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Potential of tamarind seeds (*Tamarindus indica* L.) as prebiotics on the growth of lactic acid bacteria

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Potential of tamarind seeds (*Tamarindus indica* L.) as prebiotics on the growth of lactic acid bacteria

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Abstract. The purpose of this study was to examine the potential of tamarind seeds as a prebiotic by conducting tamarind seeds with a temperature and incubation time. Prebiotic testing was carried out using *Lactobacillus casei* grown on media with the addition of tamarind seeds. The research used a completely randomized design. The temperature was divided into two levels (60 and 70°C) and the incubation time is divided into three levels (120, 180, and 240 minutes). The treatments were T1 (control), T2 (60°C, 120 minutes), T3 (60°C, 180 minutes), T4 (60°C, 240 minutes), T5 (70°C 120 minutes), T6 (70°C, 180 minutes), and T7 (70°C, 240 minutes). Each experiment was repeated twice. Data were analyzed by analysis of variance, if there was a significant effect then continued with Duncan test. Based on the results of this study: tamarind seeds which were incubated at a temperature of 60°C for 240 minutes increased the number of colonies significantly ($P<0.05$) compared to the incubation time below, incubation tamarind seeds at 70°C increased the number of bacterial colonies significantly ($P<0.05$) in all-time ranges, however the optimal time 180 minutes. Tamarind seeds have potential as prebiotics, all the parameters showed the ability of tamarind seeds as a prebiotic.

1. Introduction

Microflora imbalance in the digestive tract due to colonization of pathogenic bacteria interferes with livestock growth. Probiotic bacteria can improve the balance of microflora in the digestive tract. The main effect of probiotics in poultry is to improve growth performance, meat quality, immune response, and affect not only morphology but also gut microbiota.

Prebiotics are carbohydrates that are not digested and not absorbed by monogastric livestock, such as chickens. Several studies reported that prebiotics modulates the growth of probiotic bacteria such as Lactobacillus and Bifidobacteria by modifying the balance of intestinal microflora [1]. The main prebiotic mechanism of action is the modulation of the gut microbiota by selectively providing food for beneficial groups of probiotic bacteria [2] and reducing colonization of unwanted intestinal pathogenic bacteria, thereby increasing the integrity of the intestinal mucosa [3]. The mechanism of prebiotic activity affects the composition of bacteria in the gut and enhances immunity to chicken [4] [5].

The general requirements of prebiotics cannot be absorbed when passing through the upper gastrointestinal tract, resistant to acidic pH, stimulating the growth of non-pathogenic bacteria, and modulating the host defense system [6]. The prebiotics tried in chickens are oligosaccharides such as fructooligosaccharides, inulin [7], galactooligosaccharides [8], mannaoligosachharides [9], lactose [2], and xylooligosaccharides [10].

Some commercial prebiotics includes yeast cells [11] [12] *Saccharomyces cerevisiae* or yeast cultures showed a prebiotic effect [13]. In addition to the presence of commercial prebiotics from Saccharomyces, some plants contain polysaccharides such as oligosaccharides, inulin, which have potential as prebiotics.

Tamarind seeds (*Tamarindus indica* L), contain quite large polysaccharides around 50%-60% [14]. Fructooligosaccharides are fructose polymers that are undigested in the intestines of poultry [15] [16]. Oligosaccharides and polysaccharides undigested by non-ruminant digestive enzymes. Tamarind seeds are known to contain a large number of polysaccharides, but it is not clear whether tamarind seeds can be used for prebiotics. Therefore, this study was conducted to study the effect of tamarind seed powder on the potential prebiotics and to determine the potential of tamarind seeds as prebiotics, it is necessary to research tamarind seeds as prebiotics by observing the growth of probiotic bacteria given tamarind seeds by calculation of bacteria with Total Plate Count (TPC).

2. Materials and Methods

2.1. Preparation of tamarind seeds powder

The tamarind seeds were mashed with a blender until the size was 60 mesh, then incubated in the divided water bath at 60 and 75°C. Each sample with a different temperature was then incubated for 120, 180, and 240 minutes. Furthermore, each sample of tamarind seeds with temperature and time determination is taken 2% by dissolving in aqua dest. Proximate analysis of tamarind seeds was carried out in the laboratory of chemistry, food biochemistry, and agricultural products at the University of Jember. Proximates composition of *Tamarindus indica* (Table 1.)

Table 1. Proximates Composition of *Tamarindus indica*

Sample	Moisture (%)	Crude Protein (%)	Crude Fat (%)	Ash (%)	Carbohydrates (%)
simplo	9.73	15,61	3,26	2,70	68,71
duplo	9,28	15,60	3,31	2,62	69,19
mean	9.51	15.61	3.29	2.66	68.45

2.2 Preparation of Lactobacillus casei

The microbial culture was *Lactobacillus casei* FNCC 0090 obtained from the Center for Food and Nutrition Studies, Gadjah Mada University. The bacterial growth medium was MRSA. Dilution using (plating method). A total of 1 ml *Lactobacillus casei* placed in a test tube containing 9 ml of Sodium Chloride solution 0.9%, then diluted by taking 1 ml put in a test tube containing 9 ml of Sodium Chloride solution 0.9% so that a 10^{-1} dilution is obtained, then from the 10^{-1} dilution tube 1 ml is taken and put into the test tube containing 9 ml of Sodium Chloride 0.9% solution, so that it can be diluted 10^{-2}. The procedures were handled continuously until the level of dilution is 10^{-8}.

2.3 Treatments and trial procedure

The research used a completely randomized design. The temperature was divided into two levels (60 and 70°C) and the incubation time is divided into three levels (120, 180, and 240 minutes). The treatments were T1 (control, 27°C), T2 (60°C, 120 minutes), T3 (60°C, 180 minutes), T4 (60°C, 240 minutes), T5 (70°C 120 minutes), T6 (70°C, 180 minutes), and T7 (70°C, 240 minutes). Each experiment was repeated twice.

A total of 1 ml of each dilution treatment was put into sterile Petri dishes containing agar medium by pouring method. The percentage of tamarind seeds is divided into control (without tamarind) and 2% (dissolved in 100 ml aquadest). The pour plate was then incubated at 36°C for 24 hours. The number of colonies grown on the media was calculated using the plate count method according to [17].

2.4 Data Analysis

This study used a completely randomized design. Statistical Product of Service Solution software was used to establish the validity of the models based on analysis of variance (ANOVA), if there was a significant effect then continued with the Duncan test.

3. Results and Discussion

Prebiotics are not digested or absorbed in the gastrointestinal tract, besides providing a food source for probiotic bacteria, such as Lactobacillus, reducing the attachment of pathogenic bacteria including Salmonella, and promoting the gut microbiota. Prebiotics contain polysaccharides that inhibit the binding of pathogenic bacteria to the intestinal mucosa [18] and modulate the immune system by eliminating pathogenic bacteria from the intestinal tract [19].

Tamarind seeds are indicated to contain antinutrients in the form of tannins, free phenols, and oligosaccharides, so tamarind seeds must be processed first to be utilized [20] essential oils, and some natural polymers such as starch [14] a source of phytochemicals [21] [22] and consisting of phenolic antioxidants [23]. Tamarind seed extract exhibited potential antioxidant and anti-microbial activity [21].

One of the limitations on the use of tamarind seeds is the presence of tannins. Tannins bind proteins to form complex bonds so that these proteins are difficult to digest by protease enzymes. Tannins are compounds that can inhibit microbial growth by inhibiting the work of enzymes such as cellulose, pectinase, oxidative peroxide, and others [24]. According to [25] the phenols present in tannin compounds in high concentrations can be toxic to bacteria. The heating process can reduce the content of tannins, phytic acid, and trypsin inhibitors which are anti-nutritional substances [26]. The steaming treatment reduced the tannin content of tamarind seeds, the tamarind seeds without treatment contain 0.2849% tannin, 10 minutes of steaming reduces the tannin value to 0.2683%. 0.2663%. This showed that the steaming treatment of tamarind seeds can reduce the value of tannins contained in tamarind seeds. Tannins and phenols are water-soluble compounds. Tannins can be removed by soaking, heat treatment, or cooking [27].

The colonies number of *Lactobacillus casei* with different temperatures and time incubation of tamarind seeds are shown in Table 2.

Table 2. The colonies number of *Lactobacillus casei* (cfu/mL)

Treatments	Replications		Means
	1	2	
T1 (control)	2.85×10^{7}	1.94×10^{7}	2.39×10^{7a}
T2	1.0×10^{7}	1.0×10^{7}	1.0×10^{7a}
T3	2.45×10^{7}	1.86×10^{7}	2.15×10^{7a}
T4	387.54×10^{7}	356.85×10^{7}	372.19×10^{7b}
T5	572.47×10^{7}	536.61×10^{7}	554.54×10^{7c}
T6	917.33×10^{7}	917.16×10^{7}	917.25×10^{7e}
T7	671.87×10^{7}	656.93×10^{7}	664.40×10^{7d}

a, b, c, d, e Different superscripts in the same column show significant differences (P<0.05)

Heating tamarind seeds at 60°C for 120 minutes (T2) and 180 minutes (T3) have not shown inactivates anti-bacterial activity, from the number of bacterial colonies on T2 was the lowest of all treatments and controls, as well as on T3. Flavonoids are the largest group of tannin compounds, phenolic compounds have effective properties to inhibit the growth of viruses, bacteria, and fungi. The mechanism of flavonoid compounds in inhibiting fungal growth is by damaging the cell wall of Candida albicans which consists of lipids and amino acids. Tannin compounds with their ability to form protein complexes and damage cell membranes by denaturing protein bonds in cell membranes, resulting in cell membrane lysis [28]. However, at 240 minutes of incubation, the number of bacterial colonies increased significantly ($P<0.05$) compared to 180 minutes (T3) and 120 minutes (T2). The incubation time of 240 min is estimated to have damaged tannin in tamarind seeds, thereby reducing the inhibition of bacterial growth.

Lactobacillus casei is a lactic acid bacteria [29]. The growth and metabolic activity of lactic acid bacteria are strongly influenced by the biochemical and biophysical environment [30]. The biochemical environment is a bacterial growth medium that must provide nutrients according to the requirements of bacteria [31] [32]. A large amount of research has studied the relationship between nutritional requirements and metabolic activities of lactic acid bacteria [30].

Tamarind seeds generally contain high organic matter [33] in the form of carbohydrates, proteins, and fats [34]. Based on the results of the proximate analysis of tamarind seeds in this study, the carbohydrate content was 68.45% and the protein content was 15.61%. In addition [35] reported that tamarind seeds contain essential amino acids, such as isoleucine, leucine, lysine, methionine, phenylalanine, valine, and are a source of essential fatty acids, as well as minerals, especially calcium, phosphorus, and potassium.

The main metabolic activity of lactic acid bacteria is breaking down carbohydrates for energy [31] [32] [36] and breaking down proteins, lipids, and other compounds also essential for normal growth [30]. Tamarind seeds have phytic acid that reduces the bioavailability of certain minerals, interferes with protein utilization due to the formation of phytate protein complexes and phytate-protein minerals [37] [38]. Heating tamarind seeds can reduce the cyanogen content, reduce trypsin inhibitor activity and reduce the formation of phytate protein. Heat treatment can increase the nutritional value by destroying trypsin inhibitors and increasing the digestibility of proteins and amino acids, fats, and carbohydrates contained in feed ingredients [39]. Trypsin inhibitor activity can be reduced through the steaming process [20] so that food substances can be used for bacterial growth, this can be seen from colonies on heating 70°C (T5, T6, and T7) was significantly higher ($P<0.05$) than 60°C heating (T2, T3, and T4). The optimum incubation time at 70°C is 180 minutes, this is indicated by the number of colonies T6 higher significantly ($P<0.05$)compared to the incubation time of 120 minutes (T5) and 240 minutes (T7), but the incubation time of 240 (T7) minutes provides better conditions for bacterial growth than the incubation time of 120 minutes (T5).

4. Conclusion

Based on the results of this study:

1. Incubation of tamarind seeds at a temperature of 60°C for 240 minutes increased the number of colonies significantly compared to the incubation time below
2. Bacterial colonies using 2% tamarind seeds which were incubated at 70°C increased the number of bacterial colonies in the all-time ranges.
3. Tamarind seeds have potential as prebiotics with incubation at 70°C for 180 minutes.

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