a treatment effect will be further tested by the Duncan test [18].

III. Results and Discussion

In-Vitro fermentation, experimental research was conducted to measure feed ingredients' digestibility and microbial activity during fermentation in the rumen. The variables observed included DMD, OMD, crude protein digestibility, and digestion of fiber fractions, including Neutral Detergents Fiber (NDF) and Acid Detergents Fiber (ADF). The fermentation characteristics include pH, Ammonia (NH3), 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-NH3) 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$_3$, and VFA Total)

Treatment	pH	N-NH$_3$ (mM)	VFA Total (mM)
P0	6.69	8.67^c	63.76^a
P1	6.61	7.30^b	115.25^c
P2	6.72	6.44^b	86.16^b
P3	6.75	5.31^a	127.70^c
SEM	0.02	0.45	7.00
P-Value	0.153	0.038	<0.0001

^{a,b,c,d} 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^a	16.15^a	9.64^a	2.36^c
P1	65.11^b	32.95^b	17.19^c	1.97^b
P2	40.39^a	30.34^b	15.43^b	1.35^a
P3	73.43^b	39.93^c	14.34^b	1.84^b
SEM	4.53	2.39	0.80	0.12
P-Value	<0.0001	<0.0001	<0.0001	<0.0001

^{a,b,c,d} 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^a	52.15^a
P1	58.37^b	60.65^c
P2	53.41^a	56.43^b
P3	59.76^b	61.58^c
SEM	1.08	1.00
P-Value	<0.0001	<0.0001

^{a,b,c,d} 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^a	50.69^a	49.59^a
P1	64.25^b	54.83^{bc}	51.57^b
P2	63.36^b	54.04^b	50.96^b
P3	64.37^b	55.22^c	52.06^b
SEM	0.49	0.48	0.33
P-Value	0.007	<0.0001	0.030

^{a,b,c,d} 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^a	6175.79^b	3.77^a
P1	34.75^b	537.97^a	5.37^b
P2	36.00^b	415.11^a	5.48^b
P3	36.15^b	736.45^a	6.47^c
SEM	1.01	6.62	0.26
P-Value	0.011	<0.0001	<0.0001

^{a,b,c,d} 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-NH3, 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]. Khan *et al.* [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-NH3, can also stabilize the rumen pH; N-NH3 is produced from microbes' protein degradation. The N-NH3 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-NH3 as a source of nitrogen for rumen microbes. The increased activity sees this of rumen microbes in digesting nutrients; Furthermore, Suryani *et al.* [21] stated that microbes used the rumen N-NH3 compound for its growth to increase feed digestibility. Furthermore, the indicated low N-NH3 concentration was suspected because it was used for microbial growth in the rumen; high levels saw VFA produced in P1, P2, P3 compared to controls.

This study also showed an increase in VFA; As for the increase is suspected of having 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 Riswandi *et al.* [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 McDonald [20], the optimal VFA content for rumen microbial growth is 80 - 160 mM. Bannink *et al* [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 Zhang *et al.* [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. This study's low C2 / C3 ratio 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]. Zhang *et al.* [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)

$$R\text{-}CH\ (NH_2)\ COOH + H_2O \rightarrow RCOCOOH + NH_3 + 2H^+$$
$$RCOCOOH + H_2O \rightarrow RCOOH + CO_2 + 2H^+$$

Where:
$R = (CH_3)_2\ CH$ - (valine)
$= (CH_3)_2\ CHCH_2$ - (leucine)
$= CH_3\ CH_2\ CH\ (CH_3)$ - (isoleucine)

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. The 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]. Mitsumori and Sun [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, methane gas production will decrease because the supply of hydrogen methanogenesis is limited [35]. According to Martin *et al.* [36], an increase in fiber content in the ration can occur due to the high C2 / C3 ratio, resulting in higher CH4 production. Furthermore, unsaturated fatty acids and crude protein content can also reduce methane gas following the study's results in [37], [38]. Also, yeast supplementation 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 NH3 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.

IV. CONCLUSION

This study concludes 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.

ACKNOWLEDGMENT

The authors are grateful to Sriwijaya University for providing research funding through a competitive flagship scheme.

REFERENCES

[1] A. I. M. Ali, S. Sandi, Muhakka, Riswandi, and D. Budianta, "The Grazing of Pampangan Buffaloes at Non Tidal Swamp in South Sumatra of Indonesia," *APCBEE Procedia*, vol. 8, no. CAAS 2013, pp. 87–92, 2014, doi: 10.1016/j.apcbee.2014.03.006.

[2] R. Ullah Khan *et al.*, "Direct-fed microbial: Beneficial applications, modes of action and prospects as a safe tool for enhancing ruminant production and safeguarding health," *International Journal of Pharmacology*, vol. 12, no. 3, pp. 220–231, 2016, doi: 10.3923/ijp.2016.220.231.

[3] D. S. Kumar, C. S. Prasad, and R. M. V. Prasad, "Effect of Yeast Culture (Saccharomyces Cerevisiae) on Ruminal Microbial Population in Buffalo Bulls," in *Buffalo Bulletin*, vol. 32, no. 2, S. Sophon, Ed. Bangkok, Thailand: International Buffalo Information Centre, Office of University Library, Kasetsart University, 2013, pp. 116–119.

[4] E. Pinloche, N. McEwan, J. P. Marden, C. Bayourthe, E. Auclair, and C. J. Newbold, "The Effects of a Probiotic Yeast on the Bacterial Diversity and Population Structure in the Rumen of Cattle," *PLoS ONE*, vol. 8, no. 7, 2013, doi: 10.1371/journal.pone.0067824.

[5] A. I. M. Ali, S. Sandi, . R., A. Imsya, A. Prabowo, and N. Rofiq, "Evaluation of Yeast Supplementation with Urea-Molasses in Rice Straw-Based Diets on In-Vitro Ruminal Fermentation," *Pakistan Journal of Nutrition*, vol. 14, no. 12, pp. 988–993, Dec. 2015, doi: 10.3923/pjn.2015.988.993.

[6] S. X. Tang *et al.*, "Effects of yeast culture and fibrolytic enzyme supplementation on In-Vitro fermentation characteristics of low-quality cereal straws1," *Journal of Animal Science*, vol. 86, no. 5, pp. 1164–1172, May 2008, doi: 10.2527/jas.2007-0438.

[7] N. Nurhaita, L. Warly, Z. Mardiati, R. S.-J. P. U. J. Seri, and undefined 2010, "Sintesis protein mikroba pada domba yang mendapat ransum daun sawit amoniasi yang disuplementasi mineral S, P dan daun ubi kayu," *Jurnal Penelitian Universitas Jambi Seri Sains*, vol. 12, pp. 107–114, 2010.

[8] H. L. Zhang, Y. Chen, X. L. Xu, and Y. X. Yang, "Effects of Branched-chain Amino Acids on In-Vitro Ruminal Fermentation of Wheat Straw," *Asian-Australasian Journal of Animal Sciences*, vol. 26, no. 4, pp. 523–528, 2013, doi: 10.5713/ajas.2012.12539.

[9] A. Hayyan *et al.*, "Production of Biodiesel from Sludge Palm Oil by Esterification Process," *Energy and power engineering*, vol. 4, no. 1, pp. 11–17, 2010, doi: 10.17265/1934-8975/2010.01.002.

[10] A. MacHmüller, D. A. Ossowski, and M. Kreuzer, "Comparative evaluation of the effects of coconut oil, oilseeds and crystalline fat on methane release, digestion and energy balance in lambs," *Animal Feed Science and Technology*, vol. 85, no. 1–2, pp. 41–60, 2000, doi: 10.1016/S0377-8401(00)00126-7.

[11] J. M. A. Tilley and R. A. Terry, "A Two-Stage Technique for the In-Vitro Digestion of Forage Crops," *Grass and Forage Sci Grass and Forage Science*, vol. 18, no. 2, pp. 104–111, 1963.

[12] H. K. Goering and P. J. Van, "Forage fiber analyses," *U.S. Department of Agriculture*, no. 379, pp. 387–598, 1975.

[13] E. J. Conway and E. O'Malley, "Microdiffusion methods. Ammonia and urea using buffered absorbents (revised methods for ranges greater than 10μg. N)," *Biochemical Journal*, vol. 36, no. 7–9, pp. 655–661, Sep. 1942, doi: 10.1042/bj0360655.

[14] AOAC, *Official Methods of Analysis of AOAC INTERNATIONAL*, 18th ed., no. February. Gaithersburg, MD: AOAC International, 2005.

[15] Menke and KH, "Estimation of the energetic feed value obtained from chemical analysis and In-Vitro gas production using rumen fluid," *Animal Research and Development*, vol. 28, pp. 7–55, 1988.

[16] K. Ogimoto and S. Imai, *Atlas of rumen microbiology*. Tokyo : Japan scientific societies press, 1981.

[17] P. J. Van Soest, *Nutritional ecology of the ruminant : ruminant metabolism, nutritional strategies, the cellulolytic fermentation and the chemistry of forages and plant fibers*. Ithaca [N.Y.]: Comstock Pub. Associates, 1988.

[18] R. G. D. Steel and J. H. Torrie, *Principles and procedures of statistics.* New York: McGraw-Hill, 1980.

[19] D. N. Kamra, "Rumen microbial ecosystem," *Current Science*, vol. 89, no. 1, pp. 124–135, May 2005.

[20] P. McDonald, *Animal nutrition*. Harlow, England; New York: Prentice Hall/Pearson, 2011.

[21] N. N. Suryani, I. G. Mahardika, S. Putra, and N. Sujaya, "Enhancement Provision Of Gliricidia Sepium In Diet Increase Nitrogen Balance And Population Of Rumen Proteolitik Microorganism Of Bali Cattle)," *Jurnal Veteriner*, vol. 16, no. 1, pp. 117–123, 2015.

[22] R. Riswandi, L. Priyanto, A. Imsya, and M. Nopiyanti, "In-Vitro Digestibility of Fermented Hymenacne Acutigluma-Based Rations Supplemented with Different Legumes," *Jurnal Veteriner*, vol. 18, no. 2, p. 303, 2017, doi: 10.19087/jveteriner.2017.18.2.303.

[23] A. Bannink *et al.*, "Modelling the implications of feeding strategy on rumen fermentation and functioning of the rumen wall," *Animal Feed Science and Technology*, vol. 143, no. 1–4, pp. 3–26, 2008, doi: 10.1016/j.anifeedsci.2007.05.002.

[24] L. Riyanti, Suryahadi, and D. Evvyernie, "In-Vitro fermentation characteristics and rumen microbial population of diet supplemented with Saccharomyces cerevisiae and rumen microbe probiotics," *Media Peternakan*, vol. 39, no. 1, pp. 40–45, 2016, doi: 10.5398/medpet.2016.39.1.40.

[25] E. R. Ørskov and M. Ryle, *Energy nutrition in ruminants.* Barking: Elsevier Science Publishers Ltd, 1990.

[26] R. I. Rahayu, A. Subrata, and J. Achmadi, "In-Vitro Ruminal Fermentability on Ammoniated Rice Straw Based Diet With Supplementation of Banana Tree Root Ball and Molasess," *Indonesian Journal of Animal Science*, vol. 20, no. 3, p. 166, Oct. 2018, doi: 10.25077/jpi.20.3.166-174.2018.

[27] B. Vlaeminck *et al.*, "Milk odd- and branched-chain fatty acids in relation to the rumen fermentation pattern," *Journal of Dairy Science*, vol. 89, no. 10, pp. 3954–3964, 2006, doi: 10.3168/jds.S0022-0302(06)72437-7.

[28] L. O. Tedeschi, D. G. Fox, M. A. Fonseca, and L. F. L. Cavalcanti, "Models of protein and amino acid requirements for cattle," *Revista Brasileira de Zootecnia*, vol. 44, no. 3, pp. 109–132, Mar. 2015, doi: 10.1590/S1806-92902015000300005.

[29] V. Fievez, B. Vlaeminck, T. Jenkins, F. Enjalbert, and M. Doreau, "Assessing rumen biohydrogenation and its manipulation in vivo, In-Vitro and in situ," *European Journal of Lipid Science and Technology*, vol. 109, no. 8, pp. 740–756, 2007, doi: 10.1002/ejlt.200700033.

[30] W. F. Pellikaan, W. H. Hendriks, G. Uwimana, L. J. G. M. Bongers, P. M. Becker, and J. W. Cone, "A novel method to determine simultaneously methane production during In-Vitro gas production

using fully automated equipment," *Animal Feed Science and Technology*, vol. 168, no. 3–4, pp. 196–205, 2011, doi: 10.1016/j.anifeedsci.2011.04.096.

[31] M. M. Y. Elghandour, A. E. Kholif, A. Z. M. Salem, O. A. Olafadehan, and A. M. Kholif, "Sustainable anaerobic rumen methane and carbon dioxide productions from prickly pear cactus flour by organic acid salts addition," *Journal of Cleaner Production*, vol. 139, pp. 1362–1369, 2016, doi: 10.1016/j.jclepro.2016.08.075.

[32] I. Tapio, T. J. Snelling, F. Strozzi, and R. J. Wallace, "The ruminal microbiome associated with methane emissions from ruminant livestock," *Journal of Animal Science and Biotechnology*, vol. 8, no. 1, pp. 1–11, 2017, doi: 10.1186/s40104-017-0141-0.

[33] P. Mor *et al.*, "Effect of ammonia fiber expansion on the available energy content of wheat straw fed to lactating cattle and buffalo in India," *Journal of Dairy Science*, vol. 101, no. 9, pp. 7990–8003, 2018, doi: 10.3168/jds.2018-14584.

[34] M. Mitsumori and W. Sun, "Control of rumen microbial fermentation for mitigating methane emissions from the rumen," *Asian-Australasian Journal of Animal Sciences*, vol. 21, no. 1, pp. 144–154, 2008, doi: 10.5713/ajas.2008.r01.

[35] F. X. Philippe and B. Nicks, "Review on greenhouse gas emissions from pig houses: Production of carbon dioxide, methane and nitrous oxide by animals and manure," *Agriculture, Ecosystems and Environment*, vol. 199, pp. 10–25, 2015, doi: 10.1016/j.agee.2014.08.015.

[36] C. Martin, D. P. Morgavi, and M. Doreau, "Methane mitigation in ruminants: From microbe to the farm scale," *Animal*, vol. 4, no. 3, pp. 351–365, 2010, doi: 10.1017/S1751731109990620.

[37] Y. Widiawati, M. Winugroho, E. Teleni, and A. Thalib, "Fermentation kinetics (In-Vitro) of Leucaena leucocephala, Gliricidia sepium and Calliandra callothyrsus leaves (3) the pattern of gas production, organic matter degradation, pH, NH_3 and VFA concentration; estimated CH4 and microbial biomass production," *Indonesian Journal of Animal and Veterinary Sciences*, vol. 12, no. 3, pp. 202–211, 2007.