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Effects of Tannin-containing Supplement on Enteric Methane Emissions, Total Digestible Nutrient, and Average Daily Gain of Local Indonesian Beef Cattle

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ABSTRACT

Reducing methane (CH4) emissions is one of the most critical goals in ruminant nutrition. This study aimed to evaluate the effect of concentrate and tannin supplementation on the mitigation of methane gas in Indonesian local beef cattle. The current study was conducted *in vivo* using 12 Bali cattle using a completely randomized design with four treatments and three replicates. Cattle were fed a basal ration with field grass (control), the addition of concentrate 25% dry matter (DM) ration no tannin as well as tannin supplemented in concentrate at levels of 0.12% and 0.18% of DM concentrate. The concentrate contains 7.5% crude protein and 71.25% total digestible nutrients and tannin supplementation using gambir (*Uncaria gambir* Indonesia) tannin extract. The parameters measured were apparent digestibility, total digestible nutrients, methane production, and average daily gain. The results showed that concentrate addition significantly increased DM consumption, crude protein digestibility, and total digestible nutrients. Supplementation of 0.18% tannin in concentrate can mitigate 49.7% methane gas production resulting in energy efficiency, which was reflected in the weight gain rate of 0.75 kg/day. In conclusion, present results suggest that the supplementation of 0.18% gambier tannin extract in concentrate could be considered a suitable feed additive to mitigate methane gas production and increase the average daily gain of Indonesian local beef cattle.

Keywords: Digestibility, Feed supplement, Gambir, Methane, Tannin

INTRODUCTION

The population of beef cattle in Indonesia in 2021 was 18 million heads, increasing about 2.3% since 2016 (BPS, 2021). The increase in greenhouse gases in the atmosphere from components of carbon dioxide (CO2), methane (CH4), and nitrous oxide (N2O) is a challenge behind the increase in livestock population. Methane production in ruminants contributes 56% of total agricultural greenhouse gas emissions and 93% of total livestock emissions globally (Watts et al., 2021). In addition to impacting the environment as a greenhouse gas, methane emissions are a waste of energy that livestock should use for production. About 3-12% of the gross energy of feed consumed by ruminants is lost as methane (Ku-Vera et al., 2020). Strategies to reduce methane emissions are grouped into animal breeding, dietary manipulation, and rumen manipulation (Arndt et al., 2021). Meta-analysis showed that the addition of concentrate and tannin supplementation decreased CH4 Yield (g/kg DMI) by 13.7-17.4% and CH4/Gain by 20.1% (Congio et al., 2021). Thus, in the current study, the concentrate was added to tannin supplementation from gambir extract (*Uncaria gambir* Indonesia) to mitigate CH4 emission.

This study hypothesized that adding concentrate supplemented with tannins could mitigate CH4 emissions and increase the total digestible nutrients, thus reflecting an increase in average daily gain. Therefore, this study aimed to determine the effect of tannin concentrate and supplementation on enteric CH4 production, total digestible nutrient, and average daily gain in local Indonesian cattle.

MATERIALS AND METHODS

Ethical approval

This study was carried out according to standard protocols without causing discomfort or injury to the cattle. Furthermore, the experimental procedure was approved by the Center for Research and Community Service at Agricultural Polytechnic of Payakumbuh, referring to Government Regulation of the Republic of Indonesia No. 95 of 2012 concerning veterinary public health and animal welfare.

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Animal and treatment diets

The experiment was conducted in June-September 2021 at the Agricultural Polytechnic of Payakumbuh, Sumatera Barat, Indonesia. The climatic data referred to in AccuWeather (2021) recorded throughout the study is the end of summer and the beginning of the entry of the rainy season with a daily temperature range of 24-34 ºC. The study used 12 male Bali cattle (*Bos sondaicus*) of similar aged 1.5-1.8 years, with initial body weights of 237.31±4.85 kg. The cattle were placed in a metabolic cage measuring 1.8×1.5 m. Newly entered cattle were given the parasite drug Wormectin 1ml/ 50 kg of body weight (Medion, 2022). The animals and treatments were allocated randomly according to a completely randomized design with four treatments and three replications. The treatments were 100% basal ration (control), the addition concentrate of 25% dry matter (DM) ration without tannins (T0), and the addition of 0.12 (T2) and 0.18 (T3) % of DM concentrate gambir tannin extract. Tannin extract was obtained from the extraction of tannins from the gambir plant with water as a solvent in the gambier industry according to the method by Fauza (2014). Basal ration using mixed field grass from the genus Paspalum and the concentrate addition was composed of 30% bran, 20% coconut cake, 30% sago pith, and 20% cassava with a protein content of 7.5% and a total digestible nutrient (TDN) of 71.25%. Supplements containing tannins are made with a mixture of 15% brown sugar, 28% bran, 9% coconut cake, 15% tapioca, 20% soybean meal, 5% urea, 3% salt, 2.5% ultra-mineral, and 2.5% gambir tannin extract with a crude protein content of 23.69%, and TDN (78.55%). The chemical composition of the experimental feed is shown in Table 1.

Table 1. Nutritional content of treatment diets

Chemical composition	Basal diet* (control)	Concentrate** Level tannin***		
		T0 = 0.00	**T1 = 0.12**	**T2 = 0.18**
Organic matter (%)	90.33	91.27	90.52	90.14
Crude protein (%)	5.14	6.04	7.65	8.46
Neutral detergent fiber (%)	58.66	52.03	51.09	50.62
Acid detergent fiber (%)	37.85	33.13	32.27	31.84

*Basal diet: mixed field grass of the Paspalum genus; **add concentrate 25% of dry matter ration; ***Level tannin (percentage of dry matter concentrate)

Sample collection and analysis

The experimental period was 40 days, 26 days for the digestibility trial, and 14 days for the daily gain measurement. The digestibility experiment was started with a 10 days preliminary period; the cows were given 100% basal feed to eliminate the effect of the last feed. The next 10 days were the adaptation period to the treatment diet and 6 days to collect digestibility data. The number of rations offered and rejected was weighed to determine the total daily dry matter intake. The excreted feces are collected in a bag tied to the cattle during the collecting period. Then the samples of feed and feces (100 g as fed) were stored in the freezer (-20°C) to be analyzed for their nutritional content. The body weight of cattle was measured at the end of the digestibility data collection as the initial weight and 14 days later as the final weight. The weighing was carried out in the late morning, 16 hours after the previous day's ration was given. After completing the research, the feed and feces samples were dried in an oven at 60°C, then mashed using a 0.5 mm sieve. The concentration of dry matter, organic matter, and crude protein were determined by the method of AOAC (2005), while the fiber fraction was analyzed by the method of Van Soest et al. (1991). Methane gas production was predicted by performing the following Formula 1 according to Jentsch et al. (2007) and Ningrat et al. (2018).

Methane (MJ/day = 1.62(DCP) – 0.38(DF) + 3.78(DCF) + 1.49(DN-fe) + 1142 (Formula 1)

Where, DCP is a digestible crude protein (g), DF denotes digestible fat (g), DCF signifies digestible crude fiber (g), and DN-fe refers to digestible N-free extract (g)

Data analysis

The resulting data were tabulated and statistically processed using the statistical package for the social sciences (SPSS, Chicago, USA) was used for analyzing the data, and the one-way ANOVA test was chosen in this software. Duncan's test was chosen to determine the mean significant differences between treatments. The $p < 0.05$ was considered a significant difference between the groups.

RESULTS AND DISCUSSION

The effects of supplementation of 25% concentrate (DM diet) and different levels of tannin supplementation on consumption, digestibility, methane production, and weight gain in Bali cattle are shown in Table 2. As can be seen in Table 2, the addition of concentrate can increase ($p < 0.05$) dry matter consumption, while tannin supplementation does not affect consumption ($p > 0.05$). The addition of concentrate caused a 6.36% reduction in neutral detergent fiber (NDF) in the ration (Control vs. T0-T3, Table 1). The decrease in NDF allows the ration to be digested more quickly in the

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rumen and accelerates the rate of rumen emptying. The NDF content can reduce dry matter intake (DMI). Souza et al. (2017) reported a negative correlation between forage NDF and DMI. Condensed tannins from various plant sources at moderate levels (1-4%) did not have a significant effect on feed intake (Bunglavan and Duta, 2013).

Regression analysis showed that organic matter digestibility and NDF digestibility had a close relationship with DM consumption ($p < 0.05$). This relationship showed that dry matter consumption is influenced by the digestibility of organic matter and NDF. Organic matter and NDF are the main components of rations in local beef cattle with 100% forage on traditional farms in Indonesia. Organic matter is all organic components of dietary protein, carbohydrates, fats, and vitamins, and NDF is a component of cell walls consisting of cellulose, hemicellulose, and lignin (Lardy, 2018).

Table 2. Dry matter intake, apparent digestibility, methane production, and weight gain measured in Bali beef cattle aged 1.5 years after supplementation of diet with different levels of tannin

Items	Basal diet	Concentrate			SEM	p value
		Level tannin **				
		0.0	0.12	0.18		
Dry matter intake						
Percentage of BW	2.17[b]	2.66[a]	2.77[a]	2.70[a]	0.04	<0.01
Metabolic body weight (g/kg)	74.20[b]	88.35[a]	93.49[a]	90.91[a]	0.09	<0.01
Apparent digestibility (%)						
DM	73.74	71.47	71.59	73.02	0.51	0.06
Organic matter	77.13	75.12	75.01	76.35	0.45	0.05
Crude protein	35.97[b]	56.30[a]	55.53[a]	57.31[a]	3.00	0.01
Crude fiber	77.36	76.28	68.89	78.29	2.03	0.05
NDF	66.97	66.59	67.08	67.09	2.83	0.36
ADF	67.55	60.94	60.17	64.38	1.84	0.05
Cellulose	73.13	69.37	67.20	68.09	1.82	0.08
Total digestible nutrients						
BW (g/kg)	15.45[b]	19.18[a]	19.19[a]	19.06[a]	0.56	0.01
Percentage of dry matter intake	67.89[b]	72.77[a]	72.02[a]	75.35[a]	0.01	0.04
Methane production (MJ/day)*	10.06[a]	10.40[a]	9.79[a]	8.41[b]	0.15	<0.01
ADG (kg/d)	0.37[c]	0.47[bc]	0.59[b]	0.75[a]	0.04	0.013

[abc]mean data with different lowercase letters on the same row show significantly different ($p < 0.05$). SEM: standard error mean, BW: body weight, DM: Dry matter, BW: Body weight, NDF: Neutral detergent fiber, ADF: Acid detergent fiber, MJ: Megajoule, TDN: Total digestible nutrient ADG: Average daily gain. *Methane is predicted using the method of Jentsch et al. (2007); **Percentage of DM concentrate

The addition of concentrate and supplementation of tannins had no effect ($p > 0.05$) on apparent digestibility except for crude protein. The digestibility of crude protein increased with tannin supplementations ($p < 0.05$). In the current study, protein digestibility increased due to an increase in the protein level of the diet after the addition of concentrates and tannin supplementation. Basal diets using tropical forages had a crude protein content of 5.14%, a value considered limiting adequate rumen microbial activity, implying sub-optimal conditions in the rumen (Sampaio et al., 2010). Rira et al. (2022) reported that condensed tannins did not affect feed degradability but interfered with microbial colonization; tannin-rich feeds had a relatively lower abundance of fibrinolytic microbes, especially *Fibrobacter* spp. Furthermore, it is supported by the report of Souza et al. (2010) that the protein content below 7 g/kg DM in the basal ration limits the degradation of rumen microorganisms. The same finding was also reported by van Kuijk et al. (2022).

Tannin compounds can bind strongly to proteins (Le Bourvellec and Renard, 2018) which causes a decrease in crude protein digestibility in the rumen. According to Ramaiyulis (2021), diet containing 0.12% tannin can reduce protein degradation in the rumen from 1.17 to 0.99% per hour *in vitro*. However, in the present study, the opposite results were obtained due to the *in vivo* nature of the study. The tannin-protein complexes formed in the rumen (pH 3.5-7) make the protein resistant to rumen microbial degradation. However, these complexes should dissociate in the pH environment of the duodenum and proximal abomasum (Getachew et al., 2006). Some studies also indicated the effect of tannins in reducing crude protein degradation in the rumen. However, it did not affect total post-rumen digestibility (Jolazadeh et al., 2015; Canadianti et al., 2020).

Total digestible nutrients showed an increase ($p < 0.05$) due to the addition of concentrate and were not affected by tannin supplementation ($p > 0.05$). The addition of concentrate plays a role in supplying additional nutrients because the basal feed is classified as low-quality grass (basal feed with crude protein < 8% and NDF > 50%). Rufino et al. (2016) stated that low-quality basal feed requires crude protein supplementation to meet the needs of rumen microbes to develop. The concentrates contain essential nutrients for rumen microbial growth, such as soluble carbohydrates, crude protein sources from urea (non-protein nitrogen), and macro and micro minerals. The fulfillment of rumen microbial nutrition can increase microbial activity to digest feed in the rumen (Ramaiyulis et al., 2019).

Table 2 shows that methane production per head of livestock ranged from 8.41 to 10.40 (MJ/d). Methane production is suppressed with 0.18% tannin supplementation ($p < 0.05$). Previous studies also found a 44-49% decrease in the proportion of methane with the addition of supplements containing tannins in forage or fermented straw basal feed

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(Liu et al., 2019; Ramaiyulis et al., 2021). A dairy cattle diet containing condensed and hydrolyzed tannin compounds can be considered a sustainable approach to reducing the environmental impact of rumination because the feed containing tannins can reduce enteric CH4 emissions (Stewart et al., 2019).

The ratio of Methane/TDN decreased simultaneously with the addition of supplements 0.12 and 0.18 % tannins ($p < 0.05$). Mitigation of methane gas is energy efficient, considering that 5-15% of energy is lost in the form of methane gas during the rumen fermentation process (Wanapat and Kang, 2015). This efficiency was reflected in the increase of body weight gain in beef cattle from 0.37kg/d (control) to 0.75kg/d with the addition of concentrate and 0.18% tannin supplementation.

CONCLUSION

The supplemented concentrate with gambir extract tannins can mitigate CH4 emissions and increase total digestible nutrients. The highest CH4 mitigation (49.7%) occurred in the basal diets of field grass with the addition of concentrate (75:25% DM) and 0.18% tannin supplementation. The highest CH4 mitigation can lead to the optimal weight gain of 0.75 kg/d in local Indonesian cattle.

DECLARATIONS

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Authors' contribution

Ramaiyulis supervised the experiment and wrote the original manuscript. Devi Kumala Sari, Dihan Kurnia, Debby Syukriani, and Irzal Irda conducted the experiment and data analysis. Yurma Metri prepared tables and finalized draft. All authors have read and agreed to the data analysis and the final version of the manuscript.

Competing interests

The authors declare that they have no conflicts of interest concerning the work presented in this report.

Ethical considerations

The authors carefully examined all ethical issues concerning plagiarism, approval to publish, errors in fabrication, double publication, and submission.

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