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Research Article

Dietary effects of *Dillenia indica* leaves on growth performances, blood parameters, carcass characteristics and economic analysis in broilers

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ABSTRACT

The purpose of the study was to assess the effect of dried *Dillenia indica* leaves (DIL) and fermented *Dillenia indica* leaves (FDIL) on growth performances, blood parameters, carcass characteristics and economic analysis in broiler. A total of 90 unsexed Ross 308 day old broiler chicks were distributed in three dietary treatment groups, T_0 = Control (basal diet), T_1 = (basal diet + 0.4% DIL on DM basis) and T_2 = (basal diet + 0.4% FDIL on DM basis). Each treatment group consists of three replications having 10 birds in a completely randomized design. Final weight and average daily gain (ADG) were increased while average daily feed intake (ADFI) and feed conversion ratio decreased significantly in treatment groups compared to control ($P<0.05$) during 3rd, 4th and overall period of the study. There was a significant reduction in blood cholesterol, tryglyceride and LDL level in all treatment groups ($P<0.0001$) whereas higher HDL was found in T1 and lower in T_0 group. Carcass parameters including breast, thigh meat, liver, and abdominal fat weight were significantly differed ($P<0.05$) relative to control. A significant increase in net profit and net profit/kg was found in treatment groups than control. In conclusion, dry and fermented *Dillenia indica* leaves might be a potential feed additives with improved growth performance, excellent lipid profile and higher net profit for poultry feed.

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1. INTRODUCTION

Uses of antibiotics and other synthetic substances as a growth promoter now become a trend around the world. In Bangladesh, uncontrolled uses of antibiotics also become a matter of concern. As a consequence, *Escherichia coli* and *Vibrio cholerea* are found resistant against almost all commonly used antibiotics in Bangladesh (Akond *et al.*, 2009, 2008).

Various dietary herbs, plant extracts, especially essential oils, have been studied for their antimicrobial and growth promoter abilities (Cross *et al.*, 2007; Acamovic and Broker, 2005; Bampidis *et al.*, 2005). So scientists are concentrating on the use of our ancient medicinal system to find beneficial herbs and plants, which can be safely used to increase the production.

Herbs normally used are garlic, cloves, slippery elm, neem fruit and leaves, elephant apple, nutmeg, cinnamon, ginger, peppermint, sage, thyme, mustard and fenugreek. These plants are used as digestive stimulants, antidiarrheal, antiseptic, anti-inflammatory, anti-parasitic and appetite stimulants in human beings as well as animals. Use of probiotics or fermentable sugars instead of antibiotics is going to be popular in birds in order to improve the useful microbial population of GIT (Kermanshahi and Rostami, 2006).

Elephant apple (*Dillenia indica*) locally known as Chalta, is an evergreen tree grows in the moist forest of Sub-Himalayan region to Assam and Asian sub-continent. Traditionally different parts of *Dillenia indica* are used for the relief of indigestion, asthma, influenza, dysentery, jaundice, weakness and rheumatic pain, but recent studies reported that extractives showed significant free radical scavenging activity. Major chemical compounds betulin (pentacyclic triterpenoid) and betulinic acid show wide spectrum of pharmacological activities like anti-HIV, anti-inflammatory, anti-cancer, anti-malarial etc. (Chandana *et al.*, 2018).

Furthermore, it's also known for its useful medicinal properties like anti-diarrheal, anti-diabetic, analgesic, antioxidant activity, free radical scavenging activity anti-hyperlipidemic, and various other properties without showing any adverse effects (Gandhi and Mehta, 2013; Sunil *et al.*, 2011; Bose *et al.*, 2010); Yeshwante *et al.*, 2009. Fruit extract of *Dillenia indica* accelerated the healing of psoriasis-like wounds in rat and reduced inflammation via a mechanism associated with protection against oxidative damage in biomolecules (Kviecinski *et al.*, 2017).

As far my knowledge, the effect of supplementation of dry and fermented elephant apple leaves (*Dillenia indica*) on growth performances, blood parameters, carcass characteristics and economic analysis in broilers has not been reported yet. The present study was conducted to evaluate the effects of dietary dry and fermented elephant apple leaves on growth performance, carcass characteristics, blood parameters and economic analysis in broiler.

2. MATERIALS AND METHODS

Study area

The experiment was carried out at Chattogram Veterinary and Animal Sciences University experimental shed and analysis was performed in Animal Nutrition laboratory, Dept. of Animal Science and Nutrition, Chattogram Veterinary and Animal Sciences University (CVASU), Khulshi, Chattogram, Bangladesh.

Leaves Preparation

Dillenia indica leaves were collected from various places of Chattogram region. After collection, the leaves were dried at well maintained ventilated condition whereas temperature and relative humidity maintained 31°C and 47% respectively. Dried leaves were then grinded and sterilized after ensuring that the moisture was 30%. Electrical grinder was used to perform the grinding, as this process has a significant effect as to invasion and utilization by microbes.

Fermentation of *Dillenia indica* leaves

Lactobacillus plantarum and *Saccharomyces cerevisiae* were selected for the fermentation process with grinded leaves because of their acid, bile and heat tolerance levels according to Hossain *et al.* (2012). MRS broth used for growth of *Lactobacillus plantarum* and yeast-malt (YM) broth for *Saccharomyces cerevisiae* as per instructions of the manufacturer. Two steps fermentation process was applied to prepare the *Dillenia indica* leaves probiotics using a laboratory incubator (LGI-150T, Labnics, USA). In the first inoculation, 1.0% of *Lactobacillus plantarum* was added to solid culture media and made it moisture content about 40% to make the fermentation process properly by adding adequate distilled water. The mixture was then fermented at 40°C for 2 days under repeating cycles of 5 hour of anaerobic and 3 hour of aerobic conditions. The second fermentation was performed by adding 1.0% of *Saccharomyces cerevisiae* strains and similarly fermented for 2 days at 40°C under aerobic conditions. The formulated probiotics mixtures were then dried for 2 days until the moisture level was less than 15% using on air dry basis. To determine the number of cells, 1 g of fermented tree leaf meal was 10-fold serially diluted with sterilized saline solution (0.85% NaCl) at room temperature and cultured in solid media. The culture plate was then incubated at 37°C for 24–48h, after which the number of colonies were counted and expressed as cfu/ml (Table 1).

Table 1. The number of microbial strains of *Dillenia indica* leaves probiotics

Microbial strains in *Dillenia indica* leaves probiotics	Microbes number (cfu/ml)
Lactobacillus plantarum KCTC 3099	3.2×10^8
Saccharomyces cerevisiae KCTC 7928	1.7×10^9

Experimental birds

Strain of birds selected for the experiment was Ross 308. Day-old unsexed broiler chicks of Ross 308® were purchased from Nahar Agro Limited, Chattogram, Bangladesh. All the chicks were examined for abnormalities and uniform size. Average body weight of the chicks were almost similar (about 40.74±0.26 g).

Design of experiment

There were 90 birds randomly distributed into three dietary treatment groups demonstrated as, T_0: Control (basal diet), T_1: (basal diet + 0.4% DIL on DM basis) and T_2: (basal diet + 0.4% FDIL on DM basis) consisting 3 replications having 10 birds per replicates in a completely randomized design. The experiment was conducted under two periods. First 2 weeks were the adjustment periods and next 2 weeks were the treatment periods. Birds were kept in adjustment period to cope up with adverse condition and outgrow stress condition. Birds were provided basal diet for the first 2 weeks for adjustment and after 2 weeks, treatment diet was supplied as planned.

Table 2. Layout of the experiment

Treatments	Birds/ replication		Birds/ treatment
T_0 = Control (Basal diet)	R_1	10	30
	R_2	10	
	R_3	10	
T_1 = 0.4% DIL (Basal diet + 0.4% DIL on DM basis)	R_1	10	30
	R_2	10	
	R_3	10	
T_2 = 0.4% FDIL (Basal diet + 0.4% FDIL on DM basis)	R_1	10	30
	R_2	10	
	R_3	10	
Total		90	

DIL = Dried *Dillenia indica* leaves; FDIL = Fermented *Dillenia indica* leaves.

Housing and brooding

The experimental shed was brick made with one side open for ventilation which is guarded by collapsible metal gate. Floor space for each bird was 0.17 square feet in brooding box and 0.75 square feet in the cage. Cages for birds were randomly selected, to ensure a uniform distribution of treatments and replications. The replicate cages for each treatment were distributed randomly in different locations of the house. Birds were kept in a wired battery rearing cages, measured (3.5 ft. × 1.63 ft. for 10 birds). Therefore, floor space for each bird in the cage was 0.57 sq. ft. respectively. Each cage had a round feeder and drinker to provide feed *ad libitum* and free access to water. Room temperature and humidity was maintained using 200 watt incandescent lamps and ceiling fans. The birds were exposed to continuous lighting. During brooding period, chicks were brooded at a temperature of 95°F, 90°F, 85°F and 80°F for the 1st, 2nd, 3rd and 4th weeks, respectively with the help of incandescent bulbs. Temperatures were measured by using thermometer.

Cleaning and sanitation

The shed was thoroughly cleaned and washed by using tap water with caustic soda. For disinfection, phenyl solution (1% v/v) was sprayed on the floor, corners and ceiling. Following spray, cleaning was done by using brush and clean water. Brooding boxes, rearing cages and pens were cleaned in the same manner. After cleaning and disinfection, the house was left one week for proper drying. After drying, all doors and windows were closed. The room was fumigated (Adding 35 ml of formalin to 10 g potassium permanganate per cubic meter) and sealed for 24 hours. On the next day, lime was spread on the floor and around the shed. Footbath containing potassium permanganate (1% w/v) was kept at the entrance of the poultry shed and changed daily. Feeders were cleaned and washed weekly before being used further. Drinkers were washed with potassium permanganate (1% w/v) and dried up daily in the morning.

Experimental diets

Basal diets were supplied for all birds from 1-14 days as adjustment period. Then experimental diets were supplied to treatment groups from 15-28 days, when control groups received basal diets. The ingredients and composition of the experimental diets are shown in Table 3.

Table 3. Ingredients and chemical composition of experimental diet

Ingredients (as % feed basis)	Starter (0-14 days)	Grower (15-28 days)
Corn	52.00	53.00
Wheat	2.00	2.00
Rice polish	2.14	2.98
Soybean meal	32.00	29.20
Fishmeal	4.00	3.50
Palm oil	3.50	5.00
DCP	1.79	1.79
Limetsone	1.15	1.15
NaCl	0.30	0.30
Choline clholide	0.06	0.06
Vitamin min premix1	0.15	0.15
L-lysine	0.40	0.40
DL-methionine	0.22	0.22
Toxin binder	0.25	0.25
Enzymes	0.04	0.04
Total	100.00	100.00
Chemical composition (as fed basis)		
ME (kcal/kg)	3001.65	3104.85
Crude protein %	22.09	20.71
Crude fiber %	3.76	3.68
Ether extract %	3.67	3.68
Lysine%	0.72	0.80
Calcium%	1.30	1.26
Phosphorus%	0.72	0.70

[1] Vitamin-mineral mixture provided the following nutrients per kg of diet: Vitamin A 15,000 IU, Vitamin D3 1500 IU, Vitamin E 20.0mg, Vitamin K3 0.70mg, Vitamin B12 0.02mg, Niacin 22.5mg, thiamin 5.0mg, folic acid 0.70mg, pyridoxine 1.3mg, riboflavin 5mg, pantothenic acid 25mg, choline chloride 175mg, Mn 60mg, Zn 45mg, I 1.25mg, Se 0.4mg, Cu 10.0 mg, Fe 72mg, Co 2.5mg.

Feeding of birds

All feed ingredients were bought from Alif traders, khatunganj, Chattogram and mixed thoroughly as per formulation stated earlier. Feed was supplied at round feeder from 0-7 days and after 7 days linier feeder (2.21 ft × 0.25 ft) was installed in cage. Round waterer (3-liter capacity) were used for supplying fresh water.

Vaccination

According to vaccination schedule all birds were vaccinated against Newcastle disease on the 4th and 21th day whereas for infectious bursal disease on 12th day. After each vaccination, multivitamin (Rena-WS, Renata; 1g/5liter of drinking water) was supplied with one lemon/5 litre water to overcome the stress effect of vaccination.

Data collection

All required parameters for experiment were recorded at weekly intervals. Carcass characteristics examined at 4th week. Weight gain was calculated by deducting initial body weight from the final body weight of the birds. Feed intake was calculated by deducting leftover from the total amounts of feed supplied to the birds. FCR was calculated dividing feed intake by the weight gain.

Carcass characteristics

Three birds were selected and slaughtered randomly from each replication on 28 day. After adequate bleeding out, birds were de-feathered and dressed as recommended. During evisceration process, abdominal fat, liver, spleen, bursa, gizzard were excised separately and weighed. Dressed birds were weighed to obtain a dressed carcass weight. Total breast meat, thigh meat and thigh bone weight were recorded.

Serum biochemical analysis

Blood samples were collected from the brachial vein of three birds from each replicate using a 5ml sterile syringe and a 23-gauge needle. From each bird, 5ml

blood sample was transferred immediately into a sterile tube without anticoagulant. Clotted blood in the vacutainer tube was centrifuged at 3000 rpm for 20 minutes and prepared serum was collected into the eppendorf tube by micropipette. Different blood parameters (cholesterol, triglyceride, LDL and HDL) were measured in the Post Graduate Laboratory under the department of Animal Science and Nutrition, CVASU using standard kits (BioMereux, France) and automatic analyzer (Humalyzer 300, Merck®, Germany according to the manufacturer's instruction.

Data analysis

All the data were entered into MS excel (Microsoft office excel-2010, USA). All data were analyzed using the General Linear Model (GLM) procedure of SAS Institute Inc. (2009). Duncan's multiple range tests were used to examine significant differences among the treatment means. The level of statistical significance was preset at $P<0.05$.

3. RESULTS

Growth performance

A significant increase ($P<0.05$) in average daily gain (ADG) was found in all treatment groups compared to control (Table 4). Overall highest average daily weight gain (82.70g/d) was observed in T_2 group and the lowest average daily weight gain (77.08 g/d) was recorded in control (T_0). Overall ADG also increased significantly in treatment groups than control group ($P<0.05$). No significant variation was observed in average daily feed intake (ADFI) among dietary treatment groups during trial period ($P>0.05$). The highest and lowest overall ADFI was recorded in T_0 and T_2 groups respectively (Table 4). Remarkable difference was obtained for FCR among the treatment groups. On 3rd week, T_2 treatment group showed the lowest FCR where T_0 and T_1 group showed nearly similar FCR which is higher than T_2 group. At 4th week of age, T_2 group showed lowest FCR and T_0 group showed higher FCR. The overall FCR value was also significantly decreased ($P<0.05$) in T_2 group compared to control (Table 4).

Table 4. Dietary effect of *Dillenia indica* leaves on growth performance of broiler

Parameter	Dietary Treatments			SEM	P-value
	T_0	T_1	T_2		
3rd week					
Initial weight (g/b)	273.05	272.33	271.95	0.68	0.64
Final weight (g/b)	743.76^{b}	758.19^{a}	767.30^{a}	3.34	0.01
ADG(g/b/d)	67.25^{b}	69.41^{a}	70.76^{a}	0.55	0.01
ADFI(g/b/d)	76.98	75.71	69.15	2.79	0.30
FCR	1.15^{a}	1.09^{a}	0.99^{b}	0.02	0.003
4th week					
Initial weight (g/b)	743.76^{b}	758.19^{a}	767.30^{a}	3.34	0.01
Final weight (g/b)	1352.11^{c}	1402.68^{b}	1429.76^{a}	3.99	<.0001
ADG(g/b/d)	86.91^{b}	92.07^{a}	94.64^{a}	0.85	0.002
ADFI(g/b/d)	111.08	109.66	104.80	1.50	0.06
FCR	1.28^{a}	1.19^{b}	1.10^{c}	0.01	0.0001
Overall (15-28 days)					
Initial weight (g/b)	273.05	272.33	271.95	0.68	0.64
Final weight (g/b)	1352.11^{c}	1402.68^{b}	1429.76^{a}	3.99	<.0001
ADG(g/b/d)	77.08^{c}	80.74^{b}	82.70^{a}	0.24	<.0001
ADFI(g/b/d)	94.03	92.69	86.97	1.46	0.08
FCR	1.22^{a}	1.15^{a}	1.05^{b}	0.02	0.006

^{a,b,c} Means in a row with no common superscripts significantly differ ($P<0.05$).
Data presented as the mean value of 3 replicate groups with 10 birds per replication (n=30).
T_0 = Control (basal diet); T_1 = 0.4% DIL on DM basis; T_2 =0.4% FDIL on DM basis
ADG = Average daily gain; ADFI = Average daily feed intake; FCR= Feed conversion ratio;
SEM = Standard error of mean.

Blood serum parameters

Different blood serum parameters estimated have been presented in Table 5. Results indicated that, there is a significant reduction in blood cholesterol, TRG and LDL level in all treatment groups relative to control group (P<0.0001), however HDL value was found higher in T_1 and T_2 groups and lower in T_0 group (Table 5).

Table 5. Dietary effect of *Dillenia indica* leaves on blood serum parameters

Parameter	Dietary Treatments			SEM	P-value
	T_0	T_1	T_2		
Cholesterol (mg/dl)	133.20[a]	110.81[b]	99.94[c]	1.50	<.0001
TRG (mg/dl)	88.29[a]	70.44[b]	46.08[c]	2.28	<.0001
HDL (mg/dl)	74.52[b]	83.04[a]	77.08[ab]	1.80	0.04
LDL (mg/dl)	41.02[a]	13.68[b]	13.64[b]	1.40	<.0001

[a,b,c] Means in a row with no common superscripts significantly differ (P<0.05).
Data presented as the mean value of 3 replicate groups with 3 birds per replication (n=9).
T_0 = Control (basal diet); T_1 = 0.4% DIL on DM basis; T_2 =0.4% FDIL on DM basis
SEM = Standard error of the mean

Carcass Characteristics

The carcass parameters significantly differed (P<0.05) in terms of breast, thigh meat, liver and abdominal fat weight (Table 6). However, it did not differ significantly (P>0.05) in other parameters of all dietary treatments compared to control. Other carcass parameters were statistically observed almost similar (P>0.05).

Table 6. Dietary effect of *Dillenia indica* leaves on carcass characteristics and organ weight of broiler

Parameter	Dietary Treatments			SEM	P-value
	T_0	T_1	T_2		
Dressed weight	61.90	56.09	61.21	2.61	0.51
Relative breast meat wt	15.93[c]	18.17[a]	17.00[b]	0.25	0.004
Thigh meat (with bone)	17.24	18.15	19.22	0.48	0.12
Thigh meat wt	14.11[c]	15.39[b]	17.26[a]	0.28	0.001
Thigh bone wt	3.13	3.42	3.96	0.28	0.24
Liver	2.40[b]	2.62[a]	2.24[c]	0.02	0.0001
Gizzard	1.74	2.41	2.19	0.25	0.25
Heart	0.54	0.61	0.63	0.07	0.67
Spleen	0.10	0.11	0.09	0.02	0.75
Bursa	0.18	0.21	0.20	0.03	0.84
Abdominal fat	0.35[b]	0.51[a]	0.57[a]	0.02	0.0004

[a,b,c] Means in a row with no common superscripts significantly differ (P<0.05).
Data presented as the mean value of 3 replicate groups with 3 birds per replication (n=9).
T_0 = Control (basal diet); T_1 = 0.4% DIL on DM basis; T_2 =0.4% FDIL on DM basis
SEM = Standard error of the mean

Cost-benefit analysis

A significant increase in net profit/kg was observed in T_1 (20.34) groups which is close to T_2 (20.31) but significantly (P<0.05) differed from control group which is lowest (15.50)

Table 7. Cost-benefit analysis of broiler fed diets supplemented with *Dillenia indica* leaves

Parameter	Dietary Treatments			SEM	P-value
	T_0	T_1	T_2		
Live weight (g/b)	1.35[b]	1.40[a]	1.43[a]	0.01	0.004
Total feed intake (g/b/d)	1.68	1.64	1.71	0.02	0.07
Total feed cost (BDT)	72.24	70.52	73.53	1.15	0.27
Other cost (BDT)1	55.00	55.00	55.00	1.40	1.00
Total cost (BDT)	127.24	125.52	128.53	1.12	0.27
Selling price (BDT)	148.13[b]	154.00[a]	157.67[a]	1.19	0.004
Net profit (BDT)	20.89[b]	28.48[a]	29.13[a]	1.24	0.007
Net profit/kg (BDT)	15.50[b]	20.34[a]	20.31[a]	0.77	0.01

[a,b,c] Means in a row with no common superscripts significantly differ (P<0.05).
[1] Other cost includes labour, vaccination, medicine and electricity cost.
Data presented as the mean value of 3 replicate groups with 10 birds per replication (n=30).
T_0 = Control (basal diet); T_1 = 0.4% DIL on DM basis; T_2 =0.4% FDIL on DM basis
BDT = Bangladeshi taka; SEM = Standard error ofthe mean.

4. DISCUSSION

In this study effect of *Dillenia indica* leaves on live weight gain, FCR, blood serum parameter, carcass characteristics and cost-benefit analysis were investigated. The average body weight is remarkably increased in treatment groups in comparison to control groups. The highest body weight gain found at treatment groups supplied fermented leaves might be due to increased protein content for fermentation. Study conducted in India stated that daily oral administration *Dillenia indica* methyl extract (250 and 500 mg/kg body weight) in mice showed beneficial effects on blood glucose level and body weight gain (Yeshwante *et al.*, 2009). Endo and Nakano (1999) reported a notable improvement in the productivity and meat quality of broiler in supplementation of probiotics with feed due to improved intestinal flora and the raising environment. Between the dry leaves and probiotics treated groups, the groups with 1% probiotics mixture (Lactobacillus and Saccharomyces with spondias leaf of 30% of the composition of probiotics and 35% DDGS of the probiotics) showed the more improved weight gain (Awad *et al.*, 2015). Improved intestinal micro flora enhances digestion as well as protein portion of yeast in fermented supplement may increase ADG in this current study. Remarkable difference also obtained in FCR among the treatment groups other than control group might be for improved digestion effect of probiotics. Similarly, reduced feed intake and improved FCR value was observed in an experiment supplied essential oil produced from lemon peel oil in breeder flock (Cabuk *et al.*, 2006). It can be explained as the better FCR was achieved in treatment group due to increased ADG in this experiment. FCR might more effective in treatment groups after three weeks than the control group stated by Frankic *et al.* (2009).

The carcass parameters significantly differed in terms of breast, thigh meat, liver and abdominal fat weight, however other carcass characteristics found almost similar, which shows resemblance with the study using sweet orange peels conducted in Nigeria by Orayaga *et al.* (2016). No similar study was found in literature.

In blood serum parameter it was found that, decrease in LDL and TRG and increased HDL level in treatment groups than the control group which is similar to the result found by Yang *et al.* (2009). Anti-oxidant and lipolytic substances present in elephant apple leaves may diminish LDL and triglyceride level. The net profit/kg broiler showed a significant increase in treatment groups compared to control. It might be due to the increased ADG and improved FCR of the broiler which accelerated the profit of this research.

5. CONCLUSIONS

The study sought the effects of dry and fermented *Dillenia indica* leaves supplements on the growth performances including carcass characteristics, blood parameter and economic analysis. Statistically remarkable result found on ADG and FCR of broiler in treatment groups supplied with fermented leaves other than control group. The blood HDL level also increased in treatment groups where LDL level found lower compared to control group. The net profit value of treatment groups showed higher than control group of this study. Therefore, based on this findings dry and

fermented *Dillenia indica* (elephant apple) leaves might be a potential feed supplement with basal diet as it is grow everywhere in Bangladesh. However, a long term investigation with larger sample size and multi-dimensional temporal pattern is suggested for increasing sensitivity and validity of the study under field condition.

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