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IN VITRO NUTRITION VALUE OF SLOW RELEASE UREA PRODUCTS FROM THE COMBINATION COOKING RESULTS OF UREA WITH GEWANG (*CORYPHA UTAN* LAMK.) STEM CONTENT (UMBUT) STARCH

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ABSTRACT

Utilization of slow-release urea products can increase nitrogen and energy utilization and rumen fermentation. The purpose of this study was to evaluate the nutritional value of the slow-release urea product (combined product) as a result of in vitro cooking of the combination of urea and stem content (umbut) starch of the gewang stem. The fermentation products evaluated were pH, concentration of N-NH_3, total VFA and microbial protein synthesis. The treatments applied were 2 factors, namely cooking factor (P) in a rotating autoclave with 3 times namely respectively 0 hours (P0), 1 hour (P1) and 2 hours (P2) and urea level factor with 3 levels namely respectively 0% (U0), 2% (U2) and 4% (U4), as well as control treatment without cooking (p_0) at 3 levels of urea. The experimental design used was a completely randomized design with a 4 x 3 factorial pattern with 3 replications. Statistical analysis using analysis of variance (Anova) and Duncan's Multiple Range Test. The relationship between the two treatment factors with each variable used orthogonal polynomial regression analysis. The research results showed that there was an interaction between cooking time and urea level on the variables of pH value, N-NH3 concentration and microbial protein synthesis, while total VFA was not the result of treatment interaction. The highest in vitro nutritional value was achieved by the combined product of urea-stem content (umbut) starch of gewang stem which was cooked for 2 hours with the urea level of 4% (P2U4) namely, the value of pH 6.77; N-NH_3 9.49 mg/100 mL, total concentration of VFA 141.79 mMol and microbial protein synthesis 8.29 mg/g/4 hours. It was concluded that the combined product of cooking result of urea and stem content (umbut) starch of gewang stem produced fermentation products according to the needs of in vitro rumen microorganisms with the best cooking time of 2 hours and urea level of 4%. This combined product can be categorized as a slow release urea product.

KEYWORDS: In vitro, combination, nutritional value, cooking, stem content starch of gewang, slow release urea.

INTRODUCTION

The fulfillment of feed requirements for ruminants in the tropics has always been an obstacle both in quantity and quality because it relies on forage sources which mostly come from agricultural land and pastures. It is necessary to utilize various types of feed ingredients that can meet the needs of livestock to produce high production. Nitrogen and energy are nutrients in feed ingredients that are needed for the needs of ruminants, especially microorganisms in the rumen. Urea is a source of Non Protein Nitrogen (NPN) which is commonly used in rations for ruminants in the tropics, where grass forage as basal feed for livestock contains low easily degradable protein. Urea is one of the non-protein nitrogenous feeds whose optimal utilization in feed can reduce a number of costs for protein rations.[1]

The use of urea in ruminant feed is quite widespread and is universally expected to be a cheap material to replace plant protein sources, can increase carbohydrate utilization and increase feed conversion in ruminant rations.[2,3] Urea in the ration will be used by rumen microbes to increase growth and its activity remodel the low quality basal feed. According to Karsli and Russel,[4] Microbial protein synthesis and its growth are highly dependent on the adequacy of energy (ATP) produced from the fermentation of organic matter in the rumen and nitrogen produced from the degradation of protein and non-protein nitrogen sources in the ration. The problem with urea utilization is its rapid degradation in the rumen, resulting in the supply of ammonia exceeding the capacity of rumen bacteria to assimilate it into amino acids. The rate of ammonia release results in inefficient utilization of N in the rumen.[5]

Efforts to slow down the release and increase the utilization of urea nitrogen in livestock rations are by controlling the rate of its release so that it is more consistent with carbohydrate digestion. One source of carbohydrates (starch) that can be combined with urea is the stems content (umbut) of the gewang plant (*Corypha utan* Lamk.). According to Witono et al.[6] The carbohydrate content of the stem of the gewang plant is 86.59%, amylose 32.73%, amylopectin 51.11% and protein 0.69%. This stem content (umbut) starch of gewang stem has the potential to be combined with urea to produce products that are suitable for fermentation in the rumen. Acceleration of the starch degradation along with urea requires pretreatment and one of the pretreatments that can be applied is cooking. The cooking process causes the molecular structure of starch could be changed, so that it becomes easier to be attacked by microbial enzymes and when cooked with urea, the problem of time mismatch in the supply of energy and ammonia can be overcome. According to Bloomfield et al.[7] the process of formation of urea-carbohydrate complex at a temperature of 170^{o} C causes the release of nitrogen is more suitable for bacterial growth. The purpose of this study was to study the in vitro nutritional value of the cooking product from the combination of urea with the stem content (umbut) starch of gewang stem using a "rotating autoclave" as a slow-release urea product.

MATERIAL AND METHOD

The cooking process of the mixture of gewang stem content (umbut) with urea is carried out using a "rotating autoclave". The gewang stem content was obtained from the community in Kupang regency, Timor island, East Nusa Tenggara Province, Indonesia. Urea is obtained from a farm supply store. Rotating autoclave used to cook a mixture of gewang stem content and urea belonging to Center for Pulp and Paper / Balai Besar Pulp dan Kertas (BBPK) in Dayeuh kolot, Bandung, West Java. The combined product of urea-stem content (umbut) starch of gewang stem is obtained by cooking at 170^{o}C in a rotating autoclave, according to the procedure of Chicco et al.[8]. The treatment applied consisted of two factors, namely the length of cooking time (P) and the dose of urea (U). The cooking time factors were 0 hours (P0), 1 hour (P1) and 2 hours (P2), respectively. The factor doses of urea were 0% (U0), 2% (U2) and 4% (U4), respectively. The combination of treatments formed is as shown below:

P0U0	= cooking time 0 hours with 0% urea level
P0U2	= cooking time 0 hours with 2% urea level
P0U4	= cooking time 0 hours with 4% urea level
P1U0	= cooking time 1 hours with 0% urea level
P1U2	= cooking time 1 hours with 2% urea level
P1U4	= cooking time 1 hours with 4% urea level
P2U0	= cooking time 2 hours with 0% urea level
P2U2	= cooking time 2 hours with 2% urea level
P2U4	= cooking time 2 hours with 4% urea level

The procedure for cooking a mixture of urea with stem content (umbut) starch of gewang stem was according to the procedure of Chicco et al.[8] as follows

1. As much as 300 grams of stem content (umbut) starch of gewang stem (in dry ingredients) are mixed evenly with urea according to the level of treatment and put into an autoclave container.
2. Water is added until it reaches 75% of the water content of the ingredients. The water content is determined from the dry ingredients of the stem content (umbut) starch of gewang. The container is tightly closed and put into the autoclave room.
3. The rotating autoclave room is heated to 170^{o}C. The cooking time is calculated from the moment the temperature of the autoclave container reaches 170^{o}C.
4. After reaching the unit of treatment time of the cooking time duration, the autoclave room temperature was lowered, the container was removed and cooled in a cooling water bath for 30 minutes. The container is then opened and the cooking product is dried in an oven at 50^{o}C for 48 hours. The product of this cooking is called the combined product.

The control treatment used was a mixture of urea with stem content (umbut) starch of gewang stem without cooking treatment (p_0), namely

p_0U0 = 0% urea in dry ingredients of the stem content (umbut) starch of gewang stem without cooking treatment

p_0U_2 = 2% urea in dry ingredients of the stem content (umbut) starch of gewang stem without cooking treatment

p_0U_4 = 4% urea in dry ingredients of the stem content (umbut) starch of gewang stem without cooking treatment

To study the rumen microbial biofermentation on the combined product of urea-stem content (umbut) starch of gewang stem and controls, samples were incubated in a fermenter for 4 hours according to the procedure of Owens and Goetsch.[9] The research procedure is as follows

1. Weigh 1.0 g of dry material sample and add 10 mL of McDougall's buffer solution with pH 6,9 and sheep rumen fluid inoculum 10 mL.
2. The fermenter is put into a "water bath" at 40^{o}C and CO_2 gas flowed into the fermenter, then covered with a ventilated rubber prop.
3. After 4 hours of incubation, the fermentation process was stopped by adding 0,2 mL of saturated $HgCl_2$ to kill microbes. The fermentation product was centrifuged at a speed of 16,400 rpm for 20 minutes to separate the precipitate and supernatant.

The variables measured were rumen pH, total concentration of VFA (total volatile fatty acids), N-NH3 (ammonia) and microbial protein synthesis.

1. pH was measured using a pH meter of "Chemtrix type 40" immediately after the fermentation was stopped with $HgCl_2$.
2. Total-VFA concentration was measured using the distillation method.[10]. 10 mL of rumen fluid was acidified with 1 mL of 1 N H_2SO_4 then centrifuged at 3000 rpm for 15 minutes. The conical tube is placed in the cooler of the distiller. 5 mL of the supernatant from the rumen fluid was taken with a pipette and then put into the chimney at the top of the distiller, and 2 mL of $H_2SO_4/MgSO_4$ solution was added, washed with distilled water and immediately carried out distillation. Distillation was carried out for 20 minutes to obtain 250 mL of distillate and added 5 mL of phenolphthalein indicator solution and titrated with 0.02 N NaOH to get a pink color as the endpoint (pH = 8.3). Total VFA is known by the formula :

$$\text{Normalization (NaOH) N} = \frac{\text{Vol. HCl x Normality (HCl) (N atomic weight)}}{\text{Titer (NaOH) (mL)}}$$

$$\text{Mmol VFA/liter} = \frac{\text{Total titer (mL) x NaOH normality x 1000}}{\text{Rumen fluid volume (5 mL)}}$$

1. Concentration of $N\text{-}NH_3$. The analysis of the concentration of N-NH3 was carried out using the Conway microdiffusion technique,[10] Conway's cup and lid are smeared on the edges with Vaseline. In the middle of the cup, put in 1 ml of boric acid and 1 drop of the indicator mixture of red methyl and green bromcreso. The left side of the cup is put in 1 ml of the supernatant and 1 ml of saturated sodium carbonate (NaCO3) solution is put in the right side. The cup was closed and shaken slowly so that the supernatant and sodium carbonate were mixed homogeneously, then allowed to stand for 24 hours at room temperature. After 24 hours the cup was opened and then titrated using 0.0055 N H2SO4 until the purple color changed to pink.

Ammonia concentration calculation: N-NH3 = (ml of H2SO4 titration x N H2SO4 x 1000) mM

Information: N-NH3= The obtained N-NH3 concentration

N H2SO4 = Normality of H2SO4 solution

2. Measurement of microbial protein synthesis. Measurements used the "tungstic acid" method, namely the centrifuged precipitate was washed successively with 5 mL H2SO4 1.07 N and 5 mL NaWO4 2H2O in a ratio of 2:1:1, by centrifuging at a speed of 16.400 rpm for 20 minutes; then the supernatant was removed and the precipitate was dried in an oven and then analyzed for its protein content by the "mikroKjeldahl" method.[11]. The calculation is as follows:

$$\text{PM (mg/g/hour)} = \frac{\text{mL titran x N HCl x fp x 14 x 6.25}}{\text{Gram sample}} \times 100$$

Information: fp = factor pengencer / diluent factor.

Data Analysis

The experimental design used was a completely randomized design with a factorial pattern.[12] Analysis of variance is used to see the effect of treatment on the measured variables. To study the relationship between the two treatment factors and the observed variables, the orthogonal polynomial regression test was used and Differences between treatments were tested by Duncan's multiple-range test.[12]

RESULTS AND DISCUSSION

Acidity Degree (pH)

The pH value is an important and comprehensive indication in reflecting the fermentation process in the rumen. The pH value was measured 4 hours after incubation of the combined product of urea-stem content (umbut) of gewang stem in a fermenter. The acidity degree or pH of the rumen fluid is a balance between the buffer capacity and the alkaline or acidic nature of the fermentation product. The average rumen fluid pH values of these products are listed in Table 1.

Table 1: Average pH value of the combined product of Urea-stem content (Umbut) starch of Gewang stem due to Treatment of Cooking Time and Urea Level.

Cooking Time, Hours P	Urea Level (U)		
	0 % (U0)	2% (U2)	4% (U4)
0 (p_0)	C 6,90[a]	B 6.97[b]	B 6.97[b]
0 (P0)	A 6.55[a]	A 6.73[b]	A 6.80[c]
1 (P1)	A 6.57[a]	A 6.72[b]	A 6.78[c]
2 (P2)	B 6.62[a]	A 6.73[b]	A 6.77[b]

Information: 1) the same lowercase letters in the same line indicate not significantly different (P>0.05)
2) the same capital letters in the same column indicate not significantly different (P>0.05)

The results of the analysis of variance showed that the treatment interacted significantly ($P<0.05$) on the changes in pH. The largest pH value with the narrowest pH change was indicated by the control treatment (without cooking) at all urea levels. The pH value of the combined product of urea-stem content (umbut) starch of gewang stem in this study ranged from 6.5-6.80. This pH value is still in the range of normal pH values, namely ranges from 6.4 to 6.8 [13] meanwhile according to Alizadeh et al.[14], Rumen pH in the range of 6.22-6.99 is very suitable for the activity of cellulolytic bacteria and fiber digesters. The highest pH value with a narrow change in the treatment without cooking (6.90-7.00) will reduce the utilization of urea and the stem content (umbut) starch of gewang stem. The narrow changes in the control treatment indicated the low utilization of carbohydrates in the stem content ((umbut) starch of gewang stem due to the high production of ammonia (13.90-20.07 mg/100 mL) from urea was not able to be utilized properly by microorganisms. Cooking treatment with increased urea level resulted in a combined product with a wide change in rumen pH.

The research results showed that the treatment with urea levels of 0%, 2% and 4% in the treatment without cooking (po) from a mixture of urea-stem content (umbut) starch of gewang stem, the pH value was significantly higher than the treatment of cooking results of 0 hours, 1 hour and 2 hours. The use of 0% urea level with cooking time of 0 hours and 1 hour was significantly different with cooking time of 2 hours. 2% and 4% urea levels did not show a significant difference. The pH values in this study did not differ much from those reported by Shiehzadeh and Harbers[15] of 6.6 and 6.5 in the combined product of urea-potatoes and sorghum starch cooked at high temperature.

The analysis results of orthogonal polynomial regression showed the treatment interaction of cooking time and urea level, resulting in a combined product of urea-stem content (umbut) starch of gewang stem on the pH value during the fermentation process according to the equation, $Y = 6.551 + 0.008P + 0.111U - 0.012U^2 - 0.013\ PU$ ($R^2 = 0.95$). Changes in the pH value in vitro are expected to reach the optimum at the value of 6.76 in dry matter of stem content (umbut) starch of gewang stem. This result is almost the same as that obtained by Males et al.[16] in vivo in sheep treated with gelatinized urea-starch, namely equal to 6.78.

Concentration of N-NH3 (Ammonia)

The concentration of ammonia in the rumen is an important element to be controlled because it determines the optimization of rumen microbial growth.[17] The average concentration of ammonia in the combined product of urea-stem content (umbut) starch of gewang stem due to the treatment of urea level and cooking time is shown in Table 2.

Table 2: Average Concentration of N-NH3 in Combined Products of Urea-Stem Content (Umbut) Starch of Gewang Stem Due to Treatment of Cooking Time and Urea Level (mg/100 mL).

Cooking Time, Hours P	Urea Level (U)		
	0 % (U0)	2% (U2)	4% (U4)
0 (p_0)	A 0.38[a]	C 14.03[b]	B 20.17[c]
0 (P0)	A 0.62[a]	A 6.92[b]	A 8.65[c]
1 (P1)	A 0.65[a]	B 8.68[b]	A 9.54[b]
9.49[b] 2 (P2)	A 0.65[a]	B 8.59[b]	A 9.49[b]

Information: 1) the same lowercase letters in the same line indicate not significantly different (P>0.05)
2) The same capital letters in the same column indicate not significantly different (P>0.05)

The results of the analysis of variance showed that cooking time and urea level interacted very significantly ($P<0.01$) on the ammonia concentration. This interaction illustrates that the ammonia concentration of the combined product of urea-stem content (umbut) starch of gewang stem is influenced by the dependence relationship between the factor of cooking time and urea level.

The rate of ammonia concentration obtained in the combined product of urea-stem content (umbut) starch of gewang stem in this study was lower than the control treatment. This shows that by cooking at a temperature of 170^0C in a rotating autoclave, the hydrolysis of urea can be controlled so that it can be utilized effectively by microorganisms. These results illustrate that this product is categorized as a slow-release urea product.

The analysis results of orthogonal polynomial regression showed the relationship between cooking time (P, hours) and urea level (U, %) following the equation: $Y = 0.263 + 1.164P - 0.471P^2 + 5.175U - 0.783U^2 + 0.102PU$ ($R^2 = 0.99$). This equation illustrates that, the maximum concentration of ammonia is 10.02 mg/mL at the optimum cooking treatment of 1.605 hours and the optimum urea level is 3.410%. This result is still within the range of ammonia concentration required by rumen microbes to digest feed optimally, namely 6-12 mM,[18]

5-20 mg/dL, equivalent to 3.57-14.28 mM,[19] 2-13 mg/dL.[20] The average N ammonia found in tapioca flour based slow release urea products was 8.74 mg/100 mL.[21]

The results of Duncan's multiple range test on the treatment of the mixture of urea-stem content (umbut) starch of gewang stem without cooking (po) at 0% urea level was significantly lower than 2% and 4% urea levels. Likewise between 2% and 4% urea levels. The results obtained in the 1 hour cooking time treatment with urea levels of 0%, 2%, and 4% were the same as without cooking (po). This result is in accordance with that obtained by Shultz et al.[22] where the level of rumen ammonia obtained was higher in sheep that consume uncooked urea-cassava supplements than cooked urea-cassava supplements. The cooking of 1 hour and 2 hours had the same pattern, where with an urea level of 0%, the ammonia concentration was significantly lower than the levels of 2% and 4%. The results of this study showed that the mixture of urea-stem content (umbut) starch of gewang stem without cooking (p_0) had a significantly higher ammonia concentration than that cooked for 0 hours, 1 hour and 2 hours.

Total VFA Concentration

The average concentration of the total VFA of the mixture of urea-stem content (umbut) starch of gewang stem (control) and the combined product of urea-stem content (umbut) starch of gewang stem is shown in Table 3.

Table 3: Average Concentration of the Total VFA of the Mixture of Urea-Stem Content (umbut) Starch of Gewang Stem and the Combined Product of Urea-Stem Content (umbut) Starch of Gewang Stem Due to the Treatment of Cooking Time and Urea Levels (mMol/l).

Cooking Time, Hours P	Urea Level (U)		
	0 % (U0)	2% (U2)	4% (U4)
0 (p_0)	A 50.20[a]	A 51.32[a]	A 54.19[b]
0 (P0)	B 118.53[a]	B 120.23[a]	B 121.24[b]
1 (P1)	C 139.72[a]	C 140.32[a]	C 140.70[a]
9.49[b] 2 (P2)	C 138.40[a]	C 139.49[a]	C 141.79[a]

Information: 1) the same lowercase letters in the same line indicate not significantly different (P>0.05)
2) The same capital letters in the same column indicate not significantly different (P>0.05)

The range of the total VFA concentration in this study was 50.20 mMol/l – 141.79mMol/l. The average concentration of total VFA in the cooking treatment (0 hours, 1 hour and 2 hours) increased in line with the increase in urea level and this value was considered optimal for microbial growth, namely in the range of 70-150 mMol.[23]

The results of the analysis of variance showed that there was no significant interaction effect (P>0.05) between the length of cooking time and the level of urea on the total VFA concentration. This shows that there is no dependent relationship between the factor of cooking time and urea level, and this is in line with the opinion of Shiehzadeh and Harbers[15] that the use of nitrogen sources in the carbohydrate cooking process has no effect on the VFA concentration. The value of the total VFA concentration obtained shows that the cooking time treatment has a very significant effect following a linear pattern then becomes quadratic, while the effect of urea level significantly follows a linear pattern. Cooking time of 2 hours (P2) with the urea level of 4% (U4) showed the best results on the value of the total VFA concentration. The relationship between cooking time and urea level on total VFA concentration follows the equation: $Y = 118.887 + 31.324P - 10.820P^2 + 0.601U$ ($R^2 = 0.98$). These results indicate that the maximum value of the total VFA concentration is 138,16 mMol at the optimum cooking time of 1.56 hours.

Duncan's test results showed that the total value of the total VFA concentration from the mixture of urea-stem content (umbut) starch of gewang stem without cooking (p_0) with urea levels of 0% and 2% was not different, but the urea level of 4% was significantly higher than 0% and 2%. The same results were shown in the 0 hour cooking treatment. On the cooking for 1 hour and 2 hours, all levels of urea used did not show a difference in the value of the total VFA concentration. The value of the total VFA concentration from the cooking treatment (0, 1 and 2 hours) was significantly higher than the mixture of the uncooked urea-stem content (umbut) starch of gewang stem. The high total VFA concentration produced in the combined product is made possible by the presence of a carbon skeleton which is essential for the formation of fatty acid branched chains provided by non-cellulytic microbes to synthesize proteins from nitrogen bonds in the product. The average value of the total VFA concentration from 2 and 4 hours of cooking at all urea levels did not show a significant difference. These results indicate that the combined product of these treatments is very easily fermented by bacteria,

according to the opinion of Suherman et al.[24] that the VFA content in the rumen fluid is an indication of the efficiency of the feed fermentation process in the rumen.

Microbial Protein Synthesis

The average microbial protein content of the combined product of urea-stem content (umbut) starch of gewang stem and control is shown in Table 4.

Table 4: Average of Microbial Protein Synthesis of Combined Product of Urea-Stem Content (Umbut) Starch of Gewang Stem due to the Treatment of Cooking Time and Urea Level (mg/g/4 hours).

Cooking Time, Hours P	Urea Level (U)		
	0 % (U0)	2% (U2)	4% (U4)
0 (p_0)	A 1.15[a]	A 3.72[b]	A 3.91[b]
0 (P0)	B 1.78[a]	B 5.11[b]	B 6.01[c]
1 (P1)	C 4.46[a]	C 6.68[b]	C 7.53[c]
9.49[b] 2 (P2)	D 5.11[a]	D 7.10[b]	D 8.29[c]

Information: 1) the same lowercase letters in the same line indicate not significantly different (P>0.05)
2) the same capital letters in the same column indicate not significantly different (P>0.05)

The results of the analysis of variance showed that there was a very significant interaction (P<0.01) on microbial protein synthesis due to the treatment combination of cooking time and urea level. These results indicate that through cooking occurred a synchronization between nitrogen from urea and starch from stem content (umbut) starch of gewang stem. According to Herera-Saldana et al. [25] and Salami et al.[26], synchronization of energy and nitrogen in the ration increases the growth and efficiency of microbial protein production, even microbial protein synthesis can be maximized[27; 28]. The highest average microbial protein synthesis was achieved by the treatment of 2-hour cooking time with the urea level of 4% (P_2U_4), namely equal to 8.29 mg/g/4 hours. If it is associated with the variables of ammonia concentration and total VFA, then the control treatment (without cooking), resulted in the highest ammonia concentration (20.17 mg/100 mL) and a total VFA of 54.19 mMol (P_0U_4) but resulted in low microbial protein synthesis (1.15-3.91 mg/g/4 hours). The high concentration of ammonia because it is not used by microbes. The synchronization of carbohydrate degradation and the rate of protein degradation in the rumen enables efficient microbial protein production[4]. If the rate of protein degradation exceeds carbohydrates, a large amount of N will be lost in the form of ammonia, on the other hand if the rate of carbohydrate degradation exceeds protein, protein production can be disrupted.[29]. In the treatment of cooking time of 0 hours, 1 hour, and 2 hours with urea levels of 0%, 2%, and 4% there was a significant difference, where microbial protein synthesis increased with the increase in the urea levels. These results indicate that the urea level up to 4% in the combined product will provide more N for microorganisms to form body proteins.

The analysis results of orthogonal polynomial regression of the treatment relationship between cooking time (P, hours) and urea level (U, %) from the combined product of urea-stem content (umbut) starch of gewang stem on microbial protein synthesis (Y, mg/g/4 hours) followed the equation: $Y = 2.039 + 2.837P + 1.772U - 0.192U^2 - 0.131PU$ ($R^2 = 0.98$). Microbial protein synthesis in this study is expected to reach a maximum at 1.762 hours of cooking with the urea level of 4.012%, namely amounted to 8.091 mg/g/4 hours. According to Hogan and Weston[30] about 15 grams of microbial protein are synthesized from every 100 grams of organic matter utilized in the rumen. So that by providing organic material derived from the combined product of urea-stem content (umbut) starch of gewang stem, it is hoped that a lot of microbial protein will be formed. Optimal microbial protein synthesis requires a supply of nitrogen and organic acids. Nitrogen supply comes from the production of ammonia, while organic acids are met from the production of VFA which is the result of carbohydrate fermentation. The combined product in this study provides an adequate balance of ammonia and VFA for microbial protein synthesis.

CONCLUSION

The combined product from the cooking result of urea-stem content (umbut) starch of gewang stem produces a fermentation product according to the needs of rumen microorganisms in vitro with the best cooking time of 2 hours and the urea level of 4%. This combined product can be categorized as a slow release urea product.

CONFLIC OF INTEREST

The study have no conflict of interest.

REFERENCES

1. Getahun D, Alemneh T, Akeberegn D, Getabalew M, Zewdie D. Urea Metabolism and Recycling in Ruminants. Biomed J Sci & Tech Res., 2019; 20(1). BJSTR. MS.ID.003401.
2. Patra AK.and Aschenbach JR. Ureases in the gastrointestinal tracts of ruminant and monogastric animals and their implication in urea-N/ammonia metabolism: A review. J of Advanced Res., 2018; 13: 39-50.
3. Ding L, Lascano G, Heinrichs AJ. Effect of precision feeding high-and low-quality forage with different rumen protein degradability levels on nutrient utilization by dairy heifers. *Journal of Animal Science*, 2015; *93*(6): 3066-3075.
4. Karsli M. and Russel JR. Effects of some dietary factors on rumen microbial protein synthesis. Turk J Vet Anim Sci., 2001; 25: 681-686.
5. Xin HS, Schaefer DM, Liu QP, Axe DE, Meng QX. Effects of polyurethane coated urea supplement on in vitro ruminal fermentation, ammonia release dynamics and lactating performance of Holstein dairy cows fed a steam-flaked corn-based diet. Asian-Australasian Journal of Animal Sciences, 2010; 23(4): 491-500.
6. Witono JR, Kusuma YWC, Naiola BP, Jamal Y, Agusta A. Gewang (Corypha utan Lamk.) as local food in Timor island and its nutritional properties. In: Current Issues and Challenges in Food Safety. Proceeding of the International Seminar. Southeast Asian Food & Agricultural Science & Technology (SEAFAST) Center, Bogor Agricultural University. http://www.seafast.ipb.ac.id, 2010.
7. Bloomfield RA, Harris DW, Muhrer ME. A reacted NPN-carbohydrate complex from urea. J. Anim. Sci., 1968; 27: 976-981.
8. Chicco CF, Shultz E, Shultz TA. Pressure cooked urea-cassava meal for lambs consuming low quality hay. J. Anim. Sci., 1972; 35: 865-870.
9. Owens FN, Goestch AL. Ruminal Fermentation. In: The Ruminant Animal Digestive Physiology and Nutrition. Editor by D.C. Church. A Reston Book. Prentice Hall. Englewood Cliffs, New Yersey, 145-171.
10. General Laboratory Procedures. Departement of Dairy Science. University of Wisconsin, Madison, USA., 1966.
11. AOAC. Official Methods Of Analysis of the Association of Official Analitical Chemist, 11th Ed. Washington D.C., 1980.
12. Steel RGD, Torrie JH. Prinsip dan Prosedur Statistika. Suatu Pendekatan Biometrik, PT Gramedia Pustaka Utama, Jakarta. Hal, 209-235.
13. Erdman RA. Dietary buffering requirements of the lactating dairy cows. A Review. D. Dairy Science, 1986; 71: 3246-3250.
14. Alizadeh Z, Yansari AT, Chasnidal Y, Kazemifard M, Azarpajouh S. Effect of soybeanmeal replacement by slow-release urea on ruminal parameter, blood metabolic, and microbial protein synthesis in Zel ram. Acta Scientiarum, Animal Science, 2021; 43: e48684.
15. Shiehzadeh SA, Harbers LA. Soybean meal, urea and extruded starch-urea products compared as protein supplements in high-roughage lamb rations. J. Anim. Sci., 1974; 38: 206-212.
16. Males JR, Munsinger RA, Johnson RR. In vitro and in vivo ammonia release from "slow-release" urea supplements. J. Anim. Sci., 1979; 48: 887-892.
17. Arora SP. Pencernaan Mikrobia pada Ruminansia. Gadjah Mada University Press, Yogyakarta, 1989.
18. McDonald JW, Howard MJ. Repairing the damaged spinal cord: a summary of our early success with embryonic stem cell transplantation and remyelination. In Progress in brain research, 2002; 137: 299-309. Elsevier. https://doi.org/10.1016/S0079-6123(02)37023-7.
19. Paengkoum P, Liang JB, Jelan ZA, Basery M. Utilization of Steam-treated Oil Palm Fronds in Growing Saanen Goats: II. Supplementation with Energy and Urea. Asian-Aust. J. Anim. Sci., 2006; 19(11): 1623-1631.
20. Boucher SE, Ordway RS, Whitehouse NL, Lundy FP, Kononoff PJ, Schwab CG. Effect of incremental urea supplementation of a conventional corn silage-based diet on ruminal ammonia concentration and synthesis of microbial protein. J. Dairy Sci., 2007; 90(12): 5619-5633.
21. Rimbawanto EA, Suhermiyati S, Hartoyo B. Effects of Slow Release Urea Supplementation of Sheep Protein Source Feed Protected with Condensed Tannin from Leucaena on Protein Degradation in Rumen and Post-rumen In Vitro. Animal Production, 2017; 19(2): 119-126.
22. Shultz TA, Shultz E, Chicco CF. Pressure cooked urea-cassava meal for lambs consuming low quality hay. J. Anim. Sci., 1972; 35: 865-870.
23. McDonald P, Edwards RA, Greenhalgh JFD, Morgan CA, Sinclair LA, Wilkinson RG. Animal Nutrition. 7th Ed. Prentice Hall, Pearson, Harlow, England, 2010.
24. Suherman K, Suparwi, Widayastuti. Konsentrasi VFA total dan amonia pada onggok yang difermentasi dengan Aspergillus niger secara in vitro. J. Ilmiah Peternakan, 2013; 1(3): 827-834.
25. Herrera-Saldana R, Gomez-Alarcon R, Torabi M, Huber J. Influence of Synchronizing Protein and Starch Degradation in the Rumen on Nutrient Utilization and Microbial Protein Synthesis. Journal of Dairy Science, 1990; 73(1): 142-148. doi: 10.3168/jds.S0022-0302(90)78657-2.
26. Salami SA, Warren H, Moran CA. A meta-analysis of the effects of slow-release urea supplementation on the performance of beef cattle. Animal, 2020; 10: 657. Doi: 10.3390/ani10040657.
27. Ørskov ER. Protein Nutrition in Ruminants. 2nd Edition. Academic Press. New York, EUA, 1982.
28. Zadeh JB, Moradikor Z, Moradikor N. Synchronization of energy and protein on supply

synthesis microbial protein. Int J Adv Biol Biom Res., 2013; 1: 594-600.
29. Nocek JE, Russell JB. Protein and energy as an integrated system. Relationship of ruminal protein and carbohydrate availability to microbial synthesis and milk production. J. Dairy Sci., 1988; 71: 2070.
30. Hogan JP and RH Weston. The digestion of two diets of differing protein content but with similar capasities to sustain wool growth. Australian Journal Agricultural Res., 1967; 18: 973-981.