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Impact of Concentrate Supplementation on Biodegradability and Acidosis in In-Vitro Rumen Fermentation of Forage

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ABSTRACT

The present study aimed to evaluate degradability of various substrates supplemented with concentrates in the rumen culture and their potential for generating acidosis. Concentrates used in this study is the commercial concentrates, which mainly consist of carbohydrates. The proportion of the concentrates added in the rumen culture fermenting forage was varied from 0 to 100%. Low proportion of concentrates in the feed may lower the risk of acid build-up in the rumen culture since concentrates typically contains a significant amount of biodegradable substances. The present investigation revealed that a 5 to 10% supplement of concentrates to the rumen culture fermenting forage grass could enhance biodegradation efficiency, achieving between 65% and 80%. This concentration range also maintained the culture's pH at a neutral level (6.7-7.0), potentially averting acidosis induced by acid accumulation. However, the incorporation of a minimum of 20% concentrates in the rumen culture fermenting grass led to acid accumulation and subsequent acidosis within 24 hours of incubation, as the culture's pH plummeted from 7.0 to 6.2. A statistical analysis was performed using an ANOVA test at a 5% significance level, revealing a statistically significant correlation between the amount of concentrate supplementation and the accumulation of Volatile Fatty Acids (VFAs) in the rumen culture fermenting forage. This study underscores the importance of optimal concentrate supplementation for efficient biodegradation while preventing acidosis, offering insights for the enhancement of rumen fermentation processes.

1. INTRODUCTION

Rumen fermentation, a critical process in the health of ruminants, is facilitated by complex microbial communities that metabolize ingested feed into energy sources for the host [1]. The composition of the feed significantly influences these microbial communities, thus determining the metabolic pathways and the resulting end-products of fermentation [2]. The escalating demand for cattle products has prompted a transition in the ruminant industry from fiber-based to concentrate-rich feed [3]. Concentrates, which are rich in degradable carbohydrates, are chosen to augment ruminant energy intake, thereby enhancing productivity for milk and meat production [4]. Given the high degradability of concentrates, they readily undergo fermentation in the rumen culture. However, this increased productivity is accompanied by potential risks associated with the buildup of organic acids [5]. Organic acids, produced during carbohydrate fermentation, can accumulate to high levels, reducing rumen pH and potentially disrupting ruminant metabolism [5-7]. This imbalance can lead to a metabolic disorder known as acidosis, resulting from excessive consumption of degradable carbohydrates [8].

On the other hand, ruminants fed with dietary fiber can reap specific benefits, including sufficient salivation, plentiful energy supply, and essential nutrients [9]. Furthermore, dietary fiber helps maintain the normal function of the rumen as part of the digestive system [10]. Common sources of dietary fiber for cattle nutrition include agricultural residues and lignocellulosic biomass, such as grasses, corn stover, and rice straw. Dietary fibers can optimize the pH for cellulolytic microbes in the rumen, maintain metabolic balance, and potentially mitigate the risk of ruminal acidosis [11].

However, feeding ruminants a high proportion of forages like straw and grasses may result in decreased nutrient utilization. While forages are high in fiber, they are not rich in crude protein and exhibit low digestibility [12, 13], potentially resulting in restrained ruminant productivity in terms of body weight and milk production [14]. A balanced feed, combining forage and degradable carbohydrates, is crucial for supporting ruminant growth. During rumen fermentation, degradable carbohydrates are readily converted into energy sources, enhancing nutrient utilization [15]. A common issue in cattle feeding is the excessive addition of concentrates to the feed, often without awareness of the potential risk of acidosis. This current study would provide a significant approach in terms of formulation cattle feed of forages supplemented with an appropriate proportion of concentrates that could prevent acid accumulated in the rumen.

In-vitro rumen fermentation of forage combined with degradable carbohydrates would be carried out in this current study in which the substrates used for the experiment included forage grass (elephant grass) and concentrates. The current study would investigate the feasible feed management and

supplementation in in-vitro rumen fermentation as healthy rumen function would be derived from the feasible method for avoiding rumen acidosis. The objective of the current study is to find as well as evaluate an optimal ratio of concentrate to forage in order to prevent acidosis. Further, the current study aimed to evaluate any potential acidosis and substrate degradability during in-vitro rumen fermentation. Various proportion of concentrates added in the rumen culture fermenting grass would thoroughly be investigated to evaluate its feasibility as supplemental feed. The relevance and significance of this study is to obtain applied method for formulating cattle feed consisting of forage and concentrates, which are not only feasible to lower the risk of acidosis but also to maximize the ruminants' productivity.

2. MATERIALS AND METHODS

2.1 Collection of rumen liquor

Rumen liquor was collected at a slaughterhouse from culled beef cattle previously fed under controlled condition in the feedlot in which the cattle were fed regularly with the feed consists of some kind of grasses and lignocellulosic biomass.The slaughterhouse is situated in Peunayong, Banda Aceh City, Aceh Province, Indonesia. The fresh collected rumen fluid was then immediately kept in warm circumtances under the temperature of 38 ± 0.5℃ before starting of the experiments in order to activate the rumen microflora.

2.2 Substrates preparation

The substrates used in this current study were some substrates that were normally used as beef cattle feed including lignocellulosic material (i.e., grass) and concentrate as an additional feed. The type of forage grass utilized in this experiment was Elephant grass (*Pennisetum purpureum).* The grass was collected from a pasture grass located in Pango, Banda Aceh, Indonesia, and it was chopped to reduce its size into 0.2 ± 0.1 cm before applying it in the rumen fermentation. Concentrate utilized in this experiment was a commercial concentrate that was normally used for cattle feed, which typically consisted of some materials such as cassava starch, tofu pulp, coconut cake, rice bran and molasses. The concentrate was acquired from the cattle feed store located at Banda Aceh.

2.3 Experimental design and procedures

2.3.1 Experimental setup

The research consisted of a sequence of batch experiments with different concentrations of concentrates. The batch experiments were performed *at* mesophilic temperature, which was about 39 ± 0.5℃ using thermostatic water bath. The duration of fermentation process was 48 hours in order to provide sufficient time for rumen microbes to acclimate and adjust in the anaerobic condition and also to ensure that all substrates could completely be digested [6, 16]. The concentration of substrate applied in in-vitro rumen fermentation was about 10 g/100 ml or 100 g/L in which each batch reactor was added with 10 g substrate and topped-up with 100 mL of fresh rumen fluid. In this experiment, the fermentation was performed in batch reactor with a working volume of 100 mL. In this experiment there were no acid and/or basic solution supplemented to the rumen culture [6, 8].

2.3.2 Evaluation of various substrate concentrations

In the experiment of in-vitro rumen fermentation, some trials included rumen fluid only as a blank reactor (T0), rumen fluid containing merely grass (T1), and rumen fluid containing concentrate (T2). To assess the effects of concentrate addition to the rumen fermentation of forage grass, some proportion of concentrate were varied from 5 to 20% based on weight. This range was chosen as to avoid an excessive amount of concentrates supplemented to the feed that may cause sudden acid build-up in the rumen culture [16]. The experimental design set up was based on the feed tested including 5% concentrates and 95% grass (T3), 10% concentrates and 90% grass (T4), 20% concentrates and 80% grass (T5). In this experiment, pH culture was not controlled in a specific level. Therefore, no chemicals including acid and/or alkaline solutions were added to the reactors [6, 17].

2.4 Analytical methods

All samples taken before, after and during fermentation processes were regularly analyzed for monitoring their pH level using a Laboratory Benchtop pH Meter Multifunction Complete Probe [18]. The buffer capacity of rumen fluid was determined to evaluate its capability of neutralizing proton (H^+) and/or hydroxide (OH^-) generated from acid accumulation and/or ammonia build-up during the fermentation processes [19]. Samples obtained from rumen fermentation were measured for the ammonia content using a colorimetric procedure of NH_3 test kit and reagent [20]. The sample was dried for around 24 hours at the temperature of 105 ± 0.5℃ using a laboratory drying oven to determine its total solid (TS) and moisture content (MC) [21, 22].

To evaluate the formation of organic acids as the fermentation end-products, the titratable acidity (TTA) was determined to assess the total acids generated during the in-vitro rumen fermentation. The measurement was conducted by using a Laboratory Benchtop Standard pH meter [6, 23]. The titrant used in the burette tube was 100 mmol/L sodium hydroxide standard solutions. Prior to the start of the titration, the analyte or the analyzed sample was given a few drop of phenolphthalein as an indicator [18, 24]. Further,Volatile fatty acid (VFA) as the common metabolites in the rumen fermentation was also determined by titrimetic method. All the procedures performed for VFA analysis were based on the method developed and written by Lützhøft et al. [25]. To generate and confirm reproducible data, each sample analyzed was replicated.

2.5 Statistical analysis

Data obtained from the experiments were statistically analyzed with the method of one-way analysis of variance (ANOVA). The sample analysis was carried out in replicate. Further, the data analyzed with ANOVA single factorial were tested with 5% (α=0.05) level of significance to assess the influence of various concentration of concentrates in the feed containing grass for the potential characteristics of acidosis in the rumen fermentation.

3. RESULTS AND DISCUSSION

In-vitro rumen fermentation of forage grass supplemented with various concentration of concentrate was assessed with a series of batch trial. Table 1 showed that pH of the rumen liquor utilized in in-vitro study was about 7.13, which was quite closed to a normal pH of rumen [26]. This indicated that the rumen fluid used for the experiments was derived from the ruminant that was not suffering acidosis or no organic acids accumulated in the rumen. As presented in Table 1, pH of forage grass was somewhat neutral (7.4) while pH of the concentrate used was quite acidic (6.6). Hence, in-vitro study conducted to evaluate potential acidosis in the rumen culture fermenting forage grass with the addition of concentrate as a supplemental feed would be significant.

Study mentioned that the normal pH of forage grass fed-ruminants was around 6.0 to 7.0 [27] in which those pH ranges were considered to be the optimum pH for cellulolytic bacteria [28]. When pH of rumen drops below 6.2 the digestibility of fiber tends to be restricted [29]. Acidosis occurs when rumen pH drops below 6.2, and could be extended to the sub-acute acidosis (SARA) when the pH depresses below 6.0 [30]. This condition may significantly affect the health of ruminants in which the problem ending-up progressively worse as the pH declines [30, 31].

Table 1. Physicochemical properties of rumen fluid and substrates used for the batch experiments

Parameters	Unit	Rumen	Forage Grass	Concentrate
Total solids	%	9.62	16.81	93.7
Moisture content	%	90.38	83.19	6.3
pH	-	7.13	7.40	6.6
Total Ammonia	mg/L	10.1	5	9.98
Titratable acidity	%	0.2	0.27	0.18

The results of the study revealed that the use of solely concentrate as the feed would be somewhat too risky for the ruminants. This is because during the rumen fermentation concentrate was easily degraded and/or converted into metabolites especially organic acids. As shown in Figure 1, in vitro rumen fermentation of concentrate (T2) had organic acid accumulation represented in the low pH of the rumen culture. The results showed that within 2 hours of incubation pH of the rumen culture fermenting concentrate dropped extremely from 6.6 to 5.7 indicating that acidosis occurred quickly. This is quite contrast with the rumen fermentation of forage grass in which during the incubation process pH of the culture was somewhat stable in the range between 7.0 and 6.7. The pH ranges of the rumen culture fermenting forage grass were somewhat close to the optimal pH for microbes degrading fiber specifically cellulolytic bacteria that have the optimal pH range of 6.5-7.0 [28, 32].

As depicted in the Figure 2, the buffer capacity of forage grass (6.4 mmol/L) was about two times higher than that of concentrate (2.92 mmol/L). This indicated that the grass having high fiber content was strong enough to prevent pH drop during the rumen fermentation due to its higher buffer capacity. This suggested that the feed containing a significant amount of concentrate would be quite vulnerable to ruminal acidosis due its low buffer capacity and high biodegradability. Hence, combination of forage grassand concentrate as a feed would be potential to stabilize rumen pH, minimize the risk of rumen acidosis, and also at the same time could maintain the balanced energy supply for the ruminants. The results of the study revealed that rumen culture fermenting the feeds consisting 5% concentrate (T3) and 10% concentrate (T4) did not experience acidosis in which within 24 hours of incubation pH of the culture was stable between 7.0 and 6.7. On the other hand, rumen culture fermenting feed containing 20% of concentrate (T5) had a significant drop of pH in which within 24 hours of incubation the pH decreased from around 7 to 6.2 (Figure 1).

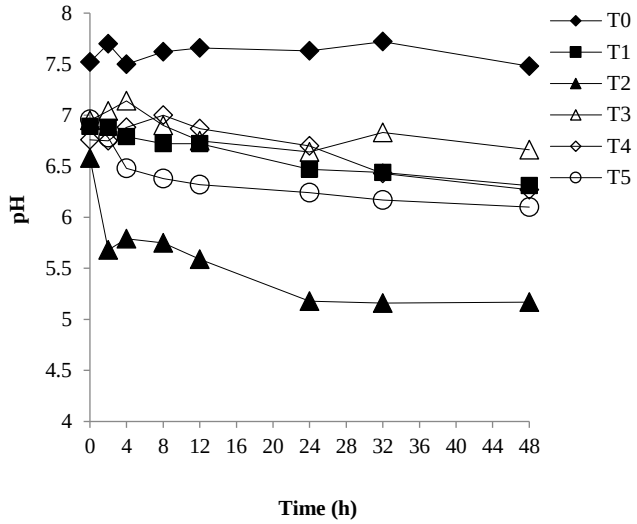

Figure 1. The pH of various substrates tested during the rumen fermentation

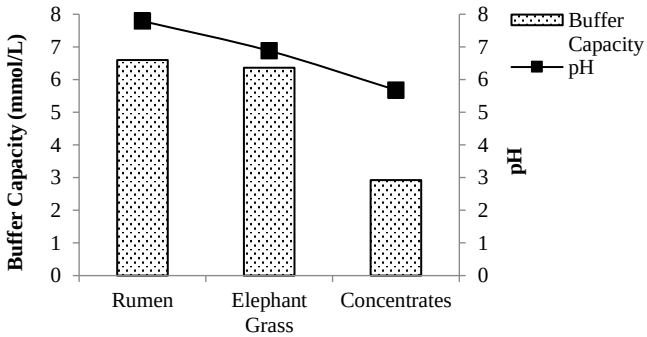

Figure 2. Relationship between buffer capacity and pH drop (after 2 h incubation period)

Results of the study showed that the rumen culture fermenting solely concentrates generated much more VFA compared to that of the tested substrates (Figure 3). The study also revealed that the more proportion of concentrates added to the rumen culture the more VFA were accumulated. In this study, the proportion of concentrates introduced was increased from 5% (T3) to 20% (T5). This occurs since concentrates are highly biodegradable substrates containing mainly carbohydrates that were easily degraded during the fermentation [6]. Hence, supplementing a significant amount of concentrates to the rumen culture would most likely generate organic acid build-up including VFA accumulated in the culture [33, 34]. This finding also indicated that using concentrates as the main feed for ruminants would be prone to

the ruminants running into acidosis or digestive disorder. To alleviate VFA accumulation in the rumen culture, the feed containing concentrates should be mixed with fibers such as various types of grasses and/or lignocellulosic biomass.

Results revealed that the rumen fermenting merely grass generated lower VFA (1 g/L of VFA) than that of other substrates used. Besides, rumen fermenting merely concentrates (T2) produced around 4 g/L of VFA while the rumen fermenting other substrates containing concentrates and grass (T3 - T5) generated slightly low VFA (1.5-2.2 g/L). Statistical analysis carried out using ANOVA test with 5% level of significance revealed that there is statistically significant between the proportion of concentrates added and VFA accumulation in the rumen culture fermenting forage grass (p value=$7.2x10^{-3}$; F_{test}=12.8; F_{crit}=5.31; df=1). This indicated that there is a close relationship between supplementation of concentrates and VFA build- in which an increase amount of concentrates would potentially increase organic acids in the rumen that may lead to acidosis. Besides, the supplementation of forage grassas a fiber substrate to the rumen culture fermenting concentrates would lower the VFA accumulation. This is because by adding grass to the rumen culture fermenting concentrate may enhance buffer capacity of the culture, prevent a drop of pH, and thereby could minimize the risk of rumen acidosis [35, 36].

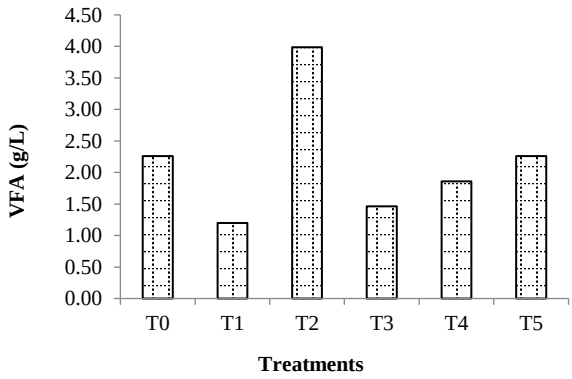

Figure 3. VFA formation after rumen fermentation

Results revealed that the rumen culture fermenting solely grass (T1) as substrate generated low acidity (0.2%) while the culture fermenting only concentrates (T2) produced high titratable acidity (0.4%). As shown in Figure 4, only within 2 hours of incubation an accumulation of acid occurred in the rumen culture fermenting concentrates (T2). An accumulation of acids were restricted when the proportion of concentrates and/or easily degradable feeds were reduced [37, 38]. In this study, the proportion of grass was varied from 100% to 80% containing concentrates from 5 to 20%.

Results of the current study showed that low proportion of concentrates supplemented to the rumen culture fermenting grass did not generate accumulation of titratable acidity. Study mentioned that highly fermentable diets, such as concentrates may need the addition of sufficient amount of fiber to lower the risk of acid accumulation leading to acidosis [39]. Besides, to prevent acid accumulation in the rumen culture supplementing 5-10% of concentrates might still be feasible to maintain the energy intake of ruminants by using concentrates as additional feeds. This is because within 24 hours of incubation an accumulation of acids did not occur in the culture of T2 and T3 (5-10% of concentrates) in which their titratable acidity ranged from 0.2 to 0.3%. On the other hand, 20% of concentrates supplemented to the rumen culture fermenting forage grass (T5) generated acids accumulated in the culture. Within 24 hours of incubation its titratable acidity was about 0.5%. In this trial, the culture began to experienced acids build-up in which its pH also dropped from 7.0 to 6.2 suggesting that the rumen culture had a sub-acute ruminal acidosis.

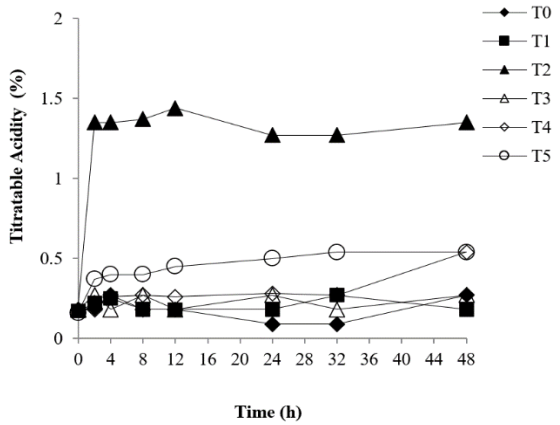

Figure 4. Titratable acidity of various substrates during the rumen fermentation

Ruminal ammonia nitrogen (NH_3-N) is a significant nutrient, which could support efficient rumen fermentation. The current study assessed the concentration of ammonia nitrogen before and after rumen fermentation of various substrates tested (Figure 5). Results of the current study revealed that the concentration of ammonia nitrogen of all substrates tested had increased significantly from 7.5 to 15.2 mg/L after 48 h of incubation. This suggested that fermentation would enhance ruminal NH_3-N that would be utilized as the source of nutrients for rumen microflora. Study mentioned that at higher ruminal NH_3-N level may induce the growth of various populations of microbes in the rumen, such as protozoa, fungi and bacteria [40]. The study also revealed that high level of ammonia nitrogen in rumen may enhance digestibility, rumen ecology and intake of feeds especially fiber materials, such as grass and straw [40].

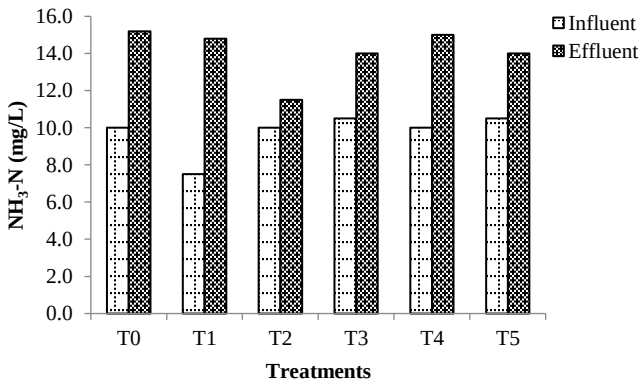

Figure 5. Ammonia profile of various substrates after rumen fermentation

The current study revealed that fermentation process in the rumen culture had been able to reduce solid content of the substrates (Figure 6). This is because during the fermentation solid content of the substrates was degraded by rumen

microbes and converted into metabolites [41]. Biodegradation efficiency or the percentage of solid conversion might highly depend on the types and/or composition of the substrates fermented [6, 42]. Typically high degradable substrates would have higher degradation rate than that of the low degradable substrates [41, 43]. In this study the grass (T1) had slightly low biodegradation efficiency (30%) than that of the concentrate (T2), which was around 38%. The low biodegradation efficiency indicated that the feed supplemented was not completely digested in the rumen system. This implied that lack of energy received by ruminants once feeding merely grass, and thereby could lower their productivity. Besides, feeding solely concentrate also generated low degradation efficiency since this substrate cannot be fully degraded since during the fermentation organic acid build-up may lead a drop of pH that could restrict the digestion process. Both grass and concentrates fermented in the rumen culture still reached low biodegradation efficiency in which both were still below 50% degradation (Table 2).

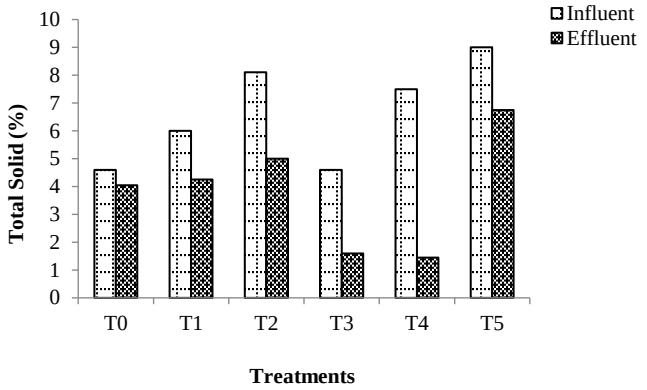

Figure 6. Solid concentration of various substrates before and after rumen fermentation

Table 2. Biodegradation efficiency

Treatments	Biodegradation Efficiency (%)
T0	11.96
T1	30.00
T2	38.30
T3	65.22
T4	80.70
T5	25.00

Combining both substrates (concentrate or grass) would be potential to generate high biodegradation efficiency than fermenting solely a single substrate [44]. This is because using the grass only as the substrate may slowly the fermentation and conversion process due to low degradability. On the other hand, using solely concentrate may potentially generate rumen acidosis since it was easily degraded and converted to organic acids leading to a drop of pH culture [44-46]. As presented in Table 2, the current study showed that 5 to 10 percent of concentrates (T3, T4) supplemented to the rumen culture fermenting grass would effectively enhance the conversion rate and biodegradation efficiency (65-80%). The applied treatment or composition (5-10% of concentrates) was quite feasible to enhance energy intake for ruminants since the composition did not generate acidosis and/or acid accumulation. Further, the pH of those treatments was somewhat stable in the range of 6.8 and 7.1 (Figure 1) suggesting that the rumen did not experience acidosis.

4. CONCLUSIONS

The results of the current study revealed that the addition of a lot of concentrates to the rumen culture may reduce pH level of the culture due acid accumulation. The acid condition in the culture may induce to rumen acidosis. On the other hand, fermentation of forage grass using elephant grass in the rumen culture did not cause rumen acidosis due to organic acid build-up. The study aims to evaluate the various proportion of concentrates (5-20%) supplemented in the rumen fermenting forage, and results of the study showed that supplementation of 10% of concentrates could increase biodegradation efficiency at about 80%. Also, this proportion could prevent a drop pH in the culture, restrict the acid accumulation and prevent rumen acidosis. The result of the current study would be significant for feeding management of cattle, and would be more applicable when the best treatment specified are tested in in-vivo study.

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REFERENCES

[1] Castillo-González, A.R., Burrola-Barraza, M.E., Domínguez-Viveros, J., Chávez-Martínez, A. (2014). Rumen microorganisms and fermentation. Archivos de Medicina Veterinaria, 46(3): 349-361.

[2] Lee, M., Jeong, S., Seo, J., Seo, S. (2019). Changes in the ruminal fermentation and bacterial community structure by a sudden change to a high-concentrate diet in Korean domestic ruminants. Asian-Australasian Journal of Animal Sciences, 32(1): 92-102. https://doi.org/10.5713/ajas.18.0262

[3] Webb, E.C., Erasmus, L.J. (2013). The effect of production system and management practices on the quality of meat products from ruminant livestock. South African Journal of Animal Science, 43(3): 413-423. https://doi.org/10.4314/sajas.v43i3.12

[4] Selemani, I.S., Eik, L.O. (2016). The effects of concentrate supplementation on growth performance and behavioral activities of cattle grazed on natural pasture. Tropical Animal Health and Production, 48: 229-232. https://doi.org/10.1007/s11250-015-0935-z

[5] Wen, K., Zhao, M.M., Liu, L., Khogali, M.K., Geng, T.Y., Wang, H.R., Gong, D.Q. (2021). Thiamine modulates intestinal morphological structure and microbiota under subacute ruminal acidosis induced by a high-concentrate diet in Saanen goats. Animal, 15(10): 100370. https://doi.org/10.1016/j.animal.2021.100370

[6] Darwin, Humaira, T., Muliawati, A. (2022). Identification and characterization of acidosis on in vitro rumen fermentation with feeds based on grass, rice bran, concentrate, and tofu pulp. Journal of Applied Biology and Biotechnology, 10(1): 53-58. https://doi.org/10.7324/JABB.2022.10s108

[7] Elmhadi, M.E., Ali, D.K., Khogali, M.K., Wang, H.

(2022). Subacute ruminal acidosis in dairy herds: Microbiological and nutritional causes, consequences, and prevention strategies. Animal Nutrition, 10: 148–155. https://doi.org/10.1016/j.aninu.2021.12.008

[8] Darwin, Blignaut, D. (2019). Alkaline treatment for preventing acidosis in the rumen culture fermenting carbohydrates: An experimental study in vitro. Journal of Advanced Veterinary and Animal Research, 6(1): 100-107. https://doi.org/10.5455/javar.2019.f319

[9] Kim, S.H., Sung, H.G. (2022). Effects of different fiber substrates on in vitro rumen fermentation characteristics and rumen microbial community in Korean native goats and hanwoo steers. Fermentation, 8(11): 611. https://doi.org/10.3390/fermentation8110611

[10] Govil, K., Yadav, D.S., Patil, A.K., Nayak, S., Baghel, R.P.S., Yadav, P.K., Malapure, C.D., Thakur, D. (2017). Feeding management for early rumen development in calves. Journal of Entomology and Zoology Studies, 5(3): 1132-1139.

[11] Stefańska, B., Nowak, W., Komisarek, J., Taciak, M., Barszcz, M., Skomiał, J. (2017). Prevalence and consequence of subacute ruminal acidosis in Polish dairy herds. Journal of Animal Physiology and Animal Nutrition, 101(4): 694-702. https://doi.org/10.1111/jpn.12592

[12] Taye, D., Etefa, M. (2020). Review on improving nutritive value of forage by applying exogenous enzymes. International Journal of Veterinary Sciences and Animal Husbandry, 5: 72-79.

[13] Geleti, D., Hailemariam, M., Mengistu, A., Tolera, A. (2013). Nutritive value of selected browse and herbaceous forage legumes adapted to medium altitude subhumid areas of western Oromia, Ethiopia. Global Veterinaria, 11(6): 809-816.

[14] Malik, K., Tokkas, J., Anand, R.C., Kumari, N. (2015). Pretreated rice straw as an improved fodder for ruminants-An overview. Journal of Applied and Natural Science, 7(1): 514-520. https://doi.org/10.31018/jans.v7i1.640

[15] Klevenhusen, F., Zebeli, Q. (2021). A review on the potentials of using feeds rich in water-soluble carbohydrates to enhance rumen health and sustainability of dairy cattle production. Journal of the Science of Food and Agriculture, 101(14): 5737-5746. https://doi.org/10.1002/jsfa.11358

[16] Darwin, Charles, W., Cord-Ruwisch, R. (2019). Anaerobic acidification of sugar-containing wastewater for biotechnological production of organic acids and ethanol. Environmental Technology, 40(25): 3276-3286. https://doi.org/10.1080/09593330.2018.1468489

[17] Darwin, Mahyudi, R., Sundari, D., Muliawati, A. (2021). Influence of solid state fermentation on Robusta coffee (Coffea cannephora) beans inoculated with microbial culture of civet fecal suspensions. Research on Crops, 22(4): 968-976. http://dx.doi.org/10.31830/2348-7542.2021.158

[18] Darwin, Muliawati, A., Bulan, R. (2022). Performance of mixed-microbial culture from civet fecal suspensions on physicochemical composition of wet fermented arabica coffee. Current Research in Nutrition and Food Science, 10(3): 918-927.

[19] Urbansky, E.T., Schock, M.R. (2000). Understanding, deriving, and computing buffer capacity. Journal of Chemical Education, 77(12): 1640. https://doi.org/10.1021/ed077p1640

[20] Baethgen, W.E., Alley, M.M. (1989). A manual colorimetric procedure for measuring ammonium nitrogen in soil and plant Kjeldahl digests.Communications in Soil Science and Plant Analysis, 20(9-10): 961-969. https://doi.org/10.1080/00103628909368129

[21] Rice, E.W., Baird, R.B., Eaton, A.D. (2017). Standard methods for the examination of water and wastewater. 23rd edition. American Public Health Association, Washington, D.C., U.S.A.

[22] Gontupil, J., Darwin, M., Liu, Z., Cheng, J.J., Chen, H. (2012). Anaerobic co-digestion of swine manure and corn stover for biogas production. In Annual International Meeting Conference. Dallas, Texas: American Society of Agricultural and Biological Engineers 2012. https://doi.org/10.13031/2013.41776

[23] Tyl, C., Sadler, G.D. (2017). pH and titratable acidity. Food Analysis, 389-406. https:// doi.org/10.1007/978-1-4419-1478-1

[24] Vahl, K., Kahlert, H., von Mühlen, L., Albrecht, A., Meyer, G., Behnert, J. (2013). Determination of the titratable acidity and the pH of wine based on potentiometric flow injection analysis. Talanta, 111: 134-139. https://doi.org/10.1016/j.talanta.2013.02.057

[25] Lützhøft, H.C.H., Boe, K., Fang, C., Angelidaki, I. (2014). Comparison of VFA titration procedures used for monitoring the biogas process. Water Research, 54: 262-272. https://doi.org/10.1016/j.watres.2014.02.001

[26] Zenker, W., Clauss, M., Huber, J., Altenbrunner-Martinek, B., Fidgett, A., Hatt, J.M., Plowman, A. (2009). Rumen pH and hoof health in two groups of captive wild ruminants. Zoo Animal Nutrition, (4): 247-254.

[27] Grünberg, W., Constable, P.D. (2009). Function and dysfunction of the ruminant forestomach. In Current Veterinary Therapy, 12-19.

[28] Abdela, N. (2016). Sub-acute ruminal acidosis (SARA) and its consequence in dairy cattle: A review of past and recent research at global prospective. Achievements in the Life Sciences, 10(2): 187-196. https://doi.org/10.1016/j.als.2016.11.006

[29] Jamarun, N., Pazla, R., ARief, A., Jayanegara, A., Yanti, G. (2020). Chemical composition and rumen fermentation profile of mangrove leaves (Avicennia marina) from West Sumatra, Indonesia. Biodiversitas Journal of Biological Diversity, 21(11): 5230-5236. https://doi.org/10.13057/biodiv/d211126

[30] Li, S., Danscher, A.M., Plaizier, J.C. (2013). Subactue Ruminal Acidosis (SARA) in dairy cattle: new developments in diagnostic aspects and feeding management. Canadian Journal of Animal Science, 94(1): 353-364.

[31] Jaramillo-López, E., Itza-Ortiz, M.F., Peraza-Mercado, G., Carrera-Chávez, J.M. (2017). Ruminal acidosis: strategies for its control. Austral Journal of Veterinary Sciences, 49(3): 139-148. https://doi.org/10.4067/S0719-81322017000300139

[32] Sari, W.N., Fahrimal, Y. (2017). Isolation and identification of a cellulolytic Enterobacter from rumen of Aceh cattle. Veterinary World, 10(12): 1515. https://doi.org/10.14202/vetworld.2017.1515-1520

[33] Zhang, Y., Wang, C., Peng, A., Zhang, H., Wang, H. (2021). Metagenomic insight: dietary thiamine

supplementation promoted the growth of carbohydrate-associated microorganisms and enzymes in the rumen of saanen goats fed high-concentrate diets. Microorganisms, 9(3): 632. https://doi.org/10.3390/microorganisms9030632
[34] Darwin, Cord-Ruwisch, R. (2019). Thermodynamics of anaerobic digestion: Mechanism of suppression on biogas production during acidogenesis. INMATEH-Agricultural Engineering, 57(1): 287-301.
[35] Llonch, L., Castillejos, L., Ferret, A. (2020). Increasing the content of physically effective fiber in high-concentrate diets fed to beef heifers affects intake, sorting behavior, time spent ruminating, and rumen pH. Journal of Animal Science, 98(6): 1-9. https://doi.org/10.1093/jas/skaa192
[36] Humer, E., Petri, R.M., Aschenbach, J.R., Bradford, B.J., Penner, G.B., Tafaj, M., Zebeli, Q. (2018). Invited review: Practical feeding management recommendations to mitigate the risk of subacute ruminal acidosis in dairy cattle. Journal of Dairy Science, 101(2): 872-888. https://doi.org/10.3168/jds.2017-13191
[37] Salfer, I.J., Morelli, M.C., Ying, Y., Allen, M.S., Harvatine, K.J. (2018). The effects of source and concentration of dietary fiber, starch, and fatty acids on the daily patterns of feed intake, rumination, and rumen pH in dairy cows. Journal of Dairy Science, 101(12): 10911-10921. https://doi.org/10.3168/jds.2018-15071
[38] Darwin, Pratama A., Mardhotillah. (2021). Organic Waste conversion via continuous anaerobic co-digestion of oil Palm empty fruit bunches and cow manure: Evaluation of feeding regime on methane Production. Acta Technologica Agriculturae, 24(1): 8-13. https://doi.org/10.2478/ata-2021-0002
[39] Zebeli, Q., Aschenbach, J.R., Tafaj, M., Boguhn, J., Ametaj, B.N., Drochner, W. (2012). Invited review: Role of physically effective fiber and estimation of dietary fiber adequacy in high-producing dairy cattle. Journal of Dairy Science, 95(3): 1041-1056. https://doi.org/10.3168/jds.2011-4421
[40] Wanapat, M., Pimpa, O. (1999). Effect of ruminal NH_3-N levels on ruminal fermentation, purine derivatives, digestibility and rice straw intake in swamp buffaloes. Asian-Australasian Journal of Animal Sciences, 12(6): 904-907. https://doi.org/10.5713/ajas.1999.904
[41] Darwin, Barnes, A., Cord-Ruwisch, R. (2018). In vitro rumen fermentation of soluble and non-soluble polymeric carbohydrates in relation to ruminal acidosis. Annals of Microbiology, 68(1): 1-8. https://doi.org/10.1007/s13213-017-1307-x
[42] Xie, C., Yan, L., Gong, W., Zhu, Z., Tan, S., Chen, D., Peng, Y. (2016). Effects of different substrates on lignocellulosic enzyme expression, enzyme activity, substrate utilization and biological efficiency of Pleurotus eryngii. Cellular Physiology and Biochemistry, 39(4): 1479-1494. https://doi.org/10.1159/000447851
[43] Darwin, Purwanto, S, Rinaldi, R. (2019). Removal of organic pollutants from Tofu-processing wastewater through anaerobic treatment process with short hydraulic retention time. Environmental Research, Engineering and Management, 75(1): 34-42. https://doi.org/10.5755/j01.erem.75.1.21532
[44] Kröger, I., Humer, E., Neubauer, V., Reisinger, N., Zebeli, Q. (2019). Feeding diets moderate in physically effective fibre alters eating and feed sorting patterns without improving ruminal pH, but impaired liver health in dairy cows. Animals, 9(4): 128. https://doi.org/10.3390/ani9040128
[45] Darwin, Ilham M, Fazil, A. (2018). Performance and kinetic study of the anaerobic co-digestion of cocoa husk and digested cow manure with high organic loading rate. INMATEH-Agricultural Engineering, 55(2): 131-140.
[46] Banakar, P.S., Shashank, C.G., Lakhani, N. (2018). Physically effective fibre in ruminant nutrition: A review. Journal of Pharmacognosy and Phytochemistry, 7(4): 303-308.