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A new strategy for improvement of tamarind seed based chicken diet after microbial detannification and assessment of its safety aspects

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ABSTRACT Tamarind seed, an abundant agricultural waste, contains a large amount of naturally occurring anti-nutrient, tannin. This study aimed at evaluating the detannification efficiency of tannase producing *Bacillus subtilis* PAB2 in comparison with enzymatic and conventional physical techniques, and assessment of its health promoting effect in chicken model. Microbial fermentation resulted in lowering of 80.8% tannin, whereas enzymatic and physical treatment removed only 48 and 28.6%, respectively. To assess the health beneficial effects, experimental schedule for poultry chicken model was designed viz., (T1) commercial food, (T2) T1+tamarind seeds, (T3) T1+enzymatic detannified seeds and (T4) T1+ fermented seeds. It was found that physiological indices of chicken in T4 group improved more than T2, but those were comparable with T1 and T3. The level of total plasma protein, albumin and other haematological parameters also significantly ($P<0.05$) improved in T4 more than T2. The quantities of small intestinal indicator microbes like *Escherichia coli*, *Clostridium perfringens* and *Yeast sp.* in T4 group increased more than T2. Thus, fermentation led to quality improvement of raw tamarind seed which suggested its employment as an alternative feedstock for poultry chicken.

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KEY WORDS

Tamarind seed
Detannification
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Introduction

Global consumption of poultry products, especially poultry meat, has been consistently increasing over the years, and this trend is expected to be continued in developing countries. Poultry production is usually associated with large investment in poultry feed. Due to commercialization, there is a need to get better yield within a reasonable period which demands formulation of cost effective, balanced diet that can supply nutrient and energy together. In this respect, traditional feed ingredients like maize, soybean meal, fish meal and meat meal are very popular, but the costs of these are increasing day by day. Since traditional food source is very limited, search for non-traditional foodstuff is very essential which would be locally available, cheap, safe and nutritious.

Till now, several reports have asserted on the possible implementation of sorghum (Rodgers et al. 2012), mango kernel (Diarra and Usman 2008), shea nuts (Dei et al. 2008a), chest nut (Jamroz et al. 2009), cassava (Zanu and Dei 2011), etc. as poultry feed. Although, these alternatives are potential, their

uses are restricted because of their scarcity as raw materials and for other technical and socio-economic reasons. In India, the seed of tamarind (*Tamarindus indica* L.) is an abundant agro-industrial by-product. It is rich source of carbohydrate (50-57%), protein (13.3-26.9%), fat (4.5-16.2%) and also essential minerals such as Ca, P, Mg and K (Kumar and Bhattacharya 2008) and amino acids (Shankaracharya 1998) including lysine. In the nutritional point of view, tamarind seed has immense potential to be used as feed for animal. Whereas, commercial poultry feed contain 19%, 7% and 2.5% of protein, fat and fiber respectively. So, on nutritional point of view, supplementation of poultry feed with tamarind seeds may open a new opportunity for profitable poultry farming in a cost-effective way. But the major bottleneck of this product is the presence of high quantity of tannin as a major anti-nutrient. Tannin in high quantity also lowers the nutrient availability, inhibits enzymatic reaction and causes indigestion that retards the growth of chick (Teguia 1995).

But the feed value and taste of a tannin rich material can be improved by various processes of detannification viz., physical, chemical and biological (Makkar 2003). Though many attempts have been made to reduce the tannin content in different forages by physical treatments such as drying,

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wilting and boiling (Makkar and Becker 1996; Ben-Salem et al. 2005) or chemical treatment with urea, calcium hydroxide, hydrochloric acid, ammonium hydroxide and wood ash (Price et al. 1979; Waichungo and Holt 1995; Vitti et al. 2005) but scant attention has been paid to the degradation of tannins by microbial process. Therefore, the present investigation has been performed to focus on the detannification of tamarind seed by physical, enzymatic and microbial cell mediated pretreatment. Then the detannified tamarind seed has been exploited as effective poultry diet. Parameters like physical growth performance, carcass and organ weight, hematological and serum biochemical components, microbial load etc. have been considered for this study.

Materials and Methods

Collection of tamarind seed and estimation of tannin

Tamarind seeds were collected from local resources of forest area of West Midnapore, West Bengal, India and dried in hot-air oven at 60 °C for 24 h. Different experimental sets were designed each with whole seeds, testa, kernel, broken seed etc. to find out the total tannin content. After overnight soaking followed by boiling of seed in water, filtrate obtained was used as source of crude tannin. Tannin content was estimated by Folin-Denis method (Schanderi 1970) with commercial tannic acid (Sigma, India) as a standard.

Detannification of tamarind seeds by physical treatment

Tamarind seeds were broken (particle size: ~1 mm × 2 mm) and soaked in water 1:10 (w/v) for overnight and boiled for 10 min. After soaking and shaking, the seed kernel was separated by scrubbing followed by wire net filtration and considered as pre-treated tamarind seed. Tannin content in the filtrate was measured by the Folin-Denis method (Schanderi 1970).

Detannification of tamarind seed by microbes

Five grams of pretreated and raw tamarind seed were taken separately and moistened with 5 ml of mineral salt solution (% w/v: NH₄Cl, 3.0; MgSO₄, 0.1 and mannose, 0.1) at pH 5.0 were taken in 250 ml Erlenmeyer flasks. The contents were autoclaved and inoculated with 1 ml (~3.2 × 10⁴ cells) of *Bacillus subtilis* PAB2 (GenBank accession number-HM853662) and allowed for 72 h fermentation at 35 °C. The contents were mixed thoroughly with distilled water (1:6 w/v) and incubated at 35 °C for 1 h. The residual tannin content

of pretreated tamarind seed was determined before and after fermentation.

Detannification of tamarind seed by enzymatic treatment

Tannase produced by *B. subtilis* PAB2 during fermentation was subjected to ammonium sulphate precipitation, dialysed and freeze-dried and used as partial purified enzyme for detannification. Five grams of pretreated seed were mixed with 10 ml of tannase (456.24 U/mg) and incubated at 40 °C for 72 hour. The degree of detannification was determined by assaying the tannin before and after the enzymatic treatment.

Birds and housing

A total of ten day-old 24 male broiler chicks (Star Boro) with average body weight (350 ± 5 g) were purchased from a commercial hatchery and housed according to the recommendations of Vidyasagar University (West Bengal, India) Guide for the Care and Use of Laboratory Animals (Recommendation No. VU/R/776/12 dt. 26.7.12). Chicks were randomly divided into four groups (6 chicks in each) and kept in separate well-ventilated wire cages (80 cm × 50 cm × 63 cm) to avoid the danger of suffocation. The commercially marketed feed was used and the birds were acclimatized in laboratory environment having temperature 28 ± 2 °C, light-dark cycle of 16: 8 h. Feed and water was offered for *ad libitum* consumption.

Animal treatment and feed

According to the mode of feeding, groups were numbered as T1 (treatment group 1), T2 (treatment group 2), T3 (treatment group 3) and T4 (treatment group 4). Group T1 was maintained as control and was fed only with commercial broiler diet throughout the experimental period. Group T2, T3 and T4 were treated with mixture (1:1) of commercial feed and raw tamarind seed, tannase treated tamarind seed and fermented tamarind seed, respectively.

Analysis of physical parameters

Prior to segregation, initial body weight of each bird was recorded. Final body weight (FBW), body weight gain (BWG), average feed intake (AFI), gain to feed ratio (GFR) and mortality were recorded throughout the entire experiment. After 30 days of experiment, the birds were sacrificed by cervical dislocation. For the carcass measurements, the birds were randomly selected and weighed individually before slaughter. The slaughter weight (SW) and plucked weight (PW) were recorded. The dressing percentages were calculated and cut-up

Table 1. Estimation of tannin content of tamarind seed, kernel (cotyledon) and testa (seed coat).

| | No. of seeds | Weight (g) | Average weight of seed (g) | Amount of tannin content (g) | | Percentage of tannin (%) |
|-------------|--------------|------------|----------------------------|------------------------------|-------------------|--------------------------|
| | | | | Soaking | Soaking + boiling | |
| Whole seed | 50 | 33.58 | 0.67 | 0.11 | 0.44 | 1.31 |
| Seed kernel | 50 | 24.00 | 0.48 | 0.01 | 0.04 | 0.17 |
| Testa | - | 9.00 | 0.18 | 0.20 | 0.37 | 3.93 |

parts (head, neck, wings, thighs, breast, back) measurements were carried out and expressed as percentages of the SW.

Haematological and biochemical data collection

Blood samples were collected from the brachial vein in heparinized tubes prior to slaughtering and centrifuged at 3000 g for 5 minutes. The serum samples are collected and then stored in the screw capped serum vial and preserved at -20 °C for further analysis. The haematological parameters like total red blood cell (RBC), packed cell volume (PCV), haemoglobin (Hb) and white blood cell (WBC) were measured. Different biochemical parameters like total protein, albumin, globulin, sodium, potassium, calcium, chlorides, urea, creatine and cholesterol were also assessed.

Microbiological data collection

The define part (2-3 cm) of intestine (small and large) was excised out and the luminal contents were suspended in normal saline (0.9% w/v NaCl) and preserved at 4 °C till analysis. Standard pour plate technique was adopted to enumerate the different microbial population. Selective media (HiMedia, India) were used and indicator microbial group detected following the protocol set out in the HiMedia Manual (www.himedialabs.com). Different bacterial populations like total aerobes and anaerobes were cultured on single-strength trypticase soya agar (TSA, HiMedia, India) and reduced Wilkins Chalgren agar (WCA, Micromaster, India) with G.N. Spore anaerobic supplement (sodium succinate, hemin, vancomycin, menadione and nalidixic acid). *Bifidobacterium spp.*, *Bacteroidetes spp.* and *Lactobacillus spp.* were estimated respectively on bifidobacterium, bacteroids and man, rogosa and sharp agar (MRS, HiMedia, India). *Clostridium perfringens* was cultured on perfringens agar media supplemented with sodium sulphadiazine (100 mg/L). Population density of *Escherichia coli* was estimated by using Mac-Conkey agar (HiMedia, India). Yeast mould agar (HiMedia, India) was used for the cultivation of yeast. The anaerobic condition was maintained in the CO₂ incubator (Heal force Air jacket, HF 151 UV), filled with 10% CO₂ and H₂ for the cultivation of anaerobic bacteria. Population density was transformed into 10 base of log value and expressed as log₁₀ CFU/ g of intestinal

content before statistical analysis.

Gas formation ability

Faecal aliquot of 50 µl was added in 20 ml of Mac-Conkey broth and volume of the gas was measured by volumetric Durham's tube after 24 h incubation at 37 °C. Gas formation ability was expressed in ml/g of wet intestinal content and acid formation by composite microflora was expressed in change of colour of Mac-Conkey broth.

Study of some selective enzyme production ability in bacterial population

Amylolytic, proteinolytic, tanninolytic and phosphate-solubilizing bacteria were enumerated on nutrient agar supplemented with 1% (w/v) of starch, casein, tannic acid and tricalcium phosphate respectively. After incubation at 37 °C for 48 h the amylolytic population was detected by formation of clear zone around the colony, after flooding with potassium iodide 10% (w/v) whereas hollow zone formation with 10% (w/v) trichloro acetic acid denoted the proteinolytic bacteria. Tannase and phosphatase producers were counted by clear zone formation on respective plates.

Statistical analysis

All data obtained were evaluated statistically by one or two-factorial ANOVA using Sigma Plot (V. 11.0) statistical software.

Results

Extraction and estimation of tannin in tamarind seed

The tannin contents in different parts of the tamarind seed were analyzed and represented in Table 1. Extraction of tannin was much higher in soaking-boiling than only soaking technique. The recorded tannin contents in whole tamarind seed, seed kernel and testa were as 1.31 (g%), 0.175 (g%) and 3.93 (g%) respectively.

Table 2. Effect of tamarind seed based diet on growth performance, carcass and organ of broiler chickens after 30 days treatment.

| Parameters | Treatment group | | | | RSD | P value |
|--|----------------------|---------------------|---------------------|----------------------|------|---------|
| | T1 | T2 | T3 | T4 | | |
| Initial body weight (g) | 353.83 ^{bc} | 368.00 ^a | 357.50 ^c | 361.20 ^{bc} | 1.38 | <0.05 |
| Final body weight (g) | 1216.33 ^a | 762.00 ^d | 906.00 ^c | 1139.00 ^b | 0.55 | <0.05 |
| Body weight gain/day | 28.75 ^a | 13.13 ^c | 18.28 ^{bc} | 25.92 ^{ab} | 1.24 | <0.05 |
| Average feed intake/day/bird | 151.78 ^a | 90.47 ^d | 110.75 ^c | 130.97 ^b | 1.26 | <0.05 |
| Gain to feed ratio | 0.18 ^a | 0.14 ^b | 0.16 ^c | 0.19 ^a | 1.80 | <0.05 |
| Slaughter weight | 1124.00 ^a | 682.85 ^d | 821.86 ^c | 1072.71 ^b | 0.50 | <0.05 |
| Plucked weight | 1016.46 ^b | 551.82 ^d | 771.99 ^c | 1045.48 ^a | 1.09 | <0.05 |
| Cut-up parts as percentage of slaughter weight (%) | | | | | | |
| Head | 2.21 ^a | 2.20 ^{ab} | 2.17 ^{ab} | 1.70 ^c | 1.31 | <0.05 |
| Neck | 5.10 ^a | 4.61 ^b | 4.40 ^b | 4.61 ^b | 3.61 | <0.05 |
| Wings | 6.75 ^a | 6.46 ^b | 6.11 ^c | 6.48 ^b | 3.34 | <0.05 |
| Thighs | 15.44 ^a | 12.61 ^d | 13.57 ^b | 13.13 ^c | 2.19 | <0.05 |
| Breast | 19.55 ^a | 16.11 ^d | 16.91 ^c | 18.79 ^b | 1.79 | <0.05 |
| Back | 10.65 ^a | 9.87 ^b | 9.54 ^c | 9.42 ^c | 2.04 | <0.05 |

Values within row followed by upper case letter (a, b, c, d) are significantly different. T1, T2, T3 and T4 represents treatment groups 1 to 4 respectively.

Table 3. Effect of tamarind seed based diet on haematological components of broiler chickens after 30 days treatment.

| Parameters | Treatment group | | | | RSD | P value |
|--|--------------------|--------------------|--------------------|-------------------|------|---------|
| | T1 | T2 | T3 | T4 | | |
| RBC count (x10 ⁹ /mm ³) | 2.72 ^b | 1.65 ^c | 2.83 ^a | 2.78 ^b | 2.51 | <0.05 |
| PCV (%) | 30.98 ^c | 25.53 ^d | 31.21 ^a | 31.1 ^b | | |
| Hb conc. (g/dl) | 4.59 ^a | 2.84 ^c | 4.47 ^a | 4.06 ^b | 2.26 | <0.05 |
| WBC Count (x10 ³ /mm ³) | 4.16 ^a | 3.83 ^b | 4.16 ^a | 4.14 ^a | 1.51 | <0.05 |
| Differential Counts | | | | | | |
| Monocytes (%) | 13.00 | 7.33 | 10.65 | 12.34 | | |
| Lymphocytes (%) | 49.00 | 59.00 | 52.00 | 47.00 | | |
| Basophils (%) | 0 | 0 | 0 | 0 | | |
| Neutrophils (%) | 37.00 | 46.4 | 36.78 | 38.00 | | |
| Eosinophils (%) | 8.00 | 7.00 | 5.00 | 7.56 | | |

Values within row followed by upper case letter (a, b, c, d) are significantly different. T1, T2, T3 and T4 represents treatment groups 1 to 4 respectively.

Detannification of tamarind seed

Detannification of tamarind seed was performed by various processes and found that physical treatment could remove 28.6% of tannin from raw tamarind seed. Detannification of pretreated tamarind seed by whole cell (*B. subtilis* PAB2) fermentation resulted in 68% removal of tannin after 72 h of fermentation. Direct fermentation of raw tamarind seed (without any pretreatment) by PAB2 could remove 76.24% of tannin after 72 h of fermentation and upgrade up to 80.8% after 120 h. Enzymatic detannification of pretreated tamarind seed with partial purified tannase reduced the tannin level of 0.020 g (60% removal) from tamarind seed after 72 h of incubation at 40 °C.

Physical growth parameter analysis

The comparison of the physical growth parameters of chicken of different groups (T1, T2, T3 and T4) were studied (Table 2) as per experimental design. It was noted that FBW, BWG, AFI and GFR values were significantly different from each other. Tamarind seed (untreated) as diet showed decreased body weight at experimental trial. FBW (1139 g), BWG (25.92 g/day), AFI (130.97 g/day) and GFR (0.19) values were comparatively higher in T4 fed group than other groups, except control (T1). It was also observed that feed intake capacity was affected vastly among the treated groups (Table 2). Six birds receiving the tamarind seed diet (90.47 g/day) consumed less amount of feed (P<0.05) than other groups. There was no mortality observed during the experimental period.

Table 4. Effect of tamarind seed based diet on serum biochemical indices of broiler chickens after 30 days treatment.

| Parameters | Treatment group | | | | RSD | P value |
|----------------------|--------------------|--------------------|--------------------|--------------------|------|---------|
| | T1 | T2 | T3 | T4 | | |
| Total protein (g/dl) | 15.73 ^a | 11.64 ^c | 13.64 ^b | 15.77 ^a | 6.83 | <0.05 |
| Albumin (g/dl) | 13.76 ^a | 7.85 ^d | 10.57 ^c | 12.81 ^b | 4.45 | <0.05 |
| Globulin (g/dl) | 2.70 ^{bd} | 4.15 ^a | 3.15 ^{bc} | 3.41 ^c | 1.87 | <0.05 |
| Sodium (mmol/L) | 151.08 | 152.38 | 152.06 | 152.30 | 0.71 | 0.246 |
| Potassium (mmol/L) | 6.31 | 6.01 | 5.83 | 6.20 | 5.40 | 0.179 |
| Calcium (mmol/L) | 3.24 | 3.36 | 3.53 | 3.48 | 6.27 | 0.128 |
| Chlorides (mmol/L) | 119.43 | 119.29 | 119.02 | 118.74 | 2.15 | 0.973 |
| Urea (mmol/L) | 3.54 | 3.54 | 3.67 | 3.51 | 6.34 | 0.922 |
| Creatine (mmol/L) | 63.24 | 62.79 | 62.71 | 63.59 | 3.58 | 0.899 |
| Cholesterol (mmol/L) | 3.55 | 3.61 | 3.57 | 3.57 | 5.98 | 0.975 |

Values within row followed by upper case letter (a, b, c, d) are significantly different. T1, T2, T3 and T4 represents treatment groups 1 to 4 respectively.

Table 5. Effect of tamarind seed based diet on different microbial population of large and small intestinal content of broiler chickens after 30 days treatment.

| | Treatment group | | | |
|--------------------------------|---------------------|--------------------|--------------------|--------------------|
| | T1 | T2 | T3 | T4 |
| Large intestine | | | | |
| Total aerobes | 3.35 ^b | 2.14 ^d | 3.98 ^a | 2.96 ^c |
| Total anerobes | 11.15 ^{ns} | 9.61 ^{ab} | 6.37 ^b | 13.38 ^a |
| <i>Escherichia coli</i> | 6.23 ^d | 7.28 ^a | 6.79 ^b | 6.41 ^c |
| <i>Bifidobacterium</i> sp. | 4.42 ^a | 3.53 ^b | 3.68 ^a | 4.21 ^{ns} |
| <i>Bacteroidetes</i> sp. | 8.27 ^d | 13.72 ^a | 10.75 ^b | 10.42 ^c |
| <i>Lactobacillus</i> sp. | 8.31 ^b | 5.65 ^d | 7.41 ^c | 10.96 ^a |
| <i>Clostridium perfringens</i> | 1.73 ^d | 2.42 ^b | 3.32 ^a | 2.19 ^c |
| Yeast sp. | 6.11 ^b | 5.01 ^d | 7.27 ^a | 5.87 ^c |
| Small Intestine | | | | |
| Total aerobes | 3.56 ^a | 1.78 ^d | 2.67 ^c | 3.12 ^b |
| Total anerobes | 10.91 ^a | 9.65 ^c | 8.92 ^d | 10.5 ^b |
| <i>Escherichia coli</i> | 5.41 ^c | 7.23 ^a | 6.51 ^b | 4.78 ^d |
| <i>Bifidobacterium</i> sp. | 3.21 ^a | 1.25 ^d | 2.22 ^c | 2.97 ^b |
| <i>Bacteroidetes</i> sp. | 8.42 ^a | 6.78 ^c | 6.76 ^c | 7.82 ^b |
| <i>Lactobacillus</i> sp. | 7.77 ^a | 6.63 ^c | 5.96 ^d | 6.79 ^b |
| <i>Clostridium perfringens</i> | 0.98 ^c | 1.9 ^a | 1.21 ^b | 0.96 ^d |
| Yeast sp. | 5.78 ^b | 5.98 ^a | 4.92 ^d | 5.31 ^c |

Values within row followed by upper case letter (a, b, c, d) are significantly different and ns-no significant difference among different groups. T1, T2, T3 and T4 represents treatment groups 1 to 4 respectively.

Carcass weight of different parts of the body (head, neck, wings, thighs, breast and back) was determined and shown in Table 2. It was found that weight of main muscular part of chicken like thighs, breast and back was significantly ($P<0.05$) lower in tamarind seed fed group. No fat was deposited in the lower abdomen resulting in a quality meat production.

Haematological and serum biochemical study

The haematological parameters like RBC, PCV and WBC

values were significantly lowered in T2 feeder than T1, T3 and T4 feed consuming groups (Table 3). In relation to RBC and haemoglobin concentration, the values were also significantly ($P<0.05$) lower in T2 diet feeding group than the other. Results of serum biochemical profile after 30 days of trial is enlisted in Table 4. Serum biochemical indices like sodium, potassium, calcium, chloride, urea, creatine and cholesterol were not significantly ($P<0.05$) altered except total protein and albumin which were significantly ($P<0.05$) lowered, whereas, globulin content elevated in the T2 fed group.

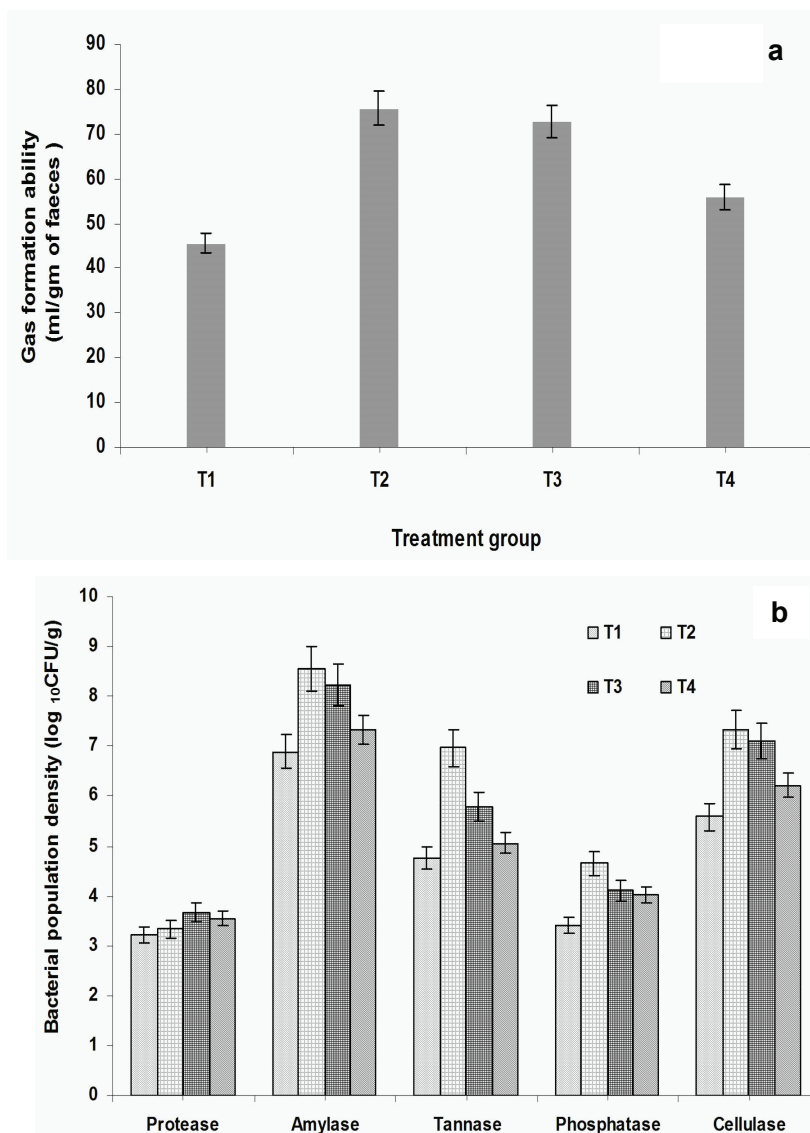


Figure 1. (a) *In vitro* gas formation ability of different feed treated group during experimental period (b) Alteration of different enzyme producing bacterial population in large intestine of different fed group after 30 days of treatment.

Microbial status of gastrointestinal tract

The population density of total aerobes, total anaerobes, *E. coli*, *Bifidobacterium* sp., *Bacteroidetes* sp., *Lactobacillus* sp., *C. perfringens* and yeast species were respectively 3.35, 11.15, 6.23, 4.42, 8.27, 8.31, 1.73 and 6.11 (log₁₀CFU/g) in T1 group (Table 5). The population size of total aerobes, total anaerobes, *Bifidobacterium* sp., *Lactobacillus* sp. and yeast species significantly decreased to 2.14, 9.61, 3.53, 5.65 and 5.01 (log₁₀CFU/g) but *E. coli*, *Bacteroidetes* sp. and *C. perfringens* increased in population size in T2 group. But after 4 weeks, the population density of total anaerobes, *Bifidobacterium* sp. and *Lactobacillus* sp. respectively declined to 6.37, 3.68 and 7.41 (log₁₀CFU/g) in T3 group. But

the microbial population of total anaerobes, *Bifidobacterium* sp. and *Lactobacillus* sp. exhibited higher population density with respect to T1 group. It showed that the nutrient composition in composite feed helped to resettle the microbial population for better endorsement of gastrointestinal health like commercial feed.

The total aerobic population in the small intestine was 3.56 (log₁₀CFU/g) which was reduced to 1.78, 2.67 and 3.12 respectively in T2, T3 and T4 group (Table 5). But the total anaerobic bacterial population conquered with population 10.91 that ultimately reached to 9.65, 8.92 and 10.5 (log₁₀CFU/g) after 4 weeks of feeding. The higher population density of total aerobes, anaerobes, *Bifidobacterium* sp., *Lac-*

tobacillus sp., *Bacteroidetes* sp. and *Yeast* sp. were noted in T2 respectively with higher growth with respect to T1 group. But the *E. coli*, *C. perfringens* and *Yeast* sp. were expanded to 7.23, 1.9 and 5.98 in T2 group. The growth trend of the microbial population in T3 group was noted like T2 group except the *Yeast* sp. which showed negative growth. In T4 group the population of total aerobes, anaerobes, *Bifidobacterium* sp. *Bacteroidetes* sp., *Lactobacillus* sp. and *Yeast* sp. increased expect the population of *E. coli* and *C. perfringens*.

Gas formation

Besides, *in vitro* gas formation ability of T1 was 45.5 ml/g that increased to 75.6 ml/g, 72.8 ml/g and 55.8 g/ml respectively by the altered microflora in T2, T3 and T4 (Fig. 1a).

Enzyme producing bacterial population

The population of protease producer was 3.21 (\log_{10} CFU/g) and reduced to 2.34 after tamarind seed feeding (T2) which was significantly ($p < 0.05$) different from T1 group (Fig. 1b). But the population sizes of this group in T3 and T4 were respectively 3.19 and 3.54. The amylase, phosphatase and cellulase producers also decreased in T2 group, but tannase producers were increased significantly. In case of T3 group, these enzyme producers slightly increased but in T4 tannase, phosphatase and cellulase producers significantly ($p < 0.05$) increased after 3 weeks than T1 group.

Discussion

The major tannin residues of tamarind seed mainly remain in the seed coat and may vary from place to place and season to season. Bhattacharya and Kumar (2008) reported the presence of 20.2% of tannin in tamarind seed coat, whereas Panigrahi et al. (1989) reported 5.62% of tannin. Detannification of tamarind seed by microbial fermentation was found to be most suitable among other physico-chemical techniques. The process is mainly associated with the decortication of tamarind seed from its kernel. Presoftening of seed followed by soaking overnight made the grinding process easier. Bhattacharya and Kumar (2008) reported decortication of tamarind seed from its kernel either by roasting or by soaking the seed in water followed by drying. Previously a number of physical techniques have been reported but with unsatisfactory result. Diarra and Usman (2008) reported detannification of mango kernel by boiling which resulted in reduced protein content.

Schons et al. (2012) also reported 66% detannification of sorghum after five successive days of fermentation with *Peciliomyces variotii*. *B. subtilis* PAB2 being a potential tannase producer, can break down the hydrolysable tannin in 72 h into gallic acid and glucose resulting in reduction of tannin

content. Previously, Dei et al. (2008b) reported the 52.9% and 79% detannification of shea nut meal fermented with *Aspergillus niger* in solid state fermentation after 8 successive days. More intimate contact between substrate and organism in solid state resulted in better substrate utilization than submerged fermentation. Sabu et al. (2005) and Jana et al. (2013) successfully used tamarind seed for production of tannase by *A. niger* and *B. subtilis*. Tannase (partially purified) mediated degradation of tannin gave misleading result as compared to fermentation. This may be due to substrate specificity and water activity of enzyme. Partial purified tannase of PAB2 can degrade 60% of tannin after 72 h of incubation. Schons et al. (2012) reported 48% detannification of sorghum after 5 days of incubation with *P. variotii* tannase. However, this is the first reporting of *in vitro* digestion of tannin by tannase.

Results of growth performance are partially in accordance with total feed consumed, which support the data of TFBW and TMG. Higher mass gain of the body was noted in commercial feed followed by composite feed, enzyme treated tamarind seed and tamarind seed only. High tannin content of the tamarind seed decreased growth rate of the chicken whereas detannified food enhanced the mass gain. The mechanisms by which tannins influence feed intake are not clearly known. Jamroz et al. (2009) found no response of chickens to the tannin supplement in terms of feed intake and conversion. Researchers have suggested that effect of tannins is related to their astringency, which involved their combination with salivary proteins to cause dryness in the mouth (Butler 1989). Our findings are in agreement with previous evidence that feeding of tannin containing diets to poultry diminishes the feed intake and feed efficiency (Olorrede et al. 1999). On the contrary, Zombade et al. (1979) reported increased feed intake in chicks fed sal seed (*Shorea robusta* Gaertn f.), which contains high level of tannin. The reduction in tannin content has a good effect for the yields that were confirmed from the weight of different parts. In the contrary, Jamroz et al. (2009) reported no significant differences caused by increased tannin level in chicken diet.

Tannins have been reported to adversely affect the feed intake and feed efficiency in broiler chickens. It is also reported that tannins lower dry matter and protein digestibility (Douglas et al. 1993). That is why the low haematological count was recorded in T2 group. The total count of WBC reflected that tannin consumption induced the stress that led to induction of immunological reaction. It was also observed in our experiment that the level of tannin content of the feed directly correlated with total and differential count of WBC. Increase in neutrophil numbers (neutrophilia) was observed generally in T2 group which indicated infection and the case of this study could be associated with the depressive effect of the high tannin level in the seeds (Medugu et al. 2010). But the enzyme treated tamarind seeds feeder and the fermented food consuming group did not show such adverse immuno-

logical effect with respect to control group.

The protein value in the seeds was very low compared to marketed feed but lowering of tannin by fermentation from the seeds might enhance the feed value. The total protein in the serum of the composite feed consuming group was not significantly different from the control. Serum protein is usually a reflection of the protein quality fed (Eggum 1970). Lowering the serum protein concentration in tamarind seed fed group may be due to tannic acid mediated protein precipitation and denaturation of protein which is believed to be responsible for the reduction of protein digestibility. On the contrary, sodium, potassium, calcium, chlorides, urea, creatine and cholesterol level were not significantly altered in all fed group with compared to the T1. Higher level of fluctuation was noted in high tannin containing tamarind seed feeders. Medugu et al. (2010) reported high level of fluctuation in serum biochemical indices after administration of chest nut extract in broiler diet. Marzo et al. (1990) reported tannic acid mediated primary and secondary immunological changes in developmental period of chickens. Results from this study therefore reveal that fermented tamarind seed could be supplemented in the marketed feed as it had no such adverse effect on serum parameters.

The intricate network of commensal bacterial flora and GI tract is co-evolved towards a mutually beneficial state, i.e. essential for physiological consequences. Any change of the nutrient status of host drastically alters the microbial population which may result in good or bad gastrointestinal health. The gastrointestinal tract of chicken is inhibited by vast and dynamic array of bacteria. Without estimating the types of the bacteria it is impossible to estimate their true physiological impact *in vivo*. The diet of chicken altered the microbial imprint that may result in good or bad gastrointestinal health. Due to indigestion and antagonist effect of tannin on enzymatic system it accumulates in large intestine and acts as growth suppresser on microbial community. But detannification of tamarind seeds and its supplementation with commercial feed encourages the growth of total anaerobes, *E. coli*, *Bacteroidetes* sp. and *Lactobacillus* sp. It may be that reduction of tannin level after enzymatic treatment that lowers the redox environment of the large intestine and induces the growth of microbial population. On the contrary, Jamroz et al. (2009) reported presence of greatest number of *Lactobacillus* when diet was supplemented with 1000 mg/kg of sweet chest nut tannin.

The 300-500 different microbial species remain associated in the proximal part of small intestine with predominance of gram-negative organisms and anaerobes. After 4 weeks of feeding of tamarind seeds in T2 group it was observed that *E. coli*, *C. perfringens* and *Yeast* population drastically increased in small intestine. It indicated that tannin of tamarind seeds suppressed the growth of other predominant bacterial population. But after feeding of detannified tamarind

seeds, no such significant alteration of microbial population was noted. But the composite feeding group (T4) resettled the microbial population and enhanced the growth of total aerobes, total anaerobes, *Bifidobacterium* sp., *Lactobacillus* sp. and *Bacteroidetes* sp. in small intestine. Our findings are in contrast with those of other authors, which indicated the distinct influence of tannin on population of microorganisms (Scalbert 1991; Bento et al. 2005).

The proliferation of coliform bacterial population and modified metabolic activity of composite microflora in T2, T3 and T4 were responsible for such higher gas formation in large intestine. The gut microflora enzymatically digested the undigested feed (cellulose, starch, pectin, xylan, etc.), provided the energy to the gut epithelial cells and maintained the integrity of the gut that ensured the immunity of gastrointestinal tract. In each group accumulation of tannin through seed feeding enhanced the growth of tannase producing organisms. This polyphenol hydrolase degraded the tannin into glucose and gallic acid. The glucose thus acted as the primary energy source. Reduction of the amylase, protease, phosphatase and cellulase producers in T2 group reflected that tannin also acted as inhibitor of enzyme producing bacterial population. Tannase treatment declined the level of tannin content and its supplement in commercial feed encouraged the growth of amylase, protease, tannase, phosphatase and cellulase producing bacterial population like marketed commercial feed.

Conclusion

Tamarind seed, which is an abundant agro-industrial waste with high calorific and nutritional values, contains a large amount of anti-nutrient, tannin. The results obtained in the present study indicated that detannification of tamarind seed by microbial technique is more suitable than the existing physico-chemical techniques. Supplementation of fermented tamarind seed consistently improved health of broiler chicken. So, fermented tamarind seed may be considered as an alternative nutrient source in broiler chicken diets, which has no adverse effect.

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