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Effect of addition cattle feed supplement on in vitro fermentation, synthesis of microbial biomass, and methane production of rice straw fermentation basal diets

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Abstract. The objective of this study was to evaluate the influence of supplementation of cattle feed supplement (CFS) and concentrate in ruminant diets based on rice straw fermented (R) on in vitro rumen fermentation, microbial biomass synthesis, and enteric methane production. Five experimental diets were evaluated, consist of R = rice straw fermented 100%, RS = R + CFS 10%, RSC1, 2 and 3 = RS + Concentrate levels 10, 20 and 30 (%DM). Supplementation of CFS increased the gas production ($P < 0.05$) and highest in treatments RSC1 and 2 (44.09 and 44.87 ml/ g substrate, respectively) and was decreased proportions of methane by inhibition rate until 49.80%. Ruminal protozoa population increased by CFS dan concentrate supplementation ($P<0,05$) and was dominated (>80%) of Entodinium genus. The treatments RS dan RSC1 promoted greater ($P < 0.01$) microbial biomass synthesis (386.32 and 312.39 mg/ g substrate, respectively). In conclusion, the supplementation of CFS and concentrate in ruminant diets based on rice straw fermented can promote a greater synthesis of microbial biomass and mitigation of methane production.

Keywords: Feed supplement, methane, microbial protein, microbial biomass

## 1. Introduction

Processing agricultural waste into quality animal feed has supported the development of beef cattle farming in Indonesia. Beef cattle fattening, known as the “Kreman” system [1], is a feedlot fattening with fermented rice straw as the main feed with high concentrate supplementation of up to 40%. Fermentation is one way of biologically processing rice straw to improve nutrition and digestibility in ruminants [2]. Concentrate supplementation provides an adequate supply of nutrients to achieve optimal livestock production [3].

Feed nutrient supply is expected to be used efficiently in the metabolism of ruminants. For example, condensed tannins were reported to increase feed efficiency in increasing rumen fermentation rate, microbial biomass production, and mitigating methane production in rice straw basal diets [4]. One of the potential sources of condensed tannins in West Sumatra, Indonesia, is the gambier plant (Uncaria gambir RoxB). Cattle feed supplements containing gambier leaf residue formulated with feed ingredients containing high soluble carbohydrates, nitrogen (CP=23%), and minerals were reported to optimize rumen microbial growth [5]. Efforts to increase fermented rice straw as a source of forage for beef cattle need to be supported by developing feed supplements and feed concentrate on producing optimal feed efficiency. Therefore, this study aims to obtain the composition of fermented straw, animal feed supplements, and concentrates that can increase rumen fermentation, microbial biomass synthesis, and mitigate methane production. 2. Materials and Methods 2.1. Treatment diets Fermented rice straw is made from rice straw (Oryza sativa, variety IR64) taken from the leftover rice harvest chopped with a chopper machine to cut and bruise the straw. Then added bran 5%, urea 1% (fresh basis), and sprinkled with Rhizopus spp yeast flour. Fermentation was carried out in an airtight plastic sack for two weeks at room temperature. Cattle feed supplements (CFS) are made from a mixture of brown sugar 15% dissolved in 1 liter of water and add to a mix of bran 27%, coconut cake 12%, soybean meal 15%, tapioca 15%, urea 5%, salt 5%, minerals 3% and gambier (Uncaria gambir RoxB) leaves 5%. In contrast, the concentrate consists of a mixture of sago pith 30%, bran 30%, cassava 20%, and coconut pulp 20%. The treatment diets is shown in Table 1, consisting of R: fermented rice straw 100% (control), RS: R + CFS 10%, RSC1, 2 and 3: RS + Concentrate10%, 20% and 30% respectively. Table 1. Ingredients and chemical composition of treatment diets. Items Treatment Diets R CFS C R RS RSC1 RSC2 RSC3 Ingredients (%DM)    Rice straw fermented (R) 100 90 80 70 60  CFS - 10 10 10 10  Concentrates (C) - - 10 20 30  Chemical composition (%DM)    Organic matters   87.06 88.26 94.12 Crude protein   9.82 23.31 11.64 BETN   43.53 52.36 69.21 NDF   70.35 27.16 36.56 Lignins   8.99 0.82 0.96 Tannins   - 1.17 - CFS = cattle feed supplement, C = Concentrates, R = Rice straw fermented, DM = dry matter. 2.2. In vitro fermentation study In vitro gas production test (IVGPT) follows the method [6]. Exactly 1 g of air-dried sample (1.0 mm size) according to the treatment was put into a 100 ml serum bottle, then added 100 ml of a mixture of artificial saliva and rumen fluid (4: 1) and incubated 24 hours at 39 °C. The fermentation gas is collected in a plastic bag connected to the bottle cap and measured with 100 ml glass syringes (Fortuna, Haberle, Germany) at the end of incubation. 100 µl of collected gas used as sampled injected for methane estimation with gas chromatography (Nucon-5765). The bottle contents were removed and centrifuged at 1,500 rpm for 3 minutes, and the filtrate was used to analyze VFA, ammonia-N, and TCA soluble N [7]. Rumen content was also prepared following the procedure [8] for counting the population and genus of protozoa using the Neubauer chamber at 400x microscope magnification. 1The residue is washed with 100 ml of neutral detergent solutions, refluxed for one h, and filtered through Whatman 41 is called NDF residue. Truly degradable organic matter in the rumen (TDOMR) = initial OM substrate-NDF residue. Partitioning factor (PF) = TDOMR (mg) / gas production (ml). Microbial biomass production (MBP) (mg) = TDOMR (mg) - (2.2 * gas production), where 2.2 is the stoichiometric factor. The efficiency of microbial biomass production (EMP) = MBP / 100 mg TDOMR. 2.3. Statistical analysis Statistical analysis of all data generated used The Statistical package for the social sciences (SPSS, Chicago, USA) by one-way ANOVA. The effects were considered significant at $P < 0.05$ and continued with Duncan's test to determine the mean difference between treatments.   3. Results and Discussion In Table 2, the results of the measurement 2of in vitro gas production variables are presented. In vitro rumen dry matter (DMD) degradability and TDOMR of fermented rice straw increased with the addition of CFS and concentrate ($P < 0.01$), and the highest was found in RS feed followed by RSC1-3. The fermented straw diets (R) showed the lowest degradability of dry matter (DMD) and organic matter (TDOMR) due to the high lignin content (8.99%) in fermented straw, which binds cellulose so that it is not available for degradation by rumen microbes [9]. The addition of 10% CFS increased the degradability of fermented rice straw. That is due to an increase in microbial biomass (MBP) 93% from control which plays a role in producing cellulase enzymes to break down cellulose into VFA. The content of tannins in CFS did not appear to harm the digestibility 1of dry matter and organic matter. This result is different from the report of other researchers [10], who reported that tannins bound to organic compounds in feed ingredients decreased the digestibility of DMD and OMD in the rumen. 2Microbial biomass production (MBP) was found to be lowest in control (R diets) and increased 93% with the addition of CFS (RS diets). CFS and concentrate combined in the RSC1-3 diets resulted in lower MBP than CFS alone in the RS diets. The diets indicate that microbial biomass production in fermented straw diets needs supplementation to produce optimally. The rumen environment and substrate availability influence the growth of 5microbial biomass in the rumen. CFS contains high soluble carbohydrates (BETN =52.36%) plus nitrogen from urea (NPN) (CP=23.31%) plus macro and micro minerals, essential nutrients for rumen microbial growth [11]. Microbial biomass production in this study is in line with research results [5] which reported optimal microbial biomass production of 111-285 mg with supplements. In contrast, without supplements, Bretschneider researchers reported low microbial biomass production between 170-191 mg in maize silage diets [12]. Microbial production efficiency (EMP) increased significantly (P = 0.031) after the addition of CFS and concentrate, but there was no significant difference (P>0.05) between the combination of CFS and concentrate. This efficiency states the amount of organic matter digested in the rumen, converted into microbial biomass. This efficiency value is higher than the report [13], 27.9 mg/g BOT in a mixed straw-concentrate diet. In addition, CFS contains condensed tannins 1.17% DM, where tannins can inhibit methane production 49.80% in this study and is in line with the statement [14] that tannins can increase the efficiency of energy use and microbial biomass synthesis. Table 2. Effect of Supplementation on in vitro rumen degradation, microbial production, methane inhibition, and fermentation metabolites. Parameters Treatment diets SEM P-value R RS RSC1 RSC2 RSC3 DMD, % 24.20e 42.89a 39.06b 33.94c 29.19d 1.06 0.001 TDOMR, mg/ g substrate 280.36c 485.03a 409.38b 394.05b 315.95c 13.30 0.002 TDOMR, % 28.04c 48.50a 40.94b 39.40b 31.59c 1.33 0.002 MBP, mg 199.75d 386.32a 312.39bc 321.27b 262.60c 16.35 0.004 EMP 67.34b 79.64a 76.06a 81.49a 80.78a 2.26 0.031 PF 8.74 14.30 10.96 12.33 15.10 1.36 0.057 Gas production (per g substrate) Total gas, ml 24.25c 36.64b 44.09a 44.87a 33.08b 3.60 0.037 Methane, ml 4.10b 3.11b 4.74a 4.52ab 5.48a 0.39 0.030 % methane 16.94a 8.49b 10.76b 10.08b 16.57a 1.16 0.006 % inhibition 0.00d 49.80a 36.41b 40.42b 24.02c 3.17 0.029 Fermentation metabolites pH 6.99 6.98 6.92 6.98 6.99 0.01 0.181 Total VFA, mM 146 141 144 110 135 5.47 0.280 Ammonia-N, mg/dL 8.87c 21.44a 11.99b 12.22b 10.43b 2.24 0.042 Total N, g/dL 122.50b 170.63a 203.44a 196.88a 157.50ab 13.72 0.036 TCA-Soluble N 60.74c 114.30ab 155.53a 130.91a 92.77b 13.34 0.028 Non-protein N 61.76 56.32 47.91 65.96 64.73 3.56 0.054 R = Fermented rice straw 100%, RS = R+10% CFS, RSC1, 2 and 3 = RS+Concentrate levels 10, 20 and 30%. DMD = in vitro dry matter degradability. TDOMR = truly degradable organic matter in the rumen. MBP = microbial biomass production. EMP = efficiency of microbial production. PF = Partitioning factor. abc different superscripts of means in a row differ significantly ($P<0,05$) The lowest in vitro fermentation total gas production was found in control (R diets) and the highest in the RSC1 and RSC2 diets. Total gas production shows the level of feed fermentation by microbes in the rumen. The rice straw is difficult to ferment, producing lower total gas production than mixed straw, CFS, and concentrate diets. The total gas composition consists of Oxygen 0.5%, Hydrogen 0.2%, Nitrogen 7.0%, methane 26.8% and $CO_2$ 64.4% [15]. In this study, the highest methane composition of the total gas was 16.94% in control (R diets), and the lowest in the RS diets was 8.49% ($P<0.01$). The highest methane production inhibition of 49.80% was found in RS diets with CFS addition. The condensed tannin content in CFS has affected the work of rumen microbes, thereby reducing methane formation. The same thing was reported [16] that condensed tannins (catechins and sinapic acid) reduced methane production without changing the total production gas. The mechanism of reducing methane gas by tannins occurs due to the inhibition of fiber digestion which reduces the production of Hydrogen and inhibition of growth and activity of methanogens bacteria[17]. Therefore, reducing the proportion of methane in the total gas is an advantage of CFS, considering that methane emissions represent the loss of energy intake (5-15% of the total) generated during the rumen fermentation process [4]. Furthermore, methane production is closely related to the acetate/propionate balance. Therefore, the decrease methane production is in line with the increase in propionate formation in rumen fermentation [18]. CFS and concentrate supplementation had no significant effect (P>0.05) on rumen pH and VFA production. The highest Ammonia-N was found in the RS diets, followed by RSC1-3 and the lowest in control (R diets). The highest TCA soluble N was found in the RSC1-3 diets, and the lowest was in the R diet. TCA soluble N indicates the amount of protein or peptides and amino acids from diets and microbial protein. Although the diet contains high grains and is easy to ferment, it does not lower the rumen pH. Rumen pH needs to be maintained because the activity of cellulolytic bacteria will be inhibited if the rumen pH is below 6.0 [19]. The concentration of VFA in the rumen is closely related to the degradation of non-nitrogen organic matter as the end product of carbohydrate fermentation (cellulose, pectin, and xylan) by rumen microbes, bacteria, and Archae [20]. Therefore, the VFA obtained was optimal to support rumen microbial growth, namely 80-160 mM [11]. VFA balance: ammonia N is required by rumen microbes in synthesizing microbial proteins [21]. Table 3. Effect of supplementation on in vitro rumen protozoa population and genus composition. Parameters Treatment diets SEM P-value R RS RSC1 RSC2 RSC3 Protozoa, x105 2.79c 4.68b 7.86a 7.27a 7.17a 0.30 0.001 Genus, % of total Entodinium 82.3 88.8 89.6 87.9 89.3 0.71 0.167 Diplodinium 11.3 5.9 5.3 5.6 4.6 0.52 0.171 Ophryscolex 2.5 1.1 0.7 1.3 0.9 0.22 0.126 Isotricha 1.3 1.2 0.8 1.6 1.4 0.18 0.143 Dasytricha 2.6 3 3.6 3.6 3.8 0.30 0.238 R = Fermented rice straw 100%, RS = R+10% CFS, RSC1, 2 and 3 = RS+Concentrate levels 10, 20 and 30%. The effect of the treatment diets on the composition and genus of rumen protozoa is shown in Table 3. The lowest protozoa population was found in control (R diets), while the highest population was found in the diet with the addition of concentrate in the RSC1-3 diets. The composition of the protozoan genus was not affected (P>0.05) by the treatment diets, but the composition was dominated (>80%) by the Entodinium genus. The protozoa population increased 68% of the control by addition of CFS and increased 68% after the addition of the concentrate. The protozoa population increased because CFS and concentrated contained high soluble (non-structural carbohydrates) with a BETN of 69.21%. Rumen protozoa are more effective in using non-structural carbohydrates by consuming three times faster than bacteria (0.14 vs. 0.04 mol/g protein/min), using them for growth, and storing them as carbohydrate reserves [22]. Other investigators also reported that the population and flow of protozoan cells into the duodenum increased by 25% when animals were fed a diet rich in soluble carbohydrates and decreased when fed a diet rich in cellulose material [23]. The content of condensed tannins in CFS did not harm protozoa, in contrast to other researchers who reported decreased protozoa population due to tannins [24]. 4. Conclusion Supplementation of CFS and concentrate in rice straw fermented basal diets can increase in vitro rumen fermentation, microbial biomass synthesis, and methane production mitigation. The optimal diet composition is 80:10:10% DM of rice straw fermented, cattle feed supplement, and concentrate. Acknowledgments We acknowledge Politeknik Pertanian Negeri Payakumbuh for facilitating funding from DIPA 2021 and nutrition and feed technology laboratory facilities. References [1] Anggraeni AS, Istiqomah L, Damayanti E. 2019. Trop.J.Trop.Anim.Prod. 20 100–110. [2] Huyen NT, Tuan BQ, Nghie NX, Bich NT, Tuyet NT. 2019. Asian J.Anim.Sci. 13 1–7. [3] Ramaiyulis, Yulia E, Fati N, Salvia, Nilawati. 2020. Sch.J.Agric.Vet.Sci. 07 35–40. [4] Polyorach S, Wanapat M, Cherdthong A, Kang S. 2016. Trop. Anim.Health Prod. 48 593–601. 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