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ENHANCING BROILER CHICKEN GROWTH AND GUT HEALTH THROUGH *MANGIFERA INDICA, LAWSONIA INERMIS*, AND *CARICA PAPAYA* SUPPLEMENTATION

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Abstract
This study investigated the effect of dietary supplementation of *Mangifera indica (MILP), lawsonia inermis (LILP)* and *papaya carica (CPLP)* leaves powder supplementation on growth performance and gut health of broiler chickens. Six hundred female broiler chickens were divided into five groups: Control group, 0.5% MILP, 0.15% LILP, 0.5% CPLP and 0.58 % mix leaves powder (mixLP) herbs groups. The measured parameters included average daily feed intake (ADFI), body weight gain (BWG), feed conversion ratio (FCR), carcass characteristics and intestinal length. The results showed that (MILP) treated group had significantly higher BWG (61.16 g) and FCR (1.44) compared to the control group. (LILP) treated group had the lowest growth performance but the highest percentage of abdominal fat. (MILP) treated group had improved carcass quality with the highest percentage (72.83%) and percentage of leg muscle (16.81%). (Mix LP) treated group had improved gut health and the longest gut length (191.67 cm). The results of the study indicate that (MILP) promote growth, health and carcass quality, and the (mix LP) supplement has a positive effect on gut health, providing a potential natural alternative to synthetic supplements in poultry production. The (CPLP) and (mix LP) treated groups' improved intestinal health by showing a favorable balance of gut microflora to propagate Lactobacillus while reducing the population of E. coli. The evidence further supports that (CPLP) supplementation can be an effective natural growth promoter, although the (mix LP) and (CPLP) also contributed positively to gut health and meat quality.

Keywords: *Mangifera Indica* (MILP), *lawsonia inermis* (LILP), *Carica papaya* (CPLP) and Mix Herbs leaves powder (Mix LP) broiler chickens, growth performance, intestinal health

INTRODUCTION
There is a global demand for increased meat production from animals. The population is projected to reach 9 billion by the year 2050. Consequently, the production of animal meat must correspondingly increase. One of the most effective strategies to achieve sustainable and rapid meat production is through poultry farming, which delivers high-quality meat to meet the growing demand for animal protein. Though, the primary constraint in poultry farming continues to be the high cost and limited availability of feed ingredients. Poultry farming expenses are significantly impacted by high feed

costs. To address this, scientists and innovative farmers have introduced locally produced non-traditional feeds, by-products, and various herbs in poultry diets. These measures aim to enhance performance, immune response, and health status while reducing costs (Aroche et al., 2018; Sugiharto et al., 2018; Gerzilov et al., 2015; Rahman et al., 2022). Additionally, plant extracts are used to improve meat quality (Bithi et al., 2020; Boby et al., 2021; Disha et al., 2020; Akter et al., 2022; Ali et al., 2022). Mangifera indica leaf powder (MILP) is known for its antimicrobial, anti-inflammatory, antioxidant, and medicinal properties due to phytochemicals like mangiferin (Okwu & Ezenagu, 2008; Sultana et al., 2012; Kumar et al., 2021). Despite its potential, MILP has not been widely commercialized, with only a few studies exploring its benefits (Marsiglia-Fuentes et al., 2023). The antimicrobial and antioxidant potential of mango leaves as alternatives to antibiotics and synthetic antioxidants, and their effects on gut microbiota, immunity, and meat quality in broilers, are not well-studied. Various leaves have significant bioactive compounds, such as vitamins, phenolic acids, flavonoids, isothiocyanates, tannins, and saponins, beneficial for chicken health (Plaatjie et al., 2023; Vergara-Jimenez et al., 2017). Using leaf powder can reduce feed costs and improve chicken health, though its high crude fiber content limits use in broiler diets (Ogunsipe et al., 2020). Broilers have low tolerance for dietary fiber, which reduces nutrient digestibility and growth performance (Tanimo et al., 2020). Papaya (CPLP), rich in nutrients, is widely cultivated in tropical countries and is known for its proteolytic enzymes used in conventional and alternative medicines (Ude et al., 2018; Bhaskaran et al., 2016; Oloruntola et al., 2020). Papaya seeds and leaves are traditionally used medicinally and incorporated into poultry feed as a botanical supplement to replace synthetic chemicals and reduce unnecessary drug costs. Papaya seed and leaf powder serve as protein sources and may enhance growth performance, immune response, and poultry health due to their proteolytic enzymes (Bolu et al., 2009; Soedji et al., 2017). Their antioxidant activity is linked to improved growth and immune system stimulation (Asghar et al., 2016; Sugiharto, 2020). Additionally, these enzymes improve performance, antioxidant capacity, and digestibility in animals (Oloruntila et al., 2018). Research primarily focuses on the nutritional and phytogenic potential of papaya seeds and leaves in poultry diets, demonstrating their benefits in enhancing dietary performance (Bolu, 2009; Saleh et al., 2018). The growing demand for efficient and sustainable poultry production has led to an interest in natural feed additives. Medicinal plants, such as (Soetan and Oyewole, 2009) Mangifera indica (MILP), Lawsonia inermis (LILP), and Carica papaya (CPLP), contain bioactive compounds like flavonoids and antioxidants, making them potential growth promoters for broiler chickens. This study examines the influence of these plant supplements on growth performance and gut health(Semwal et al., 2014). Lawsonia inermis, the only member of its genus in the Lythraceae family, contains bioactive compounds like 2-hydroxy-1,4-naphthoquinone (Coordinators, 2018). LILP exhibits health benefits, including anti-inflammatory and antimicrobial effects. Various plant extracts have been found to inhibit Gram-positive and Gram-negative bacteria (Niroumand et al., 2015). The use of functional additives as natural dietary supplements in poultry production aims to enhance efficiency, reduce health problems, and improve meat quality. Bridging this knowledge gap is essential not only for enhancing the body of literature in poultry nutrition and management but also for supplying farmers and the industry with valuable, practical information (Bhamra, 2016). This study aims to address the existing knowledge void by examining the effects of herbal dietary supplements, specifically Mangifera indica leaf powder (MILP), Lawsonia inermis leaf powder (LILP), and Carica papaya leaf powder (CPLP), on growth performance, health indicators, and meat quality attributes in broiler chickens.

Materials and Methods:

Ethical Endorsement The process of sample collection received official authorization from the Faculty of Animal Husbandry and Veterinary Science, Sindh Agriculture University, Tandojam. The established protocols obtained approval from the Directorate of Advanced Study and Research at Sindh and Animal Welfare Committee Agriculture University under reference No-DAS- 1728/2023.

Study Area: A total of 600 male day-old Cobb-700 broiler chickens were procured from a commercial hatchery. The chicks were uniformly weighed and randomly assigned to five treatment groups, each comprising six replicates of 20 chicks. The room temperature was maintained at 35°C for the first three days post-hatch, gradually reducing to 23 ± 2°C by the end of 21 days (approximately three weeks). This temperature was maintained until the experiment's conclusion. All birds were provided with ad libitum access to food and water. Daily checks were conducted, and deceased birds were removed after recording mortality according to replicates.

Treatment and Experimental Design: The broiler chicks were divided into five groups, each receiving different doses of plant powders: control (no treatment), 0.5% MILP (Mangifera indica leaf powder), 0.15% LILP (Lawsonia inermis leaf powder), 0.5% CPLP (Carica papaya leaf powder), and 0.58% Mix LP (a mixture of all powders). These doses were determined based on preliminary studies.

Measured Parameters:
Average Daily Feed Intake (ADFI): Calculated as cumulative feed intake divided by the number of birds and days.
Feed Conversion Ratio (FCR): Determined as feed intake multiplied by 100 and divided by live body weight.
Body Weight Gain (BWG), and immune organ weights of spleen, thymus, and bursa of Fabricius were measured weekly. Organ weight was measured using a digital weighing balance. Bursa and thymus weights were measured with a Vernier caliper, and intestine length was measured using a measuring tape.

Antibody Titer against ND and IB: Antibody titers were assessed to evaluate bird immunity. Blood samples (3 ml) were collected from the wing vein and centrifuged at 1500 × g for 15 minutes at 4°C to separate serum. Serum was diluted to measure ND and IB virus antibodies using ELISA kits (AFG Bioscience). Plates were coated with antigen, and diluted serum samples were added, incubated for 2 hours at 37°C, followed by adding the conjugate and incubating for an additional 2 hours. Substrate was then added, and absorbance was measured at 450 nm using an ELISA reader (MPR-A200HT, China).

Bacterial Culture: Ingesta samples from the ileum were cultured in universal broth, and suspected E. coli and Acidophilus colonies were isolated on Luria Bertani agar and MRS agar, respectively.
Statistical Analysis: Data were analyzed using JMP software by SAS USA and all of them are presented as means ± SD values. Comparisons of the mean values were done using one-way analysis of variance, and differences among means were evaluated by Tukey's comparison test at $P<0.05$.

Results:
Growth Performance: The study assessed the impact of dietary supplementation with Mangifera indica leaf powder (MILP), Lawsonia inermis leaf powder (LILP), and Carica papaya leaf powder (CPLP) on various growth parameters of broiler chickens, including average daily feed intake (ADFI), average daily weight gain (ADWG), final weight gain, weekly feed intake, and feed conversion ratio (FCR). ADFI and ADWG were similar ($p > 0.05$) among the control (88.49 g), MILP (88.017 g), and LILP (88.21 g) groups, with no significant differences observed. ADFI was significantly lower in the CPLP (85.70 g) and Mix LP groups (83.64 g) compared to control, MILP, and LILP groups ($p = 0.0014$). MILP supplementation resulted in the highest weight gain (61.16 g), significantly greater than all other groups ($p = 0.0012$). No significant differences in weight gain were noted among the control, LILP, CPLP, and Mix LP groups. The final weight gain was highest in the MILP group (2568.7 g), significantly exceeding that of other groups ($p = 0.0015$). While control, LILP, CPLP, and Mix LP groups had lower final weight gains, no significant differences were found among them. LILP, CPLP, and MILP groups exhibited the highest feed consumption, with significant

differences among groups (p = 0.0014). Significant differences in FCR values were observed between the MILP and Mix LP groups compared to other groups, with the best FCR in the MILP group and the poorest in the control group. Thus, the results indicated improved FCR in all treated groups ($p < 0.05$).

Table 1 Effect of MILP, LILP and CPLP supplementation on growth performance of broiler chicken

ADFI (g)	Groups						
	Control	***0.5%*** **(MILP)**	**0.15% (LILP)**	***0.5%*** **(CPLP)**	**0.58% (Mix LP)**	**P-Value**	**LSD**
	88.49±0.39 [a]	88.01±0.60[a]	88.21±0.18[a]	85.70±0.69[b]	83.64±1.04[c]	0.0014	2.0551
ADWG(g)	55.71±0.39[b]	61.16±1.06[a]	56.81±0.53[b]	55.55±0.39[b]	57.47±0.67b	0.0012	2.2564
FWG (g)	2340.0±0.025[b]	2568.7±0.01 [a]	2386.0±0.027[b]	2333.3±0.016[b]	2413.7±0.034[b]	0.0015	0.0717
FI (g)	3717±16.66 [a]	3696.6±253[a]	3705±7.63 [a]	3599.6±29.2[b]	3513±43.82 [C]	0.0014	86.340
FCR	1.58±0.014[a]	1.44±0.03[b]	1.55±0.012[a]	1.54±0.057[a]	1.45±0.02[c]	0.0014	0.0630

Legends: Effect of different leaves on the growth performance of broiler chicken. Significant difference was observed among different groups i.e *Control*, *0.5%* (MILP), *0.15%* (LILP), *0.5%*,(CPLP) *0.58%* (Mix LP)/100 kg feed

Besides, treated groups also revealed significant effect on the carcass traits of broiler chickens (P=0.000) (Table 3.2). The highest carcass weight (1870.7g) was observed in MILP treated group followed by Mix LP group with the least weight in control group (1510.0g). In addition to this treated groups also showed significant difference in dressing percentage (P=0.0000) among different groups. MILP treated group indicated highest dressing percentage (72.83%), followed by mix LP group (70.64%), CPLP (69.93%), LILP (65.22%) and control group (64.56%).

Abdominal relative fat percentage was also significantly difference among the different groups (P=0.0000). LILP had the highest abdominal fat rate (1.46%), MILP treated group had the lowest (0.83%) percentage. Highest chest muscle rate was observed in MILP treated group 31.69%), CPLP treated group and control group.

Chest muscle rate: Group E had the highest chest muscle rate followed by Group B (31.26%). Group D (30.56%) and Group A (30.47%) were statistically similar, while Group C had the lowest value (29.62%). The difference was close to significant (P=0.0567).

Leg muscle ratio: The leg muscle percentage was the highest in MILP (16.81%), the lowest in LILP (12.45%), and intermediate values were found in control group (15.74%), CPLP (14.71%), and in Mix LP treatment (15.81%). The differences among the groups were significant (P=0.0000).

Through MILP, LILP, and CPLP significantly impacted broiler carcass quality. MILP excelled, showing improved carcass weight, dressing percentage, and muscle yield, with lower abdominal fat. LILP increased abdominal fat and reduced muscle yield. Mix LP and CPLP had moderate effects. Overall, MILP demonstrated great potential for enhancing broiler production and carcass quality, offering a natural alternative for optimizing poultry meat production.

Table 2 Effect of MILP, LILP and CPLP supplementation on Carcass characteristics of broiler chicken

Carcass weight (g)	Groups						
	Control	***0.5%* (MILP)**	**0.15% (LILP)**	***0.5%* (CPLP)**	**0.58% (Mix LP)**	**P-Value**	**LSD**
	1510.0±0.05[d]	1870.7±2.0 [a]	1556.0±0.04[d]	1631.7±6.00 [c]	1705.0±0.03[b]	0.0000	0.0329
Dressing (%)	64.56±1.06 [c]	72.83±0.25[b]	65.22±0.89[c]	69.93±0.25[b]	70.64±0.50 [b]	0.0000	2.1490
Abdominal fat (%)	1.10±0.07[b]	0.83±0.04 [d]	1.46±0.01[a]	0.98±0.04 [bc]	0.89±0.012[dc]	0.0000	0.1327
Breast muscle (%)	30.47±0.60 [ab]	31.26±0.96 [a]	29.62±0.043 [b]	30.56±0.25 [ab]	31.69±0.46 [a]	0.0567	1.3820
Leg muscle (%)	15.74[b]±0.16	16.81[a]±0.15	12.45[d]±0.35	14.71[c]±0.19	15.81[b]±0.08	0.0000	0.6617

Legends: Legends: Effect of different leaves on the growth performance of broiler chicken. Significant difference was observed among different groups i.e *Control*, *0.5%* (MILP), *0.15%* (LILP), *0.5%* ,(CPLP) *0.58%* (Mix LP)/100 kg feed

Immune organ weights: The effect of Mangifera *indica*, *Lawsonia inermis*, and *Carica papaya* leaf powders significantly impacted the relative weights of various immune organs in broiler chickens, except the spleen. This suggests that while some herbs influence the development of specific organs, the overall immune organ weights remained stable across most treatment groups.

Spleen: There was no significant difference in spleen weight (P = 0.4163), ranging from 0.06% to 0.09%.

Thymus: There was a significant difference in thymus weight (P = 0.0386). The relative thymus weight was highest in group C (0.15%), while the lowest in group D (0.11%).

Bursa: There was a highly significant effect for bursa weight (P = 0.0011). The bursa weights of groups A (0.11%) and C (0.12%) were significantly higher than those of groups B, D, and E, ranging from 0.08% to 0.09%.

Table 3 Effect of MILP, LILP and CPLP supplementation on Relative weight of different immune organs of broiler chicken

Spleen %	Groups						
	Control	***0.5%* (MILP)**	**0.15% (LILP)**	***0.5%* (CPLP)**	**0.58% (Mix LP)**	**P-Value**	**LSD**
	0.07 ±0.01	0.09±0.06	0.08±0.01	0.09±0.011	0.06±0.04	0.4163	0.0262
Thymus %	0.13 ±0.01[bc]	0.14±0.10[ab]	0.15±0.01 [a]	0.11 ±0.01[d]	0.12 ±0.00 [bc]	0.0386	0.0244
Bursa %	0.11±0.07[a]	0.08±0.06[b]	0.12±0.06[a]	0.09 ±0.002[b]	0.08±0.04[b]	0.0011	0.0169

[abcd] Superscripts with different letters in the same column varied statistically to each other.

Legends: Legends: Effect of different leaves on the growth performance of broiler chicken. Significant difference was observed among different groups i.e *Control*, *0.5%* (MILP), *0.15%* (LILP), *0.5%*, *(*CPLP) *0.58%* (Mix LP)/100 kg feed

Intestinal Health: Additionally, supplementation of MILP, CPLP and LILP results on the health of broiler revealed significant difference among groups. It was observed that intestinal length showed significant change among different groups. Length of intestine was greater in the treatment group MILP, Mix LP supplementation, CPLP and control group in comparison to LILP supplementation (P<0.000). Mix LP supplementation group (191.67a±1.66 cm), and MILP (182.00[b]±4.35cm) had the longest intestinal length and LILP treated group had the shortest intestinal length, which was significantly shorter than the other groups.

Table 4 Effect of MILP, LILP and CPLP supplementation on Intestine length (cm) of broiler chicken

Intestine length (cm)	Groups				
	Control	***0.5%* (MILP)**	**0.15% (LILP)**	***0.5%* (CPLP)**	**0.58% (Mix LP)**
	179.33 ±2.96^{b}	182.00±4.35^{b}	113.33±3.333^{c}	181.67 ±1.20^{b}	191.67±1.66^{a}

P-VALUE: - 0.0000
LSD: - 9.2527

Legends: Legends: Effect of different leaves on the growth performance of broiler chicken. Significant difference was observed among different groups i.e *Control, 0.5%* (MILP), *0.15% (*LILP), *0.5%, (*CPLP) *0.58%* (Mix LP)/100 kg feed.

Antibody titers: The effect of MILP, LILP and CPLP powder supplementation on the broiler chickens are shown table no. 5. The results indicate, the impact of different treatments on titers of antibodies against Newcastle Disease (ND) and Infections bronchitis (IB) in broiler chickens.

Titers of Newcastle Disease: The titers of antibodies for (ND) varied significantly across the treatment groups, P = 0.026. ND titers were highest in Group B and were significantly higher than all the other groups. Moderate titers were shown by Group E, which was similar to Group D (6.20bc) and Group C (6.22bcd). Group A presented the lowest titers at 5.93e, with a significantly weaker immune response compared to the other groups.

Titers of IB Disease: Titers against Infections bronchitis (IB) also exhibited significant variations (P = 0.047). Group B again had the highest titers: 6.77a, which was higher compared to the other groups. Titer level in group A was the lowest: 5.86d. Groups C, D and E showed an intermediate response with a titer of 6.44bc, 6.14bcd, and 6.31bc respectively.

The results showed that Group B had high antibody titers against ND and IB, indicating strong immunity compared to other groups. Statistical analysis confirmed significant differences in antibody titers for all tested pathogens. These findings suggest Group B effectively enhanced broiler chickens' immunity against Infectious Bronchitis and Newcastle Disease.

Table 5 Effect of MILP, LILP and CPLP supplementation on the Antibody titters of broiler chickens

Treatment groups	**Newcastle and Infections Bronchitis Disease Titters**	
	ND	**IB**
Control	5.93^{e}	5.86^{d}
MILP	6.72^{a}	6.77^{a}
LILP	6.22^{bcd}	6.44^{bc}
CPLP	6.20^{bc}	6.14^{bcd}
Mix LP	6.31^{bc}	6.31^{bc}
P-Value	0.026	0.047

^{abcd} Superscripts with different letters in the same column varied statistically to each other.

Legends: Legends: Effect of different leaves on the growth performance of broiler chicken. Significant difference was observed among different groups i.e *Control, 0.5% (*MILP), *0.15% (*LILP), *0.5%, (*CPLP) *0.58%* (Mix LP)/100 kg feed

Effect of MILP, LILP and CPLP supplementation on the broiler chicken microflora is presented in Table 6. The study is based on the counts of E. coli and Lactobacillus (log cfu/g) in various supplemented groups.

E. coli Counts: The E. coli counts varied significantly between treatment groups; P = 0.042. The counts in control (7.82 ± 0.09a) were significantly higher than all other group. Among supplemented groups, different leaves elicited a statistically significant E. coli decline. MILP (5.10 ± 0.11cd) - was the least among supplementation groups. LILP was at 5.40 ± 0.40c, while CPLP and mix LP group had virtually the same E. coli counts of 5.72 ± 0.24bc and 5.77 ± 0.16bc, respectively.

Lactobacillus Counts: The Lactobacillus counts also differed appreciably between the treatments (P = 0.053). The lowest count of Lactobacillus was recorded in the control group (4.80 ± 0.13e). The count of Lactobacillus in the groups supplemented with MILP a higher count (7.20 ± 0.16b) indicated a better proliferation of the beneficial bacteria. Counting of the bacteria from the LILP-supplemented was at 7.03 ± 0.23b while CPLP and Mix LP recorded counts at 6.90 ± 0.20bc and 6.86 ± 0.17bc, respectively. The results show that supplementation of broiler chickens with different types of leaves significantly alters the intestinal micro flora of the chickens. The control had the highest count of E. coli and lowest count of Lactobacillus, thus demonstrating a less favorable gut balance of the micro flora. On the other hand, the supplemented groups demonstrated significant reduction of E.coli these would have decreased coli counts and thereby an induced drastic increase in Lactobacillus counts wherein MILP will demonstrate efficacy at increasing the number of helpful bacteria but to the expense of dangerous bacteria. In this respect, the gut health can be positively changed by providing dietary leaves as supplementary toward healthy broiler chicken well-being.

Table 6, Effect of MILP, LILP and CPLP supplementation on the intestinal micro flora of broiler chickens

Different Supplemented groups of broiler chicken	**Intestinal micro flora (log cfu/g)**	
	E. coli	***Lactobacillus***
Control	7.82 ±0.09[a]	4.80 ±0.13[e]
MILP	5.10±0.11[cd]	7.20±0.16[b]
LILP	5.40±0.40[c]	7.03±0.23[b]
CPLP	5.72±0.24[bc]	6.90±0.20[bc]
Mix LP	5.77±0.16[bc]	6.86±0.17[bc]
P-Value	0.042	0.053

[abcd] Superscripts with different letters in the same column varied statistically to each other.

Legends: Legends: Effect of different leaves on the growth performance of broiler chicken. Significant difference was observed among different groups i.e *Control, 0.5% (*MILP), *0.15% (*LILP), *0.5%, (*CPLP) *0.58%* (Mix LP)/100 kg feed

Discussion

Enhancing broiler chicken growth is a primary focus for poultry industrialists, who traditionally use synthetic growth promoters. However, plants like Mangifera indica (MILP), Lawsonia inermis (LILP), and Carica papaya (CPLP) contain biomolecules essential for growth. These commonly available plants not only promote growth but also improve the health of broilers, presenting a natural alternative to synthetic options. In this study, dietary supplementation with mango (MILP), henna (LILP), and papaya (CPLP) leaf powders in broiler feed significantly enhanced growth performance, carcass characteristics, and intestinal health. The 0.5% MILP group exhibited the highest final weight gain and improved feed conversion ratio (FCR) compared to other groups. This improvement is attributed to bioactive compounds in mango leaves such as mangiferin, flavonoids, and phenolic compounds, known for their antioxidant, anti-inflammatory, and antimicrobial properties. As observed by Plaatjie et al. (2015), supplementation with mango leaf extract and powder enhances growth performance and feed efficiency, likely due to improved gut health and metabolism resulting from high concentrations of bioactive compounds. The inclusion *0.5%* MILP led to lower feed intake and weight gain than the control but had a positive effect on lowering abdominal fat. The findings further imply that application of mango leaves could be effective in improving meat quality through lower fat deposition as already reported by Niroumand et al. (2015) that this medicinal plant has a role in improving lipid metabolism by anti-inflammatory and antimicrobial agents. Nevertheless, this group showed lower growth performance which could be the consequence of low inclusion rate or presence of certain compound which tends to limit nutrient digestibility (Bhamra, 2016; Semwal et al., 2014). The feed intake and weight gain were lower compared to control in the 0.5% inclusion rate of CPLP, while a positive effect by decreasing abdominal fat had been observed in Mix LP. CPLP could enhance meat quality due to a reduction in fat deposition as supported by the report of Niroomand et al. 2015), which contributes to the enhancement of lipid metabolism mediated by its anti-inflammatory and antimicrobial properties. However, growth performance was lower in this group, possibly due to limited intake rates or the presence of certain compounds that may reduce nutrient digestibility (Bhamra, 2016; Semwal et al., 2014). Papaya leaves are rich in proteolytic enzymes such as papain, which can improve protein digestion and nutrient absorption (Ueda et al., 2018; Baskaran et al., 2016). Although this group did not achieve the highest growth rate, improvements in gut health parameters suggest that CPLP may have a positive impact on nutrient metabolism and intestinal integrity (Asghar et al., 2016; Sugiharto, 2020). Group E broiler chickens, which received a combination of the three herbal powders, had better intestinal health and the longest intestinal length, indicating improved intestinal morphology and nutrient absorption. These results are consistent with previous studies showing that combinations of herbal supplements can have a synergistic effect, promoting better intestinal health and immune responses (Valenzuela-Grijalva et al., 2017). However, the overall growth performance was not higher than that of Group B, suggesting that the effect of the combination may not be as effective as the effect of MILP alone in improving growth performance (Saleh et al., 2018 ; Soetan and Oyewole, 2009). According to the data, the group supplemented with 0.5% MILP supplement showed significantly greater final weight along with an improved feed conversion ratio (FCR) contributed by the bioactive mangiferin, flavonoids, and phenolic acids from MILP. These compounds had antioxidant, anti-inflammatory, and antimicrobial actions that were aligned with previously enhanced metabolism and gut health that was found in studies done prior to this one (Plaatjie et al., 2015). Reduction of abdominal fat deposition happened in the use of MILP. This supports previously seen information that mango enhances lipid metabolism along with anti-inflammatory action that previously was reported in (Niroumand et al., 2015). Though not to its maximum growth potential, the 0.5% CPLP supplement proved to be beneficial in terms of gut health through its proteolytic enzymes and effects on protein digestion and nutrient absorption. The combination of all three herbs distinctly improved the gut health profile as illustrated through augmented intestinal length and favorably modified gut microbiota profile, with increased Lactobacillus counts and decreased E. coli counts. It thus helps support the results that herbal blends can have synergistic effects on poultry gut health (Valenzuela-Grijalva et al., 2017). LILP-supplemented birds showed lesser growth performance, apparently as a result of its bioactive composition that could adversely affect nutrient utilization. Nevertheless, greater abdominal fat deposition was noticed by LILP supplementation, results that are compatible with others already known concerning lipid metabolism. The findings suggest MILP as the best single supplement, whereas CPLP and the mixed LP herbal group provide additional gut health benefits. This may also be considered an eventual use of natural growth promoters in poultry diets.

Conclusion

The study demonstrated that dietary supplementation with herbal powders, particularly Mangifera indica leaf powder (MILP), significantly enhanced growth, health performance, feed efficiency, and carcass quality in broiler chickens. Among all treatments, 0.5% MILP yielded the most notable improvements in weight gain, feed conversion ratio, and reduction in abdominal fat, highlighting its potential as a natural growth promoter. Lawsonia inermis leaf powder (LILP) reduced fat deposition but had limited growth stimulation, while Carica papaya leaf powder (CPLP) positively impacted gut health, making it an effective digestive booster. The combination of Mix LP herbs exhibited moderate effects, primarily benefiting gut health. Overall, MILP emerges as the optimal natural supplement for improving broiler performance, positioning it as a viable alternative to synthetic growth promoters.

Conflict of interest. The authors confirm the absence of any conflicting financial or non-financial concerns.

Author's Contributions: All authors contributed to the study and approved.

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References:

1. Akter, R., Hossain, M. A., Khan, M., Rahman, M. M., Azad, M. A. K., & Hashem, M. A. (2022). Formulation of value added chicken meatballs by addition of Centella leaf (Centella asiatica) extracts. *Meat Research*, *2*(2).
2. Ali, M. S., Rahman, M. M., Habib, M., Kabir, M. H., Hashem, M. A., & Azad, M. A. K. (2022). Quality of spent hen sausages incorporated with bee honey. *Meat Research*, *2*(1).
3. Aroche, R., Martinez, Y., Ruan, Z., Guan, G., Waititu, S., Nyachoti, C. M., ... & Lan, S. (2018). Dietary inclusion of a mixed powder of medicinal plant leaves enhances the feed efficiency and immune function in broiler chickens. *Journal of Chemistry*, *2018*.
4. Asghar, N., Naqvi, S. A. R., Hussain, Z., Rasool, N., Khan, Z. A., Shahzad, S. A., ... & Jaafar, H. Z. (2016). Compositional difference in antioxidant and antibacterial activity of all parts of the Carica papaya using different solvents. *Chemistry Central Journal*, *10*(1), 1-11.
5. Baskaran, R., Mohan, P. M., Sivakumar, K., & Kumar, A. (2016). Antimicrobial activity and phylogenetic analysis of Streptomyces parvulus DOSMB-D105 isolated from the mangrove sediments of Andaman Islands. *Acta Microbiologica et Immunologica Hungarica*, *63*(1), 27-46.
6. Bithi, M. A. A., Hossain, M. A., Rahman, S. M. E., Rahman, M. M., & Hashem, M. A. (2020). Sensory, nutritive, antioxidant and antimicrobial activity of Telakucha (Coccnia cordifolia) leaves extract in broiler meatball. *Journal of meat Science and Technology*, *8*(2), 23-31.
7. Boby, F., Hossain, M. A., Hossain, M. M., Rahman, M. M., Azad, M. A. K., & Hashem, M. A. (2021). Effect of long coriander leaf (Eryngium foetidum) extract as a natural antioxidant on chicken meatballs during at freezing temperature. *SAARC Journal of Agriculture*, *19*(2), 271-283.
8. Bolu, S. A. O., Sola-Ojo, F. E., & Olorunsanya, O. A. (2009). Effect of Graded Levels of Dried Pawpaw (Carica papaya) Seed on the Performance, Haematology, Serum Biochemistry and. *International Journal of Poultry Science*, *8*(9), 905-909.
9. Coordinators, N. R. (2018). Database resources of the national center for biotechnology information. *Nucleic acids research*, *46*(Database issue), D8.
10. Disha, M. N. A., Hossain, M. A., Kamal, M. T., Rahman, M. M., & Hashem, M. A. (2020). Effect of different level of lemon extract on quality and shelf life of chicken meatballs during frozen storage. *SAARC Journal of Agriculture*, *18*(2), 139-156.
11. Gerzilov, V., Nikolov, A., Petrov, P., Bozakova, N., PENCHEV, G., & Bochukov, A. (2015). Effect of a dietary herbal mixture supplement on the growth performance, egg production and health status in chickens. *Journal of Central European Agriculture*.
12. Kumar, A., Patyal, A., & Panda, A. K. (2018). Sub-therapeutic use of antibiotics in animal feed and their potential impact on environmental and human health: a comprehensive review. *Journal of Animal Feed Science and Technology*, *6*(15), 25.
13. Kumar, M., Saurabh, V., Tomar, M., Hasan, M., Changan, S., Sasi, M., ... & Mekhemar, M. (2021). Mango (Mangifera indica L.) leaves: Nutritional composition, phytochemical profile, and health-promoting bioactivities. *Antioxidants*, *10*(2), 299.
14. Marsiglia-Fuentes, R., Chiralt, A., & García-Zapateiro, L. A. (2023). Investigating the water relations in aqueous extract powders of mango (mangifera indica) peel and seed waste for their use in food matrices as a value-added by-product. *Foods*, *12*(18), 3497.
15. Niroumand, M. C., Farzaei, M. H., & Amin, G. (2015). Medicinal properties of Peganum harmala L. in traditional Iranian medicine and modern phytotherapy: a review. *Journal of Traditional Chinese Medicine*, *35*(1), 104-109.

16. Nishimwe, G. (2019). *Characterization of Morphological and Quality Characteristics of New Papaya (Carica papaya L) Hybrids Developed at JKUAT* (Doctoral dissertation, JKUAT-AGRICULTURE).
17. Ogunsipe, M. H., Doloruntola, O., Agbede, J., & Igbasan, F. (2020). Influence of Azadirachta indica L as phytogenic feed supplement on the growth performance and immunomodulatory response of broiler chickens. *Journal of Biology and Nature*, *12*(1), 57-66.
18. Okwu, D. E., & Ezenagu, V. I. T. U. S. (2008). Evaluation of the phytochemical composition of mango (Mangifera indica Linn) stem bark and leaves. *Int J Chem Sci*, *6*(2), 705-16.
19. Oloruntola, O. D., Ayodele, S. O., & Oloruntola, D. A. (2018). Effect of pawpaw (Carica papaya) leaf meal and dietary enzymes on broiler performance, digestibility, carcass and blood composition. *Revue d'élevage et de médecine vétérinaire des pays tropicaux*, *71*(3), 121-129.
20. Oloruntola, O. D., Ayodele, S. O., Adeyeye, S. A., Jimoh, A. O., Oloruntola, D. A., & Omoniyi, I. S. (2020). Pawpaw leaf and seed meals composite mix dietary supplementation: effects on broiler chicken's performance, caecum microflora and blood analysis. *Agroforestry Systems*, *94*(2), 555-564.
21. Plaatjie, M., Onyiche, T., Legoabe, L., Ramatla, T., Nyembe, N., Suganuma, K., & Thekisoe, O. (2023). Medicinal plants as potential therapeutic agents for trypanosomosis: A systematic review. *Advances in Traditional Medicine*, *23*(4), 1011-1033..
22. Saleh, A. A., Ragab, M. M., Ahmed, E. A., Abudabos, A. M., & Ebeid, T. A. (2018). Effect of dietary zinc-methionine supplementation on growth performance, nutrient utilization, antioxidative properties and immune response in broiler chickens under high ambient temperature. *Journal of applied animal research*, *46*(1), 820-827.
23. Semwal, R. B., Semwal, D. K., Combrinck, S., Cartwright-Jones, C., & Viljoen, A. (2014). Lawsonia inermis L.(henna): Ethnobotanical, phytochemical and pharmacological aspects. *Journal of ethnopharmacology*, *155*(1), 80-103.
24. Soedji, K., Teteh, A., Gbeassor, M., Tona, K., Nideou, D., & Decuypere, E. (2017). Effect of carica papaya seeds on gastro-intestinal parasites of pullet and production parameters. *International Journal of Probiotics & Prebiotics*, *12*(2).
25. Soetan, K. O., & Oyewole, O. E. (2009). The need for adequate processing to reduce the anti-nutritional factors in plants used as human foods and animal feeds: A review. *African Journal of food science*, *3*(9), 223-232.
26. Sugiharto, S., Widiastuti, E., Isroli, I., Wahyuni, H. I., & Yudiarti, T. (2020). Effect of a Fermented Mixture of Papaya Leaf and Seed Meal on Production Traits and Intestinal Ecology of the Indonesian Indigenous Crossbred Chickens. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis*, *68*(4).
27. Sugiharto, S., Yudiarti, T., Isroli, I., & Widiastuti, E. (2018). The potential of tropical agro-industrial by-products as a functional feed for poultry. *Iranian Journal of Applied Animal Science*, *8*(3), 375-385.
28. Tanimo, H. A., Oshibanjo, D. O., Adelowo, V. O., Akwashiki, M. A., Azi, I. W., James, O. C., ... & Sunday, A. Y. (2020). Growth performance, carcass characteristics and heamatology indices of broiler chicken fed graded levels of (Mangifera indica) mango Leaf Meal. *Asian Journal of Research in Animal and Veterinary Sciences*, *6*(2), 20-27.
29. Uda, M. N. A., Shaari, N. H., Said, N. S., Ibrahim, N. H., Akhir, M. A., Hashim, M. K. R., ... & Gopinath, S. C. (2018, March). Antimicrobial activity of plant extracts from aloe vera, citrus hystrix, sabah snake grass and zingiber officinale against pyricularia oryzae that causes rice blast disease in paddy plants. In *IOP Conference Series: Materials Science and Engineering* (Vol. 318, No. 1, p. 012009). IOP Publishing.
30. Valenzuela-Grijalva, N. V., Pinelli-Saavedra, A., Muhlia-Almazan, A., Domínguez-Díaz, D., & González-Ríos, H. (2017). Dietary inclusion effects of phytochemicals as growth promoters in animal production. *Journal of animal science and technology*, *59*, 1-17.

31. Vergara-Jimenez, M., Almatrafi, M. M., & Fernandez, M. L. (2017). Bioactive components in Moringa oleifera leaves protect against chronic disease. *Antioxidants*, *6*(4), 91.
32. Zhang, J., Wang, Y. D., Xue, Q. W., Zhao, T. R., Khan, A., Wang, Y. F., ... & Cheng, G. G. (2022). The effect of ultra-high pretreatment on free, esterified and insoluble-bound phenolics from mango leaves and their antioxidant and cytoprotective activities. *Food Chemistry*, *368*, 130864.