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*by* Sri Rahayu

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To cite this article: M Bata *et al* 2022 *IOP Conf. Ser.: Earth Environ. Sci.* **1001** 012003

# Impact of ammoniation rice straw treatment with direct-fed microbials and *Hibiscus tiliaceus* leaf meal supplemented in concentrate on local sheep performances

**M Bata[1*], S Rahayu[1], E A Rimbawanto[1] and M Fita[2]**

[1]Faculty of Animal Science, University of Jenderal Soedirman, Indonesia.
[2]Master Program of Animal Science, Faculty of Animal Science, University of Jenderal Soedirman, Indonesia.

[*]E-mail: muhamadbata@yahoo.com

**Abstract.** This study aimed to determine the interaction between ammoniation rice straw treatment using direct-fed microbial (DFM) and *Hibiscus tiliaceus* leaf meal (HTLM) supplementation on local sheep performance. Thirty-six local male sheep aged 2 years (28.01 ± 2.61 kg) fed with ammoniated rice-straw (ARS) treated with DFM and concentrate supplemented with HTLM were assigned randomly to receive nine treatments in an experiment of 3 x 3 factorial design. The first factor was the use of DFM with P0 = control, P1 = $DFM_{AMS}$, and P2 = $DFM_{RK}$, while the second was level of HTLM with W0 = 0%, W1 = 0.24% and W2 = 0.48% DM concentrate. Concentrates were fed 3% of sheep body weight, and ARS was prepared *ad-libitum*. The variables measured were final weight (FW), average daily gain (ADG), dry matter intake (DMI), dry matter digestibility (DMD), crude fiber digestibility (CFD), feed conversion ratio (FCR), and consumption rate (CR). There was significant interaction (P<0.05) between DFM and HTLM supplementation on the final FW, DMI, and CR (P<0.01), while ADG and FCR were not significant. Furthermore, DFM supplementation (P<0.05) on FCR, DMD, and CFD but not ADG. The lowest FCR was achieved in a combination of W2 and $DFM_{RK}$, and ADG increased with the dose of HTLM. The highest ADG achieved was in the combination of $DFM_{RK}$ and W2. For DFM and HTLM, 0.48% of $DFM_{RK}$ and the level of HTLM improved local sheep performance and feed efficiency. It had a positive impact on the environment by minimizing methane production.

## 1. Introduction

The sustainability of ruminant livestock production can be maintained through efforts to increase feed conversion efficiency and suppress methane production. However, high fibre contents of diets such as rice straw are negatively correlated with voluntary intake, rate of organic matter fermentation, microbial cell yield per unit organic matter fermented, and propionates such as acetate ratio and high methane production in fermentation [1]. Also, rice straw contains low protein or nitrogen and fermentable carbohydrates; therefore, nitrogen and energy needs for rumen microbes are not readily supplied since several efforts are needed to improve quality animal feed from agricultural residues.

One method developed to upgrade crop residues, such as rice straw, is urea treatment. However, the method is uneconomical and causes environmental pollution because of escaping of $NH_3$-N from urea-treated rice straw [2]. Furthermore, most of the nitrogen was water-soluble, which was released rapidly in the rumen in the form of N-$NH_3$ and resulted in nutrient loss [3]. This is caused by the limitation of fermentable carbohydrates of rice straw used to improve nitrogen retention in ammonia, such as corn steep liquor [4], molasses [3,5,6], and tapioca industrial byproducts [7]. However, the ammonia produced is still high with a pungent smell. Therefore, it is not safe for farmers and their livestock due to the high possibility of poisoning and environmental pollution.

One way to overcome excess ammonia is by administering Direct-fed microbial (DFM). Ammonia and probiotic fungal supplementation provide favorable results on agricultural waste [8,9]. The use of DFM, which contains various types of microbes such as $DFM_{AMS}$ (P1) produced by PT. AMS Korea contains 17 kinds of microbes and $DFM_{RK}$ produced by PT. Banyumas Raya Indonesia contains 15 microbes and 1 yeast for fixing ammonia produced. However, the use of DFM increased the population of protozoa [10,11] in positive correlation with methane production. Ningrat et al. [12] stated that supplementation of *Saccharomyces cerevisiae* (SC) and *Uncaria gambir* Leaves waste containing ammoniated palm fronds using urea can increase nutrient digestibility average daily gain but reduce methane production in Simental cattle. Bata et al. [7] reported that *Hibiscus tiliaceus* leaf extract containing saponins reduced protozoa and methane in vitro. This study determines the effect of using $DFM_{AMS}$ and $DFM_{RK}$ in the ammoniating process of straw and hibiscus leaf flour as a defaunation agent on the digestibility, feeding speed, and performance of local sheep.

## 2. Materials and methods

### *2.1. Animals, feeds, and experimental design*

A total of 36 local male sheep (2 years) with an initial body weight of 28.01 ± 2.61 kg were placed randomly in individual cages of stage type. The concentrate was fed as much as 3% of body weight, and ARS was prepared *ad libitum*. Furthermore, the concentrated feed was administered twice daily at 07.00 in the morning and at 15.00 in the evening, respectively. The composition used was tapioca by-product, pollard, oil palm cake, coconut, soybean, molasses, dolomite, salt, urea, and minerals (Table 2).

**Table 1.** Composition and nutrient content of diets in various combinations of DFM and HTLM.

| Diets | P0 | | | P1 | | | P2 | | |
|---|---|---|---|---|---|---|---|---|---|
| | W0 | W1 | W2 | W0 | W1 | W2 | W0 | W1 | W2 |
| | (%) | | | | | | | | |
| ARS | 9 | 16 | 11 | 15 | 14 | 13 | 11 | 24 | 24 |
| Concentrates | 91 | 84 | 89 | 85 | 86 | 87 | 89 | 76 | 76 |
| HTLM | 0 | 0.24 | 0.48 | 0 | 0.24 | 0.48 | 0 | 0.24 | 0.48 |
| $DFM_{AMS}$ | - | - | - | 0.50 | 0.50 | 0.50 | - | - | - |
| $DFM_{RK}$ | - | - | - | - | - | - | 0.50 | 0.50 | 0.50 |
| *Nutrient* | | | | | | | | | |
| DM | 78.83 | 75.23 | 78.20 | 75.18 | 75.98 | 76.78 | 77.48 | 70.28 | 70.51 |
| Ash | 12.15 | 13.26 | 12.50 | 12.95 | 12.82 | 12.70 | 12.54 | 14.68 | 14.70 |
| EE | 12.30 | 11.48 | 12.08 | 11.59 | 11.72 | 11.85 | 12.06 | 10.54 | 10.56 |
| CF | 26.50 | 27.08 | 26.75 | 27.35 | 27.30 | 27.25 | 26.95 | 28.34 | 28.39 |
| CP | 8.95 | 8.86 | 8.95 | 8.86 | 8.88 | 8.91 | 8.78 | 8.43 | 8.44 |
| TDN | 72.07 | 70.15 | 71.59 | 70.39 | 70.71 | 71.03 | 71.51 | 67.92 | 67.96 |

ARS, Rice Straw Ammoniated; HTLM, Hibiscus tileaceus leaf meal; DFM, *Direct-Fed Microbials*; DM, Dry Matter; EE, Ethyl Extract; CF, Crude Fiber; CP, Crude Protein; TDN, Total Digestible Nutrients

The two kinds of DFM used are $DFM_{AMS}$ (P1) produced by PT AMS Korea, containing 17 microbes and $DFM_{RK}$ produced by PT Banyumas Raya Indonesia, containing 15 microbes and 1 type of yeast. The application method on ARS was DFM mixed with water and molasses of about 2% of the amount of water to be used, then stirred to become homogeneous. Subsequently, it is put into a spray bottle, sprayed in ARS with a 0,5% DM rice straw of DFM.

*2.2. Supply of Hibiscus tiliaceus leaf meals (HTLM) and ammoniated rice straw (ARS)*

*Hibiscus tiliaceus* leaf meals were obtained from several points in the Sokaraja Animal Market, Banyumas Regency, and Cilacap Market, Cilacap Regency, Central Java. First, they were removed from the stems and dried in the sun for 3-6 days. After that, they were ground into flour, and the HTLM was mixed according to the treatment dose.

Rice straw was obtained from the fields around Banyumas Regency, Central Java, and they were chopped using a chopping machine. The ammoniated straw used 5% urea and tapioca byproduct as additives 2.5% of the air-dry weight of rice straw. Furthermore, urea and cassava were dissolved in water, stirred, and then sprayed on rice straw. The mixture was put in a plastic bag, compacted, and tied to anaerobic conditions, and the ammonia process ended after two weeks. Then the ammoniated straw was sprayed with DFM and left for one day before being fed to the sheep.

This study was designed using a completely randomized design with a 3 x 3 factorial pattern. The first factor was the type of DFM (P0 = control, P1 = $DFM_{AMS}$, P2 = $DFM_{RK}$), while the second was the level of supplementation of HTML for 0, 0.24, and 0.48% of DM concentrate for W0, W1, and W2, respectively. Each treatment was repeated four times, and the variables measured were slaughter weight (SW), daily body weight gain (ADG), dry matter intake (DMI), feed conversion ratio (FCR), and consumption rate (CR). In addition, dry matter digestibility (DMD) and crude fiber digestibility (CFD) was measured using the total collection method [13].

*2.3. Sampling and chemical analysis procedures*

Data collection was conducted during the feeding and digestion trial. Then the data collected were body weight at the beginning of the study (after preliminary) and every month. In addition, data on feeding and leftover feed were weighed in the feeding and the digestion trial. Consumption rate observations were carried out for 5 days during the collection period in the digestion trial.

The feces sampling, feeding, and remaining feed were 5, 3, and 5% of the total weight, respectively. The samples were dried in an oven at $65^0$ C for three days. The dried samples were ground and sieved using a 3.5 mesh sieve and were composited based on the day of collection and the treatment. Analysis of moisture, ash, and crude fiber content of feces, feeding, and residual feed was carried out according to the [14]

*2.4. Statistical analysis*

Average daily gain and SW were analyzed using covariance analysis. In addition, data on DMI, FCR, and CR, DMD, and CFD were analyzed using analysis of variance and continued with the Orthogonal Polynomial test.

## 3. Results and discussion

The increase in feed efficiency shown by the low FCR is one indicator of a decrease in methane production in the rumen. Feed conversion ratio (FCR) of P0 was higher ($P<0.05$) than P2 but similar to P1 (Table 2), and P2 treatment resulted in lower feed conversion ($P<0.05$) than P1. The low FCR in F2 was due to DFM P2 containing yeast of *Saccharomyces ceriviceae,* which can reduce oxygen in the rumen to increase the fermentation process, digestibility of crude fiber and organic matter. Furthermore, this will lead to high feed efficiency or a low feed conversion ratio [15]. Suryani et al. [16] explained that *Saccharomyces ceriviceae* secretes galactosidase and glucosidase in breaking saccharides and oligosaccharides into simple sugars (di- and mono-saccharides). *Saccharomyces ceriviceae* uses oxygen for metabolism to increase oxygen utilization by yeast cultures in the rumen. Mwenya et al. [17] reported that the addition of yeast can stimulate acetogenins that compete with methanogens in utilizing hydrogen, thereby reducing methane formation. This reduction improves feed efficiency and positively impacts the environment or inhibits ozone depletion. Yeast of *Saccharomyces ceriviceae* contains micronutrients as a source of nutrients for rumen microbes, thereby increasing rumen microbial growth and feed fermentation [18].

**Table 2.** Average of final body weight, average daily gain (ADG), dry matter intake (DMI), dry matter digestibility (DMD), organic matter digestibility (OMD), feed conversion (FC), and feeding rate of local sheep fed on various combinations of Hibiscus leaf meal (HTLM) and DFM.

| Variables | Treatments | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | P0 | | | P1 | | | P2 | | |
| | W0 | W1 | W2 | W0 | W1 | W2 | W0 | W1 | W2 |
| DMI (kg) | 1.04 ± 0.07 | 1.13 ± 0.06 | 1.09 ± 0.10 | 1.03 ± 0.04 | 0.92 ± 0.04 | 1.06 ± 0.14 | 0.97 ± 0.08 | 0.82 ± 0.04 | 0.99 ± 0.08 |
| OMD (%) | 72.44 ± 1.68 | 70.56 ± 4.57 | 70.81 ± 2.47 | 72.19 ± 6.13 | 73.60 ± 6.36 | 71.09 ± 3.42 | 71.49 ± 3.55 | 73.26 ± 6.40 | 72.10 ± 3.33 |
| DMD (%) | 67.96 ± 1.78 | 65.10 ± 1.44 | 65.02 ± 1.84 | 67.43 ± 7.31 | 69.11 ± 7.77 | 66.61 ± 4.11 | 66.45 ± 4.11 | 68.85 ± 7.64 | 66.76 ± 3.94 |
| CFD (%) | 40.44 ± 1.68 | 45.56 ± 4.57 | 41.81 ± 2.47 | 62.23 ± 4.04 | 57.05 ± 7.50. | 49.71 ± 2.45 | 60.11 ± 2.37 | 57.89 ± 1.66 | 62.80 ± 1.80 |
| Final BW (kg) | 40.44 ± 1.68 | 45.56 ± 4.57 | 41.81 ± 2.47 | 41.43 ± 2.74 | 39.05 ± 2.64 | 43.60 ± 6.19 | 39.41 ± 2.28 | 38.23 ± 1.55 | 46.25 ± 3.65 |
| ADG (kg) | 0.113 ± 0.02 | 0.118 ± 0.04 | 0.125 ± 0.04 | 0.111 ± 0.02 | 0. 107 ± 0.03 | 0.127 ± 0.03 | 0.110 ± 0.01 | 0.119 ± 0.02 | 0.146 ± 0.03 |
| Feed Convertion | 9.09 ± 2.15 | 9.69 ± 2.99 | 9.99 ± 1.45 | 9.15 ± 1.61 | 8.21 ± 1.95 | 9.06 ± 1.82 | 8.22 ± 1.19 | 7.15 ± 0.85 | 6.81 ± 1.68 |
| Feeding Rate (kg/hour | 0.46 ± 0.22 | 0.67 ± 0.40 | 0.61 ± 0.27 | 0.92 ± 0.17 | 0.33 ± 0.07 | 0.38 ± 0.18 | 0.41 ± 0.16 | 0.67 ± 0.24 | 0.70 ± 0.20 |

[1]P0, without of DFM; P1, $DFM_{AMS}$; P2, $DFM_{RK}$; W0, without of HTLM; W1=HTLM of 0.24 %; W2, HTLM of 0.48 %

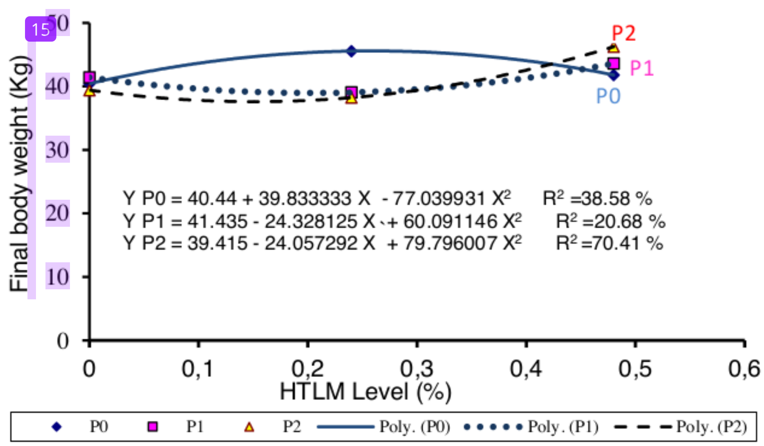


**Figure 1.** The relationship between the level of hibiscus leaf meal (HTLM) and final body weight of local sheep on various DFM (P0, P1, and P2).

Increasing the dose of hibiscus leaf powder resulted in different SW responses for three types of DFM supplementation (Figure 1). At P0, an increase in HTLM increased the SW until the optimal weight was at 0.26% HTLM with a maximum SW of 45.59 Kg and decreased with the increase in hibiscus HTLM. This was due to the content of saponins in HTLM, which function as a protozoa defaunation agent to increase the bacterial population and the digestibility of CFD, DMD, and OMD (Table 2). Furthermore, it increases dry matter consumption (Figure 2) and reduces methane production, increasing propionate, a precursor for glucose formation. Excess glucose will be stored in body fat, resulting in a higher final weight than the control. An increase in the dose of HTLM from more than 0.26% to 0.48% caused a decrease in SW due to an anti-bacterial content of sulfonamide [19]. Saponins are toxic to protozoa and bacteria in the rumen when the dose is high [20]. The increasing anti-bacterial compounds and saponins in HTLM suppressed the rumen bacteria population. Feed digestibility is directly related to SW, and at P1 and P2, there was a decrease in SW at 0.20% HTLM level with an SW of 38.97 Kg (P1) and 0.15% hibiscus leaf powder with an SW of 37.60 Kg (P2). The addition of HTLM, then, level up to 0.48% can increase the SW since the DFM in P1 and P2 contained lactic acid-producing bacteria (LAB). Besides that, the feed provided was relatively high, and almost 80% of DMI comes from concentrate. It triggers the production of lactic acid, which causes the rumen pH to be low, thus disturbing the microbial activity in digesting producing energy for the host, and it had a negative impact on sheep performance. Supplementation of high doses of HTLM on W2 can improve the growth/performance of sheep. This is because the flavonoids of HTLM can stimulate the growth of lactate user bacteria (LUB), and LUB can directly convert lactate into propionate [21]. This caused the weights on W2 for P1 and P2 to be higher than W0 and W1.

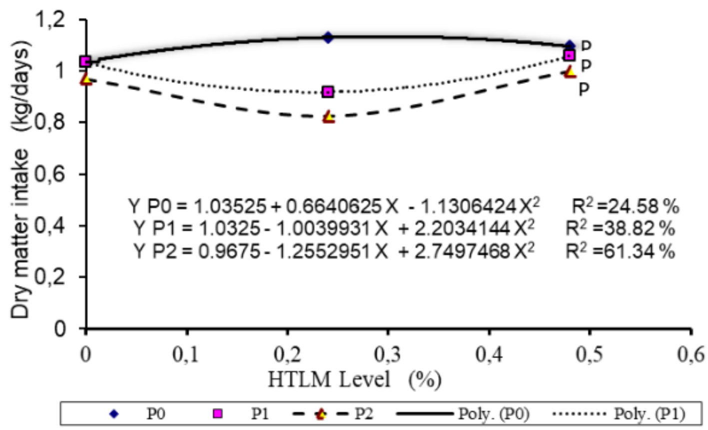


**Figure 2.** The relationship between the level of hibiscus leaf meal (HTLM) and dry matter intake of local sheep on various DFM (P0, P1, and P2).

The result showed that there was a tendency to increase ADG at P0, P1, and P2, respectively 0.125 ± 0.042 (kg/day), 0.127 ± 0.03 (kg/day), and 0.146 ± 0.031(kg/day) at high doses of HTML (W2) due to the flavonoid content in HTLM acts as a buffer in maintaining rumen pH. Flavonoids also had a role in increasing the population of LUB. Balcells et al. [22] further explained that flavonoids could increase rumen fermentation. Flavonoids had a role in increasing rumen pH, reducing methane production, and increasing the proportion of propionate [23]. In addition, the content of HTLM in the form of malate and fumarate were the essential ingredients for propionate formation. Therefore, increasing the dose of hibiscus flour can increase the supply of glucose, which plays a role in metabolism and body fat accumulation.

Dry matter Intake of P0 increased along HTLM up to the level of 0.29% with a maximum DMI of 1.132 kg per day; therefore, the addition of HTLM did not affect the diet's palatability. On the contrary, there was a decrease in DMI to a minimum level of 0.22% with a DMI of 0.918 Kg and 0.23% and a DMI of 0.824 Kg after increasing HTLM level up to 0.48% to increase DMI. The increase and decrease in consumption were due to the palatability of livestock to saponins, and the ratio of ARS to the concentrate was not balanced (18:82). Patra & Saxena [24] reported that the feeding of high concentrations caused the protozoa to die. This is due to the decrease in rumen pH caused by the fermentation of starch to lactic acid. Marhaeniyanto and Susanti [25] stated that the rumen condition influences consumption. Good rumen conditions will provide an optimum environment for the bacteria to ferment and increase the feed degradation rate. However, unfavorable rumen conditions will inhibit the performance of bacteria, causing a decrease in consumption. In addition, feeding with a high concentration will cause the pH to become acidic. An acidic pH will cause the protozoa to die before degrading starch, causing a decrease in consumption. The increase in dry matter consumption at a dose of 0.48% was because hibiscus leaves contain flavonoids, which neutralize rumen pH due to high concentrates. Bata and Rahayu [19] reported that hibiscus leaves contain flavonoids that can maintain rumen pH (buffer), increase the population of bacteria, and lactate utilizer to increase consumption.

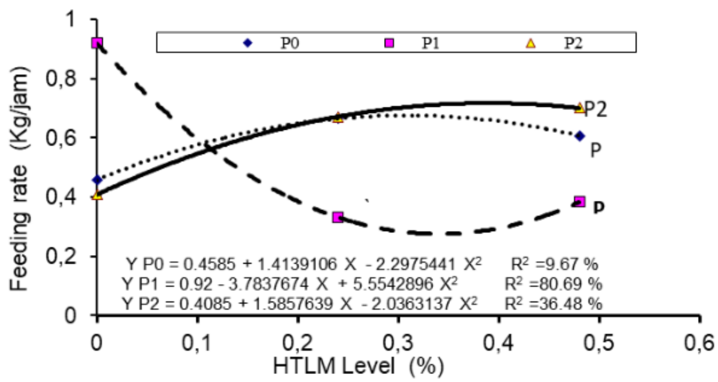


**Figure 3.** The relationship between the level of HTLM and the feeding rate of local sheep on various DFM (P0, P1, and P2)

Feeding rates were influenced by the type of DFM and the level of HTLM. The increase in the level of HTLM showed different feeding rate responses at P0, P1, and P2 (Figure 3). Meanwhile, the P0 treatment increased the feeding rate and the level of HTLM. This is presumably because HTLM also contains amino acids in the form of glutamic acid, increasing the palatability of animal feed. The glutamic acid content in small type HTLM is 0.25% [19]. The P2 treatment increased feeding rate and HTLM due to yeast preparation in Saccharomyces cerevisiae, which can produce glutamic acid to increase animal feed and consumption palatability. Suryani et al. [16] and Wikanastri et al. [26] stated that Saccharomyces cerevisiae can produce glutamic acid, increasing feed palatability. However, at P1, an increase in the level of HTLM caused a decrease in the feeding rate. The DFM does not contain *Saccharomyces cerevisiae*, but contains LAB bacteria, as previously discussed; therefore, rumen microbial activity reduced feeding rate. Meanwhile, the addition of high HTLM can increase feed speed because the flavonoid content is sufficient to stimulate the growth of lactate user bacteria. Therefore, the rumen pH, microbial activity, and the rate of feeding are increased.

## 4. Conclusions

In conclusion, 0.48% DM concentrates of $DFM_{RK}$ and HTLM level improved local sheep's performance and feed efficiency to minimize environmental pollution.

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