Evaluation of using multi-strain probiotics in fermented coffee husks as a potential growth promoter in poultry diets

Dian Hartatie[1], *Merry Muspita Dyah* Utami[2*], *Niati* Ningsih[2], *Aryanti Candra* Dewi[2]

[1] Department of Agricultural Production, Politeknik Negeri Jember, Jl. Mastrip PO Box 164, Jember, 68121, Indonesia

[2] Department of Animal Science, Politeknik Negeri Jember, Jl. Mastrip PO Box 164, Jember, 68121, Indonesia

Abstract. Antibiotics as growth promoters have adverse effects due to antibiotic resistance and residues in poultry products. Probiotics, as a natural alternative to antibiotics, improve animal health and growth. The growth of probiotic colonies is carried out through a fermentation process that uses a medium as a nutrient source. The study aims to validate the potential of coffee husks as a growth medium using single-strain and multi-strain probiotics, using a completely randomized design. Differences were tested using Duncan's test: T0 (control): coffee husks without probiotics; T1: coffee husks + single-strain *Bacillus sp*.; T2: coffee husks + single-strain *Lactobacillus sp*.; and T3: coffee husks + multi-strain *Bacillus sp*. and *Lactobacillus sp*. The fermentation method involved culturing the samples for 21 days and then analyzing the lactic acid content, acidity (pH), total dry matter, and total plate count (TPC). The results showed highest lactic acid (7.08 ± 0.46 log cfu/g) and TPC (9.61 ± 0.39 log cfu/g), as well as the lowest pH of 5.84 ± 0.152, were obtained in the multi-strain of *Bacillus sp*. and *Lactobacillus sp*. The total dry matter did not differ among the treatments. Fermented coffee husks have the potential as a medium for multistrain probiotics, as growth promoters.

1 Introduction

Research into poultry feed currently focuses on finding alternatives to antibiotics, including probiotic bacteria that promote growth [1]. These bacteria can be used as growth promoters because they produce enzymes that enhance metabolic processes and stabilize the gastrointestinal barrier function on the surface of the intestinal tract [2] and also improve nutrient absorption and produce amylase and protease enzymes that enhance the metabolism of nutrients into simple molecules, making them easier for the body to absorb for optimal growth and development in poultry [3][4][5].

*Corresponding author: merry.mdu@polije.ac.id

Probiotic bacteria can colonise the gastrointestinal tract, attach to the intestinal epithelium, produce antimicrobials, modulate the mucosal immune system, stimulate metabolic activity, and are non-pathogenic [6], do not change or get damaged during processing [7], and are resistant to bile and stomach acid [8].

The development and colonisation of probiotics must be supported. supported by a probiotic bacterial fermentation medium, which must also provide enough nutrients. Large amounts of probiotic bacteria can be produced using various techniques, such as creating a fermentation medium that encourages bacterial growth [9]. These media must have water, salt, micronutrients, and sources of carbon and nitrogen [10]. Probiotics have been made with fine bran medium, which is somewhat costly. On the other hand, agricultural activities, particularly the processing of plantation produce, generate substantial quantities of organic waste each year [11]. This waste takes the form of litter, fruit peels, pulp, and husks. If not managed properly, it can cause environmental pollution [12].

Zero-waste agriculture uses waste as input to maximize resource efficiency. One example is converting agricultural and plantation waste into poultry feed [13]. One way to achieve this is to convert agricultural and plantation waste into poultry feed [14]. Some of the main advantages of utilizing waste for poultry feed include: reduced feed costs through the substitution of expensive commercial raw materials with inexpensive local materials [15], adding economic value to previously unused waste, increasing feed supply chain resilience (through source diversification), and reducing emissions and waste through the waste-to-value principle [16][17]. Publications and case studies from meta-analyses show the potential for economic savings and environmental benefits when waste processing is carried out in accordance with animal feed safety standards.

Coffee processing typically yields 65% coffee beans and 35% coffee husk waste. One hectare of coffee plantation is estimated to produce 1.8 tons of fresh waste [8]. Coffee husks are a type of agricultural waste that could be used as animal feed or as a feed additive. However, there are some limitations to using coffee husks as animal feed, including their relatively low protein content, high crude fibre content, and limited mineral content (calcium and phosphorus) [9][18][19]. The following are the nutritional values of dried coffee husks: 38.61% moisture, 12.00% crude protein, 3.67% crude fat, 28.26% crude fibre, and 4.91% ash [20].

A fermentation method is used to enhance the nutritional value of coffee husks used as animal feed [21]. Numerous biochemical processes, including microbial fermentation, break down complex substances such as cellulose, lignin, and tannins, generate enzymes, and increase the availability of micronutrients [10]. Fermentation can lower some crude fibre and increase protein content while maintaining or altering mineral levels (Ca and P), due to the release of organic nitrogen and increased microbial biomass [22].

It has been demonstrated that microbial fermentation, ensiling, and bioconversion technologies increase digestibility, lower anti-nutritional factors, and improve the nutritional quality of waste [23]. According to experimental research and meta-reviews, fermented substances produced by bioconversion can replace some traditional protein or energy sources in chicken feed without reducing performance. Therefore, this approach provides two solutions: environmental sustainability and economic efficiency.

According to the literature, the use of fermented coffee husks in animal feed remains limited. Fermentation using *Bacillus sp*. effectively breaks down complex structures in the initial stage [1], while *Lactobacillus sp*. provides stability through lactic acid production [2]. Combining the two strains offers synergistic potential, but optimising the conditions is necessary to prevent microbial competition.

This study has the benefit of evaluating fermentation utilizing a double strain of Bacillus-Lactobacillus, as opposed to a single strain of *Bacillus sp*. and a single strain of *Lactobacillus sp*. The fermented coffee husk with this double strain has the potential as a growth promoter

for poultry, and an alternative to replace the antibiotic growth promoter. The combination of *Bacillus sp*. and *Lactobacillus sp*. is expected to utilize the synergy between the enzymatic capabilities of *Bacillus sp*. and the capabilities of *Lactobacillus sp*. in lactic acid fermentation and substrate stabilization. Fermented coffee husk with this double strain has not been previously studied; therefore, this research will provide new insights about poultry nutrition.

This study was conducted to obtain empirical data on fermented coffee husks with the best strains for animal feed and recommendations on the potential of fermented coffee husks as a natural growth promoter.

2 Materials and Methods

2.1. Preparation of coffee husks as a fermentation medium

The coffee husks were dried, ground using a disk mill, and weighed at 1 kg for each experimental unit. Then, 3% of the weight of the coffee husks was added in the form of molasses, along with 492.5 ml of distilled water, according to the method of [24].

The preparation of the starter culture begins with the rejuvenation of the Bacillus and Lactobacillus culture stocks. This involves taking 100 µl of the culture stock stored in glycerol at 5°C and adding it to 5 ml of MRSB medium. The mixture is then incubated for 24 hours at 37°C. After incubation, the growth of the culture is observed; positive results are evidenced by turbidity in the medium.

2.2. The treatments of fermentation

The treatment was the use of single-strain and multi-strain probiotics. The probiotics were used as follows: T0 (control): coffee husks (without probiotics), T1: coffee husks + single-strain probiotic *Bacillus sp*., T2: coffee husks + single-strain probiotic *Lactobacillus sp*., and T3: coffee husks + multi-strain probiotics (*Bacillus sp*. and *Lactobacillus sp*.). Each treatment was repeated five times.

All the ingredients are mixed until homogeneous and placed in a plastic sealer and compressed as tightly as possible to eliminate any air for oxygen. This prevents oxygen from entering and inhibiting the fermentation process. Once compacted, the plastic is sealed tightly using a vacuum sealer. The plastic is placed in a closed container to protect it from heat and rain. Storage is conducted at room temperature for 21 days according to the method [25], and the product is analysed for lactic acid content according to the method [26], acidity level (pH) measured using a pH meter that has been standardised using pH 7 and pH 4 buffer solutions. [27]. The total dry matter was determined and calculated using the proximate method, which involves evaporating all the water in the sample at a temperature of 105°C. [28], and total plate count (TPC) by [27].

2.3 Statistical analysis

The data were analyzed using analysis of variance (ANOVA) to determine the effect of treatment on the observed variables. If the ANOVA results show a significant difference ($P< 0.05$), then Duncan's Multiple Range Test (DMRT) is used to compare the differences in means between treatments. All statistical analyses were performed using statistical software SPSS version 22.

3 Results and Discussion

Antibiotics as growth promoters have been banned due to their detrimental effects, including antibiotic resistance and residues in products that are harmful to consumers [29]. Therefore, alternative growth promoters have become a strategic issue to be resolved. Probiotics can be used as an alternative to antibiotics to improve health and growth. Multiplying probiotic colonies is through a fermentation process that requires a medium to supply the nutrients of the probiotics, using coffee husks, a by-product of coffee production.

The zero-waste agriculture programme involves two actions: converting agricultural waste into feed to reduce costs and environmental pollution through circularity. This circular model is related to supply chain resilience, emission reduction, and local value creation [30]. It has also been endorsed by numerous reviews and policy studies emphasizing the importance of strategies to recover value from agricultural waste. From a zero-waste agriculture perspective, integrating waste management and utilizing waste as feed reduces the amount of waste sent to landfill sites [31][32].

The lactic acid content, pH, total dry matter, and total plate count (TPC) of the treatments are shown in Table 1. For more details, the results for each parameter are shown in a figure. Figure 1 shows the results of Lactic acid content, pH, and total plate count (TPC), and Figure 2 shows the total dry matter of the treatments.

Table 1. Lactic acid content, pH, total dry matter, and total plate count (TPC) of treatments

Parameters	T0 (control)	T1 single-strain *Bacillus sp*	T2 single-strain *Lactobacillus sp*	T3 multi-strain *Bacillus+Lactobacillus*
Lactic acid (log cfu/g)	3.27 ± 0.55^{a}	5.96 ± 0.36^{b}	6.77 ± 0.50^{c}	7.08 ± 0.46^{c}
pH	6.66 ± 0.114^{c}	6.18 ± 0.192^{b}	6.12 ± 0.193^{b}	5.84 ± 0.152^{a}
Total dry matter (%)	89.76 ± 0.937	91.13 ± 1.273	89.81 ± 0.858	90.99 ± 0.668
Total plate count (log cfu/g)	5.54 ± 0.82^{a}	8.32 ± 0.55^{c}	7.65 ± 0.39^{b}	9.61 ± 0.39^{c}

[a,b,c] Different superscripts on the same row indicate significant differences

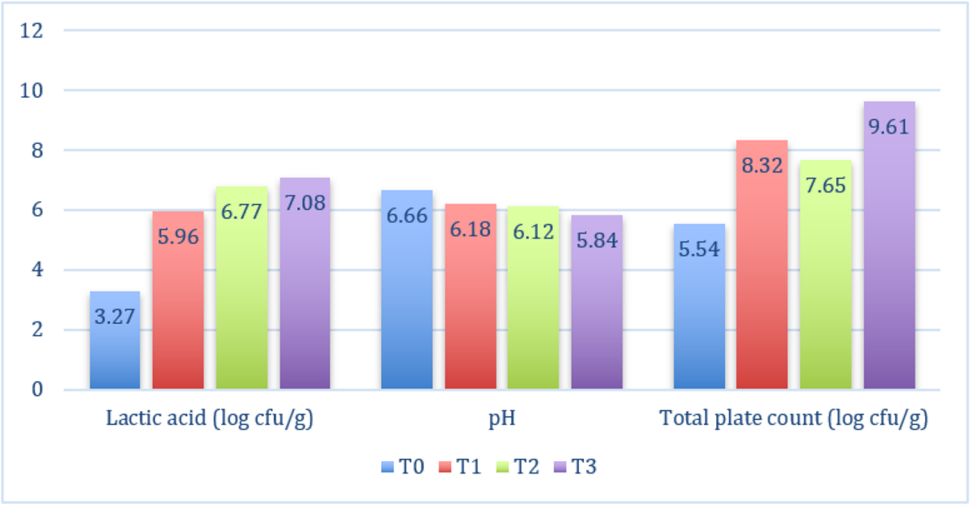

Fig. 1. Chart of Lactic acid content, pH, and total plate count (TPC) of treatments

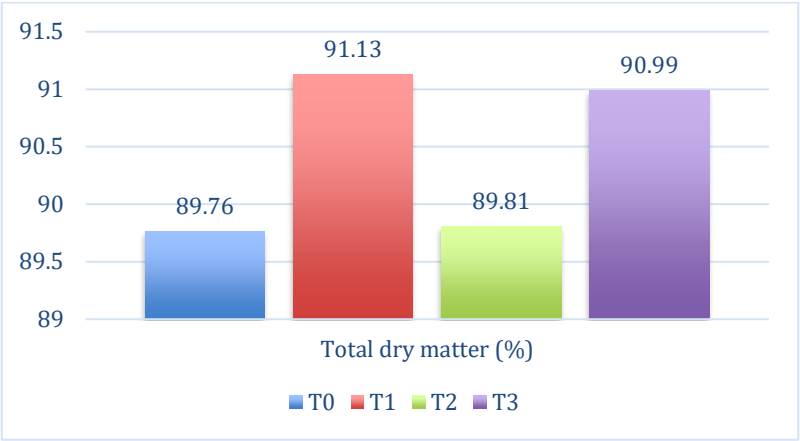

Fig. 2. Chart of total dry matter (%) of treatments

Currently, various conversion technologies have been introduced, such as enzymatic microbial fermentation, which can increase nutritional value and reduce anti-nutritional factors [10]. Several research reports indicate that fermentation treatment produces various enzymes (cellulase, protease, xylanase, and amylase) that aid the decomposition of complex components in lignocellulose substrates [33][34][35][36].

3.1 Lactic acid content

Lactic acid is formed when macromolecules, particularly carbohydrates, are broken down into simpler molecules by degradative enzymes [37][32]. Complex enzymes that play a role in the formation of lactic acid include glucokinase, fructose-1,6-bisphosphate, aldolase, glyceraldehyde-3-phosphate dehydrogenase, pyruvate kinase, and lactate dehydrogenase. The complex enzymes that play a role in the formation of lactic acid include glucokinase, fructose-1,6-bisphosphate, aldolase, glyceraldehyde-3-phosphate dehydrogenase, pyruvate kinase, and lactate dehydrogenase [38]. These enzymes convert one mole of glucose into two moles of lactic acid. Similarly, fructose can be converted into two moles of lactic acid during fermentation [39].

The lactic acid level in the treatment using *Lactobacillus sp*. was higher than that in *Bacillus sp*. An increase in lactic acid level was also observed in the multi-strain Bacillus-Lactobacillus treatment because the use of *Lactobacillus sp*. stimulated lactic acid formation in the medium that also contained *Bacillus sp*. The lowest lactic acid level was observed in the control (non-probiotic). *Lactobacillus sp*. is the main bacterium responsible for creating acidic conditions at the beginning of the fermentation process. This prevents spoilage by stopping bacteria from developing rapidly, thus maintaining feed quality. According to [24] found that an increase in *Lactobacillus sp*. is directly proportional to an increase in lactic acid content.

3.2 pH

The pH in fermentation using *Lactobacillus sp*. is lower than that of *Bacillus sp*.. Lactobacillus microbes are widely used in feed fermentation to produce lactic acid, which lowers the pH level, inhibits the growth of harmful bacteria**,** and maintains the stability of the substrate [40]. Lactic acid bacteria growth during fermentation will increase lactic acid production and result in acidic conditions characterized by a decrease in pH [41]. High lactic acid production indicates probiotic bacterial activity that causes a decrease in pH [42]. Lactobacillus is widely used in feed fermentation to produce lactic acid, which lowers the pH

level, inhibits pathogenic microbes, and maintains substrate stability [43]. This can be seen in multi-strain probiotics (T3), which have the lowest pH compared to single strains (T1 and T2) and controls (T0).

3.3 Total dry matter

Statistical analysis of the dry matter content showed that the use of probiotics did not have a significant effect (Table 1). The highest dry matter content was found in *Bacillus sp*. at 91.13%, followed by the multi-strain Bacillus-Lactobacillus at 90.99%, *Lactobacillus sp*. at 89.81% and the control at 89.76%. As shown in Table 1, there is a negative correlation between the high dry matter content in fermentation with *Bacillus sp*. and the lactic acid content. It is thought that the low lactic acid content is due to the relatively high crude fibre content of coffee husks [44]. Lactic acid bacteria that digest easily soluble carbohydrates rather than crude fibre were found to grow during the fermentation process [45]. Fermentation by *Lactobacillus sp*. produces an acidic environment that can soften cell tissue and modify fibre structure [46].

3.4 Total plate count

Total plate count testing is used to calculate the number of microbes by counting the bacterial colonies that grow on the medium [47]. The principle of this method is that the microbes will develop into visible colonies when grown on the medium [48].

The total plate count results of the fermentation showed that there were the most bacteria in the multi-strain (T3) sample, followed by the Bacillus sp. (T1) sample, which had a significantly higher number of bacteria than the Lactobacillus sp. (T1) sample. The control (T1) sample had the fewest bacteria.

Studies on the use of *Bacillus sp.* in fermentation show an increase in the content of the chemical composition (moisture content) of fermentation products. *Lactobacillus sp*. reduces moisture content through fermentative activity and relative drying, increases crude protein through bacterial growth, reduces crude fibre through softening and partial degradation, and causes slight changes in calcium and phosphorus depending on microbial metabolism and produces an acidic environment that can soften cell tissue and modify fibre structure**.**

4 Conclusion

The highest lactic acid content and total plate count (TPC), as well as the lowest pH, were obtained in multi-stain, but the total dry matter did not differ among the treatments. Coffee husk waste has the potential to provide nutrients for probiotics, leading to viability as a natural growth promoter for poultry, and can be used as an alternative to replace the antibiotic growth promoter. Additionally, using coffee husk as fermentation media can contribute to zero-waste agriculture.

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