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Comparison of Sorghum Straw (*Sorghum bicolor* (L.) *Moench*) Digestible Quality With and Without Indigofera (*Indigofera sp*) By In Vitro Technique

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ABSTRACT
The purpose of this research was to study the comparison of sorghum straw (Sorghum bicolor (L.) Moench) digestible quality with or without Indigofera (Indigofera sp) by in vitro analysis. The sorghum were added at 5% dry matter to 100% sorghum straw (T0), 70 % sorghum straw + 30 % Indigofera (T1) and 30 % sorghum straw + 70 % indigofera (T2) respectively. Together with a control for each (without any addition of indigofera), these diets were incubated in an in vitro rumen fermentation system, performed for three runs, with each run represented by two incubation units. The data were analyzed using two-way variance analysis (ANOVA), followed by Duncan's test. The experiment design of completely randomized (CRD) was applied for DMD, N-NH_3, and TVFA. The results showed that the effect of the treatments was not significant ($P>0.05$) on the observed variables such as DMD, the concentration NH_3, and TVFA. This research indicated that sorghum straw with or without Indigofera in ration did not affect the concentration of DMD, N-NH_3, and Total VFA. Thus, it can be concluded that the use of sorghum straw with or without indigofera is not recommended as a ration for ruminants.

Keywords: *Sorghum Straw, Digestibility, In vitro, Indigofera.*

1. INTRODUCTION

Sorghum (*Sorghum bicolor* (L.) Moench) and Indigofera (*Indigofera* sp) can be relied on as a source of animal feed, especially in marginal and dry areas in Indonesia, because they have good adaptability and nutritional quality. Moreover, The addition of feed with sorghum rations in cows increased daily body weight gain (DBWG) and the performance of reproductive organs [1] [2].

Sorghum straw can increase dry matter digestibility (DMD) because it has a positive effect on feed consumption and digestibility. On the other side, the concentration of NH_3 and the concentration of Total Volatile Fatty Acid (TVFA) produced also increased, which could be helpful in the process of degradation and protein synthesis by rumen microbes[3]. Furthermore, previous studies showed that the use of sorghum in buffaloes could increase the concentration of NH_3 [4]. Indigofera (*Indigofera sp*) is a forage that contains structural and non-structural carbohydrates. The nutritional value is quite good, especially for high protein, which potentially be used as livestockfeed

Indogofera rich of nitrogen, phosphorus, potassium, and calcium. The nutritional values of Indigofera leaf are 25.77% crude protein, 12.40% crude fiber, 13.08 mg/100g calcium, and 0.22 mg/100g phosphor [5]. The level of Indigofera production is relatively good. The average production per harvest reaches 2.6 ton of DM/ha/year in acidic soil conditions [6], while in red and yellow podzolic soil conditions or soils formed from high rainfall with a neutral pH reaching 4.096 ton DM/ha/year at the age of 88 days [7]. The use of rations containing bush leaves/legumes can improve the digestibility of dry matter [8].

The total VFA produced from rumen fermentation of animal fed Indogovera leaf indicated the good quality of feed and the feed fermentation process efficiently in the rumen [9]. The use of sorghum straw as animal feed has a high crude fiber digestibility value when complemented

with a non-nitrogen protein (NPN) source and support rumen fermentation in vitro [3]. Research on the digestibility of sorghum straw as feed is already available, but the combination with Indigofera is not available. Thus research on the utilization of Sorghum straw with and without Indigofera needs further observation.

2. MATERIALS AND METHOD

2.1. Research Method

This study used experimental Completely Randomized Design (CRD) with three treatments and five replications, namely treatments were 100% Sorghum straw as control (P0), treatment Sorghum straw 70% + Indigofera 30% (T1), and Sorghum straw 30% + Indigofera 70% (T2). The observed variable in this research was dry matter digestibility (DMD), the concentration of N-ammonia (N-NH_3), and the total volatile fatty acid (VFA).

2.2. Sample Preparation

2.2.1. Preparation of McDougall Solution (artificial saliva)

The preparation of McDougall's solution is divided into several stages. First, 2.5 liters of distilled water and a five-liter volumetric flask that has been provided are placed on a magnetic stirrer. Then, the following ingredients were added: Na_2CO_3 (49g), $Na_2HPO4_7H_2O$ (35g), KCL (2.85g), NaCl (2.35g), $MgSO_47H_2O$ (0.6g) and $CaCl_2$ solution (0.2g) were stirred with a spatula while adding 100 ml of distilled water. After the first process is complete, the resulting solution is put into a measuring flask and rinsed with distilled water until the water level reaches the mark then Autoclave at 121°C at 1 atm for 24 hours.

2.2.2. In vitro Digestibility Test

*2.2.3.*The rumen fluid as rumen microbe inoculum was taken from livestock and filtered through 4 layers of gauze. Then the rumen fluid was diluted (1: 2) with McDougall solution during continuous frushed CO_2 gas. A grinding process mashed feed materials, then used as a substrate. The sample was put into a fermentor bottle, and 20 ml of rumen liquid was added while CO_2 gas flowed. The fermenter bottle was closed with a butyl rubber stopper and then anaerobically incubated for 24 hours at a temperature of 39°C using a 180 rpm water bath shaker. At the end of incubation, the sample was then transferred into a 50 ml centrifuge tube, centrifuged at 2000 rpm for 10 minutes, then cooled to stop the fermentation process. The supernatant was used to determine the total VFA and ammonia concentration, while the residue was used to determine the dry matter digestibility. Dry matter digestibility was calculated by placing the residue in an oven at 105°C for 72 hours. The residual mass obtained was calculated as a percentage of dry matter in vitro.*Dry Matter Digestibility (DMD) Concentration Measurement*

Fill the tube with one gram of sample and 40 ml Mcdougall liquid. The fermentor tube was then put into a shaker water bath with a temperature of 39°C, then filled with 10 ml of rumen fluid. The tube was then shaken and flowed with CO_2 for 30 seconds. Then covered with ventilated rubber and fermented for 48 hours. After 48 hours of fermentation, $HgCl_2$ was added with about 2-3 drops to kill the microbes. The fermenter tube was then put into a centrifuge and centrifugated at 4,000 rpm for 10 minutes. The results of the centrifugation were filtered using filter paper. The supernatant was collected, and the residue was added with 50 ml of 0.2% pepsin-HCl solution. The sediment on the filter paper is put on a porcelain dish after putting it in a 105^0C oven for 48 hours. After 48 hours, the porcelain cup containing the filter paper with the residue was cooling down in a desiccator and weighed to determine the residual feed dry matter.

2.2.4. The concentration of N-Ammonia (N-NH3) Measurement

The concentration of NH_3 was measured using the Conway microdifusion method. A total of 1 ml of rumen fluid supernatant was placed on one side near the Conway dish, and on the other side, 1 ml of saturated Na_2CO_3 solution was placed. The Conway plates are positioned so that they do not mix before the cup is tightly closed. In the Conway cup, 1 ml of the boric acid solution with the indicator is placed. The cup is tightly closed with Vaseline. The supernatant and Na_2CO_3 solution were evenly mixed by shaking the cup. The ammonia released from the reaction between the two materials then be captured by boric acid, shown by a change in color. After 24 hours, ammonium borate was titrated with 0.005 N of H_2SO_4 solution until the color changed to boric acid's original color.

2.2.5. VFA Total Concentration Measurement

The steam distillation method used the steam distillation method to measure the total concentration of volatile fatty acid (VFA) [10]. Five ml of the supernatant prepared for VFA analysis were then put into a distillation tube. Sulfuric acid 15% was added, then the tube was covered with a rubber cap and connected to a cooling flask. The distillation tube was put into a distillation flask containing boiling water. Hot water vapour stimulates volatile fatty acid (VFA) and will be condensed in the cooling flask. The distillate was collected in an Erlenmeyer flask containing 5 ml of 0.5 N NaOH until 250 ml. Then 3 to 4 drops of the phenolphthalein (P.P.) indicator was added, followed by

titration with 0.5 N HCl until the color changed from pink to colorless.

2.3. Data Analysis

The data were analyzed using analysis of variance (ANOVA) with a factorial arrangement, in which the first factor was the substrate. The following statistical model was employed: When a parameter was statistically significant at $p < .05$, it was followed by a Duncan's Multi Range Test (DNMRT) test. The statistical analysis was conducted employing ms. excel [11].

3. RESULT AND DISCUSSION

3.1.1. Dry Matter Digestability (DMD)

Dry matter digestibility (DMD) is an indicator of ration quality. The higher the digestibility, the higher the chances of nutrition being digested by livestock. The digestibility of dry matter can be used to determine the quality of feed. The value of dry matter digestibility shows how much rumen microbes utilize nutrients in the feed. The average dry matter digestibility values in this study can be seen in Table 1.

Table 1. Average DMD of Sorghum straw and Indigofera

Treatments	Average DMD %
Sorghum straw 100 % (P0)	40,62±5,52
Sorghum straw 70 % + Indigofera 30 % (T1)	37,37±7,56
Sorghum straw 30 % + Indigofera 70 % (T2)	33,84±4,49

Based on Table 1, it can be seen that the average dry matter digestibility from the highest to the lowest values was the 100% sorghum straw (P0): 40.62 ± 5.52, 70% sorghum straw + 30% Indigofera (T1): 37,37 ± 7.56, and 30% sorghum straw + Indigofera 70% (T2): 33.84 ± 4.49, the average percentage of dry matter digestibility for all treatments ranged from 33.84% to 40,62%.

The feed ratio used in this study was forage-based feed, in which the dry matter digestibility value was not as high as of concentrate-based feed. The dry matter digestibility value reaches of concentrate-based feed is 74.50% [12]. The variance analysis showed that the treatment of Sorghum straw with or without Indigofera was not significantly affected (P> 0.05) on the digestibility of dry matter. This finding indicates that the use of sorghum straw with or without Indigofera in the feed did not affect the rumen fermentation process. The low value of DMD might be due to the existence of the anti-nutritional substances in the form of tannins in the Indigofera, affecting the average digestibility of dry matter.

Several previous studies reported that tannins plants affected dry matter's digestibility [13] [14]. The anti-nutritional substances such as tannins contained in legumes negatively affected feed digestibility. Tannins can inhibit microbial activity in degrading the feed dry matter [15]. The tannin contained in the animal feed is considered anti-microbial, which causes inhibition of the degradation of dry matter digestibility by rumen microbes [16]. Another factor that possibly caused the insignificant treatment effect on the dry matter digestibility was the high crude fiber (CF) content in the form of lignin in sorghum straw. Lignin in sorghum straw was significantly higher than in Indigofera.

The high crude fiber in sorghum straw reflects the high lignin fraction that binds cellulose and hemicellulose, which causes the low digestibility [17]. The crude fiber in sorghum straw is around 34-37% [18], while the crude fiber in Indigofera is 12% [19]. The high crude fiber in sorghum straw affected the average dry matter digestibility [20]. It is suspected that this factor affects the dry matter digestibility rate. The high crude fiber in sorghum straw is closely related to ADF digestibility. ADF containing lignocellulose affects dry matter digestibility and becomes insignificant [17]. The ADF content in sorghum straw ranges from 37.12% [1].

3.1.2. Concentration of N-Amonia (N-NH_3)

N-Ammonia or N-NH_3 in the rumen is a critical intermediary in microbial degradation and protein synthesis. If the feed lacks protein or the protein in the feed is difficult to be degraded. The ammonia concentration in the rumen will decrease, causing decreased microbes growth in the rumen, and the consequence is a slower breakdown of carbohydrates. The N-NH_3 concentration data in this study can be seen in Table 2.

Table 2. Concentration of N-Ammonia

Treatments	Average (mM)
Sorghum straw 100% (P0)	1,47±0,98
Sorghum straw 70 % + Indigofera 30 % (T1)	1,17±0,59
Sorghum straw 30% + Indigofera 70 % (T2)	1,72±0,47

Based on the data in Table 2, it can be seen that the highest to the lowest average value of N-NH_3were at treatments of 30% Sorghum straw + 70% Indigofera (T2): 1.72 ± 0.47 (mM), 100% Sorghum straw (P0): 1.47 ± 0.98 (mM), and 70% Sorghum straw + Indigofera 30% (T1): 1.17 ± 0.59 (mM). The variance results showed that the treatment did not significantly affect the concentration (P> 0.05). N-NH_3.

Sorghum straw with or without Indigofera did not affect the process of feed degradation by rumen microorganisms which could illustrate that the ammonia produced was unable to have a significant impact on rumen microbial synthesis due to the imbalance in the availability of energy and protein in the rumen.. According to [21], the higher degree of synchronization of energy and nitrogen release increased microbial protein synthesis partly via influencing the bacterial community, metabolism, and enzyme activities of ammonia assimilation in the in vitro fermenters. Indigofera has 27% protein content, and sorghum straw with 13% protein affect the concentrations of NH_3 that accumulate in the rumen. The contribution of NH_3 to ruminants is significant considering that the precursor for microbial protein is NH_3. NH_3 content in the rumen affects the amount of microbial protein synthesis as a source of the cell protein [22].

Another factor that caused the N-NH_3 average was not significantly different between treatments is the absence of ammonia absorption in the in vitro system, which causes ammonia accumulation in the fermenter bottle. Residual ammonia is not used in microbial protein synthesis and the carbon framework derived from carbohydrate fermentation. It can also come from protein contribution from lysed microbes [23].

3.1.3. Total Volatile Fatty Acid (VFA)

The total volatile fatty acid is the end product of carbohydrate fermentation and is the primary energy source for ruminants. Digested feed during the rumen fermentation process will be converted into the main product as VFA, a source of energy for an animal, and microbial biomass, the primary source of protein for livestock. The fermentation of carbohydrates by rumen microbes produces energy in the form of the essential fatty acids (VFA) majority are acetic, propionic, and butyric acids. The results of the Total VFA data in this study can be seen in Table 3.

Tabel 3. Total Volatile fatty acid (TVFA) of the treatment

Treatments	Average TVFA (mM)
Sorghum straw 100% (P0)	180,0±28,28
Sorghum straw 70% + Indigofera 30 % (T1)	207,5±25,00
Sorghum straw 30% + Indigofera 70 % (T2)	217,5±43,49

Based on table 3, it can be seen that from the highest to the lowest average total VFA value were 30% Sorghum straw + 70% Indigofera (T2): 217.5 ± 43.49 (mM), 70% Sorghum straw + 30% Indigofera (T1): 207.5 ± 25.00 (mM), and 100% Sorghum straw (P0): 180.0 ± 28.28 (mM). The variance results showed that the total VFA among treatments was not significantly different ($P > 0.05$).

This result showed that the fermentation process disrupts ammonia's effectiveness to be used by bacteria for microbial protein synthesis and the growth of the microbes. One of the rumen bacteria contributions was to produce VFA, which will be used as an energy source for the host animal and a carbon source for the bacteria itself [12]. However, the high concentration of VFA can also be influenced by the concentration of ammonia in the rumen because ammonia has an essential role in the synthesis of microbes in the rumen, which can further contribute to the degradation of feed in the fermentation process to produce energy and carbon sources.. It was proved that NH3 has an essential role in VFA production in the rumen as an energy source [21].

Another factor influencing the VFA concentration is the amount of energy. VFA as the carbon framework was needed in every mole of ammonia for microbial protein synthesis under in vitro conditions [22], [23]. Total VFA in the fermentation of feed ration of sorghum straw with or without Indigofera in this study did not significantly differ among treatments. This result is the same as the research conducted that fermented Sorghum, and Indigofera with the addition of torbangun (coleus amboinicus lour) leafs shows no significant difference in the total VFA [24].

4. CONCLUSION

This study concluded that the addition of the proportion of sorghum straw with or without Indigofera in the ratio could not increase the Concentration of DMD, N-NH3, and Total VFA.

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CONFLICT OF INTEREST

The authors declare no conflict of interest

AUTHOR'S CONTRIBUTIONS

All authors contributed to the design and implementation of the research, the analysis of the results, and the manuscript's writing.

REFERENCES

[1] T. Mbahi, Z. Gworgwor, and B. Yakubu, Effect of Sorghum-Legume Intercrop on Quality and Rumen Degradability of Sorghum Stover in Adamawa State, Nigeria, Adv. Res., 2017, pp. 1–11, doi: 10.9734/air/2017/30851.

[2] A. Khaskheli and M. I. Khaskheli, Significance of Feeding Practices for Small Ruminants : A Reveiw Significance of Feeding Practices for Small Ruminants : A Reveiw, 2020, doi: 10.18805/ag.R-149.

[3] S. Singh, B. V. Bhat, G. P. Shukla, D. Gaharana, and U. Y. Anele, Nutritional evaluation of different varieties of sorghum stovers in sheep, Anim. Feed Sci. Technol., 2017, pp. 42–51, doi: 10.1016/j.anifeedsci.2017.03.011.

[4] A. Dey, S. S. Paul, A. V. Umakanth, B. V. Bhat, P. C. Lailer, and S. S. Dahiya, Nutritional potential, in vitro ruminal fermentation kinetics and methanogenesis of stover from newer cultivars of sorghum (Sorghum bicolor) in buffalo, Anim. Prod. Sci., 2020, doi: 10.1071/AN19150.

[5] A. Jhon and R. A. Oluwafemi, Hematology and Serum Biochemical Indices of Growing Rabbits Fed Diet Supplemented with Different Level of Indigofera Zollingeriana Leaf Meal, Prog. Chem. Biochem. Res., 2019, pp. 170–177, doi: 10.33945/SAMI/PCBR.2019.4.2.

[6] A. Hassen, N. F. G. Rethman, W. A. van Niekerk, and T. J. Tjelele, Influence of season/year and species on chemical composition and in vitro digestibility of five Indigofera accessions, Anim. Feed Sci. Technol., 2007, pp. 312–322, doi: 10.1016/j.anifeedsci.2006.09.010.

[7] L. Abdullah, Herbage production and quality of shrub indigofera treated by different concentration of foliar fertilizer, Media Peternak., 2010, pp. 169–175, doi: 10.5398/medpet.2010.33.3.169.

[8] S. D. Anis, M. M. Telleng, D. A. Kaligis, M. Najoan, P. O. V. Waleleng, and S. Dalie, Complete Dried Ration for Ruminant Based on Pennisetum purpureum cv. Mott Enriched Phyto-Protein of Tree Legumes Leaf, IOP Conf. Ser. Earth Environ. Sci., 2020, doi: 10.1088/1755-1315/465/1/012025.

[9] A. H. Fattah, J. A. Syamsu, A. Natsir, and S. Garantjang, VFA Concentration, Ammonia And pH Value In Vivo Ransum Rument System Made From Rice Fermentation Of Lamtoro Leaves, Gamal Leaves And Indigofera Leaves, Adv. Environ. Biol., 2019, pp. 4–9, doi: 10.22587/aeb.2019.13.12.2.

[10] AOAC, *Official Methods of Analysis*, 1990. Gaithersburg, MD: AOAC International, 1990.

[11] R. G. D. Steel and J. H. Torrie, *Principles and procedures of statistics*., 1980. New York: McGraw-Hill, 1980.

[12] K. Bünemann *et al*., Effects of different concentrate feed proportions on ruminal ph parameters, duodenal nutrient flows and efficiency of microbial crude protein synthesis in dairy cows during early lactation, Animals, 2020, doi: 10.3390/ani10020267.

[13] S. Addisu, Effect of Dietary Tannin Source Feeds on Ruminal Fermentation and Production of Cattle; a Review, Online J. Anim. Feed Res., 2016, pp. 45–56, [Online]. Available: https://ojafr.com/main/attachments/article/120/ Online J. Anim. Feed Res., 6(2) 45-56, 2016

[14] K. M. Koenig and K. A. Beauchemin, Effect of feeding condensed tannins in high protein finishing diets containing corn distillers grains on ruminal fermentation, nutrient digestibility, and route of nitrogen excretion in beef cattle, J. Anim. Sci., 2018, pp. 4398–4413, doi: 10.1093/jas/sky273.

[15] I. C. Molina-Botero *et al*., Effects of tannins and saponins contained in foliage of Gliricidia sepium and pods of Enterolobium cyclocarpum on fermentation, methane emissions and rumen microbial population in crossbred heifers, Anim. Feed Sci. Technol., 2019, pp. 1–11, doi: 10.1016/j.anifeedsci.2019.01.011.

[16] I. Mueller-Harvey *et al*., Benefits of condensed tannins in forage legumes fed to ruminants: Importance of structure, concentration, and diet composition, Crop Sci., 2019, pp. 861–885, doi: 10.2135/cropsci2017.06.0369.

[17] J. . Jibril, Y. . Gazali, M. Dantani, H. Alamin, and B. . Zannah, Performance of Balami Rams Fed Graded Levelsof Mistletoe Leaves (Viscum album)and Sorghum Stover in Semi-Arid Zone of Borno State, Nigeria. Jibril, 2020, pp. 25–31, [Online]. Available: http://njast.com.ng/index.php/home/article/view/57

[18] M. Telleng, K. G. Wiryawan, P. D. M. H. Karti, I. G. Permana, and L. Abdullah, Forage production and nutrient composition of different sorghum varieties cultivated with indigofera in intercropping system, Media Peternak., 2016, pp. 203–209, doi: 10.5398/medpet.2016.39.3.203.

[19] N. R. Kumalasari, G. P. Wicaksono, and L. Abdullah, Plant growth pattern, forage yield, and quality of indigofera zollingeriana influenced by row spacing, Media Peternak., 2017, pp. 14–19, doi: 10.5398/medpet.2017.40.1.14.

[20] S. jiang Zhang *et al*., Chemical composition and in vitro fermentation characteristics of high sugar forage sorghum as an alternative to forage maize for silage making in Tarim Basin, China, J. Integr. Agric., 2016, pp. 175–182, doi: 10.1016/S2095-3119(14)60939-4.

[21] J. Zhang, N. Zheng, W. Shen, S. Zhao, and J. Wang, Synchrony degree of dietary energy and nitrogen release influences microbial community, fermentation, and protein synthesis in a rumen simulation system, Microorganisms, 2020, doi: 10.3390/microorganisms8020231.

[22] N. N. Suryani, I. W. Suama, I. G. Mahardika, and N. P. Sarini, Rumen Fermentation and Microbial Protein Synthesis of Bali Cattle Heifers (Bos sondaicus) Fed Ration Containing Different Energy Protein Level, J. Sain Peternak. Indones., 2020, pp. 187–194, doi: 10.31186/jspi.id.15.2.187-194.

[23] C. G. Schwab and G. A. Broderick, A 100-Year Review: Protein and amino acid nutrition in dairy cows, J. Dairy Sci., 2017, pp. 10094–10112, doi: 10.3168/jds.2017-13320.

[24] H. Apriyani, Panca Dewi MHK, and D. A. Astuti, evaluate the fermentation of formulation Indigofera zollingeriana and Sorghum bicolor at different proportion of Torbangun leaves (Coleus amboinicus lour) as in vitro study., Faculty of Animal Science, IPB University, Bogor, 2014. Accessed: Jun. 06, 2021. [Online]. Available: https://repository.ipb.ac.id/handle/123456789/67842