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Assessment of nutrient digestibility in broiler finisher ration supplemented with different levels of bael *(Aegle marmelos)* fruit powder and probiotics

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Abstract

A nutrient digestibility trial was conducted to evaluate the effect of supplementation of bael (Aegle marmelos) fruit powder and probiotics on the nutrient digestibility of finisher ration of broiler chickens. A total of 192-day-old Vencobb 430 Y broiler chicks were randomly divided into six dietary treatments (T1, T2, T3, T4, T5 and T6), each having four replicates with eight birds per replicate. The birds were fed with a basal diet (T1), basal diet + 0.1 per cent bael fruit powder (BFP) (T2), basal diet + 0.2 per cent BFP (T3), basal diet + 0.1 per cent BFP + 0.01 per cent L. plantarum (1 × 10^9 CFU/Kg diet) (T4), basal diet + 0.2 per cent BFP + 0.01 per cent L. plantarum (T5), and diet with 0.01 per cent L. plantarum (T6), respectively. All the birds were fed as per BIS (2007) recommendations. One bird from each group was selected randomly for the three-day metabolism trial conducted on the 42nd day of the experiment. The results of the study revealed that supplementation of bael fruit powder and probiotics at different combinations showed a significant (P<0.05) increase in ether extract and NFE digestibility compared to the control. The dietary treatments did not affect the dry matter, crude protein, crude fibre and organic matter digestibility in birds. Further, it was concluded that 0.1 per cent bael fruit powder supplementation in finisher ration showed better digestibility of nutrients in broiler chicken.

Keywords*: Bael fruit, L. plantarum, nutrient digestibility, broiler finisher ration*

The consumption of poultry meat in India has increased over the past few decades. The rise in poultry meat production and consumption can be attributed to several factors such as low cost, high-nutritional value and lower production cost compared to other red meat sources. Over 50 per cent of India's total meat production comes from the poultry sector (BAHS, 2023). The Indian Council of Medical Research (ICMR) recommends that an adult should consume 10.80 kilograms of poultry meat annually (ICMR, 2019). However, the nation's current consumption is only 2.20 kg per person, which is significantly lower than the recommended quantity (ICAR, Directorate of Poultry Science, 2023). India needs to increase the production of broilers to meet the enormous demand for chicken meat and its byproducts. In the present scenario, the search for alternatives to antibiotic growth promoters in the poultry sector is vital. In response, more researchers are focusing on utilising plant extracts or phytobiotics in poultry diets as alternatives to antibiotics.

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These biological products have been shown to be less toxic, residue-free and effective as growth promoters for birds.

The bael (*Aegle marmelos*) is a medicinal plant belonging to the *Rutaceae* family with the leaves, fruits, flowers, stem and roots being used to treat various human ailments. The bael tree is rich in amino acids, fatty acids, organic acids, minerals, carbohydrates, vitamins, fibres (Singh *et al*., 2012) and phytochemicals (Shakyawar *et al*., 2020). The bael fruit exhibits anti-bacterial, anti-hyperlipidemic, hepatoprotective, immunomodulatory and antioxidant effects (Aneesh *et al*., 2019 and Muley *et al*., 2021). The use of bael fruit (BF) and probiotics as poultry feed additive is less exploited, by the Indian broiler industry. Hence, this study is envisaged to assess the effect of supplementation of BF alone or in combination with probiotics on the apparent total nutrient digestibility of broiler finisher ration.

Materials and methods

The present research was conducted by the Department of Animal Nutrition, College of Veterinary and Animal Sciences, Pookode at Poultry Farm of Instructional Livestock Farm complex (ILFC), Pookode, Wayanad, Kerala.

The experimental protocols were approved by the Institutional Animal Ethics Committee of Kerala Veterinary and Animal Sciences University (Ref Number: IAEC/COVAS/PKD/22/5/2024)

Collection of study material

Bael fruits were collected from Janjgir - Champa district of Chhattisgarh state. After washing and cleaning, the fruits were chopped into small pieces followed by drying at 50ºC in a hot air oven. The dried fruits were ground to powder form by using mixer grinder in Department of Animal Nutrition, Pookode. *Lactobacillus plantarum* (LAB) was purchased from Exotic Mushroom, Maharashtra, India. The chemical composition of bael fruit was analysed as per AOAC (2016). The concentration of total phenolics in bael fruit was determined by the Folin-Ciocalteus (F-C) assay (Escarpa and Gonzales, 2001) with slight modifications.

Experimental animals, housing and management

One hundred and ninety-two, day old Vencobb 430Y broiler chicks were purchased from a local hatchery. They were randomly distributed to six groups (T1, T2, T3, T4, T5 and T6) with four replicates having eight chicks each. All the chicks were wing banded and weighed individually. The experimental pens were properly cleaned and disinfected with Kohrsolin-Th (Glutaraldehyde 10 g/100ml) at 10 ml/L of water one week prior to the commencement of the experiment. The feeder, waterer and other equipment were cleaned, disinfected with Kohrsolin-Th at the dilution rate of 5 ml/ L of water and sun-dried three days before the arrival of chicks. Chicks were grown in standard management conditions under deep litter system of management throughout the experimental period in the same shed. Rice husks were used as litter material and spread to a thickness of 5 cm in each pen. The chicks were brooded using incandescent bulbs till they attained three weeks of age. Thereafter, light was provided only during night hours. Clean and fresh drinking water was provided *ad libitum* daily. All the experimental chicks were vaccinated against Ranikhet disease and Infectious bursal disease.

Experimental ration of broiler chicken

The experimental rations were prepared as per BIS (2007) specifications. Group 1 chicks were fed with the basal ration. For the other groups, the basal ration was supplemented with BF @ 0.10 per cent (T2), BF @ 0.20 per cent (T3), BF @ 0.10 per cent + 0.01 per cent LAB (T4), BF @ 0.20 per cent + 0.01 LAB (T5) and 0.01 per cent LAB (T6) respectively. The level of supplementation followed in this study is presented in table 1.

Table 1. Experimental ration of broiler chicken

Treatment Group	Experimental Diet
T1	Basal diet
T2	Basal diet + BF @ 0.10%
T3	Basal diet + BF @ 0.2 0%
T4	Basal diet + BF @ 0.10% + 0.01% LAB
T5	Basal diet + BF @ 0.20% + 0.01 % LAB
T6	Basal diet + 0.01% LAB

The birds were fed with pre-starter feed from day one to 7th day, starter feed from 8th to 21st day and finisher feed from 22nd to 42nd day. The ingredient composition (% as fed basis) of finisher ration for experimental birds are presented in table 2. The chemical composition of broiler finisher feed was analysed as per AOAC (2016).

Nutrient digestibility trial

A metabolism trial was conducted for three consecutive days at the end of 6th week (42nd day) of the experiment by taking one bird from each replicate randomly and caged individually. They were fed with respective treatment diets. An adaptation period was given for two days followed by three days collection period. During the metabolism trial, quantities of daily feed offered, droppings voided and residue left were recorded. Representative samples of feed offered were taken daily during the trial period for proximate analysis. The dry matter content of the feed offered as well as residue was determined daily. All the excreta from each bird were collected on a polythene sheet over a period of 24 hours and excreta were processed for dry matter content. A representative

Table 2. Ingredient composition of broiler finisher feed (%)

Ingredient	G1	G2	G3	G4	G5	G6
Maize	59.00	59.00	59.00	59.00	59.00	59.00
SBM	31.84	31.84	31.84	31.84	31.84	31.84
DCP	2.00	2.00	2.00	2.00	2.00	2.00
Calcite	1.02	1.02	1.02	1.02	1.02	1.02
Salt	0.38	0.38	0.38	0.38	0.38	0.38
Vegetable oil	5.76	5.76	5.76	5.76	5.76	5.76
Total	100	100	100	100	100	100
Trace Mineral Mix[1]	0.10	0.10	0.10	0.10	0.10	0.10
Vitamin Premix[5]	0.08	0.08	0.08	0.08	0.08	0.08
Lysine[1]	0.06	0.06	0.06	0.06	0.06	0.06
Methionine[2]	0.20	0.20	0.20	0.20	0.20	0.20
Choline[3]	0.10	0.10	0.10	0.10	0.10	0.10
Toxin Binder[6]	0.10	0.10	0.10	0.10	0.10	0.10
Liver powder[7]	0.03	0.03	0.03	0.03	0.03	0.03
Coccidiostat[8]	0.05	0.05	0.05	0.05	0.05	0.05
Bael Fruit Powder	0.00	0.10	0.20	0.10	0.20	0.00
Probiotic *Lactobacillu plantarum*	0.00	0.00	0.00	0.01	0.01	0.01
Calculated Analytical Values						
Metabolizable Energy (Kcal/kg)	3200	3200	3200	3200	3200	3200
Crude Protein (%)	20.00	20.00	20.00	20.00	20.00	20.00
Calcium (%)	1.00	1.00	1.00	1.00	1.00	1.00
Available Phosphorus (%)	0.45	0.45	0.45	0.45	0.45	0.45

sample (1/10th) of excreta was taken for nitrogen estimation in 10 per cent sulphuric acid solution to avoid nitrogen loss. The dried samples of three consecutive days were pooled and then thoroughly mixed, dried, powdered and used for proximate analysis as per AOAC (2016) and the digestibility coefficient (%) of nutrients were calculated.

Statistical analysis

The experimental data collected on nutrient digestibility parameters were analysed statistically for analysis of variance by One-way ANOVA for linear terms as per Snedecor and Cochran (1994). The means of other data were compared for their significance at a 99.5 per cent confidence level by Duncan's multiple range tests using the General Linear Model (GLM) of multivariate in the statistical package IBM SPSS version 24.0

Results and discussion

Chemical composition of bael fruit

The per cent chemical composition of bael fruit on dry matter basis used for the preparation of experimental ration is given in table 3. The analysis of the chemical composition of bael fruit powder revealed that it had 22.64 per cent dry matter (DM), 8.55 per cent crude protein (CP), 3.42 per cent crude fibre (CF), 0.87 per cent ether extract (EE), 3.28 per cent total ash, 83.89 per cent nitrogen free extract (NFE), 0.70 per cent acid insoluble ash, 0.58 per cent calcium (Ca), 0.38 per cent phosphorus (P), 9.61 per cent acid detergent fibre (ADF), 12.56 per cent neutral detergent fibre (NDF) and 150.02 total phenolics content (mg of tannic acid/100g) on dry matter basis.

The chemical composition of bael fruit was comparable with the study by Sarkar *et al*. (2021), who reported premature bael fruit contained 8.81 percent protein, 1.04 percent fat and 3.50 percent ash. The calcium content observed in the present study was higher compared to the findings of Sarkar *et al*. (2021), who reported a calcium level of 146.49 mg/100 g. Singh *et al*. (2012) reported that the bael fruit pulp contains 61.6 g/100g moisture, 4.7 g/100g CP, 0.5 g/100g EE 6.5 g/100g CF, 2.7 g/100g ash, 12.00 g/100g NFE and 12.00 g/100g ADF on dry matter basis.

Sawale *et al*. (2018) found that the nutrient content in bael fruit powder were 62.04 per cent moisture, 1.57 per cent CP, 3.07 per cent CF, 0.39 per cent EE and 1.7 per cent ash. Biswas *et al.* (2023) reported that the chemical analysis of bael fruit pulp has 61.20 per cent moisture, 2.48 per cent CP, 3.04 per cent CF, 0.47 per cent EE and 1.29 per cent total ash. Ullikashi *et al*. (2017) reported that the 100 g edible portion of bael fruit contains 85 mg calcium and 50 mg phosphorus. The variation may be due to the different environmental conditions, *viz*. salinity, soil composition, pH, the medium of cultivation and stage of maturity of bael fruit.

Chemical composition of broiler finisher ration

The per cent chemical composition broiler finisher ration on dry matter basis used for the preparation of experimental ration are given in table 4. The per cent of dry matter (DM), crude protein (CP), crude fibre (CF), ether extract (EE), total ash (TA), acid insoluble ash (AIA) and nitrogen free extract (NFE) in finisher ration were 89.05 to 89.10, 20.14 to 20.20, 4.46 to 4.49, 8.17 to 8.32, 7.35 to 7.58, 1.11 to 1.22, 59.58 to 59.84, respectively. It was in accordance with BIS, (2007) specifications.

Table 3. Chemical composition of bael fruit

Parameters	Chemical composition (%, on DM basis)
DM	22.64 ± 0.28
CP	8.55 ± 0.53
CF	3.42 ± 0.01
EE	0.87 ± 0.01
Total ash	3.28 ± 0.01
NFE	83.89 ± 0.52
Acid insoluble ash	0.70 ± 0.07
NDF	12.56 ± 0.33
ADF	9.61 ± 0.61
Ca	0.58 ± 0.03
P	0.38 ± 0.01
Total phenolics content (mg of tannic acid/g)	150.02 ± 6.23

*Average of four values with SE

Digestibility of nutrients

The chemical composition of droppings voided by the experimental broilers on six dietary treatments T1, T2, T3, T4, T5 and T6 during the three days of the metabolism trial are presented in table 5. The DM, CP, CF, EE, TA, AIA NFE and OM varied from 20.74 to 24.47, 10.79 to 12.08, 10.65 to 12.35, 5.62 to 7.05, 16.74 to 18.03, 3.44 to 4.00, 51.93 to 54.62 and 81.97 to 83.27 per cent on dry matter basis, respectively.

The digestibility coefficient of nutrients is given in table 6. The per cent digestibility of nutrients in finisher feed fed to broilers belonging to six groups T1, T2, T3, T4, T5 and T6 were 71.69, 72.72, 71.82, 73.47, 71.21 and 73.32 for dry matter, 82.18, 84.13, 84.76, 85.34, 85.02 and 84.69 for crude protein, 29.85, 26.36, 27.73, 31.52, 24.13 and 35.56 for crude fibre, 75.32, 81.88, 75.56, 80.14, 78.61 and 80.80 for ether extract, 73.62, 77.03, 74.37, 75.51, 74.02 and 75.49 for nitrogen free extract, 73.53, 76.58, 74.52,

Table 4. Chemical composition of finisher* broiler ration (%, on DM basis)

Parameters	T1	T2	T3	T4	T5	T6
DM	89.06 ± 0.15	89.06 ± 0.42	89.10 ± 0.22	89.07 ± 0.42	89.09 ± 0.25	89.05 ± 0.19
CP	20.15 ± 0.11	20.14 ± 0.38	20.18 ± 0.11	20.18 ± 0.43	20.20 ± 0.10	20.15 ± 0.09
CF	4.48 ± 0.14	4.47 ± 0.02	4.49 ± 0.16	4.47 ± 0.18	4.46 ± 0.18	4.49 ± 0.19
EE	8.27 ± 0.19	8.17 ± 0.28	8.18 ± 0.08	8.17 ± 0.09	8.20 ± 0.10	8.32 ± 0.14
Total ash	7.35 ± 0.13	7.39 ± 0.19	7.50 ± 0.18	7.57 ± 0.16	7.58 ± 0.22	7.36 ± 0.12
Acid insoluble ash	1.11 ± 0.11	1.16 ± 0.07	1.15 ± 0.07	1.16 ± 0.10	1.22 ± 0.09	1.11 ± 0.06
NFE	59.75 ± 0.33	59.84 ± 0.28	59.66 ± 0.22	59.61 ± 0.59	59.58 ± 0.41	59.67 ± 0.36
OM	92.65 ± 0.13	92.61 ± 0.19	92.50 ± 0.18	92.43 ± 0.16	92.43 ± 0.22	92.64 ± 0.12
Ca	1.09 ± 0.03	1.08 ± 0.02	1.08 ± 0.12	1.04 ± 0.10	1.16 ± 0.08	1.12 ± 0.04
P	0.78 ± 0.02	0.78 ± 0.02	0.81 ± 0.02	0.81 ± 0.02	0.82 ± 0.02	0.79 ± 0.02

* Average of four values with SE

Table 5. Chemical composition of excreta of chicks in six treatment groups (% on DM basis)

Parameters	T1	T2	T3	T4	T5	T6
DM	23.56 ± 1.28	24.04 ± 1.32	24.47 ± 0.44	20.74 ± 1.11	23.94 ± 1.90	22.94 ± 2.04
CP	12.05 ± 0.70	12.08 ± 0.78	10.82 ± 0.54	11.06 ± 0.32	10.79 ± 0.20	11.41 ± 0.73
CF	10.54 ± 0.23	12.35 ± 0.08	11.20 ± 0.44	11.17 ± 0.50	11.84 ± 0.13	10.65 ± 0.20
EE	6.89 ± 0.44	5.62 ± 0.25	7.05 ± 0.35	6.04 ± 0.48	6.13 ± 0.43	5.85 ± 0.14
Total ash	17.03 ± 0.29	18.03 ± 0.10	17.40 ± 0.03	17.71 ± 0.51	16.74 ± 0.50	17.47 ± 0.23
Acid insoluble ash	3.58 ± 0.02	4.00 ± 0.03	3.44 ± 0.01	3.54 ± 0.03	3.60 ± 0.03	3.56 ± 0.24
NFE	53.49 ± 0.75	51.93 ± 1.01	53.53 ± 0.92	54.02 ± 0.85	54.51 ± 0.80	54.62 ± 1.13
OM	82.97 ± 0.29	81.97 ± 0.10	82.60 ± 0.03	82.30 ± 0.51	83.27 ± 0.50	82.54 ± 0.23

* Average of four values with SE

Table 6. Digestibility coefficient of nutrients in broilers having six treatment groups, (%)

Parameters %	T1	T2	T3	T4	T5	T6	F-value (P-value)
DM	71.69 ± 2.02	72.72 ± 1.09	71.82 ± 1.29	73.47 ± 1.16	71.21 ± 1.18	73.32 ± 2.62	0.316^{ns} (0.897)
CP	82.18 ± 1.80	84.13 ± 1.38	84.76 ± 0.98	85.34 ± 0.63	85.02 ± 0.69	84.69 ± 1.38	0.877^{ns} (0.516)
CF	29.85 ± 3.99	26.36 ± 2.92	27.73 ± 4.47	31.52 ± 5.13	24.13 ± 2.61	35.56 ± 2.97	1.143^{ns} (0.374)
EE	75.32^{b} ± 1.29	81.88^{a} ± 1.15	75.56^{b} ± 0.67	80.14^{a} ± 1.60	78.61^{ab} ± 2.18	80.80^{a} ± 1.08	3.819* (0.016)
NFE	73.62^{b} ± 0.82	77.03^{a} ± 0.77	74.37^{b} ± 0.94	75.51^{ab} ± 0.40	74.02^{b} ± 0.71	75.49^{ab} ± 0.39	3.172* (0.032)
OM	73.53 ± 1.09	76.58 ± 0.86	74.52 ± 0.84	75.99 ± 0.62	74.47 ± 0.89	76.06 ± 0.69	1.965^{ns} (0.133)

* Significant at 0.05 level (P<0.05); ns non-significant. Means having different letters as superscripts differ significantly within a row.

75.99, 74.47 and 76.06 for organic matter, respectively on dry matter basis. The data on the digestibility coefficient of nutrients revealed that there was a significant (p<0.05) difference among treatments, related to the digestibility of EE and NFE. Improved EE and NFE digestibility enhance energy and nutrient absorption, leading to better growth, feed efficiency and overall production performance. However, the digestibility of DM, OM, CP and CF were similar (p>0.05) among six treatment groups.

In the present study, T2, T4 and T6 had the significantly higher (p<0.05) digestibility of EE whereas T1 and T3 were similar among the groups. The T5 group also showed improved digestibility. The birds in group T2 had the highest (p<0.05) NFE digestibility in the current study, followed by T4 and T6, with T1, T3, and T5 having similar digestibility.

The presence of active chemicals in bael fruit that supports microbial balance and greater enzyme activity, which improves nutrient absorption and digestion, might be the source of the enhanced digestibility coefficient of EE and NFE. Similarly, probiotic supplementation increases the digestive process and absorption of nutrients by stimulating the digestive system to secrete natural enzymes. Probiotics support to maintain the integrity of the intestinal barrier by promoting the production of mucus and tightening junctions between epithelial cells. This ensures optimal nutrient absorption while reducing the risk of gut permeability, which can lead to nutrient loss.

According to Sobolewska *et al*. (2017), use of synbiotics which improved nutrient absorption, significantly altered gut structure. Similar results were obtained by Ambili (2022), who reported that dried and steam-treated banana inflorescence supplementation to broilers at 5 g/Kg significantly (P<0.001) increased the digestibility of ether extract and nitrogen-free extract. Moreover, the digestibility of crude protein was non-significant among the treatment groups. Dinsha (2023) also found that the supplementation of essential oil from *Glycyrrhiza glabra* at 200 mg/Kg significantly increased the digestibility of NFE compared to other groups. Similar findings were previously reported by Sampath *et al*. (2021), who stated that the *L. plantarum* supplementation at 0.1 per cent to broilers showed no significant (P>0.05) difference in the nutrient digestibility of dry matter and nitrogen. Elangia *et al.* (2024) also reported that there were no notable distinctions in the ileal digestibility of DM, CP, CF and organic matter by feeding *L. plantarum* to broilers at 0.6 per cent level in finisher ration.

In contrast to the current study, Hidayat *et al.* (2021) found that a combination of probiotics (*Lactobacillus acidophilus*) and phytobiotics (garlic, onion and bay leaves) at 1.2 mL and 2 per cent, 1.2 mL and 4 per cent, or 1.2 mL and 6 per cent for probiotics and phytobiotics, respectively, increased ileal protein digestibility compared to the control group.

The combination of phytobiotics and probiotics have a synergistic effect, enhancing gut health and nutrient digestibility more effectively than either additive alone. Phytobiotics may also enhance the survival and colonization of probiotics in the gut, further improving digestive efficiency.

Conclusion

The overall critical evaluation of the results from the present study indicated that the birds supplemented with bael fruit powder alone or in combination with probiotics had improved EE and NFE digestibility compared to the control group, which could help in better nutrient absorption and growth in broiler chickens. Although phytobiotics and probiotics are thought to be superior substitutes, more research is still needed on a number of aspects such as optimal dosages, complex composition, stability, weather, harvesting period, extraction method, storage conditions, antagonistic effects, anti-nutritional factors, and microbial contamination.

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Conflict of interest

The authors declare that they have no conflict of interest.

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