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Growth performance of broiler starter chicks fed diets containing different levels of aqueous *Citrus aurantium* stem bark extracts

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ABSTRACT

Two hundred, 1-day old broiler chicks (Arbo acres) were used to evaluate the growth performance of broiler starter fed diets having different levels of aqueous *Citrus aurantium* stem bark (CASB). Birds were randomly distributed into 5 groups, each with 5 replicates consisting of 10 birds each in a completely randomized design. Treatment 1 (T1) basal diet + Ciprofloxacin 0.2 mL/litre of water, T2 (basal diet + 10 mL/litre CASB), T3 (basal diet + 20 mL/litre CASB), T4 (basal diet + 30 mL/litre CASB) and T5 (basal diet + 40 mL/litre CASB). Feed and water were provided *ad libitum* throughout the experiment which lasted for 21 days. Gas chromatography –mass spectrometry (GC-MS) was also carried out on CASB and the result revealed the presence of 23 bioactive compounds which accounts for 96.76 %. The most abundant secondary metabolite is D-limonene (50.06 %) followed by α-cubebene (8.49 %), linalool (6.02 %), β-citrylidene ethanol (4.30 %), myrcene (3.44 %), α-longipinene (2.75 %), β-santalene (2.50 %), terpinen-4-ol (2.04 %), γ-eudesmol (1.93 %), α-pinene (1.71 %), Cis-4-thujanol (1.67 %), β-fenchol (1.40 %), γ-terpinene (1.10 %), carvenone (1.10 %), β-cayrophyllene (0.77 %), 4-methyl-2,3-hexadien -1-ol (0.17 %), capraldehyde (0.16 %), phytol (0.10 %), spathulenol (0.10 %), torreyol-α-cadinol (0.07 %), 3-methoxy-p-cymene (0.01 %) and β-Elemene (0.01 %) respectively. Average daily weight gain and feed conversion ratio were significantly (P <0.05) different among the treatments. Feeding birds CASB 10 mL to 40 mL/liters increased the average daily feed intake though not significantly (P >0.05). It was concluded that CASB is rich in several bioactive compounds with therapeutic properties and it could be fed to broilers up to 40 mL per liter of water without causing any deleterious effect on the performance of birds.

Keywords: *Citrus aurantium*, broiler chicks, growth, gas chromatography, performance

1. INTRODUCTION

Antibiotics used as a prophylactic agent have shown to have positive effects on the growth performance of birds as a presumed result of reduced pathogen load, and reduction in competition for nutrients in the small intestine, reduction of inflammation, and improvement of digestion (Thomke and Elwinger, 1998;

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Olafadehan *et al*., 2021). Antibiotics are chemical compounds that kill or inhibit bacterial growth, however, their prolonged use lead to antimicrobial resistance and deposit of harmful residue in animal products such as meat, egg and milk (Agubosi *et al*., 2022). This prompted the European Union in 2006 to place a ban on the prophylactic use of antibiotics in animal feed to promote food safety and livestock production.

Presently, the use of medicinal plants is gaining more interest from consumers because of the increasing awareness on the dangers of synthetic additives (Oluwafemi *et al*., 2020; Alagbe, 2017). Plants naturally contains phytochemicals which are cheap, effective and are generally recognised as safe (GRAS) which make them good candidates to be used as feed additives in poultry production in comparison with antibiotics (Hashemi *et al*., 2008; Alagbe, 2017). Plant extracts contains bioactive compounds (phytochemicals) which are capable of improving animal's health and performance because of their anti-bacterial (Shittu *et al*., 2021), antioxidant, antiviral and hepatoprotective properties (Olafadehan *et al*., 2021), and their ability to modulate gut microbiota (Hashemi *et al*., 2009) and enhance immune responses (Chowdhury *et al*., 2018). There are several species of medicinal plants that have been reported to be reservoir of biologically active compounds with therapeutic properties (Dilfuza *et al*., 2015). Among the potential medicicinal plants is *Citrus aurantium*.

Citrus aurantium also known as sour orange is a fruit-bearing tree native to Asia. It belongs to the family Rutaceae and the tree is characterized by a greenish-white and glabrous shoot (Periyanayagam *et al*., 2013). It is also rich in several bioactive compounds like; alkaloids, tannins, flavonoids, oxalates, saponins and glycosides which have been reported to perform multiple biological activities (Simon *et al*., 2013; Jeong *et al*., 2002). The dried stems and roots traditionally known to be useful for the treatment of stomach ache, vomiting, blood pressure, cough, cold, bronchitis, ear ache, dysentery, diarrhea, abdominal pain and fever (Jeong *et al*., 2002; Gutierrez *et al*., 2008).

Previous studies have shown that phytogenic feed additives exhibits high biological activities resulting in growth promotion when used as feed additives in poultry (Lee, 2002; Platel and Srinivasan, 2000). Another study showed that aqueous Moringa and *Daniellia oliveri* leaf extract contains a high phenolic content that exhibits antioxidant and antimicrobial properties (Alabi *et al*., 2016; Adewale *et al*., 2021; Singh *et al*., 2021). However, there are inconsistencies in the results due to variation in their chemical composition, dosage, anti-nutrients, influences of locations, climatic conditions, harvest and storage conditions (Alagbe, 2022).

Therefore, this experiment was carried to examine the growth performance of broiler starter chicks fed diets containing different levels of aqueous *Citrus aurantium* stem bark extracts.

2. MATERIALS AND METHODS

Experimental site

The experiment was carried out at Division of Animal Nutrition, Sumitra Research Institute, Gujarat, India with a coastline of 1,600 Km, 23° 13′N 72°41′E.

Source, extraction and identification of bioactive compounds in *Citrus aurantium* stem bark

Fresh stem of *Citrus aurantium* were obtained within Sumitra Research Institute, India in the month of March, 2022. It was authenticated by a qualified taxonomist Dr. Kumar, cut into pieces and thoroughly washed with running tap water to remove sand and other dirt's. It was then shade dried for 10 days and the dried samples were pulverized into powder using pestle and mortar, thereafter 200 grams of the sample was soaked into 1000 litres of water for 72 hours and stirred 3 hours interval and kept in the refrigerator at 4°**C**. All mixtures were filtered using Whatman filter paper and the filtrates (CASB) were collected into a clean labeled plastic container for further analysis.

GC-MS analysis of aqueous CASB was carried out using a Perkin-Elmer GC clarus 500 system and gas chromatograph interfaced to a mass spectrometer equipped with an Elite-I fused silica capillary column (30m × 0.25 mm × ID × 1μm). Injection temperature was maintained at 25°C, helium flow rate as 1.5ml/min and ion source temperature at 230°C. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Identifications of the compounds were based on mass spectral matching with standard compounds in National Institute of Standard and Technology (NIST) having more than 62000 patterns.

Birds, housing and experimental diets

Two hundred, 1-day old broiler chicks (Arbo acres) were used for the experiment. The birds were purchased from a commercial hatchery in India and randomly distributed into 5 groups, each with 5 replicates consisting of 10 birds each. A battery cage with dimension 200 cm × 100 cm × 80 cm (length × breath × height) of 100 cm above the ground was used for the experiment. The cages

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were placed in a semi closed well ventilated pens which was properly fumigated and labeled for easy identification before the commencement of the experiment. Birds were given anti-stress on arrival; feeds were formulated based on NRC (1994) requirements for broilers. 200 watts bulb was installed in the pens to supply heat to birds; foot dip was placed at the entrance of the pen for proper biosecurity. Feed and water were offered *ad libitum* all other management practices were strictly adhered to throughout the experimental period which lasted for 21 days.

Experimental design

Completely randomized design was used for the experiment. The treatments were assigned to receive treatment diet as follows: Treatment 1 (T1) basal diet + Ciprofloxacin 0.2 mL/litre of water, T2 (basal diet + 10 mL/litre CASB), T3 (basal diet + 20 mL/litre CASB), T4 (basal diet + 30 mL/litre CASB) and T5 (basal diet + 40 mL/litre CASB).

Measurements

Daily feed intake (g) was calculated as a difference between feed offered and left-over.

Body weight gain (g) was calculated by subtracting the final live weight from the initial weight.

Average daily weight gain (g) was calculated by dividing the body weight gain by the number of experimental days.

Average total feed intake was calculated by dividing the total feed intake by the number of experimental days.

Proximate compositions of experiment diet were determined by using official method of analysis by AOAC (2000).

Statistical analysis

All data were subjected to one -way analysis of variance (ANOVA) using SPSS (23.0) and significant means were separated using Duncan multiple range tests (Duncan, 1955). Significant was declared if $P \leq 0.05$.

The model: $Txy = \mu + \alpha x + \beta xy$ was used in this experiment:

Where Txy = any of the response variables; x = the overall mean; αx = effect of the xth treatment and βxy = random error due to experimentation

Table 1: Composition of experimental diets (0-21 days)

Materials	Quantity
Maize	50.00
Wheat offal	6.00
Soya meal	30.55
Groundnut cake	10.00
Fish meal (72%)	2.00
Bone meal	0.60
Limestone	0.30
Lysine	0.20
Methionine	0.20
*Premix	0.25
Salt	0.30
Total	**100.0**
Determined analysis	
Crude protein (%)	23.64
Ether extract (%)	5.03
Crude fibre (%)	3.17
Calcium (%)	0.98
Phosphorus (%)	0.46
Lysine (%)	1.17
Meth +Cyst (%)	0.87
ME (Kcal/kg)	2940.5

*Starter premix supplied per kg diet: - vit A, 13,000 I.U; vit E, 5mg; vit D3, 3000I.U, vit K, 3mg; vit B2, 5.5mg; Niacin, 25mg; vit B12, 16mg; choline chloride, 120mg; Mn, 5.2mg; Zn, 25mg; Cu, 2.6g; folic acid, 2mg; Fe, 5g; pantothenic acid, 10mg; biotin, 30.5g; antioxidant, 56mg.

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Table 2: GC-MS compositions of *Citrus aurantium* stem bark

Compounds	Area (%)	R.T (min)	Activities
Myrcene	3.44	9.21	Antioxidant and anti-inflammatory
Linalool	6.02	13.16	Anti-bacterial, antivial and antifungal
γ-terpinene	1.10	13.73	Hepato-protective and cytotoxic
β-fenchol	1.40	15.74	Antifungal, antipyretic and antiviral
Cis-4-thujanol	1.67	10.33	Antioxidant and anti-inflammatory
Carvenone	1.10	9.67	Antioxidant and anti-proliferative
β-santalene	2.50	10.12	Anticancer, antioxidant and antiviral
α-cubebene	8.49	17.38	Antioxidant and anticancer
β-cayrophyllene	0.77	19.80	Anti-inflammatory, antitumor, antifungal
D-limonene	50.06	20.57	Anti-inflammatory, antipyretic, analgesic
α-longipinene	2.75	25.41	Hypo-cholesterolemic, antimicerobial
Terpinen-4-ol	2.04	24.33	Anti-fibrotic, antioxidant, antibacterial
α-pinene	1.71	27.06	Anti-tumor and anti-inflammatory
γ-terpinene	0.94	27.55	Anti-helmithic, antifungal and antiviral
γ-eudesmol	1.93	29.10	Anti-coagulant and anti-inflammatory
Capraldehyde	0.16	29.55	Anti-proliferative, cytotoxic, antifungal
Torreyol-α-cadinol	0.07	30.80	Anti-malarial, antioxidant, cytotoxic
β-citrylideneethanol	4.30	30.51	Antiviral, anti-inflammatory, cytotoxic
Caryophyllene	5.50	12.81	Anti-tumor and anti-inflammatory
Phytol	0.10	18.93	Anti-helmithic, antifungal and antiviral
4-methyl-2,3-hexadien -1-ol	0.17	33.19	Antioxidant and anti-inflammatory
Spathulenol	0.10	29.16	Anti-inflammatory, antitumor, antifungal
3-methoxy-p-cymene	0.01	34.05	Anti-fibrotic, antioxidant, antibacterial
β-Elemene	0.01	34.01	Antioxidant and anti-inflammatory
Total	96.76		

Table 3: Performance traits of broiler chickens fed different level of *Citrus aurantium* stem bark

Parameters	T1	T2	T3	T4	T5	SEM	*P*-value
(1-21 days)							
IBW (g)	51.61	50.93	51.11	52.02	51.88	0.81	0.25
FBW (g)	600.8^{b}	788.7^{a}	800.7^{a}	807.3^{a}	811.7^{a}	16.94	0.17
BWG (g)	549.19^{b}	737.77^{a}	749.59^{a}	755.28^{a}	759.82^{a}	15.27	0.44
ADWG (g)	26.15^{a}	35.13^{a}	35.69^{a}	35.97^{a}	36.18^{a}	5.22	0.71
TFI (g)	972.1	988.7	990.1	995.6	965.1	18.10	0.04
ADFI (g)	46.29	47.08	47.15	47.41	45.96	0.81	0.06
FCR	1.77^{c}	1.34^{b}	1.32^{b}	1.32^{b}	1.27^{a}	0.06	0.01

Means in the same row with different superscripts differ significantly ($P<0.05$); IBW: initial body weight; FBW: final body weight; BWG: body weight gain; ADWG: average daily weight gain; TFI: total feed intake; ADFI: average daily feed intake; FCR: feed conversion ratio

3. RESULTS AND DISCUSSION

GC-MS compositions of *Citrus aurantium* stem bark

The GC-MS analyses of *Citrus aurantium* stem bark (CASB) reveals the presence of 24 bioactive compounds which accounts for 96.46 %. The most abundant secondary metabolite is D-limonene (50.06 %) followed by α-cubebene (8.49 %), linalool (6.02 %), β-citrylideneethanol (4.30 %), myrcene (3.44 %), α-longipinene (2.75 %), β-santalene (2.50 %), terpinen-4-ol (2.04 %), γ-eudesmol (1.93 %), α-pinene (1.71 %), Cis-4-thujanol (1.67 %), β-fenchol (1.40 %), γ-terpinene (1.10 %), carvenone (1.10 %), β-cayrophyllene (0.77 %), 4-methyl-2,3-hexadien -1-ol (0.17 %), capraldehyde (0.16 %), phytol (0.10 %), spathulenol (0.10 %), torreyol-α-cadinol (0.07 %), 3-methoxy-p-cymene (0.01 %) and β-Elemene (0.01 %) respectively. The bioactive compounds or phytochemicals present in CASB agrees with the findings of Mohammad *et al*. (2019); Periyanayagam *et al*. (2013). Several reports have also shown that phytochemicals in plants varies according to age of plants, storage methods, extraction procedure, geographical location and antinutrients (Shittu *et al*., 2021; Alagbe, 2019; Alagbe, 2021). Phytochemicals used in livestock production performs several functions including being anti-oxidant, anti-estrogenic, anti-inflammatory, immunomodulatory, hypolipidemic and anticarcinogenic (Agubosi *et al*., 2022). Some of the bioactive components of CASB could be classified as alkaloids, flavonoids, phenols, saponins, oxalates as well as terpenoids (Oluwafemi *et al*., 2021). These compounds are capable of improving animal's health and performance because of their anti-microbial, anti-stress (Dilfuza *et al*., 2015) and anti-oxidant properties (Agubosi *et al*., 2022; Olafadehan *et al*., 2021), and their ability to modulate gut microbiota (Alagbe, 2019) and enhance immune responses (Chowdhury *et al*., 2018; Alagbe, 2022). Phytochemicals originates from natural sources and are generally recognised as safe (GRAS) which make them excellent alternatives to be used as feed additives in poultry production in comparison with antibiotics (Cushnie *et al*., 2014; Kurimoto *et al*., 2012).

Performance characteristics of broilers fed different levels of *Citrus aurantium* stem bark

Table 3 reveals the performance traits of broiler chickens fed different level of *Citrus aurantium* stem bark. Initial body weight (g) ranged from 50.93 – 52.02 g, final body weight [FBW; 600.8 – 8.11.7 g], body weight gain/bird [BWG; 549.19 – 759.82 g], average daily weight gain [ADWG; 26.15 – 36.18 g], total feed intake [TFI; 972.1 – 965.1 g], average daily feed intake [ADFI; 46.29 – 45.96 g] and feed conversion ratio [FCR; 1.27 – 1.77]. BWG, ADWG and FCR were significantly (P<0.05) different among the treatments. Conversely, TFI and ADFI were not significantly (P>0.05) influenced by the treatments. Birds fed CASB (T2, T3, T4 and T5) had a significant higher weight compared to the other treatments. This could be due to the presence of bioactive compounds in CASB especially D-limonene (50.06 %) which is significantly abundant in the extract thereby stimulating the activities of digestive enzymes and efficient nutrient utilization in birds (Shittu *et al*., 2021). D-limonene could also act as an antioxidant promoting good health in birds (Alagbe, 2022). The synergistic combination of limonene with other bioactive compounds could actively suppress the growth of pathogenic bacteria (Cencic and Chingwaru, 2010). Thus, they reward the host by shaping gut microbiota in a beneficial way which would translate to a better weight gain among birds (Laparra and Sanz, 2010). It was observed that average body weight increase as the level of CASB increases; this result is in agreement with the findings of Alagbe (2019); Cazares *et al*. (2019) who reported that birds fed plant extracts recorded a higher growth performance compared to that of an antibiotic growth promoter. This is a clear indication that CASB can act as probiotic and could also be used to bridge the gap between food safety and livestock production. This result is however, contrary to the findings of Jang *et al*. (2003) who recorded a non-significant (P>0.05) difference in body weights of broilers starters fed essential oil. This variation in results could possibly be attributed differences in dosage, variations in chemical composition in the test material, differences in environmental conditions as well as processing methods (Huyghebaert *et al*., 2011; Adewale *et al*., 2021; Musa *et al*., 2020). Average daily feed intake values slightly increases as the level of CASB increased though not at a significant (P<0.05) level. This shows that CASB is capable of improving palatability and better feed conversion ratio among birds. The outcome of this result agrees with the findings of Alabi *et al*. (2016) when moringa leaf extracts were fed to broiler chicks.

4. CONCLUSION

Citrus aurantium stem bark (CASB) is loaded with various bioactive compounds of pharmacological benefits. It is effective, environmental friendly and generally regarded as safe and it could be fed to broilers up to 40 mL per liter of water without causing any deleterious effect on the performance of birds.

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Conflicts of interests

The authors declare that there are no conflicts of interests.

Data and materials availability

All data associated with this study are present in the paper.

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