In vitro fermentability and digestibility of seedless noni waste (*Morinda citrifolia* L.) as a concentrate substitute in lactating dairy goat diet

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Abstract. Noni waste (*Morinda citrifolia* L.) is waste from noni juice that contain high lignin but still having bioactive compounds such as polyphenols and saponins. In this study, around 75% of lignin content was reduced by removal the seeds from noni waste. The aims of this research were to evaluate by in vitro's method the potency of seedless noni waste (SNW) as a concentrate substitute in lactating dairy goat diet. Randomized Block Design were applied with 3 times rumen fluid intake and 6 diets treatment i.e. R0 = 60% Napier Grass (NG) + 40% Concentrate (control); R1 to R5 = 60% NG + 5 to 25% SNW + 35 to 15% Concentrate. The variables were pH, fermentability (NH\textsubscript{3} and total VFA), microbial protein synthesis, total bacteria and protozoa population, dry matter and organic matter digestibility. The results showed there were no differences among diets for all variables except the fermentability (NH\textsubscript{3} and total VFA) and microbial protein synthesis (P<0.05) that showed increasing quadratically (P<0.01) by addition of SNW and achieved the peak at 15% SNW. It can be concluded that 15% of seedless noni waste (SNW) is able to substitute 37.5% of concentrate in lactating dairy goat diet with ratio of 60% forages and 40% concentrate. The diet is able to support fermentability and microbial protein synthesis without interfere the microbial population as well as in vitro digestibility.

1. Introduction
The goat population in Indonesia increase every year, 17.9 million head in 2012 to 19.6 million head in 2016 [1]. The milk productivity of local diary goats was still very low, which was around 0.5-1.2 l/ head / day in PE goats [2]. The low productivity is due to the low quality of the feed provided by farmers. This is because the quality of the forage is not consistent with the changing seasons and the high cost of concentrate feed ingredients. Tropical climate possesses high humidity that facilitates the growth of pathogenic microorganisms, so that helminth disease and mastitis are quite high in Indonesia. The frequency of occurrence in sheep or goats in helminthiasis was 80%, especially in areas with high rainfall. The prevalence rate in the area of West Java was reported to vary, namely 87.5-100%. The incidence of mastitis in dairy goats in Indonesia often occurs, but epidemiological data have not been widely reported. Based on somatic cell counts in milk, the incidence of subclinical mastitis in goat ranges from 9-50%, where the clinical mastitis by 25.5% occurred after delivery or 40 days after delivery with consequence losses the milk production by around 10-25% [3-5]. Noni waste
is a waste from noni fruits processing to be noni juice, which still contains bioactive compounds such as polyphenols and saponins [6]. The total production of noni fruits in 2014 amounted to 8,577,347 kg [7]. Based on our in vitro researches, noni waste could be used 15% in animal diet without interfere the growth of rumen microbial. Moreover, noni waste was also identified function as an anti-helminthic and as anti-subclinical mastitis. Unfortunately, the decreasing of rumen fermentability occurred if the use of noni waste exceeded than 15%. This might relate to the high lignin content of noni seed in waste (24.51%) [8]. Therefore, in this research study, seedless noni waste was used as a concentrate substance. The study aims were to evaluate the capacity of seedless noni waste (SNW) to substitute the concentrate in lactating dairy goat diet through in vitro analysis of fermentability, microbial protein synthesis, microbial population and digestibility.

2. Materials and Methods

The main ingredient in this study, namely seedless noni waste that obtained from Sentul and diet ingredient, namely elephant grass, fine rice bran, corn, soybean meal, coconut meal, tempeh waste, CaCO3, DCP and premix. The preparation of the experimental diets was guided by nutritional requirements for lactating dairy goats containing 12-17% crude protein and 53-66% Total Digestible Nutrients (TDN). The nutrient composition of the experimental diets can be seen in Table 1.

| Item                        | R0     | R1     | R2     | R3     | R4     | R5     |
|-----------------------------|--------|--------|--------|--------|--------|--------|
| Ingredients                 |        |        |        |        |        |        |
| Napier Grass (NG)           | 60.00  | 60.00  | 60.00  | 60.00  | 60.00  | 60.00  |
| Seedless Noni Waste (SNW)   | 0.00   | 5.00   | 10.00  | 15.00  | 20.00  | 25.00  |
| Rice Bran                   | 7.50   | 5.28   | 3.75   | 2.50   | 3.00   | 0.36   |
| Corn                        | 7.50   | 4.91   | 3.75   | 2.50   | 0.35   | 0.38   |
| Soybean meal                | 14.00  | 14.00  | 14.00  | 14.00  | 13.00  | 12.50  |
| Coconut meal                | 5.00   | 4.91   | 3.75   | 2.50   | 1.25   | 0.38   |
| Tempe Waste                 | 5.00   | 4.91   | 3.75   | 2.50   | 1.40   | 0.38   |
| CaCO3                       | 0.80   | 0.80   | 0.80   | 0.80   | 0.80   | 0.80   |
| DCP                         | 0.10   | 0.10   | 0.10   | 0.10   | 0.10   | 0.10   |
| Premix                       | 0.10   | 0.10   | 0.10   | 0.10   | 0.10   | 0.10   |
| Nutrient composition        |        |        |        |        |        |        |
| Crude Protein               | 15.07  | 15.02  | 14.85  | 14.66  | 14.14  | 13.80  |
| Crude Fiber                 | 24.30  | 24.87  | 24.92  | 24.94  | 25.19  | 25.20  |
| TDN                          | 63.70  | 63.19  | 62.79  | 62.38  | 61.68  | 61.38  |

Note: R0 = 60% NG + 40% Concentrate (control); R1 = 60% NG + 5% SNW + 35% Concentrate; R2 = 60% NG + 10% SNW + 30% Concentrate; R3 = 60% NG + 15% SNW + 25% Concentrate; R4 = 60% NG + 20% SNW + 20% Concentrate; R5 = 60% NG + 25% SNW + 15% Concentrate.

2.1.1. In vitro fermentation test

In vitro rumen fermentation test [9] used control diets that consists of 60% Napier grass and 40% concentrate. The treatment diets contained seedless noni waste with levels of 5%, 10%, 15%, 20% and 25%. Fresh rumen liquid with a temperature of 39 °C taken from a slaughterhouse is stored in a thermos to maintain the stability of temperature and pH. A total solution comprised of 0.5 g of sample, 40 ml of the McDougall buffer solution and 10 ml of fresh rumen liquid was put into the fermenter tube. Samples were incubated for 4 hours under anaerobic conditions at 39 °C and pH 6.5-6.8 in a water shaker bath. Before fermentation process is stopped by the addition of a saturated HgCl2 solution, then centrifuged at 5000 rpm for 15 minutes to take the supernatant, as much as 0.5 ml and 1
ml of samples are taken for the total bacterial and protozoa population counting, respectively. After that, the sample supernatants are used for analysis of fermentability that consist of total VFA (using the steam distillation technique) and NH₃ concentration (using the Conway Micro 3 diffusion method), and also for the microbial rumen synthesis [10].

2.1.2. In vitro digestibility
For analysis of digestibility [9], the fermentation process is conducted as long 2 x 48 hours, which is divided into 48 hours anaerobically for measurement the coefficient digestibility of dry matter and 48 hours aerobically for measurement the coefficient digestibility organic matter. Similar to fermentation for fermentability measurement, the fermentative digestion process after 48 hours ends with 2 drops of saturated HgCl₂ solution. After that, for next 48 hours of the enzymatic digestion process aerobically, samples are centrifuged at 5000 rpm for 15 minutes, then supernatant is removed and the residue is added with 0.2% pepsin-HCl solution. The mixture is then filtered with Whatman no. 41 using a vacuum pump. The filtrate put into a porcelain cup of known weight, then dried in a 105 °C oven for 24 hours for determination of dry matter content by weighing the and sample in the cup, after that, the cup was put back into the 600 °C furnaces for determination of ash content and organic matter calculation. Dry matter content and organic matter are used to determine the coefficient digestibility of dry and organic matter.

2.1.3. Viability test bacteria and protozoa
The counting of total bacteria and protozoa population are done to measure the viability [11]. The principle of counting is the rumen fluid diluted serially, then cultured in Brain Heart Infusion (BHI) media. Brain Heart Infusion media (BHI) is made by mixing BHI flour 3.7 g, glucose 0.05 g, CMC (1%) 1 ml, starch 0.05 g, cysteine-HCl 0.05 g, 0.5 ml hemin, resazurin 0.05 ml and distilled water to 100 ml. The mixture is heated until there is a colour change from fawn to reddish brown and changes again to yellowish brown and cooled with CO₂ gas. A 5 ml medium was put into a Hungate tube containing Bacto 0.15 g and sterilized using an autoclave (temperature 121°C, 15 minutes, pressure 1.2 Kgf.cm⁻²). Before culturing, the sample is diluted with a diluent medium. Dilution was carried out as follows: 0.5 ml of rumen liquid was put into 4.5 ml of the first diluent medium, then from the first dilution was taken 0.05 ml and put in 4.95 ml of the second diluent medium and so on with the same procedure for the third, fourth and subsequent dilution. Dilution is carried out three times, including 10⁻², 10⁻⁴, 10⁻⁶. Each sample from the diluent tube was taken 0.1 ml, then inoculated into agar medium. The bacteria were grown in BHI agar medium and incubated at 39°C for 24 hours, to counting the number of colonies.

The protozoa’s population counting is done by entering 2 drops of samples which have been mixed with Blue Formalin Copy (TBFS) solution with a ratio of 1:1 on Counting Chamber. As much as 100 ml solution of TBFS is made from a mixture of 4% formalin and 0.9% NaCl as physiological solution. The total protozoa counted from 16 boxes of Counting Chamber under 10 times magnification of the microscope.

2.1.4. Microbial protein synthesis test
Analysing the microbial protein synthesis is continued by Lowry method [10]. In the first step, three solutions for Lowry’s method are made i.e. the complex reagents, the 2 N NaOH solution and the Folin-Ciocalteu reagents. For sample preparation, 20 ml of rumen fluid is distilled at 400 rpm for 45 seconds using a magnetic stirrer. After that, the sample is centrifuged within 5 minutes at 408 gravities to reduce the population of protozoa in the rumen fluid and removal the remaining feed particles. Aliquots (a rumen liquid which has been centrifuged at 408 gravities) were taken as much as 10 ml and added to 2.5 ml of 64.5% Trichloro Acetic Acid (TCA), then the supernatant is discarded and the precipitate is taken, then washed it with distilled water. Secondly centrifuge is done in the sample at 15,000 rpm for 20 minutes to obtain the precipitate. Then, the precipitate is added with 30 ml of 0.25 N NaOH solution and boiled as long 10 minutes. After cool, one ml of sample solution is taken for
analysis of microbial protein by following the Lowry’s method by using the complex reagents and Folin-Ciocalteu reagents. Protein concentration of samples is measured using Spectrophotometer with absorbance of 660 nm, if protein concentration is below 500 μg / ml or 550 nm, if the protein concentration is between 100-2000 μg /ml.

2.1.5. Statistical analyses
Randomized Block Design was used in this research with 6 treatment diets and 3 groups of rumen fluid taken. Data were analysed by the variance (ANOVA), further using orthogonal polynomial and Duncan test.

3. Results and Discussion

3.1. Fermentability and microbial protein synthesis
The results of fermentability and microbial protein synthesis can be seen in Table 2. The addition of seedless noni waste (SNW) in all diets did not influence the pH fermentation (6.93-6.98). The value of rumen pH has an important role in regulating the rumen process in describing microbial populations and rumen fermentation products [12].

In further tests using orthogonal polynomial, addition of SNW in diets shown a very significant difference (P<0.01) in NH3 concentration with quadratic pattern in accordance with the equation y = -0.0135x² + 0.3982x + 10.118 and R²= 0.8676 (where y was average value of NH3 concentration and x was SNW percentage). By following this equation, the maximum percentage of SNW addition in diets was 15% to produce 13.05 mM of NH3 concentration. Based on Duncan’s test, the addition of SNW in diets significantly increased NH3 concentration to 15% of SNW, then decreased (P<0.05). If using the equation, addition of SNW achieved 30% to produce almost similar to NH3 concentration of control (R0). Moreover, all of NH3 concentration that produced in this research are able to support the microbial protein synthesis due to the NH3 concentration still in normal range of 6-21 mM [13].

| Diets       | pH       | Fermentability (mM) | Microbial protein synthesis (mg/mL) |
|-------------|----------|---------------------|-------------------------------------|
|             |          | NH3                |          | Total VFA |          |                        |
| R0          | 6.93±0.23| 9.72±0.67c          | 102.15±1.83c | 18.810±1.438a |
| R1          | 6.97±0.16| 12.52±0.29ab        | 121.64±0.17b | 18.461±0.189a |
| R2          | 6.98±0.24| 12.67±0.36ab        | 136.62±2.30a | 17.730±1.210a |
| R3          | 6.93±0.18| 12.79±0.48a         | 136.86±3.25a | 19.057±0.343a |
| R4          | 6.98±0.28| 12.47±0.90ab        | 107.42±2.86c | 15.567±0.147b |
| R5          | 6.95±0.18| 11.88±1.15b         | 104.28±3.53c | 14.959±0.352b |
| Liner       |          | **                  |  *       |            | **       |
| Quadratic   |          | **                  |  **      |            | **       |

Note: R0= 60% NG + 40% Concentrate (control); R1= 60% NG + 5% SNW + 35% Concentrate; R2= 60% NG + 10% SNW + 30% Concentrate; R3=60% NG + 15% SNW + 25% Concentrate; R4=60% NG + 20% SNW + 20% Concentrate; R5=60% NG + 25% SNW + 15% Concentrate. (NG: Napier grass; SNW: Seedless Noni Wastes). Means with different superscripts in the same column differ significantly (P<0.05). *Significant (P<0.05). **Highly significant (P<0.01).

Total VFA as the main product of providing energy and carbon for growth and maintaining microbial survival, so that VFA formed is strongly influenced by digestibility and quality of the fermented ration [14]. Addition of 5-25% SNW increased total VFA production until certain percentage and after that decreased gradually. The orthogonal polynomial test for total VFA production as well as microbial protein synthesis also form a same pattern with the NH3 concentration’s equation by addition of SNW in the diets. The quadratic equation of total VFA production was y = -0.2077x² + 5.0117x + 103.12 and R²= 0.8062 (where y was average value of total VFA and x was SNW percentage). From that equation and also Duncan’s test, the maximum addition
of SNW in diets around 10-15% to produce around 136.74 mM of total VFA (P<0.05). According to McDonald (2002), the normal range of total VFA production was 70-150 mM. That normal range was achieved in this research, so that NH₃ and VFA are able to fulfil carbon and nitrogen sources of rumen microbes to synthesize the microbial protein.

Microbial protein synthesis depends on the speed of nitrogen feed breakdown, the speed of ammonia absorption and amino acids, the flow rate of material out of the rumen, the need of microbes to amino acids and the type of rumen fermentation based on the type of feed [15]. Microbial protein synthesis follows a quadratic equation $y = -0.0088x^2 + 0.0684x + 18.596$ and $R^2 = 0.78$ (where $y$ was average value of microbial protein synthesis and $x$ was SNW percentage). From that equation and also Duncan’s test, addition of SNW in diets until 15% were able to synthesize the microbial protein significantly similar to control that around 18.515 mg/ml (P<0.05).

### 3.2. Microbial population and in vitro digestibility

The results of microbial population and digestibility were shown in Table 3. The addition of SNW in diets did not influence the population of total bacteria as well as protozoa. The averages of total bacteria were 6.33-6.97 log CFU ml⁻¹ or equivalent to 10⁶-10⁷ CFU ml⁻¹. There were some factors that influenced bacteria population, such as type of forage or concentrate or type of feed nutrient, genetics of animal. The average protozoa yield in each treatment was 4.36-4.44 log CFU ml⁻¹ or equivalent to 10⁴ CFU ml⁻¹. These results are still in the normal range that is 10⁴-10⁶ CFU ml⁻¹ [14].

| Diets | Microbial population (log CFU ml⁻¹) | Digestibility (%) |
|-------|------------------------------------|-------------------|
|       | Bacteria                           | Protozoa          | Dry Matter | Organic Matter |
| R0    | 6.50±0.53                          | 4.36±0.11         | 56.37±1.83 | 55.20±2.83     |
| R1    | 6.55±0.63                          | 4.42±0.22         | 52.83±2.91 | 51.34±3.96     |
| R2    | 6.86±0.09                          | 4.44±0.22         | 54.77±1.57 | 53.28±2.86     |
| R3    | 6.97±0.09                          | 4.44±0.26         | 55.62±2.61 | 56.50±2.86     |
| R4    | 6.33±0.73                          | 4.43±0.29         | 57.57±2.90 | 57.93±3.96     |
| R5    | 6.42±0.51                          | 4.37±0.31         | 57.88±2.70 | 55.96±3.96     |

Note: R0= 60% NG + 40% Concentrate (control); R1= 60% NG + 5% SNW + 35% Concentrate; R2= 60% NG + 10% SNW + 30% Concentrate; R3=60% NG + 15% SNW + 25% Concentrate; R4=60% NG + 20% SNW + 20% Concentrate; R5=60% NG + 25% SNW + 15% Concentrate. (NG: Napier grass; SNW: Seedless Noni Waste).

The addition of SNW in diets did not influence the dry and organic matter digestibility (DMD and OMD). The average of DMD and OMD of ration contained SNW increased by increasing the SNW percentage in diets due to increasing the amount of SNW organic matter in diets as long 48 hours of incubation. Compared to previous studies, the average DMD of 15% SNW was lower (55.62%) than the average DMD of 15% native noni juice waste (64%) [8]. This might be caused by the large loss of organic matter from noni juice waste in the fermentation process of the fresh noni fruit (more than one month) before the noni fruit is pressed into noni juice. Thus the organic matter content of SNW has resulted in low of digestibility.

### 4. Conclusions

It can be concluded that 15% of seedless noni waste (SNW) is able to substitute 37.5% of concentrate in lactating dairy goat diet with ratio of 60% forages and 40% concentrate. The diet is able to support fermentability and microbial protein synthesis without interfere the microbial population as well as in vitro digestibility.
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References
[1] Directorate General of Livestock and Animal Health 2017 Goat Population in Indonesia. Jakarta (ID): Ministry of Agriculture. Ditjennak Jakarta Indonesia. [In Indonesian]
[2] Supriyati P W, Sutama I K, Budiarsana I G M, Mathius I W, Lubis D 2008 Proceedings of Internasional Seminar on Production Increase in Meat and Dairy Goats by Incremental Improvements in Technology and Infrastructure for small-scale Farmers in Asia. Bogor (ID): The FFTC-Asian and Pasific region, IRIAP Indonesia, LRI/COA-Taiwan ROC p. 21-24.
[3] Sanchez J, Montes P, Jimenez A, Andres S 2007 Prevention of clinical mastitis with barium selenite in dairy goats from a selenium deficient area J. Dairy Sci. 90 2350-2354.
[4] McDougall S, Pankey W, Delaney C, Barlow J, Patricia A M, Scruton D 2002 Prevalence and incidence of subclinical mastitis in goats and dairy ewes in Vermont USA Small Rumin Res. 46 115-121.
[5] Leitner G, Silankove N, Merin U 2008 Estimate of milk and curid yield loss of sheep and goats with intramammary infection and its relation to somatic cell count Small Rumin Res. 74 221-225.
[6] Bintang I A K, Sinurat A P, Purwadaria T 2007 Addition of noni waste as a bioactive compound to the performance of broiler chickens JITV 12(1) 1-5. [In Indonesian]
[7] Directorate General of Horticulture 2015 Horticultural Production Statistics in 2014. Ministry of Agriculture. Ditjen Hortikultura Jakarta Indonesia. [In Indonesian]
[8] Evvyernie D, Permana I G, Toharmat T, Fakhirudin, Hilal M, Wicaksono M A 2018 In vitro evaluation of noni juice waste as forage substitute, anti mastitis and anthelmintical candidates in dairy goat ration [In the publication process]
[9] Tilley J M A, Terry R A 1963 A two stage technique for the in vitro digestion of forage crops J. British Grassland Soc. 18 104-111.
[10] Makkar H P S, Sharma O P, Dawra R K, Negi S S 1982 Simple determination of microbial protein in rumen liquor Journal Dairy Sci. 65 2170-2173.
[11] Ogimoto K, Imai S 1981 Atlas of Rumen Microbiology Tokyo (JP): Japan Sci. Soc. Pr.
[12] Istiqomah L, Herdian H, Febrisantosa A, Putra D 2011 Waru leaf as saponin source on in vitro ruminal fermentation characteristic J. Indonesian Trop Anim. Agric. 36 43-49.
[13] McDonald P, Edwards R A, Greenhalgh J F D, Morgan C A 2002 Animal Nutrition 6th Ed. Ashford Colour Pr. New York USA.
[14] Kamra D N 2005 Rumen Microbial Ecosystem J. Indian Veterinary Research Institute 89(1) 124-135
[15] Arora S P 1995 Microbial Digestibility in Ruminant. Translation by R Murwani. Yogyakarta (ID): Gadjah Mada University Press Yogyakarta Indonesia. [In Indonesian]