

Dokumen Bukti Korespondensi untuk karya penelitian dengan judul artikel : **The Effect of Supplementation of Cassava Leaves, Palm Oil Sludge and Yeast in Kumpai Grass-Based Rations on Ruminal Fermentation and Gas Methane Concentration In-Vitro**

Penulis : *Riswandi\**), Arfan Abrar, Agus Wijaya, Basuni Hamzah, Nama Jurnal : International Journal on Advanced Science Engineering and Information Technology, Volume Jurnal : 11, Nomor Jurnal : 5, Tahun Terbit Jurnal : 2021, Halaman : 1921-1927, Print-ISSN : 2088-5334, Online-ISSN : 2460-6952, Yang terdiri dari :

- 1) Surat Permohonan Publikasi ke IJASEIT (18 Mei 2020)
- 2) Surat IJASEIT Reviewer 1 (18 Oktober 2020)
- 3) Surat IJASEIT Reviewer 2 (20 Oktober 2020)
- 4) Surat Tanggapan Penulis dan Perbaikan Manuscript (29 Oktober 2020)
- 5) Our Revised Manuscript based on two IJASEIT Reviewers (29 Oktober 2020)
- 6) Surat Accepted IJASEIT dan Journal Processing Fee (2 April 2021)
- 7) Surat Bukti Pembayaran Publikasi (6 April 2021)

# 1. Surat Permohonan Publikasi ke IJASEIT (18 Mei 2020)

Submission article for Publication in IJASEIT

 Riswandi Wandi <riswandi\_dya@yahoo.com>  
To: **Rahmat Hidayat** [mr.rahmat@gmail.com](mailto:mr.rahmat@gmail.com)

Mon, 18 May 2020

Dear Mr. Rahmat Hidayat

Editor in Chief

Following we send you our manuscript with title: "Effect Of Branched-Chain Amino Acids and Unsaturated Fatty Acids Contained In Cassava Leaves and Palm Oil Sludge Combined With Yeast On Digestive Quality And Methane Gas Concentration In Vitro" to be published in International Journal on Advanced Science, Engineering and Information Technology

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We look forward to hearing from you at your earliest convenience.

best regards

Riswandi

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## 2. Surat IJASEIT Reviewer 1 (18 Oktober 2020)

[IJASEIT] Revision Required

Yahoo/Email inbox



**Rahmat Hidayat** <mr.rahmat@gmail.com>

To: **Riswandi Wandu** <riswandi\_dya@yahoo.com>

Sun, 18 Oct 2020

Dear Mr. Riswandi Riswandi,

We have reached a decision regarding your submission to International Journal on Advanced Science, Engineering and Information Technology, "EFFECT of BRANCHED-CHAIN AMINO ACIDS and UNSATURATED FATTY ACIDS CONTAINED in CASSAVA LEAVES and PALM OIL SLUDGE COMBINED with YEAST on DIGESTIVE QUALITY and METHANE GAS CONCENTRATION IN VITRO". Our decision is to: Revision Required

Please submit your revision in 10 days. More than 10 days of paper will be rejected from the system. Re-upload your revision into journal system NOT via email.

Editor

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Reviewer A :

Title:

The title is not appropriate with journal format, please consider to change it according to the format.

Abstract:

Some sentences in the abstract section are needed to revise and rearrange, some are difficult to understand. Please write down the chemical formula with suitable format

Introduction:

Please revise how to write the species names, it should be written in italic.

Please do not simply taken the results from language translator, there are so many sentences which are difficult to understand and many grammatical errors are found in the manuscript.

Please revise some sentences in the introduction section.

The authors are also needed to follow the manuscript writing guide according to the IJASEIT Format, for example the way to written citation in the text body.

What author means with yea-sacc? Is it Saccharomyces yeast? Is it

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appropriate? Please explain, if it is not please consider to change it with the appropriate form.

The objective in the abstract section as well as in the introduction are not match. In the abstract, author talks about kumpai grass, however, in the Introduction section non of sentences talk about kumpai grass, in fact the authors talk about cassava leaves. Please explain!!

The importance of the issue are not highlighted in the introduction sufficiently.

The story line in the Introduction section are not well organized and confusing.

The author are needed to rewrite the Introduction section with clearly mention the background and objective of the research.

Materials and Method:

Please make a brief description regarding each method not only refers to the references

What method did author used for GLC, (gas flow, injection temperature, etc.)

Results and Discussion:

Please use formal/official English on the preparation of the manuscript. Do not simply use the translation results from the translator. It should be rectified.

There are many mistakes throughout the text and some phrases are difficult to understand.

The use of chemical formula in the whole text are not in the correct manner, please revised!

How many replication (duplicate, triplicate??) of data collection? Please give standard error/error boundaries.

In discussion section, I guess there are many improper evaluation of the literature.

Conclusion:

-

References:

Please check your References section, are they suitable with the Journal format?

Some references are out of date. Please convert the references into suitable format of Journal requirement.”

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International Journal on Advanced Science, Engineering and Information Technology

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### 3. Surat IJASEIT Reviewer 2 (20 Oktober 2020)

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Yahoo/Email inbox



**Rahmat Hidayat** <mr.rahmat@gmail.com>

To: **Riswandi Wandu** <riswandi\_dya@yahoo.com>

Tue, 20 Oct 2020

Dear Mr. Riswandi Riswandi,

We have reached a decision regarding your submission to International Journal on Advanced Science, Engineering and Information Technology, "EFFECT of BRANCHED-CHAIN AMINO ACIDS and UNSATURATED FATTY ACIDS CONTAINED in CASSAVA LEAVES and PALM OIL SLUDGE COMBINED with YEAST on DIGESTIVE QUALITY and METHANE GAS CONCENTRATION IN VITRO".

Our decision is to: Revision Required

Please update your abstract into 220-250 words and your reference 70% in (2017-2020) from journal indexed by Scopus. Citation and Reference in Paper must using Mendeley with IEEE Style.

Please submit your revision in 10 days. More than 10 days of paper will be rejected from the system. Re-upload your revision into journal system NOT via email.

Editor

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Reviewer B:

Improve the title to be shorter and more concise, and in the title, the novelty of the research should be described. Refine the method by showing the flow chart. Improve Table 1 and Table 2. Refine the table under discussion, and replace it in the form of a graphic that describes the changes that have occurred during the study. Improve conclusions by providing accurate and precise data on the results of the research. Add primary references from journals within the last 3 years.

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International Journal on Advanced Science, Engineering and Information Technology

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## 4. Surat Tanggapan Penulis dan Perbaikan Manuscript (29 Oktober 2020)



Riswandi Wandi <riswandi\_dya@yahoo.com>

To: **Rahmat Hidayat** [mr.rahmat@gmail.com](mailto:mr.rahmat@gmail.com)

Thu, 29 Oct 2020

Dear Mr Rahmat Hidayat

Editor in Chief

We thank you for the criticisms and suggestions given by editors and reviewers to improve our articles, all suggestions and criticisms given will be corrected and sent on schedule

best regards

Riswandi

## 5. Our Revised Manuscript based on two IJASEIT Reviewers (29 Oktober 2020)



Riswandi Wandu <riswandi\_dya@yahoo.com>

To: **Rahmat Hidayat** [mr.rahmat@gmail.com](mailto:mr.rahmat@gmail.com)

Thu, 29 Oct 2020

Dear Mr Rahmat Hidayat

Editor in Chief

Following we send you our revised manuscript entitled "Effect Of Branched-Chain Amino Acids and Unsaturated Fatty Acids Contained In Cassava Leaves and Palm Oil Sludge Combined With Yeast On Digestive Quality And Methane Gas Concentration In Vitro " to be published in International Journal on Advanced Science, Engineering and Information Technology. The revision was done based on recommendation of the two reviewers.

best regards

Riswandi

# Revised Manuscript based on Two Reviewers



Riswandi Wandu <riswandi\_dya@yahoo.com>  
To: Rahmat Hidayat [mr.rahmat@gmail.com](mailto:mr.rahmat@gmail.com)

## EFFECTS OF SUPPLEMENTATION OF CASSAVA LEAVES, PALM OIL SLUDGE AND YEAST IN KUMPAL GRASS-BASED RATIUNS ON RUMINAL FERMENTATION AND GAS METHANE CONCENTRATION IN VITRO

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**Abstract**— Swamp forage (kumpai grass), waste products from plantations, and agricultural industries are alternative feed for ruminants. As an effort to increase the value of its availability, yeast supplementation is needed. This study aimed to determine the effect of kumpai grass-based ration supplemented with cassava leaves, palm oil sludge, yeast on digestibility, rumen fermentation characteristics, and methane gas production. This study was determined using a Completely Randomized Design consisting of four treatments. The treatment is described as follows 70% grass + 30% Concentrate (P0, Con), 55% grass + 30% cassava leaves + 15% Concentrate + 0.05% yeast (P1), 55% grass + 30% palm oil sludge + 15% concentrate + 0.05% yeast (P2), and 55% kumpai grass + 15% cassava leaves + 15% palm oil sludge + 15% concentrate + 0.05% yeast (P3). Each treatment was incubated in vitro with a buffer solution (pH 6.9) and rumen of four replications (each repetition represented by three incubation bottles) for 48 hours at 39 °C. The data obtained were analyzed for variance; if there were a treatment effect, the Duncan test would be further tested. The results showed that supplementation affected the increase (P <0.05) of dry matter digestibility, organic matter, crude protein, neutral detergent fiber, acid detergent fiber, total volatile fatty acids (total VFA), partial VFA, and total bacteria. Whereas, N-ammonia (N-NH<sub>3</sub>), the ratio of acetate-propionate and methane gas has decreased. **It was concluded that supplementation of 15% cassava leaves, 15 % palm oil sludge, and 0.05% yeast in kumpai grass-based rations gave the best increase in the ration digestibility, total rumen bacterial count, rumen fermentation characteristics, and reduced methane concentration.**

**Keywords**— ruminal fermentation; methane gas; feed supplements; kumpai grass; volatile fatty acids.

Manuscript received.....; revised.....; accepted ..... Date of publication.....  
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### INTRODUCTION

Swampland has the potential as a source of forage, kumpai grass (*Hymenachne acutigluma*), which can function as a fiber source. However, some problems occur, namely the high content of grass fiber fraction, to



reduce the digestibility of feed and increase methane gas production [1]. Furthermore, high methane gas production has a negative impact in the form of a decrease in the value of feed efficiency; therefore, we need a solution to answer these problems. In this study, the solution needed to overcome this problem is supplementing a combination of probiotics, cassava leaves, and oil palm mud as concentrate feed replacement.

Nutritional supplementation with a balance of energy and protein and probiotics (yeast) aims to optimize rumen microbial growth by maximizing the use of fiber fractions in high fiber feed. *Saccharomyces cerevisiae* is a type of yeast probiotic that has been widely used as a dietary supplement in ruminants. Its effect in increasing cellulolytic bacterial populations reduces lactic acid accumulation [2]. Furthermore, *Saccharomyces cerevisiae* causes an increase in oxygen concentration in the rumen fluid and utilization of starch provided in the ration, which positively impacts livestock performance [3],[4]. The results prove that *Saccharomyces cerevisiae* supplementation increases fiber digestibility and increases crude protein degradation, fiber fraction, and microbial efficiency [5],[6].

~~Even though probiotic supplementation cannot achieve maximum results without balanced nutrition, feed with good nutrient content is needed.~~ The addition of cassava leaves in the ration; was reasonably caused by high branched amino acid content; The branched-chain amino acid (BCAA) content in cassava leaves consists of Valine 0.45%, leucine 0.63%, and isoleucine 0.46% [7]. Reference [8] reported the addition of BCAAs in the ration could increase the growth of bacteria, which is reflected in the increased digestibility of dry matter, neutral detergent fiber (NDF), and acid detergent fiber (ADF).

Furthermore, to increase the ratio's nutrient content and reduce methane gas production, the addition of oil palm mud to the ration is essential. Palm sludge is a waste of the palm oil processing industry and is a source of unsaturated fatty acids in 39.58% oleic acid and 9.92% linoleic acid [9]. The unsaturated fatty acids can affect the decline in methane gas production to increase propionic acid production. The increase in propionic acid is manipulation for the formation of ruminant livestock meat [10]. **Based on this, it is necessary to research the effect of supplementation of cassava leaves, palm oil sludge, and yeast on digestive quality, rumen fermentation characteristics, and methane gas concentration in vitro.**

## THE MATERIAL AND METHOD

### *Experimental Design and Sample Preparation*

This research was conducted using a completely randomized design with four treatments; described as follows 70% kumpai grass + 30% concentrate + 0% (control, P0), 55% kumpai grass + 30% cassava leaves + 15% concentrate + 0.05% yeast (P1), 55% kumpai grass + 30% Palm oil Sludge + 15% concentrate + 0.05% yeast (P2), and 55% kumpai grass + 15% cassava leaves + 15% Palm oil Sludge + 15% concentrate + 0.05% yeast (P3).

The sample preparation in this study was carried out through 2 stages: first, the collection of feed ingredients composing the treatment ration consisting of kumpai grass, cassava leaves, palm oil sludge, yeast, and concentrate. The kumpai grass obtained came from swampland in Rambutan village, Indralaya district. Cassava leaves are obtained from cassava crop farmers in Tanjung Pering village, Indralaya district. Previously, the process of reducing water content has been carried out with the hay method. Furthermore, Palm mud was obtained from the waste of PT Ben's palm oil processing factory in Rambutan Indralaya village. In contrast, yeast was obtained from the Bogor Ciawi Animal Research Institute.

Second, feed ingredients that have been collected; they are chopped and mashed using a blender; Thus, the continued process of mixing a small amount of material in advance, such as yeast and concentrate. It is mixed with feed ingredients with larger particle sizes such as cassava leaves, palm oil, and kumpai grass after mixing evenly. Mixing is done until homogeneous; As for the flowchart of the process of making treatment samples can be seen in Figure 1.

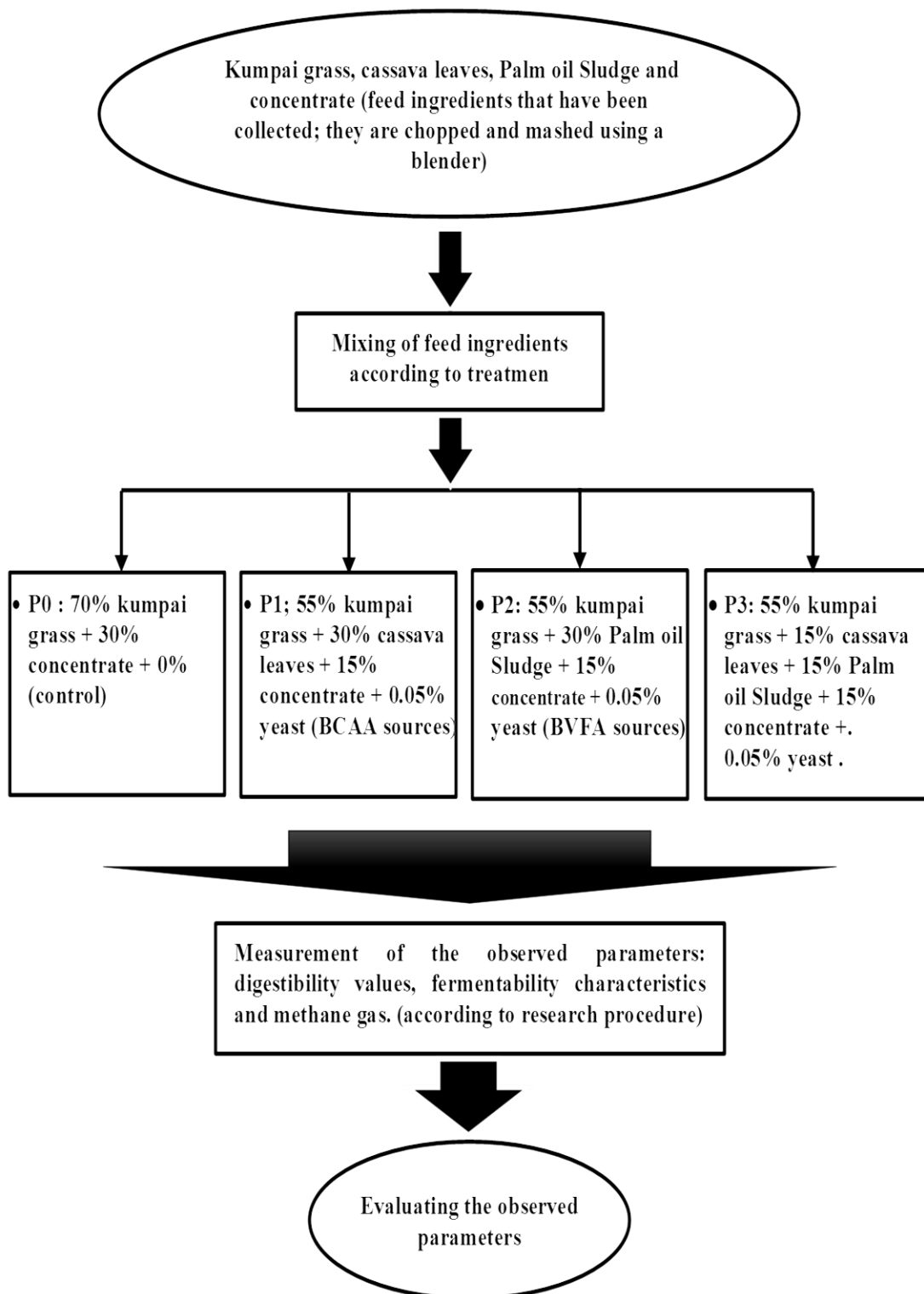


Fig. 1 flowchart of the process of making treatment samples

Furthermore, the ration nutrition quality measurement was carried out based on proximate analysis, fiber fraction, total rumen microbes, digestibility, and rumen fermentation ability in vitro. The study of the constituent feed ingredients' chemical composition and the ratio of treatment rations can be seen in Table I and Table II.

TABLE I.  
THE CHEMICAL COMPOSITION OF KUMPAI GRASS (KG), CASSAVA LEAVES (CL), PALM OIL SLUDGE (POS), AND CONCENTRATE (CON)  
(% DM)

Nutrient	KG	CL	POS	CON
Dry matter	91.86	88.75	90.76	80.59
Organic matter	88.52	78.96	83.23	63.21
Crude protein	8.43	20.34	18.85	15.62
Crude fiber	32.85	23.64	13.54	11.24
Ether extract	4.64	7.92	18.93	6.99
Neutral detergent fiber	58.43	30.58	46.37	37.71
Acid detergent fiber	46.35	22.34	35.32	21.66
Hemicellulose	12.08	8.24	11.14	16.05
Cellulose	26.64	16.72	27.21	15.86
Lignin	13.51	4.62	7.25	3.52
Oleate	0.05	0.13	42.18	1.42
Linoleic	0.22	0.38	11.24	1.58
Valine	0.03	0.63	0.39	0.43
Leucine	0.07	0.75	0.48	0.37
Isoleucine	0.12	0.67	0.43	0.28

TABLE II.  
INGREDIENTS AND CHEMICAL COMPOSITIONS OF THE EXPERIMENTAL DIETS (% DM)

Ingredients	Diet			
	P0	P1	P2	P3
Kumpai grass (%)	70	55	55	55
Cassava leaves (%)	0	30		15
Palm oil sludge (%)	0		30	15
Concentrate (%)	30	15	15	15
Yeast (%)	0	0.05	0.05	0.05
TOTAL	100	100	100	100
Dry matter	88.48	88.81	89.76	89.54
Organic matter	80.87	83.19	84.09	82.47
Crude protein (%)	11.29	13.63	13.18	13.41
Crude fiber (%)	28.37	25.85	23.81	25.33
Ether extract (%)	5.79	6.15	9.62	7.93
Neutral detergent fiber (%)	52.21	46.97	51.33	49.35
Acid detergent fiber (%)	39.94	35.44	38.84	36.65
Hemicellulose (%)	13.27	11.71	12.39	12.05
Cellulose (%)	23.41	22.05	25.19	23.62
Lignin (%)	10.51	9.35	10.13	9.13
Oleate (%)	0.46	0.28	12.89	6.59
Linoleic (%)	0.63	0.47	3.73	2.10
Valine (%)	0.15	0.27	0.20	0.23
Leucine (%)	0.16	0.32	0.24	0.28
Isoleucine (%)	0.08	0.27	0.24	0.27

#### IN VITRO INCUBATION, RUMEN FERMENTATION CHARACTERISTICS, AND GAS PRODUCTION.

According to the Tilley and Terry method in many previous studies, objectivity observations were based on in vitro process [11]. Rumen fluid is taken from slaughterhouses and filtered with four layers of cheesecloth. One part of rumen fluid (10 mL) is mixed with four pieces of media (40 mL) consisting of buffer solution, macro and micro mineral solution, resazurin, and reduction solution [12]. One gram of sample is put into a 100mL incubation tube and then added with 50 ml of the mixed solution before the tube is closed; CO<sub>2</sub> gas is drained for 30 seconds and is incubated for 24 hours. After the incubation time is finished, add two drops of HgCl<sub>2</sub>.

The sample and incubation media were centrifuged in tubes at 4000 rpm for 10 minutes to separate the supernatant and the residue. The supernatant was taken to analyze further the concentration of N-Ammonia (N-NH<sub>3</sub>), total volatile fatty acid (VFAT), partial VFA, total gas production, methane gas, and calculation of the total number of bacteria. The concentration of N-NH<sub>3</sub> in the modified Conway procedure [13]; The VFA content was analyzed using Gas-Liquid Chromatography (GLC Bruker Scion 436-GC, Bruker Daltonik GmbH, Bremen, Germany) using columns (BR-Wax fame, mmlD 0.32, 0.25 lm df) and FID detectors. Supernatant preparations obtained from in vitro incubation were added with 3 mg of sulfosalicylic acid dehydrate, centrifuged for 10 minutes at 12 000 rpm at 7°C, and then injected into the GLC column.

VFA quantification in acetate, propionate, and butyrate was obtained by comparing them with external standards. It was determined as  $\mu\text{mol} / \text{ml}$  or mM. The total VFA content was obtained by the sum of the respective VFA constituents [14]. Gas production and methane concentration were carried out after incubation for 24 hours by taking fermented gas samples of 3 mL using a syringe glass. Thus, gas samples stored in

venoject tubes analyzed methane gas concentration using Gas-Liquid Chromatography (GLC, HEWLETT PACKARD, 3700, USA) [15]. Total bacteria, according to the Ogimoto procedure [16]. Analysis of food content using incubated residue, then added with 50 mL pepsin-HCl 0.20% and incubated for 48 hours. The solution was then filtered using Whatman filter paper No. 41, then dried for 48 hours at 60 °C to analyze food substances' digestibility value.

#### ANALYSIS OF CHEMICAL COMPOSITION AND DIGESTIBILITY.

This study also analyzed the chemical composition according to AOAC procedures [14], which includes dry matter (DM), organic matter (OM), and crude protein (CP) as the analysis of fiber fraction content includes NDF and ADF, according to the van Soest procedure [17]. Furthermore, the observations on the quality of digestion include digestion of dry matter (DMD), digestibility of organic matter (OMD), and digestion of fiber fractions, including NDF and ADF. They were measured based on the amount of nutrient content and the fraction of fiber lost. The difference between the sample before incubation in vitro with the residual sample from in vitro incubation.

#### STATISTICAL ANALYSIS.

The data obtained were statistically analyzed according to the SAS 9.4 User Guide (SAS Inst, Inc, Cary, NC). If there is a treatment effect will be further tested by the Duncan test [18].

### RESULTS AND DISCUSSION

In vitro fermentation, experimental research was conducted to measure the digestibility and microbial activity of feed ingredients during fermentation in the rumen. The variables observed included DMD, OMD, crude protein digestibility, and digestion of fiber fractions, including Neutral Detergents Fibre (NDF) and Acid Detergents Fibre (ADF). The fermentation characteristics include pH, Ammonia (NH<sub>3</sub>), and volatile Fatty Acid (VFA). Furthermore, observations of the byproducts of fermentation production were also carried out to determine the quality of fermentation results, including total gas production, methane gas concentrations, and total bacteria.

The results of observations of the fermentation characteristics showed exciting results, as presented in Table III. At the pH value, there were no significant differences between treatments ( $P > 0.05$ ). However, there was a decrease in ammonia concentration (N-NH<sub>3</sub>) in P1, P2, and P3 treatments compared to P0 ( $P < 0.05$ ). The lowest concentration was found in the P3 treatment, followed by P1 and P2 (P1 vs. P2,  $P > 0.05$ ). Furthermore, the reverse results are shown in the total value of VFA; where the increase occurred in the P3 treatment with the highest VFA value compared to other treatments; which were then followed sequentially in the treatments P1, P2, and P0 (P1 vs. P2 vs. P0,  $P < 0.05$ ). The results can be seen in Table IV.

TABLE III.  
EFFECT OF TREATMENTS ON FERMENTATION CHARACTERISTIC (PH, N-NH<sub>3</sub>, AND VFA TOTAL)

Treatment	pH	N-NH <sub>3</sub> (mM)	VFA Total (mM)
P0	6.69	8.67 <sup>c</sup>	63.76 <sup>a</sup>
P1	6.61	7.30 <sup>b</sup>	115.25 <sup>c</sup>
P2	6.72	6.44 <sup>b</sup>	86.16 <sup>b</sup>
P3	6.75	5.31 <sup>a</sup>	127.70 <sup>c</sup>
SEM	0.02	0.45	7.00
<i>P-Value</i>	0.153	0.038	<0.0001

<sup>a,b,c,d</sup> SIGNIFICANTLY DIFFERENT IN A COLUMN ( $P < 0.05$ ).

TABLE IV.  
EFFECT OF TREATMENTS ON VFA PRODUCTION IN RUMEN

Treatment	Acetate (mM)	Propionate (mM)	Butyrate (mM)	Ratio A/P
P0	37.96 <sup>a</sup>	16.15 <sup>a</sup>	9.64 <sup>a</sup>	2.36 <sup>c</sup>
P1	65.11 <sup>b</sup>	32.95 <sup>b</sup>	17.19 <sup>c</sup>	1.97 <sup>b</sup>
P2	40.39 <sup>a</sup>	30.34 <sup>b</sup>	15.43 <sup>b</sup>	1.35 <sup>a</sup>
P3	73.43 <sup>b</sup>	39.93 <sup>c</sup>	14.34 <sup>b</sup>	1.84 <sup>b</sup>
SEM	4.53	2.39	0.80	0.12
<i>P-Value</i>	<0.0001	<0.0001	<0.0001	<0.0001

<sup>a,b,c,d</sup> SIGNIFICANTLY DIFFERENT IN A COLUMN ( $P < 0.05$ ).

Based on the analysis results in Table V, it was shown that treatments P1 and P3 had the highest DMD and OMD values compared to other treatments ( $P < 0.05$ ), which was then followed by P2 treatment compared to P0 treatment (P2 vs. P0,  $P < 0.05$ ). Furthermore, the same pattern was shown in the NDF digestibility value ( $P < 0.05$ ). Meanwhile, the digestibility value of crude protein and ADF showed contrast results. The increased digestibility was established in the P1 and P3 treatments compared to the P0 treatment ( $P < 0.05$ ); However, an increase in P1 was not followed by a significant difference compared to the P2 treatment ( $P > 0.05$ ). The digestibility values of crude protein, NDF, and ADF can be seen in Table VI.

TABLE V  
EFFECT OF TREATMENTS ON DIGESTIBILITY OF DRY MATTER (DM) AND ORGANIC MATTER (OM)

Treatment	Digestibility (%)	
	DM	OM
P0	50.65 <sup>a</sup>	52.15 <sup>a</sup>
P1	58.37 <sup>b</sup>	60.65 <sup>c</sup>
P2	53.41 <sup>a</sup>	56.43 <sup>b</sup>
P3	59.76 <sup>b</sup>	61.58 <sup>c</sup>
SEM	1.08	1.00
<i>P-Value</i>	<0.0001	<0.0001

<sup>a,b,c,d</sup> SIGNIFICANTLY DIFFERENT IN A COLUMN ( $P < 0.05$ ).

TABLE VI  
EFFECT OF TREATMENTS ON DIGESTIBILITY OF CRUDE PROTEIN, NEUTRAL DETERGENT FIBER (NDF), AND ACID DETERGENT FIBER (ADF).

Treatment	Digestibility (%)		
	CP	NDF	ADF
P0	60.65 <sup>a</sup>	50.69 <sup>a</sup>	49.59 <sup>a</sup>
P1	64.25 <sup>b</sup>	54.83 <sup>bc</sup>	51.57 <sup>b</sup>
P2	63.36 <sup>b</sup>	54.04 <sup>b</sup>	50.96 <sup>b</sup>
P3	64.37 <sup>b</sup>	55.22 <sup>c</sup>	52.06 <sup>b</sup>
SEM	0.49	0.48	0.33
<i>P-Value</i>	0.007	<0.0001	0.030

<sup>a,b,c,d</sup> SIGNIFICANTLY DIFFERENT IN A COLUMN ( $P < 0.05$ ).

TABLE VII  
EFFECT OF TREATMENTS ON GAS PRODUCTION (GP), METHANE CONCENTRATION, AND TOTAL BACTERIA.

Treatment	Gas production (ml)	Methane Concentration (ppm)	Total microbial ( $10^{10}$ cfu)
P0	28.00 <sup>a</sup>	6175.79 <sup>b</sup>	3.77 <sup>a</sup>
P1	34.75 <sup>b</sup>	537.97 <sup>a</sup>	5.37 <sup>b</sup>
P2	36.00 <sup>b</sup>	415.11 <sup>a</sup>	5.48 <sup>b</sup>
P3	36.15 <sup>b</sup>	736.45 <sup>a</sup>	6.47 <sup>c</sup>
SEM	1.01	6.62	0.26
<i>P-Value</i>	0.011	<0.0001	<0.0001

<sup>a,b,c,d</sup> SIGNIFICANTLY DIFFERENT IN A COLUMN ( $P < 0.05$ ).

Based on the analysis results in table VII, there was a significant increase in gas production in the treatments P1, P2, and P3 compared to the P0 treatment ( $P < 0.05$ ). Conversely, methane gas concentration showed a significant decrease in P1, P2, and P3 treatments compared to the P0 treatment ( $P < 0.05$ ). Furthermore, the total microbial observation showed a significant increase in the microbial population in P3 treatment compared to other treatments ( $P < 0.05$ ).

#### A. Effect of cassava leaves, palm oil sludge, and yeast supplementation on rations based kumpai grass on the characteristics of fermentation (pH, N-NH<sub>3</sub>, Total VFA, and partial VFA)

This study's pH value ranged between 6.61-6.75 so that it did not interfere with microbial growth in the rumen. The reasonable condition of the rumen pH is 6.0-6.9 [19]. This ideal pH value is achieved because the yeast activity reduces lactic acid accumulation in the rumen [3],[4]. Reference [2] stated that yeast *Saccharomyces cerevisiae*'s supplemented to a high-energy ration could stabilize rumen pH and prevent acidosis. Besides, the balance of fermented products, VFA and N-NH<sub>3</sub>, can also stabilize the rumen pH; N-NH<sub>3</sub> is produced from microbes' protein degradation. The N-NH<sub>3</sub> produced is then used as a source of nitrogen for microbial protein synthesis [20].

Low levels of ammonia in dietary supplements (P1, P2, and P3) indicate the high utilization of N-NH<sub>3</sub> as a source of nitrogen for rumen microbes. The increased activity sees this of rumen microbes in digesting nutrients; Furthermore, Reference [21] stated that microbes used the rumen N-NH<sub>3</sub> compound for its growth to increase feed digestibility. Furthermore, the indicated low N-NH<sub>3</sub> concentration was suspected because it was used for microbial growth in the rumen; high levels saw this of VFA produced in P1, P2, P3 compared to controls.

This study also showed an increase in VFA; As for the increase suspected to have a relationship with increased nutritional digestibility. As a result, the higher the ratio's digestibility value, the level of TVFA also increases. TVFA levels in this study are still in the normal range to guarantee rumen microbial activity and growth, ranging from 63.76 - 127.70 mM. This TVFA value is lower than the research results in Reference [22], which reported that supplementation of different legumes consisted of Kemon (*Neptunia olerancia*), lamtoro leaf (*Leucaena leucocephala*), and acacia. Each 5% in the grass-based ration produced TVFA ranging from 70.02 - 158.84 mM. According to Reference [20], the optimal VFA content for rumen microbial growth is 80 - 160

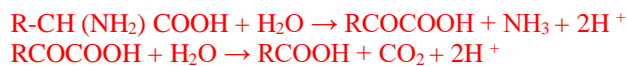
mM. Reference [23] stated that the composition of VFA formed in the rumen is influenced by the fermented substrate, microbial population, and rumen ecology.

The results showed that dietary supplements showed an increase in C2, C3, and C4 compared to control treatments. An increase also followed an increase in C2 and C4 in C3. Partial VFA can predict the value of ration efficiency in the form of a comparison between C2 and C3 (C2 / C3). Our results are in Reference [8], which found C2, C3, and C4 production increased with the BCAA diet. The results showed that dietary supplements showed a decrease in the C2 / C3 ratio in the rumen.

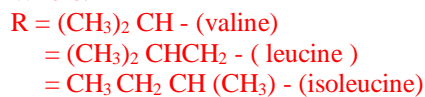
This decrease also has to do with yeast activity, as reported by [24]. The smaller the C2 / C3 ratio value, the higher the ration efficiency [25]. This study's results were lower than [26], showed that supplementation of banana humps and molasses in ammoniated rice straw-based feed produced a C2 / C3 ratio of 3.32-3.45. The low C2 / C3 ratio in this study is thought to be due to the high efficiency of livestock use. The low ratio or C2 / C3 ratio is due to the high fermentable organic matter in dietary supplementation; this allows the propionic acid formation to be higher than acetic acid. Propionic acid is the primary precursor for blood glucose formation and is glucogenic [27]. The C2 / C3 ratio value is closely related to methane gas production; the lower the C2 / C3 ratio value, the lower the methane production.

#### B. *The effect of cassava leaves, palm oil sludge, and yeast supplementation on nutrient digestibility.*

The effect of dietary supplements increases ( $P < 0.05$ ) the value of nutrient digestibility; the activity of yeast causes this as a rumen controller [3], [4]; thus being able to support the growth of rumen microbes to degrade nutrients [5], [6]. The lower fiber fraction content and high crude protein from dietary supplements will also increase degradation [20]. The effect of cassava leaf supplement (P1) on rumen degradation equals P3, which consists of a combination of cassava leaves and oil palm sludge; this is due to the high BCAA content in cassava leaves (Table 1), which functions as a carbon frame for the growth of fiber digesting bacteria [28]. Reference [8] stated that the addition of BCAAs increased the digestibility of DM and NDF. **The carbon frame source was obtained by decarboxylation and deaminase of branched-chain amino acids. Which were Valine, leucine, and isoleucine transformed into isobutyric, 2-methyl butyric, and isovaleric acids. The process of deamination and decarboxylation of BCAAs into BCVFA can be described as follows (29)**



Where:



Oil palm sludge supplement (P2) has a degradation effect on dry matter, organic matter, and rumen. NDF lower than P1 and P3. this is due to the high content of unsaturated fatty acids from oil palm sludge (Table 1). It affects the activity of cellulolytic bacteria in digesting fiber [30]. The availability of BCAAs and unsaturated fatty acids together in P3 (Table 2) is an optimal N and energy source for rumen microbial growth to degrade nutrients.

C. Effect of cassava leaves, palm oil sludge, and yeast supplementation on rations based on kumpai grass on gas production, methane gas concentration, and total microbes.

Gas production is a picture of fermented organic matter in the rumen [31] to evaluate the nutritional value of feed [32]. The total gas production due following increased rumen's microbial activity and it was related to increased digestibility and rumen fermentability [33]. Reference [34] stated that the total level of gas production in vitro fermentation depends on the composition of nutrient content such as (fiber, carbohydrates, protein), the presence of inhibitors for gas production, the quality of the ruminant diet, and the fermentation activity of microorganisms in rumen fluid.

Dietary supplements produce methane gas concentrations lower than control; low methane gas concentrations illustrate an increase in the value of hexose conversion efficiency, which means the rumen fermentation system, which leads to the synthesis of propionate acid [35]. The reduction in methane gas concentration correlates with the amount of C2 / C3 ratio in P1, P2, and P3 treatments and has lower than controls. These relationships have an attachment to the C2 production process, which produces carbon dioxide [20]; thus, the lower C2 than C3, the production of methane gas will decrease because of the supply of hydrogen methanogenesis is limited [35]. According to Reference [36], an increase in fiber content in the ration can occur due to the high C2 / C3 ratio, resulting in higher CH<sub>4</sub> production. Furthermore, the content of unsaturated fatty acids and crude protein can also reduce methane gas following the study's results in [37],[38]. Also, The supplementation of yeast was thought caused by stimulating acetogenins to compete using hydrogen with methanogens to reduce methane emissions [35].

The effect of dietary supplementation on treatment (P3) results in higher total rumen microbes than other treatments. This increase occurred positively related to digestibility value and rumen fermentability characteristics. VFA and NH<sub>3</sub> compounds obtained from fermented carbohydrates and proteins play an essential role as energy sources and N for rumen microbes [20]. This increase was also due to yeast activity, as reported by [3]. Finally, supplementation of cassava leaves, palm mud, and treated yeast has shown promising initial results for digestive coefficients, rumen fermentability characteristics, and methane gas. This experiment's results can be applied in in vivo experiments on goats and cattle, which are expected to improve feed efficiency and livestock performance.

## CONCLUSIONS

It was concluded that supplementation of 15% cassava leaves, 15 % palm oil sludge, and 0.05% yeast in kumpai grass-based rations gave the best increase in the ration digestibility, total rumen bacterial count, rumen fermentation characteristics, and reduced methane concentration.

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