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Effect of cooking time and urea level on dry matter loss, nitrogen fixation and digestibility of dry matter, organic matter in vitro in the combined products of urea-pith Gewang (*Corypha Utan* Lamk.) starch

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Abstract

The purpose of this study was to evaluate dry matter loss, nitrogen fixation and dry matter and organic matter digestibility in vitro from the cooking product of pith Gewang with urea. The treatments applied consisted of cooking time (P) namely 0 hours (P0), 1 hour (P1) and 2 hours (P2) respectively, and the treatment of urea levels namely 0% (U0), 2% (U2) and 4% (U4) respectively, which were combined into 9 treatment combination units, to determine the dry matter loss and nitrogen fixation of the combined product. Determination of digestibility of dry matter and organic matter in vitro of the combined product is the combination of treatment of pith Gewang with urea before adding the control treatment, namely a combination of pith Gewang with urea level without cooking (p_0). The research results showed that the dry matter loss of the combined product increased with an increase in the cooking time, but the increase in the urea level decreased the dry matter loss. Nitrogen fixation increased with an increase in the cooking time with urea level. Digestibility of dry matter and organic matter increases with an increase in the cooking time and urea level. It is concluded that, the cooking of pith Gewang with urea level resulted in the combined product that was low in dry matter loss and high in nitrogen fixation, dry matter and organic matter digestibility. Cooking time of 2 hours with urea level of 4 percent is the best combination in producing the combined product.

Keywords: *In vitro* digestibility; Dry matter loss; Nitrogen fixation; Urea level; Pith Gewang

1. Introduction

The need for feed protein for ruminants in the tropics is still a problem because the main feed that is generally provided is low-quality forage and agricultural waste. Supplementation of feed sources containing nitrogen becomes the priority in solving this problem [1]. Sources of non-protein nitrogen (NPN) such as urea, are commonly used in protein supplementation because ruminants are able to utilize it, have low nitrogen density and low cost per unit nitrogen [2]. In the diet of ruminants, it can be used as an alternative source to true protein, since it is capable of replacing it with the advantage of being less expensive [3].

Urea is converted to ammonia by rumen microbes and used to form body proteins (4); However, the amount of urea that can be used in feed is limited by the rapid hydrolysis to ammonia in the rumen by microbial enzymes [5;6]. Efforts to increase the utilization of urea as a supplement is to try to slow down its release in the rumen so that it can be utilized efficiently by rumen microbes, and this can be done by synchronizing the energy from carbohydrate source feed with

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urea. Through synchronization of nitrogen and energy availability, the efficiency of microbial protein synthesis can be increased [7; 8].

The degradation of carbohydrates in the rumen and microbial growth is a very slow process; therefore, the synchronization of the process will increase the efficiency of the incorporation of NPN into microbial proteins further improving the efficiency of the N use. The presence of O-H groups in starch that captures N-H groups in urea so that interactions occur through hydrogen bonds so that the release of the product becomes synchronized. According to Chanjula and Ngampongsai [9], sago from the stem content of palm plants is an excellent source of easily degradable starch and has the potential to improve the performance of ruminants. Gewang (*Corypha utan Lamk*.) plant is one of the palm plants whose contents in the stem (pith) have the potential as a source of easily available carbohydrates which can be combined with urea to produce a slow-release urea product. This process like of extrusion of urea with corn, in addition to controlling the release of nitrogen, improves the use of urea due to a better synchronization during the degradation of these ingredients [10]. Even according to Inacio et al. [11], controlled-release urea is already a product used in ruminant feed.

This product is produced by urea cooking with pith Gewang (Gewang stem content) in an autoclave, where through this process there is a change in the molecular structure of starch from pith Gewang so that it becomes easier for urea to imbibite into starch granules and form a mixed product of gelatinized starch with urea. The purpose of this study was to evaluate the impact of cooking time in an autoclave and urea level on the combined product of urea and pith Gewang starch that was measured by dry matter loss and nitrogen fixation as well as dry matter and organic matter digestibility *in vitro*.

2. Material and methods

The combined product of urea-pith Gewang starch is produced from the process of cooking the mixture of pith Gewang with urea in a "rotating autoclave". Pith of the Gewang stem was obtained from the community in Kupang Regency, Timor Island, and East Nusa Tenggara Province, Indonesia. Urea is obtained from a farm supply store. Rotating autoclave used to cook a mixture of pith Gewang with urea belonging to the Center for Pulp and Paper/Balai Besar Pulp dan Kertas (BBPK) in Dayeuh kolot, Bandung. The purpose of this study was to study the incorporation of non-protein nitrogen compounds (urea) with carbohydrates (pith of Gewang stem) through cooking at a temperature of 170^{o}C in a rotating autoclave, according to the procedure of Chicco et al. [12]. The treatment in this study consisted of two factors, namely the length of cooking time (P) and the dose of urea (U). Cooking time factors, respectively, 0 hours (P0), 1 hour (P1) and 2 hours (P2). The urea dose factors were 0% (U0), 2% (U2) and 4% (U4), respectively. The treatment combinations formed are as shown below:

- P0U0 = cooking time 0 hour with 0% urea level
- P0U2 = cooking time 0 hour with 2% urea level
- P0U4 = cooking time 0 hour with 4% urea level
- P1U0 = cooking time 1 hour with 0% urea level
- P1U2 = cooking time 1 hour with 2% urea level
- P1U4 = cooking time 1 hour 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

Each treatment combination conducted 3 times repetition. The procedure for cooking a mixture of urea with pith Gewang starch according to the procedure of Chicco et al. [12] as follows:

- As much as 300 grams of pith Gewang starch (in dry matter) is mixed evenly with urea according to the treatment level and put into an autoclave container.
- Water is added until it reaches a water content of 75%. The water content is determined from the dry matter of pith Gewang starch. The container is tightly closed and put in the autoclave chamber.
- The autoclave chamber is heated to 170^{o}C. The length of cooking time is calculated from the moment the temperature of the autoclave container reaches 170^{o}C.
- After reaching the unit of treatment time of the length of cooking time, 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 a temperature of 50^{o}C. The dry matter loss was obtained by calculating the difference between the dry matter of the mixture ingredient before cooking and the dry matter of the cooking product with the formula.

$$\text{Dry Matter (DM) Loss (\%)} = \frac{A - B}{A} \times 100\%$$

Where

A = Dry matter from ingredients before cooking (g)
B = Dry matter of cooking product (g)

Nitrogen fixation was measured by analyzing the nitrogen content of cooking products according to the Kjeldahl method [13].

2.1. Determination of Digestibility of Dry Matter and Organic Matter *in Vitro*

The aim was to study the digestibility of dry matter and organic matter from cooking products of urea-pith starch of Gewang stem by rumen microbial enzymes for 24 hours and pepsin enzymes from post-rumen digestive organs for 24 hours. The treatments applied were all treatments on the determination of dry matter loss and nitrogen fixation plus control treatment, namely a mixture of urea-pith starch of Gewang stem without cooking treatment (po), i.e :

- p_0U0 = 0% urea in dry matters of pith starch of Gewang stem without cooking
- p0U2 = 2% urea in dry matters of pith starch of Gewang stem without cooking
- p_0U_4 = 4% urea in dry matters of pith starch of Gewang stem without cooking

The experimental design used was a completely randomized design with a 4 x 3 factorial pattern with 3 replications. The first factor is the length of cooking time (p_0, P0, P1, P2) and the second factor is the level of urea (U0, U2, U4). The relationship between the two treatment factors and each observed variable used orthogonal polynomial regression analysis.

The working procedure in this experiment is a modification of Tilley and Terry's two-stage method [14], as follows :

- Weigh 1.0 g of dry matter sample and put in 1 mL of McDougall buffer solution with pH 8.9 and sheep rumen fluid inoculum 10 mL.
- The fermenter is put into a "water bath" with a temperature of 40oC and CO2 gas flowed into the fermenter, then covered with a ventilated rubber prop.
- After 24 hours of incubation, the fermentation process was stopped by adding 0.2 mL of saturated HgCl2 to kill microbes.
- The fermentation product was then centrifuged at a speed of 16.400 rpm for 25 minutes, then the precipitate was incubated again with 20 mL of 0.2% pepsin solution for 24 hours in an open state. The rest of the digestion product was then filtered through Whatman filter paper number 41 with the assistance of a vacuum pump and washed with 25 mL of hot water.
- The filter results and filter paper are put into a porcelain dish. The residual water content was evaporated in an oven at 105°C for 24 hours to analyze the residual dry matter. Organic matter was analyzed by means of igniting the residue in an electric furnace for 8 hours at a temperature of 650o C. As a blank is the fermentation residue without a substrate. The dry matter digestibility coefficient and organic matter digestibility coefficient are obtained using the formula:

$$\text{Dry Matter Digestibility (DMD) (\%)} = \frac{\text{Sample Dry Matter} - (\text{Remaining DrMatter} - \text{Blank Dry Matter})}{\text{Sample Dry Matter}} \times 100$$

$$\text{Organic Matter Digestibility (OMD) (\%)} = \frac{\text{Sample Organic Matter} - (\text{Remaining Organic Matter} - \text{Blank Organic Matter})}{\text{Sample Organic Matter}} \times 100$$

2.2. Data Analysis

The experimental design used was a completely randomized design with a factorial pattern [15]. 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, an orthogonal polynomial regression test was used and the differences between treatments were tested using Duncan Multiple Range Test.

3. Results and discussion

3.1. Loss of Dry Matter and Nitrogen Fixation/Bonding

The average dry matter loss due to cooking treatment and urea level in the combined product of urea-pith Gewang starch produced are listed in Table 1.

Table 1 Average Dry Matter Loss in the Cooking Product of Urea with Pith Gewang Starch (%)

Cooking Time, Hour P	Urea Level (U)		
	0% (U0)	**2% (U2)**	**4% (U4)**
0 (P0)	A	A	A
	6.61^{a}	5.07^{b}	4.39^{b}
1 (P1)	B	B	B
	12.93^{a}	9.60^{b}	7.55^{c}
2 (P2)	C	C	C
	9.60^{b}	13.19^{b}	10.62^{c}

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

The data in Table 1 shows that the increase in cooking time tends to increase dry matter loss but the increase in urea content in fact reduces dry matter loss from the combined product of urea-pith Gewang starch. The results of the analysis of variance showed that there was a very significant interaction (P<0.01) between the factor of cooking time and the level of urea on the dry matter loss of the combined product of urea and pith Gewang starch. The increase in the level of urea in the dry matter of pith Gewang starch will reduce the dry matter loss due to cooking time and this results in a linear pattern becoming quadratic at 0 to 2 hours of cooking time. According to Chantaro and Pongsawatmaint [16], heating is an important step in preparing starch-based products, because it will affect product quality due to gelatinization and starch degradation.

Changes in dry matter loss followed a linear pattern for the cooking time factor, while for the urea level factor the linear pattern changed to a quadratic pattern. The results of orthogonal polynomial regression analysis of the relationship between cooking time (P, hours) and urea level (U, %) on dry matter loss (Y, %) follow the equation: $Y = 6.825 + 6.132P – 1.498U + 0.237U^2 – 0.811PU$ ($R^2 = 0.98$). According to Harper [17], granule expansion occurs when starch is heated with water and the hydrogen bonds that stabilize the double helix structure in the crystal are broken and replaced by hydrogen bonds with water. The existence of this development will suppress the granules from the inside so that the granules will break and starch molecules, especially amylose will come out. The more amylose molecules that come out of the starch, then the higher the solubility. This is what causes when the cooking time is longer at high temperatures, the dry matter that is lost increases. In the gelatinization mechanism, water slowly imbibites back and forth into the granules and with increasing temperature the granules will expand rapidly and the birefringence properties will be lost so that the starch molecules diffuse out. When starch molecules are heated in excess of water, the semi-crystalline structure will break and the water molecules will be bonded by hydrogen bonds with hydroxyl groups that are broken down from amylose and amylopectin molecules [18] and [19]. The level of urea given can still inhibit the rate of dry matter loss to be smaller until the cooking time is 2 hours. The graph of the relationship between cooking time and urea level on dry matter loss of the combined product urea-pith Gewang starch is shown in Figure 1.

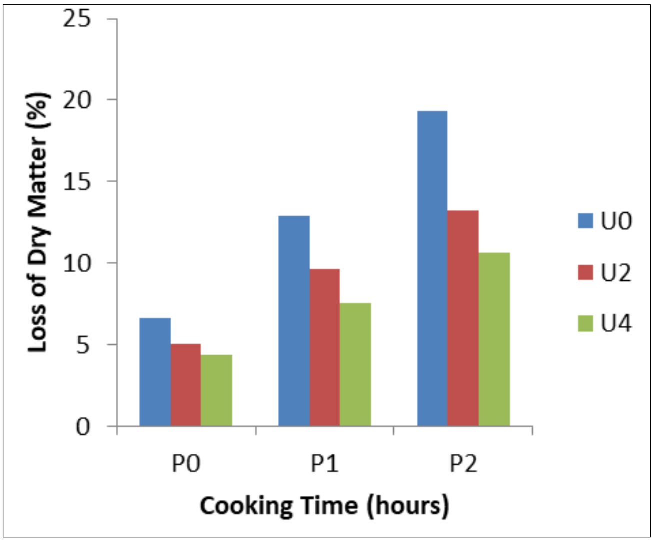

Figure 1 The relationship between cooking time (P, hour) and urea level (U, %) on dry matter loss of the combined product of urea-pith Gewang starch

In the process of cooking a mixture of urea with pith Gewang starch, urea will experience hydrolysis due to heat becoming ammonia and cyanide acid. Nitrogen compounds from urea will react with starch from pith Gewang starch and form carbamate starch compounds [19]. The level of carbamate increases according to the increase in urea concentration and also the temperature and length of reaction time. According to Khalil et al. [20], the solubility of carbamate starch samples depends on the concentration of urea, temperature and reaction time according to the type of starch used. The solubility that occurs with an increase in the heating causes amylose experiencing depolymerize. This condition will cause the amylose molecules produced to be simpler, namely there are short straight chains so that they are easily soluble in water.

An increase in the level of urea will reduce the loss of dry matter in the combined product of urea-pith Gewang starch. This means that the nutrients present in the dry matter can also be more retained in this combined product. In this study, the highest dry matter loss was indicated by the cooking treatment for 2 hours. During this cooking time, the addition of urea level was not able to inhibit the loss of dry matter of the combined product compared to the lower cooking time. The results of this study differ from those reported by Hangewa [21], where sago pulp cooked for more than 3 hours in a rotating autoclave experienced the highest dry matter loss. The existence of differences with this study, it is suspected that the starch granules between sago pulp and pith Gewang starch have different qualities [22].

The cooking treatment 0 hour with 0% urea level (P_0U_0) resulted in a combined product that had a significantly higher dry matter loss than the use of 2% and 4% urea levels. The use of urea levels of 2% and 4% dry matter loss of the combined product is not significantly different. In the treatment of 1 hour and 2 hours of cooking time with urea levels of 0%, 2%, and 4%, the dry matter loss of the combined product was significantly different. These results illustrate that the combined product of cooking results in the absence of urea, a lot of dry matter is lost. The loss is caused by the breakdown of starch granules of pith Gewang starch so that a lot of amylose and other nutrients that are soluble in water are lost. The increase in urea level in the dry matter of pith Gewang starch allows a lot of nitrogen from urea to enter together with water into the starch granules and inhibits the release of easily soluble nutrients into the solution system so that less dry matter is lost. Treatment of cooking time of 2 hours at each level of urea resulted in the highest dry matter loss in the combined product. According to Mcready [23], in the gelatinization mechanism, water slowly imbibites back and forth into the granules and with increasing temperature, the granules expand rapidly and the birefringence properties are lost so that the starch molecules diffuse out.

The average nitrogen fixation due to cooking treatment and the level of urea in the combined product of urea-pith Gewang starch produced are listed in Table 2.

Table 2 Average Nitrogen Fixation of Cooking Product of the Urea with Pith Gewang Starch (%)

Cooking Time, Hour P	Urea Level (U)		
	0% (U0)	2% (U2)	4% (U4)
0 (P0)	A	A	A
	0.86a	1.05b	1.14c
1 (P1)	B	B	B
	0.94a	1.69b	1.88c
2 (P2)	C	C	C
	1.09a	1.93b	2.15c

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

The research results showed that an increase in urea levels and cooking time resulted in an increase in the bound nitrogen in the combined product of urea-pith Gewang starch. Cooking at high temperatures, some nitrogen can be obtained through the reaction of urea with the carbonyl group of starch molecules and the amount of nitrogen bound in the combined product of urea and starch can reach the equivalent of 40% crude protein. In this study, the nitrogen bound in the combined product was highest in the 2-hour cooking treatment with 4% urea level, namely amounted to 2.15% N or equivalent to 13, 44% crude protein. Similar to the process of extruding urea with starch where urea in the form of a crystalline structure changes to non-crystalline and is found in gelatinized starch [24].

The purpose of cooking these two materials is to improve the quality of pith Gewang starch and the utilization of urea can be achieved because nitrogen bound from urea in the combined product causes the release of ammonia to be slow and in accordance with the availability of carbon frame and energy from pith Gewang starch. This combined product can later be utilized efficiently by rumen microorganisms for the synthesis of its body proteins. The lack of synchronization between rumen ammonia production and fermented energy availability has a negative effect on the efficiency of microbial protein production [25]. The relationship between cooking time and urea level on nitrogen fixation in this combined product is shown in Figure 2.

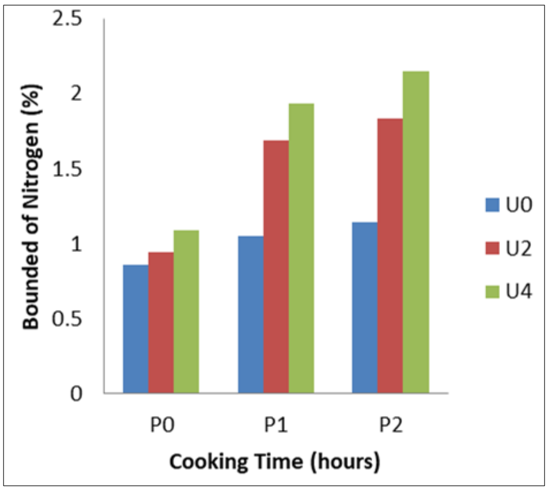

Figure 2 Relationship between cooking time (P, hours) and urea level (U, %) on the nitrogen fixation of the combined product of urea and pith Gewang starch

From the figure, it can be seen that the treatment of cooking time and urea level affected the nitrogen fixation of the combined product of urea – pith Gewang starch following a quadratic pattern, namely the nitrogen bound in the combined product of urea – pith Gewang starch increased in line with the increase in cooking time and urea level. The results of polynomial regression analysis showed the relationship between urea level and cooking time on the nitrogen fixation of the combined product of urea - pith Gewang starch following the equation: $Y = 0.759 + 0.422P – 0.307U – 0.054U + 0.098PU$ ($R = 0.97$). In this study, the highest nitrogen bound in the combined product was 2.15% at a cooking time of 2 hours (P2U4). The results of this study are lower than those obtained by Shultz, et al. [26] on a mixture of urea - cassava starch with an urea level of 5% which was cooked for 4 hours at a temperature of 170° C in a rotating autoclave produced bound nitrogen of 2.5%. The difference is due to the different levels of urea and starch granules.

The results of the variance test showed that the interaction of the treatment of cooking time and urea level was very significant (P<0.01) on nitrogen fixation in the combined product of urea – pith Gewang starch. From Table 2, it can be seen that in the treatment for cooking time of 0 hours, 1 hour and 2 hours with urea levels of 0%, 2% and 4% showed significant differences on the nitrogen fixation of the combined product of urea – pith Gewang starch. At each cooking time, it was seen that the urea level of 4% showed the highest nitrogen fixation. This will be advantageous because the release of energy and carbon frame from starch will coincide with the hydrolysis of urea and synchronization of the release rate of the two products will result in optimal utilization of ammonia. This result is similar to the extrusion process [27; 28] which causes the incorporation of urea into the starch structure, reducing its solubility.

3.2. Dry Matter Digestibility

The dry matter digestibility of the combined product of urea-pith starch of Gewang stem was measured during the fermentation of microorganism enzymes in the first 24 hours and pepsin enzymes for the next 24 hours. The average dry matter digestibility value of the combined product is listed in Table 3.

Table 3 Average Digestibility of Dry Matter of the Cooking Product of Urea with Pith Gewang Starch due to the Treatment of Cooking Time and Urea Level (%)

Cooking Time, Hour P	Urea Level (U)		
	0% (U0)	2% (U2)	4% (U4)
0 (p0)	A	A	A
	32.39^{a}	35.54^{b}	37.38^{b}
0 (P0)	B	B	B
	44.73^{a}	50.17^{b}	57.40^{c}
1 (P1)	B	C	C
	47.03^{a}	67.30^{b}	71.40^{c}
2 (P2)	B	C	C
	48.28^{a}	68.58^{b}	73.09^{c}

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

The dry matter digestibility value obtained from this study showed that the increase in dry matter digestibility of the combined product of urea-pith Gewang starch was in line with the increase in cooking time and urea level. The results of statistical analysis showed a very significant interaction (P<0.01) on the dry matter digestibility of the combined product. As the cooking time increases, the urea level increases, the dry matter digestibility of the combined product increases. The results of this study are similar to those reported by Hangewa [21], that cooking time and urea level in the urea-sago pulp complex product increase dry matter digestibility.

The dry matter digestibility value in the treatment of a mixture of uncooked urea-pith Gewang starch (p_0) with an urea level of 0% was lower than an urea level of 2% and 4%; while between 2% and 4% urea levels did not show a difference. Treatment of cooking time 0 hours, 1 hour and 2 hours with urea levels 0%, 2%, and 4%, dry matter digestibility showed a significant increase. Viewing from the treatment of cooking time and without cooking, the dry matter digestibility of the combined product increased with the increase in the urea level. It can be explained that through the cooking process of urea-pith Gewang starch, dry matter loss can be inhibited, especially those from soluble carbohydrates so that a lot of dry matter is available for microorganisms to digest. Treatment of 2% and 4% urea levels with a cooking time of 0 hours, the dry matter digestibility was significantly lower than the cooking time of 1 hour and 2 hours. This shows that with increasing cooking time, starch granules expand so that it becomes easier for microbial enzymes, especially α-amylase and β-amylase enzymes from amylolytic bacteria to penetrate into starch granules and utilize amylose and ammonia which have been bound in the granules so that the dry matter digested increases. According to Van Soest [22], the cooking process of plant starch causes gelatinization which will increase the rate of digestion and increase the efficiency of non-protein nitrogen. During the gelatinization process, the starch granules absorb water so that they become swollen and some of the amylose then radiates out and the "birefringence" will be lost so that it becomes easier for the enzymes of microorganisms to degrade it.

The highest dry matter digestibility of the combined product of urea-pith Gewang starch was shown by the treatment with a cooking time of 2 hours with an urea level of 4% (P2U4), namely equal to 73.09%. The greater dry matter digestibility of the combined product than the control indicates that the combination of urea with the cooked pith Gewang can increase the synchronization of the resulting products (ammonia and energy) which will stimulate an increase in digestibility by microorganisms in the rumen. This dry matter digestibility number corresponds to the digestibility of starch in the rumen calculated by Waldo [29] namely an average of 72.3%, but this value varies depending on the type of processing of the starch source. In general, an increase in processing will increase the digestion of starch in the rumen, decrease the rate of starch to the small intestine, and decrease the percentage of starch available for digestion in the small intestine; Thus the digestion products of starch will be available for rumen microorganisms. According to Owens et al. [30], starch digested in the small intestine provides 42% higher energy than starch digested in the rumen. However, the decrease in starch digestibility in the rumen will reduce the amount of easily available energy for rumen microbes and will reduce the supply of microbial protein for livestock. Figure 3 shows the relationship between cooking time and urea level on dry matter digestibility of the combined product of urea-pith Gewang starch.

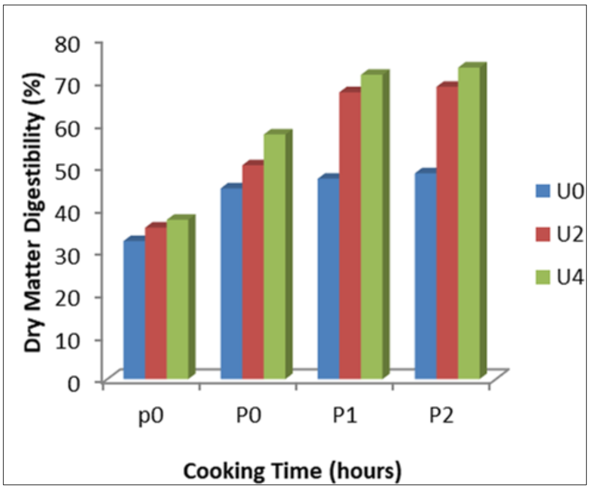

Figure 3 The relationship between cooking time (P, hours) and urea level (U, %) on dry matter digestibility of the combined product of urea-pith Gewang starch

Figure 3 shows that the factor of cooking time and urea level have shown the same response to the increase in dry matter digestibility. The increase in dry matter digestibility of the combined product is also in line with the increase in urea level. The results of orthogonal polynomial regression analysis of the relationship between cooking time (P, hours) and urea level (U, %) on dry matter digestibility (Y, %) follow the equation: $Y = 42.131 + 14.776P – 6.135P^2 + 8.507U – 1.257U^2 + 1.673PU$ ($R^2 = 0.95$). The change in the linear pattern of cooking time to a quadratic pattern is caused by a linear to quadratic change in the urea level used. From this regression equation, it is assumed that at the optimum urea level of 3.384% and the optimum cooking time of 1.832 hours will produce a maximum dry matter digestibility of 73.395%.

3.3. Organic Matter Digestibility

The digestibility value of organic matter of the combined product of urea-pith Gewang starch from the combination of cooking time and urea level is shown in Table 4.

Table 4 Average Digestibility of Organic Matter of Cooking Products of Urea with Pith Gewang Starch due to the Treatment of Cooking Time and Urea Level (%)

Cooking Time, Hour P	Urea Level (U)		
	0% (U0)	2% (U2)	4% (U4)
0 (p_0)	A	A	A
	31.11[a]	32.46[a]	36.37[b]
0 (P0)	B	B	B
	40.20[a]	46.51[b]	49.14[c]
1 (P1)	B	C	C
	42.37[a]	60.26[b]	61.26[b]
2 (P2)	B	C	C
	43.63[a]	60.56[b]	62.03[b]

Information: 1) the same lowercase letters in the same row 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 statistical analysis showed that there was a very significant interaction effect (P<0.01) from the treatment of cooking time and urea level on the digestibility of organic matter of combined product of urea-pith Gewang starch. The highest average organic matter digestibility was achieved by the 2-hour cooking time treatment with a urea level of 4% (P2U4), namely equal to 62.03%.

The results of Duncan's test showed that in the mixture of uncooked urea-pith Gewang starch (p_0) with an urea level of 0%, the digestibility of organic matter was not significantly different from the 2% urea level, but between 0% and 2% urea levels compared to 4% urea levels, there was a significant difference. In the 0 hour cooking time (P0) treatment with 0% urea level, the digestibility of organic matter produced was significantly lower than 2% and 4% urea levels, while 4% urea levels were significantly higher than 2% urea levels. In the treatment of 1 hour and 2 hour cooking time with 0% urea level, the organic matter digestibility was significantly lower than the 2% and 4% urea levels, while the 2% and 4% urea levels did not differ. With the increase in the level of urea, the digestibility of organic matter of the mixture of urea-pith Gewang starch is higher at various cooking times.

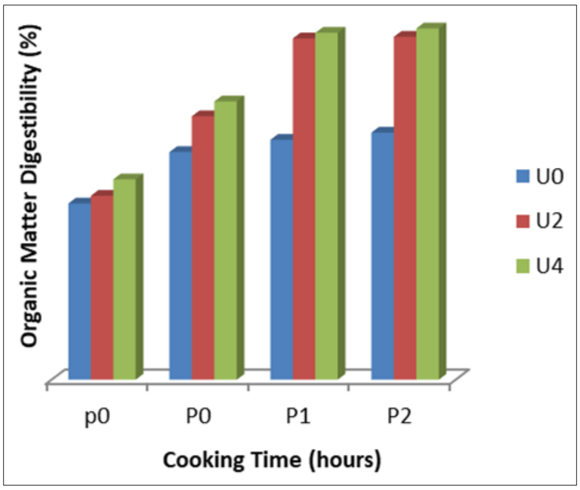

Figure 4 The relationship between cooking time (P, hours) and urea level (U, %) on the digestibility of organic matter of the combined products of urea-pith Gewang starch

The treatments for urea levels of 0%, 2%, and 4% without cooking (p_0) as well as the cooking time of 0 hours, 1 hour and 2 hours, had the same pattern of differences, namely between the cooking time of 0 hours with 1 hour and 2 hours the digestibility of organic matter is significantly different while between 1 hour and 2 hours of cooking time did not show a significant difference. However, the digestibility of the organic matter of all combined products was higher than the mixture of uncooked urea-pith Gewang starch (p_0). This shows that the resulting combined product is more capable of providing the products needed by microorganisms compared to the control treatment and especially in providing products containing ammonia and energy in harmony.

The results of this study are in accordance with those reported by Shultz et al. [26], by "in vivo" (in sheep) where the digestibility of organic matter from a mixture of urea cassava cooked in an autoclave at 170° C was higher than a mixture of the two ingredients that were not cooked or cassava alone without a mixture of urea. From the research results, it can also be explained that the carbon frame which is essential for the formation of branched-chain fatty acids can be obtained through the synthesis of non-cellulolytic microbial protein from carbohydrates supplemented with urea.

Organic matter digestibility of the combined product of urea-pith Gewang starch resulting from the relationship between the treatment of cooking time and urea level is illustrated in Figure 4.

In Figure 4, it can be seen that there is a change in the urea level from linear to quadratic caused by linear and quadratic changes in the cooking time treatment. The results of orthogonal polynomial regression analysis showed the relationship between cooking time (P, hours) and urea level (U, %) on organic matter digestibility following the equation: $Y = 38.260 + 12.242P – 5.061P^2 + 8.513U – 1.501U^2 + 1.341PU$ (R^2= 0.96).

The Increase in organic matter digestibility of the combined product of urea-pith Gewang starch will increase the digestibility of starch not only in the rumen but also in the small intestine, as stated by Owens et al. [30]. The low digestibility of organic matter from the control (without cooking) is probably due to the fact that ammonia from urea is hydrolyzed faster than the carbon frame provision by pith Gewang starch, so that microorganisms are unable to take advantage of the ammonia produced. So even though a lot of N from urea is available but cannot be utilized by microorganisms because of the unavailability of energy or carbon frame from carbohydrate.

4. Conclusion

The cooking of urea with pith Gewang starch in an autoclave resulted in the combined product that was low in dry matter loss and high nitrogen fixation, as well as high in vitro dry matter and organic matter digestibility. Cooking time of 2 hours with urea level of 4 percent is the best combination in producing in vitro nutritional value of the combined product of urea-pith Gewang starch. This combined product is categorized as a slow-release urea product.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no competing interests.

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