Unlocking the potential of fermented turmeric (*Curcuma longa L.*) as a potential natural feed additive for poultry

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Abstract. This study investigated the fermentation process of Turmeric (Curcuma longa L.) using Lactobacillus casei and its impact on Total Plate Count (TPC), pH, and antibacterial activity. The research followed an experimental design, examining antibacterial activity across six treatments with four replications, analyzed through Analysis of Variance (ANOVA). TPC and pH data were compared between non-fermented and fermented Turmeric, with 12 replications for each treatment, analyzed using T-Tests with IBM SPSS version 26 (IBM, New York, NY, USA) to identify significant differences ($p<0.05$). The research results showed a significant pH change to 3.95 and TPC analysis revealed an increase in bacteria count in fermented turmeric, with the bacterial growth curve peaking on day 3. Antibacterial analysis demonstrated fermentation time's impact on the inhibition zone, with increased inhibition observed against Salmonella typhimurium, Escherichia coli, and Lactobacillus casei. In summary, fermentation alters curcuminoid and flavonoid content, TPC, pH, and the zone of inhibition, thereby enhancing the quality of turmeric fermentation.

1 Introduction

Turmeric (*Curcuma longa L.*) holds a significant place as a spice in India, China, and Southeast Asia [1]. Characterised by its yellow hue, the turmeric plant contains three curcuminoid components: curcumin (77%), demethoxycurcumin (17%), and bisdemethoxycurcumin (3%), alongside essential oils like turmerone, aloe vera, and white wood oil [2]. It serves as a potential feed additive for chickens, offering an alternative to antibiotics. Feed additives, be they natural or synthetic substances, are incorporated in small quantities, typically around 1%, into basal feed or drinking water to enhance feed quality, boost animal performance, and bolster animal health [3]. However, turmeric's efficacy as a feed additive is hindered by its low bioavailability [23], potentially limiting its beneficial effects when administered directly to animals.

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One promising approach to enhance the efficacy of turmeric is through fermentation. Fermentation is a natural process that elevates the levels of vitamins, essential amino acids, anti-nutrients, protein content, appearance, taste, and aroma of food. Lactic acid bacteria (LAB) play a pivotal role in the fermentation process, acting as primary agents that drive the fermentation and significantly influence the final characteristics of the fermented product [20]. Moreover, a study by Fideler et al. [4] also underscores the importance of LAB in the fermentation industry, highlighting their role in influencing taste, texture, and aroma and fostering beneficial bacterial diversity.

A commonly employed starter in fermentation is the *Lactobacillus casei*. This microorganism exhibits potential as a beneficial microbe, guarding against pathogenic bacteria during fermentation, thus preserving product quality and extending shelf life [24]. However, the application of *Lactobacillus casei* in turmeric fermentation remains largely unexplored. One advantage of *Lactobacillus casei* lies in its ability to enhance LAB diversity and optimize microbial performance during fermentation [13]. Bacterial growth during fermentation is greatly influenced by metabolic activities throughout the process, underscoring the importance of fermentation in bacterial growth and its role in antibacterial activity [24]. This study aims to investigate the fermentation process of turmeric (Curcuma longa L.) using *Lactobacillus casei* and its effect on Total Plate Count (TPC), pH, and antibacterial activity.

2 Materials and Methods

2.1 Materials

Turmeric aged approximately 1 year, specifically the rhizome or tuber, was gathered [26]. They were preparing turmeric liquid extract involved cleaning the turmeric rhizome to remove any adhering soil using clean water. Subsequently, the rhizome was blended until smooth and then transferred into 1.5-liter bottles, to which *Lactobacillus casei* bacteria were added. The bottles were sealed to maintain anaerobic conditions. The freshly prepared turmeric liquid extract then was transported to the laboratory and left at room temperature for seven days under anaerobic fermentation conditions. Bacterial counting, isolation, and pH determination were carried out on the seventh day of fermentation.

2.2 Optical density analysis

According to Wu *et al*. [25] Analysis of the number of *Lactobacillus casei* bacteria in fermented turmeric samples was performed by measuring the optical density (OD) of the fermentation liquid. A spectrophotometer was used, with a wavelength of 400 nm to measure absorbance values and bacterial cell counts.

2.3 Antibacterial activity, microbial analysis, and pH

Antibacterial activity was assessed using disc diffusion on agar plates containing solid media infused with bacteria. The test substance was applied to the discs. Petri dishes were divided into four quadrants, each featuring discs loaded with non-fermented turmeric liquid extract, fermented turmeric, and fermented turmeric + Microbes. The petri dishes were then incubated at 37°C for 24 hours [5]. Preparation of turmeric liquid extract before and after fermentation was carried out for *Total Plate Count* (TPC) analysis [6]. pH testing was conducted using a calibrated pH meter.

2.4 Statically analysis

The analysis of antibacterial activity testing, comprising six treatments with four replications each, was conducted using Analysis of Variance (one-way ANOVA) with IBM SPSS version 26 (IBM, New York, NY, USA) to ascertain significant differences (p <0.05). Total Plate Count (TPC) and pH data, contrasting non-fermented and fermented turmeric with 12 replications for each treatment, underwent analysis using T-Tests with IBM SPSS version 26 to identify significant differences (p <0.05) using the following [3] models:

$$Y_{ij} = \mu + T_i + e_{ij} \quad (1)$$

Where Y_{ij} was the parameters observed, μ was the overall mean, Tithe effect level of various onion meal, and e_{ij} was the amount of error number.

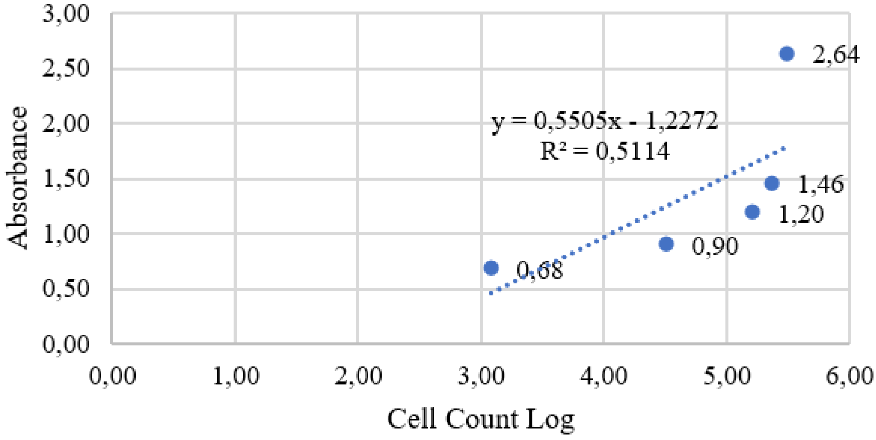
3 Results

3.1 Optical density of turmeric liquid fermentation and bacterial standard curve

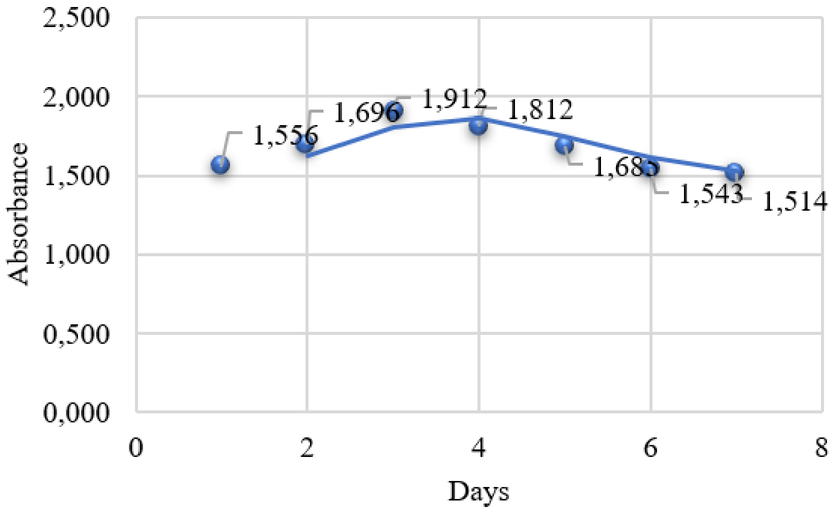
The bacterial standard curve serves as a graph utilised to estimate the number of bacterial cells indirectly. The results of the descriptive data of *Lactobacillus casei* concentration in turmeric 1:1 showed the highest values of uptake and cell count, which were 2.644 (OD) and 3.1 x 105, respectively. In contrast, the lowest values were recorded at a concentration of 1:8, with an uptake of 0.678 (OD) and a cell count of 1.2 x 103. The results of the descriptive data of optical density (OD) calculation of Lactobacillus casei in turmeric are depicted in Table 1 and Figures 1a and 1b.

Table 1. Standard Curve Data for the Cell Count of *Lactobacillus casei*

***Lactobacillus casei* : Turmeric**	**Absorbance (OD)**	**Cell Count (CFU/ml)**	**Log Cell Count (CFU/ml)**
1:1	2.64	3.1×10^5	5.49
1:2	1.45	2.3×10^5	5.36
1:4	1.19	1.6×10^5	5.20
1:6	0.90	3.2×10^4	4.51
1:8	0.67	1.2×10^3	3.08



(a)



(b)

Fig. 1. (a) Optical Density (OD) of *Lactobacillus casei* and (b) growth Curve of Fermented Turmeric Bacteria.

3.2 Inhibition zone of fermented turmeric

According to the research findings, the fermented turmeric liquid extract displayed inhibition zones against *Salmonella Typhimurium*, *Escherichia coli*, and *Lactobacillus Casei*, as depicted in Table 2. The table indicates that fermented turmeric forms inhibition zones with variations in fermentation time. However, at a 95% confidence level ($P>0.05$), these differences did not statistically significantly impact the inhibition zones formed against *Salmonella Typhimurium*, *Escherichia coli*, and *Lactobacillus Casei*.

Table 2. *Zone of Fermented Turmeric*

Treatments	*Salmonella Thypimurium*	*Escherichia coli*	*Lactobacillus Casei.*	P-value
Lactobacillus Casei	5.36 ± 1.17	6.49 ± 0.58	0.0	NS
Turmeric	2.28 ± 0.34	2.74 ± 0.15	2.7 ± 0.20	NS
Turmeric + *Lactobacillus Casei* (0 day)	2.40 ± 0.22	2.84 ± 0.42	2.3 ± 0.27	NS
Turmeric + *Lactobacillus Casei* (Day 1)	2.64 ± 0.11	3.15 ± 0.32	3.6 ± 0.89	NS
Turmeric + *Lactobacillus Casei* (Day 2)	2.74 ± 0.30	3.25 ± 0.11	2.9 ± 0.35	NS
Turmeric + *Lactobacillus Casei* (Day 3)	3.49 ± 0.42	3.34 ±0.28	3.4 ± 0.33	NS

Antibacterial activity tests conducted on turmeric, turmeric + *Lactobacillus Casei* (0), and turmeric + *Lactobacillus Casei* (day 3) reveal numerical discrepancies. Turmeric demonstrates a 12% inhibition of *Salmonella Typhimurium*, whereas turmeric + *Lactobacillus Casei* (0) and turmeric + *Lactobacillus Casei* (day 3) show 13% and 19% inhibition, respectively. Similarly, for *Escherichia coli*, turmeric exhibits a 12% inhibition, while turmeric + *Lactobacillus Casei* (0) and turmeric + *Lactobacillus Casei* (day 3) display 13% and 15% inhibition, respectively. Regarding L. Casei bacteria, turmeric shows an 18% inhibition, whereas turmeric + *Lactobacillus Casei* (0) and turmeric + *Lactobacillus Casei* (day 3) demonstrate 15% and 23% inhibition, respectively. This suggests that the fermentation duration of the turmeric influences the inhibition zone.

3.3 Total Plate Count (TPC) and pH in fermented turmeric liquid

The research findings highlighted disparities in Total Plate Count (TPC) values between non-fermented turmeric and turmeric fermented with *Lactobacillus casei*. Table 3 illustrates that both types of samples underwent TPC testing to evaluate bacterial quantities, with results presented in Log cfu/ml. The study revealed a 20.27% increase in TPC after fermentation. Independent T-test analysis ($p<0.05$, 0.01, and 0.001) indicated a notable difference in turmeric liquid fermentation utilising *Lactobacillus casei*.

The research results also pointed out variations in pH values between non-fermented turmeric and turmeric fermented with *Lactobacillus casei*. Table 3 displays differing pH values in non-fermented and fermented turmeric, using *Lactobacillus casei* as a starter in fermentation. pH level indicates the acidity or alkalinity of a substance, with values ranging from 0 (very acidic) to 14 (very alkaline). Fermentation employing *Lactobacillus casei* was assessed after 7 days, resulting in a 37.5% alteration in pH value. Independent T-test analysis ($p<0.05$, 0.01, and 0.001) suggested a significant difference in turmeric fermentation using *Lactobacillus casei* concerning pH values.

Table 3. Non-fermented and fermented turmeric

Parameters	Non-Fermented	Fermented *Lactobacillus casei*	SEM	P-Value
TPC (Log cfu/ml)	5.603 ± 0.285	7.028 ± 0.554	0.09	<0.001
pH	6.32 ± 0.18	3.95 ± 0.11	0.06	<0.001

4 Discussion

Figure 1b illustrates how fermentation time affects the growth curve of bacteria in fermented turmeric liquid. Based on the descriptive data, the longer the fermentation period, the greater the impact on bacterial growth. There was an increase in growth of 8.9% and 22.8% on days 2 and 3, followed by a periodic decrease from days 4 to 7. This indicates that fermentation and bacterial growth peaked on day 3, followed by a decrease in activity on day 7. According to [7], the bacterial growth curve can be divided into four phases: the lag or adaptation phase, where bacteria adjust to the substrate and fermentation medium; the exponential (log) phase, where bacteria begin to grow and use the fermented substrate material for energy and cell division; the stationary phase, where bacterial growth slows down due to the cessation of bacterial cell division in the fermentation process; and the last is the death phase, where bacteria die along with the end of the fermentation process.

As outlined by [8], metabolic performance plays a significant role in bacterial growth during fermentation, with metabolism being a key factor influencing this growth. Within the cell, a controlled chemical reaction occurs, catalysed by enzymes, with temperature increasing the rate of this reaction. The fermentation processes allow microorganisms to break down large organic molecules into simpler compounds, such as proteins being transformed into peptides or amino acids, and sugars and starch into alcohol [9]

The composition of the turmeric substrate and the microorganisms used in fermentation both impact the outcomes. Alongside the substrate, fermentation time also holds importance as it correlates with bacterial growth during fermentation. In turmeric, fermentation reaches its peak at three days with the highest absorbance, while from day 4 to 7. This decrease indicates several things, including nutrient limitation, accumulation of fermentation products such as acids, alcohols, and other compounds, and changes in pH due to acid accumulation. Crucial metabolic activities in LAB, particularly lactobacilli, involve the breakdown of carbohydrates and related compounds to obtain energy and carbon molecules, essential for survival and growth. Most LAB can ferment monosaccharides like galactose and glucose, as well as disaccharides such as sucrose and lactose. Nutrient deficiencies, such as a lack of carbohydrate sources, inhibit both the lag and exponential growth phases of bacteria [10]. Additionally, the fermentation pathway influences the nutritional variances bacteria require throughout the fermentation process.

Longer fermentation leads to a significant increase in microorganisms, particularly LAB, and a decrease in pH, making it acidic and capable of killing and suppressing the growth of pathogenic bacteria. According to Purwanti et al. [11], a 2.5% water extract of turmeric acts as an antibacterial agent against Escherichia coli, Salmonella sp., and Lactobacillus sp., attributed to turmeric's bioactive substance, curcumin.

Bacterial resistance also contributes to these differences. Numerically, the most extensive inhibition zone is observed for Salmonella Typhimurium compared to Escherichia coli and Lactobacillus Casei bacteria, indicating that Salmonella Typhimurium and Lactobacillus Casei bacteria are more sensitive. According to Alamsyah et al. [12], Escherichia coli is a Gram-negative bacterium with a more complex cell wall structure, comprising an outer membrane of lipopolysaccharides and lipoproteins acting as a barrier against disinfectants and antibacterial compounds like antibiotics

The Total Plate Count (TPC) calculation is determined following 24 hours of sample incubation on nutrient agar (NA) media at 37-38°C, revealing differing outcomes. This variation arises from disparities in the number of bacterial colonies that develop during the 24-hour incubation period. The fermentation process significantly impacts bacterial development, involving the breakdown of substrates into simple sugars because Lactic acid bacteria (LAB) is a type of microbe that has a role in the fermentation process and produces amylase, protease, lipase, and glucoamylase enzymes [15].

Fermentation is a chemical transformation process of organic substrates into simpler compounds facilitated by enzymes produced by microorganisms like fungi, yeast, and bacteria because fermentation methods can preserve food, enhance flavor, and provide various beneficial components such as lactic acid bacteria (LAB), exopolysaccharides, vitamins, minerals, biologically active peptides, and conjugated linoleic acid (CLA) [13]. Additionally, fermentation can reduce the content of toxic components in food through aging, as microbes metabolize sugar from the medium into simpler compounds and energy for cell synthesis [14]. Fermentation using Lactobacillus plantarum and Lactobacillus fermentum strains as starter agents in blueberry juice increases the number of live microorganisms by 35% compared to the control [16].

The fermentation process is influenced by several factors, including the substrate used, starter addition adjusted to the substrate, pH, and temperature. Temperature change during fermentation is one of the most influential factors, affecting the life processes of bacteria, and is crucial for fermenting various foods [17, 18].

The pH values obtained post-fermentation indicate an acidic pH, signifying a successful fermentation process. Fermentation involves microorganisms such as bacteria or yeast breaking down organic substances into simpler forms like acids or alcohols. Fermentation can alter taste, aroma, colour, and nutritional properties, and increase acidity. Using Lactobacillus casei as lactic acid bacteria in fermentation leads to a decrease in pH. Table 3 demonstrates that fermented turmeric has a lower pH than non-fermented turmeric. The decrease in pH indicates the presence of lactic acid bacteria, preserving the quality of the fermented liquid turmeric and hydrolysing complex substrates into simpler compounds like ethanol, alcohol, and acetic acid. During fermentation, lactic acid bacteria (BAL) cause several changes in the medicinal plant's substrate composition, leading to the biotransformation of polyphenols and the production of anti-inflammatory compounds [19]. The fermentation process is time-sensitive, increasing the total titratable acid. Microorganisms convert glucose into organic acids, subsequently transformed by acetic acid bacteria into acetic acid. In addition to acetic acid, Gluconobacter sp. produces glucuronic acid, pyruvic acid, malic acid, salicylic acid, succinic acid, and lactic acid by BAL. The fermentation process's role in increasing acid content due to the microbe's ability to transform glucose into ethanol, increasing organic acids impacting pH reduction [20, 21]. At the end, these should not cause any negative or side effect to human, or animal health [22].

5 Conclusions

In terms of descriptive data, optimal density was achieved at the 3-day fermentation stage; fermented turmeric could inhibit the growth of Salmonella Typhimurium, Escherichia coli, and *Lactobacillus casei* bacteria, which correlated with fermentation duration and larger inhibition zones. Total Plate Count (TPC) increased in fermented turmeric, indicating active microbial growth during fermentation. A decrease in pH in fermented turmeric indicated an acidic environment produced during fermentation.

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