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Effect of papaya leaf juice on growth performance and bacterial load in faeces of sonali chicken

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***Abstract**: A study was undertaken to investigate the effect of papaya leaf juice on growth performance and bacterial load in the feces of Sonali chicken. A total of one hundred eighty (180) vigorous 28 days Sonali chicks were randomly divided into five treatment groups namely T_0 (Control), T_1 (growth promoter (Amino solve) @1ml/1L drinking water), T_2 (10% papaya leaf juice in drinking water), T_3 (20% papaya leaf juice in drinking water) and T_4 (5% dried leaf in feed) having three replication in each treatment group. Data on live weight and feed intake were collected and bacterial load in feces was recorded. Total feed intake was non-significant ($P>0.05$) among the treatment groups but final live weight, live weight gain, and FCR significantly differed. Total feed intake and live weight at the 1st, 2nd, 3rd, and 4th week in-significantly differed among the treatment groups but the live weight (g/bird) at the 5th and 6th week and FCR significantly differed. The final (6th week) average body weight (g) was significantly higher T_1 (605.00±7.64). The best and lowest FCR was found in T_2 (2.63±0.04) and the highest FCR was in T0 (2.98±0.07). The load of* Salmonella *sp. and* E. coli *was significantly lower at increasing concentrations of papaya leaf juice compared to the control. The study concludes that the supplementation of 10% papaya leaf juice in drinking water can be used as an alternative to commercial growth promoters for the production of Sonali chicken.*

***Keywords:**Sonali Chicken, Papaya Leaves, performance, Bacterial load.*

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I. INTRODUCTION

The most significant and developing area of agriculture in Bangladesh is poultry. Bangladesh's demand for chicken goods (meat and eggs) has increased dramatically over the years; in 2019, per capita consumption of poultry meat and eggs was 8.5 kg and 104 pieces, respectively. But more chicken production is required to meet the rising domestic demand for meat and eggs [1]. With a contribution to GDP of roughly 1.5–1.6% and an average annual growth rate of 12–15%, Bangladesh's poultry business has proven to be a lucrative economic sector. The Bangladesh Poultry Industries Central Committee (BPICC) estimates that approximately 6 million people are employed in Bangladesh's poultry business, which has received a total investment of over BDT 3500 million [2]. Bangladesh's chicken industry is a promising and significant source of income, particularly for rural residents [3].

It is anticipated that by 2021, this sector would generate work opportunities for around 10 million people, the bulk of whom will be unemployed young men and women [4]. In addition to creating jobs, the chicken sector improved food security and enhanced the availability of high-quality protein in Bangladeshi diets. The demand for meat, eggs, and related products has skyrocketed as a result of increased affluence, urbanization, and dietary changes. Notwithstanding differences in religion, economy, society, and demography, chicken meat is currently the least expensive source of animal protein in Bangladesh. Meat is an integral part of our daily diet [5, 6]. In Bangladesh, poultry meat makes up 37% of all meat production and around 22-27% of all animal protein supplies, according to [7]. To meet customer demand, the Sonali chicken population gradually grows among Bangladesh's poultry [8]. Eggs and poultry meat are easily digestible, reduce the risk of high blood pressure, heart disease, and cancer in humans [9]. Sonali chickens, which are a mix between Rhode Island Red cockatoos and Fayoumi hens, are more likely to be native to Bangladesh [10]. Sonali crossbred chicken was introduced in the northern regions of Bangladesh between 1996 and 2000 by the Department of Livestock Services (DLS), providing employment for millions of rural women. When compared to broiler chickens, they are more suited to tropical temperatures and require less care and attention to raise [11]. Sonali currently fulfills around 30% of Bangladesh's overall demand for chicken meat. Sonali chicken meat is preferred by consumers above conventional broiler chicken meat and resembles native chicken flesh. Sonali chicken preference increased from roughly 20% of all poultry meat in 2018 to 23% in 2019 [1]. Unfortunately, numerous enteric illnesses like *coccidiosis* have made it difficult to produce poultry [12].

Most frequently, some species of *Eimeria*, such as *Eimeria tenella*, *Eimeria acervulina*, *Eimeria maxima*, and *Eimeria brunette*, can infect poultry [13]. All chickens are prone to *coccidiosis*, but those that are 3 to 8 weeks old are most at risk [14]. *Coccidiosis* consequently decreased feed conversion efficiency, caused significant physiological damage, weakness, diarrhea, weight loss, and anemia, and ultimately resulted in mortality [15]. Moreover, commercial farm poultry have high rates of coccidiosis, ranging from 5% to 70%, which increases morbidity and mortality [16], driving up the production cost per chicken by £0.16[17].

Consequently, the main method for preventing *coccidiosis* in chickens is to use chemical *coccidiostats* in poultry feed [18]. Unfortunately, regular use and abuse of these medications encourage the emergence of Eimeria strains with drug-resistant characteristics, among other harmful effects [19]. Anticoccidial medication residues are also observed in poultry products together with antibiotic resistance, raising concerns about public health and food safety [20]. As an alternative, plant extracts have received much research over the past few decades to replace preventive measures for limiting avian *coccidiosis* and improving chicken performance[21]. C. *papaya* is a member of the *Caricaceae* family, which has been shown to be effective against *coccidiosis* in some states due to less bitterness and the presence of carotene, making it one of the botanical components that can serve as sustainable substitutes[22]. Also, it can improve the taste of the food, which will help the chicks grow more quickly. Moreover, it protects the caecal epithelial cells, has anti-inflammatory properties, and inhibits *coccidial* reproduction[23]. Unfortunately, only a small number of results have been published on C. papaya leaf extract as a viable treatment for *coccidiosis*, particularly in Bangladesh's Sonali hens. Moreover, current data on the relative efficacy of plant extracts are crucial since *Eimeria spp*. might alter their resistance to the traditional anticoccidial medicines on a daily basis, creating a constant need to find novel anticoccidials[24]. As a result, the current study was carried out to evaluate the effects of papaya leaf juice supplementation on the performance of sonali chicken production as well as to identify the bacterial load in sonali feces.

II. MATERIALS AND METHODS

2.1 Duration and location of the experiment

The experiment was conducted for a period of 27 July to 18 September 2019 at Hajee Mohammad Danesh Science and Technology University (HSTU) Poultry Shed, Dinajpur and the microbial load was analyzed at Microbiology lab (HSTU).

2.2 Experimental birds

One hundred eighty (180) vigorous 28 days of Sonali chicks were collected from Nourish Poultry and Hatchery Ltd.

2.3 Preparation of research shed

The shed was cleaned and washed using fresh water and disinfectant (Iodin, Non Ionic Surfectant, Sulfuric Acid, Phosphoric Acid, FAM 30®, Renata animal health, Bangladesh).Then the shed was kept free for 15 days before placing experimental birds. All necessary equipment was disinfected and set properly to care for the broiler chicks successfully.

2.4 Source and Preparation of plant extract

The leaves were selected for effectiveness as growth promoters on poultry. Mature and disease free papaya leaves were collected from the Dinajpur district. The fresh young green papaya (*Carica papaya*) leaves were picked and washed with running fresh water. Then, they were soaked with cotton and kept in a well-ventilated room for air drying. The air-dried leaves were chopped into small pieces and mashed by a pestle and mortar. Finally, the leaves extracts were obtained by squeezing and pressing mashed leaves. Then, 0.5% of the suspension was produced by dissolving the ground papaya leaves in distilled water.

2.5 Experimental materials and treatments of the experiment

A total of 180, 28 days old Sonali chicks were purchased from the dealer of Nourish Poultry and Hatchery Limited, Gazipur. All birds were randomly allocated to five dietary treatment groups as T_0 (Control fed with commercial diet), T_1 (Commercial diet with growth promoter (Amino solve) @1ml/1L drinking water), T_2 (Commercial diet with 10% papaya leaf juice in drinking water), T_3 (Commercial diet with 20% papaya leaf juice in drinking water) and T_4 (Commercial diet with 5% dried papaya leaf in feed), each group consisted of 3 replications containing 12 birds in each replication. Feed and water were supplied ad libitum throughout the trial. All birds were fed with standard commercial (Nourish Poultry & Hatchery Ltd.) ration throughout the experimental period.

2.6 Biosecurity and sanitation

Different measures were taken to maintain biosecurity: Visitors were not allowed to enter the house; all equipment in the experimental house was kept clean.

2.7 Experimental Procedure and Data Collection

The growth performance of Sonali chicken was evaluated by using changes in body weight (growth rate), feed intake, and feed conversion ratio. The live weight of each bird was measured with the help of digital balance on

initial weight, 1st to 6th week of the experiment, and recorded. The birds were weighed at the start (initial body weight) and then at the end of the experiment (final body weight). Body weight gain/loss was calculated by the difference between initial body weight and final body weight.
Body weight gain = Final body weight - initial body weight
Feed intake was determined by weighing the amount of feed offered and feed refusal in each pen (replicate) every week. Feed intake per pen was obtained by calculating the difference between the total weight of the feed given and the weight of the refused feed (leftover feed). However, the Feed conversion ratio (FCR) was obtained by dividing the total feed intake by the total weight gain of all birds in each pen. The feed intake and feed conversion ration were obtained by using the following formula:
Feed intake (FI) = Feed offered – Feed refusal
Feed Conversion ratio (FCR) = Feed intake/ Average daily gain

2.8 Collection and transportation of faecal sample

Feces from the Sonali of each group were collected aseptically and kept in phosphate-buffered saline (PBS) solution containing collecting tubes and sent to the microbiology lab, Mohammad Danesh Science and Technology University (HSTU), Dinajpur.

2.9 Bacteriological media preparation

2.9.1 Eosin Methylene Blue (EMB)

Lactose fermenting pink colony from MacConkey was subcultured into EMB agar, used selective media for *E. coli*, and incubated at 37^0C for 24 hours.

2.9.2 Salmonella-Shigella (SS) agar

The non-lactose fermenting colorless colony from the MacConkey agar was sub-cultured on SS agar media used as selective media for pathogenic *Salmonella* spp. and *Shigella* and incubated at 37^0c for 24 hours.

2.9.3 Isolation of E. coli and Salmonella in pure culture

All samples were cultured primarily in nutrient agar at 37°C for 24 h, and then subcultured onto the MacConkey and EMB agar and S-S agar by streak plate method to observe the morphology. The organism showing, the characteristic colony morphology of *E. coli* was repeatedly sub-cultured onto EMB agar until the pure culture with homogenous colonies, and the organism showing, the characteristic colony morphology of Salmonella was repeatedly subcultured onto S-S agar until the pure culture with homogenous colonies.

2.9.4 Examination of Plates (Identification of the isolates)

2.9.4.1 Gross colony study

Morphological characteristics (shape. size, surface texture, edge, elevation, color, opacity etc.) developed after 24 hours of incubation were carefully studied as described by [25] and recorded.

2.10 Statistical analysis

Data were collected from each treatment and entered into the computer database (excel sheet) ready for statistical analysis (SPSS, Version 22.0). The data were analyzed by one-way analysis of variance, followed by the Duncan post hoc test to determine significant differences in all the parameters among all groups using the SPSS computer program (Version 20.0; SPSS,). Differences with values of $P<0.05$ were considered to be statistically significant. All data were expressed as Mean±Standard Error of Mean (SEM). Differences were considered statistically significant at least $P<0.05$.

III. RESULTS AND DISCUSSIONS

3.1 Effect of papaya leaf juice on body weight (g/bird) of sonali chicken

The present study revealed that the average body weight (g) of Sonali chicken did not significantly differ among the treatment groups at 1st week, 2nd week, 3rd week, and 4th week of age but significantly ($P<0.05$) differed at 5th and 6th week of age (Table 1). The average body weight (g) in 1st week was maximum in T_2 (169.31±8.44) and minimum in T_0 (164.83±8.80). Similar findings were also obtained at the age of 2nd week [highest in T_2 (274.36±9.71) and lowest in T_0 (243.71±5.99)]. Higher average body weight (g) was found in T_4 (336.50±27.01), T_2 (404.31±19.01), and T_1 (495.56±23.41) at the 3rd and 4th week of age. The average body weight (g) was significantly highest in T_1 (502.83±19.86), followed by T_2 (494.64±7.52), T_4 (475.11±4.98), T_3 (471.00±9.93) and T_0 (444.33±7.22) respectively at 5th week of age. The final (6th week age) average body weight (g) was significantly higher in T_1 (605.00±7.64) and lower in T_0 (539.33±14.85). The present study is supported by [26] who observed that papain crude extract levels both in mash and pellet feed form were able to improve the body weight of the broiler. [27] and [28] Found that Pawpaw Leaf Meal (PLM) also increased body weight. The present result disagreed with the findings of [29] reported the use of papaya leaf extract to be mixed into the feed of poultry until a concentration of 25ml per liter of drinking water has not shown any significant influence on the performance of broiler production.

Table 1: Effect of papaya leaf juice on body weight (g/bird) of Sonalichicken

Age	Dietary treatment groups					P value
	T_0	T_1	T_2	T_3	T_4	
1st week	164.83±8.80	166.80±4.20	169.31±8.44	165.58±3.15	168.14±3.49	NS
2ndweek	243.71±5.99	265.42±8.49	274.36±9.71	261.78±5.90	268.19±7.37	NS
3rd week	289.57±4.84	330.44±10.58	336.47±13.92	327.47±15.59	336.50±27.01	NS
4th week	373.29±9.37	396.08±23.30	404.31±19.01	386.11±3.07	383.97±4.98	NS
5th week	444.33±7.22[a]	502.83±19.86[c]	494.64±7.52[c]	471.00±9.93[b]	475.11±4.98[b]	*
6th week	539.33±14.85[a]	605.00±7.64[c]	601.88±5.82[c]	572.22±8.92[b]	569.31±6.38[b]	*

Here, T_0= Control fed with commercial diet, T1=Commercial diet with growth promoter (Amino solve) @1ml/1L drinking water), T_2 = Commercial diet with 10% papaya leaf juice in drinking water, T_3=Commercial diet with 20% papaya leaf juice in drinking water and T_4=Commercial diet with 5% dried papaya leaf in feed

The effect of papaya leaf juice on the feed intake (g/bird) of Sonali chicken is shown in Table 2. The study indicated that there was no significant (P>0.05) variations among the different treatment groups in the case of feed intake (g/bird) in all ages of birds. The highest feed intake (g/bird) was found in T_0 (194.06±5.89), T_3 (227.67±1.54), T_0 (258.76±4.24), T_0 (301.70±4.32), T_2 (309.69±3.29) and T_1 (326.15±2.50) in 1st, 2nd, 3rd, 4th, 5th and 6th week of age. Feed intake (g/ bird) was more or less similar in all treatment groups. [26] Observed that papain crude extract levels both in mash and pellet feed form were able to improve feed intake of broiler

Table 2: Effect of papaya leaf juice on feed intake (g/bird) of Sonali chicken

Age	Dietary treatment groups					P value
	T_0	T_1	T_2	T_3	T_4	
1st week	194.06±5.89	192.50±1.07	184.97±4.49	189.50±3.03	191.92±1.61	NS
2nd week	223.33±8.82	222.86±2.86	219.67±2.99	227.67±1.54	220.83±4.68	NS
3rd week	258.76±4.24	253.72±2.05	247.14±2.07	255.39±3.32	251.72±1.35	NS
4th week	301.70±4.32	291.11±3.89	289.16±4.29	293.47±1.52	295.63±3.05	NS
5th week	303.57±11.96	307.22±1.87	309.69±3.29	308.19±1.84	308.22±4.15	NS
6th week	325.03±3.38	326.15±2.50	322.64±2.34	324.13±2.91	325.86±2.32	NS

Here, T_0= Control fed with commercial diet, T1=Commercial diet with growth promoter (Amino solve) @1ml/1L drinking water), T_2 = Commercial diet with 10% papaya leaf juice in drinking water, T_3=Commercial diet with 20% papaya leaf juice in drinking water and T_4=Commercial diet with 5% dried papaya leaf in feed

Effect of Papaya leaf juice on total feed intake (g/bird) and FCR of sonalichicken

Table 3 shows the effect of papaya leaf juice on the total feed intake (g/bird) and FCR of Sonali chicken. It was revealed that there was no significant difference among all treatment groups in the case of total feed intake but a significant (P<0.05) variation was found in the case of FCR. The total feed intake (g/bird) was highest in T_0 (1605.80±12.74), followed by T_3 (1598.35±3.09), T_4 (1594.18±6.26), T_1 (1593.57±1.13) and T_2 (1573.26±5.29) respectively. The best and lowest FCR was found in T_2 (2.63±0.04) and the highest FCR was in T_0 (2.98±0.07) whereas in T_1, T_3, and T_4 was 2.63±0.04, 2.79±0.05 and 2.80±0.04 respectively. The FCR in T1 and T_2 were statistically similar. A similar result is also observed by [26] and [30] who reported that papaya leaf extract improved the feed consumption and feed efficiency of the birds.

Table 3: Effect of Papaya leaf juice on total feed intake (g/bird) and FCR of Sonalichicken

Parameter	Dietary treatment groups					P value
	T_0	T_1	T_2	T_3	T_4	
Total feed intake (g)	1605.80±12.74	1593.57±1.13	1573.26±5.29	1598.35±3.09	1594.18±6.26	NS
FCR	2.98±0.07[c]	2.63±0.04[a]	2.61±0.03[a]	2.79±0.05[b]	2.80±0.04[b]	*

Here, T_0= Control fed with commercial diet, T1=Commercial diet with growth promoter (Amino solve) @1ml/1L drinking water), T_2 = Commercial diet with 10% papaya leaf juice in drinking water, T_3=Commercial diet with 20% papaya leaf juice in drinking water and T_4=Commercial diet with 5% dried papaya leaf in feed

Effect of papaya leaf juice on bacterial load of sonalichicken

Table 4 presents the effect of papaya leaf juice on bacterial load in the feces of Sonali chicken. The load of *Salmonella* sp. was significantly (P<0.05) lower in T_4 (111.00±7.23), followed by T_3 (148.33±7.26), T_2 (170.33±10.17), T_1 (244.33±26.77) and T_0 (250.33±24.91) respectively. The load of *E. coli* was significantly (P<0.05) lower in T_4 (118.67±4.10), followed by T_3 (124.33±5.49), T_2 (171.67±2.60), T_1 (230.33±22.81) and T_0 (252.67±11.29) respectively. The present study is similar to the reports of [29] who reported that papaya leaf extract helps to reduce the microbial load of birds. [31] Reported that the extract of the leaves of *C. papaya* showed potent antimicrobial activity against *Salmonella sp*., *Staphylococcus aureus*, *Streptococcus faecalis*, *Escherichia coli*, and *Proteus mirabilis*.

Table 4: Effect of papaya leaf juice on bacterial load in faecesof Sonali chicken

Parameter	Dietary treatment groups					P value
	T_0	T_1	T_2	T_3	T_4	
Salmonella sp.	250.33±24.91^{c}	244.33±26.77^{c}	170.33±10.17^{b}	148.33±7.26^{ab}	111.00±7.23^{a}	*
E. coli	252.67±11.29^{c}	230.33±22.81^{c}	171.67±2.60^{b}	124.33±5.49^{a}	118.67±4.10^{a}	*

Here, T_0= Control fed with commercial diet, T1=Commercial diet with growth promoter (Amino solve) @1ml/1L drinking water), T_2 = Commercial diet with 10% papaya leaf juice in drinking water, T_3=Commercial diet with 20% papaya leaf juice in drinking water and T_4=Commercial diet with 5% dried papaya leaf in feed

IV. Conclusion

The present study indicated that the average body weight (g) of Sonali chicken did not significantly differ among the treatment groups in 1st weekto 4th week. The final (6th week age) average body weight (g) was significantly higher T_1 (605.00±7.64). It was found that there was no significant difference among all treatment groups in the case of feed intake but significant variation was found in the case of FCR. The total feed intake (g/bird) was highest in T_0 (1605.80±12.74). The best and lowest FCR was found in T_2 (2.63±0.04). The load of *Salmonella sp.* and *E. coli* was significantly lower in T_4 (111.00±7.23) and (118.67±4.10) respectively. The result of this study suggests that the supplementation of 10% papaya leaf juice in drinking water can be used as an alternative to commercial growth promoters for the production of Sonali chicken.

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