# Performance and antioxidative status of broiler chickens consuming diets supplemented with a wild cucumber (*Momordica charantia*) leaf powder and clove basil (*Ocimum gratissimum*) leaf powder blend

**O.D. Oloruntola[1,*], S.O. Ayodele[2], A.O. Jimoh[2], A.B. Falowo[1], O.J. Olarotimi[1], O.A. Ayodele[1], O.A. Adu[3] and F.S. Oladebeye[1]**

[1] Department of Animal Science, Adekunle Ajasin University, Akungba-Akoko 342111, Nigeria
[2] Department of Agricultural Technology, The Federal Polytechnic, Ado Ekiti 360231, Nigeria
[3] Department of Animal Production and Health, The Federal University of Technology, Akure 340252, Nigeria
* Corresponding author: olugbenga.oloruntola@aaua.edu.ng; oloruntoladavid@gmail.com
Submission: 29 November 2023 Revised: 16 November 2024 Accepted: 27 November 2024

## ABSTRACT

**Background and Objective:** This study examined the effect of the composite mix of wild cucumber and clove basil leaf powder (WCLP) supplementation on the performance, blood indices, and stress biomarkers of broiler chickens.

**Methodology:** 300 one-day-old Cobb 500 broiler chicks weighing 42.06 ± 0.44 g were randomly allotted to five diets [diet 1 (negative control), diet 2 (positive control: 0.1% probiotic, prebiotic and acidifier blend (PPAB) supplementation), diet 3 (0.25% WCLP), diet 4 (0.50% WCLP), and diet 5 (0.75% WCLP)].

**Main Results:** At the starter phase, broiler chickens fed diets 3 and 4 were similar ($P > 0.05$) to those fed diet 1; from another angle, the body weight gain (BWG) of the birds fed diets 1, 2, and 3 were similar ($P > 0.05$). The least BWG was recorded in the birds fed diet 5. At the finisher phase and overall period (1–42 days), the BWG recorded in broiler chickens fed diets 2 and 3 were similar ($P > 0.05$) but significantly ($P < 0.05$) higher than those fed diets 1, 4, and 5. Broiler chickens fed diets 3, 4, and 5 recorded the lowest cholesterol levels, compared to birds on diets 1 and 2. Broiler chickens fed diets 3, 4, and 5 recorded higher concentrations of serum glutathione peroxidase at 150.50, 214.52, and 168.51 mg/mL, respectively, compared to diet 1 (96.32 mg/mL) and diet 2 (144.50 mg/mL).

**Conclusions:** WCLP could be used as a feed addition at 0.25% without affecting broiler chicken growth performance. Beyond 0.25% WCLP would increase stress biomarkers and lower serum cholesterol concentrations in broiler chickens.

**Keywords:** Antioxidant enzymes, broiler, clove basil, performance, wild cucumber

Thai J. Agric. Sci. (2024) Vol. 57(4): 181−193

## INTRODUCTION

The use of conventional antibiotic feed additives to enhance performance and boost poultry health has led to the deposition of antimicrobial residues in products (such as meat and eggs) and also antimicrobial resistance in humans when such products are consumed (Falowo and Akimoladun, 2020). Thus, there is a need for alternative feed additives to eliminate or lower the usage of antibiotics in poultry production and minimize the incidence of antimicrobial resistance in animals and humans (O'Neill, 2015; Santoso *et al*., 2018). As a remedy, some herbs and medicinal plants have been discovered as a suitable alternative to replace commercial antibiotic feed additives, because of their functional components and activities (Ahmed *et al*., 2016). *Momordica charantia* and *Ocimum gratissimum* plants are medicinal plants that can be utilized as potential natural feed additives (Oloruntola *et al*., 2021a).

*Momordica charantia* plant (commonly known as the wild cucumber, bitter gourd, melon, or pumpkin) is an edible healthy vegetable that belongs to the family of Cucurbitaceae (Nagarani *et al*., 2014). The plant is rich in bioactive nutrients such as ash (22.67%), crude fiber (20.86%), crude fat (5.37%), crude protein (9.89%), nitrogen-free extract (35.69%), minerals, vitamins, carbohydrates, proteins, alkaloids (191.49 mg/g), flavonoids (225.64 mg/g), carotenoids, cucurbitane triterpenoids, phenols (21.04 mg/g), glycosides, saponin (57.36 mg/g), polypeptide, and phytosterols (Nagarani *et al*., 2014; Oloruntola *et al*., 2021a). Traditionally, the plant is used for the treatment of different disorders such as cancer insurgence, diabetes mellitus, abdominal pain, kidney (stone), fever, ulcers, inflammation, and scabies (Saeed *et al*., 2018). The *in vitro* antioxidant study has also shown that the plant is very effective in scavenging free radicals and other reactive oxygen species causing oxidative stress, lipid peroxidation, and liver damage in biological systems (Nagarani *et al*., 2014; Saeed *et al*., 2018). The anti-microbial activity of the plant has been documented against a broad spectrum of microorganisms including *Escherichia coli, Klebsiella pneumonia*, and *Bacillus subtilis* (Nagarani *et al*., 2014). Despite these potentials, little or no study has been reported on the utilization of the *Momordica charantia* plant as a natural feed additive on the performance of broiler chicken productions.

On the other hand, the *Ocimum gratissimum* plant (commonly known as African basil, clove basil, scent leaf, etc) is a herbaceous food spice plant that belongs to the family of Labiatae (Igbinosa *et al*., 2013). The plant is widely used in the tropics of Africa and Asia for both nutritional and medicinal purposes. The phytochemical screening of the plant has shown that it contains strong bioactive compounds [alkaloids (123.00 mg/g), tannins (0.61 mg/g), phytates (5.66 mg/g), phenols (14.72 mg/g), flavonoids (182.18 mg/g), and saponin (40.96 mg/g)] and exhibits high antioxidant activities against free radicals and other reactive oxygen species in the biological system (Igbinosa *et al*., 2013; Oloruntola *et al*., 2021a). The nutritional study of the plant showed that it contains 9.07% moisture, 18.98% crude protein, 12.18% crude fiber, 10.12% ether extract, and 11.15% ash content (Nte *et al.,* 2017). The use of the plant as feed additives or supplements in animal health management has shown great potential in the treatment of diseases such as gastrointestinal diseases, helminths, diarrhea, and fungal and bacterial infections (Adebayo *et al.,* 2019). Moreover, the inclusion of *Ocimum gratissimum* leaf as a supplement at concentrations 1, 2, 3, and 4 g/kg feed has been shown to improve body weight gain, feed intake, feed conversion ratio, and meat quality of broiler chickens (Olumide and Akintola, 2018).

The potential of the nutrients and bioactive variability in leaf meals to produce positive effects on the broiler chickens' performance, blood indices, and antioxidant status has been reported (Oloruntola *et al*., 2018). In the same vein, the variability of the *Momordica charantia* and *Ocimum gratissimum* leaf powder bioactive compounds could produce significant impacts on broiler chickens, particularly, when the two botanicals are processed and used as a composite mix supplement (Oloruntola *et al*., 2021a). Combining two-leaf meals with diverse chemical and nutritional compositions in feed supplements could maximize synergistic effects, potentially boosting overall health and performance, thereby offering a holistic approach to animal nutrition and fostering optimal well-being and performance (Oloruntola *et al*., 2019; Adeyeye *et al*., 2020). Relatively, low attention has been given to the effects of herbal composite mix on poultry performance. In addition, the response of broiler chickens to *Momordica charantia* and *Ocimum gratissimum* composite leaf mix dietary supplementation in terms of performance, nutrient digestibility, antioxidants, hematological indices, meat quality, and serum enzymes has not been fully researched. Hence, this study was designed to examine the effects of *Momordica charantia* and *Ocimum gratissimum* composite leaf mix dietary supplementation on the performance, blood indices, and stress biomarkers of broiler chickens.

## MATERIALS AND METHODS

### Collection, Processing, and Analysis of Phytogens After Ethical Approval

The Research and Ethics Committee of the Department of Agricultural Technology, The Federal Polytechnic, Ado Ekiti, Nigeria, accepted the experiment's requirements and criteria for animal and animal protocol. Wild cucumber (*Momordica charantia*) and clove basil (*Ocimum gratissimum*) leaves were newly plucked and cleaned in clear running water, drained, and spread lightly on polythene in the shade for two weeks.

Dried leaves of wild cucumber and clove basil were used to make wild cucumber leaf powder (WLP) and clove basil leaf powder (CLP), respectively. The WLP and CLP's proximate, phytochemical, antioxidant, and mineral components were studied and have been reported by Oloruntola *et al*. (2021). After that, the composite mix of wild cucumber and clove basil leaf powder (WCLP) was produced by blending equal parts (1:1) of WLP and CLP as earlier reported by Adeyeye *et al*. (2020). The phytochemical composition (tannins, alkaloids, phenols, saponins, flavonoids, and phytate) and antioxidant activity (2,2-diphenyl-1-picrylhydrazyl hydrate) were determined (Figure 1) as earlier reported by Oloruntola *et al*. (2021a).

### The Probiotic, Prebiotic, and Acidifier Blend

The probiotic, prebiotic, and acidifier blend (PPAB) was procured from a commercial feed mill in Akure. PPAB was prepared by Xvet, GMBH, 22529, Hamburg, Germany, and is composed of: *Bacillus licheniformis* + *Bacillus subtills* ($4 \times 10^9$ CFU), *Enterococcus foecium* ($1 \times 4 \times 10^9$ CFU), *Lactobacillus acidophilus* ($5 \times 4 \times 10^9$ CFU), *Saccharamyces cerevisae* (40%), formic acid (9,000 mg), citric acid (2,000 mg), ortho-phosphoric acid (3,000 mg), lactic acid (3,000 mg), and magnesium (5%).

### Preparation of Experimental Diets, Living Conditions, and Experimental Design

A broiler chicken's basal diet (Table 1) was compounded for the starter phase (0–21 days) and finisher phase (21–42 days) to meet the nutritional needs of the birds (NRC, 1994). For each phase, the basal diet was divided into five equal portions and named diets 1 to 5. Diet 1 was not supplemented (negative control), while diet 2 (positive control) received 0.1% PPAB supplementation. Then, in diets 3, 4, and 5, 0.25% WCLP, 0.50% WCLP, and 0.75% WCLP were introduced, respectively. The feeding trial was undertaken at the Federal Polytechnic's Teaching and Research Farm in Ado Ekiti, Nigeria.

In a completely randomized design, 300 one-day-old Cobb 500 broiler chicks weighing 42.06 ± 0.44 g were randomly assigned to all five experimental diets. Each diet was replicated six times, each time with ten birds (10 birds/replicate). To a depth of 3 cm, wood shavings were utilized to cover the floor of the experimental pen (2 m × 1 m) that housed each replicate. The experimental house was kept at 31 ± 2°C for the first week and then reduced by 2°C each week after that until the temperature reached 26 ± 2°C. The lighting was turned on for 24 hours on the first day and 23 hours on consecutive days.

**Table 1** Composition of the experimental basal diets

| Composition | Starter feed | Finisher diet |
|---|---|---|
| Ingredients (%) | | |
| Maize | 52.35 | 59.35 |
| Maize bran | 7.00 | 0.00 |
| Rice bran | 0.00 | 6.00 |
| Soybean meal | 30.00 | 24.00 |
| Fish meal | 3.00 | 3.00 |
| Soy oil | 3.00 | 3.00 |
| Limestone | 0.50 | 0.50 |
| Bone meal | 3.00 | 3.00 |
| Lysine | 0.25 | 0.25 |
| Methionine | 0.30 | 0.30 |
| Premix | 0.30 | 0.30 |
| Salt | 0.30 | 0.30 |
| Analyzed composition (%) | | |
| Crude protein | 22.18 | 20.03 |
| Crude fiber | 3.52 | 3.61 |
| Calculated composition (%) | | |
| Metabolizable energy (kcal/kg) | 3,018.89 | 3,108.10 |
| Available phosphorus | 0.46 | 0.41 |
| Calcium | 1.01 | 0.99 |
| Lysine | 1.36 | 1.24 |
| Methionine | 0.68 | 0.66 |

**Growth Performance**

Every seven days, the experimental birds' body weight (BW) and feed intake (FI) were measured and recorded. The average body weight gain (BWG) was computed using the difference between the birds' starting and final weights, and the feed conversion ratio (FCR) was calculated using the ratio of feed consumed to weight gain.

**Collection and Analysis of Blood Samples**

On day 42 of the feeding study, 18 birds (3 birds per replication) were randomly chosen from each dietary group and bled via the wing vein with a syringe and needle. A portion (3 mL) of the blood sample was dispensed into a plain blood sample bottle for serum biochemicals and enzymes (i.e. total protein, aspartate aminotransferase, creatinine, and cholesterol) and antioxidant enzymes (i.e. glutathione peroxidase, superoxide dismutase, and catalase), while the second portion (2 mL) was dispensed into the ethylenediaminetetraacetic acid bottle for hematological indices determination. Before analysis, the blood sample in each of the plain bottles was spun and the serum was decanted into another plain bottle before being frozen at -20°C. Within 2 hours of collection, the hematological indices were determined (Cheesbrough, 2000). The serum biochemical and enzyme concentrations were measured using a Reflectron ®Plus 8C79 (Roche Diagnostic, GombH Mannheim, Germany) and kits. The serum catalase, superoxide, and glutathione peroxidase were measured using the methods reported by Aebi (1974), Misra and Fridovich (1972), and Rotruck *et al*. (1973), respectively.

**Analysis of Data**

The model: $y_{ij} = \mu + \tau_i + \varepsilon_{ij}$, was used in this experiment, where $y_{ij}$ represents the response variable in this study; μ denotes the overall average; $\tau_i$ indicates the treatment effects (i = diets 1, 2, 3, 4, and 5); and $\varepsilon_{ij}$ is the random error associated with the investigation. Using SPSS version 20, all data were subjected to one-way ANOVA. Duncan's multiple range test of SPSS was used to detect the differences between the treatment means ($P < 0.05$).

## RESULTS AND DISCUSSIONS

The results presented in Figure 1 illustrate the phytochemical composition and antioxidant activity of a blend comprised of wild cucumber and clove basil leaf powders in a 1:1 ratio. The blend contains various phytochemicals, including tannins (0.94 mg/g), alkaloids (157.42 mg/g), phenols (17.97 mg/g), saponins (50.08 mg/g), flavonoids (204.59 mg/g), and phytate (6.91 mg/g). These compounds contribute to the bioactive profile of the blend and may confer potential health benefits (Oloruntola *et al*., 2021a). Additionally, the antioxidant activity of the blend was assessed using the 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH) assay, with a recorded value of 48.89 mg/kg. This indicates the blend's ability to scavenge free radicals, suggesting its potential role in combating oxidative stress and associated health conditions (Oloruntola *et al*., 2021a).

The inclusion of leaf meal as a feed additive in the diet is mainly to reduce the cost of using protein-rich feed ingredients, the side effects of using commercial antibiotics (such as antibiotic residue in meat, antibiotic resistance in animals and humans) and improve the performance and wellbeing of animal (Valenzuela-Grijalva *et al*., 2017; Oloruntola *et al*., 2021b). However, the performance responses of broilers to different dietary leaf meals may vary depending on the types of leaf meal, levels of incorporations, and bioavailability of inherent phytochemicals and other bioactive compounds (Oloruntola *et al*., 2018; 2019; Adeyeye *et al*., 2021).

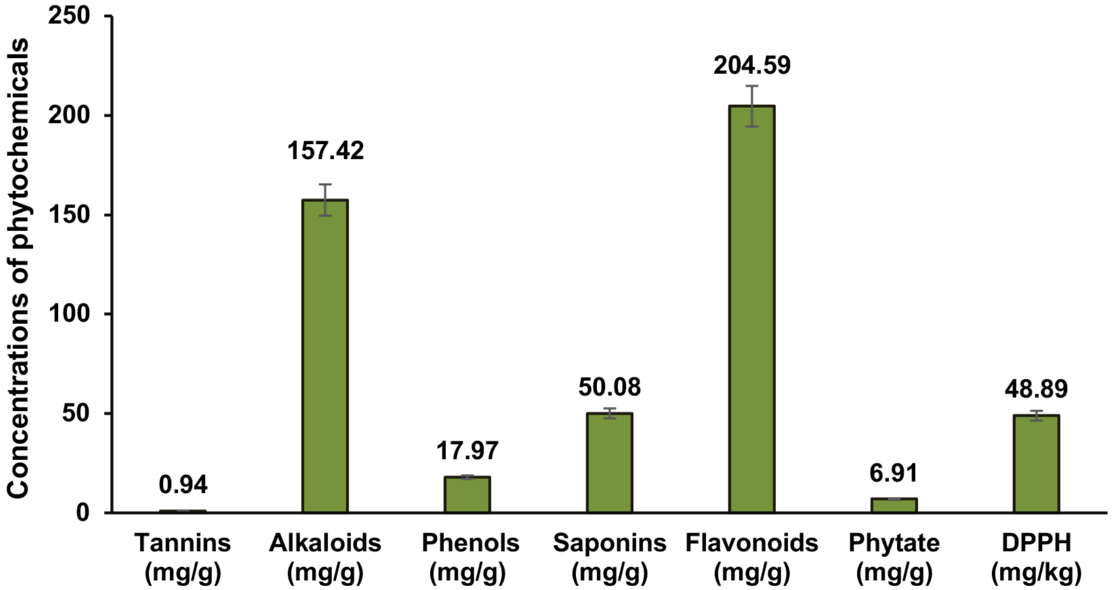


**Figure 1** The phytochemical composition and antioxidant activity of wild cucumber and clove basil leaf powder blend (1:1). DPPH = 2,2-diphenyl-1-picrylhydrazyl hydrate.

**Table 2** Effects of the wild cucumber leaf powder and clove basil leaf powder blend on the performance characteristics of broiler chickens

| Parameters | Diet 1 Control | Diet 2 0.1% PPAB | Diet 3 0.25% WCLP | Diet 4 0.50% WCLP | Diet 5 0.75% WCLP | SEM | P-value |
|---|---|---|---|---|---|---|---|
| Starter phase (1–21 days) | | | | | | | |
| Initial body weight (g/bird) | 41.49 | 43.57 | 41.84 | 41.40 | 42.02 | 0.44 | 0.57 |
| Body weight gain (g/bird) | 892.19$^{ab}$ | 918.41$^{a}$ | 862.48$^{abc}$ | 815.67$^{bc}$ | 790.17$^{c}$ | 15.87 | 0.03 |
| Feed intake (g/bird) | 1,219.29 | 1,165.77 | 1,210.51 | 1,235.44 | 1,091.01 | 29.05 | 0.43 |
| Feed conversion ratio | 1.36 | 1.27 | 1.40 | 1.51 | 1.39 | 0.03 | 0.43 |
| Viability (%) | 100.00 | 95.00 | 100.00 | 100.00 | 100.00 | 1.00 | 0.56 |
| Finisher phase (22–42 days) | | | | | | | |
| Body weight gain (g/bird) | 2,088.97$^{bc}$ | 2,461.44$^{a}$ | 2,318.70$^{a}$ | 1,949.38$^{c}$ | 1,834.66$^{c}$ | 65.99 | <0.001 |
| Feed intake (g/bird) | 2,797.62 | 2,889.42 | 2,754.03 | 2,632.32 | 2,655.86 | 55.55 | 0.62 |
| Feed conversion ratio | 1.34 | 1.17 | 1.19 | 1.36 | 1.46 | 0.04 | 0.12 |
| Viability (%) | 100.00 | 95.00 | 100.00 | 100.00 | 95.00 | 1.37 | 0.57 |
| Overall (1–42 days) | | | | | | | |
| Body weight gain (g/bird) | 2,873.65$^{bc}$ | 3,199.85$^{a}$ | 3,026.18$^{a}$ | 2,685.05$^{cd}$ | 2,544.83$^{d}$ | 66.84 | <0.001 |
| Feed intake (g/bird) | 4,016.91 | 4,055.20 | 3,964.54 | 3,867.77 | 3,746.87 | 75.65 | 0.74 |
| Feed conversion ratio | 1.39 | 1.26 | 1.31 | 1.45 | 1.48 | 0.03 | 0.26 |
| Viability (%) | 100.00 | 95.00 | 100.00 | 100.00 | 95.00 | 1.37 | 0.57 |

**Note:** Means within a row with different letters are significantly different at $P < 0.05$. PPAB = probiotic, prebiotic, and acidifier blend, WCLP = wild cucumber and clove basil leaf powder, SEM = standard error of the mean.

Table 2 shows the result of the effect of the WCLP dietary supplementation on BWG, FI, and FCR. Only the BWG of the broiler chickens at the starter (1–21 days) and finisher (22–42 days) phases as well as at the overall period (1–42 days) was significantly influenced ($P < 0.05$) by WCLP supplementation. At the starter phase, broiler chickens fed the diet supplemented with 0.25% WCLP (diet 3; 862.48 g/bird) and 0.50% WCLP (diet 4; 815.67 g/bird) had similar BWG compared to those fed the control diet (diet 1; 892.19 g/bird). Additionally, the BWG of birds fed diets 1 (892.19 g/bird), 2 (918.41 g/bird), and 3 (862.48 g/bird) were similar. The least BWG was recorded in birds fed 0.75% WCLP (diet 5; 790.17 g/bird). At the finisher phase (22–42 days) and the overall period (1–42 days), the BWG recorded in broiler chickens fed diets 2 (2,461.44 g/bird for finisher, 3,199.85 g/bird for overall) and 3 (2,318.70 g/bird for finisher, 3,026.18 g/bird for overall) were similar but significantly ($P < 0.05$) higher than those fed diets 1 (2,088.97 g/bird for finisher, 2,873.65 g/bird for overall), 4 (1,949.38 g/bird for finisher, 2,685.05 g/bird for overall), and 5 (1,834.66 g/bird for finisher, 2,544.83 g/bird for overall).

The FI and FCR were not significantly different at the starter, finisher, and overall periods across experimental treatments. In this study, similar BWG recorded in birds fed diets 1, 2, and 3 at the starter phase suggests stability in the physiological conditions in support of the normal body weight gain of the birds at that stage of their life. However, the depressed body growth at 0.50% and 0.75% WCLP dietary supplementation (diets 4 and 5) suggests that at those aforementioned levels, the concentration of the bioactive compounds in WCLP exceeds the tolerance level of the birds, particularly at the starter phase. It was reported that some phytochemicals could interfere with the toxicity of herbal plants (Hashemi *et al*., 2008). Furthermore, the BWG recorded in broiler chickens fed diets 2 and 3 containing 0.1% PPAB and 0.25% WCLP, respectively at finisher phases, and at the overall period suggest that this diet promotes better growth than diet 1 (0% supplementation), diet 4 (0.50% WCLP supplementation), and diet 5 (0.75% WCLP supplementation). This similar performance in the BWG of these birds on diets 2 and 3 suggests a similar mechanism of actions of the two supplements. Particularly, it could be attributed to the combined mechanism of action of probiotics, prebiotics, and acidifiers in PPAB which have been reported to enhance nutrient digestion and increase growth rate in animals (Mokhtari *et al*., 2015). In addition, as earlier reported by Oloruntola *et al*. (2021a), *Momordica charantia* and *Ocimum gratissimum* leaves contain some phytochemicals such as tannins, phenols, and alkaloids, that can produce enhancing effects on weight gain of animals by improving or maintaining the gut health, improving enzyme activities, and accelerating digestion (Suganya *et al*., 2016; Lillehoj *et al*., 2018).

Also in this study, WCLP showed that it should be used successively as feed additives at an inclusion rate of not more than 0.25% to improve the BWG of broiler chickens since its inclusion at 0.50% (diet 4) and 0.75% (diet 5) decreased the BWG of broiler chicken compared to the control group. This decrease in BWG of broiler chicken receiving WCLP at 0.50% and 0.75% could be due to the presence of high inherent phytochemicals in the plant leaf meal. Oloruntola *et al.* (2021a) reported that *Momordica charantia* and *Ocimum gratissimum* contained a high content of saponins (57.36 mg/g vs 40.96 mg/g), alkaloid (191.49 mg/g vs.123.00 mg/g), and phytate (7.79 mg/g vs. 5.66 mg/g). Reports have also revealed that a high intake of residual phytochemicals such as tannin, phytate, alkaloid, and saponin from plant materials could cause depressed growth performance and reduce nutrient absorption in animals (Sobayo *et al*., 2012; Sugiharto *et al.,* 2019). The result is similar to the report of Sobayo *et al.* (2012) and Gulizia and Downs (2020) who found that dietary inclusion of *Asystasia gangetica* leaf meal, kudzu (*Pueraria montana* var. lobata) leaf meal, and alfalfa leaf meal significantly decreased BWG of broiler chicken compared to the control group. Moreover, the non-significant differences recorded in feed intake and feed conversion ratio across the treatments suggest that the dietary intake of WCLP at the inclusion levels used in this study did not negatively alter the taste and palatability of the diet during consumption (Buragohain, 2016). This result is in contrast with the findings of Adeyeye *et al.* (2020) who reported that dietary inclusion of wild sunflower and goat weed leaf meals composite mix at 0.4%, 0.8%, and 1.2% significantly improved feed intake, feed conversion ratio, and BWG of broiler chickens compared to the control group.

The result of hematological indices of broiler chickens fed WCLP-supplemented diets is presented in Table 3. The WCLP supplementation did not influence the hematological indices of the experimental birds across the dietary treatments (P > 0.05). Consequently, the observed non-significant difference in hematological indices of broiler chicken in this study suggests that the dietary inclusion of WCLP did not alter the normal hematopoiesis in the birds (Adeyeye *et al*., 2020). This result is in agreement with the finding of Adeyeye *et al.* (2020) who reported no significant difference in broiler chickens fed a diet containing wild sunflower and goat weed leaf meals composite mix at 0.4%, 0.8%, and 1.2%. On the other hand, this result is in contrast with the findings of Oloruntola *et al*. (2019) who reported that the addition of neem, pawpaw, and bamboo leaf meals significantly influenced the hematological indices of broiler chickens compared to the control group.

The result of serum chemistry indices of broiler chickens fed diets supplemented with WCLP is presented in Table 4. The WCLP supplementation did not influence the serum chemistry indices of the experimental birds across the dietary treatments, except for the cholesterol concentration (P < 0.05). Broiler chicken fed the diet supplemented with WCLP at 0.25% (diet 3), 0.50% (diet 4), and 0.75% (diet 5) recorded lower cholesterol levels, compared to birds on diets 1 and 2. The hypolipidemia recorded in birds fed diets 3 and 4 could potentially be attributed to the bioactive compounds present in the phytogenic supplement (WCLP) utilized in this study. As previously documented by Darmawan *et al*. (2022), phytogenic supplementation has been shown to reduce blood cholesterol levels in animals by binding to bile acids and inhibiting liver synthesis of new cholesterol, facilitated by bioactive substances such as flavonoids and saponins. The observed similarities in values of the serum alanine aminotransferase and creatinine of the birds in this present study suggest that WCLP could be used to promote the normal functioning of the liver, heart, and kidneys in animals (Lording and Friend, 1991; Adeyeye *et al*., 2020). Alanine aminotransferase and creatinine are primarily used to monitor the course of disorder in the liver, kidney, and other organs in the body (Huang *et al*., 2006). This result is in line with the report of Adeyeye *et al.* (2020) who reported no significant difference in serum alanine aminotransferase and creatinine of the broiler chickens fed the diets containing 0.4%, 0.8%, and 1.2% wild sunflower and goat weed leaf meals composite mix compared to the control group. However, the continuous decrease in the cholesterol level of broiler chicken as WCLP increases in diet suggests the hypocholesterolemic capacity of the WCLP compared to the control and PPAB-supplemented diet. Dietary leaf meal containing strong and huge phytochemicals has been reported to inhibit hypercholesterolemia and lower cholesterol levels by delaying intestinal absorption of dietary fat and pancreatic lipase activity in animals (Adu *et al*., 2020). This result is in line with the finding of Olugbemi *et al*. (2010) who reported a significant decrease in cholesterol levels of birds fed diets containing *Moringa oleifera* at 5% and 10% and Oloruntola *et al*. (2021b), who recorded reduced blood cholesterol in broiler chickens fed 0.25% *Ocimum gratissimum* leaf powder supplemented diet.

The serum stress biomarkers concentration of broiler chickens fed diets supplemented with WCLP is presented in Table 5. The dietary supplementation of WCLP revealed a significant increase (P < 0.05) in the concentration of total serum glutathione peroxidase (GPx) and catalase (CAT) while the value of superoxide dismutase (SOD) was statistically similar across the treatments. Broiler chicken fed a diet containing WCLP at 0.25% (diet 3), 0.50% (diet 4), and 0.75% (diet 5) recorded higher concentrations of serum glutathione peroxidase at 150.50, 214.52, and 168.51 mg/mL, respectively, compared to diet 1 (96.32 mg/mL) and 0.1% PPAB treated (diet 2) birds (144.50 mg/mL).

**Table 3** Effects of the wild cucumber leaf powder and clove basil leaf powder blend on hematological indices of broiler chickens

| Parameters | Diet 1 Control | Diet 2 0.1% PPAB | Diet 3 0.25% WCLP | Diet 4 0.50% WCLP | Diet 5 0.75% WCLP | SEM | P-value |
|---|---|---|---|---|---|---|---|
| Packed cell volume (%) | 29.00 | 29.33 | 27.00 | 32.00 | 28.00 | 0.75 | 0.32 |
| Red blood cells ($\times 10^{12}$/L) | 2.03 | 1.80 | 1.56 | 1.76 | 2.06 | 0.07 | 0.21 |
| Hemoglobin concentration (g/dL) | 9.66 | 9.78 | 9.00 | 10.66 | 9.33 | 0.25 | 0.32 |
| Mean cell hemoglobin concentration (g/dL) | 33.01 | 32.91 | 33.24 | 33.01 | 32.87 | 0.10 | 0.85 |
| Mean cell volume (fL) | 145.18 | 162.78 | 174.24 | 182.01 | 137.64 | 6.76 | 0.18 |
| Mean cell hemoglobin (pg) | 48.39 | 54.26 | 58.08 | 60.66 | 45.88 | 2.25 | 0.17 |
| White blood cells ($\times 10^{9}$/L) | 2.95 | 2.10 | 2.30 | 2.20 | 2.10 | 0.16 | 0.47 |
| Granulocytes ($\times 10^{9}$/L) | 0.36 | 0.33 | 0.63 | 0.85 | 0.53 | 0.07 | 0.13 |
| Lymphocytes ($\times 10^{9}$/L) | 2.56 | 1.74 | 1.62 | 1.33 | 1.57 | 0.15 | 0.07 |
| Monocytes ($\times 10^{9}$/L) | 0.01 | 0.02 | 0.04 | 0.02 | 0.02 | 0.01 | 0.32 |

**Note:** PPAB = probiotic, prebiotic, and acidifier blend, WCLP = wild cucumber and clove basil leaf powder, SEM = standard error of the mean.

**Table 4** Effects of the wild cucumber leaf powder and clove basil leaf powder blend on serum chemistry indices of broiler chickens

| Parameters | Diet 1<br>Control | Diet 2<br>0.1% PPAB | Diet 3<br>0.25% WCLP | Diet 4<br>0.50% WCLP | Diet 5<br>0.75% WCLP | SEM | P-value |
|---|---|---|---|---|---|---|---|
| Total protein (g/L) | 54.33 | 58.00 | 63.67 | 54.68 | 52.00 | 2.46 | 0.67 |
| Aspartate aminotransferase (IU/L) | 110.68 | 106.75 | 94.50 | 123.57 | 106.57 | 5.33 | 0.63 |
| Creatinine (μmol/L) | 94.33 | 94.67 | 93.00 | 93.00 | 91.33 | 3.33 | 0.99 |
| Cholesterol (mmol/L) | $2.30^{a}$ | $1.60^{ab}$ | $1.54^{b}$ | $1.14^{b}$ | $1.27^{b}$ | 0.14 | 0.04 |
| Monocytes ($\times 10^9$/L) | 0.01 | 0.02 | 0.04 | 0.02 | 0.02 | 0.01 | 0.32 |

**Note:** Means within a row with different letters are significantly different at P < 0.05. PPAB = probiotic, prebiotic, and acidifier blend, WCLP = wild cucumber and clove basil leaf powder, SEM = standard error of the mean.

**Table 5** Effects of the wild cucumber leaf powder and clove basil leaf powder blend on the broiler chickens' stress biomarkers

| Parameters | Diet 1<br>Control | Diet 2<br>0.1% PPAB | Diet 3<br>0.25% WCLP | Diet 4<br>0.50% WCLP | Diet 5<br>0.75% WCLP | SEM | P-value |
|---|---|---|---|---|---|---|---|
| Glutathione peroxidase (mg/mL) | $96.32^{c}$ | $144.50^{bc}$ | $150.50^{bc}$ | $214.52^{a}$ | $168.51^{ab}$ | 12.11 | 0.01 |
| Catalase (U/mL) | $7.57^{c}$ | $10.66^{bc}$ | $13.03^{ab}$ | $14.97^{a}$ | $16.96^{a}$ | 0.99 | <0.001 |
| Superoxide dismutase (%) | 79.07 | 82.90 | 91.32 | 89.83 | 91.52 | 2.60 | 0.49 |

**Note:** Means within a row with different letters are significantly different at P < 0.05. PPAB = probiotic, prebiotic, and acidifier blend, WCLP = wild cucumber and clove basil leaf powder, SEM = standard error of the mean.

Similarly, the concentration of catalase was higher in broiler chickens fed a diet containing WCLP than those on diets 1 and 2. Catalase and glutathione peroxidase are among the popular antioxidant enzymes that catalytically remove the reactive oxygen species and consequently defend the body of the organism against the effects of oxidative stress (Nandi *et al*., 2019). The observed increased level of catalase and glutathione peroxidase recorded in broiler chickens supplemented with WCLP (diets 4 and 5) compared to diets 1 and 2 suggest the ability of the composite leaf mix to produce higher endogenous antioxidant enzymes to inhibit and/or scavenge free radicals and other reactive species which have been implicated in causing lipid peroxidation and organ damages in animals (Oloruntola *et al*., 2019). This result is in line with the report of Kostadinović *et al*. (2015) and Adeyeye *et al.* (2020) who found that the addition of phyto-additive in the broiler diet significantly enhanced the concentration of glutathione peroxidase and catalase compared to the control group.

## CONCLUSIONS

The findings of this study demonstrated that WCLP could be used as a feed addition at 0.25% without affecting broiler chicken growth performance. Beyond 0.25% WCLP would increase antioxidative status and lower serum cholesterol concentrations in broiler chickens, but it would also reduce body weight gain.

## REFERENCES


Adebayo, K.O., R.Y. Aderinboye, K.A. Sanwo, I.K. Oyewusi and O.A. Isah. 2019. Growth performance and fecal worm egg count of West African dwarf goats fed diets containing varying levels of *Ocimum gratissimum* (scent leaf). Livest. Res. Rural Dev. 31(8): 124.

Adeyeye, S.A., A.B. Falowo, O.D. Oloruntola, M.H. Ogunsipe and J.O. Agbede. 2021. Performance and nutrient digestibility of growing rabbits fed dietary processed kola nut (*Cola nitida*) pod-husk meal. Trop. Agric. (Trinidad). 98(1): 47–56.

Adeyeye, S.A., O.D. Oloruntola, S.O. Ayodele, A.B. Falowo and J.O. Agbede. 2020. Wild sunflower and goat weed leaf meals composite-mix supplementation in broiler chickens: Effects on performance, health status and meat. Acta Fytotechn Zootechn. 23(4): 205–212. https://doi.org/10.15414/afz.2020.23.04.205-212.

Adu, O.A., F.A. Gbore, O.D. Oloruntola, A.B. Falowo and O.J. Olarotimi. 2020. The effects of *Myristica fragrans* seed meal and *Syzygium aromaticum* leaf meal dietary supplementation on growth performance and oxidative status of broiler chicken. Bull. Natl. Res. Cent. 44(1): 149. https://doi.org/10.1186/s42269-020-00396-8.

Aebi, H. 1974. Catalase, pp. 673–684. *In*: H.V. Bergmeyer, (Ed.), Methods of Enzymatic Analysis. Verlag Chemic, Academic Press, New York, USA.

Ahmed, S.T., H.S. Mun, M.M. Islam, S.Y. Ko and C.J. Yang. 2016. Effects of dietary natural and fermented herb combination on growth performance, carcass traits and meat quality in grower-finisher pigs. Meat Sci. 122: 7–15. https://doi.org/10.1016/j.meatsci.2016.07.016.

Buragohain, R. 2016. Growth performance, nutrient utilization, and feed efficiency in broilers fed *Tithonia diversifolia* leaf meal as substitute of conventional feed ingredients in Mizoram. Vet. World. 9(5): 444–449. https://doi.org/10.14202/vetworld.2016.444-449.

Cheesbrough, M. 2000. District Laboratory Practice in Tropical Countries. 1st Edition. Cambridge University Press, UK.

Darmawan, A., W. Hermana, D.M. Suci, R. Mutia, Sumiati, A. Jayanegara and E. Ozturk. 2022. Dietary phytogenic extracts favorably influence productivity, egg quality, blood constituents, antioxidant and immunological parameters of laying hens: A meta-analysis. Animals 12(17): 2278. https://doi.org/10.3390/ani12172278.

Falowo, A.B. and O.F. Akimoladun. 2020. Veterinary drug residues in meat and meat products: Occurrence, detection and implications. *In*: S. Oppong Bekoe, M. Saravanan, R. Kwame Adosraku and P.K. Ramkumar, (Eds.), Veterinary Medicine and Pharmaceuticals. IntechOpen, London, UK.

Gulizia, J.P. and K.M. Downs. 2020. Comparison of dietary kudzu leaf meal *(Pueraria montana* var. *lobata*) and alfalfa meal supplementation effect on broiler (*Gallus gallus domesticus*) performance, carcass characteristics, and organ parameters. Animals (Basel) 10(1): 147. https://doi.org/10.3390/ani10010147.

Hashemi, S.R., I. Zulkifli, M. Hair Bejo, A. Farida and M.N. Somchit. 2008. Acute toxicity study and phytochemical screening of selected herbal aqueous extract in broiler chickens. Int. J. Pharmacol. 4(5): 352–360. https://doi.org/10.3923/ijp.2008.352.360.

Huang, X.J., Y.K. Choi, H.S. Im, O. Yarimaga, E. Yoon and H.S. Kim. 2006. Aspartate aminotransferase (AST/GOT) and alanine aminotransferase (ALT/GPT) detection techniques. Sensors 6(7): 756–782.

Igbinosa, E.O., E.O. Uzunuigbe, I.H. Igbinosa, E.E. Odjadjare, N.O. Igiehon and O.A. Emuedo. 2013. *In vitro* assessment of antioxidant, phytochemical and nutritional properties of extracts from the leaves of *Ocimum gratissimum* (Linn). Afr. J. Tradit. Complement. Altern. Med. 10(5): 292–298.

Kostadinović, L., J. Lević, S. Popović, I. Čabarkapa, N. Puvača, O. Djuragic and S. Kormanjoš. 2015. Dietary inclusion of *Artemisia absinthium* for management of growth performance, antioxidative status and quality of chicken meat. Europ. Poult. Sci. 79. https://doi.org/10.1399/eps.2015.75.

Lillehoj, H., Y. Liu, S. Calsamiglia, M.E. Fernandez-Miyakawa, F. Chi, R.L. Cravens, S. Oh and C.G. Gay. 2018. Phytochemicals as antibiotic alternatives to promote growth and enhance host health. Vet. Res. 49: 76. https://doi.org/10.1186/s13567-018-0562-6.

Lording, P.M. and S.C.E. Friend. 1991. Interpretation of laboratory results. Aust. Vet. Pract. 21(4): 188–193.

Misra, H.P. and I. Fridovich. 1972. The univalent reduction of oxygen by reduced flavins and quinones. J. Biol. Chem. 247(1): 188–192. https://doi.org/10.1016/S0021-9258(19)45773-6.

Mokhtari, R., A. Yazdani and H. Kashfi. 2015. The effect of different growth promoters on performance and carcass characteristics of broiler chickens. J. Vet. Med. Anim. Health. 7(8): 271–277.

Nagarani, G., A. Abirami and P. Siddhuraju. 2014. Food prospects and nutraceutical attributes of *Momordica* species: A potential tropical bioresources–A review. Food Sci. Hum. Wellness. 3(3–4): 117–126. https://doi.org/10.1016/j.fshw.2014.07.001.

Nandi, A., L.J. Yan, C.K. Jana and N. Das. 2019. Role of catalase in oxidative stress- and age-associated degenerative diseases. Oxid. Med. Cell. Longev. 2019: 9613090. https://doi.org/10.1155/2019/9613090.

NRC (National Research Council). 1994. Nutrient Requirements of Poultry. 9th Edition. National Academy Press, Washington, DC, USA.

Nte, I.J., V.U. Oleforuh-Okoleh and L.B. Fakae. 2017. Responses of broiler chicks to scent leaf (*Ocimum gratissimum*) aqueous extracts during different stages of growth. Delta Agriculturists 9(1): 99–105.

O'Neill, J. 2015. Antimicrobials in Agriculture and the Environment: Reducing Unnecessary Use and Waste. Wellcome Trust, London, UK.

Oloruntola, O.D., J.O. Agbede, S.O. Ayodele and D.A. Oloruntola. 2019. Neem, pawpaw and bamboo leaf meal dietary supplementation in broiler chickens: Effect on performance and health status. J. Food Biochem. 43(2): e12723. https://doi.org/10.1111/jfbc.12723.

Oloruntola, O.D., O.A. Adu, F.A. Gbore, A.B. Falowo and O.J. Olarotimi. 2021b. Performance of broiler chicken fed diets supplemented with *Irvingia gabonensis* kernel powder and *Ocimum gratissimum* leaf powder. Slovak. J. Anim. Sci. 54(1): 7–20.

Oloruntola, O.D., O.S. Ayodele and D.A. Oloruntola. 2018. Effect of pawpaw (*Carica papaya*) leaf meal and dietary enzymes on broiler performance, digestibility, carcass and blood composition. Rev. Elev. Med. Vet. Pays Trop. 71(3): 121–129. https://doi.org/10.19182/remvt.31640.

Oloruntola, O.D., S.O. Ayodele, O.P.A. Olowu, A.B. Falowo, S.A. Adeyeye, I.S. Omoniyi and C.O. Osowe. 2021a. The proximate analysis, phytochemical screening, antioxidant activity and mineral composition of *Momordica charantia* and *Ocimum gratissimum* leaf powder. Asian J. Res. Biochem. 8(4): 30–39. https://doi.org/10.9734/ajrb/2021/v8i430188.

Olugbemi, T.S., S.K. Mutayoba and F.P. Lekule. 2010. *Moringa oleifera* leaf meal as a hypocholesterolemic agent in laying hen diets. Livest. Res. Rural Dev. 22(4): 84.

Olumide, M.D. and A.S. Akintola. 2018. Effect of scent leaf meal (*Ocimum gratissimum*) supplementation on performance, carcass and meat quality of broiler chicken. Nig. J. Anim. Prod. 45(3): 228–236. https://doi.org/10.51791/njap.v45i3.436.

Rotruck, J.T., A.L. Pope, H.E. Ganther, A.B. Swanson, D.G. Hafeman and W.G. Hoekstra. 1973. Selenium: Biochemical role as a component of glutathione peroxidase. Science 179(4073): 588–590. https://doi.org/10.1126/science.179.4073.588.

Saeed, F., M. Afzaal, B. Niaz, M.U. Arshad, T. Tufail, M.B. Hussain and A. Javed. 2018. Bitter melon (*Momordica charantia*): A natural healthy vegetable. Int. J. Food Prop. 21(1): 1270–1290. https://doi.org/10.1080/10942912.2018.1446023.

Santoso, U., Y. Fenita, K. Kususiyah, O. Widiantoro and S. Kadarsih. 2018. The effect of medicinal herb on fat deposition, meat composition, amino acid and fatty acid composition of broiler meats. J. Indones. Trop. Anim. Agric. 43(1): 54–65. https://doi.org/10.14710/jitaa.43.1.54-65.

Sobayo, R.A., O.A. Adeyemi, O.G. Sodipe, A.O. Oso, A.O. Fafiolu, I.M. Ogunade, O.S. Iyasere and L.A. Omoniyi. 2012. Growth response of broiler birds fed *Asystasia gangetica* leaf meal in hot humid environment. J. Agric. Sci. Env. 12(1): 53–59. https://doi.org/10.51406/jagse.v12i1.1289.

Suganya, T., S. Senthilkumar, K. Deepa, J. Muralidharan, G. Gomathi and S. Gobiraju. 2016. Herbal feed additives in poultry. Int. J. Sci. Environ. Technol. 5(3): 1137–1145.

Sugiharto, S., T. Yudiarti, I. Isroli, E. Widiastuti, H.I. Wahyuni and T.A. Sartono. 2019. Recent advances in the incorporation of leaf meals in broiler diets. Livest. Res. Rural Dev. 31(7): 109.

Valenzuela-Grijalva, N.V., A. Pinelli-Saavedra, A. Muhlia-Almazan, D. Dominguez-Diaz and H. Gonzalez-Rios. 2017. Dietary inclusion effects of phytochemicals as growth promoters in animal production. J. Anim. Sci. Technol. 59: 8. https://doi.org/10.1186/s40781-017-0133-9.