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THE EFFECT OF NITROGEN AND SULFUR IN MAKING ONCOM OF CASSAVA PULP BY *Neurospora sitophila* AND ITS IMPACT ON *IN VITRO* DIGESTIBILITY AND FERMENTABILITY IN SHEEP DIET

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ABSTRACT: This study focused on the modification of oncom making based on cassava (*Manihot esculenta*) pulp to improve the nutritional profile through fermentation by *Neurospora sitophila* and enrichment with a mixture of urea (0, 2.5, 5, 7.5, and 10% of dry matter) and a nitrogen:sulfur ratio of 15:1 (oncom cassava pulp= OCP). Hence an investigation was carried out on the *in vitro* digestibility and fermentability of OCP when included in sheep diets. *In vitro* evaluation (diet oncom cassava pulp; DOCP) was carried out on diets consisted of a mixture of 50% grass and 50% concentrate containing 2.5%OCP (which selected based on the highest analysed crude protein and crude fiber levels when compared to the nutrient contents of other enriched OCP levels, $P < 0.001$) with compositions of 0, 10, 20, and 30% of DOCP. Results showed that the higher use of urea and nitrogen:sulfur ratio caused a decrease in crude fiber and gross energy and an increase in nitrogen-free extract (NFE) levels ($P < 0.001$). Using 2.5%OCP in the diet resulted in significantly different N-ammonia (N-NH_3), volatile fatty acid (VFA), *in vitro* dry matter digestibility (IVDMD), and *in vitro* organic matter digestibility (IVOMD) ($P < 0.05$), except for total gas and ruminal pH values. The highest IVDMD and IVOMD were obtained in 30% DOCP with a close N-NH_3 and VFA and within the normal range. In summary, CP fermentation by *Neurospora sitophila* which is enriched with a mixture of 2.5% urea and nitrogen:sulfur at a 15:1 ratio can be used in sheep diet up to 30%.

Keywords: Chemical additives, Feed, Fermentation, *Neurospora sitophila,* Rumen.

INTRODUCTION

Oncom is a traditional peanut meal-based ferment that has long been produced by the Indonesian people, especially in the West Java region (Kenyamu et al., 2014). Through the fermentation process, oncom acquires a distinctive aroma and taste, because the chemical structure of complex ingredients breaks down into compounds that are simpler and easier to digest (Firoh et al., 2024). Two types of oncom are known to Indonesian society, namely red oncom and black oncom (Mulyani and Wisma, 2016). Red oncom is fermented by *Neurospora sitophila*, a microscopic filamentous fungus of the *Ascomycota* division, which spreads rapidly in the environment through vegetative spores of orange colour formed on the lateral branches of conidiogenous hyphae (Firoh et al., 2024). Meanwhile, black oncom is made through fermentation by the mold *Rhizophus oligosporus,* with the resulting spores being black (Mulyani and Wisma, 2016). The impact of fermentation on feed processing, apart from improving the quality of oncom products as well as the development of mold, is expected to also have a positive impact on ruminants if they consume it. This is because mold can act as a probiotic (Yu et al., 2020), where when consumed early in the rumen, the mold is still alive and will consume oxygen, thereby making the rumen environment more anaerobic. This condition will enable rumen bacteria to grow better because they live in an aerobic atmosphere.

Cassava pulp (CP; *Manihot esculenta*) is a by-product of the tapioca flour manufacturing industry which comes from cassava (Norrapokea et al., 2022). However, tapioca waste, like agro-industrial waste in general, has a limiting factor in its use, namely its low protein content. This weakness can be overcome by the fermentation process. Fermentation processing is not only useful for increasing the fermentability and digestibility of feed in digestive tract, but it can also increase nutritional value and improve palatability (Roger et al., 2015). Mold as a fermentation agent requires additional supplements in the form of nitrogen and sulfur sources for growth to work optimally during fermentation (Kampen, 2014). Nitrogen and sulfur are macronutrients essential for mold growth (Perner et al., 2011). Beyond this, the addition is expected to enrich the quality of the substrate.

Previous research on the use of *Neurospora sitophila* supplemented with urea and minerals has not been widely reported. Research on *Neurospora sitophila* has been carried out regarding the activity of cellulase, protease, amylase, gluco amylase, and phytase (Li et al., 2013; Liu et al., 2016; Syed et al., 2016; Kanti and Sudiana, 2016; Kanti, 2017). In

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another study, Neurospora *sitophila* was used to ferment tapioca for laying hen feed (Nuraini et al., 2015) and banana stems as a solid substrate for commercial cellulase production (Asad et al., 2006).

The benefit of this research is as a technology to improve the quality of CP as ruminant feed. This technology can increase the nutrient content of CP as a feed ingredient for ruminants and can replace other feed ingredients, which compete for use with other types of livestock (poultry).

MATERIALS AND METHODS

Making oncom from cassava pulp

Oncom is a typical Javanese fermented food product that uses peanut cake or tofu dregs as a substrate which is inoculated with red oncom mold spores (*Neurospora sitophila)* (Kenyamu et al., 2014). The research was carried out at the home industry oncom in Pasireungit Village, Legok District, Sumedang Regency, and at the Ruminant Animal Nutrition and Feed Chemistry Laboratory, Faculty of Animal Husbandry, Universitas Padjadjaran. Cassava pulp (CP) was obtained from a tapioca flour factory in Sumedang Regency, Indonesia. Urea was used as a nitrogen source while it contains is 46%. The source of sulfur was derived from the compound $MgSO_4.7H_2O$ purchased from a chemical shop in Bandung City. This compound is easily soluble in water (Ramalingom et al., 2001). At the same time, the *Neurospora sitophila* culture was obtained from a producer in Sumedang Regenecy who usually supplies the mold inoculum needs.

Urea was weighed at 0%OCP, 2.5%OCP, 5%OCP 7.5%OCP, and 10%OCP of dry matter (DM) of the cassava pulp. $MgSO_4.7H_2O$ was weighed based on a ratio of nitrogen (urea) and sulfur at a ratio of 15:1 and dissolved with water to 200 mL. This solution was then placed in a plastic bottle for 1 treatment requirement. The cassava pulp was steamed until hot (15-20 minutes), and then mixed with a solution of urea and $MgSO_4.7H_2O$ until homogenous, according to the treatment. Then it was molded as a cube with dimensions of 11×12×2 cm^3 and weighed. It is stored on the fermentation rack. After it cooled, the *Neurospora sitophila* culture was added to the substrate surface and left for 3 days.

After 3 days, the cube molds were dried in the sun and continued with oven drying at 60°C. The nutrient content and gross energy were analyzed using the proximate analysis method (AOAC, 2010) and a bomb calorimeter. The best results at this stage are followed by *in vitro* tests. One aspect that needed to be considered in this research was the influence of the fermentation room, which was very dominant. The fermentation was successful because it was carried out in a place where OCP production fermentation is usually carried out (home industry). If conducted elsewhere, different results might be obtained. This is because the room is saturated with *Neurospora sitophila.*

In Vitro batch culture preparation

The experimental diets were then measured for fermentability and digestibility using *in vitro* techniques as described by Gosselink et al. (2004) and Despal et al. (2023). The diet consisted of a mixture of 50% grass and 50% concentrate containing 2.5%OCP with a composition of 0, 10, 20, and 30%DOCP. Based on Table 1, the 2.5% treatment produced significantly ($P < 0.001$) the highest crude protein and is most efficient in the use of urea levels and nitrogen:sulfur ratio. Concentrates were made from other feed ingredients which were arranged in such a way so that they were isonitrogenous and isocaloric. The complete feed samples were ground through a sieve with a size of 1 mm. The media solution was used as a medium for microbial development in carrying out fermentation activities and digesting feed. The media solution aimed to imitate the rumen fluid of ruminant livestock following the original conditions. The medium consisted of 3 local sheep rumen fluid and buffer solution or artificial saliva in a ratio of 4:1 mL and was maintained so that the pH value was around 6.5-7.0 (Hernaman et al., 2022). The media solution was mixed with the experimental feed sample (0.5 g) in a fermenter tube. During the filling process, CO_2 gas was supplied to maintain an anaerobic atmosphere. Previously, the fermenter tube had been placed in a water bath at a temperature of 39-40°C. Then part of it was incubated for 3 hours to take samples and measure the concentration of volatile fatty acids (VFA) using the Markam steam distillation method (AOAC, 2010) and $N\text{-}NH_3$ using the microdiffusion technique of Conway (Gosselink et al., 2004) and pH value analysis. Some samples were left for 2 × 48 hours to measure dry matter digestibility (DMD) and organic matter digestibility (OMD) as described by Despal et al. (2023). It was crucial to ensure that the rumen fluid used was teeming with live microbes and free from feed particles from the livestock before slaughter to obtain accurate results.

In vitro rumen fermentation and digestibility

The top of the Conway unit was smeared with Vaseline. A 1 mL of boric acid with the indicator was placed in the middle position of the Conway, 1 mL of rumen fluid or supernatant was fitted in the right position of the cup, and 1 mL of saturated Na_2CO_3 was fitted in the left position of the Conway. It was ensured that the components did not mix before the Conway unit was sealed. The Conway unit was then closed tightly so that no air could enter. The unit was gently moved in a figure-eight shape so that the rumen fluid and saturated Na_2CO_3 were mixed evenly and slowly, and it was then left for 24 hours at room temperature. After 24 hours, the mixture was titrated with 0.01 N H_2SO_4 until the color changed from blue to pink. The calculation formula for $N\text{-}NH_3$ concentration is as follows (Gosselink et al., 2004):

$N\text{-}NH_3$ = (b x N H_2SO_4 x 1000/L) mM

b = volume of H_2SO_4 used (mL); N= normality of H_2SO_4 solution

The steam distillation apparatus was turned on, and the sample holder was rinsed. Then, 5 mL of the supernatant was drawn up using a micropipette, placed in the sample holder, and 1 mL of 15% H_2SO_4 was added. The distillate was collected in a 250 mL Erlenmeyer flask filled with 5 mL of 0.5 N NaOH until the distillate volume reached 100 mL, followed by adding 2 drops of the phenolphthalein indicator. The distillate was then titrated with 0.5 N HCl until a color change was observed. A blank solution was prepared by titrating 5 ml of 0.5 N NaOH with 0.5 N HCl until a color change occurred. Measurement of total VFA concentration is calculated as follows (Gosselink et al., 2004):

Total VFA = (b-s) × N HCl × (1000/5) mM

b = volume of HCl used (mL); s = volume of sample titrant (mL); N= normality of HCl solution

The *in vitro* digestibility test consisted of two stages. The first stage simulated the fermentative digestion process in the rumen. At the end of the first stage, the tube lid was opened, proceeding to the second stage. In the second stage, after 48 hours of fermentation, microbial fermentation was halted. A 0.25 mL drop of $HgCl_2$ was introduced into the fermenter tube to stop microbial activity. Subsequently, 2 mL of 4 N HCl and 0.06 g pepsin were added to each tube, and then it was placed back in the incubator at 39°C for 48 hours without a rubber lid. The digested material was filtered using a filter, transferred to a crucible glass, and then placed in an oven at 105°C for 24 hours to determine the dry matter (DM) residues. After the second stage, the tube contents were filtered with sintered glass, and the residue was further processed to determine digestibility variables. The second stage replicated the enzymatic hydrolysis procedure digestion in the post-rumen.

Gas production was measured using a gas measuring cylinder which captured the gas produced (Blümmel et al., 1997). Observations of gas production occurred at intervals of 2, 4, 8, 12, 24, 48, and 72 hours of fermentation. Rumen fluid pH was measured using a pH meter, and the reading was recorded as the pH value.

Data analysis

A completely randomized design was employed for the research. The data collected were analyzed using the Duncan test using the SPSS IBM 21 data processing application.

RESULTS AND DISCUSSION

Nutrient of oncom from cassava pulp

After 3 days of fermentation process carried out by *Neurospora sitophila* on piles enriched with urea and sulfur, data on nutrient and energy content were produced which are presented in Table 1. Table 1 illustrates that fermentation by *Neurospora sitophila* and enrichment with a mixture of urea (0, 2.5, 5, 7.5, and 10% of dry matter) and nitrogen:sulfur at a ratio of 15:1 resulted in significant changes ($P < 0.001$) in the content of crude protein, crude fiber, NFE, and gross energy. In contrast, the crude fat and ash content did not show a significant difference.

Crude protein increased whereas crude fiber and gross energy decreased after CP was fermented by *Neurospora sitophila* enriched with urea and sulfur. NFE also experienced a decrease but then increased again in line with the decreasing amount of crude protein and crude fiber. The highest crude protein and gross energy were obtained in the 2.5%OCP treatment, which is fermentation enriched with 2.5% urea and sulfur at a nitrogen and sulfur ratio of 15:1.

Table 1 - Nutrient and energy content of OCP*

Parameter	0%OCP	2.5%OCP	5%OCP	7.5%OCP	10%OCP	SEM	*P* value
Ash (%)	1.63	1.39	1.52	1.28	1.28	0.822	0.631
Crude protein (%)	2.91^{c}	7.65^{a}	7.00^{b}	6.70^{b}	6.49^{b}	0.349	0.001
Crude fat (%)	1.51	1.91	2.02	2.17	2.12	0.438	0.137
Crude fiber (%)	14.90^{a}	14.42^{a}	13.84^{ab}	12.61^{b}	9.77^{c}	0.090	0.001
Nitrogen-free extract (NFE) (%)	79.05^{ab}	74.62^{d}	75.62^{cd}	77.25^{bc}	80.35^{a}	0.505	0.001
Gross energy (kkal/kg)	3266.6^{a}	3162.2^{b}	3012.2^{c}	3014.2^{c}	3009.6^{c}	22.65	0.001

Values are expressed as mean (n=5), different letters within rows represented significant differences (p <0.05). *) Fermentation of cassava pulp (CP) by *Neurospora sitophila* enriched with urea (0, 2.5, 5, 7.5, 10%) and a 15:1 ratio of nitrogen:sulfur.

The increase in crude protein in the treatment with the addition of urea and sulfur occurs because these components are utilized to form protein in cell development and the growth of *Neurospora sitophila*. Microbes in the fermentation process can produce enzymes that degrade complex compounds into simpler ones and synthesize protein, which is a

protein enrichment process (Cruz-Casas et al., 2021). Based on this, it can be inferred that some non-protein nitrogen (NPN) compounds in the form of urea undergo conversion into pure protein compounds in the form of microbial protein (Norrapokea et al., 2022). The function of sulfur minerals in the physiology of microbial cells includes being a part of the protein amino acids cysteine and methionine and being part of several enzymes (CoA, Co-enzyme A carboxylase; Kabil et al., 2014), which are essential amino acids, while the enzymes formed play a major role in metabolic processes in cells. However, the treatment using more than 2.5% urea, produces lower crude protein; this is believed to occur because the fermentation process, being aerobically conducted, allows the dissolved urea to evaporate into ammonia (NH_3), characterized by the distinctive smell of ammonia, making urea less effective if used in amounts greater than 2.5%.

Crude fat does not change significantly. This is because crude fat is not an organic material needed by *Neurospora sitophila* in the fermentation process, and the enzymes commonly produced by *Neurospora sitophila* are those that digest fiber, sugar and protein. The absolute amount of this compound remains unchanged, only because other nutrient components decrease, it appears as if there is an increase. Meanwhile, the decrease in crude fiber, NFE, and gross energy content indicates that the application of urea and sulfur has provided nutrients for the growth of *Neurospora sitophila*, ensuring that fermentation runs well as the mold requires energy obtained from crude fiber and NFE. The ash component was not significantly different, because ash is an inorganic material with a small amount and is not an organic material component that undergoes breakdown during the fermentation process, therefore, its amount remains relatively constant.

The decrease in crude fiber content alongside the addition of urea and sulfur suggests that *Neurospora sitophila* utilizes crude fiber predominantly as an energy source. Research conducted by Qingxin et al. (2014) which analyzed enzymes produced by *Neurospora sitophila*, found that the enzymes peptidase (protease), endoglucanase, exoglucanase, β-glucosidase, and cellobiose dehydrogenase play roles in the breakdown of cellulose and hemicellulose. Fermentation using *Neurospora sp.* which has cellulolytic properties can break down cellulose bonds, causing the crude fiber content in the substrate to decrease. Fermentation using *Neurospora sp*. also leads to the degradation of cellulose, hemicellulose, and polymers into simpler sugars or their derivatives, increasing the nutrient content of the substrate material (Znameroski et al., 2012). Endo-β-1,4-glucanase cuts chain bonds in cellulose to produce shorter cellulose molecules. Exo-1,4-glucanase cuts the end of the cellulose chain to produce a cellobiose molecule, while β-glucosidase further breaks down the cellobiose molecule into two glucose molecules (Romero et al., 1999).

The NFE value decreased then increased gradually in the next treatment. This condition does not indicate an increase in NFE value. The NFE is also fermented as an energy source, as evidenced by the decreasing gross energy in treatment along with increasing NFE value. The fermentation process not only utilized crude fiber as an energy source but also other organic components, including NFE (Hernaman et al., 2017). The NFE value is obtained from calculation and depends on other nutrient components. So, changes in NFE values are caused by changes in the values of other nutrient components.

In vitro fermentability and digestibility of diet containing OCP

The results at stage 1 showed that 2.5% OCP treatment produced the highest protein, so it was used in diets for *in vitro* testing with the composition of diets and nutrient content presented in Table 2. Table 2 shows that the use of 2.5% OCP can replace the role of corn and rice bran, the higher the use of 2.5% OCP, the lower the utilization of these two conventional feed ingredients.

Table 2 - Composition of experimental diets (DOCP)*

Ingredients	0%DOCP	10%DOCP	20%DOCP	30%DOCP
Napier Grass cv Taiwan (%)	50.00	50.00	50.00	50.00
Oncom of Cassava Pulp (2.5% OCP) (%)	0.00	10.00	20.00	30.00
Corn flour (%)	38.49	27.64	16.79	5.92
Soybean meal (%)	0.50	0.50	0.50	0.50
Pollard (%)	2.26	5.69	9.13	12.58
Rice bran (%)	7.76	5.17	2.58	0.00
Mineral mix (%)	1.00	1.00	1.00	1.00
Nutrient				
Dry matter (%)	77.24	77.16	77.08	77.00
Ash (%)	17.29	17.21	17.14	17.06
Crude protein/CPr (%)	12.00	12.00	12.00	12.00
Crude fat/CFt (%)	4.62	4.21	3.79	3.39
Crude fiber/CFr (%)	14.44	15.58	16.71	17.01
NFE (%)	51.65	51	50.36	50.54
TDN (%)	63.50	63.50	63.50	63.50

TDN was calculated using Sutardi equation as described in Hernaman et al. (2022): (70.6+0.259%CPr+1.01%CFt-0.76%CFr+ 0.0991% NFE).
*) The diet consisted of a mixture of 50% grass and 50% concentrate containing 2.5%OCP with a composition of 0% in DOCP, 10% in DOCP, 20% in DOCP, and 30% in DOCP

Table 3 - *In vitro* fermentability and digestibility of experimental diets (DOCP)*

Parameters	0%DOCP	10%DOCP	20%DOCP	30%DOCP	SEM	*P*-value
N-ammonia (N-NH_3) (mM)	4.16^{b}	4.31^{ab}	5.11^{a}	3.69^{b}	0.17	0.015
Volatile fatty Acid (VFA) (mM)	148.57^{ab}	159.63^{a}	137.28^{b}	135.85^{b}	3.46	0.036
Total gas (mM)	132.06	126.74	136.08	128.30	3.17	0.760
Ruminal pH	6.92	6.96	6.92	6.89	0.02	0.716
In vitro dry matter digestibility (IVDMD) (%)	66.4^{b}	57.29^{c}	67.13^{b}	69.49^{a}	1.08	0.0001
In vitro organic matter digestibility (IVOMD) (%)	73.62^{b}	65.08^{c}	75.36^{ab}	76.83^{a}	1.11	0.0001

Values are expressed as mean (n=5), different letters within rows represent significant differences ($P < 0.05$). *) The diet consisted of a mixture of 50% grass and 50% concentrate containing 2.5%OCP with a composition of 0% in DOCP, 10% in DOCP, 20% in DOCP, and 30% in DOCP.

Consequently, this suggests that 2.5%OCP could potentially substitute for corn and rice bran, which are commonly utilized in the poultry industry (Rohaeni et al., 2021). Subsequently, the experimental diet was evaluated *in vitro*, and the results are depicted in Table 3. In Table 3, the N-NH_3 content in the 20%DOCP treatment was significantly higher ($P<0.05$) compared to the 30%DOCP and 10%DOCP treatments yet shared the same value as the 10%DOCP treatment. Meanwhile, the highest VFA levels were found in the 10%DOCP treatment, which had a similar average value as the 0%DOCP treatment and greater than the 20% and 30% DOCP treatments, whereas between these treatments there was no significant difference. The *in vitro* dry matter digestibility (IVDMD) and organic matter digestibility (IVOMD) in the 30%DOCP treatment was superior ($P < 0.05$) compared to other treatments. Furthermore, pH and total gas measurements remained consistent across all treatments.

Overall, the N-NH_3 and VFA concentrations are 3.69-5.11 mM and 135.85-159.63 (Table 3). The N-NH_3 is generally lower than 5 mM that the minimum required for the microbial protein synthesis in the rumen (Dewhurst and Newbold 2022). Meanwhile, the VFA concentrations is still within the normal range for rumen fluid, as established by McDonald et al. (2002), at 70 -150 mM. The percentages for IVDMD and IVOMD were relatively high, ranging between 57.29-69.49% and 65.08-76.83%, respectively. These figures align closely with the findings of Tresia et al. (2024) which utilized rations composed of local feed ingredients, reporting values of 51.18-56.38% and 49.12-54.45%, respectively. The pH levels for all treatments spanned from 6.89 to 6.96 (Table 3), remaining within the physiological rumen range of 5.5-7.5 (Franzolin et al., 2010). The McDougall's artificial saliva, added as a buffer, effectively maintained pH levels within normal limits, even though significant variations were noted in fermentation products such as N-NH_3 and VFA ($P < 0.05$).

Total gas production, a by-product of the fermentation process in the rumen, remained consistent across all treatments, signifying that 2.5% OCP inclusion in the diet did not negatively impact the rumen microbial activity. Total gas production indicates a feed fermentation process by microbes in the rumen (Suassuna et al., 2022). The stable volume of total gas indicates effective feed fermentation by rumen microbes and suggests a uniform degree of feed degradation and organic material digestion.

These analytical results demonstrate that incorporating up to 30% of 2.5%OCP maintains normal fermentability and optimizes digestibility. Thus, 2.5%OCP proves to be a viable ingredient in rations, capable of substituting for other feed components. It is evidenced in Table 2 that 2.5%OCP can replace significant amounts of corn and rice bran, with increasing levels of 2.5%OCP, there is a corresponding decrease in the use of corn and rice bran. This is noteworthy because corn and rice bran are predominantly allocated to poultry feed formulations, with corn constituting more than 60% (Islam et al., 2015) and rice bran accounting for 20-30% (Rohaeni et al., 2021).

Fermented feed, having undergone a process of chemical structural transformation through the action of microbial enzymes, offers multiple benefits. Notably, it enhances the fermentability and digestibility of feed in the rumen, improves nutritional value, and increases palatability (Roger et al., 2015).

CONCLUSION AND RECOMMENDATION

Production of oncom resulting from cassava pulp (CP) fermentation by *Neurospora sitophila* enriched with 2.5% urea and sulfur at a nitrogen-to-sulfur ratio of 15:1 has been shown to produce optimal changes in nutrient composition and gross energy values. When included in sheep diets at a ratio of up to 30%, it demonstrates significant potential for nutritional enhancement. It highlights the significance of microbial fermentation, facilitated by *Neurospora sitophila*, in enhancing the nutritional profile of plant-based substrates. Such advancements not only propose an avenue for improving the efficiency of animal nutrition but also open up possibilities for addressing feed scarcity and promoting environmental sustainability. The implication of this research extends to the concept of circular economy in agriculture, where waste products are upcycled into valuable feed, contributing to waste reduction and resource optimization.

DECLARATIONS

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Data availability
The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Authors' contribution
I.Hernaman is a leader in research who, conducts research, interprets data, and writes manuscripts. T.Dhalika, U.H.Tanuwiria, R.Hidayat are the co-authors who assisted in conducts research, interpreting data, and writing manuscripts. U.Rosani, A.Budiman, and M.Rifqi are the co-authors who assisted in preparing research, interpreting data, and writing manuscripts. B.K.Mutaqin and M.R.Nugraha are the co-authors who assisted in conducting research and collecting data. Budi Ayuningsih is the co-author who assisted in conducting research and financial administration.

Ethical approval
This research does not use live animals (sheep) and the rumen fluid used comes from slaughterhouses that meet animal welfare standards of the Indonesian National Standards for Halal Slaughter of Ruminant Animals No 99003:2018

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Competing interests
The authors declare that there are no competing interests.

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