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Efficacy of Feeding of *Morinda citrifolia* Fruit Juice and *Lactobacillus acidophilus* in Broiler

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ABSTRACT

An experiment was conducted to study the efficacy of feeding of *Morinda citrifolia* fruit juice (noni) and *Lactobacillus acidophilus* (LAB) and their combination in commercial broilers. Commercial broiler chicks were fed till 7 weeks of age as per Bureau of Indian Standards (BIS). At day old age, chicks were randomly assigned to each of four water supplement treatments namely, T1 (5 mL noni juice/bird/day), T2 {5 mL (1×10^8 CFU mL⁻¹) LAB/bird/day}, T3 (2.5 mL noni+2.5 mL LAB/bird/day) and T4 (control with basal diet only). The birds were maintained under deep litter system of rearing and provided with normal basal ration throughout the experiment period. Results revealed that broilers given with noni juice recorded significantly ($p < 0.05$) higher mean body weight (451.9±17.1 and 1434.5±60.5 g) and weight gain (402.5±18.4 and 1391±60.3 g) at 21 and 49 days, respectively. Control group recorded significantly comparable body weight and weight gain. Feed efficiency was significantly ($p < 0.05$) better (1.6±0.17) in noni group. Humoral immune response in noni+LAB group (0.73) was found to be significantly higher than control group (0.36) and other treatment groups (0.53) at one week post inoculation of Goat Red Blood Cells (GRBC). Noni and LAB groups showed significant ($p < 0.05$) reduction in the coliform load. Treatment groups did not influence pH of intestine and carcass yield significantly. The feed consumption of the noni group was 10% lower than the other groups thus in turn it saved the feed cost of ₹ 5.39 per bird. Based on the present study, it is concluded that the feeding of *Morinda citrifolia* juice showed improved growth performance, high immune response, low gut microbial load and feed cost efficiency in commercial broilers and the combination of noni and LAB might be promising alternatives for antibiotic growth promoters to improve the production of safety poultry products.

Key words: *Morinda citrifolia*, *Lactobacillus*, performance, humoral immunity, broilers

INTRODUCTION

The poultry industry is one of the most leading agribusiness trade in the world. Feed supplements in poultry production has significant role to improve the productivity of poultry. Now a days the food safety and security is more seriously considered than before. Since, the ban of antibiotics as growth promoters by the European Union in 2006, several other alternatives for antibiotics such as probiotics, prebiotics and supplementation of medicinal plant extracts have been reported in the poultry diet. Over the last 5-10 years, probiotics have often been proposed as an alternative to antibiotics. The use of lactic acid bacteria in livestock poultry feed as probiotic has been well studied by many workers as feed supplements for growth, enhance production performance and immune response (Ibrahim *et al.*, 2005; Salarmoini and Fooladi, 2011; Zamanzad-Ghavidel *et al.*, 2011).

The use of medicinal plant extracts in poultry for growth and immunity has been reported by some workers (Narimani-Rad *et al.*, 2011; Javed *et al.*, 2009; Mishra *et al.*, 2008). *Morinda citrifolia* commonly known as noni has a rich history in Ayurveda and grows widely throughout the coastal regions of many countries including the Andaman and Nicobar group of Islands. Its fruit and leaves have been consumed as traditional foods in Southeast Asia and in the Pacific islands (West *et al.*, 2006). Reports suggested that the fruit has been used as a feed supplement for livestock and poultry (Sunder *et al.*, 2011a). However, feeding of *Lactobacillus* alone and noni alone have been reported by several workers. The combination of this medicinal plants and the *Lactobacillus* is not available in the broiler. Hence, the present study was designed with an objective to study the efficacy of feeding of *Morinda citrifolia* fruit juice (noni) and *Lactobacillus acidophilus* (LAB) and their combination in commercial broilers.

MATERIALS AND METHODS

Preparation of *Morinda citrifolia* juice (noni) and *Lactobacillus acidophilus* culture: The *Morinda citrifolia* ripened fruits were collected, washed with clean water, dried and packed in polythene bags for preparation of the fresh juice. The concentrated juice was extracted from the noni fruits and kept at room temperature for further use. *Lactobacillus acidophilus* NCDC-11 was used for the present study. The *Lactobacillus* was grown in the MRS agar plate and biochemical test and sugar fermentation test was carried out to check the purity of the culture. The culture was maintained in MRS broth and used for further study.

Experimental design and general management: A total of 124 days old commercial broiler chicks were randomly divided into four groups in completely randomized design with three replicates of 12 birds in each replicate. Chicks were vaccinated for Ranikhet disease and Marek's disease at the hatchery. The experiment was conducted for 49 days. The birds were kept under deep litter system of rearing and provided with standard starter and finisher ration and water *ad-lib*. No medication, deworming was given throughout the experiment. The starter feed was given to the birds till 3rd week of age and then finisher feed was given to the birds from 4-7 weeks. Broilers were assigned to each of four treatment groups:

- **T₁:** *Morinda citrifolia* juice (noni), Basal diet with 5 mL LAB/bird/day in water
- **T₂:** *Lactobacillus acidophilus* (LAB), Basal diet with 5 mL juice/bird/day in water (1×10^8 CFU mL⁻¹)
- **T₃:** (Noni+LAB), Basal diet with 2.5 mL noni+2.5 mL LAB/bird/day in water (1×10^8 CFU mL⁻¹)
- **T₄:** (Control), Basal diet with water only

The ingredients and chemical composition of the basal diets are presented in Table 1. The diets were isoenergetic and isonitrogenous and formulated to meet the minimum nutrient requirements of broilers.

The observations viz. body weight, weight gain, mortality, feed efficiency, immune response, gut microbial load, carcass quality were recorded and economics was calculated.

Humoral immune response: The method of Siegel and Gross (1980) with slight modification was followed for assaying the immune response to Goat Red Blood Cells (GRBCs). To assess the humoral immune response the haemagglutination test (HAT) was conducted with GRBC in the

Table 1: Composition of basal diet fed to broilers

Parameters	Starter	Finisher
Ingredients (%)		
Maize	59.85	65.1
Soyaoil	1.0	2.4
Soya bean meal	30.3	24.0
Fishmeal (48% CP)	6.0	6.0
DCP	1.1	0.9
Calcite	0.25	0.2
Salt	0.23	0.2
DL methionine	0.18	0.13
L-Lysine HCl	0.13	0.08
Vitamin premix	0.1	0.1
Tracemineral mix	0.15	0.15
Toxin binder	0.1	0.1
Liver powder	0.1	0.1
Phytase	0.01	0.01
Non starch polysacharides	0.05	0.05
Antitoxin	0.1	0.1
Tylosin	0.05	0.05
Cholin chloride	0.1	0.1
Antioxin	0.05	0.05
Emulsifier	0.05	0.08
Acidifier	0.1	0.1
Total (g)	100.0	100.0
Chemical composition (g kg⁻¹ dry matter)		
Crude protein (%)	21.52	17.74
ME (kcal kg ⁻¹)	3090	3150
Lysine (%)	1.17	0.95
Methionine (%)	0.54	0.45

Vitamin mineral premix: 100 g Ultra Vite-M contains, Vit: A-4,00,000 IU, Vit B2: 0.1 g, Vit D3: 69,000 IU, Vit B12: 0.6 mg, Vit E: 30 IU, Vit K: 0.04 g, Niacinamide: 0.4 g, Calcium pantothenate: 0.1 g, Choline chloride: 12 g, Calcium: 30.4 g, Copper: 0.08 g, Iodine: 0.08 g, Iron: 0.8 g, Manganese: 2.2 g, Zinc: 2.08 g and Cobalt: 4 mg, 100 g Ultra Sil-TCF contains sodium aluminosilicate 95.25%, predigested protein: 20 ppm, Cobalt and Organic acid: 2 ppm, 100 g Ultra Phos: D3 contains Calcium 21.6 g, Phosphorus: 15.6 g, Vit: D3 12,000 IU, Vit: B12: 80 Mcg, Manganese: 0.80 mg, Zinc: 1040,100 g Ultra B12: F S contains Vit B12: 10 mg, Elemental cobalt 10 mg, Elemental calcium: 22.5% , Protein hydrolysate: 5 ppm, *Mineral mixture (TANUVAS) composition Calcium: 23%, Phosphorus: 12%, Magnesium: 6.5%, Iron: 0.5%, Iodine: 0.026%, Copper: 0.077%, Manganese: 0.12%, Cobalt: 0.012%, Zinc: 0.38%, Sulphur: 0.5%, Fluorine: 0.07 (max), Selenium: 0.3 ppm

experimental birds. The goat RBC was used as an antigen and 2% suspension was prepared in PBS (pH 7.2) and injected into wing vein of the birds at the rate of 0.25 mL per bird through intravenous route. The blood samples before the injection and at every week interval were taken from each bird from upto 3rd week of post inoculation. The sera samples were separated and were assessed for presence of anti GRBC antibodies by HA test. The HA test was carried out in 96 well U shaped microtitre plate. In all the wells 25 mL of PBS was added, then in the first well 25 mL of test sera was added and double fold dilution was made in the respective wells. Then 25 mL of freshly prepared 2% GRBC was added into all the wells and mixed properly. In the control wells only 25 mL of 2% GRBC was added. The plate was then incubated at 37°C for 1-2 h. The plate was read for formation of button formation and mat formation. The formation of the button formation was considered as negative. The titer was expressed as the log₂ of the reciprocal of the highest dilution giving visual agglutination (button formation) and the data were statistically analyzed.

Carcass yield (%): At the end of the experiment, broilers were slaughtered by humane method. The head, feet and giblets were removed and eviscerated carcass weight was taken and carcass yield percent was calculated.

Gut microbial load: Approximately 1 g of the small intestinal contents was mixed with 9 mL of sterilized NSS and homogenized. From the initial 10^{-1} dilution, 10-fold serial dilutions were subsequently made in sterile NSS and were diluted to 10^{-5} , 10^{-7} and 10^{-9} . For each dilution, 0.1 mL was inoculated in EMB and Mckownkey's agar plate for coliform load. The plates were incubated at 37°C for 24 h and the bacterial colonies were counted.

pH of the gut: pH of the crop, proventriculus and gizzard were measured at 4, 5 and 6th week of age of all the broilers.

Economics: Cost of production was calculated for broiler produced for various feeding treatments taking into consideration the prevailing market rates of commercial feed, chick cost and other miscellaneous cost.

Statistical analysis: Data was analyzed in a completely randomized design using the general linear models procedures of SAS (SAS., 1991).

RESULTS

Growth performance: The results of the body weight, weight gain, feed intake and feed efficiency are presented in Table 2. The noni group recorded significantly ($p < 0.05$) higher mean body weight (451.9±17.1 and 1434.5±60.5 g) and weight gain (402.5±18.4 and 1391±60.3 g) at 21 and 49 days, respectively which was comparable to control group. However, the combination of LAB+noni produced significantly lower body weight than *Morinda* group at the respective periods. The mean cumulative feed consumption was not significantly different among treatment groups till 21 days of age; whereas, control group recorded significantly ($p < 0.05$) highest feed consumption during latter period i.e., from 21-49 days and hence, the mean cumulative feed consumption was significantly ($p < 0.05$) higher with control group. Feed efficiency was significantly ($p < 0.05$) better (1.6±0.17) in noni group compared to the other groups.

Humoral immune response: The results of the humoral immune response revealed that the antibody titer values in noni+LAB group (0.73) was found to be significantly higher than control group (0.36) at 1 week Post Inoculation (PI) whereas the antibody titer in the noni and LAB group was 0.53, respectively (Table 3). The antibody titer reached its peak at 2nd week PI in noni+LAB and noni group.

Effect on gut microbial load: The result of the microbial load especially the coliform load in the different groups showed that feeding of combination of noni and LAB showed significant ($p < 0.05$)

Table 2: Effect of *Morinda* juice and noni+LAB on growth performance of broilers

Parameters	Experimental diets (Groups)				
	Period	Noni juice	LAB	Noni+LAB	Control
Body weight (g)	1-21 days*	451.90±17.1 ^a	383.50±18.6 ^b	423.00±16.9 ^b	457.10±19.8 ^a
	1-49 days*	1434.50±60.5 ^a	1250.10±41.6 ^b	1283.30±53.5 ^b	1412.50±40.8 ^a
Weight gain (g)	1-21 days**	402.50±18.4 ^a	333.50±16.4 ^{ab}	373.70±17.9 ^a	407.20±13.3 ^a
	1-49 days**	1391.00±60.3 ^a	1194.00±41.3 ^{ab}	1243.00±53.4 ^a	1367.00±40.4 ^a
Feed Intake (g)	1-21 days ^{NS}	593.20±3.95 ^a	574.50±3.77	592.30±3.90	829.90±6.16
	1-49 days*	2266.00±2.59 ^a	2237.00±3.16 ^a	2257.00±2.81 ^a	2491.00±2.22 ^b
Feed conversion ratio	1-21 days ^{NS}	1.30±0.20	1.50±0.25	1.40±0.20	1.80±0.33
	1-49 days*	1.60±0.17 ^a	1.78±0.26 ^b	1.76±0.36 ^b	1.79±0.46 ^b

LAB: *Lactobacillus acidophilus*, Mean follow by different letter are significant different, *Significant, **, Highly significant, NS: Non significant

reduction in the coliform load (Table 4) compared to control group. Feeding of LAB also showed reduction in the coliform load however, the synergistic effect of noni and LAB was found to be more compared to the other groups. Control group showed higher microbial load than other groups. The bacteria viz. *Enterobacter* sp. *E.coli* and *Salmonella* spp. were found in the gut.

pH of intestine: No significant changes were observed in pH of crop, proventriculas and gizzard in all the groups. The pH of crop of noni+LAB group was found lower at 5th week followed by noni, LAB and control group, respectively. The pH of proventriculas of noni group was found lower at 5th week followed by noni+LAB, LAB and control group, respectively. The pH of gizzard of noni was found lower at 5th week followed by LAB, control and noni+LAB, respectively.

Carcass traits: The effect of the dietary treatments on carcass yield is summarized in Table 5. No significant difference was observed in the dressing percentage among different groups of birds; however, all the treatment groups showed higher dressing percentage (62.5%) compared to control group (Table 6).

Economics: The lowest production cost per bird and feed cost efficiency for 1 kg b.wt., and the highest feed cost benefit efficiency of ₹ 5.39 for kg b.wt., was obtained in *Morinda* juice fed group.

Table 3: Humoral Immune response of *Morinda* juice and noni+LAB at different day of interval

Experimental diets (groups)	Post inoculation (Week)			
	0	1	2	3
<i>Morinda</i> juice	0.37	0.53	0.54	0.30
LAB	0.38	0.53	0.71	0.40
Noni+LAB	0.38	0.73	0.71	0.40
Control	0.35	0.36	0.47	0.60

Table 4: Efficacy of *Morinda* juice and noni+LAB on gut microbial load (CFU mL⁻¹)

Parameters	Experimental diets (Groups)			
	<i>Morinda</i> juice	LAB	Noni+LAB	Control
<i>Enterobacter</i> sp.	12.50×10 ⁶	7.75×10 ⁶	5.25×10 ⁶	15.43×10 ⁶
<i>E. coli</i>	14.50×10 ⁶	13.00×10 ⁶	5.00×10 ⁶	16.00×10 ⁶
<i>Salmonella</i> sp.	5.00×10 ⁶	2.50×10 ⁶	4.50×10 ⁶	16.67×10 ⁶

Table 5: Carcass quality characteristics influenced by *Morinda* juice and noni+LAB

Experimental diets (groups)	Parameters					
	Live weight (g)	Dressed (%) ^{NS}	Breast yield (%) ^{NS}	Back (%) ^{NS}	leg (%) ^{NS}	Giblets (%) ^{NS}
<i>Morinda</i> juice	1630.0	62.13±0.99	18.83±0.87	14.87±0.55	19.25±0.26	6.86±0.29
LAB	1500.0	62.99±1.89	16.64±0.83	13.38±0.89	18.83±0.45	6.89±0.38
Noni+LAB	1668.0	62.61±0.69	17.91±0.87	16.44±0.56	18.85±1.05	7.42±0.59
Control	1636.0	59.74±1.79	17.12±0.30	14.19±0.68	18.71±0.50	7.03±0.38

Ns: Not significant

Table 6: Feed cost efficiency of *Morinda* juice and noni+LAB

Economic details	Noni juice	LAB	Noni+LAB	Control
Chick cost (Rs)	25	25	25	25
Total feed consumed till market age (kg)	2.23	2.24	2.26	2.5
Feed cost (Rs.) at the rate of Rs (25 kg ⁻¹)	55.75	56	56.5	62.5
Production cost per broiler (Rs.)	80.75	81	81.5	87.5
Body weight (kg)	1.434	1.25	1.283	1.412
Production cost efficiency (Rs.)	56.31	64.8	63.52	67.28
Feed cost efficiency (Rs.)	38.9	44.8	44.0	44.3
Feed cost benefit efficiency (Rs.) over control	5.39	0.50	0.3	

DISCUSSION

Growth performance and immunity: The results of the body weight, weight gain, feed intake and feed efficiency are in agreement with the findings of Khaligh *et al.* (2011) and Sharifi *et al.* (2013), who used different medicinal plants extracts for growth performance in broiler. They have found that feeding of medicinal plant extracts improved growth performance and adding it to the diet could be an alternative to the use of antibiotics as growth promoters in poultry production. Feeding of noni juice as growth promoter has also been reported by Sunder *et al.* (2007, 2011a, b). The present study also supports the finding of the earlier studies. The lactobacillus group did not significantly influence the growth performance; where as in combination with noni showed better growth than LAB alone. The synergistic effect was observed in body weight and weight gain with combination of LAB and noni. The less growth and body weight in the lactobacillus group might be due to lower potency of the *Lactobacillus* strain used in the study; however, the study clearly indicated that use of noni in combination with lactobacillus enhanced the activity of the lactobacillus and has got synergistic effect. The *Morinda citrifolia* fruit is very rich in the nutraceutical compounds, amino acids, vitamins, minerals and coenzymes which might have played an important role in uptake of the nutrients in the gut (Singh *et al.*, 2008) and in turn directly or indirectly help in metabolism of the nutrients and help in overall growth of the cell and issues, hence the *Morinda* fed group might have utilized the feed efficiently and blended well with other growth promoter and did not loss its potency. The significant increase in humoral immunity with morinda groups may be attributed to the presence of several bioactive compounds in the *Morinda citrifolia* which might have influenced the immune response. The present study corroborated with the findings of earlier studies conducted on feeding of *Morinda citrifolia* to broiler and Nicobari fowl which also showed high humoral immunity (Sunder *et al.*, 2007, 2011a) with noni juice. They have reported the high B cell and T cell response in Nicoabri fowl, broiler and Japanese quail. The fruit is reported to contain various compounds like polysaccharide rich substance which are responsible for eliciting the immune response (Hirazumi and Furusawa, 1999). Report suggested that *Morinda citrifolia* is capable of stimulating the release of several mediators from murine effector cells IL-1, IL-10, IL-12, IFNs and nitric oxide.

Effect on gut microbial load and its pH: Significant reduction in the coliform load with feeding of noni is in agreement with the earlier studies conducted by Sunder *et al.* (2007, 2011a), where the effect of noni and *Lactobacillus* showed significant reduction in the microbial load in gut when fed alone. However, the present study showed the synergistic activity of noni juice along with LAB where both showed antimicrobial activity. Broad spectrum antimicrobial activity of *M. citrifolia* has also been reported earlier (Sunder *et al.*, 2007, 2011a, b). The present studies on the effect of noni juice on the microbial load are mainly due to the presence of compounds with wide range of biochemical properties. Reports suggested that the terpenoids compounds present in the fruit extract may be responsible for the antimicrobial activity (Narimani-Rad *et al.*, 2011). Some reports also claim that the activity is due to the presence of phenolic compounds such as acubin, alizarin, acopletin and other anthraquinones (Stef *et al.*, 2009). The effect of feeding of lactobacillus on the reduction of microbial load mainly the coliform load was also reported by Jin *et al.* (1998) and Salarmoini and Fooladi (2011). The *Lactobacillus* metabolizes the feed to produce lactic acid which in turn lower the pH of gut intestinal flora and inhibit the pathogenic organisms mainly the *Salmonella* and *E. coli*. It was observed the feeding of noni juice and LAB synergistically reduced the pH of the crop, proventriculus and gizzard, however, there was no significant changes observed

in terms of different weeks of age. The feeding of noni and lactobacillus showed that they lower the pH of the intestine which showed inhibitory activity to the microbial load. The lowering in the pH is mainly due to production of the lactic acid by the lactobacillus which showed synergistic effect when fed with noni juice.

Carcass traits: The effect of the dietary treatments on carcass yield is summarized in Table 5. No significant difference was observed in the dressing percentage among different groups of birds however, all the treatment groups showed higher dressing percentage (62.5%) compared to control group. The breast meat yield was higher in noni group than all other groups. The effects of feed additives used in this study were associated with growth stimulation, enhanced nutrient digestion and absorption, though this enhancement was not converted to carcass yield.

Economics: The study revealed that supplementation of *Morinda* juice supported the bird to convert feed efficiently so that its supplementation saved 10% of feed and in turn feed cost of ₹ 5.39 per bird could be saved.

The present study on *Morinda citrifolia* juice and *Lactobacillus* combination is the first report which showed the beneficial properties of the combination of *Morinda citrifolia* and lactobacillus. However, *Lactobacillus acidophilus* alone did not show good growth performance when compared to the *Morinda* group. However, the combination with noni juice lactobacillus showed the synergistic effect in terms of growth performance, immune response and lowering the gut microbial load.

CONCLUSION

It was concluded that the *Morinda citrifolia* juice in combination with lactobacillus combination showed improved growth performance, high immune response, low gut microbial load and feed cost efficiency in commercial broilers. Further, the combination of noni and lactobacillus might be promising alternatives for antibiotic growth promoters to improve the production of safety poultry products as pressure to eliminate antibiotic growth promoters in animal feed increases.

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