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Research Article

GROWTH PERFORMANCE, CARCASS CHARACTERISTICS, NUTRIENT METABOLIZABILITY, INTESTINAL MORPHOMETRY AND SERUM BIOCHEMISTRY OF KUROILER CHICKEN FED DIET WITH MULBERRY LEAVES

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ABSTRACT: Mulberry leaves yield high-quality forage characterized by enhanced digestibility and palatability, making them a viable alternative feed resource. In this regard, the present study is designed to evaluate the effects of mulberry leaf meal (MLM) on growth performance, carcass characteristics, nutrient metabolizability, serum biochemistry and intestinal morphometry of Kuroiler chicken. 200 Kuroiler birds were divided into four dietary treatment groups with 50 birds in each in a completely randomized design (CRD). The basal diet having wheat bran was replaced with MBL at the rate of 0%, 50%, 70% and 100% in the control, T_1, T_2, T_3 groups, respectively. Each group was sub-divided into 5 replicates with 10 birds in each at random. The experiment was conducted for 6 weeks. There was significant effect ($p<0.05$) on body weight gain in T_1 group and that was comparable with control group. Compared to control, feed intake was increased ($p>0.05$) in all replacement groups. Among the treated groups, better ($p<0.05$) feed conversion ratio was observed in T_1. No remarkable changes ($p>0.05$) were observed on serum biochemical parameters. No discernible differences were observed on carcass characteristics however supplementation of 70% MBL, significantly decreased ($p<0.05$) the relative gizzard yield. Metabolizability of dry matter, crude protein and ether extract significantly decreased in T_2 and T_3 group. Profit per kg live weight was higher ($p<0.05$) in 50% MBL fed group. Thus, it is concluded that, on economic production point of view, wheat bran can be effectively replaced by mulberry leaves up to 50% level for clean Kuroiler chicken production without affecting production performances.

Keywords: Biochemical, Body weight gain, Carcass, Economic, Growth, Mulberry leaves.

INTRODUCTION

With increase of population and the improvement of people's living standards, the demand for conventional feed resources as well as livestock and poultry production is increasing day by day. The shortage of feedstuff has become gradually prominent and as a result, the price of conventional feedstuff (corn, soybean meal and fish meal) has increased. Therefore, finding cheap and reasonable feed resources to replace conventional feedstuff has now become a research hotspot.

Mulberry (*Morus alba*), a deciduous perennial woody plant generates a lot of high-quality forage, which makes it a very promising alternative feed source [1]. Mulberry leaves have a higher digestibility and better palatability in comparison with other commonly used feeds [2]. Therefore, the Food and Agriculture Organization strongly advised to use mulberry leaves as a protein-rich forage supplement for animal production in place of cereals (maize, cotton seeds, beans, etc.) in order to preserve edible foods in the animal production process [3]. Yields of about 51 tones /ha/year of fresh leaves have been reported in India [4]. Mulberry leaves are abundant in protein, minerals (Ca, P), vitamins (ascorbic acid, carotene, vitamin B1, folic acid, folinic acid, vitamin D) metabolizable energy with absence of or negligible anti- nutritional factors [5]. The amino

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acids composition of mulberry leaf meal (MLM) indicates it is rich in essential amino acids especially lysine 1.80% and leucine 2.58% [6].

Wheat bran, a by-product derived from wheat grains, comprises approximately 15.2% crude protein and 12% crude fibre, which restricts its incorporation to less than 5% in broiler feed formulations cereals in non-ruminants [8]. However, the rising cost of wheat bran necessitates the exploration of alternative, cost-effective feed ingredients. One potential substitute is mulberry leaf meal (MLM), which is both more affordable and readily accessible.

The existing strategy for the unorganized sector as outlined in the National Action Plan for Egg and Poultry-2022 aims to convert backyard poultry into a commercial economic model [9]. Specifically, the introduction of low input technology (LIT) birds is intended to facilitate the transition and subsequent scaling up to 1,000-2,000 birds for larger commercial poultry operations. Additionally, the plan emphasizes the development of alternative breeds and LIT birds to enhance family poultry systems [9]. At present, there is an increasing demand for the meat and eggs of backyard/indigenous chicken throughout the country. The prices for both indigenous chicken meat and eggs have exceeded those of broiler meat and commercially produced table eggs. In some areas, these products are even promoted as organic, resulting in heightened consumer interest. Therefore, it is essential to raise indigenous chickens for both meat and egg production by supplying them with balanced poultry feeds. Employing alternative and economical ingredients, such as MLM, to reduce production costs may serve as a practical approach for impoverished and marginal farmers.

Kuroiler is a resilient, dual-purpose indigenous chicken breed with high productivity, developed by Kegg Farm Pvt Ltd in India. Dried mulberry leaf meal (MLM) possesses notable nutritional benefits (16% crude protein, 1200 Kcal/kg metabolizable energy, 11.34% total ash, 1.44% ether extract, 12.00% crude fibre, 2.40% calcium, and 0.23% phosphorus) for poultry, suggesting that incorporating this by-product into feed may enhance farmers' economic returns (5). Numerous studies have evaluated the impact of varying levels of mulberry leaf powder on the performance of broilers [10, 11], layers [12, 13], and quails [14]. However, there remains a significant gap in systematic research concerning the use of MLM replacing wheat bran in poultry nutrition, particularly for LIT birds. Therefore, this study aims to investigate the extent to which wheat bran can be substituted with MLM in the diet of Kuroiler chickens to optimize production, carcass traits, nutrient digestibility, intestinal morphology, and serum biochemistry.

MATERIALS AND METHODS

Location and preparation of leaves

Mulberry leaves, aged between 4 to 6 months, were harvested from the Fodder Farm at the College of Veterinary Sciences & A.H. in R.K. Nagar, West Tripura. These leaves were subjected to drying in direct sunlight before being placed in an oven set at 60°C for duration of 24 hours or until a stable weight was reached. The dried samples were then processed using a grinder to achieve a particle size of 0.5 mm and were subsequently incorporated into the feed to formulate the treatment diets. Subsequently, these were sun-dried and ground using a mixer for incorporation into the experimental diet. All procedures used in the experiment on the birds were reviewed and approved by the Institutional Animal Ethics Committee of the college (F.1/GEN/IAEC/CVS/RKN/2020). The experiment was carried out in Poultry Shed of the Livestock Farm Complex, College of Veterinary Sciences and A. H., R. K. Nagar, Agartala, Tripura, India.

Birds and their management

Following a completely randomized design, 200 one-day-old Kuroiler chicks of uniform body weight were randomly allocated into four treatment groups with 50 birds in each. Each group was further sub-divided into 5 replicates with 10 birds in each replicate. Four dietary treatments were formulated: Control (no MBL), T_1 (replacement of wheat bran with MBL @50%), T_2 (replacement of wheat bran with MBL @70%) and T_3 (replacement of wheat bran with MBL@100%). The ingredient and chemical composition of all basal diet is shown in Table 1 and it was made as per BIS [1]). In a conventional poultry shed, birds were accommodated in 20 pens each measuring 10.56 sq. feet (3.25 ×3.25 feet). In the brooder cum grower house, birds were maintained in deep litter system up to 7 weeks. Vaccination was performed against Newcastle disease (5th and 21st day with NDB_1 and Lasota strain) and Infectious Bursal Disease (12th day).

Growth performance parameters

Weekly body weight and feed intake were recorded and overall (0-3 weeks, 4-6 weeks and 0-6 weeks) body weight gain, feed intake, feed conversion ratio

(FCR) was calculated. Mortality was recorded as and when it occurred. FCR was calculated on the basis of unit feed intake to unit weight gain in body weight for each replicate. FCR was adjusted according to the feed consumption of the dead birds.

Carcass charateristics

At the end of the 7-week feeding trial, 5 birds from each dietary treatment group were weighed, fasted for 12 h and ethically slaughtered, de-feathered and eviscerated. The hot carcass was weighed and chilled at 1^0C for 48 h. The chilled carcass was separated into cuts, and the weight of different body parts viz., breast, thigh, back, drumstick and giblets (gizzard, liver, lungs and heart) were recorded.

Biochemical parameters

Approximately 5 ml blood was collected at the end of the trial from the wing vein in sterilized glass tubes. Serum was separated from the whole blood at 2500 rpm for 10 minutes and stored in deep freeze at -20^0C for further analysis of different blood parameters. Serum glucose, total cholesterol, total protein, albumin, globulin, aspartate amino transferase (AST), alanine amino transferase (ALT), uric acid, uric acid and creatinine were estimated by automatic blood analyzer (Microlab 200, E-Merck India Ltd.) using Cayman Diagnostic kits. Hemoglobin level in blood was estimated by cyanmethemoglobin method using UV Spectrophotometer. Proximate principles in feed and meat samples were analyzed as per methods described in [15]. All samples and standards were measured in duplicate.

Nutrient metabolizability study

For metabolizability studies, one bird in each replicate close to the average weight was selected and housed separately in wire floor metabolism cage. After three days of adaptation period, a total collection of excreta was carried out for 7 days. Daily feed consumption as well as faecal output from each bird was quantified. The excreta samples (100 g) from each replicate was collected every day in a previous weighed clean and dry petri dish and were dried in hot air oven (100 ± 2^0C), pulled together and preserved for subsequent analysis. The pooled feed and fecal sample were subjected for analysis of dry matter (DM), organic matter (OM), crude protein (CP), crude fibre (CF), nitrogen free extract (NFE) and ether extract [15].

Intestinal morphometry

At the end of experiment, 15 birds per treatment were used to study intestinal morphometry under a high-resolution microscope with micrometry and photographic attachment (Lynx, Lawrence and Mayo Binocular Microscope). A 1 cm segment of the midpoint of the jejunum was removed, then the segments were washed with physiological saline solution, and fixed in 10% buffered formalin. Each segment was then embedded in paraffin, and a 2-mm section of each sample was placed on a glass slide and stained with haematoxylin and eosin for examination. Histological sections were examined microscopically. Villus height (measured from the tip of the villus to the villus-crypt junction), crypt depth (measured from the crypt-villus junction to the base of the crypt), villus width, villus height to crypt depth ratio, villus height to villus width ratio and villus surface area $[(\pi \times mh \times h) + (\pi \times mh/2)^2]$, where 'mh' is the width at the mid-villus height and 'h' is villus height [16], villi length and width were measured from 5 villi per chicken and only the complete, vertically oriented villi were measured. Jejunum was of particular interest because it is a major site of nutrient absorption in poultry [17].

Economics of production

The economics was calculated by considering cost of raw ingredients, additives and other supplements and then computing the cost of feed.

Statistical analysis

All analysis was done using Sigma Plot 11.0 software package. The results were expressed as the mean and pooled standard error of mean. Significance was tested by using two-way ANOVA considering treatment and period as factors with the following model:

$$Y_{ijk} = \mu + T_i + P_j + TxP_{ij} + e_{ijk}$$

where, Y_{ijk} is the observation associated with each parameter; μ is the overall mean; T_i is the effect of i^{th} treatment groups (i=1, 2, 3 & 4); P_j is the effect of j^{th} periods (j=1, 2 & 3); TxP_{ij} is the interaction effect of j^{th} periods within i^{th} treatment group; and e_{ijk} is the random error. For comparison between treatment and control groups, Holm-Sidak test was used. Probability values of $p<0.05$ were declared as significant. No statistical analysis was performed for mortality data and cost calculation of different basal diets. The specific P-values were mentioned in the text where there is a significant difference.

RESULTS AND DISCUSSION

Growth performance

The findings related to growth performance are summarized in Table 2. The data reveal that chickens receiving a 100% substitution of wheat bran with MBL exhibited significantly reduced body weight gain (BWG) during the 4-6 week and 0–6-week periods when compared to both the control and T_1 groups, while no significant differences were observed between T_1 and T_2. However, during the 0–3-week growth phase, dietary replacements did not yield any significant effects ($p > 0.05$). Previous research indicated that geese fed diets containing varying percentages of MLM (4, 8, 12, 16%) experienced lower weight gains, increased feed consumption, and a higher feed-to-gain ratio ($p < 0.05$) compared to those on a control diet [18]. The addition of mulberry leaf meal at levels of 7.5% and above resulted in diminished weight gain in broiler chickens [19]. Furthermore, [20] documented a reduction in weight gain among rabbits consuming 20% mulberry leaves. Results from another study supported these findings [21, 22]. [10] noted a decline in weight gain with increasing levels of mulberry leaves (0, 0.5, 1.0, and 2% of the diet), likely attributed to the high tannin and fibre content of the leaves. Our results align with these previous findings. Various studies have reported that incorporating mulberry leaf meal up to 9% does not adversely affect body weight in broilers [11, 23, 24] and laying hens [25, 26]. Conversely, [27] found that the highest body weight gain occurred with a 10% inclusion of mulberry leaf meal. Feed conversion ratios were slightly less favorable in chicks fed diets supplemented with mulberry leaves, which may explain the observed decrease in weight gain. The reduction in weight gain in the current study may be attributed to the percentage of insoluble fiber, which hampers the ability to effectively utilize the diet due to poor digestion and absorption.

Feed consumption was markedly ($p<0.05$) elevated in the replacement groups compared to the control during 0-3 weeks and 0-6 weeks of the growth phase.

Table 1. Ingredient and calculated composition of experimental diets.

Ingredients	Dose of MBL			
	Control	T_1	T_2	T_3
Maize	57.40	57.90	58.30	58.60
Soybean	28.50	28.25	28.00	27.90
Wheat bran	10.00	5.00	2.50	-
MBL	-	5.00	7.50	10.00
DCP	1.50	1.50	1.40	1.24
LSP	1.50	1.30	1.20	1.00
Iodized salt	0.30	0.30	0.30	0.30
Feed additive premix*	0.74	0.74	0.74	0.74
Chemical composition (g kg^{-1})[1]				
ME (Kcal kg^{-1})[2]	2756	2751	2751	2751
DM	89.50	88.96	89.98	91.80
CP	20.00	20.00	19.98	19.98
EE	3.65	3.75	3.47	3.55
CF	5.33	5.16	5.59	5.86
TA	8.28	7.87	6.78	8.37
NFE	62.74	63.22	64.18	62.24
OM	91.72	92.13	93.22	91.63
Ca	1.02	1.05	1.04	1.03
Total phosphorus	0.52	0.47	0.43	0.41
Lysine[2]	1.13	1.13	1.12	1.12
Methionine[2]	0.33	0.34	0.33	0.33

*Mineral supplement 0.15% (per kg of diet contain: Zn 60 mg; Mn 90 mg; Fe 110 mg; KI 2.5 mg), vitamin supplement 0.06% (per gram contains: vitamin A 82,500 IU; vitamin B_2 50 mg; vitamin D_3 12,000 IU; vitamin K 10 mg, vitamin B_1 4 mg; vitamin B_6 8 mg; vitamin B_{12} 40 mg; vitamin E 40 mg; calcium-D-pantothenate 40 mg; niacin 60 mg), Choline chloride 0.10%, Maduramycin 0.05%, Sodium bicarbonate 0.31%, Antioxidant (BHT) 0.01%, Biophos 0.015%, Toximar 0.05%, Probios 0.05%. [1]Assayed values. [2]Calculated on the basis of standard values applicable under Indian Condition [42, 43]

No significant differences were detected in feed intake between the control and other replacement groups during the 4–6-week period (Table 2). Previous studies have indicated that the elevated fiber levels and certain anti-nutritional components present in mulberry leaves can considerably diminish nutrient retention within the digestive system, thereby shortening digestion time and impairing nutrient absorption [24, 28]. Although our findings showed a reduction in live weights, the T_2 and T_3 groups exhibited the highest feed consumption, suggesting that the birds were compensating for their nutrient needs due to impaired nutrient digestion. These results align with those of [29], who reported no significant impact on feed intake in broilers fed a diet with 30% mulberry leaf substitution for commercial feed. Additionally, our findings are consistent with various studies involving broilers [30, 23], layers [25], indigenous chickens [31], and rabbits [20], indicating that dietary inclusion of mulberry leaves did not adversely affect the performance of kuroiler chickens. Conversely, [12] found a significant decrease in feed intake among laying hens when their diet included 10% mulberry leaf meal. Similarly, [32] noted a decline in feed intake with higher levels of dietary leaf meals in both broilers and laying hens. Mulberry has been shown to reduce food intake in a concentration-dependent manner by prolonging gut transit time [33]. Nevertheless, the high fiber content may also enhance feed intake [34]. In this study, the increased feed intake is likely attributed to the chickens' efforts to fulfill their nutritional needs in light

Table 2. Effect of dietary supplementation MBL on production performance of Kuroiler chicken.

Attributes	Dose of MBL				SEM	p value
	C	T_1	T_2	T_3		
BWG (g/bird)						
0-3 weeks	254.33	247.76	236.39	224.80	6.72	0.452
4-6 weeks	649.93^{b}	652.53^{b}	561.87^{ab}	501.71^{a}	21.71	0.020
0-6 weeks	904.25^{b}	900.29^{b}	798.26^{ab}	726.51^{a}	23.78	0.008
Feed intake (g/bird)						
0-3 weeks	617.91^{b}	786.88^{a}	837.75^{a}	838.94^{a}	32.83	0.038
4-6 weeks	1520.32	1922.33	2061.31	2080.44	88.24	0.074
0-6 weeks	2138.23^{b}	2709.21^{a}	2899.06^{a}	2919.38^{a}	107.63	0.019
Feed conversion ratio (FCR)						
0-3 weeks	2.44^{a}	3.19^{c}	3.55^{bc}	3.71^{b}	0.13	<0.001
4-6 weeks	2.38^{a}	3.00^{a}	3.68^{b}	4.19^{b}	0.22	0.006
0-6 weeks	2.38^{a}	3.04^{c}	3.62^{bc}	4.03^{b}	0.17	<0.001
Mortality%	5.00	4.00	4.00	5.00	-	-

Means bearing superscripts in the same row differ significantly (p <0.05).
SEM: Standard error of mean, p: Probability.

Table 3. Effect of dietary supplementation of MBL on carcass traits, cut up parts and relative organ weight (% of dressed yield) of Kuroiler chicken.

Attributes	Dose of MBL				SEM	p value
	C	T_1	T_2	T_3		
Carcass yield	483.94	449.63	438.35	399.79	20.60	0.991
Dressed yield	62.27	61.36	64.33	59.23	1.09	0.475
Breast	20.90	22.07	23.04	22.64	0.53	0.568
Neck and back	26.45	26.52	25.88	27.55	0.57	0.829
Thigh	14.09	12.29	14.54	14.08	0.44	0.303
Drumstick	14.74	14.60	12.69	14.15	0.39	0.223
Wings	13.58	13.36	13.61	15.11	0.39	0.404
Liver	4.42	4.59	4.30	4.79	0.30	0.959
Gizzard	4.90^{b}	4.72^{b}	4.93^{b}	4.35^{a}	0.19	0.001
Heart	0.90	1.20	1.02	1.00	0.07	0.507
Pancreas	0.39	0.57	0.50	0.59	0.04	0.323
Spleen	0.63	0.50	0.48	0.44	0.05	0.662

Means bearing superscripts in the same row differ significantly (p<0.05).
SEM: Standard error of mean, p: Probability.

Table 4. Effect of dietary supplementation of MBL on serum biochemical parameters of Kuroiler chicken.

Attributes	Dose of MBL				SEM	p value
	C	T_1	T_2	T_3		
Hemoglobin (g/dl)	15.29	12.83	13.88	13.83	0.49	0.052
Glucose (mg/dl)	187.34	205.74	202.41	201.20	4.99	0.612
Total Protein (g/dl)	4.21	3.81	3.49	3.52	0.12	0.113
Albumin (g/dl)	1.95	1.88	1.77	1.82	0.04	0.445
Globulin (g/dl)	2.26	1.92	1.73	1.70	0.09	0.152
A:G ratio	0.898	0.997	1.032	1.122	0.05	0.433
Cholesterol (mg/dl)	119.19	131.24	124.39	101.27	4.52	0.075
HDL Cholesterol (mg/dl)	72.98	68.75	69.96	61.56	2.41	0.408
LDL Cholesterol (mg/dl)	22.98	25.43	30.01	22.20	1.52	0.273
Triglyceride (mg/dl)	43.87	50.21	41.51	32.77	2.52	0.092
Serum enzymes						
Aspertate amino transferase (IU/L)	270.97	308.15	326.74	307.80	12.25	0.466
Alanine amino transferase (IU/L)	22.79	23.90	22.75	28.85	2.42	0.811
Kidney function test						
Uric acid (mg/dl)	4.02	3.65	3.22	3.65	0.30	0.857
Creatinine (mg/dl)	0.817^a	0.800^a	0.950^a	1.130^b	0.033	0.002

Means bearing superscripts in the same row differ significantly (p<0.05).
SEM: Standard error of mean, p: Probability.

Table 5. Effect of dietary supplementation of MBL on metabolizability of nutrients in Kuroiler chicken.

Attributes	Dose of MBL				SEM	p value
	C	T_1	T_2	T_3		
DM	58.48^b	43.77^b	36.27^a	41.74^a	2.86	0.030
EE	58.48^b	43.77^b	35.84^a	39.44^a	2.64	0.028
CP	89.43^b	87.45^a	78.64^a	78.56^a	1.72	0.008
CF	42.69	40.40	36.41	37.42	2.25	0.772

Means bearing superscripts in the same row differ significantly (p<0.05).
DM: Dry matter; EE: Ether extract; CP: Crude protein; CF: Crude fibre;
SEM: Standard error of mean, p: Probability.

Table 6. Effect of dietary supplementation of MBL on intestinal morphometry in Kuroiler chicken.

Attributes	Dose of MBL				SEM	p value
	0	T_1	T_2	T_3		
VH (µm)	87.98	85.74	84.81	61.83	4.44	0.122
VW (µm)	24.17^b	24.42^b	16.82^a	12.55^a	1.27	<0.001
CD (µm)	12.85	12.68	11.67	11.81	0.71	0.744
VA (mm^2)	0.168^a	1.619^b	0.744^a	0.405^a	0.14	<0.001
VH/CD (µm)	8.23	6.92	7.77	5.72	0.63	0.287
VH/VW (µm)	3.87	3.71	5.39	5.08	0.29	0.088

VH: Villi height; VW: Villi width; CD: Crypt depth; VA: Villi area, VH/CD: ratio of Villi height and crypt depth; VH/VW: Ratio of villi height and villi width; SEM: Standard error of mean, p: Probability.
Means bearing superscripts in the same row differ significantly (p<0.05).

of reduced feed digestibility caused by the high fiber content. Furthermore, the proteins and carbohydrates present in diets rich in crude fiber are not efficiently digested, as the mixing of digesta is hindered, leading to decreased digestibility and nutrient absorption within the gastrointestinal tract [35].

Among the three levels of dietary replacement, the feed conversion ratio (FCR) was notably poor in the T_3 groups; however, a significantly improved FCR was observed in the T_1 group during the periods of 0-3, 4-6, and 0-6 weeks. These results align with the findings of [21] in hybrid chickens, [20] in rabbits, and [19] in broiler chickens, who reported a significant reduction in FCR. In contrast, [24, 25, 29] found no significant impact on FCR with 3% and 9% MLP supplementation in broiler chickens. Additionally, contrary to our findings, [27] reported the lowest FCR values in broiler chickens fed a diet containing mulberry leaves. The improved FCR in the 50% replacement group may be attributed to a relatively lower feed intake and higher body weight gain (BWG) compared to the 70% and 100% replacement groups, suggesting that this level of replacement could effectively reduce production costs.

Carcass characteristics

Slaughter performance serves as an important index for assessing the carcass quality of meat-producing livestock and poultry. It not only provides a direct indication of the proportion of various tissue types relative to the total mass but also highlights the disparities in nutrient deposition across different tissues. With the exception of gizzard weight, dietary replacement did not significantly influence carcass characteristics (p>0.05) as shown in Table 3. This aligns with the findings of [24], who indicated that diets enriched with fermented mulberry leaves did not affect dressing percentage, eviscerated carcass yield, or thigh muscle yield in broilers, corroborating the results of [36] and [20]. Conversely, [19] and [21 observed significant reductions in carcass weight, carcass yields, breast weight, thigh plus leg weight, and abdominal fat with increased levels of mulberry foliage supplementation (10, 20, and 30 In this experiment, though there was no difference on breast yield but numerically it showed 10.04% improvement on breast yield in T_2 group which may potentially attributed to enhanced digestibility of crude protein.

Biochemical parameters

No statistically significant differences (p >0.05) were detected in serum biochemical parameters among the dietary treatment groups (Table 4). However, the group with 100% replacement exhibited a notable increase in creatinine levels. Previous research has demonstrated that incorporating mulberry leaves into broiler diets can lead to a reduction in serum cholesterol levels. In the current study, treatment group T_3 showed a 17% decrease (p>0.05) in serum cholesterol. [37] reported that administering a 5% extract of *Morinda citrifolia* mulberry leaves in drinking water significantly lowered serum cholesterol compared to the control group. Similarly, [10] found that including 0-2% mulberry leaves in broiler diets resulted in significant reductions in both serum cholesterol and triglycerides, particularly with higher dietary inclusions. These findings corroborate our results.

Nutrient metabolizability

The impact of substituting wheat bran with mulberry leaves (MBL) on the nutrient metabolizability in Kuroiler chickens is illustrated in Table 5. The metabolizability of dry matter (DM), ether extract (EE), and crude protein (CP) significantly declined in the groups with 70% and 100% replacement compared to the control and T_1 groups. Conversely, no significant differences were observed in the metabolizability of crude fibre (CF) across all groups, which is corroborated by [24]. In this study, the T_1 group exhibited comparatively greater body weight and FCR compare to T_2 and T_3 groups among the treatment groups, likely due to enhanced DM, EE, and CP metabolizability. Our results align with those of [22], who noted that the inclusion of 10% and 20% mulberry leaves in

Table 7. Effect of dietary supplementation of MBL on economics of production in Kuroiler chicken.

Attributes	Dose of MBL				SEM	p value
	C	T_1	T_2	T_3		
Intake of feed/bird (kg)	2.14^a	2.71^b	2.90^b	2.92^b	0.11	0.019
Cost of feed/kg	41.75	40.53	40.04	39.38		
Total cost of feed/bird	89.27	109.80	116.08	114.97	4.05	0.052
Fixed cost/bird	37	37	37	37		
Total expenditure/bird	126.27	146.80	153.08	151.97	4.05	0.052
Final BW (kg)	0.941^b	0.939^b	0.836^{ab}	0.764^a	0.02	0.008
Cost of live bird@400/kg	376.45^b	375.49^b	334.29ab	305.72^a	9.53	0.008
Return/bird	250.18^b	228.68^b	181.21^a	153.75^a	10.88	<0.001
Profit/kg live weight	265.03^b	242.14^b	216.69^a	200.82^a	6.92	<0.001

Means bearing superscripts in the same row differ significantly (p<0.05).
SEM: Standard error of mean, p: Probability.

broiler diets significantly diminished final body weight and dietary DM and CP digestibility. Similarly, [26] reported reduced protein and fat digestibility in laying hens supplemented with mulberry. The digestibility of mulberry leaves is relatively low (35-37%) in poultry, attributed to their high neutral detergent fiber (NDF) content. Despite this low utilization, DM, CP, and EE are highly digestible (73% and 88%, respectively) in poultry [38]. Furthermore, digestibility tends to decrease with higher supplemental doses, likely due to an accelerated digestion rate resulting from increased dietary fibre content, which shortens nutrient digestion time and affects nutrient absorption in the gastrointestinal tract, thereby influencing the retention of nutrients such as DM, organic matter, and nitrogen [35].

Intestinal morphometry

The micrometric measurements of jejunal sections across various treatments are presented in Table 6. It was noted that an increase in the level of MBL replacement led to a significant decrease ($p<0.05$) in villi width for the T_2 and T_3 groups. Conversely, no significant differences ($p>0.05$) were found in villi height, crypt depth, the ratio of villi height to crypt depth, or the ratio of villi height to width, despite a trend of decrease in these parameters within the treatment groups compared to the control group. The area of the villi (mm^2) showed a significant increase ($p<0.05$) in the T_1 group when compared to the control and other replacement groups. There exists a positive correlation between villus length, nutrient absorption, and digestibility [39]. Consequently, it was evident that the control group, which exhibited the highest weight gain, also possessed the largest villi. Shorter villi and deeper crypts are associated with diminished nutrient absorption. [40] indicated that deeper crypts reflect a rapid renewal of villi in response to tissue inflammation or toxins from pathogens. Therefore, it is plausible that the alterations in crypt depth and epithelial thickness contributed to the reduced weight gain observed in the T_3 group. Villi height, crypt depth, and the ratio of villi height to crypt depth are critical indicators for assessing intestinal digestion and absorption in animals, with greater villi height correlating with enhanced intestinal digestive and absorptive capabilities [41].

Economics of production

The impact of substituting wheat bran with mulberry leaf (MBL) on production economics is detailed in Table 7. Within the replacement groups, the total cost per bird was highest in the T_2 group and lowest in the T_1 group. However, the return per bird and profit per kilogram of live weight were significantly greater ($p<0.05$) in the 50% replacement group, followed by the T_2 and T_3 groups. Consistent with our findings, [29] indicated that incorporating mulberry leaves up to 30% reduced starter and grower feed costs by 24.82% and 26.09%, respectively, without any negative effects. The T_1 group exhibited the highest economic benefit, likely due to lower feed consumption coupled with greater body weight gain. Therefore, for optimal economic returns, we recommend a 50% replacement of wheat bran with MBL in the basal diet.

CONCLUSION

Mulberry leaves serve as an effective feed component for Kuroiler chickens, enhancing weight gain, feed efficiency, and overall chick performance. At 50% replacement of wheat bran with MLM has no adverse effect and it is comparable with non-supplementation of MLM (control) on production performance of Kuroiler chicken. Consequently, it is advisable to incorporate mulberry leaf meal at a 5% level with 5% wheat bran in total feed to optimize the performance of Kuroiler chicks.

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