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Supplementation Effectivity of Cassava and *Indigofera zollingeriana* Leaves Extraction on Rumen Fermentation System of in Vitro

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Abstract. The function of ruminant feed supplements is to fulfil nutritional requirements to optimize rumen performance. Rumen microbes work optimally if the nutrient sources in the feed are always available and easily degraded. The source of the nutrients can be obtained from agricultural products, plantation products, agricultural industries and food [1]. Tanin and saponins from the plant are defaunation agents that are widely used in several studies. Tanin can be used as a defaunation agent which can reduce the protozoa population and suppress methane emissions in the rumen. Observations were using Randomized Block Design of 4 treatments and 4 groups with 3 replications in each group. The treatments observed were as follows: R0: base ration without extract (control), R1: base ration + 2% extract of cassava leaves, R2: base ration + 2% extract of *Indigofera zollingeriana* leaves, R3: base ration + 1% extract of cassava leaves + 1% extract of *Indigofera zollingeriana* leaves. The results showed that treatments of R3 significantly effective for fermentation system of in vitro by measurement at the degradation of dry matter and organic matter also at total gas production and methane production.

INTRODUCTION

The function of ruminant feed supplements is to fulfil nutritional requirements to optimize rumen performance. Rumen microbes can work optimally if the nutrient sources contained in the feed are always available and easily degraded. The source of these nutrients can be obtained from agricultural products, plantation products, agricultural industries and food [1]. The use of feed ingredients that do not compete with humans is the main requirement so that the availability of feed always continuous. The right ration formula would improve feed efficiency [2]. The method that developed as optimization of rumen performance is by applying defaunation technology, by reducing the population of protozoa in the rumen. Several methanogenic bacteria live in the rumen by attaching to the surface of the protozoa cell, so that defaunation of protozoa will cause the population of methanogenic bacteria to decrease in the rumen. The reduction in methanogenesis bacteria has implications for increasing feed efficiency and optimizing rumen metabolic performance thereby increasing digestibility because nutrients are absorbed properly.

Tanin and saponins from plants are defaunation agents that are widely used in several studies. Tanin can be used as a defaunation agent which can reduce the protozoa population so it can suppress methane emissions in the rumen [3]. A condensed tannin feed of 25.9 g kg-1 BK in *Lotus corniculatus* was reported to be able to reduce methane emissions in cattle [4]. The addition saponin to the ration can reduce the population of rumen protozoa partially or completely [5]. Several studies on the effect of giving tannin and saponin compounds had a beneficial effect on livestock and the environment by reducing the production of methane [6].

Cassava leaves beside as defaunation can pass feed proteins from rumen fermentation [7] and contained high crude protein, which is equal to 23.4% of dry matter [8]. So the use of sources of dafauntaor agents from cassava leaves as a supplement can increase digestibility and total gas production.

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One of the defaunation agents in *Indigofera zollingeriana* is saponin which is contained in 2.24% [9]. Saponins consumed by ruminants will serve to suppress the population of protozoa in the rumen so as to increase the population of the rumen and propionic acid [10] and reduce the production of methane in the rumen [11] so that digestibility will highly increase.

Provision of tannin and saponin supplementation of plants is expected to be able as a defaunation agent that has the potential to suppress the growth of protozoa, so that rumen performance is optimal. The research hypothesis is the supplementation of cassava, and *Indigofera zollingeriana* leaves extraction on rumen fermentation system of *in vitro*.

EXPERIMENTAL DETAILS

The tools used in this study are analytic scales, ovens 60 ºC and 105 ºC, porcelain saucers, CO_2 gas cylinders, hoses, vial tubes, ventilated rubber caps, vacuum pumps, spatulas, bulb pipettes, sectors, volumetric pipettes, magnetic stirrers, centrifuge, burette, water bath shaker. The materials for this study were using concentrates (Table 2) that processed for, *in vitro* observations, cassava and *Indigofera zollingeriana* leaves as extraction objects, McDougall buffer solutions, rumen fluids from fistula cattle taken at LIPI Cibinong, CO_2, water, NaOH 6N, and H_2SO_4.

The extraction of Cassava and *Indigofera zollingeriana* leaves prepared. Plant samples as defaunation used were cassava and *Indigofera zollingeriana* leaves then each plant sample was collected and cleaned. Each plant sample was dried first by aerating, after that it was opened for 24 h at 50 ºC, to obtain a dried plant sample for extraction. Dried plant samples (Cassava and *Indigofera zollingeriana* leaves) were separated into jars, then extracted by maceration (minimal heat) using ethanol 96% for 24 h using a shaker. After 24 h, the ethanol filtrate was separated and then concentrated with a vacuum evaporator. The ethanol-concentrated extract was then weighed to obtain the amount of raw material content from each of the processed plant samples.

TABLE 1. Nutrient content of feed ingredients

Nutrient Component	Content (%)						
	DM	**CP**	**F**	**CF**	**TDN**	**Ca**	**P**
Pollard	88	16.9	4.1	7.4	70	0.09	0.75
Onggok	83.8	7.8	0.4	14.9	78.3	0.02	0.05
Molasses	87.5	3.1	0.9	0.6	70.7		
Palm Kernel	88.5	18.58	12.55	15.38	81	0.08	0.52
Soybean Kernel	88	47.12	3.8	8.69	83.2	0.27	0.68
Rice Bran	89.6	6.53	2.36	29.81	67	0.14	0.6
$CaCO_3$						40	
Sawit Leaves*	4.05	11.39	2.20	23.26			
Sawit Fronds*	7.29	2.38	0.87	39.33			

Source: Nurfitriani [27], *PAU IPB (2018)

TABLE 2. Feed formulation

Feed Materials	Formulation (%)
Pollard	9
Onggok	9
Molasses	1.8
Palm Kernel	4
Soybean Kernel	7
Rice Bran	9
$CaCO_3$	0.2
Sawit Leaves	30
Sawit Fronds	30
Total	**100**

TABLE 3. Nutrition content of feed formulation

Treatment	DM	CP	F	CF	TDN	Ca	P
R0-R3	38.40	11.04	2.32	24.70	29.71	0.12	0.25

TABLE 4. Supplementation of Cassava and *Indigofera zollingeriana* leaves

Extraction	R0	R1	R2	R3
Cassava leaves	-	2%	-	1%
I. zollingeriana leaves	-	-	2%	1%

Observations using Randomized Block Design, namely 4 treatments and 4 groups with 3 replications in each group. The treatments observed are as follows: R0: base ration without extraction (control), R1: base ration+2% cassava leaves extraction, R2: base ration+2% *Indigofera zollingeriana* extraction and R3: base ration+1% cassava leaves extraction+1% *Indigofera zollingeriana* extraction

The observation of in vitro started bythe sample was put into an incubation tube as much as 0.5 g then added 33 ml of Mc Dougall buffer solution and 16 ml of rumen liquid. Previous rumen fluid was taken from fistula cattle donors on the morning before feeding, cassava and *Indigofera zollingeriana* extraction were added according to the treatment. Mc Dougall buffer solution flowed CO_2 gas during the process before being inserted into the tube to keep anaerobic conditions in the reaction. After that each in vitro tube was immediately tightly closed with a rubber cap and reinforced with an aluminum cap and put into a water bath (Master Lab, Anax-Pty, Limited) with a temperature of 39 ºC and incubated for 48 hours (taking total gas production in every 2, 4, 6, 8, 10, 12, 24, 30 and 48 h of observation) [25]. The result data will be analyzed by variance (ANOVA); if there were significant differences there would be further testing [12].

RESULTS AND DISCUSSION

Digestibility of Dry Matter and Organic Matter

The digestibility value of dry matter and organic matter is one indication for the feed quality, the higher digestibility potentially more nutrients are absorbed in the body [13]. The formulation of R3 treatment, namely 1% supplementation of cassava leaves extraction and 1% *Indigofera zollingeriana* extraction leaves extraction in base rations significant on the digestibility of dry matter and organic matter. This happens because in cassava leaves extraction and *Indigofera zollingeriana* leave extraction, there are secondary compounds including tannins and saponins, which can help the process of degradation of feed fibers in the digestive process. The results of measuring the digestibility of dry matter and organic matter are shown in Tables 5 and 6.

TABLE 5. Effect of treatment on Digestibility of Dry Matter

Groups	Digestibility of Dry Matter (%)			
	R0	R1	R2	R3
1	39.67	44.10	44.74	52.23
2	39.34	45.21	45.60	59.85
3	40.48	38.80	38.06	41.39
4	40.13	48.79	49.48	60.55
Average	39.90[a]	43.73[a]	44.47[a]	66.37[b]

[a,b,] superscripts on the same line are significant differences (P<0.05)

Secondary compounds of tannins and saponins contained in cassava leaves and *Indigofera zollingeriana* leaves extraction affected the growth of rumen microorganisms, especially fiber degrading microorganisms. Saponin is a defaunation agent that will inhibit the growth of protozoa [14]. If the protozoa are inhibited, the digestibility value will increase because the digesting bacteria of the fiber eaten by the population protozoa will increase. This is supported by the results of research that the use of tannin extract from the *Leucaena* plant was able to reduce the population of protozoa in vitro [15].

TABLE 6. Effect of treatment on Digestibility of Organic Matter

Group	Digestibility of Organic Matter (%)			
	R0	R1	R2	R3
1	45.56	52.90	51.55	58.99
2	44.74	53.16	52.84	63.86
3	48.11	43.63	43.48	55.59
4	46.30	56.85	58.10	64.16
Average	46.17[a]	51.64[a]	51.49[a]	60.65[b]

[a,b,] superscripts on the same line are significant differences (P<0.05)

The decrease in protozoa population causes the rumen microorganisms to work optimally so as to increase dry matter digestibility which is directly proportional to the digestibility of organic matter. Because the digestibility value of dry matter and organic matter is high due to the total of nutrients that can be digested by rumen microbes [16].

Total Gas and Methane Production

Determination of gas production kinetics in vitro is information about fermented feed consumed by ruminants [17]. Gas production is the result of fermentation processes that occur inside the rumen which can show microbial activity in the rumen and describe the amount of undigested organic matter. Gas production has a close relationship with the digestibility value of ruminant feed ingredients [18]. The increase in total gas production and decrease in methane gas in this study was generated by R3 treatment, supplementation of 1% cassava leaves extraction and 1% *Indigofera zollingeriana* leaves extraction in base rations as defaunation because it contains secondary compounds of tannins and saponins. The total kinetics of gas production and methane production are illustrated in Graphs 1 and 2.

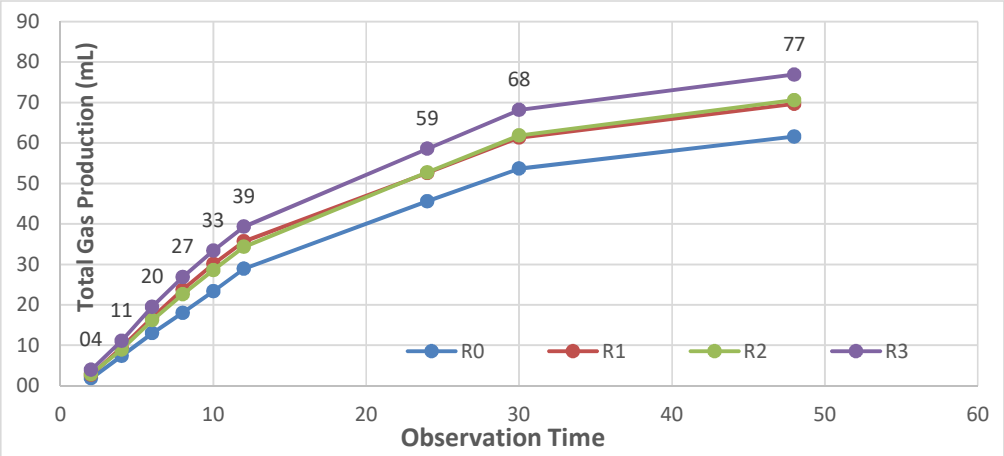

FIGURE 1. Kinetics of Total Gas Production

The total gas production kinetics of R3 significantly increase throughout incubation time, which reached 77 mL at 48 hours of observation (Fig.1). There is a correlation between total gas production and the activity of microorganisms in the rumen [19]. In addition, the production of gas from fermented feed can reflect the quality of the feed [20]. So that the higher the digestibility of feed ingredients, the higher the total gas production.

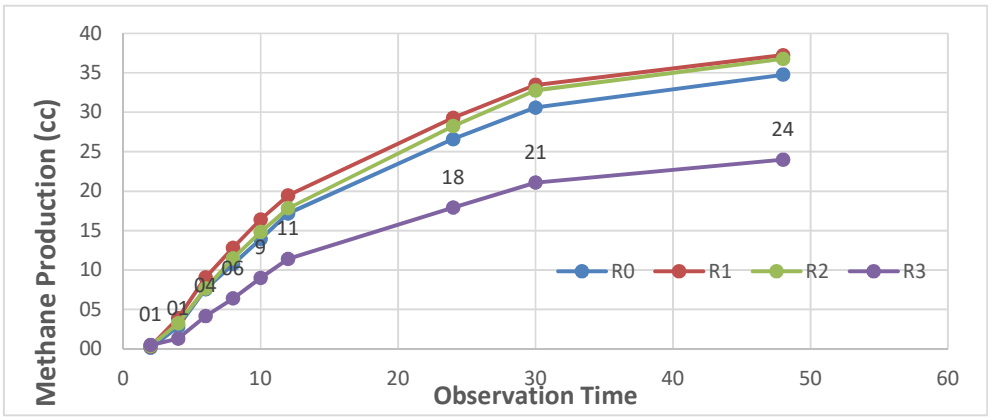

FIGURE 2. Kinetics of Methane Production

Methane gas (CH_4) released from the rumen indicates the energy lost from the body of ruminants with a variation of 7% -12% of the energy consumed [21]. The digested parts of feed energy will be lost as methane energy, which causes decreased livestock production and environmental pollution. The strategy to suppress methane production is to utilize feed ingredients that contain secondary compounds of tannins and saponins because to reduce methane gas production in ruminants is feeding, which contains tannin [22].

The lowest methane production rate was shown in R3 treatment, reaching 24 cc at 48 h of observation (Fig. 2). This indicates the operation of secondary compounds contained in cassava leaves and *Indigofera zollingeriana* leaves extraction. Supported by research results that legumes containing tannins such as *Calliandra calothyrsus* or *Hemingra macrophylla* can reduce the production of methane by ruminants [23].

The effect of saponin on reducing methane emissions is through the mechanism of reduction of the rumen protozoa population [14]. The population of protozoa in the rumen is directly proportional to the production of methane gas, meaning that the production of methane gas decreases if the protozoa population decreases [24]. Saponins consumed by ruminants function to suppress protozoan populations in the rumen and increase the population of rumen bacteria [25] and reduce the production of methane gas in the rumen [26].

SUMMARY

Supplementation of 1% cassava leaves extraction and 1% *Indigofera zollingeriana* leaves extraction contained secondary compounds of tannin and saponin effectively as defaunation agents in improving the quality of feed in the rumen of *in vitro*. This is indicated by the high digestibility of dry matter and organic matter, indicating the number of nutrients in the feed material absorbed in the digestion process. In addition, it is indicated by high total gas production but low methane gas production so that it will increase livestock production and environmental pollution by methane gas from livestock can be reduced.

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