^a]  | 2395.0±3.50[^a] | 188.29±9.29[^a] | 2.99±0.04[^a]        |
| GC     | 684.5±3.55[^b]  | 2349.5±3.50[^b] | 192.51±11.08[^b]| 3.37±5.01[^b]        |
| CN     | 692.5±2.50[^c]  | 2302.5±1.50[^c] | 147.75±6.76[^c] | 2.01±3.01[^c]        |
| BC     | 675.0±2.50[^d]  | 210.00±7.19[^d] | 136.75±9.30[^d] | 2.01±3.01[^d]        |

Note: CO= control; GC= diet supplemented with 4 mg/kg garlic; CN= diet supplemented with 4 mg/kg cinnamon; BC= diet supplemented with 4 mg/kg black cumin. Means in the same column with different superscript differ significantly. *p<0.05; ** highly significant (p<0.01); NS= not significant.

**Table 3. PCV value and some serum biochemical parameters of broilers fed on different sources of plant extracts (mean±standard error)**

| Groups | PCV(%) | Glucose (mg/100 mL) | Protein (g/100 mL) | Cholesterol (mg/100 mL) | Triglyceride (mg/100 mL) |
|--------|--------|---------------------|-------------------|------------------------|-------------------------|
| CO     | 24.75±2.55[^a] | 188.29±9.29[^a] | 2.99±0.04[^a] | 210.00±7.87[^a] | 136.75±9.30[^a] |
| GC     | 33.57±1.60[^c] | 150.25±5.40[^c] | 3.40±0.20[^c] | 155.75±5.03[^c] | 81.75±4.94[^c] |
| CN     | 39.80±1.55[^d] | 170.00±3.39[^d] | 4.69±0.56[^d] | 192.51±11.08[^d] | 100.25±2.90[^d] |
| BC     | 30.92±1.08[^a] | 176.00±7.19[^a] | 4.98±0.15[^a] | 147.75±6.76[^a] | 97.25±5.39[^a] |

Note: CO= control; GC= diet supplemented with 4 mg/kg garlic; CN= diet supplemented with 4 mg/kg cinnamon; BC= diet supplemented with 4 mg/kg black cumin; PCV= Packed cell volume. Means in the same column with different superscript differ highly significant (p≤0.01).
biological compounds may help to enhance gut digestion. They can also be utilized as beneficial phytotherapeutic compounds to modify the healthy microflora, reduce harmful microflora population counts such as coliforms, E. coli, and C. Perfringen, stimulate endogenous secretions (enzymes) and lower pH in birds’ gut (Wenk, 2003; Khan et al., 2012a; Arif et al., 2019). This condition can bring about a cascade of alterations in birds’ responses to disease challenges and nutrients. Even though these metabolic changes did not influence feed intake among groups except in the GC group from 1 day till 6 weeks, which reduced feed intake, which in turn, is of importance from the economic point of view. As a result, this may enable the consumption of the same or lower level of food mass with more metabolizable nutrients via histological optimization in villi length and villi surface area with a decrease of crypt depth (Adibmoradi et al., 2006; Purwanti et al., 2014). Findings of our data are similar to the results of Faghani et al. (2014), who reported a significant increase in body weight and weight gain with a decrease in FCR and feed intake in broiler chickens at 42 days by adding 0.02% of each GC and CN in the diet. Also, these results are partly in line with the results of Varmaghany et al. (2015) who stated that diet containing garlic bulbs at 5, 10, or 15 g/kg fed to broilers exposed to standard and cold temperature conditions had increased final body weight but without the impact upon FI and FCR. On the other hand, it was found that the inclusion of 0.5% and 1.0% dietary GC powder (Puvac et al., 2014) and liquid GC extract in the diet at 1, 1.5, and 2.25 ml/kg (Brzoska et al., 2015) increased feed intake, and did not affect FCR. However, it improved body weight significantly at 42 days in broilers. The results reflect those of Topchyani et al. (2011) and Abo El-Maaty et al. (2014), who found that every 4 g/kg of GN or GC and 0.5 g/kg of GN respectively increased body weight and improved FCR without influencing feed intake. Our data are inconsistent with the results obtained by Sang-Oh et al. (2013) and Naderi et al. (2014) who stated that (3, 5, and 7%) and (2.5 and 7.5 g/kg of dietary CN respectively did not affect body weight and FCR but with a similar amount of consumed food as well. Similarly, concerning the use of dietary BC, it was proven that there was a significant increase in body weight gain accompanying a decrease in FCR for broilers when fed diet supplemented with BC at 2.5 and 5.0% in 28 and 42 days (Khan et al., 2012b), at 10 and 20 g/kg from 0-42 days (Ghasemi et al., 2014) and at 10 and 15% in 6 weeks (Singh et al., 2018).

## Haematological and Biochemical Blood Parameters

Multiple aspects of hematological and biochemical blood parameters could be positively altered by feeding birds with these plant extracts. The plant extracts and their essential oils possess physiological properties, which may stimulate the erythropoietic system to produce red blood cells. These plant-based substances may play an immunizing role in the function of organs pertaining to blood cell synthesis, such as spleen, thymus, and bone marrow. Allicin in GC, cinnamaldehyde in CN, and multiple polyphenols presented in BC affect the performance of intestinal absorption of certain electrolytes such as iron (Abbas & Ahmed, 2010; Khan et al., 2012a; Tajodini et al., 2015; Kumar & Patra, 2017), which in turn, might enhance its role in erythropoiesis leading to the increase in the number of red blood cells and enhance PCV as a final result. Therefore, the increase of red cells in the blood stream is correlated with the greater PVc values in the current data (Al-Shammari et al., 2011) and Naderi et al., 2017). Another possible reason for the high value of PCV might be associated with high levels of thyroid hormones (T3 and T4) and TSH (Table 4) secreted from the pituitary gland in the majority of our treated groups because there is a positive correlation between PCV and thyroid hormones (Sturkie, 2015) accompanying high metabolic rate and a clear increase in serum protein (Table 3) which was reflected by the greater body weights in treated groups (Table 2). Various substances such as alkaloids, glycosides, flavonoids, carotenoids, terpenoids, etc. are documented to have antidiabetic influence, which can act as safe and substitutional drugs for diabetes mellitus. The hypoglycemic functions of GC and CN in our data may be due to their activities to regulate blood glucose concentrations by decreasing gluconeogenesis or increasing pancreatic insulin secretion from beta cells (Arif et al., 2014). There is no doubt that numerous plant materials, e.g. GC (Khan et al., 2012a), and essential oils derived from plants (Lee et al., 2004) have hypcholesterolemia properties by their abilities to inhibit the key regulatory enzymes in cholesterol and lipid syntheses, such as hepatic

**Table 4. Some hormonal values in serum of broilers fed on different sources of plant extracts (mean±standard error)**

| Groups | T3* (µg/mL) | T4** (µg/mL) | TSH ** (µg/mL) |
|--------|-------------|--------------|----------------|
| CO     | 1.12±0.01b  | 10.12±0.27c  | 0.20±0.008b    |
| GC     | 2.73±0.23b  | 17.27±0.27c  | 0.43±0.013b    |
| CN     | 1.75±0.20ab | 13.14±0.36b  | 0.25±0.030b    |
| BC     | 2.75±0.46ab | 18.01±0.25b  | 0.44±0.020b    |

Note: CO= control; GC= diet supplemented with 4mg/kg garlic; CN= diet supplemented with 4 mg/kg cinnamon, BC= diet supplemented with 4 mg/kg black cumin; T3= Triiodothyronine T4: Thyroxine; TSH+ Thyroid stimulating hormone. Means in the same column with different superscripts differ significantly; *=p<0.05; **= highly significant (p<0.01).

**Table 5. Antibody titers against Newcastle and Gumboro diseases virus of broilers fed on different sources of plant extracts (mean±standard error)**

| Groups | Antibody titer against Newcastle (Log2) | Antibody titer against Gumboro (Log2) |
|--------|----------------------------------------|----------------------------------------|
| CO     | 887.50±33.50c                          | 1131.50±31.50d                         |
| GC     | 4110.50±33.50c                         | 2128.50±12.50c                         |
| CN     | 1365.50±32.50c                         | 4227.50±25.50c                         |
| BC     | 2948.50±15.00c                         | 5090.00±26.50c                         |

Note: CO= control; GC= diet supplemented with 4 mg/kg garlic; CN= diet supplemented with 4 mg/kg cinnamon; BC= diet supplemented with 4 mg/kg black cumin. Means in the same column with different superscripts differ highly significantly (p<0.01).
3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, malic enzyme, glucose 6 phosphate dehydrogenase, cholesterol 7α-hydroxylase, and fatty acid synthetase. Similar data indicated there was a decrease in total cholesterol and triglycerides by 0.5% dietary GC (Puvaca et al., 2014), decrease in cholesterol, glucose, and triglycerides by 1, 2, and 4% dietary GC (Hossain et al., 2014), increase in total protein and high PCV by 2.5 and 5.0% dietary BC (Khan et al., 2012b), increase in total protein and decrease in total cholesterol and triglyceride values without a change in glucose level by 1 and 1.5% dietary BC (Singh et al., 2018) of broiler chickens serum. On the other hand, it was stated that the PCV value was unaffected by 2.5 and 7.5 g/kg dietary CN (Naderi et al., 2014). In addition, contrary to the current findings, Brzóska et al. (2015) mentioned that using liquid garlic extract as feed supplements by 1, 1.5, and 2.25 ml/kg of diet for 42 days did not influence cholesterol and glucose levels. However, there was a clear increase in the total protein content of broiler serum for 42 days. Conflicting data was recently achieved by Arif et al. (2019) who stated that using different concentrations of herbal mixtures consisting of black cumin, Moringa oleifera, and chicory seeds at 0.2%, 0.4% or 0.6% of diet did not change PCV and triglycerides, but it decreased total serum cholesterol in the serum of Cobb broiler chicks.

Thyroid Hormones

So far, there has been a scarcity of studies pertaining to the impact of the specific plant materials upon thyroid hormonal levels. It was concluded that garlic powders in the diets of broilers reared under normal and cold environmental conditions had no impact on T3 and T4 levels in blood plasma (Varmaghany et al., 2015). Apart from that, Berrama et al. (2017) found that thyroid hormones (T3, T4, and T3/T4 levels) measured in broilers’ plasma were not significantly affected by 0.2% dietary cumin during heat thermal stress conditions at 49 days.

Antibody Titer against Newcastle Disease and Gumboro Disease

Plant extracts can affect the gut-associated lymphoid tissue which plays the main role in cellular immunomodulation and adjustment of immunoglobulins’ secretion in animals (Vidaranarchi et al., 2005) with the increased weight of functional immunological organs such as the spleen, Bursa, and thymus (Khan et al., 2012b; Sang-Oh et al., 2013), and decreased heterophil to lymphocyte ratio (Naderi et al., 2014). Faghan et al. (2014) offered similar observations and stated that antibody titer against Newcastle Disease vaccine of broilers’ serum increased in the treated diet with GC or CN powder. However, based on previous works, no impact of dietary GC or CN was reported on antibody titers against the Newcastle, influenza (Toghyani et al., 2011) Gumboro, and infectious bronchitis in broilers at 21 days (Naderi et al., 2014).

CONCLUSION

Adding the dietary garlic, cinnamon, and black cumin powders as natural additives in the feed at level 4 mg/kg diet of broiler chickens resulted in a clear increase in birds’ performance, improve blood biochemistry, increase thyroid hormones, and increase Newcastle and Gumboro antibody titers. Based upon the premise, the disturbance of broiler production and physiology may be reduced by dietary supplementation of these safe (plant) extracts through their biological properties and the ability to ameliorate the adverse influence of productive and physiological traits of birds.

CONFLICT OF INTEREST

The authors declare no conflict of interest with any financial and personal issues or organization in relation to the materials used in this paper.

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