Effect of synchronizing the rate degradation of protein and organic matter of feed base on corn waste on fermentation characteristic and synthesis protein microbial

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Abstract. Synchronization of feed sources of energy and protein can produce a positive influence on microbial protein synthesis, especially in ruminants that are fed low quality feed such as corn waste. Corn waste, including corn straw and corn peri carp are widely used as beef cattle feed. The objective of this research was to determine the effect of synchronizing the rate degradation of protein and energy of feed base on corn waste on rumen fermentation characteristic and synthesis protein microbial on 24 hours in vitro condition. The three synchronization index levels in corn-based feed waste are 0.4 (low); 0.5 (moderate) and 0.6 (high) were used in this study. Characteristics of rumen fermentation observed were pH, individual volatile fatty acids (acetic acid, propionic acid and butyric acid), NH3, CO2, CH4, degradation of organic matter, microbial protein production, and efficiency of microbial protein synthesis. Increasing the synchronization index level caused a significant increase (P<0.05) of NH3 (53.60 to 59.07 mg/L) and efficiency of microbial protein synthesis (14.83 to 17.87), but high synchronization index decrease (P<0.05) the concentration of acetic acid (22.45 mM to 15.95 mM), estimated CO2 (18.69 to 15.50 mol) and CH4 (11.49 to 7.98) in rumen fluid. This result indicated that the improvement of synchronization index can improve the productivity of livestock through increase efficiency of microbial protein synthesis and it reduce global warming.

1. Introduction
Corn plant is one of the food crops in Indonesia besides rice, soybeans, peanuts, sweet potatoes and cassava. Corn is the second crop with the highest production and planting area after rice. The planted area, production and productivity of maize in Indonesia in 2018 were respectively 5,734,326 Ha, 30,055,623 tons and 52.41 quintals / ha [1]. Corn has long been an important component of animal feed. Corn grain are an important source of energy for poultry. Corn crop waste such as corn straw, corn bran, tumpi, corn peel and corn cobs [2]. The feed material from corn plant waste is used as ruminant animal feed both as a source of fiber and as a source of energy. Maize straw, corn peel and corn cobs can be used as a source of fiber, while corn bran and tumpi can be used as an energy source. Food plant waste has considerable potential as animal feed even though it has limitations in the form of low crude protein content and organic matter and high crude fiber [3]. The problem with using corn straw as a feed source of fiber in a single form is its low palatability so that it can only be consumed at 1.5% of body weight.
in the form of dry matter. This will result in a lower consumption of other nutrients and have a negative impact on growth. The productivity of beef cattle with corn straw feed as a source of fiber can be increased by providing protein source feed ingredients and high organic matter content [3].

The use of corn straw as the sole feed causes low dry matter consumption due to less capability to support the growth of rumen microbes that play in fermentative digestion especially in feedstuffs containing high crude fiber. One of the ways to support the growth of rumen microbes is by supplementation. Tumpi can be used as a supplement on maize straw as forage. Supplementation of fiber source feed aims to provide nutrition for livestock and microorganisms that live in the rumen. Supplementation for rumen microorganisms through synchronizing degradation of protein and energy can have a positive effect on the growth of rumen microorganisms and microbial protein synthesis, especially in livestock with agricultural waste-based feed [4,5]. The synchronization degradation rate of energy and protein has been proposed as a method to increase ruminal MPS, improve efficiency of N usage and animal performance, decrease urinary N excretion [6,7] and fermentative carbon losses in CO₂ and CH₄ [8]. Synchronous supply of energy and protein to the rumen enhanced the efficiency of microbes in capturing N and use of ATP for microbial growth [9], which implied synchronized feeds increased microbial protein production in the rumen and enhanced rumen fermentation efficiency, and thereby improved feed utilization and animal performance [8].

Formulation of feed with synchronization of availability of protein and energy in the rumen was developed by [6]. [6] developed a parameter that called synchronization index. Synchronization index expressed as the ratio between the hourly degradability of nitrogen (g N) with organic matter (kg OM) or carbohydrates in the rumen where the highest value for the synchronization index is 1.0.

This research was a study to evaluate the supplementation on of corn waste in ruminant animals with pay attention to synchronizing supply of protein and energy in the rumen on 24 hours of in vitro condition.

2. Material and methods

This research was conducted in Beef Cattle Research Station, Grati-East Java. The study consisted of two stages, the first stage was the determination of nutrients and the value of feed degradation parameter while the second stage was a research that examined the effect of synchronization index on feed based on corn plant waste and by product to the fermentation characteristics and rumen microbial protein production on 24 hours in vitro condition.

2.1. Determination of nutrients and degradation parameters of feedstuffs

Feedstuffs that used to formulate a rations were maize straw, native grass, tumpi, cassava flour, palm kernel cake, coconut cake and molasses. The feed ingredients have been analyzed to determine the nutrient content including dry matter (DM), crude protein (CP), ash, extract ether (EE), crude fiber (CF) based on [10]. Nitrogen free extract (NFE) was calculated according to [11]. Total Digestible Nutrient (TDN) value was determined according to [12].

Measurement degradation parameters in the rumen has been carried out using the nylon bag technique after two weeks adaptation period in three males of Ongole Crossbred fitted with permanent rumen cannula which is fed in the form of elephant grass and concentrate with forage ratio to concentrate 60:40 that was calculated approximately at 3% DMI of live weight and given twice per day. Measurement of degradation parameters in the rumen for each feed ingredient was repeated 4 nylon bags for each of degradation time in every cattle. As much as 5 mg of feed material sample is put into a nylon bag, then the nylon bag is tied using a nylon rope. Then, all the nylon bags filled with the feed material are tied to a nylon rope with a weight in the form of anti-rust metal and then inserted into the rumen through the canula before feeding in the morning. The nylon bags were taken from the rumen after a time interval incubation 2, 4, 6, 8, 12, 24, and 48 hours for feed concentrates; 2, 4, 6, 12, 24, 48, and 72 hours for forage. The nylon bags were then washed on tap water until the water is clean. Nylon bag containing feed material residue was dried in an oven 60°C. The residue was analysed of dry matter.
(DM), organic matter (OM) and crude protein (CP). To determine the content of water soluble material, bags representing 0 hours degradation also underwent the same washing procedure as the incubated bags. The value of feedstuffs degradation were the percentage of the sample weight that were missing after incubation with the initial sample weight. Further the value of feedstuffs degradation were used for the determination of degradation parameters using the formula developed by [13] using a computer program developed by [14]. The formula developed by [13] is as follows:

\[ P = a + b (1 - e^{-ct}) \]

Description:
- \( P \) = cumulative amount degraded at time t.
- \( a \) = fraction of feed material that rapidly soluble in the rumen (%).
- \( b \) = potentially degradable fraction in the rumen (%).
- \( c \) = rate of degradation (% / h).
- \( t \) = time (hours).

The variables observed in this study were 1) the fraction of protein and organic matters of feed that rapidly soluble in the rumen or \( a \) value, 2) protein and organic matter of feed ingredients that insoluble but potential for degradation or \( b \) value and 3) the rate of degradation of protein and organic matter or \( c \) value.

2.2. Effect of synchronization of degradation of protein and organic matter of feed based on corn plant waste and by product to fermentation characteristics and rumen microbial protein production on 24 hours in vitro condition

The material of this study was three of feed based on corn plant waste and by product using feed ingredients such as corn straw, native grass, tumpi, cassava flour, coconut cake and palm kernel cake with synchronization index low (0.4) as P1, medium (0.5) as P2 and high (0.6) as P3. Determination of the value of synchronization index using the formula developed by [6], as follows:

\[ \text{synchrony index} = 25 \sum_{t=24}^{25} \sqrt{\frac{(25\text{-hourly N/OM})^2}{24}} \]

Description:
- Twenty five (25) is 25 g N-protein/kg OM digested in the rumen, is assuming the optimum ratio for efficiency of the synchronization of microbial protein synthesis in the rumen [15].
- Hourly N/OM is the quantity of nitrogen and organic matter degraded per hour.
- Synchronization with the index value of 1.0 indicates perfect synchronization between the supply of N - protein and energy during the day and the value of < 1.0 indicated unsynchronization.
- Computer programs are modified by [6] was used to calculate the N and OM supply from feed, this program requires input data including:
  a. Content of DM, CP and OM of feedstuffs.
  b. Value of soluble fraction (a) potentially degradable fraction (b) and the rate of degradation (c) of OM and CP of feedstuffs.
  c. The proportion of each constituent in the diet.
  d. DM intake per day, in this study assumed 3% of live weight.
  e. Feeding frequency, in this study it was assumed 2 times
  f. The outflow rate of solid (k) from the rumen, in this study it was assumed 0.05/h.
2.3. Determination of rumen characteristics and microbial protein production

Determination of rumen characteristics and microbial protein production using in vitro digestibility test developed by [16] with some modifications the procedure developed by [17]. This procedure has been done according to [18]. Rumen fluid used for testing the in vitro digestibility was taken from male Ongole cross breed that fitted with permanent canula in the rumen. A 500 mg of sample inserted into the in vitro bottle and it was added 50 ml of McDougal buffer solution and rumen fluid (pH 6.9 and temperature of 39°C) with a ratio of 4:1. The bottles were saturated with CO2, then they were closed with a rubber and put into a water bath in 39°C of temperature for fermentation process. The bottle containing a mixture of McDougal and rumen fluid without samples was used as a blank.

All treatments were fermented for 24 hours. Shaking the bottle fermentors performed every 4 hours. Fermentation of each feed treatment performed on 4 bottles, 2 bottles fermentor which were used to measure the apparent degradability and the others were used to measure the true degradability. After 24 hours, the fermentation was stopped by immersion in ice water for 15 minutes. Apparent degradability was determined using two bottles that were centrifuged at 2500 g for 30 min. Supernatant was used to measure pH (Hanna 301) then it was used for measurement of NH3 (preserved with 1 ml of H2SO4 1 N for 10 ml sample) and individual VFA (acetic acid, propionic acid and butyric acid) was preserved with HgCl2 and H3PO4 as much as 1 ml for 10 ml of sample. The residue was placed into a porcelain cup that had been weighed, then porcelain cup and the residue dried at 105°C for 12 hours followed by burning at a constant temperature of 600°C for 2 hours to determine the degradability of organic matter.

True degradability determined using a sample that has been fermented in two other bottles. They reflux with 100 ml of Neutral Detergent Solution (NDS) for 1 hour then they were filtered using crucible filter. Crucible and the residue were dried at 105°C for 12 hours. Microbial biomass production (MBP) was calculated as the difference of digested weight of sample from the measurement of true and apparent degradability. Fermented organic matter in the rumen (FOM) is determined by calculating the organic matter content of feed multiplied by the organic matter degradability. NH3 concentration measurements were using steam destilasion while individual VFA (acetic acid, propionic acid and butyric acid) concentration measurements carried out according with the instructions of [19]. Concentration of CH4 and CO2 were estimated according to [20]. The efficiency of microbial protein synthesis (EMPS) was determined as follows:

\[
EMPS (\text{g N/kg FOM}) = \frac{\text{MBP (g) x 7\%}}{\text{FOM (kg)}}
\]

Note: 7% is an average content of N in microbial cell.

Variables that observed in this research were rumen fermentation characteristics (pH, NH3 concentrations, individual VFA, estimated the concentration of CO2 and CH4, the ratio of C2: C3, organic matter degradability, fermented organic matter in the rumen (FOM), microbial biomass production (MBP) and efficiency of microbial protein synthesis (EMPS). The study was used a randomized complete design with 3 treatments of synchronization index (SI). Three level of synchronization indexes were P1 (0.4); P2 (0.5) and P3 (0.6). Data were analyzed using a program GENSTAT 14.2 (2011).

3. Result and discussion

3.1. Determination of nutrients and degradation parameters of feedstuffs

Nutrient content and degradation parameters of feedstuffs used in the experiment were presented in table 1 and table 2, respectively. The feedstuffs varied widely in term of nutrient content and the degradation parameters. The nutrient content of diets was presented in table 3.
Table 1. Nutrient content of feedstuffs.

| Feedstuffs      | DM(%) | OM (% DM) | CP (% DM) | EE (% DM) | CF (%DM) | NFE (%) | TDN (% DM) |
|-----------------|-------|-----------|-----------|-----------|----------|---------|------------|
| Maize straw     | 28.15 | 83.14     | 8.80      | 1.76      | 22.54    | 50.04   | 58.55      |
| Native grass    | 26.71 | 78.30     | 10.50     | 1.35      | 21.42    | 45.03   | 55.00      |
| Tumpi           | 91.41 | 92.49     | 8.08      | 1.47      | 19.97    | 62.97   | 50.44      |
| Cassava flour   | 90.03 | 95.54     | 3.18      | 1.22      | 4.20     | 86.94   | 74.00      |
| Coconut meal    | 84.59 | 94.27     | 23.83     | 2.54      | 14.79    | 53.11   | 72.89      |
| Palm kernel cake| 91.91 | 91.89     | 17.37     | 7.69      | 24.42    | 25.81   | 81.91      |

Nutrient value (DM, OM, CP, EE, CF and TDN) of feedstuffs was varied. Dry matter of forage (rice straw and native grass) ranged from 26.86% (native grass) to 45.82% (rice straw). Dry matter value of concentrate varied between 88.19% (cassava) to 95.39% (palm kernel cake). Organic matter value varied between 76.97% (rice straw) to 93.58% (cassava flour). Crude protein value (CP) varied between 2.29% (cassava) to 24.91% (coconut meal). Value of ether extract (EE) varied between 1.25% (cassava) to 9.20% (palm kernel cake). Value of Total Digestible Nutrients (TDN) varied between 43.77% (rice bran) to 68.72% (cassava).

Table 2. The value of parameter degradation of organic matter (OM) and crude protein (CP).

| Feedstuffs      | a (%) | b (%) | c (fraction/hour) | a (%) | b (%) | c (fraction/hour) |
|-----------------|-------|-------|-------------------|-------|-------|-------------------|
| Maize straw     | 23.40±0.8 | 51.53±3.7 | 0.03±0.01 | 33.30±0.80 | 60.30±0.8 | 0.03±0.01 |
| Native grass    | 19.70±1.4 | 49.27±11.7 | 0.05±0.03 | 34.70±2.17 | 44.73±2.2 | 0.09±0.06 |
| Tumpi           | 18.53±6.5 | 53.16±0.7 | 0.05±0.01 | 27.80±10.62 | 55.80±10.6 | 0.04±0.01 |
| Cassava flour   | 48.90±2.9 | 37.80±8.2 | 0.05±0.03 | 22.50±22.63 | 36.60±22.6 | 0.01±0.01 |
| Coconut meal    | 26.50±2.7 | 65.73±6.8 | 0.06±0.02 | 40.30±11.36 | 41.37±11.4 | 0.04±0.01 |
| Palm kernel cake| 17.70 | 63.60 | 0.03 | 35.80 | 49.80 | 0.04 |
| Molasses        | 100   | 0     | 0     | 100   | 0     | 0     |

Research on the determination of the degradation parameters of rice straw and cassava by [8], and [21] and copra meal conducted by [22] gives different results with the results in this study. According to [23] the difference in the degradation parameter values the same feed ingredients caused by harvesting of feed ingredients, methods of measurement and factors specific to the measuring method is in sacco digestibility.

Table 3. Feed formulation and nutrient content of dietary treatment.

| Feedstuffs      | P1 (%) | P2 (%) | P3 (%) |
|-----------------|--------|--------|--------|
| Maize straw     | 35     | 15     | 2.5    |
| Native grass    | 5      | 25     | 37.5   |
| Tumpi           | 20     | 20     | 20     |
| Cassava flour   | 20     | 20     | 12.5   |
| Coconut meal    | 10     | 10     | 2.5    |
| Palm kernel cake| 10     | 10     | 20     |
| Molasses        | 0      | 0      | 5      |
| Nutrient        |        |        |        |
| DM (%)          | 91.93  | 90.38  | 91.20  |
| OM (%DM)        | 92.0   | 93.7   | 94.4   |
| TDN (%DM)       | 70.85  | 69.35  | 66.89  |
| CP (%DM)        | 9.6    | 9.6    | 9.7    |
| CF (%DM)        | 17.8   | 17.4   | 17.0   |
| EE (% DM)       | 3.4    | 5.0    | 6.64   |
| synchronization index | | | |
Value of dry matter and organic matter respectively were 90.78% -93.05% and 89.50% - 89.90%. There was an increase in total digestible nutrient of feed due to increased synchronization index. Content of crude fiber of the diet decreased with the increasing of synchronization index. Improved synchronization index caused a decrease in the use of feed ingredients with high crude fiber content of rice straw. Ether extract content of the diet increased with the improvement in the synchronization index, it is because of the increased use of palm kernel cake and native grasses.

Table 4. Effect of synchronization index against concentration of acetic acid, propionic acid, butyric acid, NH3 and C2:C3 ratio.

| Variable         | Dietary treatment | SEM | P Value |
|------------------|-------------------|-----|---------|
|                 | P1                | P2  | P3      |
| pH               | 6.8±0.13          | 6.8±0.11 | 6.8±0.03 | 0.063 | 0.949 |
| Acetic acid (mM) | 21.31b±0.12       | 22.45ab±1.41 | 15.95b±4.80 | 0.728 | 0.007 |
| Propionic acid (mM)| 5.28b±0.67       | 6.20ab±0.26 | 5.25b±1.23 | 0.603 | 0.020 |
| Butyric acid (mM)| 4.31b±0.14        | 4.18±0.30  | 4.89±0.17  | 0.347 | 0.029 |
| C2:C3 Ratio      | 5.27±3.68         | 3.52±0.26  | 2.88±1.20  | 0.796 | 0.013 |
| CO2              | 18.44b±2.71       | 18.69ab±0.16 | 15.50±1.34 | 0.765 | 0.300 |
| CH4              | 11.49b±0.31       | 11.41ab±0.25 | 7.98±1.78  | 0.470 | 0.031 |
| NH3 (mg/l)       | 54.50b±3.33       | 53.60b±3.55 | 59.07b±3.42 | 0.787 | 0.145 |
| OM degradability |                   | 3.15       | 0.773   |
| FOM (g)          | 0.23±0.03         | 0.22±0.01  | 0.22±0.02  | 0.013 | 0.478 |
| MBP (mg)         | 0.23±0.03         | 0.22±0.01  | 0.22±0.02  | 2.01  | 0.373 |
| EMPS             | 0.23±0.03         | 0.22±0.01  | 0.22±0.02  | 1.21  | 0.133 |

Synchroization index did not affect the pH of the rumen fluid. Synchroization index affected to the concentration of acetic acid (P = 0.007), propionic acid (P = 0.020), butyric acid (P = 0.029), CH4 (P = 0.031) and ratio of C2: C3. Improved synchronization index led to a decrease in crude fiber in the diet (see table 3). This caused a decrease in acetic acid and CH4 [20]. Based on this result, it was shown that the feed formulation according to synchronization index can improve feed efficiency and reduce global warming.

High concentrations of acetic acid led to a decrease in feed intake in vivo conditions. The formation of acetic acid also results in the formation of methane. Acetic acid formation produces hydrogen ions which are precursors for the formation of methane gas. Methane gas production on the use of agricultural waste as ruminant feed becomes a problem because methane is one of the greenhouse gases that contribute to global warming [24].

Synchroization index did not affect the concentrations of NH3. Ammonia is a major and important source of nitrogen for microbial protein synthesis, the range of NH3 in this study was 50.04 mg / l – 52.27 mg / l. This concentration is sufficient to support microbial growth that is 50 mg N-NH3 / l [25]. Concentration of NH3 in the rumen is influenced by the quantity, the solubility, rate of degradation, endogenous N sources, the use of NH3 by rumin microbes and recycling of NH3 [26]. Low levels of NH3 in the rumen can be caused higher microbial protein synthesis [27]. The level of use of NH3 for microbial protein synthesis is affected by the availability of energy. If the energy is limited then NH3 cannot be used in the synthesis of microbial protein that is indicated by accumulation of high amounts of NH3 [28,29].

Synchroization index did not affect to degradation of organic matter, organic matter fermented in the rumen, microbial biomass production and efficiency of microbial protein synthesis. Production of microbial biomass and efficiency of microbial protein synthesis tends to increase with the improvement of synchronization index. They have shown that the better synchronization index of the feed can increase the original microbial protein contribution to the host. The condition is expected to improve the productivity of livestock. This is consistent with results from [30] who reported that improvement of the index of synchronization can increase the efficiency of protein synthesis and fermentation of microbes
in the rumen. The impact of the improvement of the efficiency of microbial protein synthesis is a decreased production of methane [31].

4. Conclusion

Improvement of synchronization Index on feed based on rice by products causing a decrease in the concentration of acetic acid, CH₄ concentration, the ratio of C₂: C₃ so it can reduce global warming and increase on the efficiency of microbial protein synthesis. This can have an impact on improving livestock production.

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