Effect of aqueous Mutingia calabura milled leaves extract on the hematological properties, serum biochemistry, and intestinal morphology in broiler chickens.

.

ABSTRACT

|  |
| --- |
| **Aims:** This experiment evaluated the effects of different levels of Mutingia calabura leaves (MCL) on hematology parameters, serum parameters, and small intestine histomorphology of broiler chickens at 42 days of age.  **Study design:** The study design was a completely randomized design.  **Place and Duration of Study:** Department of Animal, Aquaculture and Range Sciences, Sokoine University of Agriculture, between September 2024 and March 2025.  **Methodology:**  A total of 250 Day old chick broiler chicks were randomly assigned into six treatments with four replications of 10 chicks each. Commercial feed and aqueous MCL extract were supplied ad libitum throughout the experiment. The treatment groups consisted of T1 (positive control), T2 (0g/L), T3 (2g/L) T4 (4g/L), T5 (6g/L), and T6 (8g/L). Parameters measured were small intestine (duodenum, jejunum, and ileum) histomorphology (villus height, villus width, crypt depth, and villus height to crypt depth ratio), blood serum biochemistry, and hematology parameters. The recorded data were analyzed under one-way analysis of variance using SAS, and the comparison of means was done using Turkey-test.  **Results:** Results show that MCL significantly (*P=*0.05) affects hematological parameters while lymphocytes, RDW, and MCHC showed no significant differences. Birds treated with T5-6g/L were observed to have higher values of WBC, followed by T4-4g/L, T1-positive control, T3-2g/L, T6-8g/L the least being T1-positive control. RBC and hemoglobin were higher in T5-6g/L, T4-4/g/L, and T3-2g/L while T2-negative control showed least value. Significant differences were observed in total protein and AST. Total protein was high in T5 followed by T4, T6, T3, T2, and least in T1 while, AST was high in T1 but still in the normal range. MCL showed a significant difference in histomorphology parameters and was observed to increase VH, VW and VH/CD as the level of dose increases up to 6g/L (T5).  **Conclusion:** Conclusively, MCL can be used as feed additive to improve intestinal and blood parameters which increase overall growth performance of broiler. |

*Keywords: broiler, serum, hematology, intestinal morphology.*

1. INTRODUCTION

Currently broiler industry is increasing in its potential, especially in most developing countries. Broiler chickens are more prone to diseases therefore, antibiotics are usually used to overcome disease challenges and also in some cases as growth promoter. Overuse of antibiotics is associated with antimicrobial resistance (AMR) which is currently of a global concern as far as human health is concerned. AMR is the natural process that occurs in bacteria in response to the use of antimicrobials either for therapeutic or non-therapeutic purposes. AMR in animal production is a silent phenomenon in which its effect intensity depends on the quantity of antimicrobial used, duration and frequency of exposure and antimicrobial residue exposure (Wall *et al.,* 2016). However, some organizations have approved a continuous use of antimicrobials in subtherapeutic levels as growth promotion, prophylaxis or metaphylaxis. It is a known fact that the existing interaction between animals, human, plants and environment tend to accelerate the spread of resistant pathogens globally, which results to more spread of infectious diseases that are hard to treat (Ahmad *et al.,* 2021). To ameliorate the problem, the use of natural antimicrobials that are deemed to be safer to both animal health and humans who consumes the products has been advocated to reduce the use of synthetic antibiotics (Khare *et al.,* 2021). Some plants are good source of natural antimicrobials and growth promoter agents mainly phytochemicals that are believed to be less AMR causing agents (Demir *et al.,* 2003; Aziz & Karboune., 2018) some of these plants include; garlic, neem, moringa and have been observed to affect feed intake, growth parameters, carcass parameters, blood parameters and boiler immunity (Fadlalla *et al.,* 2010; Gobezie., 2022; Modisaojang-Mojanaga *et al.,* 2019).

Herbal plants used in broiler as feed ingredients or as feed additives has shown to affect blood parameters as well as intestinal structures. A review done by Sadid & Anam (2025) on Moringa oleifera observed significance improvement in average daily gain, red blood cells, and haemoglobin in 32 studies. Another study on neem and moringa supplementation recorded improved effect in haemoglobin levels, lymphocytes and heterophils. Mutingia calabura leaves (MCL) is another plant product that has been reported to have various phytochemicals including sterols, flavonoids, alkaloids, saponins, glycosides, and tannins. MCL has been used in fish, pig, dairy cow, and goats to promote growth (Febrianti, 2021; Silverio & Ramoran, 2022; Preethi *et al.,* 2012 and John, 2024). These authors observed a positive effect of MCL on growth performance parameters, immunoglobulins, breast muscle fatty acids, increase in the level of unsaturated fatty acids and lowering of the level of saturated fatty acids which led to increase in customer acceptability of broiler meat. Due to the limited information of MCL this study aims to determine the effect of MCL extract on hematological properties, serum biochemistry, and intestinal morphology in broiler chickens.

2. material and methods

**2.1 Location and duration of study.**

The study was conducted at Department of Animal science, Range and Aquaculture in lower farm poultry houses at Sokoine University of Agriculture Morogoro (SUA) Tanzania that lies on Uluguru mountains slope at an altitude of 500-600 meters above the sea level and the annual rainfall is 600-1000mm.

**2.2 Management of experimental units and housing.**

250-day-old broiler chicks (DOC) were bought from Silverland company. After their arrival chicks were measured for weight and wing tagged, then brooded separately according to the treatment provided for two weeks. After brooding, broiler birds were raised under a deep litter system using rice husks. During brooding, chicks were fed a commercial compounded starter diet (crumbles) followed by a grower diet (pellet form) for three weeks. Anticoccidials and vaccinations were provided as per recommended schedules.

**2.3 Experimental Design**

The 250 DOC were randomly subjected to six (6) treatments (T1, T2, T3, T4, T5, and T6) using a completely randomized design. T1 being a positive control supplied with compounded feed and antibiotic & anticoccidials (prophylactic), T2 a negative control supplied with feed and plain water, T3 supplied with feed and 2g/1L of MCL in water, T4 supplied with commercial feed and 4g/1L of MCL in water, T5 supplied with commercial feed and 6g/1L MCL in water and T6 supplied with commercial feed and 8g/1L MCL in water. Each of the six (6) treatment had forty (40) birds with four (4) replicates for each treatment, that is ten (10) bird per replicate.

**2.4 Preparation of aqueous mutingia calabura leaf extract.**

MCL were harvested from SUA farm, air dried under the shade at normal room temperature for three (3) weeks, then milled to obtain the leaf powder. The leaf powder was soaked in water for 24 hours according to treatment concentration and provided to chickens daily until the end of the experiment.

**2.5 Data collection and measurement**

**2.5.1 Hematological and serum data**

At the age of six weeks 24 broilers were selected randomly 4 from each treatment followed by taking blood sample from the brachial wing vein using a vacutainer tube. The blood containers were inverted gently 6-10 times and placed into a rack ready for analysis. The analysis of hematology and serum blood parameters was done using an automatic hematology analyzer MS4s from MELET SCHLOESING Laboratories.

**2.5.2 Histomorphology parameters**

The selected broilers from each treatment were anesthetized through exsanguination and intestine was pooled to section duodenum, jejunum and ileum. All procedures followed the guideline for animal use in experimentation of Sokoine University of Agriculture. The duodenum segment was differentiated from jejunum through gizzard to bile duct, the jejunum segment was differentiated from ileum through Meckel's diverticulum and the ileum was differentiated from large intestine through ileo-cecal colonic junction. The tissue samples were dissected from the middle part of duodenum, jejunum and ileum followed by fixation in 10% neutral buffered formalin for 72 hours at room temperature. Small section (4 mm thick) of duodenum, jejunum and ileum were cut transversely and processed for histomorphometric analysis. The tissues were processed in ascending ethanol series concentration, cleared in chloroform and impregnated in paraffin wax. The processed tissues were embedded to form tissue blocks for sectioning. The tissue blocks were cut at 3μm thick (using microtome machine) to produce serial tissue sections. The sections were transferred into cold water then into hot water (45ºC) and then mounted on a clean microscope glass slide. The tissue sections were dried in hot air oven overnight.

Tissue sections were deparaffinized in xylene followed by rehydration through a descending ethanol series concentration to distilled water. The sections were stained in Hematoxylin for 4 minutes, washed in distilled water then differentiated in acid – alcohol and later washed again in distilled water. The tissues were blued in alkaline (lithium carbonate) solution and washed in distilled water. Later, the tissues were counterstained with eosin for 5 minutes, dehydrated in methanol, cleared in xylene and mounted by microscope glass coverslip using mounting solution (dibutylphthalate polystyrene xylene, DPX). Stained tissues were visualized, measured and photographed on Olympus Light Microscope (Olympus Corporation, Model D21-CB, SN 0010842A2, Tokyo, Japan) equipped with an adjusted digital camera (Olympus U-TV0.5XC-3, SN OK73198, Tokyo Japan). All histology/histomorphometric images and measurements were made in μm, at ×40 magnification.

Measurements of intestinal villus height, width and crypt. The following intestinal histomorphometric parameters were measured in all sections: villus height, villus width and crypt depth. These parameters were measured on well-aligned (straight) villi and corresponding crypts from each section of the intestinal segments. The heights of the villi were measured from their tip to the base and the widths were measured at the mid part of the villus. Lastly, the depth of intestinal crypts was measured as the distance from the top of villus crypt to the muscularis mucosa.

**2.6 Statistical Analysis**

All data were analyzed using one-way analysis of variance (ANOVA) implemented in SAS software to evaluate treatment effects. Tukey’s Honest Significant Difference (HSD) test was employed for multiple comparisons among treatment means. Results were expressed as means ± standard error of the mean (SEM), with statistical significance set at *P*= 0.05. This approach ensured reliable identification of significant differences in hematological, serum, and histomorphology parameters across treatments.

3. results and discussion

**3.1 Results**

The proximate composition of the starter and grower ration are presented in Table 1. Both diets had adequate major nutrients that can support the growth of birds as reported by Dozier *et al.,* (2010) and Agah, & Norollahi, (2008).

The effect of MCL on hematological parameters in broilers is shown in Table 2. Results show that MCL significantly (*P*=0.05) affected the hematological parameters. The lymphocytes, RDW, and MCHC showed non-significant differences. Birds treated with T5-6g/L were observed to have higher values of WBC followed by T4-4g/L, T1-positive control, T3-2g/L, T6-8g/L then T1-positive control. RBC and hemoglobin were higher in T5-6g/L, T4-4/g/L, and T3-2g/L while T2-negative control showed the lowest value.

The effect of MCL on serum parameters in the broiler is shown in Table 3. Highly significant differences were observed in total protein and AST. Total protein was high in T5, T4, T6, T3, T2, and least in T1 while AST was high in T1 but still within the normal range. MCL showed a significant difference in histomorphology parameters, MCL was observed to increase VH and VW on jejunum, duodenum, and ileum as the level of dose increases up to T5.

Table 1: Proximate composition of starter and grower

|  |
| --- |
| Nutrients/ parameters starter grower |
| DM (%) 96.47 96.81  Ash 14.36 14.32  Crude protein (%) 23.64 20.06  Crude fiber (%) 4.63 3.03  Ether extract (%) 5.9 6.04  Nitrogen-free extract 51.47 56.55  ME MJ/kg (%) 12.10 12.42 |

Table 2: Least Square Means (±SE) on the effect of MCL on hematological parameters

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Treatment | T1 | T2 | T3 | T4 | T5 | T6 | SEM | P-value |
| WBC(m/mm3) | 75.31bc | 42.83e | 71.32c | 80.59ab | 84.73a | 64.26d | 1.3242 | 0.0001⁎⁎ |
| Lym(%) | 86.775ab | 83.875b | 91.21a | 91.27a | 91.72a | 91.51a | 1.5311 | 0.007⁎ |
| Mon(%) | 7.95 | 6.1 | 6.5 | 6.925 | 6.75 | 5.8 | 0.76237 | 0.4618 |
| Neu(%) | 5.275a | 3.375ab | 2.6b | 2.8b | 2.8b | 2.75b | 0.5207 | 0.0154⁎ |
| RBC(M/mm3) | 2.27bc | 1.88d | 2.40a | 2.48a | 2.53a | 2.15c | 0.04423 | 0.0001⁎ |
| MCV(ft) | 119.79a | 115.64b | 120.96a | 121.013a | 121.43a | 121.4a | 1.3275 | 0.0465⁎ |
| Hct(%) | 26.11a | 17.96b | 25.84a | 26.40a | 25.99a | 25.76a | 0.29024 | 0.0001⁎ |
| MCH(pg) | 50.00a | 48.55b | 50.51a | 50.80a | 51.04a | 48.73b | 0.26266 | 0.0001⁎ |
| MCHC(g/dl) | 41.0475 | 41.2825 | 40.77 | 41.41 | 40.6625 | 40.345 | 0.34715 | 0.2926 |
| RDW | 8.2 | 8.225 | 8.225 | 8.2 | 8.25 | 8.15 | 0.0433 | 0.6848 |
| Hb(g/dl) | 10.27c | 8d | 10.36c | 11.31b | 12.37a | 10.75c | 0.10675 | 0.0001⁎ |
| THR(M/mm3) | 7.5c | 4.75d | 7.75c | 10.25b | 11.25b | 16.5a | 0.4564 | 0.0001⁎ |
| MPV(fl) | 6.1a | 5.72b | 6.075a | 6.05a | 6.12a | 6.05a | 0.05303 | 0.0005⁎ |

*Mean with different superscripts are significant differences at a P= 0.005.*

*WBC= white blood cell, Lym= lymphocyte, Mon= monocytes, Neu= neutrophils, RBC= red blood cells, MCV= mean* *corpuscular volume, Hct=* *haematrocrit, MCH= mean concentration haemoglobin, MCHC= mean corpuscular haemoglobin concentration RDW= red cell distribution width, Hb= haemoglobin, THR= thyrotropin releasing hormone, MPV= mean platelet volume*

Table 3: Least Square Means (±SE) on the effect of MCL on serum biochemistry of broiler

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Treatment | T1 | T2 | T3 | T4 | T5 | T6 | SEM | P-value |
| Total protein (g/d/) | 2.46c | 2.55c | 2.89b | 3.28a | 3.43a | 3.02b | 0.052 | 0.0001⁕ |
| ALT (U/L) | 2.780 | 2.629 | 2.799 | 2.811 | 2.826 | 2.786 | 0.051 | 0.1347 |
| AST (U/L) | 76.86a | 54.81b | 56.31b | 55.96b | 54.47b | 55.40b | 1.232 | 0.0001⁕ |
| CREATININE (mg/dl) | 0.359 | 0.322 | 0.308 | 0.314 | 0.297 | 0.291 | 0.034 | 0.750 |
| UREA (mg/dl) | 5.492 | 5.542 | 5.85 | 5.404 | 5.983 | 5.542 | 0.290 | 0.6897 |

*Mean with different superscripts are significant differences at a P= 0.005.*

AST= *aspartate aminotransferase,* ALT= *alanine transaminase*

Table 4: Least Square Means (µm) (±SE) on the effect of MCL on histomophometry

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | T1 | T2 | T3 | T4 | T5 | T6 | SEM | P-VALUE |
| **Deudonum** |  |  |  |  |  |  |  |  |
| VH | 1905.45f | 1923.8e | 2104.87c | 2129.11b | 2144.81a | 1949.85d | 1.635 | 0.0001⁎ |
| VW | 210.316f | 216.046e | 226.046c | 235.432b | 243.814a | 220.646d | 0.818 | 0.0001⁎ |
| CD | 274.717a | 263.101b | 242.805d | 236.312e | 226.029f | 253.704c | 0.506 | 0.0001⁎ |
| VH/CD | 6.93f | 7.312e | 8.669c | 9.009b | 9.48a | 7.68d | 0.019 | 0.0001⁎ |
| **Jejunum** |  |  |  |  |  |  |  |  |
| VH | 1498.45e | 1595.87d | 1791.23b | 1830.2a | 1836.43a | 1603.21c | 1.344 | 0.0001⁎ |
| VW | 152.07f | 161.53e | 170.57c | 185.41b | 195.94a | 166.29d | 0.729 | 0.0001⁎ |
| CD | 268.78a | 257.756b | 251.369c | 236.924d | 237.246d | 253.892bc | 1.413 | 0.0001⁎ |
| VH/CD | 5.57d | 6.19c | 7.12b | 7.73a | 7.740a | 6.314c | 0.046 | 0.0001⁎ |
| **Ileum** |  |  |  |  |  |  |  |  |
| VH | 995.95f | 1043.76e | 1134.32c | 1158.03b | 1285.93a | 1099.64d | 2.135 | 0.0001⁎ |
| VW | 86.66f | 97.33e | 121.49c | 139.69b | 151.35a | 113.71d | 0.418 | 0.0001⁎ |
| CD | 217.546a | 201.07b | 199.30d | 196.62e | 191.90f | 209.76b | 0.428 | 0.0001⁎ |
| VH/CD | 4.57f | 5.19e | 5.69c | 5.88b | 6.70a | 5.24d | 0.018 | 0.0001⁎ |

*Mean with different superscripts are significant differences at P = 0.005.*

*VH= villi height, VW= villi weight, CD= crypt depth*

**3.2 Discussion**

This study evaluated the effect of *Muntingia calabura* leaf extract on hematological properties, serum biochemistry, and intestinal morphology, demonstrating its potential as a natural growth promoter in broiler production. The results of this study highlight the significant potential of *Muntingia calabura* leaf (MCL) extract in improving hematological parameters, serum biochemistry, and intestinal morphology in broiler chickens.

**3.2.1 Hematological Parameters**

The observed improvements in hematological parameters, particularly in treatments supplemented with 6 g/L MCL (T5), indicate enhanced physiological status and immune function. White blood cell (WBC) counts were significantly higher in T5 compared to other treatments, suggesting that the phytochemicals in MCL, such as flavonoids, alkaloids, and saponins, may have immunomodulatory effects. Flavonoids, for instance, are known for their antioxidant properties, which reduce oxidative stress and enhance immune cell activity (Upah *et al.,* 2024). Antioxidants present in MCL includes; 20, 40- dihydroxy chalcone (isoliquiritigenin (cabreuvin), (2S)-50-hydroxy-7, 8, 30, 40-tetramethoxyavan 20, 40-dihydroxydihydrochal-cone and 3, 4, 5-trihydroxybenzoic acid, (Preethi *et al.,* 2010). Also, phytochemicals may enhance the release of anti-inflammatory cytokines which work together with transforming growth factor beta TGF- B to suppress inflammatory responses, (John, 2024). This aligns with findings by Febrianti (2021), who reported improved WBC levels in fish supplemented with MCL extract.

Red blood cell (RBC) counts and hemoglobin (Hb) levels were also highest in T5, reflecting improved erythropoiesis. This may be attributed to the presence of iron and bioactive compounds in MCL that enhance iron absorption and utilization, as supported by Johnson (2013). Additionally, the ability of flavonoids to increase ferroportin expression and inhibit eryptosis (premature RBC death) may contribute to the observed outcomes (Restivo *et al.,* 2022); (Nkukwana *et al.,* 2015). The lower RBC and Hb values in the negative control group (T2) highlight the importance of supplementation to mitigate nutritional deficiencies.

As the level of MCL dosage increased, so did the TRH value. TRH is responsible for growth hormone production and regulates thyroid function by stimulating the release of thyroid-stimulating hormone. Birds under T6 were observed to have an abnormally high level of TRH, which may have resulted in weight loss by affecting the brain feeding center, which reduced feed intake (Fröhlich & Wahl, 2019). There is no clear explanation of how phytochemicals enhance TRH production however, it may be caused by; modulation of hormonal signaling through affecting the hypothalamic pituitary thyroid axis and influencing gene expression of TRH or related proteins, anti-oxidant effect, and interaction with thyroid hormone metabolism by enhancing iodine uptake, binding with estrogen receptors, and interaction with enzyme (Solís *et al.,* 2017; Lillehoj *et al.,* 2018; Smeriglio *et al.,* 2018).

**3.2.2 Serum Biochemistry**

The study revealed significant improvements in total protein levels among broilers supplemented with MCL. Total protein was highest in T5, suggesting enhanced protein synthesis and metabolic efficiency. Phytochemicals in MCL, such as tannins and alkaloids, may play a role in modulating liver function and protein metabolism (Preethi *et al*., 2012). Phytochemicals also accelerate synthesis of growth factors that are protein hence increasing total protein in blood (Murakami, 2013). These findings align with Purwanti *et al*., (2019) and Jin *et al*., (2020), who demonstrated that herbal extracts enhance dietary protein uptake and support overall metabolic processes.

Aspartate aminotransferase (AST) levels were within the normal range across treatments, with the highest levels observed in the positive control group (T1). Elevated AST in T1 might indicate hepatic stress associated with synthetic antibiotics, as suggested by Frohlich and Wahl (2019). The lower AST levels in MCL-treated groups imply hepatoprotective effects of MCL, likely mediated by its antioxidant properties that reduce oxidative damage in hepatocytes (Tamilvanan *et al*., 2017). According to the review documented by Prajapati *et al.,* (2024), it was concluded that phytochemicals and herbal extracts can effectively change liver damage biochemical markers, accelerate antioxidation, and modulate inflammatory activities.

**3.2.3. Intestinal Morphology**

The histomorphological analysis revealed substantial improvements in villus height (VH), villus width (VW), and villus height-to-crypt depth (VH/CD) ratios in the duodenum, jejunum, and ileum of broilers supplemented with MCL. The T5 treatment demonstrated the highest VH and VH/CD ratios, indicating enhanced nutrient absorption and gut health. These findings are consistent with those of Samanya and Yamauchi (2002), who reported that herbal supplements improved intestinal morphology by promoting epithelial cell proliferation and reducing crypt depth.

The observed improvements in intestinal morphology can be attributed to the phytochemicals in MCL. Flavonoids and saponins are known to have anti-inflammatory and antimicrobial properties that protect the intestinal mucosa from damage and pathogenic colonization (Preethi *et al.,* 2012). Also, phytochemicals may increase the number of proliferating stem cells which add count of mucin-producing goblet cells (Qaid *et al.,* 2021). Additionally, tannins in MCL may promote gut health by binding to proteins and forming protective layers on the intestinal epithelium, reducing inflammation and enhancing nutrient absorption (Nkukwana *et al.,* 2015).

The improvements in intestinal morphology observed in this study are critical for broiler performance. Increased villus height and surface area enhance nutrient absorption efficiency, leading to better growth performance and feed conversion ratios. These results align with Tamilvanan *et al.,* (2017), Liu *et al.,* (2021), Elbaz *et al.,* (2025) who reported similar findings in broilers supplemented with herbal extracts.

**3.2.4 Growth Performance and Health Benefits**

The cumulative effects of MCL supplementation on hematological, serum, and intestinal parameters translate into improved overall growth performance (Daud *et al.,* 2025 unpublished). The significant improvements in RBC, Hb, and total protein levels, coupled with enhanced intestinal morphology, indicate that MCL supports both systemic and gastrointestinal health. The observed effects are consistent with findings by Silverio and Ramoran (2022), who demonstrated that natural plant extracts improve growth performance by modulating immune responses and enhancing nutrient utilization.

The reduction in oxidative stress, as evidenced by improved hematological parameters and reduced AST levels, underscores the antioxidant potential of MCL. Antioxidants in MCL, such as flavonoids and phenolic compounds, scavenge free radicals, reduce lipid peroxidation, and protect cellular integrity (Preethi *et al.,* 2010). These properties are particularly beneficial in mitigating the adverse effects of oxidative stress, which is common in broilers raised under intensive systems.

**3.2.5 Implications for Tropical Poultry Production**

The findings of this study are particularly relevant for poultry production in tropical regions, where heat stress and antimicrobial resistance (AMR) pose significant challenges (Fletcher, 2015, Founou *et al*., 2021, Aberu *et al*., 2023, Bukari *et al*., 2025). The immunomodulatory and antioxidant properties of *Muntingia calabura* (MCL) provide a natural and sustainable alternative to synthetic antibiotics, helping reduce AMR risks (Febrianti, 2021; Silverio & Ramoran, 2022). Additionally, MCL's hepatoprotective and gut health-promoting effects improve intestinal morphology, nutrient absorption, and overall broiler productivity (Nkukwana *et al.,* 2015; Preethi *et al.,* 2012), making it an effective feed additive under stressful environmental conditions.

4. Conclusion

This study demonstrates that aqueous Muntingia calabura leaf (MCL) extract has significant potential as a natural feed additive to enhance the growth performance, intestinal morphology, and hematological and serum parameters of broiler chickens. Birds supplemented with MCL, particularly at 6 g/L (T5), exhibited improved white blood cell counts, hemoglobin levels, and villus height-to-crypt depth ratios, which indicate better nutrient absorption and immune modulation. Furthermore, the increase in total protein and reduced oxidative stress highlight the phytochemicals’ role in promoting overall health and growth. These findings underline MCL’s potential as an alternative to synthetic antibiotics in broiler production, addressing challenges such as antimicrobial resistance. Future research should focus on optimizing dosage levels and evaluating long-term effects to establish MCL as a cost-effective, sustainable solution for improving broiler productivity and welfare in tropical poultry systems. More investigation is required to determine how specific phytochemicals affect serum parameters, hematology parameters, and intestinal structures.

References

Aberu, R., Semedo-Lemsaddek, T., Cunha, E., Tavares, L., & Oliveira, M. (2023). Antimicrobial drug resistance in poultry production: Current status and innovative strategies for bacterial control. Microorganisms, 11(4), 953.

Ahmad, I., Malak, H. A., & Abulreesh, H. H. (2021). Environmental antimicrobial resistance and its drivers: a potential threat to public health. Journal of Global Antimicrobial Resistance, 27, 101-111.

Aziz, M., & Karboune, S. (2018). Natural antimicrobial/antioxidant agents in meat and poultry products as well as fruits and vegetables: A review. Critical reviews in food science and nutrition, 58(3), 486-511.

Bukari, Z., Emmanuel, T., Woodward, J., Ferguson, R., Ezughara, M., Darga, N., & Lopes, B. S. (2025). The Global Challenge of Campylobacter: Antimicrobial Resistance and Emerging Intervention Strategies. Tropical Medicine and Infectious Disease, 10(1), 25.

Demir, E., Sarica, Ş., Özcan, M. A., & Sui Mez, M. (2003). The use of natural feed additives as alternatives for an antibiotic growth promoter in broiler diets. British poultry science, 44(S1), 44-45.

Elbaz, A.M., Ashmawy, E.S., Farahat, M.A.A., Abdel‑Maksoud, A., Amin, S.A. and Mohamed, Z.S., (2025). Dietary Nigella sativa nanoparticles enhance broiler growth performance, antioxidant capacity, immunity, gene expression modulation, and cecal microbiota during high ambient temperatures. Scientific Reports, 15(1), pp.1-13.

Fadlalla, I. M. T., Mohammed, B. H., & Bakhiet, A. O. (2010). Effect of feeding garlic on the performance and immunity of broilers. Asian Journal of Poultry Science, 4(4), 182-189.

Febrianti, D. (2021). Preliminary study of dietary Muntingia calabura leaf on the hematology status of Clarias sp. IOP Conference Series: Earth and Environmental Science, 718(1). https://doi.org/10.1088/1755-1315/718/1/012001

Fletcher, S. (2015). Understanding the contribution of environmental factors in the spread of antimicrobial resistance. Environmental health and preventive medicine, 20, 243-252.

Founou, L. L., Founou, R. C., & Essack, S. Y. (2021). Antimicrobial resistance in the farm-to-plate continuum: more than a food safety issue. Future science OA, 7(5), FSO692.

Fröhlich, E., & Wahl, R. (2019). The forgotten effects of thyrotropin-releasing hormone: Metabolic functions and medical applications. Frontiers in neuroendocrinology, 52, 29-43.

Gobezie, E. (2022). Effect of Neem (Azadirachta indica) leaf powder on the growth performance and carcass quality of Broiler Chicken-A Review. Journal of Livestock Science, (13).

Jin, L.Z., Dersjant-Li, Y. and Giannenas, I., (2020). Application of aromatic plants and their extracts in diets of broiler chickens. Feed Additives, pp.159-185.

John, A. O. (2024). Growth Performance, Carcass Characteristics, Immune Response and Meat Quality of Broiler Chickens Fed Muntingia Calabura Leaf Extract. Journal of Drugs Addiction & Therapeutics. SRC/JDAT-172. J Drug Addi Ther, 5(4), 2-8.

Johnson, I. T. (2013). Phytochemicals and health. Handbook of plant food phytochemicals: Sources, stability and extraction, 49-67.

Khare, T., Anand, U., Dey, A., Assaraf, Y. G., Chen, Z. S., Liu, Z., & Kumar, V. (2021). Exploring phytochemicals for combating antibiotic resistance in microbial pathogens. Frontiers in pharmacology, 12, 720726.

Liu, S.J., Wang, J., He, T.F., Liu, H.S. and Piao, X.S., (2021). Effects of natural capsicum extract on growth performance, nutrient utilization, antioxidant status, immune function, and meat quality in broilers. Poultry science, 100(9), p.101301.

Modisaojang-Mojanaga, M. M., Ogbuewu, I. P., Oguttu, J. W., & Mbajiorgu, C. A. (2019). Moringa leaf meal improves haemato-biochemical and production indices in broiler chickens: a review. Comparative Clinical Pathology, 28, 621-632.

Nkukwana, T.T., Muchenje, V. Masika, P.J. & Mushonga, B. (2015). Intestinal morphology, digestive organ size and digesta pH of broiler chickens fed diets supplemented with or without Moringa oleifera leaf meal. South African Journal of Animal Science 45. 4: 362-370.

Prajapati, J., Bhatt, N., & Rawal, R. (2024). Hepatoprotective effects of phytochemicals and plant extracts against chemotherapy-induced liver damage in animal models: a systematic review. Archives of Toxicology, 1-28.

Preethi, K., Premasudha, P., & Keerthana, K. (2012). Anti-inflammatory activity of Muntingia calabura fruits. Pharmacognosy Journal, 4(30), 51–56. https://doi.org/10.5530/pj.2012.30.10

Preethi, K., Vijayalakshmi, N., Shamna, R., & Sasikumar, J. M. (2010). In vitro antioxidant activity of extracts from fruits of Muntingia calabura Linn. from India. Pharmacognosy Journal, 2(14), 11-18.

Purwanti, S., Agustina, L., Syamsu, J. A., & Putra, R. D. (2019). Histology of the liver and small intestine broiler using phytobiotic in the ration infected Salmonella pullorum. In IOP Conference Series: Earth and Environmental Science (Vol. 247, No. 1, p. 012054). IOP Publishing.

Qaid, M. M., Al-Mufarrej, S. I., Azzam, M. M., Al-Garadi, M. A., Albaadani, H. H., Alhidary, I. A., & Aljumaah, R. S. (2021). Growth performance, serum biochemical indices, duodenal histomorphology, and cecal microbiota of broiler chickens fed on diets supplemented with cinnamon bark powder at prestarter and starter phases. Animals, 11(1), 94.

Restivo, I., Attanzio, A., Tesoriere, L., Allegra, M., Garcia-Llatas, G., & Cilla, A. (2022). Anti-eryptotic activity of food-derived phytochemicals and natural compounds. International Journal of Molecular Sciences, 23(6), 3019.

Sadid, M. M., & Anam, M. S. U. (2025). Dietary effect of Moringa oleifera leaf meal on growth performance, blood lipids, and haematological parameters in broiler chickens: a meta-analysis. World's Poultry Science Journal, 81(1), 205-226.

Samanya, M. & Yamauchi, K. (2002). Histological alterations of intestinal villi in chickens fed dried Bacillus subtilis var. Natto. Comp. Bioch. Physio133:95–104.

Silverio, K. I., & Ramoran, R. (2022). Kerson Fruit Muntingia calabura linn and Golden Apple Snail Pomacea canaliculata (KFGAS) as Foo d Pellet Additives for the Growth Performance of Chicken Broiler Gallus gallus domesticus. ASEAN Journal of Agriculture and Food Engineering 1(1) (2022) 11-18.

Tamilvanan, S., Sivasankar, A., Jai, S., Marudhai, T. and Anandamoy, K. (2017). Efficacy of early chick nutrition with Aloe vera and Azadiractaindica on gut health and histomorphometry in chicks. Vet world10.6: 569–573.

Upah, S. O., Orayaga, K. T., Yakubu, R. N., Gyang, I. Y., Odeh, O. M., & Magaji, S. T. (2024). Haematological and biochemical indices of finisher broiler chickens fed graded levels of spurge weeds (Euphorbia heterophylla) leaf meal. Open Journal of Animal Sciences, 14(3), 204-218.

Wall, B. A., Mateus, A. L. P., Marshall, L., Pfeiffer, D. U., Lubroth, J., Ormel, H. J., et al. (2016). Drivers, dynamics, and epidemiology of antimicrobial resistance in animal production. Food and Agriculture Organization of the United Nations.

Murakami, A. (2013). Modulation of protein quality control systems by food phytochemicals. Journal of clinical biochemistry and nutrition, 52(3), 215-227.

Solís‐S, J. C., García‐Solís, P., Robles‐Osorio, L., & Hernández‐Montiel, H. L. (2017). Beneficial effects of phytochemicals on the endocrine system. Fruit and Vegetable Phytochemicals: Chemistry and Human Health, 2nd Edition, 67-84.

Lillehoj, H., Liu, Y., Calsamiglia, S., Fernandez-Miyakawa, M. E., Chi, F., Cravens, R. L., ... & Gay, C. G. (2018). Phytochemicals as antibiotic alternatives to promote growth and enhance host health. Veterinary research, 49, 1-18.

Smeriglio, A., Denaro, M., & Trombetta, D. (2018). Dietary Phytochemicals and endrocrine-related activities: An update. Mini Reviews in Medicinal Chemistry, 18(16), 1382-1397.