Study on The Quality of Fermented Feed Using Thua-nao as a Fermentation Supplementation

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Abstract

The objective of this study was to evaluate the effect of Thua-nao and fermentation duration on the quality of fermented feed. The Thua-nao was prepared, the soybean seeds were soaked, boiled and incubated for 48 hours before used. Fermented feed was prepared by adding distilled water to the mash feed at a ratio of 1:1.5 in a plastic container. Treatments were designed as follows: fermented feed without Thua-nao, fermented feed with 0.5% and 1% of Thua-nao. Fermentation durations were set at 24, 48 and 72 hours. The supplementation of Thua-nao in the fermented feed did not show a significant difference in pH value. The pH value at 48 hours of fermentation was the lowest compared to both 24 and 72 hours of fermentation. The addition of Thua-nao to the fermented feed led to a significance increase in lactic acid production ($p < 0.05$). The increased fermentation duration was found to significantly increase the lactic acid ($p < 0.05$). The fermented feed incubated with Thua-nao did not affect the total number of lactic acid bacteria (LAB), and the fermentation duration also did not alter the total number of LAB. In conclusion, Thua-nao supplementation in fermented feed increased lactic acid production, and the extended fermentation duration resulted in a decreased pH level and increased lactic acid.

Keywords: Fermented feed, Thua-nao, lactic acid bacteria

Introduction

Employing fermented feed for animal rearing provides several benefits, including the reduction of toxins and anti-nutrients in feed stuff. Additionally, fermented feed is rich in lactic acid bacteria, offering health advantages. By incorporating organic acids and lowering the pH value, this feature acts as a protective barrier against disease-causing germs. To enhance efficiency, lactic acid bacteria may be added to expedite the fermentation process. It has been reported that the fermentation of feed not only decreases the total fiber content but also concurrently enhances the crude protein levels (Khempaka, et al., 2013). There have also been reports of a reduction in mycotoxins in animal feed ingredients. Okeke, et al. (2015) reported that fermenting maize grain for 48-72 hours resulted in a reduction of mycotoxins such as Aflatoxin B1, B2, Citrinin, Cyclopiazonic acid, Fumonisin B1, B2, B3 and Zearalenone compared to unfermented maize. Fermentation also reduces antinutrients in animal feed, such as phytate. This reduction is a result of the phytase enzyme produced by miccroorganisms occurring in fermented feed (Sokrab, et al., 2014). In addition, fermentation contributes to the reduction of cyanide in the cassava peel (Suranindyah & Astuti, 2012).

Thua-nao is a local food of the northern region of Thailand. It has been reported that microbes playing an important role in its fermentation include *Bacillus sp*. (mainly *B.subtilis*). These microbes produce protein-digesting enzymes, resulting in the formation numerous peptides (Chukeatirote, 2015). The crude protein of fermented sacha inchi residue with Thua-nao was increased (Thaikosa & Phinyo, 2021). Recently, Phinyo, et al. (2024) found that incorporating Thua-nao fermented soybean meal into the diet of tilapia enhances survival rates. Therefore, Thua-nao is an intriguing fermented additive to include in the present study.

Objective

To study the effect of using Thua-nao as a source of microorganisms on the quality of fermented feed.

Scope of research

The study examines the changes in pH levels, lactic acid concentration, and the number of lactic acid bacteria in fermented feed with Thua-nao and varying fermentation durations.

Research Methodology

1. Thua-nao preparation

The soybean seeds were soaked in water overnight, followed by boiling for 2-3 hours until the beans softened. Afterward, the boiled beans were placed in a basket to separate excess water. The container was then covered with plastic wrap, ensuring to poke holes, and incubated at 37 degrees Celsius for 48 hours.

2. Fermented feed

In this study, microorganisms from fermented spoiled beans were incorporated along with varying levels of fermented feed. The fermented feed used will be a mixed feed for chickens at each stage, adjusted according to the requirements of chicken. The feed mixture is then taken, and Thua nao are added in amounts of 0%, 0.5%, and 1% (dry matter). Fermentation is initiated by combining the mixture with tap water at a ratio of 1:1.5 in a plastic container. The fermentation process occurs at 38 degrees Celsius for 24, 48, and 72 hours. The pH of the fermented feed, the quantity of lactic acid, and the count of lactic acid bacteria are measured.

3. Measurement of fermented feed quality

3.1 pH

The fermented feed from each treatment was weighed at 1 g, mixed with 9 ml distilled water, and then the pH was measured.

3.2 Lactic acid measurement

The fermented feed was blended and weighed at 10 g, mixed with 10 ml of distilled water, and then a 1% phenolphthalein solution in ethanol was added as an indicator. The sample was titrated with 0.1 N sodium hydroxide until reaching the endpoint, at this point the solution turned pink. The quantity of 0.1 N sodium hydroxide was determined using the following equation:

$$\text{Lactic acid (\%)} = \frac{C \times V \times MW \times DF \times 100}{W \times 1000}$$

C = Concentration of sodium hydroxide
V = Volume of sodium hydroxide
MW = Molecular weight of lactic acid
DF = Dilution factor
W = Amount of fermented feed used

3.3 LAB Measurement

To prepare MRS agar, suspend the components, dehydrated powder, in distilled water. Bring the medium to a boil with constant stirring for at least 1 minute. Autoclave the mixture at 121 degrees Celsius and a pressure of 15 pounds/square inch for 15 minutes. Subsequently, pour the sterilized medium into a Petri dish in preparation for inoculation. For inoculation: Weigh 1 gram of the fermented feed. Dilute it with 9 ml of distilled water until the fermented feed reaches a concentration of 10^{-5}. Transfer the diluted sample onto a streak plate in the prepared culture medium. Place the plate in a CO_2-containing box and incubate it at 37 degrees Celsius for 78 hours. After incubation, count the number of colonies specifically for lactic bacteria, and observe a clear yellow zone around the colonies. Count the number of colonies within the range of 30-300 colonies. Calculate the number of lactic bacteria colonies per gram, then convert it to log10.

Results

pH value

The effect of Thua-nao supplementation and fermentation duration on pH level is shown in Figure 1. The fermented feed supplemented with Thua-nao did not show a significant difference on pH value (Table 1.). However, the duration of fermentation (24, 48, and 72 hours) significantly affected the pH value ($p < 0.05$). The pH value at 48 hours of fermentation was the lowest compared to both 24 and 72 hours of fermentation.

Lactic acid

The effect of Thua-nao supplementation and fermentation duration on lactic acid production is shown in Table 1. The addition of Thua-nao at concentrations of 0.5 and 1% into fermented feed resulted in a significant increase in lactic acid production compared to the control ($p < 0.05$). In addition, an extended fermentation duration was found to significantly increase lactic acid production ($p < 0.05$).

Lactic acid bacteria

There was no significant difference in the total number of LAB observed in the fermented feed incubated with Thua-nao compared to the control group ($p > 0.05$), and, the fermentation duration did not have a significant effect on the total number of LAB ($p >0.05$).

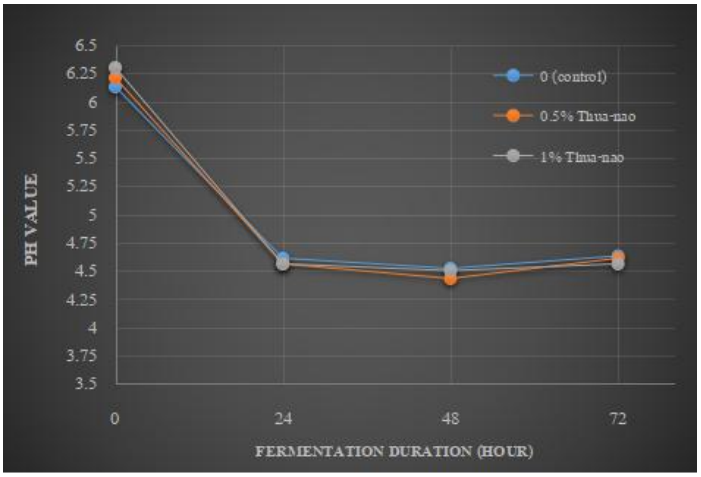

Figure 1: The pH value of fermented feed incubated with Thua-nao and the fermentation duration

Table 1: Effect of supplementation of Thua-nao in fermented feed and fermentation duration on pH value, percentage of lactic acid, and total number of LAB

Thua-nao	Fermentation duration	pH	% Lactic acid	Total no. of LAB CFU (log_{10} CFU/g)
0	24	4.68±0.06^{c}	1.35±0.06^{e}	8.40±0.21
	48	4.44±0.005^{ab}	1.74±0.49^{d}	8.43±0.13
	72	4.52±0.04^{b}	2.23±0.13^{c}	8.40±0.10
0.5	24	4.75±0.04^{c}	1.34±0.08^{e}	8.40±0.08
	48	4.52±0.11^{b}	2.03±0.19^{cd}	8.51±0.05
	72	4.38±0.04^{a}	2.66±0.17^{ab}	8.43±0.07
1	24	4.76±0.02^{c}	1.39±0.05^{e}	8.45±0.05
	48	4.43±0.34^{ab}	2.37±0.13^{bc}	8.45±0.19
	72	4.40±0.07^{a}	2.78±0.06^{a}	8.44±0.09
			P-value	
Thua nao		0.814	0.001	0.514
Hour		0.000	0.000	0.411
Thua nao x Hour		0.008	0.112	0.825

$^{a-e}$Mean within each column with different superscripts are statistically significantly different ($p < 0.05$)

Discussion

The benefits of fermented feed have been reported to enhance the nutritive value of feed such as reducing fiber, while increasing crude protein (Engberg, et al., 2009; Sugiharto & Ranjitkar, 2019). In addition, the LAB in fermented feed resulted in a low pH and produced the lactic acid in the feed (Engberg, et al., 2009; Zhu, et al., 2023). Our present study found that pH levels were decreased in fermented feed compared with the diet before fermentation (0 H). The supplementation of Thua nao in fermented feed did not affect the pH value, while the fermentation duration was the main factor influencing the pH value. Engberg, et al. (2009) reported that the pH level of fermented feed for 24 hours at 20°C was 4.5. Extended fermentation for 48 hours at 26°C resulted in a pH range of 3.9-4.1 (Missotten, et al., 2013). In our study, Thua nao supplementation and fermentation duration affected the percentage of lactic acid. Increasing Thua nao in fermented feed improved lactic acid production. Using Thua nao as a fermentation starter in fermented soybean meal enhance the protein content (Phinyo, et al., 2024). Pakwan, et al. (2020) reported that LAB were found in Thua nao at 1 and 2 days at high level. The major of LAB found in Thua nao was *Bacillus sp.*, especially *B.subtilis* (Inatsu, et al., 2006). These bacteria degraded the carbohydrate into small particles such as organic acid, resulting increased lactic acid. In addition, the increasing fermentation incubation of feed increased the lactic acid (Beal, et al., 2005).

Conclusion

Thua-nao supplementation in fermented feed increased lactic acid, and the extended fermentation duration resulted in decreased pH level and increased lactic acid. As a results of this finding, Thua nao can used as an additive substance to enhance the quality of fermented feed.

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