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# Rumen un-degraded dietary protein and TCA soluble protein with gambier leave residue supplementation as a source of tannins in cattle feed supplement

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Abstract. This study aims to obtain high rumen undegraded dietary protein and TCA soluble protein in a rations combination of forage, concentrates, and supplements. Gambier leaf residue (GLR) supplementation in cattle feed supplement contains 29.53% crude protein and 75.01% TDN energy. There were 4 treatment rations, forage (F), F + concentrate (FC), F + supplement (FS), and FCS, where the tannin concentration of 0.117% was found in the FS and FCS rations. Rumen microbial degradation and microbial protein synthesis were evaluated in vitro method using cattle rumen fluid. The results showed that GLR supplementation as a source of condensed tannins significantly reduced the rate of protein degradation in the rumen from 1.17 to 0.99% per hour. This effect has implications for the increase in rumen undegraded dietary protein (RUDP) and Peptide-N as rumen fermentation products. Rations containing supplements (FS and FCS significantly produced higher microbial biomass and microbial protein. TCA soluble N was superior in the FCS combination ration which produced the highest amount of protein for intestine sourced from RUDP and microbial protein.

## 1. Introduction

Beef cattle farming in

Indonesia has rapid development in recent years, in line with the government's program to achieve self-sufficiency in beef and buffalo by 2022. Cattle farming in Indonesia 82.73% are small business farmers that difficulties with low livestock productivity with daily weight gain less than 0.5 kg/day due to the low quality of the ration. The rations usually consist of forage and concentrate such as bran, sago pith, cassava, and coconut pulp. Gambir (Uncaria gambir. RoxB) belongs to the Rubiaceae family, which is a specific shrub from West Sumatra in Indonesia. Gambier plant processing produces gambier products as a superior export product from West Sumatra. The main components of gambier are Tannat catechu acid (20-50%), catechin (7-33%), and pyrocatechol (20-30%) [1]. Gambier processing leaves gambier leaf residue (GLR) that has not been utilized, in terms of containing 9.98% tannins of the catechin type which is classified as condensed tannins. The role of tannins in ruminant nutrition has been widely reported that tannin can bind to a protein in the rumen so that it is protected from rumen microbial degradation which causes a decrease in the biological value of protein [2,3]. The application of tannins in rations can increase the daily weight gain of beef cattle [4]. This study evaluated the protective power of tannins against protein through supplementation of the GLR and to obtain high protein fermentation products. The hypothesis is that ICALS 2020 IOP Conf. 7Series: Earth and Environmental Science 759 (2021) 012045 IOP Publishing doi:10.1088/1755-1315/759/1/012045 2 the supplementation of GLR as a source of tannins can protect protein thereby increasing RUDP and TCA-soluble protein as total protein available to the small intestine. 2. Material and Methods 2.1 Ration Treatment rations refer to rations provided by the small business farmers, 2 types of rations are commonly found, namely 100% grass and a mixture of grass and concentrate (60: 40%). Both types of rations are supplemented with the GLR which have been formulated in the cattle feed supplement with a relatively equal tannin content of 0.117% (dry basis). The rations content and nutritional analysis are shown in Table 1. Table 1. Ration composition Item F FC FS FCS Ration ingredient, %DM Roughage 100 60 90 60 Concentrate 0 40 0 30 Supplement 0 0 9.50 9.50 Gambier leaf residue - - 0.50 0.50 Nutrient

composition, %DM Organic matter 90.33 91.58 89.89 91.02 Crude protein 5.41 6.24 7.24 7.99 Neutral Detergen Fiber (NDF) 56.47 49.82 55.51 47.55 Tannin - - 0.117 0.117 F = forage; FC = F + concentrate; FS = F + supplement; FCS = F + C + S; DM = dry matter 2.2 In vitro analysis In vitro rumen analysis followed the Krishnamoorty procedure [5]. The treatment ration was mashed with a 1 mm sieve and then weighed 2.5 grams and then put it in 250 ml Erlenmeyer tubes. Then add 250 ml of a mixture containing buffer solution (9.8 g NaHCO3, 4.62 g Na2HPO4, 0.57 g KCl, and 0.12 g MgSO4/ liter) and rumen fluid with a ratio of 4:1 (v/v). To create an anaerobic condition in the rumen, CO2 gas is sprayed for 5 seconds to the Erlenmeyer then is closed with a rubber which is given a nipple. The incubation was carried out for 48 hours at 39 °C using a shaker water bath (Precision, USA). Fermentation was stopped at the end of the incubation time by immersing Erlenmeyer in cold water 3-5 °C for 5 minutes. All contents of Erlenmeyer were separated by centrifuge at 3,000 rpm for 5 minutes. The filtrate was used for NH3-N analysis using the Conway micro diffusion method [6] and the residue was washed 3 times with 0.85% NaCl solution, then dried at 60 °C. Protein content was measured by the Kjeldahl method [6] and the difference between the sample protein and the residual protein was protein degraded. Microbial biomass was measured following the Griswold method [7] by centrifuging the filtrate at 15,000 rpm for 30 minutes at 4 °C and the biomass obtained was analyzed for protein by the biuret method using a 540 nm UV spectrophotometer with bovine serum albumin (BSA) as standard. TCA-Soluble protein was measured by the ICARDA method [8] using trichloroacetic acid (Brand Darmstadt, Germany). 2.3 Statistic Data obtained from laboratory analysis were tabulated and analyzed using SPSS 22 software (IBM, USA) with the F test comparing the mean value on one-way ANOVA. The difference in the mean value is significant if the P-value is <0.05 then a further test is carried out with the turkey test. ICALS 2020 IOP Conf. Series: Earth and Environmental Science 759 (2021) 012045 IOP Publishing doi:10.1088/1755-1315/759/1/012045 3 3. Results and Discussion Table 2. Protein degradation, microbial protein, and protein to the intestine of ration by rumen in vitro

Parameter F FC FS FCS SEM P-value Protein Degradation Degradation rate, %/ hr 0.82b 1.17a 1.12a 0.99b 0.03 0.003 Degraded, % 38.34c 55.01a 52.38a 46.31b 1.64 0.003 Ruminal NH3-N, mg/dl 2.34b 2.94b 4.51a 4.57a 0.17 0.002 Rumen Microbial Protein Protozoa, cellx104 9.485b 18.970a 9.462b 19.874a 3.26 0.047 Microbial Biomassa, mg/dl 120b 114b 189a 169a 9.12 0.004 MPSE, mg MP/ g BOT 8.37c 6.84c 13.51a 10.60b 0.65 0.003 Nett Protein to Intestine Peptide N, mg/dl 9.63c 26.17a 15.00b 24.54a 1.33 0.002 RUDP, mg/ml 31.67a 24.17b 37.03a 34.19a 1.22 0.003 Microbial protein, mg/dl 63.45b 92.40a 103.35a 92.62a 4.92 0.006 TCA-soluble N, mg/dl 74.80c 151.89b 121.98b 181.93a 1.19 0.002 Superscript = significant (P<0.05); SEM = standard error of means; F = forage; FC = F + concentrate; FS = F + supplement; FCS = F + C + S; DM = dry matter; DOM = digestible organic matter; MPSE = Microbial protein syntesis efficiency; RUDP = Rumen undegraded dietary protein 3.1 Protein degradation Tannins in the rumen form a tannin-protein complex that is resistant to microbial proteolysis enzymes [9]. The tannin content of the FS and FCS rations was found to be effective in inhibiting protein degradation in the combined ration of forages, concentrates, and supplements (FCS). The rate of protein degradation by microbes in the rumen was significantly higher in the FC and FS rations compared to F and FCS which had implications for the amount of degraded protein. Degradation of crude protein into peptides and 10amino acids which are then deaminated to ammonia, the high rate of protein degradation in FC and FS does not cause high ammonia in the rumen but high ammonia in FS and FCS is related to the urea content in the supplement on FS and FCS rations. 3.2 Rumen microbial protein The supply of soluble carbohydrates in concentrate (FC and FCS) has been a higher protozoan population in the rumen. Supplementation of GLR containing tannins in FS and FCS rations (0.117% tannin content) was not able to suppress the protozoa population in the rumen. Microbial biomass is bacterial biomass in rumen fluid, significantly higher in rations with the addition of a supplement both on forages (FS) and concentrate (FCS), but the efficiency of microbial protein synthesis was significantly the highest in the FS ration. Other researchers reported the microbial biomass

in the rumen fluid of cattle normally ranges from 118 to 148 mg/dl [10]. In connection with the lower NH3N concentrations in the F and FC rations, it resulted in lower efficiency of microbial protein synthesis in both rations. 3.3 Nett Protein to Intestine The role of tannins in protecting proteins from microbial degradation in the rumen is indicated by the high peptide N found in the FCS ration, this is also seen in the high RUDP, and both going to be available to the intestine. The lowest microbial protein was found in rations of 100% forage (F) and significantly increased when added with concentrates and or supplements. The final results of both RUDP and microbial protein with bound to TCA-soluble protein as a total protein that enters the small intestine were found to be the most superior in rations with a combination of forage, concentrate, and  supplement (FCS). The tannin-protein complex can be broken down again due to changes in 11pH in the abomasum and intestine [11] so that it can be digested and absorbed by ruminants. 4. Conclusion supplementation of gambier leaf residue as a source of condensed tannins in the ration at a concentration of 0.117% can reduce the rate of protein degradation by microbes in the rumen. The addition of supplements can increase the microbial biomass and supply of microbial protein along with RUDP available to the intestines of ruminants. TCA soluble N derived from RUDP and microbial protein as total protein available in the intestine was found to be the highest in rations with a combination of forages, concentrates, and supplements 5. References [1] Ferdinal N 2014 IJASEIT 4 53-5 [2] Ani AS, Pujaningsih RI and Widiyanto 2015 Veteriner 16 439-47 [3] Ningrat RWS, Zain M, Erpomen and Suryani H 2017 Asian J. Anim . Sci. 11 47-53 [4] Ramaiyulis, Zain M, Ningrat RWS and Warly L 2019 Int .J. Zool . Res. 15 6-12 [5] Krishnamoorthy UK 1983 Br. J. Nutr. 50 555-68 [6] AOAC 1980 Official Methods of Analysis Association of Official Agriculture Chemist. 13th 552 p. [7] Griswold KE, Apgar GA, Bouton J and Firkins JL 2003 J. Anim. Sci. 81 3298-337 [8] Zaklouta M, Hilali ME, Nefzaoui A and Haylani M 2011 Animal Nutrition and Product Quality Laboratory Manual 92 p. [9] Alonso-Díaz MA, Torres-Acosta JFJ, Sandoval-Castro CA and Capetillo-

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