Unripe banana meal *vs.* *Lactobacillus casei*-fermented unripe banana meal: Which are better as feed ingredient for broiler chicken?

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Unripe banana meal *vs. Lactobacillus casei*-fermented unripe banana meal: Which are better as feed ingredient for broiler chicken?

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Abstract

The present study confirmed whether unripe banana meal or *Lactobacillus casei*- fermented unripe banana meal that are better as feed ingredient for broiler chickens. The experiment was designed as 4×2 factorial with unripe banana cultivars (Klutuk, Norowito, Raja Bandung, and Green bananas) and *L. casei*-fermentation or not as the factors. *L. casei*-fermentation increased the number of lactic acid bacteria (LAB) and decreased pH values of all cultivars of unripe banana meals. *L. casei*-fermentation increased ($p<0.05$) crude fibre and moisture, while decreasing ($p<0.05$) carbohydrates, total energy and antioxidant activity of unripe banana meals. In conclusion, irrespective of banana cultivars, *L. casei*-fermentation adversely affected the energy and crude fibre content as well as the antioxidant activity of unripe banana meals. Unfermented unripe banana meal was better to be included in broiler feeds as energy source compared to *L. casei*-fermented unripe banana meal.

Keywords: *antioxidant, fermentation, prebiotics, unripe banana*

Introduction

Feed is the main factor in broiler chicken production, which accounts for about 80% of production costs. In addition to protein, energy is a very important nutritional component in feed ingredients for growth so that nutritionists pay great attention to it. Conventionally, yellow corn is the main energy source for broilers. However, recently the price of corn often fluctuates so that it can disrupt the sustainability of broiler chicken production. Several studies have been conducted to find alternative energy sources for broilers, including Dumorné et al. (2020) which used unripe banana meal. Apart from being rich in energy, unripe banana meal is also reported to have high content of fructo-oligosaccharides (FOS), galacto-oligosaccharides (GOS) and resistant starch (Anyasi et al 2013; de Andrade et al 2021), which can act as prebiotic for broilers.

Bananas are widely consumed in Indonesia and come in a variety of cultivars. There are, however, numerous banana cultivars whose use is still limited or whose price is quite low. Some of these cultivars available include Klutuk, Norowito, Raja Bandung, and Green bananas. Klutuk banana is not preferred due to their high seed content (Prihhartanti 2014), while consumers do not accept Norowito banana because it has a little sour and bitter taste (Faozan and Sugiharto 2018). Raja Bandung banana is not generally consumed due to its slightly sour flavour (Slamet 2010; Noor 2020). Despite the fact that Green bananas are generally consumed, this banana cultivar is widely grown in Indonesia, making it abundantly available (Muztniar et al 2018). Taken all the facts together, using the above-mentioned banana cultivars as energy rich-feed ingredients for broilers may be expected to gain the additional values to these cultivars of bananas while also contributing to the long-term sustainability of broiler farming.

Lactic acid bacteria (LAB)-fermentation is generally thought as a simple method to improve the nutritional content of feed ingredients. In this respect, LAB-fermentation increased the energy content of feedstuffs (Adebo et al 2022). Also, LAB-fermentation increased some beneficial components in feedstuffs such as probiotic bacteria, antioxidants, etc. (Curiel et al 2015; Sugiharto and Ranjitkar 2019). For these reasons, it was interesting to fermenting unripe banana meals using LAB in order to confirm whether LAB-fermentation can increase the energy value and other beneficial components in the unripe banana meals or not, since there has never been any publication regarding this matter. Fermentation with LAB has been reported to increase energy, protein and ash content, whereas reducing the content of crude fibre of brewer's spent grain (Mladenović et al 2020). In addition, LAB-fermentation increased antioxidant activity of *Myrtus communis* berries in the study of Curiel et al (2015).

In this present study, LAB particularly *Lactobacillus casei* was used to ferment the unripe banana meals. The aim of the study was to investigate the effect of *L. casei*-fermentation on the LAB counts, nutritional composition and antioxidant activity of different cultivars of unripe bananas. The results of this study were expected to confirm whether unripe banana meal or *L. casei*-fermented unripe banana meal that are better as feed ingredient for broiler chickens.

Materials and methods

Preparation of fermented unripe banana meals

Four cultivars of unripe bananas (i.e., Klutuk, Norowito, Raja Bandung, and Green bananas) were collected from the banana plantation in Pati district, Central Java province. The unripe bananas were peeled, washed, chopped into small pieces and sun-dried. The unripe banana pieces were then mashed and sifted to produce an unripe banana meal. To prepare the fermentation starter, the rejuvenation of *Lactobacillus casei* bacteria from stock culture was carried out on De Man, Rogosa and Sharpe (MRS; Merck KGaA, Darmstadt, Germany) agar and incubated at 38°C for 48 hours. The bacteria were regrown and subsequently harvested using 100 mL MRS broth (Merck KGaA, Darmstadt, Germany). The broth solution was then diluted in 1000 mL distilled water and used to inoculate the unripe banana meal. For each cultivar, a 100 g of unripe banana meal was autoclaved, allowed to cool at room temperature and then placed in anaerobic jar and inoculated (1:4, g:mL) with suspension containing *L. casei* (around 1×10^{8} cfu/mL). The mixture was incubated for 5 days at 38°C. Following the fermentation, sample was obtained for the determination of LAB and coliform counts and pH values. The rest of the fermented unripe banana was then sun-dried and used for proximate, antioxidant activity and short chain fatty acids (SCFA) analyses.

Laboratory analysis

Laboratory analysis was conducted both on unripe banana meals and fermented unripe banana meals. The numbers of LAB were enumerated on MRS agar after a 48 hours anaerobic incubation at 38°C. Coliform was counted as red colonies on MacConkey (Merck KGaA, Darmstadt, Germany) agar after a 24 hours aerobic incubation at 38°C. The pH values was determined with the help of a pH meter (Portable pH Meter OHAUS ST300). Proximate analysis was conducted based on the standard AOAC method (AOAC, 1995). The antioxidant activity of the sample was determined by the 2,2-diphenylpicrylhydrazyl (DPPH) method according to Wu et al (2009) with minor modifications. The absorbance was measured at 515 nm. Antioxidant activity is indicated by the percentage of inhibition. For the measurement of SCFA level, a 0.5 g sample was suspended in 1.5 mL 2.5% metaphosphoric acid solution. The suspension was immediately immersed in ice water for 30 minutes, occasionally homogenized with a vortex, then centrifuged for 10 minutes at 14,000 *g* at 4°C. Gas chromatography (Ailgent 7890A, Wilmington, NC) was used to determine the levels of SCFA (acetic acid, propionic acid, and butyric acid) in the supernatant, as described previously by Garca-Villalba et al (2012).

Statistical analysis

The experiment was designed as 4×2 factorial with unripe banana cultivars and fermentation or not as the factors. The data obtained were analysed using the General Linear Models Procedure based on a 2×2 factorial design (SAS Inst. Inc., Cary, NC, USA). The data are presented in the form of means and standard error of the means.

Results and discussion

Table 1 shows the numbers of LAB and coliform as well as the pH values of fermented unripe banana meals. There was interaction ($p<0.05$) between banana cultivars and fermentation in term of LAB counts. It was apparent that fermentation increased the number of LAB in all cultivars of unripe banana meals. This finding was in accordance with Sugiharto and Ranjitkar (2019) revealing that fermentation increased LAB counts of feed ingredients. Also, our finding suggested that, irrespective of banana cultivars, unripe banana meals could support the growth of LAB especially *L. casei*. Hence, unripe banana meals could be exploited as prebiotic for broiler chickens. Indeed, unripe banana meals contain high amounts of FOS, GOS and resistant starch that can be substrate for the growth of LAB in the intestine of chickens (Anyasi et al 2013; de Andrade et al 2021). In this study, the increased LAB counts in fermented unripe banana meals varied across cultivars. The exact reason for such variations remains unclear. The differences in the contents of FOS, GOS and resistant starch seemed to be responsible for the different capacity of each unripe banana meal cultivar in supporting the growth of LAB. Yet, this inference should be interpreted with caution as we did not determine the levels of FOS, GOS and resistant starch in unripe banana meals in the present study. In this study, coliform bacteria was not detected in raw unripe banana meals, which was sterilized (autoclaved) prior to fermentation. The absent coliform bacteria in the fermented unripe banana meals confirmed the antibacterial activity of LAB against coliform bacteria as suggested by Sugiharto (2016). No notable interaction between cultivars and fermentation was observed in respect to pH values. In contrast to LAB counts, the pH values decreased with *L. casei*-fermentation on unripe banana meals. This seemed to be attributed to the increased LAB counts, which can increase lactic acid production and thereby decreased pH values.

Table 1. Selected bacteria population and pH values of fermented unripe banana meals

Items	Green		Klutuk		Norowito		Raja Bandung		SE	*p* value		
	−	+	−	+	−	+	−	+		C	F	C*F
LAB (log cfu/g)	0.00^{d}	9.24^{a}	0.00^{d}	8.39^{c}	0.00^{d}	9.34^{a}	0.00^{d}	8.89^{b}	0.09	<0.01	<0.01	<0.01
Coliform (log cfu/g)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	NA	NA	NA
pH	6.71^{ax}	4.75^{ay}	6.25^{ax}	4.98^{ay}	6.13^{abx}	4.67^{aby}	5.92^{bx}	4.19^{by}	0.24	0.04	<0.01	0.50

^{a,b,c,d} *Means within the row of LAB marked by superscript letters differ substantially (p<0.05)*

^{a,b} *Means among banana cultivars marked by superscript letters differ substantially (p<0.05)*

^{x,y} *Means between non-fermented and fermented marked by superscript letters differ substantially (p<0.05) LAB: lactic acid bacteria, cfu: colony forming unit, "−": non-fermented, "+": fermented, C: banana cultivar, F: fermentation, NA: not analysed, SE: standard error of the means*

There was no interaction (*p>0.05*) between banana cultivars and fermentation in respect to chemical composition of unripe banana meals (Table 2). Different cultivars were associated with the variations (*p<0.05*) in crude fibre, moisture, ash, carbohydrate and total energy of unripe banana meals. This was in line with Arvanitoyannis et al (2009) revealing that chemical compositions of banana are greatly affected by genotype and the conditions of cultivation. In addition, the ripening stages may also influence the chemical and functional properties of banana. Irrespective of banana cultivars, crude fibre increased following the fermentation with *L. casei*. Similarly, Sugiharto et al (2020) reported that fermentation (using shrimp paste rich in *Lactobacillus* sp.) increased crude fibre content in rice bran. The latter authors suggested that fermentation break down complex carbohydrates into oligosaccharides (simpler carbohydrates) resulting in increased crude fibre proportion. However, the latter inference should be interpreted with careful as we did not determine the levels of complex and simple carbohydrates in the banana meals in the present study. Another possibility could be that LAB-fermentation reduced the content of more soluble carbohydrates (Negrete-Romero et al 2021), which consequently increased the relative proportion of crude fibre in the unripe banana meals. In line with Sugiharto et al (2020), fermentation increased moisture content of unripe banana meals. This seemed to be associated with the inoculation of unripe banana meal with the suspension containing *L. casei* prior to fermentation. In this study, carbohydrates proportion decreased with *L. casei*-fermentation, which is in line with Sugiharto et al (2020) when fermenting rice bran. It was most likely that LAB used carbohydrate as an energy source during fermentation process resulting in decreased carbohydrates content in unripe banana meals. This inference was supported by the increased LAB counts following fermentation. Indeed, the decrease in carbohydrates due to fermentation was attributed to the decreased total energy in unripe banana meal, which is also found by Sugiharto et al (2020).

Table 2. Chemical compositions of fermented unripe banana meals

Items	Green		Klutuk		Norowito		Raja Bandung		SE	*p* value		
	−	+	−	+	−	+	−	+		C	F	C*F
Crude protein (% DM)	3.49	3.40	4.79	3.94	3.23	3.11	2.72	3.56	0.49	0.06	0.87	0.41
Crude fibre (% DM)	2.73^{cy}	4.71^{cx}	6.11^{ay}	7.92^{ax}	3.50^{cy}	5.36^{cx}	5.26^{by}	6.16^{bx}	0.45	<0.01	<0.01	0.62
Moisture (% DM)	14.1^{by}	15.2^{bx}	16.3^{ay}	25.3^{ax}	11.6^{by}	16.6^{bx}	10.9^{by}	16.1^{bx}	1.73	<0.01	0.01	0.19
Ash (% DM)	3.43^{b}	3.00^{b}	4.14^{a}	4.28^{a}	3.86^{ab}	3.57^{ab}	2.54^{c}	2.23^{c}	0.35	<0.01	0.36	0.86
Crude fat (% DM)	0.49	0.53	0.50	0.57	0.27	0.25	0.23	0.68	0.15	0.29	0.23	0.42
Carbohydrates (% DM)	79.1^{ax}	77.8^{ay}	74.5^{bx}	66.0^{by}	81.1^{ax}	76.5^{ay}	83.6^{ax}	78.2^{ay}	1.56	<0.01	<0.01	0.17
Total energy (kcal/100 g)	335^{ax}	329^{ay}	320^{bx}	285^{by}	340^{ax}	321^{ay}	348^{ax}	330^{ay}	6.85	<0.01	<0.01	0.25
Energy from fat (kcal/100 g)	4.43	4.75	4.46	5.07	2.46	2.25	2.08	4.55	1.28	0.18	*0.42*	0.77

^{a,b} *Means among banana cultivars marked by superscript letters differ substantially (p<0.05)*

^{x,y} *Means between non-fermented and fermented marked by superscript letters differ substantially (p<0.05) DM: dry matter, "−": non-fermented, "+": fermented, C: banana cultivar, F: fermentation, SE: standard error of the means*

There were interaction (*p<0.05*) between banana cultivars and fermentation on acetic acid level and DPPH scavenging activity of unripe banana meals (Table 3). Whereas no significant changes in acetic acid content were observed in other cultivars, the concentration of acetic acid decreased in fermented unripe banana meals for Klutuk cultivar. During the fermentation, acetic acid can be produced from carbohydrate (glucose) and can be oxidized (in the presence of oxygen) to form carbon dioxide and water (Wusnah et al 2018). Such oxidation of acetic acid may therefore decrease the level of acetic acid in the fermented unripe banana meals from Klutuk cultivar. In this study, unripe banana meals from Klutuk cultivar had the lowest carbohydrates content (Table 2). This may consequently be associated with the lower acetic acid production in Klutuk unripe banana meal during the fermentation process. Indeed, the moisture content was higher in the fermented unripe banana meals from Klutuk cultivar as compared to other groups. This condition may indicate the presence of oxygen during anaerobic fermentation that may cause acetic acid oxidation. The latter condition resulted in increased water and decreased acetic acid contents in the fermented product. In this study, the difference in propionic and butyric acids were not observed across the treatment groups.

Table 3. Short chain fatty acids content and antioxidant activity of fermented unripe banana meals

Items	Green		Klutuk		Norowito		Raja Bandung		SE	p value		
	−	+	−	+	−	+	−	+		C	F	C*F
Acetic acid	1.92^{abc}	2.32^{ab}	3.00^{a}	0.37^{c}	1.32^{bc}	1.75^{abc}	3.19^{a}	1.99^{ab}	0.54	0.22	0.06	0.03
Propionic acid	0.02	0.00	0.06	0.14	0.03	0.00	0.09	0.00	0.05	0.23	0.64	0.36
Butyric acid	0.15	0.97	0.00	0.02	0.28	0.00	0.00	0.00	0.36	0.37	0.58	0.46
DPPH scavenging activity (%)	8.65^{cd}	4.15^{d}	92.2^{a}	66.7^{b}	94.2^{a}	4.26^{cd}	23.9^{c}	3.26^{d}	6.57	<0.01	<0.01	<0.01

a,b,c,d Means within the same row marked by superscript letters differ substantially (P<0.05)
DPPH: 2,2-diphenylpicrylhydrazyl, "−": non-fermented, "+": fermented, C: banana cultivar, F: fermentation, SE: standard error of the means

It was reported in this current study that antioxidant activity of unripe banana meals decreased with the *L. casei*-fermentation process (Table 3). This finding was in accordance with Sugiharto et al (2020) showing the decrease in antioxidant activity of rice bran with LAB-fermentation. The latter investigators further suggested that fermentation may damage the phenolic compounds resulting in poor scavenging activity of phenolics against free radicals. It was shown in this investigation that the extent of reduction in antioxidant activity varied among the banana cultivars. Apart from the difference in the antioxidant activity across the raw unripe banana cultivars (that are greatly affected by genotype, growing condition and ripening stages [Arvanitoyannis et al 2009]), the different degree in phenolic destruction during fermentation may be responsible for the different extent of reduction in antioxidant activity of the unripe banana meals.

As a general discussion, fermentation using *L. casei* reduced the energy content and increased crude fibre in unripe banana meal without affecting the protein, fat and ash contents. These data indicate that *L. casei*-fermentation adversely affected the nutritional values of unripe banana meal. The *L. casei*-fermentation process (showing the increased LAB counts with fermentation) proved that unripe banana meal was able to support the growth of LAB *in vitro*. This data was expected to be the basis for the *in vivo* condition that unfermented unripe banana meal (without being fermented prior to provision to chickens) can be a prebiotic in the intestines of broiler chickens. Regardless of the cultivars of bananas, fermentation actually reduced the antioxidant activity in unripe banana meals. This can decrease the functional values of the unripe banana meal for broiler chickens. Overall, it seemed that unfermented unripe banana meal was better to be used as feed ingredient for broiler chickens as compared to *L. casei*-fermented unripe banana meal.

Conclusions

- Irrespective of banana cultivars, *L. casei*-fermentation adversely affected the energy and crude fibre content as well as the antioxidant activity of unripe banana meals.
- Unfermented unripe banana meal was better to be included in broiler feeds as energy source compared to *L. casei*-fermented unripe banana meal.

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