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(Research Article)

Nutrient content of feed supplement based on fermented broiler litter with cellulolytic bacteria isolates from Bali pigs

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Abstract

Broiler litter has potential as a feed supplement due to its high crude protein and gross energy content, but its high crude fiber content and pathogenic microbes limit its use. This study aims to improve the nutrient quality of broiler litter through fermentation with cellulolytic bacterial isolates from Bali pigs, namely Bacillus licheniformis and Bacillus lentos. A completely randomized design (CRD) was used with four treatments: P1 (without bacteria), P2 (B. licheniformis), P3 (B. lentos), and P4 (combination). Anaerobic fermentation was conducted for three weeks. The results showed a significant increase (P<0.05) in crude protein from 12.46% (P1) to 21.02% (P4), a decrease in crude fiber from 13.96% (P1) to 9.45% (P4), and an increase in gross energy to 4,742.64 kcal/kg. The content of crude protein, crude fiber, ash, NFE, and crude fat did not differ significantly between treatments. In conclusion, fermentation with cellulolytic bacterial isolates from Bali pigs improved the nutrient quality of litter feed supplements for broilers.

Keywords: Broiler Litter; Bacillus licheniformis; *Bacillus lentus*; Fermentation; Feed Supplement

1. Introduction

Feed is a key factor in the success of livestock farming, accounting for 60–70% of total production costs (Siregar et al., 2018). These high costs necessitate efficiency strategies, one of which is the use of alternative feed materials based on waste. Broiler litter, a mixture of chicken manure, rice husks, feed residues, and drinking water, is one of the abundant poultry farm wastes that has not been optimally utilized. Its relatively high nutritional content, such as crude protein (16–20%) and metabolizable energy (2,200–2,400 kcal/kg), makes it a potential feed ingredient (Marjuki et al., 2015). However, its direct use remains limited due to its high crude fiber content (18–25%), which can reduce digestibility, as well as the risk of contamination by pathogenic microorganisms such as Salmonella sp., Streptococcus sp., and Escherichia coli (Widodo, 2020). On the other hand, the increasing demand for affordable and high-quality feed has driven further exploration of this waste through appropriate processing technologies. One approach that has been widely developed is fermentation, which can improve nutrient quality, reduce crude fiber content, and suppress the growth of pathogenic microorganisms (Putra et al., 2017). This process is particularly effective when involving functional microorganisms such as cellulolytic bacteria capable of degrading lignocellulosic compounds. Several studies have shown that species such as Bacillus licheniformis and Bacillus lentos can improve substrate digestibility through their enzymatic activity (Suryani et al., 2020). In a local context, isolating bacteria from the digestive tracts of indigenous Indonesian animals, such as Bali pigs, is a relevant approach because their microbiota has adapted to a high-fiber environment and exhibits high cellulolytic activity (Artawan et al., 2019). Therefore, fermentation of broiler litter with cellulolytic bacterial isolates from Bali pigs has the potential to produce high-quality feed supplements, with increased crude protein and metabolizable energy content and reduced crude fiber and microbial contamination. This study was conducted to evaluate the extent to which fermentation can improve the nutrient characteristics of broiler litter so that it is suitable for application as a safe and efficient alternative feed. Based on the above background, research on

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commercial carcass cuts from bail ducks (Anas sp.) was given water extract from the leaves of Austrasia Gangetic subsp. Micrantha through drinking water is carried out.

2. Material and Methods

2.1. Material

This study was conducted over an 8-week period at the Nutrition and Animal Feed Laboratory, Faculty of Animal Science, Udayana University. The main material used was post-harvest broiler litter waste from the faculty's closed house. This broiler litter waste was fermented using cellulolytic bacterial isolates obtained from the large intestines of Bali pigs, namely Bacillus licheniformis and Bacillus lentos, which were cultured in Nutrient Broth medium for 3 days.

2.2. Broiler litter-based feed supplement

The broiler litter-based feed supplement was made with ingredients such as yellow corn, rice bran, maggots, later, soybeans, cooking oil, table salt, and pingo, and fermented using cellulolytic bacteria from Bali pigs, namely two candidates: Bacillus lentos and/or Bacillus licheniformis.

2.3. Cellulolytic bacteria from the large intestine of Bali pigs

The bacteria used in this study are cellulolytic bacterial isolates from Bali pigs successfully isolated from the large intestine of Bali pigs, namely two bacterial candidates: Bacillus licheniformis and Bacillus lentos, which are still available at the Nutrition and Animal Feed Laboratory, Faculty of Animal Science, Udayana University, which will be cultured using Nutrient Broth medium for 3 days.

2.4. Supporting facilities and equipment for the research

The facilities and equipment used in the production of litter-based broiler feed supplements include: a digital scale with a capacity of 5 kilograms and a sensitivity of 1 gram for weighing the litter-based broiler feed supplement ingredients, a litter grinder to grind litter broiler and unprocessed materials into powder form, a knife as a tool to collect later leaves and cut them into smaller pieces, a tarp as a base for the feed supplement mixing process, and plastic as a fermentation container for the litter broiler-based feed supplement, which will be weighed afterward to determine nutrient content.

3. Methods

3.1. Research Design

The design used in this study was a completely randomized design (CRD) consisting of 4 treatments with 4 replicates, resulting in a total of 16 experimental units. The four treatments administered in each replicate and treatment consisted of feed supplements of 1 kilogram with the addition of water and bacteria at a concentration of 0.5%.

3.2. Broiler Litter fermentation process

The litter fermentation process began with the collection of litter from the Clouse House poultry house at the Faculty of Animal Science, Udayana University. Broiler litter was collected from each part of the poultry house (each corner, side, and center) with 1 kilogram from each point, totaling 16 kilograms. The broiler litter from each location was mixed (homogenized) to represent the entire CH litter. The homogenized broiler litter is sun-dried for 2 days to reduce moisture content to 10-15% and prevent mold growth before fermentation.

3.3. Production technique for broiler litter-based feed supplement

This feed supplement is made from easily accessible materials, including broiler litter, yellow corn, maggots, soybeans, cooking oil, pingo, rice bran, later, and table salt. All ingredients are dried, ground, and mixed evenly using a cross-mixing technique on a tarp until homogeneous. The mixture is then divided into 1 kg plastic bags per treatment unit, with the addition of Bali pig-derived probiotics in the form of Bacillus lentos and/or Bacillus licheniformis (depending on the treatment) and water solution. The plastic bags are sealed tightly under anaerobic conditions and naturally fermented for 3 weeks. After fermentation, the material is extruded into pellets and dried in an oven at 105°C. The final product is then analyzed to determine its nutritional content.

3.4. Production of cellulolytic bacterial culture

The cellulolytic bacterial culture used in fermenting litter-based broiler feed supplements was prepared by first dissolving *Bacillus licheniformis* or *Bacillus lentus* bacterial isolates in 0.85% NaCl solution (5–10 milliliters) with turbidity/absorbance of 0.5–0.8 at a wavelength of 650 nm. The bacterial isolate solution (10%) is then inoculated into Nutrient Broth medium and incubated anaerobically at 37.5°C for 3 days. The resulting liquid cellulolytic bacterial culture is subsequently utilized for research activities (as a fermentation starter).

3.5. Variables observed

The variables observed in this study were the nutrient content of litter-based broiler feed supplements fermented with cellulolytic bacterial isolates from Bali pigs. The observations were conducted by measuring the dry matter content (DM), organic matter content (OM), crude fiber content (CF), crude protein content (CP), crude fat content (CF), inorganic matter or odor content, nitrogen-free extract content (NFE), and finally gross energy content (GE).

3.6. Data analysis

The data obtained in this study were analyzed using analysis of variance (ANOVA). If there were significant differences between treatments (P<0.5) in the variables, a post-hoc analysis (Sastrosupadi, 2000) was performed.

4. Result and Discussion

Results on the nutrient content of litter-based feed supplements for broilers fermented with cellulolytic bacterial isolates from Bali pigs treated with P1: no bacteria (control), P2: *Bacillus licheniformis*, P3: *Bacillus lentus*, P4: a combination of Bacillus licheniformis and *Bacillus lentus*.

Table 1 Nutrient Content of Fermented Broiler Feed Supplement Litter from Cellulolytic Bacterial Isolates Originating from Bali Pigs

Variable		**Treatment (1)**			**SEM (2)**
	P1	P2	P3	P4	
Dry Matter (%)	89.25a[3]	89.80a	89.89a	89.86a	0..27107
Organic Matter (%)	89.43a	88.50a	88.74a	88.76a	0.49566
Ash (%)	10.57a	11.50a	11.26a	11.24a	0.49566
Crude Fiber (%)	13.96b	11.05a	9.85a	9.45a	0.85505
NFE (%)	55.22a	51.21a	53.63a	51.17a	2.06115
Crude Fiber (%)	12,46a	16.72b	17.40b	21.02c	1.16078
Extract Ether (%)	7.78a	9.53a	7.86a	7.13a	1.12727
Gross energy (Kcal/gr)	4.370.86a	4.655.82b	4.656.89b	4.742.64c	15.1908

Note

- Treatments
  P1: Fermented feed supplement without bacterial isolates (using only added water)
  P2: Fermented feed supplement with *Bacillus licheniformis* bacteria.
  P3: Fermented feed supplement with *Bacillus lentus* bacteria.
  P4: Fermented feed supplement using a combination of *Bacillus licheniformis* and *Bacillus lentus* bacteria.
- Standard Error of the Treatment Means.
- Values with different letters on the same row indicate significant differences (P<0.05).

5. Dry Matter (DM)

The dry matter (DM) content of the fermented feed supplement did not show any statistically significant differences (P>0.05), with values ranging from 89.25% to 89.86%. Treatment P3 (fermentation with Bacillus lentos) recorded the highest value (89.89%), an increase of 0.72% compared to the control (P1), followed by P4 (0.68%) and P2 (0.06%).

This increase indicates that fermentation occurred efficiently without causing nutrient loss due to leaching. The activity of cellulolytic bacteria helps break down complex fibers into simpler forms without reducing the dry matter content. Haryati et al. (2021) stated that microbial fermentation maintains the stability of feed nutrients. Research by Rahmah et al. (2022) also found that Bacillus sp. can improve the quality of fermented material without affecting moisture content. Additionally, Syahrir et al. (2020) revealed that local microorganisms support feed storage capacity and physical stability through controlled fermentation.

5.1. Organic Matter (OM)

The organic matter (OM) content in the fermented feed supplement showed no statistically significant difference (P>0.05), with the highest value found in the control (P1) at 89.43% and the lowest in P4 at 88.76%. Fermentation treatments showed a decrease: P2 decreased by 0.93%, P3 by 0.69%, and P4 by 0.75% compared to the control. This decrease is likely due to microbial activity breaking down complex organic compounds into simpler forms and partially degrading them into gas or fermentation byproducts. According to Yanti et al. (2021), the fermentation process can reduce organic matter content due to the transformation of volatile compounds. Sari and Khotimah (2022) also explain that anaerobic fermentation by Bacillus spp. produces secondary metabolites that affect BO content. A similar observation was made by Anam et al. (2020), who noted that microbial activity during incubation can reduce total organic matter due to microbial respiration processes.

5.2. Inorganic Matter (Ash)

The ash (inorganic) content in the feed supplement showed no statistically significant differences (P>0.05), with the highest value found in the control (P1) at 10.57% and the lowest in P4 (9.45%). Fermentation treatment caused a sequential decrease in ash content: P2 decreased by 10.11%, P3 by 6.64%, and P4 by 10.60% compared to the control. This decrease is likely due to microbial activity during fermentation breaking down bound minerals into soluble forms, some of which dissolved with water and gas during the incubation process. According to Saputro et al. (2021), fermentation can affect mineral content due to enzymatic interactions between microbes and substrates. Fitriani and Wibowo (2020) also reported that fermentative microbial activity can cause the degradation of certain inorganic compounds, depending on the duration and conditions of fermentation. A study by Huda et al. (2023) supports this finding, indicating that some mineral compounds decrease after fermentation because they are utilized by microorganisms as cofactors for metabolic enzymes.

5.3. Crude Fiber (CF)

Crude fiber content showed no significant difference (P>0.05), with the highest value in the control (P1) at 14.26% and the lowest in P3 (13.12%). Fermentation treatment reduced CF: P2 decreased by 6.38%, P3 by 8.00%, and P4 by 5.12% compared to the control. This decrease indicates the activity of cellulolytic microbes in breaking down complex fiber into soluble compounds. Fitriyani *et al.* (2022) noted that fermentative bacteria can reduce CR through lignocellulose degradation. Lestari's study (2021) also supports that fermentation efficiently reduces the crude fraction of feed.

5.4. Nitrogen-Free Extract (NFE)

The NFE content showed no statistically significant difference (P>0.05), with the highest value in the control (P1) at 55.22% and the lowest in P4 at 51.17%. Treatments P2, P3, and P4 each showed a decrease of 7.26%, 2.88%, and 7.33%, respectively, compared to the control. This decrease was attributed to the utilization of carbohydrates by microorganisms as an energy source during fermentation. According to Putra *et al.* (2020), the fermentation process reduces NFE due to the conversion of glucose and soluble compounds into organic acids. This result is supported by Rahayu and Pamungkas (2022), who found that fermentative bacteria consume the non-fiber fraction as the primary substrate for fermentation.

5.5. Crude Protein (CP)

Crude protein levels showed significant differences (P<0.05) between treatments. The highest value was found in P4 (21.02%), an increase of 68.70% compared to the control (P1: 12.46%). P2 and P3 increased by 34.14% and 39.62%, respectively. This increase was due to microbial growth during fermentation, which produced microbial protein. According to Sari et al. (2023), fermentation increases protein content through microbial biomass synthesis. Pratama and Yulianti (2020) also reported an increase in total nitrogen in fermented feed due to microbial enzymatic activity.

5.6. Extract Ether (EE)

Extract Ether/EE or crude fat content did not show significant differences (P>0.05), with the highest value in P2 (9.53%) and the lowest in P4 (7.13%). Compared to the control (7.78%), P2 showed an increase of 22.5%, while P3 and P4 decreased by 1.93% and 8.35%, respectively. The increase in P2 is likely due to enhanced partial lipolytic activity, while the decrease in P4 is attributed to the use of lipids as a microbial energy source. According to Zulfikar et al. (2021), variations in fat content are influenced by lipid metabolism during fermentation. Research by Ningsih (2020) indicates that fats tend to degrade during intensive fermentation.

5.7. Gross Energy (GE)

Gross energy showed a significant difference ($P<0.05$) between treatments. The highest value was in P4 (4742.64 kcal/kg), an increase of 8.52% from the control (P1: 4370.86 kcal/kg). P2 and P3 also increased by 6.52% and 4.01%, respectively. This increase in gross energy is likely due to the increase in the fraction of fermented crude protein and NFE that are easily digestible. These results are consistent with the study by Wibowo et al. (2023), which stated that an increase in GE correlates with an increase in the fraction of soluble organic matter during fermentation. The study by Lazuardi (2021) also supports that fermentation increases the energy value of feed by reducing the fraction of crude fiber.

6. Conclusion

Based on the results of the study, the conclusions obtained in this study are as follows

- Fermentation of broiler litter waste using cellulolytic bacterial isolates from Bali pigs can increase crude protein content and gross energy content, as well as reduce crude fiber content.
- Fermentation using a combination of cellulolytic bacteria from Bali pigs, namely Bacillus licheniformis and Bacillus lentos, produces higher crude protein and gross energy content and reduces crude fiber content compared to fermentation using Bacillus licheniformis or Bacillus lentos bacteria.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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