



Addition of Fermented Biomass Combination of Pineapple Waste and Indigofera Leaves in Broiler Rations on Nutrient Digestibility in Vitro and In Vivo

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ABSTRACT. This study aimed to evaluate the effect of adding fermented biomass from a combination of pineapple waste and Indigofera leaves on nutrient digestibility in broiler chickens, both in vitro and in vivo. The materials used included 21-day-old broiler chickens, finisher-phase broiler rations, pineapple waste, and Indigofera leaves. A completely randomized design (CRD) was applied, consisting of five treatments with three replications each. The treatments included different levels of fermented biomass addition to the broiler rations: P1= 100% pineapple waste, P2= 98% pineapple waste + 2% Indigofera, P3= 96% pineapple waste + 4% Indigofera, P4= 94% pineapple waste + 6% Indigofera, and P5= 92% pineapple waste + 8% Indigofera. The results showed that the addition of fermented biomass from pineapple waste and Indigofera leaves had a significant effect ($P < 0.05$) on increasing crude protein digestibility and crude fiber digestibility. In conclusion, incorporating fermented biomass of pineapple waste and Indigofera into broiler rations improves protein and fiber digestibility, with the optimal combination being 92% pineapple waste + 8% Indigofera

Keywords: Indigofera, nutrient digestibility, pineapple waste, probiotic

INTRODUCTION

High broiler productivity can be achieved by maintaining digestive tract health, ensuring optimal nutrient absorption from feed. One effective way to improve gut health is by incorporating probiotics into broiler rations. Probiotics are beneficial microbes that help balance intestinal microflora (Safitri et al., 2019). They work by enhancing gut microbial balance and increasing the population of beneficial microbes (Luthfiana et al., 2015). Additionally, probiotics produce enzymes such as amylase, protease, and lipase, which aid in the digestion of specific nutrient substrates and improve nutrient absorption (Haryati, 2011).

A promising alternative probiotic source is fermented biomass derived from pineapple peel and Indigofera leaves, utilizing *Lactobacillus* bacteria from yogurt fermentation. Pineapple peel contains essential nutrients such as protein, glucose, and fructose, which serve as substrates for fermentation (Andriani, 2013). Indigofera leaves (*Indigofera* sp.) are rich in protein and have a low non-protein nitrogen (NPN) content, making them an ideal food source for *Lactobacillus* bacteria during fermentation (Palupi et al., 2014).

Fermenting pineapple peel and Indigofera leaves produces biomass and supernatant, which are incorporated into broiler rations. The liquid fermented biomass contains *Lactobacillus bulgaricus*, which enhances nutrient absorption in the digestive tract. Palupi et al. (2020) reported that

fermented biomass from pineapple peel and Indigofera leaves contained 8.4×10^8 CFU/mL of *Lactobacillus* bacteria. The introduction of *Lactobacillus* into broiler feed has been shown to lower intestinal pH, increase beneficial bacteria populations (e.g., lactic acid bacteria (LAB)), suppress pathogenic bacteria growth, and improve digestive tract function (Kurniasih et al., 2019). Furthermore, *Lactobacillus* probiotics promote intestinal villi development, increasing their height and density, which enhances nutrient absorption efficiency (Hartono et al., 2016).

The addition of fermented biomass from pineapple peel and indigofera leaves is expected to improve nutrient digestibility in broiler rations. This study aims to determine the optimal composition of pineapple peel and Indigofera leaves as biomass constituents that have potential as probiotic sources in broiler feed.

MATERIALS AND METHODS

Research Material

This study used 15 Lohman strain male broilers, aged 20 days, along with pineapple peels, Indigofera leaves, and commercial finisher rations. The pineapple peel was sourced from pineapple jam production waste, while Indigofera leaves were harvested from the Poultry Experimental Cage, Department of Animal Husbandry Technology and Industry, Sriwijaya University. The commercial finisher ration used was Hi-Pro-Vite 591, a non-antibiotic growth promoter (AGP) feed, purchased from PT Charoen Pokphand Indonesia. The composition of the ration is detailed in Table 1. For

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proximate analysis, the study utilized: Reagents: Distilled water (aquadest), concentrated H₂SO₄, 40% NaOH, mixed catalyst (CuSO₄ and K₂SO₄), 0.1 N NaOH, mixed indicator, 3.25% NaOH, 1.25% H₂SO₄, acetone, 0.1 N H₂SO₄, and ice cubes. For the in vitro digestibility test, the following were used: Reagents: 90 mL HCl, 1 L distilled water, 0.2% pepsin solution (1 L), and water.

The study used 15 metabolic cages, each equipped with feed and water containers. For liquid fermentation, the following equipment was used: Plastic bags, grinding machine, rubber bands, weighing scales, stove, steaming pot, jars, paper, stirrers, and isolation materials. For proximate analysis, the tools included: oven, analytical balance, filter paper, crucibles, tweezers, desiccator, spatula, furnace, glass funnel, Büchner funnel, deconstruction flask, pipettes (measuring and dropper), boiling stone, hot plate, beaker glass, measuring cup, Erlenmeyer flask, and label paper. For in vitro digestibility measurement, the following were used: Beaker glass, stirrer, Erlenmeyer flask, vial tube, shaker water bath, and filter paper.

Table 1. Nutrient content of broiler ration

Feed substances	Feed Hi-Pro Vite 591 (%)
Water content (max.)	13.0
Protein	18.0-20.00
Fat (min.)	3.0
Crude (max.)	6.0
Ash (max.)	7.0
Calcium (min.)	0.90
Phospor (min)	0.60

Source: PT. Charoen Pokphand Indonesia

Research Design

This study employed a completely randomized design (CRD) with five treatments and three replicates per treatment. The treatments consisted of fermented biomass from a combination of pineapple peel and Indigofera leaves incorporated into the broiler ration, with the following compositions:

- P1 = 100% pineapple peel biomass
- P2 = 98% pineapple peel biomass+ 2% Indigofera leaves
- P3 = 96% pineapple peel biomass+ 4% Indigofera leaves
- P4 = 94% pineapple peel biomass+ 6% Indigofera leaves
- P5 = 92% pineapple peel biomass+ 8% Indigofera leaves

This design allowed for a comprehensive evaluation of the effects of different Indigofera

inclusion levels on broiler performance and nutrient digestibility.

Fermentation Process

The fermentation process followed the method of [Nurhayati and Berliana \(2014\)](#) with modifications, incorporating Indigofera leaves at varying levels based on the treatment groups. The fermentation procedure was as follows: Substrate Fermentation: The fermentation was conducted for 72 hours. Separation of Biomass and Supernatant: After fermentation, the biomass and supernatant were separated through filtration. Processing of Biomass: The biomass was first weighed in a wet state. It was then dried before being incorporated into the broiler ration. Incorporation into Ration: The dried biomass was added to the broiler ration at a 6% inclusion rate of the total ration. The mixture was stirred until homogeneous. Digestibility Measurement: Finally, in vitro and in vivo digestibility assessments were performed.

In Vitro Digestibility Measurement

The in vitro digestibility of broiler rations was assessed using the [Tilley and Terry \(1963\)](#) Stage 2 Method, which evaluates pepsin digestibility.

Preparation of Pepsin Solution

The pepsin solution used in the [Tilley and Terry \(1963\)](#) Stage 2 Method was prepared using the following steps: Preparation of HCl Solution: 6.1 mL of HCl was dissolved in 1 litre of distilled water (aquades) to create an acidic environment. Preparation of Pepsin Solution: 0.2 g of pepsin was added to 1 litre of distilled water and mixed until homogeneous. Heating and Mixing: The HCl solution was heated on a hot plate at 420-450°C. The pepsin solution was then slowly added to the heated HCl solution while stirring to ensure complete dissolution.

In Vitro Measurement

Weighing the Ration Sample: Weigh 5 g of broiler ration, ensuring the known content of dry matter (DM), crude protein (CP), and crude fiber (CF). Add 0.30 g (6% of the ration) of biomass obtained from the liquid fermentation of pineapple peel and Indigofera leaves. Preparation for Digestion: Place the mixture into a vial bottle. Add 100 ml of pre-warmed pepsin solution (heated to 420-450°C). Incubation Process: Place the vial bottle in a water shaker bath for 16 hours to simulate digestive conditions. Filtration and Residue Collection: After 16 hours, remove the vial

bottle from the water shaker bath and let it stand for 15 minutes to allow the residue to settle. Slowly filter the supernatant using pre-weighed filter paper. Rinse the vial bottle with acetone to ensure complete transfer of residues. Final Analysis: The remaining residue will be analysed for dry matter, crude protein, and crude fiber content. These values will be used to determine the percentage of digestibility of the fermented biomass of pineapple peel and Indigofera leaves.

In Vivo Digestibility Measurement

Preparation of Metabolic Cages and Equipment: Clean the metabolic cages thoroughly and spray with a disinfectant solution to eliminate potential disease sources. Clean the feed and drink containers before placing them inside the metabolic cages. **Preparation of Experimental Rations:** Weigh 6 g of biomass from liquid fermentation of pineapple peel and Indigofera leaves (equivalent to 6% of the daily ration consumption). Add the biomass to the broiler ration according to each treatment and mix well until homogeneous.

Feeding Process: Broiler chickens were initially fasted for 8 hours before being given the treatment ration. After fasting, rations and drinking water were provided ad-libitum (free access). The amount of given and remaining rations were weighed to calculate total ration consumption during the study.

Excreta Collection: Excreta collection started when broilers reached 20 days old and were housed in metabolic cages. The excreta expelled into the reservoir was sprayed every 2 hours with 0.1 N H₂SO₄ solution to preserve nitrogen content. Excreta collection continued for 3 days, with samples collected every 24 hours. Collected excreta were then dried and weighed to determine the daily excreta weight (Guzman-Cedillo et al., 2017). **Proximate Analysis of Excreta:** The dried excreta were analyzed for dry matter (DM), crude protein (CP), and crude fiber (CF). The obtained data were used to calculate the digestibility of dry matter, crude protein, and crude fiber.

Parameters Observed

The parameters observed in this study included: pH of the small intestine measurement was conducted by feeding the chickens for six hours, followed by slaughtering. After slaughter, the small intestine was separated from the digestive organs, and its digesta was removed. The pH of the small intestinal digesta was then measured using a pH meter (Rahmawati et al., 2014). Dry matter digestibility was determined using in vitro and in

vivo methods, following Tillman et al. (2005). It was calculated as the difference between the dry matter content of the ration consumed and the dry matter excreted in the feces. Crude protein digestibility was analysed in vitro and in vivo, referring to Tillman et al. (2005). It was calculated based on the difference between the crude protein in the ration consumed and the crude protein excreted in the feces. Crude fiber digestibility was assessed in vitro and in vivo, based on Tillman et al. (2005). It was determined by measuring the difference between the crude fiber content in the ration and that excreted in the feces.

$$\text{DMD} = \frac{\text{DM intake feed} - \text{DM excreta}}{\text{DM intake feed}} \times 100\%$$

$$\text{DMD} = \frac{\text{CP intake feed} - \text{CP excreta}}{\text{CP intake feed}} \times 100\%$$

$$\text{DMD} = \frac{\text{CF intake feed} - \text{CF excreta}}{\text{CF intake feed}} \times 100\%$$

Data Analysis

The data were analyzed using analysis of variance (ANOVA) following a completely randomized design (CRD). If a significant difference was observed among treatments, the analysis was further examined using the Duncan's Multiple Range Test (DMRT) to determine differences between treatment means (Steel and Torrie, 1991).

RESULTS AND DISCUSSION

pH of the Small Intestine

The impact of feeding fermented biomass of pineapple peel and Indigofera leaves on the pH of the small intestine in broiler chickens is presented in Table 2. The analysis of variance results indicates that the use of pineapple peel biomass and Indigofera leaves had a significant effect ($P < 0.05$) on the pH level of the small intestine in broiler chickens. The higher the proportion of Indigofera leaves in the substrate composition, the lower the pH of the small intestine. This reduction in pH is attributed to the increased metabolic activity of bacteria, driven by the availability of nitrogen sources in the substrate, leading to higher lactic acid production. LAB (lactic acid bacteria) require nitrogen for optimal growth (Ayad et al., 2020), and lactic acid a byproduct of LAB metabolism is known for reducing pH levels (Abedi and Hashemi, 2020).

Table 2. Small intestine pH values during the study

Treatment	Small intestine pH levels
P1	6.83 ^a ±0.38
P2	6.47 ^a ±0.88
P3	5.78 ^b ±0.18
P4	5.22 ^b ±0.33
P5	5.13 ^b ±0.36

Note: P1 (100% pineapple skin), P2 (98% pineapple skin + 2% Indigofera leaves), P3 (96% pineapple skin + 4% Indigofera leaves), P4 (94% pineapple skin + 6% Indigofera leaves), P5 (92% pineapple skin + 8% Indigofera leaves). The numbers followed by the same letter in the same column means not significant at 5% level (DMRT).

The DMRT revealed that P1 and P2 had significantly different effects ($P < 0.05$) compared to P3, P4, and P5. The greater the concentration of LAB, the lower the pH in the small intestine. The addition of LAB results in a more acidic digestive tract, reducing the pH levels (Widodo et al., 2015). This decrease in pH is particularly beneficial as LAB thrive in acidic conditions, whereas pathogenic bacteria prefer neutral environments (Imam et al., 2018). A more acidic small intestine environment inhibits the growth of pathogenic bacteria, leading to better digestive tract health and enhanced nutrient digestibility. This ultimately improves the efficiency of nutrient absorption in the small intestine (Rahmawati et al., 2014).

Dry Matter Digestibility

The average in vitro dry matter digestibility of broiler rations with fermented biomass from pineapple peel and Indigofera leaves ranged from 75.42% to 75.62%. The composition of the substrate in the fermented biomass did not significantly affect the dry matter digestibility value of broiler rations. This finding suggests that the ability of LAB from different substrate compositions of pineapple peel and Indigofera leaves was similar in digesting dry matter under in vitro conditions. Research by Palupi et al. (2020) reported that fermented biomass of pineapple peel and Indigofera leaves contained LAB concentrations ranging from 6.5×10^4 to 2.3×10^8 CFU/mL. It is suspected that the fermented biomass used in in vitro digestion did not contain LAB capable of utilizing these prebiotics effectively. As a result, there was no increase in metabolic activity related to dry matter digestion in vitro. Patterson and Burkholder (2003) stated that prebiotics selectively enrich specific beneficial bacteria in limited amounts and modify gut microbiota composition and activity.

The analysis of variance results indicates that the addition of biomass from liquid

fermentation of pineapple peel and Indigofera leaves had no significant effect ($P > 0.05$) on in vitro dry matter digestibility. However, the treatment had a significant effect ($P < 0.05$) on in vivo dry matter digestibility. The effect of the treatment on dry matter digestibility values is presented in Table 3.

Table 3. Average dry matter digestibility (DMD) values obtained in the study

Treatment	Dry matter digestibility in vitro (%)	Dry matter digestibility in vivo (%)
P1	75.42±0.66	79.02 ^a ±2.53
P2	75.42±0.29	78.52 ^a ±3.54
P3	75.42±0.32	78.33 ^a ±3.61
P4	75.53±0.75	80.23 ^a ±3.57
P5	75.62±0.61	83.03 ^b ±3.63

Note: P1 (100% pineapple skin), P2 (98% pineapple skin + 2% Indigofera leaves), P3 (96% pineapple skin + 4% Indigofera leaves), P4 (94% pineapple skin + 6% Indigofera leaves), P5 (92% pineapple skin + 8% Indigofera leaves). The numbers followed by the same letter in the same column means not significant at 5% level (DMRT).

The average in vivo dry matter digestibility of broiler rations supplemented with fermented biomass from pineapple peel and Indigofera leaves ranged from 79.02% to 83.03%. The analysis of variance indicated that the addition of fermented biomass had a significant effect ($P < 0.05$) on the in vivo dry matter digestibility of broiler rations. The variation in biomass composition led to differences in dry matter digestibility, with an increasing proportion of Indigofera leaves in the fermentation substrate resulting in higher dry matter digestibility. This increase in digestibility was consistent with improvements in crude protein and crude fiber digestibility. The high digestibility of dry matter is influenced by the organic and inorganic components in the feed, including carbohydrates, proteins, fats, vitamins, and minerals (Hamdan et al., 2017). However, a study by Abeddargahi et al. (2022) reported that the inclusion of up to 20% fermented soybean meal as a prebiotic in broiler rations did not significantly affect the dry matter digestibility in the finisher phase.

The higher inclusion of Indigofera leaves in the fermented biomass composition of pineapple peel and Indigofera leaves resulted in a higher dry matter digestibility of broiler rations. This increase was attributed to the greater availability of protein sources for lactic acid bacteria (LAB) growth, which enhanced LAB metabolism in aiding the digestion of organic matter in vivo.

The increase in LAB concentration leads to greater mucus production in the intestine, which in turn slows down the digesta rate. As a result, nutrient absorption is optimized since the ration remains in the digestive tract for a longer period. Dry matter digestibility is closely linked to organic matter digestibility, as a significant portion of dry matter consists of organic components, and organic matter retention is influenced by dry matter retention. According to [Rompas et al. \(2016\)](#), dry matter digestibility is determined by the digestibility of its individual components, such as protein, carbohydrates (nitrogen-free extract and crude fiber), fat, and ash. Several studies have reported that probiotics provide beneficial effects on the body's antioxidative capacity, digestive enzymes, and nutrient absorption, while also reducing cell apoptosis, thereby promoting gut health and broiler production performance ([Bay et al., 2017](#); [Rodjan et al., 2018](#); [Wu et al., 2019](#); [Wang et al., 2021](#)).

Crude Protein Digestibility

The results of the analysis of variance indicated that the addition of biomass from the liquid fermentation of a combination of pineapple peel and *Indigofera* leaves had a significant effect ($P < 0.05$) on crude protein digestibility both in vitro and in vivo. The effect of the treatment on crude protein digestibility values is presented in Table 4.

The increase in protein digestibility of the fermented combination of pineapple peel waste and *Indigofera* leaves is expected due to the enhanced performance of protease enzymes, which hydrolyse proteins into simpler molecules, facilitating digestion and absorption in the digestive tract. The composition of the fermented biomass significantly influences small intestine pH higher inclusion of *Indigofera* leaves in the fermentation substrate results in lower small intestine pH. This environment enhances the activity of protease enzymes, optimizing protein breakdown and nitrogen production. Protease enzymes in pancreatic secretions function efficiently at low pH, breaking down proteins into amino acids, which are then absorbed by the small intestine ([Svendsen, 2016](#)). Additionally, pineapple contains bromelain, a protease enzyme capable of breaking down proteins ([Noviandi et al., 2017](#)).

The digestibility value of crude protein increased due to the higher inclusion of *Indigofera* leaves, which also led to an increase in LAB concentration in the fermented biomass. The fermentation duration and higher *Indigofera* levels further promoted LAB growth. Research by [Palupi](#)

[et al. \(2020\)](#) reported that increasing *Indigofera* leaf levels to 8% in the fermentation substrate resulted in LAB concentrations reaching 2.3×10^8 CFU/mL. The LAB produced from fermented pineapple peel and *Indigofera* leaves plays a crucial role in improving crude protein digestibility in broiler rations. The addition of LAB to the ration enhances protease enzyme activity, which facilitates protein digestion. These bacteria can survive in the digestive tract, inhibiting the growth of pathogenic and harmful bacteria ([Usman et al., 2018](#)). This finding aligns with [Mubarak et al. \(2018\)](#), who suggested that probiotics increase the acidity of the digestive tract, making it inhospitable for pathogenic bacteria. Consequently, proteins are efficiently digested and absorbed by the intestines. Probiotics contribute to digestion by producing protease enzymes, which break down proteins into amino acids, leading to better protein utilization and energy absorption compared to digestion without probiotic supplementation.

Table 4. Average crude protein digestibility (CPD) values obtained in the study

Treatment	In vitro digestibility of crude protein (%)	In vivo digestibility of crude protein (%)
P1	74.55 ^a ±0.82	76.58 ^a ±3.09
P2	75.62 ^a ±0.44	77.22 ^{ab} ±3.55
P3	77.04 ^b ±0.31	78.31 ^b ±3.49
P4	79.18 ^b ±0.81	81.96 ^c ±2.97
P5	80.37 ^c ±0.36	85.56 ^c ±3.01

Note: Different superscripts in the same column indicate significant differences ($P < 0.05$), P1 (100% pineapple peel), P2 (98% pineapple peel + 2% *Indigofera* leaves), P3 (96% pineapple peel + 4% *Indigofera* leaves), P4 (94% pineapple peel + 6% *Indigofera* leaves), P5 (92% pineapple peel + 8% *Indigofera* leaves). The numbers followed by the same letter in the same column means not significant at 5% level (DMRT).

Crude Fiber Digestibility

The effect of fermented biomass of pineapple peel and *Indigofera* leaves on crude fiber digestibility is presented in Table 5. The results of variance analysis indicated that the addition of liquid fermented biomass had a significant effect ($P < 0.05$) on crude fiber digestibility, both in vitro and in vivo. This effect is attributed to the variation in substrate composition used to produce fermented biomass. Higher levels of *Indigofera* leaves in the substrate contributed to an increase in nitrogen sources, which enhanced LAB growth. According to [McNaught and MacFie \(2000\)](#), probiotic microbes from *Lactobacillus* species, which produce lactic acid, can also synthesize cellulase enzymes that aid digestion. These enzymes help break down crude fiber, which is otherwise difficult

to digest in the poultry digestive tract. Additionally, as the amount of LAB in the ration increases, it enhances feed metabolism and improves the digestive process, thereby facilitating nutrient digestion and absorption in poultry (Nurhayati, 2014).

Table 5. Average crude fiber digestibility values (CFD) obtained in the study

Treatment	In vitro crude fiber digestibility (%)	In vivo crude fiber digestibility (%)
P1	73.91 ^a ±0.44	85.65 ^a ±1.85
P2	74.68 ^{ab} ±0.41	85.15 ^a ±2.35
P3	74.90 ^b ±0.79	85.02 ^a ±2.48
P4	75.20 ^b ±0.53	85.92 ^a ±2.52
P5	75.63 ^b ±0.91	87.66 ^b ±2.75

Note: P1 (100% pineapple peel), P2 (98% pineapple peel + 2% Indigofera leaves), P3 (96% pineapple peel + 4% Indigofera leaves), P4 (94% pineapple peel + 6% Indigofera leaves), P5 (92% pineapple peel + 8% Indigofera leaves). The numbers followed by the same letter in the same column means not significant at 5% level (DMRT).

The variation in substrate composition in the fermentation of pineapple peel and Indigofera leaves did not result in significant differences in the crude fiber digestibility of broiler rations in this study. This is likely because the amount of LAB produced was similar across all treatments, leading to consistent cellulase enzyme activity, which maintained a similar ability to digest crude fiber in the digestive tract. According to Palupi et al. (2020), fermented biomass from a combination of pineapple peel and Indigofera leaves contained 8.4×10^8 CFU/mL of *Lactobacillus* bacteria. *Lactobacillus* species play a crucial role in improving broiler performance by: enhancing digestive function increasing the bioavailability of micronutrients modulating intestinal microflora boosting immunomodulation improving overall broiler health (Lee et al., 2010; Kim et al., 2019; Palupi et al., 2023).

The average crude fiber digestibility from the addition of fermented biomass from pineapple peel and Indigofera leaves in vivo ranged from 85.65% to 87.66%. These results are higher compared to previous studies, such as: Dara (2016), who reported that using fermented soybean juice pulp with *Neurospora cytophila* in broiler rations resulted in crude fiber digestibility of 53.03%. Kurniasih et al. (2019), who found that Dayak onion extract as a prebiotic in broiler rations produced crude fiber digestibility of 21.24-24.54%. The higher crude fiber digestibility observed in this study is likely due to the symbiotic role of the fermented biomass of pineapple peel and

Indigofera leaves. This symbiosis functions as both a probiotic and prebiotic, contributing to: Providing LAB nutrients in the digestive tract Enhancing digestive enzyme activity According to Dev et al. (2020), supplementation of 0.2% MOS (mannan-oligosaccharides) along with LAB at a dose of 10^6 CFU/g significantly improved growth performance, serum biochemistry, antioxidant profile, health index, meat quality, and lipid oxidative stability in broilers. Furthermore, Such et al. (2021) stated that symbiotic supplementation in broiler feed can increase the *Lactobacillus* population while reducing the Enterobacteriaceae family, improving gut health and digestion efficiency.

CONCLUSION

The results demonstrated that the addition of fermented biomass from pineapple peel and Indigofera leaves in the ration significantly increased the digestibility of dry matter, crude protein, and crude fiber both in vitro and in vivo. The optimal combination for improved digestibility was found to be 92% pineapple peel and 8% Indigofera leaves. This composition showed significant improvements: In vitro protein digestibility increased by 7.80%. In vivo protein digestibility increased by 11.72%. In vitro crude fiber digestibility increased by 2.33%, However, in vivo crude fiber digestibility was not significantly improved. The findings indicate that the addition of pineapple peel and Indigofera leaves in the ration, particularly in the optimal 92:8 ratio, plays an essential role in enhancing nutrient digestibility, especially protein. However, while in vitro crude fiber digestibility showed improvement, the in vivo results did not exhibit the same trend, suggesting that other factors, such as microbial populations or digestive enzyme activity, may play a role in the effectiveness of fiber digestion in the live birds.

REFERENCE

- Abeddargahi, F., Kuhi, H.D., Rafiei, F., Roostaie-Alimehr, M., Takalu, Z., RH Sajedi, R.H., Mohammadpour, F. 2022. The effect of probiotic and fermented soybean meal based on *Bacillus subtilis* spore on growth performance, gut morphology, immune response and dry matter digestibility in broiler chickens. *Italian Journal of Animal Science*. 21(1), 1642-1650.
- Abedi, E., Hashemi S.M.H. 2020. Lactic acid production – producing microorganisms and

- substrates sources-state of art. *Heliyon*. 6(10). e04974.doi:10.1016/j.heliyon.2020.e04974.
- Andriani, R.D. 2013. Pemanfaatan limbah buah nenas sebagai media pertumbuhan *xanthophyllomyces dendrorhous* untuk produksi lipid. *Jurnal Teknologi Pertanian*. 14(3), 193-200.
- A.O.A.C. 2005. Official Method of Analysis of The Association of Official Analytical Chemists. 18th edition. Published by The Association of Official Analytical Chemists, Benjamin Franklin Station. Washington D.C.
- Ayad, A.A., Deiaa, A., El-Rab, G., Salam, A., Ibrahim, Leonard, L.W. 2020. Nitrogen sources effect on *Lactobacillus reuteri* growth and performance cultivated in date palm (*Phoenix dactylifera* L.) By-Products Fermentation. 6(3), 64.
- Bai, K., Huang, Q., Zhang, J., He, J., Zhang, L., Wang, T. 2017. Supplemental effects of probiotic *Bacillus subtilis* fmbj on growth performance, antioxidant capacity, and meat quality of broiler chickens. *Poult Sci*. 96, 74–82.
- Dara, H.Y. 2016. Pengaruh Penggunaan Ampas Sari Kedelai Fermentasi dengan *Neurospora Sitophila* Dalam Ransum Broiler Terhadap Daya Cerna Serat Kasar, Retensi Nitrogen Dan Energi Metabolisme. Tesis. Fakultas Peternakan. Universitas Andalas.
- Dev, K., Nasir, A.M., Avishek, B., Jyoti, K., Jubeda, B., Rajiv, K., Asitbaran, M. 2020. Dietary symbiotic supplementation improves the growth performance, body antioxidant pool, serum biochemistry, meat quality, and lipid oxidative stability in broiler chickens. *Animal Nutrition*. 6, 325-332.
- Guzman-Cedillo, A.E., Corona, L., Castrejon-Pineda, F., Rosiles-Martínez, R., Gonzalez-Ronquillo, M. 2017. Evaluation of Chromium Oxide and Titanium Dioxide as Inert Markers for Calculating Apparent Digestibility in Sheep. *J. Appl. Anim. Res*. 45(1), 275–279.
- Hamdan, H., Amiluddin, Widhi, K., Amrullah, P. 2017. Efektivitas Metode Pengolahan Kulit Pisang (*Musa Paradisiaca*) Terhadap Kecernaan Nutrien Ayam Kampung Fase Grower. *JITP*. 5(2), 86-89.
- Hartono, E.F., Iriyanti, N., Suhermiyati, S. 2016. Efek penggunaan sinbiotik terhadap kondisi mikroflora dan histologi usus ayam sentul jantan. *Jurnal Agripet*. 16(2), 97-105.
- Haryati, T. 2011. Probiotik dan prebiotik sebagai pakan imbuhan non ruminansia. *Wartazoa*. 21(3), 125-132.
- Imam, S., Mahfudz, L.D., Suthama, N. 2018. Perkembangan mikrobia usus ayam broiler yang diberi pakan stepdown protein dengan penambahan asam sitrat sebagai acidifier. *Jurnal Litbang Provinsi Jawa Tengah*. 16(2), 13-21.
- Jin, L.Z., Ho, Y.W., Abdullah, N., Jalaludin, S. 1998. Growth performance, intestinal microbial populations, and serum cholesterol of broilers fed diets containing *Lactobacillus* cultures. *Poultry Science*. 77, 1259–1265.
- Kim, W.H., Lillehoj, H. 2019. Immunity, immunomodulation, and antibiotic alternatives to maximize the genetic potential of poultry for growth and disease response. *Animal Feed Science and Technology*. 250, 41–5.
- Kurniasih, N., Yuanita, I., Suthama, N., Wahyuni, H.I. 2019. Pengaruh ekstrak bawang dayak (*Eleutherine Palmifolia*) dikombinasikan dengan *Lactobacillus acidophilus* terhadap pemanfaatan energi dan pencernaan serat kasar pada ayam broiler. *Prosiding Seminar Nasional 2019*. Universitas Tidar.
- Lee, K.W., Lillehoj, H.S., Siragusa, G.R. 2010. Direct fed microbials and their impact on the intestinal microflora and immune system of chickens. *Jpn. Poult. Sci*. 47, 106–114.
- Luthfiana, K., Kurtini, T., Hartono, M. 2015. Pengaruh pemberian probiotik dari mikroba lokal terhadap gambaran darah ayam petelur. *Jurnal Ilmiah Peternakan Tropika*. 3(3), 151-156.
- McNaught, C.E., MacFie, J. 2000. Probiotics in clinical practice: a critical review of the evidence. *Nutr. Research*. 21, 343-353.
- Mubarak, P. R., Mahfudz, L.D., Sunarti, D. 2018. Pengaruh pemberian probiotik pada level protein pakan berbeda terhadap perlemakan ayam kampung. *Jurnal Sain Peternakan Indonesia*. 13(4), 357-364.
- Noviandi, I., Yaman, M.A., Rinidar. 2017. Efek Pemanfaatan Kulit Nanas (*Ananas Comosus*

- (L). Merr) Dalam Pakan Fermentasi Terhadap Kandungan Protein Daging Ayam Potong. Prosiding seminar Nasional Biotik 2017. Program Studi Kesehatan Masyarakat Veteriner, Program Pasca Sarjana Universitas Syiah Kuala Darussalam.
- Nurhayati, N. 2014. Pengaruh tingkat yoghurt fermentasi terhadap pencernaan *in vitro* bahan kering, bahan organik, protein dan serat kasar kulit nanas fermentasi. Buletin Peternakan. 38(3), 182-188.
- Nurhayati, N., Berliana., 2014. Perubahan kandungan protein dan serat kasar kulit nanas yang difermentasi dengan plain yoghurt. Jurnal Ilmiah Ilmu-Ilmu Peternakan. 15(1), 31-38.
- Palupi, R., Abdullah, L., DA, A., Sumiati. 2014. Potensi dan pemanfaatan tepung pucuk indigofera sp. sebagai bahan pakan substitusi bungkil kedelai dalam ransum ayam petelur. Jurnal Ilmu Ternak dan Veteriner. 19(3), 210–219.
- Palupi, R., Verawaty, M., Lubis, F.N.L., Oktarinah, N. 2020. Total lactic acid bacteria, phenolic compounds and antioxidant activities of pineapple waste and indigofera zollingeriana leaves by liquid fermentation. Jurnal Ilmu Ilmu Peternakan. 30(1), 1-9.
- Palupi, R., Lubis. F.N.L., Pratama, N.T.P., Muhakka. 2023. Effects of lactobacillus-fermented feed on production performance and carcass quality of broiler chickens. J. World Poult. Res. 13(1), 127-135.
- Patterson, J.A., Burkholder, K.M. 2003. Application of prebiotics and probiotics in poultry production. Poult sci. 82(4), 627-631.
- Rahmawati, D.P., Mulyono, Mangisah, I. 2014. Pengaruh level protein dan asam asetat dalam ransum terhadap tingkat keasaman (pH) usus halus, laju digesta dan bobot badan akhir ayam broiler. Anim. Agric. J. 3(3), 409-416.
- Rodjan P., Soisuwan, K., Thongprajukaew, K., Theapparatt, Y., Khongthong, S., Jeenkeawpieam, J. 2018. Effect of organic acids or probiotics alone or in combination on growth performance, nutrient digestibility, enzyme activities, intestinal morphology and gut microflora in broiler chickens. J Anim Physiol Anim Nutr. 102, e931–e940.
- Rompas, R., Tulung, B., Mandey, J.S., Regar, M. 2016. Penggunaan eceng gondok (*Eichhornia Crassipes*) terfermentasi dalam ransum itik terhadap pencernaan bahan kering dan bahan organik. Jurnal Zooteek. 36(2), 372-378.
- Safitri, S.R.I., Suprijatna, E., Sarengat, W. 2019. Pengaruh Pemberian Limbah Kulit Singkong (*Manihot esculenta L.*) dan Bakteri Asam Laktat Sp. sebagai Aditif Pakan terhadap Performa Ayam Kampung Super. Seminar Nasional Dalam Rangka Dies Natalis UNS Ke 43 Tahun 2019. 3(1), 25-33.
- Setiyowati, S., Sartono, T.A., Yudiarti, T. 2016. The addition of crude papain in the chicken diet on the digestion rate and the total number of microbes in the broiler intestine. Animal Agriculture Journal. 3(4), 599-605.
- Sionek, K., Szydłowska, A., Trzaskowska, M., Krajewska, K.D. 2024. The Impact of physicochemical conditions on lactic acid bacteria survival in food products. Fermentation. 10(6), 298
- Such, N., Farkas, V., Molnár, A., Csitári, G., Pál, L., Rawash, M. A., Koltay, I. A., Husveth, F., Károly, D. 2021. The effect of diet composition, a probiotic and a symbiotic treatment on the ileal microbiota composition of one-week-old broiler chickens. Acta Agraria Debreceniensis. 1, 213-220.
- Steel, R.G.D., Torrie, J.H. 1991. Prinsip dan Prosedur Statistika Suatu Pendekatan Biometrik (Terjemahan: Bambang Sumatri). Jakarta: PT. Gedia.
- Svendsen, A. 2016. Understanding Enzymes: Function, Design, Engineering and Analysis. Florida (US): CRC Press.
- Tilley, J.M.A., Terry, R.A. 1963. A Two stage Technique for the *In Vitro* Digestion of Forage. J. British Grassland Soc. 18, 104–111.
- Tillman, A.D., Reksohadiprodjo, S., Prawirokusumo, S., Lebosoekojo, S. 2005. Ilmu Makanan Ternak Dasar. Gadjah Mada University Press, Yogyakarta.
- Usman, N.A., Suradi, K., Gumilar, J. 2018. Pengaruh konsentrasi bakteri asam laktat

- Lactobacillus Plantarum* dan *Lactobacillus casei* terhadap mutu mikrobiologi dan kimia mayones probiotik. Jurnal Ilmu Ternak. 18(2), 79-85.
- Wang, Y., Chianning, H., Zhou, X., Cao, G., Jiang, L., Wang, J., Li, K., Wang, D., Xiuan Zhan, X. 2021. Supplemental Bacillus Subtilis DSM 29784 and enzymes, alone or in combination, as alternatives for antibiotics to improve growth performance, digestive enzyme activity, anti-oxidative status, immune response and the intestinal barrier of broiler chickens. Br J Nutr. 125(5), 494–507.
- Widodo, T. S., Sulistianto, B., Utama, C. S. 2015. Jumlah bakteri asam laktat (BAL) dalam digesta usus halus dan sekum ayam broiler yang diberi pakan ceceran pakan pabrik yang difermentasi. J. Agripet. 15(2), 98 – 103.
- Wu, Y., Wang, B., Zeng, Z., Liu, R., Tang, L., Gong, L., Li, W. 2019. Effects of probiotics *Lactobacillus Plantarum* 16 and *paenibacillus polymyxa* 10 on intestinal barrier function, antioxidative capacity, apoptosis, immune response, and biochemical parameters in broilers. Poult Sci. 98, 5028–5039.
- Wu, L., Tang, Z., Chen, H., Ren, Z., Ding, Q., Liang, K., Sun, Z. 2021. Mutual interaction between gut microbiota and protein/amino acid metabolism for host mucosal immunity and health. Animal Nutrition, 7, 11-16.