Efficacy of *Rumex nervosus* leaves or *Cinnamomum verum* bark as natural growth promoters on the growth performance, immune responsiveness, and serum biochemical profile of broiler chickens

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**ABSTRACT**

This study was conducted to evaluate the potential growth and health promotion of *Rumex nervosus* leaves (RNL) and *Cinnamomum verum* bark (CVB) supplementation as phytogenic growth promoters on broilers. For 34 days, 540 day-old broiler chicks were randomly assigned to nine groups. Birds were fed the basal diet supplemented with 1, 3, or 5 g/kg RNL, 2, 4, or 6 g/kg CVB. In addition, birds were fed the basal diet complemented with antibiotic (AGP), or basal diet without feed additive as controls. All bird vaccinated for IBV, IBDV, and NDV except negative control (NC). The selected indicators were measured and analysed. Broilers given 1 g RNL were numerically heavier at 34 days and gained more to a degree comparable to the AGP group ($p = .053$). The addition of CVB at 2 g resulted in the best-feed conversion up to 21 d ($p = .04$). Throughout the study, dietary treatments had no impact on feed intake. Except for ALT, none of the immune-related parameters or serum biochemical profile differed statistically between treatments when compared to vaccinated control group. When comparing the non-vaccinated un-treated group on day 34, vaccination increased significantly antibody titres to NDV and IBDV. In conclusion, dietary RNL and CVB, especially at low doses, attempted to maximise the performance without compromising health in broiler chickens under typical conditions. Further research into the use of natural herbs under challenging conditions is required to maximise performance, maintain animal productivity, and achieve the ultimate goal of reducing antibiotic use in the poultry industry.

**HIGHLIGHTS**

- *Cinnamomum verum* bark at a dose of 2 g/kg diet improved feed conversion ratio during the starter period. However, both selected herbs result unsatisfactory improvements in broiler performance at overall period under the ideal hygienic conditions.
- If broilers are exposed to bacterial challenges, phytogenic feed additives or even antibiotics may manifest their activity in promoting animal health and, as a result, growth performance.
- Low levels of hepatic enzymes at day 34 of age showed that birds’ health was good.
- Cinnamon powder improves kidney function at day 21 by lowering serum creatinine levels with increasing dose ($p < .05$).
- *Rumex nervosus* leaves or *Cinnamomum verum* bark have hypoglycaemia activity on day 31 by lowering serum glucose levels as the dose is reduced.

**Introduction**

Producing simple feeds that contain no or fewer drugs and reducing the impact of egg and meat products on human health are becoming the goals of researchers, organisations, and companies (Leeson 2008; WHO 2014; Andrew Selaledi et al. 2020). Antibiotics are used therapeutically to medications or subtherapeutically to promote growth or health in humans or animals. Antibiotics are used in poultry and other livestock diets to increase body weight, maximise feed efficiency or reduce feed conversion ratio, control pathogenic bacteria, and improve the quality of livestock products (Shao et al. 2021; Swaggerty et al. 2022). However, the excessive or indiscriminate use of antibiotics in livestock has been associated with an increase in multidrug-resistant germs (Shao et al. 2021).
Consumers could be affected directly by residues of antibiotic growth promoter (AGP) in meat or indirectly by the development of antibiotic-resistant pathogens in meat (Chowdhury et al. 2018). Therefore, many countries ban the use of antibiotics in livestock nutrition, EU ban under Council Regulation (EC) No 2821/98 in 2006 (Teymouri et al. 2021; Swaggerty et al. 2022).

Medicinal plants and their extracts or phyogenic agents have effective economic and excellent therapeutic effects, so they are used to treat a wide range of diseases in humans and livestock, improve zootechnical indices, optimise feed efficiency and increase animal production (Swaggerty et al. 2022). The therapeutic effects of spices and herbal feed additives are attributed to combinations of active phytochemicals and secondary metabolites such as organic acids, surfactants, terpenoids, chitin, enzymes, phenolic compounds, and aldehydes, which may exert antimicrobial, anti-inflammatory, antioxidative activities, etc. (Ahmadi and Shahri 2020; Gemechu et al. 2021). Some researchers have studied the growth performance, gut health, and ingredients utilisation of broilers (Attia et al. 2011; Attia and Al-Harthi 2015; Attia et al. 2017) or rabbit (Attia and Kamel 2012; Attia et al. 2019) whose diets were supplemented with natural herbs or their extracts.

*Rumex nervosus* has been reported as a traditional herbal medicine in Arab and African countries (Al-Sunafi 2016; Gemechu et al. 2021). There is insufficient or no evidence to support the use of RNL as an antibiotic replacement in broiler diets. Thus, the study proposed to use RNL as a supplemental feed for poultry to improve flock health and productivity due to its anti-inflammatory, antibacterial, and antioxidative properties, etc. In addition, RNL is rich in antioxidant enzymes and scavenges free radicals that can protect the intestinal mucosa and cells from lipid peroxidation (Ahmadi and Shahri 2020; Gemechu et al. 2021).

Cinnamon is an aromatic ethnomedicinal spice gotten from the inner bark of the *Cinnamomum verum* tree (Ali et al. 2021). The leaves and bark of cinnamon herb and their metabolites are receiving more attention as phyogenic feed additives replacing AGP as they are natural antibiotics, readily available, non-toxic and residue free (El-Hack et al. 2020). Cinnamon contains various phytochemicals such as cinnamaldehyde, eugenol, carvacrol, camphor and numerous macro and micronutrients (Ali et al. 2021). These bioactive compounds can contribute to herd health and performance through several pathways. For example, cinnamon can be used as an appetite stimulant and food preservative, it promotes digestion by stimulating digestive enzymes secretion (Ali et al. 2021). It has potent hypoglycaemic, hypcholesterolemic, antimicrobial and antioxidant properties (El-Hack et al. 2020). Although there are numerous scientific literature that have investigated the effect of cinnamon supplementation on performance, and health of broiler chickens. However, the doses used varied considerably, ranging from 0.2 to 70 g/kg (i.e., 0.02% to 7%), and the results were not consistent (Toghyani et al. 2011; Sang-Oh et al. 2013; Faghani et al. 2014; Hussein et al. 2016). Therefore, an appropriate dosage of cinnamon was chosen in this study to obtain satisfactory results consistent with previous studies.

The study hypothesised that the studied herbs could be used to improve feed conversion ratio, liver and kidney function, hypoglycaemia activity, and broiler chicken health because they contain a wealth of phytochemicals such as bioactive components and an abundance of macro- and micronutrients (Al-Sunafi 2016; Lusiana et al. 2019; El-Hack et al. 2020). To test this hypothesis, different dietary levels of RNL and CVB were investigated. Hence, this study aimed to assess the effects of adding dietary RNL and CVB as a substitute for antibiotics on growth performance, serum biochemical profile, and immune status of Ross broiler chickens fed a broiler diet till 34 days of age. The study also compare the effects of the vaccinated/herbs-treated groups on the selected variables with the vaccinated/antibiotic-treated group (AGP) and the vaccinated/untreated group (PC) and the nonvaccinated/untreated group (NC).

**Materials and methods**

Recently, we tested the bioactive constituents of RNL and CNB extracts by GC-MS and HPLC analysis. The constituents of RNL (gallic acid and other 11 volatile compounds) and of CNB (cinnamaldehyde and other 25 volatile compounds) were detected in our previous pre-starter study that presented HPLC and GCMS analysis (Azzam et al. 2020; Qaid et al. 2021). In addition, proximity analysis showed that the macronutrients of these plants are present in different ratios (Azzam et al. 2020; Qaid et al. 2021).

**Ethical approval**

The research was conducted at Poultry Production Experimental unit, King Saud University (KSU), in Riyadh, Saudi Arabia. Parameters were taken in accordance with Saudi Arabian standards for the use of...
animals and with the approval of the KSU Animal Care and Welfare Committee (KSU-SE-20-44).

**Bird husbandry, dietary treatments, and experimental design**

A total of 540 one-day-old chicks (Ross 308) were obtained from Al Khomasia Hatchery, Riyadh, Saudi Arabia. On delivery, chicks were sexed using feathers, weighed individually and allocated to nine treatments. From day 1 to 21, each treatment comprised ten replicates with six birds (3♂ & 3♀) for each replicated cage based on a completely randomised design (CRD). For days 21 to 34, the number of birds and replicates was reduced to half (270 birds; each group contain only five replicates). The chickens were kept under the same housing conditions in a cage with a length of 58 cm, a width of 50 cm and a height of 35 cm for a total of 34 days. Stocking density were 6 bird/0.30 m². The average outdoor temperature during the experimental period was around 26.4°C, and it was slightly to moderately humid. A lighting program (23L:1D) ‘23 h on:1 h off’ was used as the photoperiod.

RNL were harvested from the mountains and valleys around the village of Bait Al-Aqra, Ibb city, Yemen. The harvested RNL were air-dried every five days for 15 days under sunlight. A taxonomist at King Saud University, Department of Botany validated the plant botanical identity. Moreover, CVBs were purchased in Riyadh, Saudi Arabia, from a local store. Then, the dried leaves and bark were ground into a fine powder (particle size; 0.25–0.30 mm) using a blender in nutrition laboratory. Birds in treatment groups 1–3 were fed a basal diet supplemented with 1, 3 and 5 g RNL/kg feed, respectively. Birds in groups 4–6 received the basal diet plus 2, 4 and 6 g CVB/kg feed, respectively. The birds in group 7 were given the basal diet plus antibiotic growth promoter (AGP: Colimox ‘a combination of the antibiotics amoxicillin and colistin at 200 and 150 mg/kg, respectively’ based on Elbadawy and Aboubakr 2017). As control groups, birds in groups 8 and 9 were fed a basal diet (the positive control group was vaccinated and the negative control group was not vaccinated).

All chicks were vaccinated at the hatchery against avian infectious bronchitis virus (IBV), Newcastle disease virus (NDV), and infectious bursal disease virus (IBDV) except those in the negative control. Then, birds were immunised by instillation of a 0.03 mL drop by oral nasal route using booster dose against IBDV at 14 d and against NDV and IBV at 21 d of age according to the manufacturer’s directions (Fort Dodge Animal Health, USA). The following vaccinal strains were used: HB1 (106.5) to Newcastle disease, H120 (103.5) to infectious bronchitis and D78 (101.0) to infectious bursal disease. The supplemental herbs were included in the starter (days 1–21) and finisher (21–34 d) diets. Chickens were fed a mash diet and had *ad libitum* access to feed and water (nipple drinkers). The ingredients for the basal diet were obtained from ARASCO Company, Riyadh, Saudi Arabia. The experimental diets met or exceeded the requirements of the National Research Council (NRC 1994). Experimental diets were formulated for the starter (d 1-21 d) and finisher (21–34 d) phases (Table 1).

**Growth performance indices**

Birds were weighed individually at days 0, 21, and 34 during the study period. The average live body weight for each treatment were recorded and the average body weight gain were calculated using the equation body weight gain = final body weight – initial body weight. The average daily gain = body weight gains/period.

**Table 1.** The nutritional matrix of the control diet for the starter (1–21 d) and finisher (21–34 d) phases of broilers.

| Ingredient                        | Starter | Finisher |
|-----------------------------------|---------|----------|
| Yellow corn                       | 53.22   | 58.09    |
| Soybean meal                      | 37.85   | 32.15    |
| Wheat bran                        | 2.00    | 2.20     |
| Corn gluten meal                  | 1.40    | 0.00     |
| Choline chloride CL 60            | 0.05    | 0.05     |
| Corn oil                          | 1.50    | 4.20     |
| Dicalcium phosphate DCP           | 1.98    | 1.62     |
| Ground limestone                  | 0.90    | 0.79     |
| Salt                              | 0.40    | 0.30     |
| DL-methionine                     | 0.29    | 0.25     |
| Lysine-HCL                        | 0.21    | 0.11     |
| Vitamin-mineral premixa           | 0.20    | 0.20     |
| Total                             | 100.00  | 100.00   |
| Metabolic energy, kcal/kg         | 3200.00 | 3200.00  |
| Crude protein, %                  | 23.00   | 20.00    |
| Non phytate P, %                  | 0.48    | 0.41     |
| Calcium, %                        | 0.96    | 0.81     |
| Lysine, %                         | 1.28    | 1.06     |
| Sulphur amino acids, %            | 0.95    | 0.83     |
| Threonine, %                      | 0.86    | 0.71     |

*Vitamin-mineral premix comprises the following per kg: vitamin B1, 3200 mg; vitamin B2, 8600 mg; vitamin B3, 65,000 mg; pantothenic acid, 20,000 mg; vitamin B6, 4300 mg; biotin 220 mg; antioxidant ‘butylated hydroxyanisole (BHA) + butylated hydroxytoluene (BHT)’, 50,000 mg; B9, 2200 mg; B12, 17 mg; vitamin A, 12,000,000 international unit (IU); vitamin D3, 5,000,000 IU; vitamin E, 80,000 IU; vitamin K3, 3200 mg; copper, 16,000 mg; iron, 20,000 mg; iodine, 1250 mg; manganese, 120,000 mg; zinc, 110,000 mg; and selenium, 300 m. The basal diet supplemented with 1, 3, 5 g/kg diet *Rumex nervosus* leaves; 0, 2, 4, and 6 g/kg cinnamon; and antibiotic growth promoter ‘Colimox’ on top of feed.
Number of days. Feed intake was calculated weekly by weighing the remaining feed at the end of each period and subtracting it from the total amount offered at the beginning of the period, taking into account the number of dead birds and the number of days they were fed. Feed conversion ratio (g/g) was calculated as Feed intake/body weight gains.

**Lymphoid organs relative weights**

The birds (1 birds per replicate) were slaughtered in accordance to the official standards of King Saudi Arabia. After slaughter by severing the jugular vein, the chickens were bled for approximately two minutes before having their feathers plucked and viscera eviscerated. The carcases of the birds were then opened and the lymphoid organs were carefully removed and weighed separately. Percent weights of lymphoid organs were calculated relative to live body weight. For example, spleen yield was calculated as the spleen weight in relation to body weight. Thus, spleen yield = (spleen weight/live body weight) × 100).

**Blood sampling and safety assessment through biochemical analysis**

At 21 and 34 days of age, blood samples were taken from the chickens (1 birds per replicate) via the wing vein. The number of blood samples collected for each treatment ranged from four (Shabani et al. 2015), five (Attia et al. 2019), six (Attia et al. 2011; Hussein et al. 2016), and higher. Blood was allowed to clot completely at room temperature for two hours before serum was separated by centrifugation at 3000 xg at 30 °C for 15 min, and stored in clean, sterile tubes at −20 °C until biochemical parameters estimation.

A spectrophotometer and commercial diagnostic kits were used to determine the concentrations of biochemical parameters in serum; the test was performed according to the manufacturer’s instructions. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), and creatinine were used to evaluate liver and kidney function. In addition, glucose, cholesterol, total protein, and albumin levels were measured, while total globulin level was calculated (globulin = total protein − albumin).

**Determination of antibody titres against IBV, IBDV and NDV**

The antibody titres in chicken serum against IBV, IBDV and NDV virus were determined by enzyme-linked immunosorbent assay ‘ELISA test’, this methods as described by reagents manufacturers (IDEXX Laboratories Corporation, Inc., Columbia, MO). In briefly, one μL of test sample was diluted with 500 μL of sample diluent prior to being assayed. Samples were mixed thoroughly prior to dispensing into the antigen-coated plate. The reagents was allowed to come to 18–26 °C then was mixed gently by inverting and swirling. In duplicate wells, 100 μL of undiluted negative control was dispensed. In duplicate wells, 100 μL of undiluted positive control was dispensed. The 100 μL of diluted sample was dispensed into appropriate wells and then was incubated for 30 min at 18–26 °C. Liquid content of all wells was aspirated into appropriate waste reservoir. Each well was washed with approximately 350 μL of distilled or deionised water 3–5 times and was aspirated completely. Each well received 100 μL of conjugate. Incubation was repeated and liquid content was aspirated and washed. Each well received 100 μL of TMB substrate. Incubate for 15 min at 18–26 °C. To stop the reaction, 100 μL of stop solution was dispensed into each. The absorbance values were measured and record at 650 nm. The relative level of antibody in the sample was determined by calculating the sample to positive (S/P) ratio. Endpoint titres were calculated using the following equation:

\[
\log_{10} \text{titre} = 1.09 (\log_{10} S/P) + 3.36
\]

**Statistical analysis**

All data were subjected to one-way ANOVA using the general liner model (PROC GLM) of Statistical Analysis System (SAS Institute, Inc., Cary, NC) software (SAS 2012). The model equation is described as follows:

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

where \( Y_{ij} \) is the individual observation, \( \mu \) is the overall mean, \( T_i \) is the effect of the \( i \)th dietary treatment as a fixed main effect, and \( e_{ij} \) is the random normally
distributed error with mean zero and variance \( \sigma^2 \) \( \epsilon \sim \mathcal{N} (0, \sigma^2) \). Each cage represents an experimental unit in terms of performance parameters. For the other parameters chosen, one bird from each cage serves as an experimental unit. Duncan’s Multiple Range Test was used for comparisons between the means, and results were considered statistically significant at \( p < .05 \).

## Results

### Growth performance indices

Data on performance parameters of broilers are summarised in Table 2. During the early stages of broiler development, treatments had no effect on broiler body weight; however, as broiler age progressed, there was an intended to be (\( p = .072 \)) impact of treatments on broiler body weight. Treatments had no effect on body weight gain at the beginning (days 1–21) or end (days 22–34) of the rearing period, but treatments intended to have an effect (\( p = .073 \)) on gain throughout the growth period (days 1–34). Therefore, broilers receiving 1 g RNL were intended to be heavier at slaughter age (34 days) and gained more during overall period to a degree comparable to the AGP group. The treatments had no effect on cumulative feed consumption at starter (days 1–21), finisher (days 22–34) or even over the entire period (days 1–34). Thus, the experimental treatments had no effect on daily feed intake (\( p > .05 \)).

Birds fed 2 g/kg CVB during the starter period (days 1–21) had significantly better feed conversion ratio than the antibiotic and both control groups (\( p < .05 \)) and were not differ from other groups. However, as broilers aged, treatments had no effect on feed conversion ratio in the finisher period or total period (days 1–34). Thus, supplementing the diet with 2 g/kg CVB significantly (\( p < .05 \)) improved feed conversion ratio in the starter period, but did not affect broiler feed conversion ratio in other periods (finisher and total period). All chickens had a healthy status, with no mortality being recorded even in negative control group.

### Lymphoid organ development and immune status

The effects of the control and treatment diets on the relative weights of the lymphoid organs are shown in Table 3. At day 34, there was no difference (\( p > .05 \)) between treatments in the percent weights of the lymphoid organs.

No significant effect of the treatments on IBV, IBDV and NDV antibody titres was observed (\( p > .05 \)) at day 21 and 34 of age compared to vaccinated/control group (Table 4). However, when comparing the vaccinated/treated groups to the non-vaccinated/untreated group at day 34, vaccination against IBDV and NDV titres produces enhance responsiveness of antibody titres against IBDV and NDV.

### Serum biochemistry profile

Tables 5 and 6 summarise the effects of the dietary treatments on the biochemical serum parameters of broilers at days 21 and 34, respectively. Addition of selected herbs to the diet of birds at days 21 and 34...
had no effect \((p > 0.05)\) on tested serum biochemical parameters. However, the addition of selected herbs to the diet of broilers has effect \((p < 0.05)\) on serum glucose and ALT concentration at 34 day and creatinine levels at day 21 of age.

### Discussion

The birds were in good health and had no health problems during this experiment. Colimox, amoxicillin and colistin combination, was used here as AGP based on Elbadawy and Aboubakr (2017). As far as we know, there are few if any reports investigating the performance of broiler chickens fed RNL. Most of the work published in the literature shows in vitro activity of RNL rather than in vivo effects. Furthermore, a wide range of CVB concentrations used in previous studies from 1 g (Hussein et al. 2016) to 70 g (Safa Eltazi 2014) was required to see it from this angle in this study.

The primary goal of the starter diet, on the other hand, is to provide sufficient calories to manage potential stress during the initial post-hatching period. The beneficial effect of cinnamon supplements on feed conversion ratio was more noticeable at younger ages, as body weight gains and feed intake were not negatively affected. This was not reflected in slaughter age, probably because the nutritional requirements of older birds decrease with age and they have a better-developed digestive tract and organs. In addition, RNL at a concentration of 1 g/kg resulted in an insignificant increase in target body weight (day 34), and an insignificant increase in body weight gains during period 1–34. The beneficial effect of AGP inclusion on body weight gains persists until slaughter age. Although the body weight gains of the chicks in the RNL group was lower at 1 g/kg in the starter phase, this was compensated in the finisher phase, so they intended to have the highest gains in the whole period. Toghyani et al. (2011) suggested that 2 g/kg CVB can be used in broilers diets as AGP substitutions, which is partially similar to our findings. Their findings indicated that chicks had increased body weight, decreased feed

### Table 3. Relative lymphoid organ weights at 34 d of birds fed control and treatment diets.

| TRT  | Live weight | Bursa% | Spleen% | Thymus% |
|------|-------------|--------|---------|---------|
| T1 (1 RNL) | 1795* | 0.16 | 0.10 | 0.41 |
| T2 (3 RNL) | 1801 | 0.14 | 0.11 | 0.35 |
| T3 (5 RNL) | 1794 | 0.23 | 0.11 | 0.43 |
| T4 (2 CVB) | 1816 | 0.19 | 0.11 | 0.49 |
| T5 (4 CVB) | 1777 | 0.19 | 0.08 | 0.52 |
| T6 (6 CVB) | 1752 | 0.24 | 0.08 | 0.55 |
| T7 (AGP) | 1832 | 0.20 | 0.09 | 0.43 |
| T8 (NC) | 1759 | 0.21 | 0.08 | 0.42 |
| T9 (PC) | 1667 | 0.18 | 0.09 | 0.40 |
| SEM | 0.912 | 0.235 | 0.448 | 0.781 |

*RNL: Rumex nervosus leaves, \(\cdot\)CVB: Cinnamomum verum bark, \(\cdot\)AGP: antibiotic growth promoter ‘Colimox’, \(\cdot\)PC: positive control group = basal diet only, vaccinated. \(\cdot\)NC: negative control group = basal diet only, no vaccinated. \(\cdot\)Mean of five birds per treatment.

### Table 4. Humoral immune responses (log \(_{10}\) antibody titres) against infectious bronchitis virus (IBV), Newcastle disease virus (NDV), and infectious bursal disease (IBDV) on days 21 and 34 in birds fed control and treatment diets.

| Antibody titres at d 21* | Antibody titres at d 34* |
|--------------------------|--------------------------|
| Treatments (g/kg diet)   | IBV | NDV | IBDV | IBV | NDV | IBDV |
| T1 (1 RNL) | 3.00 | 3.16 | 2.98 | 3.04 | 3.25* | 2.20* |
| T2 (3 RNL) | 3.20 | 3.05 | 2.87 | 3.04 | 3.48* | 2.44* |
| T3 (5 RNL) | 3.18 | 3.18 | 2.75 | 2.80 | 3.28* | 2.40* |
| T4 (2 CVB) | 3.25 | 3.13 | 2.94 | 3.15 | 3.59* | 2.14* |
| T5 (4 CVB) | 3.23 | 3.06 | 2.94 | 3.03 | 3.45* | 2.29* |
| T6 (6 CVB) | 3.08 | 3.09 | 2.66 | 2.93 | 3.43* | 1.97ab |
| T7 (AGP) | 2.95 | 3.12 | 3.13 | 2.89 | 3.45* | 1.62ab |
| T8 (NC) | 3.05 | 2.81 | 2.71 | 2.93 | 1.62* | 1.26 |
| T9 (PC) | 3.16 | 2.93 | 2.88 | 2.98 | 2.72* | 2.02ab |
| SEM | 0.027 | 0.033 | 0.046 | 0.027 | 0.140 | 0.094 |
| Probability | 0.189 | 0.187 | 0.214 | 0.001 | 0.017 |

*RNL: Rumex nervosus leaves, \(\cdot\)CVB: Cinnamomum verum bark, \(\cdot\)AGP: antibiotic growth promoter ‘Colimox’, \(\cdot\)PC: positive control group = basal diet only, vaccinated. \(\cdot\)NC: negative control group = basal diet only, no vaccinated. \(\cdot\)Mean of ten and five birds per treatment at day 21 and 34 days, respectively; \(\cdot\)Probability in the same column with different superscripts differ significantly \((p < 0.05)\).

### Table 5. Serum biochemical parameters at d 21 of birds fed control and treatment diets.

| Treatments (g/kg diet) | TP (g/dL) | Albumin (g/dL) | Globulin (g/dL) | Glucose (mg/dL) | Cholesterol (mg/dL) | Creatinine (mg/dL) | ALT (U/L) | AST (U/L) |
|------------------------|-----------|----------------|----------------|----------------|---------------------|-------------------|----------|----------|
| T1 (1 RNL) | 4.02* | 2.20 | 1.82 | 263.44 | 112.03 | 0.61a | 13.37 | 149.03 |
| T2 (3 RNL) | 3.96 | 2.09 | 1.87 | 247.11 | 119.76 | 0.51ab | 17.93 | 151.71 |
| T3 (5 RNL) | 4.50 | 2.43 | 2.07 | 273.89 | 130.12 | 0.56ab | 16.04 | 152.01 |
| T4 (2 CVB) | 4.14 | 2.34 | 1.80 | 244.86 | 134.51 | 0.55ab | 13.02 | 149.71 |
| T5 (4 CVB) | 4.15 | 2.39 | 1.76 | 270.67 | 133.61 | 0.50ab | 19.65 | 148.94 |
| T6 (6 CVB) | 4.31 | 2.16 | 2.15 | 250.89 | 130.03 | 0.36b | 16.73 | 151.26 |
| T7 (AGP) | 4.30 | 2.26 | 2.04 | 269.33 | 141.29 | 0.45ab | 15.67 | 186.87 |
| T8 (NC) | 3.27 | 1.74 | 1.53 | 280.56 | 135.05 | 0.63a | 16.02 | 144.85 |
| T9 (PC) | 3.77 | 2.35 | 1.42 | 251.78 | 138.20 | 0.59ab | 15.94 | 147.96 |
| SEM | 0.10 | 0.09 | 0.09 | 4.37 | 3.21 | 0.03 | 0.66 | 3.89 |
| Probability | 0.177 | 0.11 | 0.44 | 0.46 | 0.51 | 0.02 | 0.40 | 0.31 |

*RNL: Rumex nervosus leaves, \(\cdot\)CVB: Cinnomomum verum bark, \(\cdot\)AGP: antibiotic growth promoter ‘Colimox’, \(\cdot\)PC: positive control group = basal diet only, vaccinated. \(\cdot\)NC: negative control group = basal diet only, no vaccinated. \(\cdot\)Mean of ten birds per treatment. TP: Total protein; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase.
Also, Hussein et al. (2016) indicated that birds fed cin-

Table 6. Serum biochemical parameters at d 34 of birds fed control and treatment diets.

| Treatments (g/kg diet) | TP (g/dL) | Albumin (g/dL) | Globulin (g/dL) | Glucose (mg/dL) | Cholesterol (mg/dL) | Creatinine (mg/dL) | ALT (U/L) | AST (U/L) |
|-----------------------|-----------|----------------|-----------------|----------------|---------------------|-------------------|-----------|-----------|
| T1 (1 RNL<sup>1</sup>) | 2.80<sup>s</sup> | 1.50           | 1.30            | 184.3<sup>s</sup> | 92.1                | 0.53              | 13.52<sup>k</sup> | 130.53    |
| T2 (3 RNL)           | 3.59      | 1.85           | 1.74            | 188.5<sup>h</sup> | 85.5                | 0.63              | 10.61<sup>bc</sup> | 133.39    |
| T3 (5 RNL)           | 3.70      | 1.89           | 1.81            | 236.0<sup>h</sup> | 83.7                | 0.46              | 8.01<sup>c</sup>  | 85.09     |
| T4 (2 CVB<sup>3</sup>)| 3.36      | 2.05           | 1.31            | 170.4<sup>h</sup> | 105.0               | 0.50              | 8.86<sup>d</sup>  | 110.63    |
| T5 (4 CVB)           | 3.52      | 1.88           | 1.64            | 196.3<sup>k</sup> | 104.1               | 0.44              | 9.81<sup>c</sup>  | 54.38     |
| T6 (6 CVB)           | 2.94      | 1.55           | 1.39            | 210.0<sup>h</sup> | 109.1               | 0.44              | 9.51<sup>c</sup>  | 127.08    |
| T7 (AGP<sup>4</sup>) | 3.91      | 2.18           | 1.73            | 239.0<sup>s</sup> | 95.2                | 0.69              | 11.27<sup>bc</sup> | 114.46    |
| T8 (NC<sup>5</sup>)  | 3.21      | 1.64           | 1.57            | 232.6<sup>h</sup> | 113.3               | 0.51              | 17.39<sup>c</sup> | 139.70    |
| T9 (PC<sup>6</sup>)  | 2.82      | 1.47           | 1.35            | 207.2<sup>h</sup> | 114.9               | 0.51              | 18.25<sup>c</sup> | 133.88    |
| SEM<sup>s</sup>       | 0.14      | 0.39           | 0.06            | 7.62            | 2.99                | 0.03              | 0.62<sup>c</sup>  | 7.21      |
| Probability           |           |                |                 |                |                     |                   |           |           |

<sup>1</sup>RNL: Rumex nervosus leaves. <sup>2</sup>CVB: Cinnamomum verum bark. <sup>3</sup>AGP: antibiotic growth promoter Colimox. <sup>4</sup>NC: negative control group = basal diet only, no vaccinated. <sup>5</sup>PC: positive control group = basal diet only, vaccinated. <sup>6</sup>SEM, standard error of mean for the effect of treatments. <sup>7</sup>Mean of five birds per treatment; a,b,cMeans in the same column with different superscripts differ significantly (p<.05). TP: total protein; ALT: alanine aminotransferase; AST: aspartic aminotransferase.

conversion ratio, and unaffected feed intake at d 28. Also, Hussein et al. (2016) indicated that birds fed cinnamom powder at concentrations of 0.25, 0.50, 0.75, and 1.00 g had significantly higher live weight and improved feed conversion ratio in the first 3 weeks compared with control, but their feed intake was lower.

The results of present study is partial consistent with the previous findings that the addition of 100 or 200 ppm cinnamon oil (Al-Kassie 2009) and 500 or 1000 mg cinnamon powder/kg (Khafaji 2018) had a significant effect on performance. However, Lee et al. (2003) did not observe any effect on the performance of female broiler chickens fed cinnamaldehyde enriched diets. Similarly, Sadeghi et al. (2012) found that cinnamon infusion did not benefit broiler performance. RNL or CVB contributed as phytogenic mixture through their secondary metabolites such as flavonoids and phenols with antioxidant and other medicinal properties (Ahmadi and Shahri 2020; Gemechu et al. 2021).

Throughout the experiment, the treatment diets had no effect on feed intake. Both herbs tested have a rather bitter taste and intense odour, which may affect appetite and feed intake. In agreement, Chowlu et al. (2019) stated that the use of CVB powder, regardless of its amount, had not affect feed intake, perhaps due to the fact that broiler chickens tolerated its smell and taste (light citrus notes and sweet, spicy, pungent perfume) better.

Because RNL and CVB potentially have broad antimicrobial activity as mentioned in previous chapter, it was expected that immune responses would be elevated. However, none of the measured immunological parameters, including antibody titres responses and lymphoid organ weight, were stimulated either positively or negatively in our study. It is probably that a higher dosage of the herbal natural feed supplements is required to stimulate the humoral immune response. Although antibody titres in broiler chickens improved significantly in PC compared to NC at day 34 in response to NDV virus vaccines, there were no differences between experimental treatments compared to PC. This could be explained by the fact that vaccination with a boosted dose of NDV virus on day 21 improves immune response on day 34, but the selected herbs do not.

Although there are few reports on the effect of Rumex nervosus or Cinnamomum verum on immune response in broilers, we agree with Toghyani et al. (2011) who found that addition of 2 and 4 g/kg of cinnamon were not boost the serological response of birds to NDV and influenza virus vaccines. In agreement with Toghyani et al. (2011), the treatments did not significantly affect the relative weights of the lymphoid organs.

The biochemical parameters in the serum can provide information about the physiological state and nutrient metabolism of the body. Feeding birds with diets supplemented with selected herbs especially the inclusion of herbs at lowest levels CVB at 2 g level and RNL at 1 g level, in diets resulted in the lowest glucose levels than those of birds fed diets containing the AGP and the NC group at day 34. Serum glucose levels were within or nearest normal published values, including the control groups. Normal glucose levels have been given as 200–250 mg/dL (Thaxton et al. 2006). Some studies suggest that cinnamon possess hypoglycaemic effects in broilers (Al-Kassie 2009; Faghani et al. 2014; Hussein et al. 2016), a mechanism attributed to the role of cinnamon and its derivatives in protecting the pancreas by improving oxidative stress and proinflammatory milieu in diabetic (Sun P et al. 2016).

Moreover, the results showed that that the differences in cholesterol concentration at 34 days of age did
not reach statistical significance, but tended to decrease in the RNL and antibiotic groups compared to the CVB and control groups. Many studies have found that cinnamon lowers cholesterol levels (Al-Kassie 2009; Ciftci et al. 2010; Nabiela et al. 2013; Faghani et al. 2014; Najafi and Taherpour 2014; Hussein et al. 2016). Cholesterol levels in all groups were within or nearest the normal range of 100–200 mg/dL (Thaxton et al. 2006). Several active compounds, including cinnamaldehyde, carvacrol and thymol can cause hypocholesterolemia by inhibiting the hepatic enzyme regulating cholesterol synthesis, 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase (Lee et al. 2003).

Albumin as the principal protein have varies affinity binding to polyphenol compounds according to the polyphenol chemical structure. The binding site of cinnamaldehyde and its major metabolite binds to serum albumin at site I and site II, respectively (Sun Q et al. 2018). The body requires albumin and total protein to fight infection and perform other functions; when their levels are below normal; this indicated liver damage or other diseases. The increase in serum albumin may be related to the improvement in protein synthesis in the liver and amino acid transport, which is associated with less oxidative stress and toxicity (Sitar et al. 2013). The values of albumin and total protein were within the normal values reported by (Campbell 2004): 0.8–2 g/dL and 2.5–4.5 g/dL, respectively. The presence or absence of an herbal effect can be attributed to the various biochemical transformations that affect the bioavailability and potency of the molecular structures of polyphenols, which affect the extent of their intestinal absorption and the nature of the circulating metabolites in plasma. Phytogenic feed additives such as thyme and cinnamon have been reported to increase serum albumin (Al-Kassie 2009; Sadek et al. 2014), total protein and globulin (Khafaji 2018). One of the active compounds detected in thyme extract and RNL was gallic acid. Gallic acid was observed to bind to human serum albumin and bovine serum albumin (Chanphai and Tajmir-Riahi 2020). However, other studies reported that feed supplements with 2 g and 4 g cinnamon had no effect on serum albumin or albumin to globulin ratio (Toghyani et al. 2011).

Broilers supplemented with RNL and CVB on days 21 and 34 showed no changes in aspartate-amino-transferase levels (AST), indicating no liver or muscle damage. Although AST is a sensitive and non-specific marker of hepatocellular disease in most birds, it did not need to be measured together with a muscle-specific enzyme such as creatine kinase to distinguish hepatic from muscular lesions because no liver or muscle lesions were observed here. Koochaksaraie et al. (2011) found that cinnamon had no effect on AST, or ALT levels. Serum ALT and AST enzymes are generally found in low levels in the bloodstream, and their levels increase when liver or muscle damage occurs. Therefore, they are considered safety indicators and may reflect either normal liver metabolic function or the degree of liver injury (Brancaccio et al. 2010; Wang et al. 2013).

Selected biochemical serum parameters of broiler chickens were not affected by the addition of RNL or CVB to the diet of broiler chickens during all sampling periods, with exception of glucose and ALT levels at day 34 of age. Although serum ALT enzyme levels differed significantly between experimental groups, they were found in low concentrations in the bloodstream. On the other hand, these results indicate that RNL or CVB at the dose used in this study has no adverse effects on blood biochemical parameters chickens particularly in the relation of blood biosafety of tested herbs, as reflected by no adverse changes in liver and kidney functions. This means that RNL or even CVB can be added to the diet of broiler chickens up to 5 g RNL and up to 6 g CVB per kg diet without any adverse effects. There is a reason for the difference between this study and others: most previous studies used either seeds or stems, leaves or a mixture of compounds or essential oils, different amounts of inclusions, different breeds of chickens, different herbal sources, or different experimental conditions and compositions. There are few or no studies that have investigated the effect of the studied herbs, especially Rumex nervosus, on blood constituents in poultry.

Conclusions
Antibiotics as growth promoters are no longer permitted, resulting in an increase in demand for natural phytogenic feed additives. Dietary supplementation with 1 g/kg RNL intended to have increase target body weight and overall gain to degree nearest to AGP. In addition, 2 g/kg CVB may be considered as a beneficial phytogenic feed additive to improved feed conversion to meat in broilers up to 21 days. The data suggest that using RNL and CVB in broiler diets did not result in satisfactory improvements in broiler performance or health status under the ideal hygienic conditions. However, if the experiment had been conducted under natural or artificial bacterial challenges,
the herbs would have had positive effects. Therefore, we suggest implemented during this status a similar study be conducted using a bacterial challenge such as Clostridium perfringens or salmonella to allow tested herbs or antibiotics to express their antimicrobial activity and promote animal health and consequently the growth performance.

**Ethical approval**

The research was conducted at Poultry Production Experimental unit, King Saud University (KSU), in Riyadh, Saudi Arabia. Parameters were taken in accordance with Saudi Arabian standards for the use of animals and with the approval of the KSU Animal Care and Welfare Committee (KSU-SE-20-44).

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No potential conflict of interest was reported by the author(s).

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