



The Effect of Fermentation of Leftover Food from Restaurants and Hotels with *Bacillus amyloliquefaciens* on Total Colony Count of *Bacillus* sp. and Nutrition Content of Leftover Food

WIZNA¹, RUSFIDRA², ROBI AMIZAR³, ROMI ANDIKA¹, MUHAMMAD HAIKAL¹, ZURMIATI^{1*}

¹Department of Animal Feed and Technology, Faculty of Animal Science, Universitas Andalas, Padang 25163, Indonesia; ²Department of Technology and Livestock Production, Faculty of Animal Science, Universitas Andalas, Padang 25163, Indonesia; ³Department of Animal Nutrition and Feed Technology, Faculty of Animal Science, Universitas Andalas, Campus II Payakumbuh, West Sumatra, Indonesia.

Abstract | Functional feed is a type of food that contain natural or additive ingredients that have positive health benefits for livestock. This study aims to the effect of fermentation of leftover food from restaurants and hotels with *Bacillus amyloliquefaciens* on total colony count of *Bacillus* sp. and nutrition content of leftover food. The research, a 3x3 complete randomized design factorial, consisted of two factors, and each treatment was repeated three times. Factor A is the dose of *B. amyloliquefaciens* (3, 4, and 5%), and factor B is the fermentation time (3, 4, and 5 days). The total colony count of *Bacillus* sp., water content, dry matter, crude protein, crude fiber, crude fat, calcium, and phosphorus were measured. The dose of *B. amyloliquefaciens* and fermentation time significantly effect ($p < 0.05$) the total colony count of *Bacillus* sp. in the fermented product, but there was no interaction. The dose of *B. amyloliquefaciens* had a significant effect ($p < 0.05$) on the water content, dry matter, crude protein, crude fat, and calcium. In addition, the dose of *B. amyloliquefaciens* and fermentation time did not affect crude fiber, phosphor, and ash. In conclusion, fermentation of leftover food with the dose of *B. amyloliquefaciens* 5% and a fermentation time of 3 days can increase the nutrition content of leftover food and founded the colony count of *Bacillus* sp. in fermented products.

Keywords | *B. amyloliquefaciens*, Fermentation, Functional feed, Leftover food, Nutrient content

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***Correspondence** | Zurmiati, Department of Animal Feed and Technology, Faculty of Animal Science, Universitas Andalas, Padang 25163, Indonesia; **Email:** zurmiati@ansci.unand.ac.id

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INTRODUCTION

Animal feed plays an essential role in determining the success of a livestock business. The feed cost takes the most significant portion, about 70%-80% of the total production cost. Efforts to reduce production costs can be made using unconventional feed matter without compromising the quality of feed and products produced. One alternative is to take advantage of leftover food in

restaurants and hotels that are still of good quality to feed poultry. Leftover food can partially substitute corn and soybean in broiler diets [Truong et al. \(2019\)](#). [Anasih et al. \(2021\)](#) reported that In Indonesia, people produce around two and a half liters of daily waste. Based on its composition, such waste is dominated by organic waste, as much as 58%, while the remaining 42% is inorganic waste. In Padang City, the capital of West Sumatra province in Indonesia, restaurants and hotels produce

an average of 132 kg of leftover food daily (Dewilda et al., 2019). Leftover food derived in restaurants contains 10.89% crude protein, 9.70% crude fat, 9.13% crude fiber, 0.08% calcium, 0.39% phosphorus, and 1780 kcal/kg metabolizable energy (Achadri et al., 2013).

Leftover food in restaurants and hotels can be processed into functional feed with maintained nutrient content. One of the efforts that can be made is through fermentation. Fermented products use *B. amyloliquefaciens* as an inoculum and can be used as a feedstuff, at the same time, it also has another function where the spores in the fermented product function as probiotics for livestock that consume them, so the fermented product can be called a functional feed. Wizna et al. (2009) states that tapioca by product fermented with *B. amyloliquefaciens* contain 40×10^{10} CFU/mL of spores *B. amyloliquefaciens*. The performance of *B. amyloliquefaciens* in fermented products in the digestive tract will match the ability of *B. amyloliquefaciens* given as probiotics through drinking water, although the method of administration is different. Leftover food in restaurants and hotels has a great potential to be used as feed matter. These matters do not compete with human needs, and they can be obtained in any season. Based on the description above, research has been conducted to determine the effect of fermentation of leftover food from restaurants and hotels with *Bacillus amyloliquefaciens* on the total colony count of *Bacillus* sp. and the nutrition content of leftover food.

MATERIALS AND METHODS

COLLECTION OF LEFTOVER FOOD FROM RESTAURANTS AND HOTELS

The method for collecting samples of restaurant waste was based on Dewilda et al. (2019). The collection began by conducting surveys and gathering information about leftover food in restaurants and hotels in Padang City. The samples were derived from the leftover food of these places. The samples were taken from 10 restaurants out of the 127 restaurants in Padang. Types of leftover food included rice, side dishes (including bones and meat), and fruit waste. The leftover food collected was then identified according to its type. Finally, the leftover food was dried under the sun; the remaining food was then ready to be fermented.

FERMENTATION OF LEFTOVER FOOD

The leftover food was fermented using the Wizna et al. (2009) method. As much as 100 g of restaurant and hotel food scraps were sterilized in an autoclave for 15 minutes, temperature 121 °C with 1 atm pressure, then the media was cooled to room temperature. Furthermore, mix *B. amyloliquefaciens* as an inoculum

with the dose according to the treatment (3, 4, and 5%) until homogeneous. The leftover food that had been mixed with *B. amyloliquefaciens* was then incubated with the length of incubation according to the treatment (3, 4, and 6 days) at the optimal temperature for the growth of *B. amyloliquefaciens* 40 °C. After incubation, the media was dried in an oven at 60 °C until dry to obtain a fermented product.

PREPARATION OF LEFTOVER FOOD AS FERMENTED PRODUCTS FOR ANALYSIS

Wet samples were taken by as much as 200 grams. The samples were then fermented by *B. amyloliquefaciens*, with the dose adjusted according to the treatment. They were stored in a plastic medium for as long as they were treated. The fermented results were placed in an oven to obtain dried samples. These dried samples were later milled, and the results were analyzed at the laboratory. The method for anaerobic fermentation referred to the processing method of probiotics according to Fardiaz (1987). Leftover food was dried in the oven, ground until smooth with a blender, then analyzed.

EXPERIMENTAL DESIGN

The research, a 3x3 complete randomized design factorial, consisted of two factors, and each treatment was repeated three times. Factor A is the dose of *B. amyloliquefaciens* (3, 4, and 5%), and factor B is the fermentation time (3, 4, and 5 days).

PARAMETERS

Total colony count of *Bacillus* sp. in fermented products, calculating the CFU using a method of dilutions and total plate counts as described by (Cappucino and Sherman, 1987; Hadioetomo, 1991). The fermented product of leftover food was weighed as much as 1 g for each treatment and replication. Successively diluted in 1:10 increments from 10⁻¹ to 10⁻¹³ in distilled water (13 total dilutions). Approx 10⁻¹³ dilution of fermented products of leftover food from each (1 mL) was inoculated onto Petri dishes filled with *Bacillus* sp., selective medium, and incubated at room temperature for 24 h. Water content, dry matter, crude protein, crude fiber, crude fat, ash, phosphor, and calcium were analyzed by proximate analysis (AOAC, 2016).

STATISTICAL ANALYSIS

All data obtained were analyzed by analysis of variance (ANOVA). Differences among treatments will be followed by an analysis of Duncan's multiple range test (P<0.05) (Steel and Torrie, 1991).

RESULTS AND DISCUSSION

Statistical analysis showed no interaction between the

dose of *B. amyloliquefaciens* and fermentation time

Table 1: The average content of total *Bacillus* sp., colony count in fermented products, total water content, dry matter, crude protein, crude fats, and calcium of leftover food fermented by *B. amyloliquefaciens*.

Variables	The dose of <i>B. amyloliquefaciens</i> (%)	fermentation time (Day)			Mean
		D3	D4	D5	
Total <i>Bacillus</i> sp., colony count in fermented products (log CFU mL ⁻¹)	W3	22,86	23,91	26,17	24,31 ^a
	W4	25,33	26,74	27,33	26,47 ^b
	W5	28,6	28,21	28,59	28,47 ^c
	Mean	25,60 ^a	26,29 ^b	27,36 ^c	
Total water content (%)	W3	66,39	66,64	66,24	66.42 ^a
	W4	68,28	68,34	68,78	68.47 ^b
	W5	71,14	70,95	71,26	71.11 ^c
	Mean	68,61	68,64	68,76	
Dry matter (%)	W3	33,61	33,36	33,76	33.58 ^a
	W4	31,72	31,66	31,22	31.53 ^b
	W5	28,86	29,05	28,74	28.89 ^c
	Mean	31,40	31,36	31,24	
Crude protein (%)	W3	15,24	16,11	15,94	15,76 ^a
	W4	16,16	16,49	16,6	16,42 ^b
	W5	20,39	16,72	16,37	17,83 ^b
	Mean	17,27	16,44	16,3	
Crude fat (%)	W3	17,03	15,75	16,64	16.47 ^a
	W4	17,80	17,43	15,58	16.93 ^a
	W5	18,06	19,72	20,98	19.59 ^b
	Mean	17,63	17,63	17,73	
Calcium (%)	W3	0,50	0,59	0,84	0.64 ^a
	W4	0,90	0,82	0,71	0.81 ^b
	W5	0,63	0,67	0,70	0.67 ^c
	Mean	0,68	0,69	0,75	

Note: W3 = 3% the dose of *Bacillus amyloliquefaciens*; W4 = 4% the dose of *Bacillus amyloliquefaciens*; and W5 = 5% the dose of *Bacillus amyloliquefaciens*. Fermentation time (D3 = 3 d; D4 = 4 d; and 5 d). The results of the data analysis indicate a statistically significant effect ($P < 0.05$) within the various lowercase superscripts found in the columns and rows.

($p > 0.05$) on total *Bacillus* sp. colony count in fermented products (Table 1). However, the dose of *B. amyloliquefaciens* and the fermentation time had a significant effect ($p < 0.05$) on the total colony count of *Bacillus* sp. in fermented products (Table 1). The highest total colony of *Bacillus* sp. in the fermented product was found at 5% *B. amyloliquefaciens* dose and 5 days of fermentation. The results showed that the higher the inoculum dose and the longer the fermentation time, the higher the total colony of *Bacillus* sp. in the fermented product. In this study, the increase of the total *Bacillus* sp. colony in the fermentation products

was caused by increasing the dose of *B. amyloliquefaciens* as inoculum. In this study, the total colony of *Bacillus* sp., which was getting higher as the doses of inoculum increased and fermentation time, indicates that there are spores of *B. amyloliquefaciens* in the fermented product. *B. amyloliquefaciens* spores contained in the fermented products are expected to serve as probiotics for the livestock that consume them. In this sense, fermented products are referred to as functional feed. Functional feed, in general, can be defined as all feed that positively affects an individual's health, physical appearance, and psychic state with the addition of its nutritional value (Goldberg, 1994). Some researchers have previously reported that *B. amyloliquefaciens* bacteria can be used as probiotics in poultry and improve poultry rations' health, performance, and efficiency (Zurmiati et al., 2017a, b; Hong et al., 2021).

Statistical analysis showed no interaction between the dose of *B. amyloliquefaciens* and fermentation time ($p > 0.05$) on the water content of fermented products (Table 1). Fermentation time had no significant effect ($p < 0.05$) on water content; however, the dose of *B. amyloliquefaciens* has a significant impact ($p < 0.05$) on the water content of fermented products (Table 1). The results of this study indicate that the higher the inoculum dose, the higher the water content of the fermented product. This is due to the microbial activity during the fermentation process that can increase the water content of the material; the fermentation process that produces metabolic water is an indicator of the sustainability of the fermentation process. Steinkrauss (1995) stated that during fermented soybean cake, water is produced due to the breakdown of carbohydrates by microbes. Furthermore, Rachman (1989) reported that water is one of the products of aerobic fermentation.

Statistical analysis showed no interaction between the dose of *B. amyloliquefaciens* and fermentation time ($p > 0.05$) on the dry matter of fermented products (Table 1). Fermentation time had no significant effect ($p < 0.05$) on dry matter; however, the dose of *B. amyloliquefaciens* has a significant impact ($p < 0.05$) on the dry matter of fermented products (Table 1). High inoculum doses can accelerate fermentation, and a high microbial population can provide high enzyme activity. Microorganisms have a unique role in breaking down complex compounds into simpler forms. During the fermentation process, microbes used nutrients from substrates as sources of carbon, nitrogens, minerals, and CO₂ release, as well as energy in the form of heat, which was evaporated with airborne particles created by catabolic processes that converted complex compounds into simpler ones (Astuti et al., 2017). In this study, the fermentation time did not affect the dry matter of the fermented products. This is because *B. amyloliquefaciens*, as a fermented inoculum, has achieved optimal growth

on the third day. The results of previous studies using *B. amyloliquefaciens* as an inoculum have reported different variations in the fermentation time. One factor that affects the fermentation time is the type of fermentation substrate. Optimal conditions of tapioca by-product fermentation with *B. amyloliquefaciens* is at a fermentation length of 6 days, inoculum dose of 2%, and fermentation temperature of 40 °C (Wizna et al., 2009). Further, Ramadan et al. (2017) reported that the fermentation of blood meal with *B. amyloliquefaciens* requires an optimal time of 5 days.

Statistical analysis showed no interaction between the dose of *B. amyloliquefaciens* and fermentation time ($p>0.05$) on the crude protein of fermented products (Table 1). Fermentation time had no significant effect ($p<0.05$) on crude protein; however, the dose of *B. amyloliquefaciens* has a significant impact ($p<0.05$) on the crude protein of fermented products (Table 1). The rise in the total colony count of *Bacillus* sp in fermented products caused the increase in crude protein. A high microbial population produces high crude protein as well. Jamila et al. (2009) reported that *Lactobacillus* sp. increases the number of microbes during fermentation, increasing protein synthesis and making amino acids. The growth of microbes used in fermentation increases biomass in the fermented products during the fermentation process (Jude-Ojei, 2010; Itelima et al., 2013).

Statistical analysis showed no interaction between the dose of *B. amyloliquefaciens* and fermentation time ($p>0.05$) on the crude fat of fermented products (Table 1). Fermentation time had no significant effect ($p<0.05$) on crude fat; however, the dose of *B. amyloliquefaciens* has a significant impact ($p<0.05$) on the crude fat of fermented products (Table 1). Almost all foods contain fats and oils, especially matters of animal origin. In plants, fat formation can be divided into three stages: the formation of glycerol, fatty acid molecules, and then the condensation of fatty acids with glycerol to form fat (Winarno, 2004). Lipase breaks fats into fatty acids and glycerol (Deliani, 2008). Based on the statistical analysis results in Table 1, there was an increase in fat levels at the dose of inoculum by 5%. The increase in fat levels was due to microorganisms producing microbial oils during fermentation, where microorganisms are living cells capable of producing fat. It is called an oily species and can be considered a source of oil. The oil obtained is called single-cell oil (SCO), a euphemism similar to the single-cell protein commonly used to denote proteins derived from single-cell microorganisms (Kurniati et al., 2012). Furthermore, Tandrianto et al. (2014) reported that most of the constituent masses of microbial cells are proteins, but there is also a tiny percentage of phospholipids. In this study, the analysis conducted was an analysis of coarse fats. The possibility of being dissolved in organic solvents is fat, glyceride, and volatile fatty acids, where the substance does

not include food substances but is dissolved in solvents, so-called crude fat. The increase in the fat content of fermentation is generally due to the high fatty acid content (Suparmo, 1989).

Statistical analysis showed no interaction between the dose of *B. amyloliquefaciens* and fermentation time ($p>0.05$) on the calcium content of fermented products (Table 1). Fermentation time had no significant effect ($p<0.05$) on calcium content; however, the dose of *B. amyloliquefaciens* has a significant impact ($p<0.05$) on the calcium content of fermented products (Table 1). This study's increase in the fermented products' calcium was caused by a dose of *B. amyloliquefaciens* as inoculum related to dry matter. In a lower proportion of dry matter in the fermented products, the mineral concentration increased when measured based on dry matter. This finding aligns with Adam (1990), who reported that fermentation resulted in a lower proportion of dry matter in the sample and increased concentrations of some minerals when measured by dry weight.

Statistical analysis showed no interaction between the dose of *B. amyloliquefaciens* and fermentation time ($p>0.05$) on the crude fiber of fermented products (Table 1). The dose of *B. amyloliquefaciens* and fermentation time had no significant effect ($p<0.05$) on crude fiber (Table 2). Leftover restaurant food is generally rice. The rice contains starch ranging from 81.23 to 88.835% (Othman and Oma, 2017). The starch content varies in rice, depending on its variety. The main difference in starch composition is the variation in the ratio of the two macromolecules, like amylose and amylopectin. Amylose is a linear molecule in which α -1,4 glucosidic bonds link D-glucose units, whereas amylopectin, a branched polymer, contains α -1.4 and α -1.6 bonds (Denardin et al., 2012). During fermentation, bacteria release enzymes influenced by the inducer on the substrate. The bacteria then secreted enzymes to degrade more superficial chemical structures. Bacteria consume easily digested carbohydrates first as an energy source (Anwar et al., 2008). The high availability of starch in the fermentation substrate caused the crude fiber content in the fermented product to remain because the crude fiber compound is more complex than starch. This study assumed the inoculum utilized starch as a carbon source, not as crude fiber. Microbes need a substrate such as carbon, nitrogen, and minerals (Hardini, 2010). Carbohydrates are used as an energy source for microbes during the fermentation process; the carbohydrate breakdown process occurs rapidly, especially in the early stages of fermentation, because carbohydrates are the primary energy source for microbes (Hu et al., 2010). The results of burning materials produced ash. Ash levels were closely related to the mineral content of organic and inorganic minerals. Organic matter in the material reached 95%, and the rest were ash and mineral salts. Based on the study results, it was found that

the dose of *B. amyloliquefaciens* and fermentation time did not affect ($p>0.05$) on the ash of fermented product (Table 2). According to Sudarmaji et al. (1989), ash levels are related to the mineral content in the material.

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Table 2: The average crude fiber, phosphor, and ash of leftover food fermented by *B. amyloliquefaciens*.

The dose of <i>B. amyloliquefaciens</i> (%)	Fermentation time (day)	Crude fiber (%)	Phosphor (%)	Ash (%)
3	3	9.34	1.28	7.88
	4	7.68	0.88	8.46
	5	7.11	0.81	8.68
4	3	9.84	1.16	8.49
	4	9.67	1.15	7.80
	5	10.94	1.12	8.13
5	3	10.49	1.25	8.57
	4	7.71	1.51	8.08
	5	8.80	0.64	9.75
	SE	0.98	0.31	0.81

Note: SE = Standard Error

Statistical analysis showed no interaction between the dose of *B. amyloliquefaciens* and fermentation time ($p>0.05$) on the phosphor of fermented products (Table 1). The dose of *B. amyloliquefaciens* and fermentation time had no significant effect ($p<0.05$) on phosphor (Table 2). The phosphor level depended on the content of inorganic materials in materials. The fermentation process did not affect the phosphor levels of the product fermented. The phosphor content in this research ranged from 0.64-1.51%. This content was similar to macronutrients in the restaurant waste found by Garcia et al. (2005), ranging from 0.81% to 2.07% phosphorus dryly.

CONCLUSIONS AND RECOMMENDATIONS

In conclusion, fermentation of leftover food with the dose of *B. amyloliquefaciens* 5% and a fermentation time of 3 days can increase the nutrition content of leftover food and founded the colony count of *Bacillus* sp. in fermented products as much as 28,6 log CFU mL⁻¹. Recommendation, it is necessary to conduct biological tests on poultry to determine the effect of using fermented products as functional feed for poultry.

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NOVELTY STATEMENT

There have been no reports regarding the fermentation of leftover food from restaurants and hotels with *Bacillus amyloliquefaciens*. Fermented products use *Bacillus amyloliquefaciens* as an inoculum can be used as a feedstuff; at the same time, it also has another function where the spores in the fermented product function as probiotics for livestock that consume them, so the fermented product can be called a functional feeds.

AUTHOR’S CONTRIBUTION

Wizna contributed to original ideas from research, research design, the conduct of experiments, the statistical analysis of data, and the writing and editing of articles. Zurmiati contributed to the article’s manuscript preparation, writing, and editing. Rusfidra and Robi Amizar contributed to the research design and editing of articles. Romi Andika and Muhammad Haikal contributed to research preparation, conducted research, and collected data.

CONFLICTS OF INTEREST

The all authors have declared no conflict of interest.

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