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Growth performance, serum biochemical parameters, serum corticosterone level and intestinal histomorphology in broiler chicken supplemented with *Kaempferia galanga* and *Curcuma longa*

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ABSTRACT

The study was conducted to evaluate the individual as well as synergistic growth promoting effects of *K. galanga* and *C. longa* in broiler chicken. Total 150 Vencobb 430Y chicks were grouped into four treatments (T_1 to T_4). The birds of T_2 and T_3 were supplemented with *K. galanga* at a level of 25 g/kg of feed and *C. longa* at 1.9 g/kg of feed respectively, and the birds of T_4 were supplemented with a combination of *K. galanga* (62.5 mg/kg) and *C. longa* (0.38 mg/kg). The weekly body weight, cumulative feed intake and feed conversion ratio were evaluated. On forty-second day, three birds from each treatment were humanely slaughtered and the carcass characteristics, serum biochemical values, serum corticosterone level and intestinal histomorphology were studied. Significantly better growth-promoting activities in terms of body weight, FCR and carcass characteristics were exhibited by birds from treatments fed with *K. galanga* alone and its combination with *C. longa*. No significant difference could be observed in serum biochemical parameters and corticosterone level, but significantly higher villi-to-crypt ratio was observed in *K. galanga* fed alone and combination fed groups. All these data are indications of the growth-promoting efficiency of *K. galanga* alone and its combination with *C. longa* in broiler chicken. A combination of these two herbs [*K. galanga* (62.5 mg/kg) and *C. longa* (0.38 mg/kg)] can thus be recommended for commercial broiler chicken considering the economic feasibility in terms of higher body weight gain and low cost of herbal additives.

Keywords: Broiler chicken, *Curcuma longa*, FCR, Growth promoter, *Kaempferia galanga*

Phytobiotic growth promoters in broiler chicken represent a natural and plant-derived approach to enhancing growth and performance in poultry production. Phytobiotics, also known as botanicals or plant-based additives, encompass a wide range of bioactive compounds derived from herbs, spices, essential oils, and other plant sources. These natural substances have gained popularity in the poultry industry as alternatives to traditional growth promoters. Phytobiotics exert various positive effects on broiler chickens, including antimicrobial and antioxidant properties, modulation of the gut microbiota, and improvements in digestion and nutrient absorption (Mohammadi Gheisar and Kim 2018). By harnessing the power of nature, phytobiotic growth promoters aim to promote sustainable and healthier poultry farming practices while addressing concerns related to antibiotic resistance and residues in poultry products. This shift towards natural additives reflects a growing emphasis on holistic and environmentally friendly approaches to poultry nutrition and production.

Kaempferia galanga (aromatic ginger) and *Curcuma longa* are Zingiberaceous medicinal plants with proven medicinal properties (Vincent *et al*. 1992, Verma *et al*. 2018). The supplementation of *K. galanga* at 2.5% in broiler feed resulted in better growth promoting activities (Raghavendra 2022). Herbal synergism is the concept that two or more herbs can work together to produce an effect that is greater than the sum of their individual effects (Williamson 2001). Synergistic mixtures require lower levels of use with superior efficacy (Lansky 2022). *K. galanga* can be combined with any other commonly available herb in order to reduce the level of inclusion in feed as well as to improve the efficacy of both herbs. The current study was conducted to evaluate the synergistic growth promoting effect of *K. galanga* and *C. longa* in broiler chicken.

MATERIALS AND METHODS

Experimental design: Day-old Vencobb 430 Y broiler chicks (n=120) were distributed equally on body weight basis into four groups (T_1-T_4) with three replicates each having 10 birds. The experimental duration was of six

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weeks from day one to the 42nd day. Isocaloric and isoprotein diets (broiler pre-starter, starter and finisher feed) were prepared as per BIS (IS 1374: 2007) for all the treatments (Supplementary Tables 1, 2 and 3). The inclusions levels of *K. galanga* and *C. longa* to the basal diet are presented in Table 1 (Reshma 2023).

Table 1. Feeding programme

Group	Feeding programme
T_1	Basal diet
T_2	Basal diet + 25 g/kg *K. galanga*
T_3	Basal diet + 1.9 g/kg *C. longa*
T_4	Basal diet + 62.5 mg/kg of feed *K. galanga* + 0.38 mg/kg of feed *C. longa*

Mean weekly body weight, mean weekly feed intake and cumulative FCR: Individual body weights of all the birds were recorded at weekly intervals in the morning hours before feeding. Daily feed consumption was calculated from the quantity of feed offered to the birds in each replicate after subtracting the quantity of feed that remained in the same feeder. Weekly feed intake was calculated by adding up the daily feed consumption for the particular week and further cumulative weekly feed intake and cumulative FCR were calculated.

Carcass characteristics and sensory evaluation of meat: After completing the feeding trial on day forty-second, three birds were randomly selected, weighed and humanely slaughtered from each treatment. The pre-slaughter weight (live weight) of the birds was taken on arrival at the slaughter hall. Birds were slaughtered by the halal method. Carcass weight and weight of giblets (heart, gizzard and liver) were recorded. The dressed weight was calculated after removing the skin, blood, head, feet and visceral organs. Carcass yield and giblet weight were calculated as the percentage of live weight. Sensory evaluation of the breast and thigh meat from respective treatments were conducted by a six-member panel consisting of semi-trained members pooled from various departments of the College of Veterinary and Animal Sciences, Pookode.

Serum biochemical parameters: Serum was separated by centrifugation at 3000 rpm for 10 min from the whole blood collected without anticoagulant on the day of slaughter and biochemical parameters were estimated using a semi-auto analyser (Microlab 300, Merck, France) using commercially available biochemical kits supplied by Agappe Diagnostics Pvt. Ltd., Kochi. Serum was analysed for total protein (direct biuret method, Gomall *et al*. 1949), albumin (bromocresol green methodology, Doumas *et al*. 1971), cholesterol (cholesterol oxidase peroxidase methodology, Flegg 1973), calcium (modified arsenazo III method, Bauer 1981), inorganic phosphorus (phosphomolybdate methodology, Taussky and Shorr 1953), creatinine (Enzymatic method, Artiss *et al*. 1984), SGPT and SGOT (IFCC recommended methodology, Thefeld *et al.*1974).

Intestinal histomorphometry: The representative pieces of the duodenum, jejunum and ileum kept in 10% neutral buffered formalin were used for histomorphometric examination. After seven days the 0.5 cm long tissue was cut from the duodenum, jejunum and ileum part and placed in a cassette and processing was done by standard tissue processing protocols (Luna 1968). Sections were taken at 5 µm thickness by using a semi-automatic M-TECH microtome. Haematoxylin and Eosin staining method was used for histological studies. The histomorphometric measurements were taken at 40× magnification for intestinal villus and crypt depth using image analysing software (Micaps Microview).

Serum corticosterone assay: Serum corticosterone concentration was determined using a commercially available Cayman's corticosterone ELISA kit (Cayman Chemical, USA). The assay was performed according to the manufacturer's information.

RESULTS AND DISCUSSION

Mean weekly body weight, feed intake and cumulative FCR: The mean weekly body weight, mean weekly feed intake, cumulative feed intake and cumulative FCR are presented in Table 2 and Supplementary Tables 4, 5 and 6, respectively. A significantly higher body weight was observed in herbal supplemented groups in comparison to basal diet fed group throughout the study except for the fifth and sixth weeks. The body weight on the forty-second day was comparable between treatments fed with 2.5% *K. galanga* and a combination of these herbs at a much lower concentration. These findings suggest that the concentration of phytobiotics that are to be incorporated in feed can be efficiently reduced by providing them in combination with another synergistic phytobiotic agent. The results obtained were in agreement with Raghavendra (2022), where 2.5% supplementation of *K. galanga* in broiler feed resulted in significantly higher mean weekly body weight compared to the basal diet-fed group. The results were also comparable with the results obtained by Sahoo *et al*. (2018) and Thomas *et al.* (2020), where both of them studied the individual as well as combination effects of turmeric and ginger and observed that the herbs in combination showed better growth performance than ginger alone and the results was next to that of turmeric at higher concentration.

The weekly feed intake was significantly lower in treatment with herbs fed in combination, at the same time, significantly higher feed intake was exhibited by basal diet fed treatment. The mean weekly cumulative feed intake was also higher in basal diet fed treatments. Durrani *et al*. (2006) and Gowda *et al.* (2009) also observed a significant reduction in feed intake of turmeric-fed broiler chicken. Similarly, supplementation of ginger in broiler feed resulted in reduced cumulative feed intake as per Herawati (2010). The pungency of the phytobiotic compounds may cause the animals to consume less feed (Brenes and Roura 2010), there are many reports to substantiate the hypothesis. But this was not the situation in the present

Table 2. Mean weekly body weight (g)

Week	T_1	T_2	T_3	T_4	P-value
DOH	40.53±0.04	40.53±0.01	40.54±0.02	40.54±0.05	0.089^{ns}
Week 1	$141.39±0.32^{b}$	$147.32±0.09^{a}$	$148.51±1.51^{a}$	$149.87±1.13^{a}$	$<0.001^{**}$
Week 2	$362.81±1.74^{c}$	$392.60±1.03^{a}$	$373.89±2.89^{b}$	$381.49±1.78^{b}$	$<0.001^{**}$
Week 3	$718.44±16.81^{d}$	$784.26±3.88^{b}$	$749.65±6.57^{c}$	$829.44±3.91^{a}$	$<0.001^{**}$
Week 4	$1152.15±21.24^{c}$	$1214.84±8.66^{b}$	$1194.03±5.26^{b}$	$1249.20±1.89^{a}$	$<0.001^{**}$
Week 5	$1803.68±7.72^{c}$	$1825.68±10.09^{b}$	$1803.42±6.38^{c}$	$1927.17±1.68^{a}$	$<0.001^{**}$
Week 6	$2330.83±16.71^{c}$	$2466.41±17.74^{b}$	$2323.50±18.31^{c}$	$2559.60±3.84^{a}$	$<0.001^{**}$

**, Significant at 0.01 level; *, Significant at 0.05 level; ^{ns}, non-significant. Means having different small letters as superscripts differ significantly within a row.

study, the low feed intake was observed in herbs fed at a minimum concentration group, therefore the reduction in feed intake was not due to any pungent odour or flavour imparted by *K. galanga* and *C. longa*.

There was a significant improvement in the FCR of herbal-fed treatments with better FCR exhibited in herbs fed in the combination group. The findings were in line with those of Raghavendra (2022), who noticed better FCR in *K. galanga* fed at a concentration of 2.5% when compared to that of the basal diet fed group in broiler chicken. Similar results were published by Lokaewmane *et al*. (2022) and Eltazi (2014). Similarly, the supplementation of turmeric in the feed also improved FCR in chicken as per the results published by Nouzarian *et al*. (2011) as well as Urusan and Bölükbasi (2017).

The better growth performance in broiler chicken supplemented with herbs could be due to the prebiotic effect causing alteration of the intestinal microbiota, an increase of enzyme secretion, improvement of the immune response, histo-morphometric changes of the gastrointestinal tract and antioxidant activity (Kamel 2000, Petrolli *et al*. 2012, Amuamuta *et al*. 2017, Kiramang *et al.* 2019). The increased growth performance in chicken supplemented with herbs might also be due to the up regulation of growth regulatory factors. Hafez *et al*. (2022) reported upregulation of Insulin-like Growth factor-1 (*IGF-1*) and Growth hormone receptor (*GHR*) genes in broiler chicken supplemented with curcumin, the active ingredient in turmeric powder.

Carcass characteristics and sensory evaluation of meat: The results of carcass characteristics and sensory evaluation of meat are represented in Table 3 and Supplementary Table 7, respectively. A significantly higher live weight, carcass weight, giblet weight and dressing percentage were noted in herbs fed in combination birds and no significant difference could be observed in immune organ weight. The results obtained were in agreement with that of Raghavendra (2022), where significantly better carcass characteristics were exhibited by *K. galanga* fed treatments in comparison to the basal diet fed group. These beneficial effects on the carcass quality parameters may be brought on by the numerous positive effects of the active ingredients present in *K. galanga* and *C. longa*. Ahmed *et al*. (2015) reported similar results in chicken supplemented with herbs. The active ingredients in phytobiotics stimulate the expression of genes linked to appetite stimulation and increased feed consumption (e.g. the *GHRL* gene), nutrient absorption and transport (e.g. the *muc* and *pept1* genes), lipid digestion (e.g. the *lpl* and *alp* genes), and other metabolic processes, all of which support growth and body weight (Chakraborty *et al*. 2014, Safari *et al.* 2020).

The sensory evaluation of broiler meat obtained from treatments supplemented with *K. galanga, C. longa* and their combination did not show any significant difference

Table 3. Carcass characteristics and immune organ weight

Variable	T_1	T_2	T_3	T_4	P-value
Live weight(g)	$2351.33±20.73^{c}$	$2489.33±1.76^{b}$	$2363.33±2.33^{c}$	$2566.00±33.60^{a}$	$<0.001^{**}$
Carcass weight (g)	$1456.00±26.50^{c}$	$1628.66±33.19^{ab}$	$1555.33±28.90^{bc}$	$1724.00±45.48^{a}$	0.003^{*}
Giblet weight (g)	$86.33±1.33^{b}$	$90.00±2.30^{b}$	$86.66±5.84^{b}$	$107.33±2.66^{a}$	0.008^{*}
Dressing (%)	$65.58±0.69^{b}$	$69.04±1.20^{a}$	$69.47±1.26^{a}$	$71.35±0.92^{a}$	0.027^{*}
Giblet yield (%)	3.67±0.06	3.61±0.09	3.67±0.24	4.18±0.15	0.099^{ns}
Thymus (g)	9.90±2.66	10.00±0.67	8.00±1.00	13.41±3.79	0.490^{ns}
Thymus (%)	0.42±0.11	0.40±0.02	0.33±0.04	0.52±0.15	0.626^{ns}
Bursa (g)	6.33±0.66	5.33±0.88	3.33±0.88	6.00±0.57	0.089^{ns}
Bursa (%)	0.27±0.03	0.21±0.03	0.14±0.03	0.23±0.02	0.092^{ns}
Spleen (g)	2.33±0.33	3.00±0.57	2.66±0.33	3.33±0.33	0.400^{ns}
Spleen (%)	0.10±0.01	0.12±0.02	0.11±0.01	0.13±0.01	0.695^{ns}

**, Significant at 0.01 level; *, Significant at 0.05 level; ^{ns}, non-significant. Means having different small letters as superscripts differ significantly within a row.

Table 4. Intestinal histometry of duodenum, jejunum and ileum

Parameter		T_1	T_2	T_3	T_4	P-value
Villi height (µm)	Duodenum	1311.82±51.95^{b}	1663.25± 65.51^{a}	1353.32±31.31^{b}	1450.80±22.47^{b}	<0.001**
	Jejunum	1151.88±71.92^{c}	1441.70±44.74^{a}	1248.40±30.64^{bc}	1343.12±72.73^{ab}	0.016*
	Ileum	1340.37±12.52^{c}	1422.15±23.33^{bc}	1644.43±62.94^{a}	1469.46±19.29^{b}	<0.001**
Crypt depth (µm)	Duodenum	385.63±8.63^{a}	233.11±6.63^{c}	308.24±20.28^{b}	303.55±7.99^{b}	<0.001**
	Jejunum	259.52±16.32^{a}	290.68±11.80^{a}	148.50±4.93^{c}	207.03±7.54^{b}	<0.001**
	Ileum	147.16±14.13^{b}	186.62±2.89^{a}	137.05±12.57^{b}	216.27±10.79^{a}	<0.001**
Villus: Crypt	Duodenum	3.40±0.14^{c}	7.15±0.32^{a}	4.44±0.23^{b}	4.79±0.15^{b}	<0.001**
	Jejunum	4.55±0.53^{c}	5.00±0.30^{c}	8.44±0.39^{a}	6.53±0.48^{b}	<0.001**
	Ileum	9.42±0.85^{b}	7.62±0.14^{bc}	12.38±1.20^{a}	6.86±0.34^{c}	0.001**

**, Significant at 0.01 level; *, Significant at 0.05 level; ^{ns}, non-significant. Means having different small letters as superscripts differ significantly within a row.

in their sensory attributes in any of the samples and results were comparable to that of control groups. These aromatic herbs did not transfer any unfavourable scent or flavour into the meat since the sensory quality criteria of the herbal fed treatments were similar to those of the basal diet fed group. The results were in line with that of Purwanti *et al*. (2019) and Raghavendra (2022), where all sensory test parameters in cooked meat had no effect following phytobiotic extract supplementation.

Serum biochemical parameters and corticosterone assay: The results of serum biochemical parameters and corticosterone level are presented in Supplementary Table 8. No significant difference in serum total protein, albumin, cholesterol, calcium, SGPT, SGOT and corticosterone could be observed. In agreement with the current study, Mehala and Moorthy (2008) and Basavaraj *et al.* (2011) also did not observe any significant change in the serum biochemical parameters in chicken supplemented with turmeric. Similarly, Qorbanpour *et al*. (2018) also reported that no significant difference in serum biochemical parameters of broiler chicken could be observed when supplemented with different levels of ginger.

Intestinal histomorphometry: The results of intestinal histomorphometric parameters are presented in Table 4. In the duodenal region of the intestine, significantly higher duodenal villi length and villi-to-crypt ratio were observed in *K. galanga* fed birds. When jejunal histomorphometric parameters were studied, a significantly higher villi height was observed in birds fed with *K. galanga* and its combination with *C. longa*. The basal diet-fed group exhibited a lower villi-to-crypt ratio. The villi height to crypt depth ratio is utilised as a predictor of the small intestine's potential capability for digestion. This ratio rises in proportion to increased digestion and absorption as per Montagne *et al*. (2003). Similarly, Sieo *et al*. (2005) reported that a greater area for nutrient absorption and a higher absorption function is indicated by increased intestinal villi height and the ratio of villi height to crypt depth. The small intestine parameters can be impacted by a variety of variables, such as feeding practices or the inclusion of feed additives (Wang *et al*. 2016). In the present study, significantly higher villi length and villi to crypt ratio of duodenal, jejunal and ileal compartments were observed in herbs-supplemented birds. The results were in agreement with Namagirilakshmi *et al*. (2010) who found an increase in the villi length of the intestine of chicken supplemented with turmeric. Similarly, Galli *et al*. (2020) observed an increased villi-to-crypt ratio in broiler chicken supplemented with a combination of curcumin, carvacrol, thymol and cinnamaldehyde.

It can be concluded that the supplementation of *K. galanga* and its combination with *C. longa* can be effectively utilised as growth promoters in broiler chicken without affecting the sensory attributes of meat. The combinations of these two herbs are more efficient when considering the economic feasibility and thus can be recommended in broiler chicken as the phytobiotic growth promoter.

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