Potential Use of Banana Peels Waste at Different Ripening Stages for Sheep Feeding on Chemical, Tannin, and In Vitro Assessments

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Abstract. This research aimed to investigate the potential use of banana peels waste for sheep feeding. Completely randomized design was used to compare the chemical, phenol, and tannin compositions between raw and ripened banana peels from 4 different varieties (Ambon, Muli, Nangka, Kapas). Moreover, a 2x4 factorial design was used to test the main effects of ripening stages (raw, ripened) and doses (10, 20, 30, 40%) of Ambon banana peels on in vitro dry matter and organic matter digestibility (DMD and OMD, %), ammonia (NH₃, mM), volatile fatty acids (VFA, mM), pH, and total gas production (TGP, ml). Raw banana peels contained less (P<0.05) dry matter (DM, %) and total digestible nutrients (TDN, %) but it contained higher (P<0.05) crude fiber (CF, %) and gross energy (GE, kcal/kg) compared with ripened banana peels. Raw and ripened banana peels contained considerable amount of total phenols (TP, %) and total tannins (TT, %) although being not different (P>0.05). Based on in vitro assessments, raw Ambon banana peels had lower (P<0.001) DMD, OMD, and VFA but higher NH₃ (P<0.001) than ripened ones. Adding Ambon banana peels from 10 to 40% replacing roughage in the diet increased (P<0.001) DMD, OMD, and VFA but decreased (P<0.001) NH₃. Both raw and ripened Banana peels have the potential for sheep feeding based on their chemical and in vitro assessments.

1. Introduction
Banana is a tropical fruit and easily grown in tropical countries such as Indonesia. The use of banana either for direct consumption or processed products i.e. banana chips creates waste products including banana peels. This waste can be used as an alternative feed or roughages for sheep. So far, one of the challenges that Indonesian sheep farmers have faced is lack of high quality roughages since many pastures have massively changed into crops, housing, and industries, especially in Java Island. Conversely, the market of lambs in this highly populated island is high, not only for daily consumption but also for Muslim-related-celebrations called Aqeqah and Iedul Adha. Wina [1] stated that banana peels waste has not been optimally used yet for ruminant feeding although it contains organic biomass including protein, fiber, minerals, and other nutrients which are needed by ruminants. Inside the rumen, banana peel waste will be fermented in anaerobic condition by rumen microbes resulting in more available nutrients to be absorbed in the small intestine and distributed in the blood for the needs of growth and production such as meat and milk [2,3].

As a tropical fruit, banana should be able to adapt to tropical climate characterized by high temperature, rain, and humidity with so many varieties of insect predators, bacteria, and fungi so that most tropical plants contain plant bioactive compounds to protect them from predators but they are not directly involved in a biochemical process of plant metabolite systems [4]. Banana peels are supposed
to contain considerable high in plant bioactive compounds to protect the inside of banana from predators’ attack. Tannin is one of the bioactive compounds that is possibly contained in banana peels so that the waste of banana peels can be used as a source of natural feed additive to improve the productivity of ruminant animals. This research was done to (1) analyze proximate, CF, nitrogen-free extract (NFE, %), TDN, GE, mineral, TP, and TT in different local varieties of banana peel (Ambon, Muli, Nangka, and Kapas) at two ripening stages (raw, ripened) and (2) tested the effect of raw and ripened Ambon banana peels at different doses on in vitro digestibility, fermentation profiles, and TGP.

2. Materials and methods

2.1. Banana peels’ sampling preparation

The sample of each variety of banana peels, either Ambon, Muli, Nangka, or Kapas, was obtained from whole bananas bought in 3 different markets: Pasar Induk Caringin Bandung, Pasar Induk Gedebage Bandung, and Pasar Tanjungsari Sumedang. Pasar Induk means a region main wet market while Pasar is a common wet market. At our laboratory, each of banana was peeled at raw and ripened stages and each peel was then sun dried, ground, and filtered at 20 mesh. After becoming dried powder, each sample was put into coded plastic and ready for the next analyses.

2.2. Nutrients, phenols, and tannins analyses

Proximate (DM, OM, CP, ash, fats), CF, NFE, TDN, GE, Ca, and P analyses were done at Laboratory of Feed Chemical and Nutrition, Faculty of Animal Husbandry, Universitas Padjadjaran using the method of AOAC [5]. Meanwhile, TP and TT analyses have followed the Folin-Ciocalteu procedure utilizing a spectrophotometer as explained in Makkar [6] using a tannic acid as a standard equivalent. Most unit measurements of analyses were calculated in % DM except DM (% fresh weight) and GE (kcal/kg).

2.3. Experimental diets

The experimental diets were formulated following a 2x4 factorial arrangement to test the effect of 2 ripening stages (raw and ripened) and 4 doses (10, 20, 30, 40%) of Ambon banana peels to replace grass in the diets on in vitro degradability and fermentation profiles. The experimental diets were iso-protein (10.7% ± 0.163) and iso-TDN (61.5% ± 0.503) and they consisted of raw or ripened Ambon banana peels, field grass, and a mixed concentrate. The nutrient contents of raw and ripened Ambon banana peels can be seen in the results of this study while field grass contained 21.9 % DM, 9.33% ash, 9.10% CP, 28.8% CF, 4.72% Fats, 48.1% NFE, 60.6% TDN, 2994 kcal.kg GE, 0.33% Ca, and 0.18% P. Concentrate contained 92.7% DM, 14.2% ash, 13.8% CP, 18.8% CF, 9.4% Fats, 43.9% NFE, 65.2% TDN, 3499 kcal.kg GE, 0.87% Ca, and 0.61% P.

2.4. DMD and OMD Measurements

In vitro DMD and OMD were measured using the procedure of Tilley and Terry [7]. Briefly, about 1 gram of each diet sample was put inside a fermenter tube and 40 ml of artificial McDougall saliva and 10 ml fresh rumen fluid added. CO2 was blown into the fermenter tube to keep an anaerobic condition and the tube was closed with a ventilated rubber and incubated in a water bath at about 39 °C for 48 hours. During incubation, each tube was gently shaken every 3 hours (phase 1). After 48 hours incubation, 0.2 ml HgCl2 was added into each tube and it was centrifuged at 4000 rpm for 15 minutes to separate supernatant and its residue. About 50 ml Pepsin-HCL 10% solution (pepsin activity 1:10,000) was added into the tube containing the residue (without supernatant) and it was incubated again at 39 °C for 48 hours in an aerobic condition (phase 2). After that, the residue was filtered using Whatman paper No. 41 and rinsed with distilled water to make sure all the residue went down into the filter paper. Filtered residue was then dried in an oven at 105°C for 24 hours, cooled in a desiccator, and weighed for calculating DMD. Dried residue was finally heated at 550 °C in a furnace, cooled in a desiccator, and weighed for calculating OMD.
2.5. VFA and NH3 measurements
A similar in vitro method of Tilley and Terry [7] was used in VFA and NH3 measurements. After 24 hours incubation, about 0.2 mL HgCl2 was added into each of fermenter tube and the tube was then centrifuged at 4000 rpm for 15 minutes to separate supernatant and the residue. The supernatant was then taken off and stored in a bottle in a refrigerator for the next analysis of VFA and NH3, respectively. About 5 mL of each supernatant was placed in a steam heated distillation tube and 1 mL H2SO4 15% added and closed the tube tightly. Heated steam forced VFA in the cooling tube to be condensed and stored in an Erlenmeyer tube containing 5 mL NaOH 0.5 N until the volume reached 200-300 mL. After that, 2 drops of phenolphthalein (pp) indicator was added into the tube and titrated with HCl 0.5 N. Titration was ended at the beginning of color change from red to no color. While for NH3 analysis, 1 mL supernatant was placed in the left side of Conway cup and 1 mL Na2CO3 was placed in the right side. Small cup in the middle was filled with 1 mL boric acid with red methyl and green brome cresol indicators. Conway cup was tightly closed with vaseline-lubricated cap and shaken so that the supernatant could be well mixed with Na2CO3. Then, it was kept at room temperature for 24 hours to let the ammonia gas bounded with boric acid. After 24 hours, it was titrated with H2SO4 0.006 N until the color changed into red.

2.6. pH and TGP measurements
TGP was measured following the procedure of Menke and Steingass [8] using measurable syringes at 24 hours of in vitro incubation. After incubation, the in vitro solution was measured for pH using a calibrated pH meter.

2.7. Data tabulation and statistical analysis
Each sample of banana peel was analyzed for nutrients, TP, and TT in duplicate while the data were presented in average. The different of chemical compositions between raw and ripened banana peels was analyzed using one-way ANOVA with different varieties of banana peels as replicates (n=4). Meanwhile, the data of a 2x4 factorial design with 5 replicates in each experimental unit, to test the main effects of ripening stages (raw, ripened) and doses (10, 20, 30, 40%) of Ambon banana peels on in vitro digestibility and fermentation profiles, were analyzed using two-way ANOVA. Here, Minitab 16 Statistical Software was used for all the statistical analyses.

3. Results and discussion
Table 1. describes nutrients, TP, and TT contents (% DM except % fresh weight for DM and kcal/kg for GE) in different varieties of banana peels at raw and ripened stages. At raw stage, there were averagely not different among varieties of banana peels (Ambon, Muli, Nangka, and Kapas) in DM, OM, ash, GE, and Ca. However, Kapas banana peels had averagely the highest CP than others while Nangka banana peels had averagely the highest fats, Ca, and P than others. Muli banana peels had averagely the highest NFE but the lowest CP compared to others whilst Ambon banana peels had averagely the highest TP and TT but the lowest fats than others. At ripened stage, the average OM, ash, TDN, and GE contents among varieties of banana peels seemed the same. However, Ambon banana peels had averagely the highest DM, TP, and TT but the lowest Ca and P in comparison with others while Kapas banana peels had averagely the highest CP, Ca, and P but the lowest NFE, TP, and TT.

Table 2. shows the different of nutrients, TP, and TT compositions (% DM except % fresh weight for DM and kcal/kg for GE) between raw and ripened banana peels. Raw banana peels had significantly (P<0.01) less DM and TDN but higher CP (P<0.01) and GE (P<0.05) in comparison with ripened banana peels. Raw banana peels had averagely higher fats but less TP than ripened banana peels although they were not significant (P = 0.073 and 0.085, respectively).

Table 3. outlines the means in vitro digestibility, fermentation profiles, and TGP, for only the main effects of raw and ripened stages of Ambon banana peels and their doses (10, 20, 30, 40%) of inclusion
as these were mostly significant (P<0.001) but not their interactions. Diets containing ripened Ambon banana peels had significantly higher DMD, OMD, and VFA but lower NH₃ and TGP compared with those containing raw Ambon banana peels. Meanwhile, adding Ambon banana peels into a sheep diet up to 40% could significantly increase DMD, OMD, and VFA but reduce NH₃. Interestingly, the highest TGP was reached by adding Ambon banana peels up 30% but not 40%. There was no significant different between ripening stages and doses on pH.

It can be seen that banana peels has considerable DM, OM, ash, CP, CF, fats, NFE, TDN, GE, Ca, P, TP, and TT that can be beneficial for sheep feeding but their contents in the peels might be different depend upon varieties and ripening stages. Mohapatra et al. [9] added that banana peels also rich in polyunsaturated fatty acids such as linoleic and α-linolenic, essential amino acids such as leucine, valine, phenylalanine, threonine, starch and pectine containing glucose, galactose, arrabinose, rhamnose, xylose, cellulose and hemicelulose. Good nutrient qualities of Ambon banana peels might be the reason why adding the peels into a sheep diet up to 40% in replacing grass could significantly increase DMD, OMD, and VFA. Ambon banana peels have also lower CF but higher GE compared with grass which was used in the current study.

Meanwhile, decreased NH₃ production due to Ambon banana peels addition is supposedly caused by considerable tannin content in the peels. Generally, tannins can reduce the solubility and rumen degradability of most leaf proteins due to their potential binding with proteins. Consequently, they can reduce rumen NH₃ production and increase the availability of by-pass protein and non-ammonia nitrogen (N) supply to be absorbed in the small intestine [10,11,12]. Although NH₃ is an important source of N for rumen microbes, its over or fast production may exceed the ability of microbes to utilize it. This can lead to an excessive NH₃ supply that, after absorption through the rumen wall, can enter the blood stream, liver, and eventually excreted in urine as an N waste [13,14]. Except increasing potential by-pass protein as previously explained, tannins can also lower CH₄ production by slowing the interspecies transfer of H₂ into methanogenic bacteria and thus depress their growth [12,15], improve animal health through their antioxidant properties to prevent bloat and break protein-rich cells of nematodes [16,17], and increase the rumenic acid and polyunsaturated fatty acids (PUFA), and decrease saturated fatty acids (SFA) in ruminant products such as meat and milk through altered bio-hydrogenation by changing the microbial population in the rumen [18,19,20].
Table 1. Nutrients, TP, and TT compositions (% DM or otherwise stated) in different varieties of banana peels at raw and ripened stages.

| Nutrients (%) DM | Raw banana peels | Ripened banana peels |
|------------------|------------------|-----------------------|
|                  | Ambon | Muli | Nangka | Kapas | Ambon | Muli | Nangka | Kapas |
| DM (%)           | 37.9  | 38.1 | 38.3   | 34.5  | 67.3  | 50.7 | 58.8   | 54.0  |
| OM               | 88.8  | 89.5 | 87.7   | 86.8  | 88.2  | 88.8 | 87.1   | 84.9  |
| Ash              | 11.2  | 10.5 | 12.3   | 13.3  | 11.8  | 11.3 | 12.9   | 15.1  |
| CP               | 6.8   | 5.3  | 5.5    | 6.0   | 5.3   | 5.1  | 5.2    | 5.0   |
| CF               | 18.7  | 17.2 | 17.2   | 17.1  | 16.2  | 15.7 | 16.3   | 15.5  |
| Fats             | 2.2   | 1.8  | 2.2    | 2.2   | 2.2   | 2.2  | 2.2    | 2.2   |
| NFE              | 58.2  | 62.1 | 58.7   | 53.2  | 61.6  | 61.9 | 62.8   | 56.3  |
| TDN              | 56.7  | 58.7 | 58.4   | 56.0  | 61.1  | 61.9 | 62.8   | 56.3  |
| GE (kcal/kg)     | 3472  | 3369 | 3494   | 3445  | 3386  | 3329 | 3368   | 3321  |
| Ca               | 0.63  | 0.65 | 0.78   | 0.78  | 0.78  | 0.78 | 0.78   | 0.78  |
| P                | 0.19  | 0.23 | 0.31   | 0.29  | 0.29  | 0.29 | 0.29   | 0.29  |
| TP               | 5.76  | 4.80 | 4.83   | 4.57  | 6.94  | 5.88 | 5.74   | 5.08  |
| TT               | 5.32  | 4.34 | 4.47   | 4.19  | 5.85  | 5.30 | 5.11   | 4.61  |

DM, dry matter; OM, organic matter; CP, crude protein; CF, crude fibre; NFE, nitrogen free extract; TDN, total digestible nutrients; GE, gross energy; TP, Total phenols; TT, total tannins.

Table 2. The different of nutrients, TP, and TT contents (% DM or otherwise stated) between raw and ripened banana peels.

| Banana Peels | DM % | OM    | Abu   | CP    | CF    | Fats  | NFE   | TDN   | GE (kcal/kg) | Ca | P | TP | TT |
|--------------|------|-------|-------|-------|-------|-------|-------|-------|--------------|----|----|----|----|
| Raw          | 38.1 | 88.2  | 11.8  | 8.20  | 19.7  | 2.13  | 58.2  | 57.4  | 3445         | 0.68| 0.26| 4.99| 4.58|
| Ripened      | 63.0 | 87.2  | 12.8  | 9.54  | 15.3  | 1.78  | 60.6  | 61.7  | 3351         | 0.65| 0.23| 5.80| 5.22|
| SEM          | 2.62 | 0.75  | 0.750 | 0.84  | 0.54  | 0.12  | 1.66  | 0.60  | 2.21         | 0.04| 0.02| 0.20| 0.26|
| P valuea     | 0.001| 0.398 | 0.398 | 0.303 | 0.001 | 0.073 | 0.337 | 0.003 | 0.024        | 0.562| 0.420| 0.085| 0.127|

aMean values were significantly different at P<0.05. DM, dry matter; OM, organic matter; CP, crude protein; CF, crude fibre; NFE, nitrogen free extract; TDN, total digestible nutrients; GE, gross energy; TP, Total phenols; TT, total tannins; SEM, standard error of mean.
Table 3. Means for the main effects of raw and ripened stages of Ambon banana peels and their doses (10, 20, 30, 40%) of inclusion into sheep diets on $in\text{ vitro}$ digestibility, fermentation profiles, and TGP.

| In vitro tests | Ripe stages (n=20) | Doses (%) (n=10) | SEM and Significances |
|---------------|------------------|-----------------|-------------------------|
|               | Raw   | Ripened | 10   | 20   | 30   | 40   | Stages | Doses | Interaction |
| DMD (%)       | 62.5  | 66.8    | 60.7D | 63.0B | 65.5B | 69.3A | 0.13*** | 0.18*** | 0.25NS      |
| OMD (%)       | 51.4  | 55.1    | 48.2D | 51.6C | 54.8B | 58.4A | 0.12*** | 0.17*** | 0.24**      |
| NH₃ (mM)      | 6.33  | 5.38    | 6.71A | 6.12B | 5.55C | 5.05D | 0.06*** | 0.09*** | 0.13NS      |
| VFA (mM)      | 135   | 142     | 119D  | 136C  | 144B  | 156A  | 0.81*** | 1.15*** | 1.62**      |
| pH            | 6.54  | 6.49    | 6.51  | 6.57  | 6.48  | 6.50  | 0.04NS  | 0.05NS  | 0.07NS      |
| TGP (ml)      | 244   | 193     | 179C  | 245A  | 244A  | 207B  | 4.60*** | 6.50*** | 9.20*       |

Here *, ** and *** represent significant differences between means at $P<0.05$ or $P<0.01$ or $P<0.001$ respectively; SEM, standard error of mean; n, number of replicates; DMD, $in\text{ vitro}$ dry matter digestibility; OMD, $in\text{ vitro}$ organic matter digestibility; NH₃, ammonia; VFA, volatile fatty acids; TGP, total gas production.

4. Conclusion
Banana peels are considerably rich in nutrients, phenols and tannins and their compositions may be different depending upon banana varieties and the levels of ripe. Based on $in\text{ vitro}$ assessments, Ambon banana peels can be included in a diet of sheep up to 40% to increase degradability, VFA production, and potential by-pass protein, but $in\text{ vivo}$ experiments to further prove their potential need to be done.

Acknowledgement
Authors would like to thank Directorate General of Higher Education, Ministry of Research, Technology, and Higher Education and Directorate of Research and Services, Universitas Padjadjaran for funding this research via PUPT Scheme 2015/2016.

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