Slow release urea nitrate with starch polymer coating technique for fermentation and rumen digestibility in vitro

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Abstract. 7KH SUHVHQW VWXG\ ZDV DLPHG WR HYDOX
VWDUFK SRO\PHU FRDWLQJ WHFKQLTXHV XVHG 7KHUH GLYLGHG LQWR
VDJR VWDUFK DQG UDQGRPL]HG EORFN GHVLJQ
ZDV XWLOL]HG LQ WKLV VWXG\ DQG FRQVLVWHG RI I
UHSOLFDWLRQV 7KH WUHDWPHQWV DUH 7 FRQWURO GLHW
XQFRDWHG XUHD 7 QLWUDWH 7 FRDWHG XUHD
QLWUDWH ZLWK VDJR VWDUFK 7 FDVVDYD VWDUFK
FRDWLQJ XUHD QLWUDWH VXSSOHPHQWDWLRQ WUHDWPHQW
FRDWLQJ ZHUH H[SHFWHG WR SURGXFH EHWWHU GLHW
FRQWURO GLHW DQG VORZ GRZQ WKH UHOHDVH
5HVXOWV UHYHDOHG WUHDWPHQWV UHGXFHG DPPRQLD
FRPSDUHG WR 7 DQG 7 7 7 3 +RZHYHU RWKHU
SDUDPHWHUV VXFK DV UXPHQ S+ SURILOH IDW
V\QWKHVLV GU\ PDWWHU GLJHVWLELOLW\ RUJDQ
JDV SURGXFWLRQ PHWKDQH SURGXFWLRQ ZHUH VLPLOD
WUHDWPHQWV ,Q FRQFOXVLRQ VXSSOHPHQWDWLRQ ZLWK
FDVVDYD VWDUFK FRDWLQJ LQ UXPLQDQW GLHW P
RI DPPRQLD LQ WKH UXPHQ LQ YLWUR

1 Introduction

Ruminant livestock ruminant livestock productivity is still not optimal in Indonesia needs to improve feed quality. Agricultural waste products it is quite abundantly used as animal feed. Feeding in the form of agricultural crop residues usually have low nutrient content, especially the protein content so that livestock obtain feed from waste agriculture over a long period of time produces livestock productivity low and disrupt the balance of livestock protein needs [1]. The main problem in providing protein source feed is that it is relatively difficult and expensive so protein supplementation is needed to make efficient use of ingredients protein source feed in increasing ruminant livestock productivity. on the other hand, the livestock sector, especially ruminants, contributes to accumulation atmospheric methane and enteric

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fermentation contributed 17% of global methane sources [2] that impacted the considerable loss of food energy that should be reduced to productivity [3].

Supplementation urea high non-protein nitrogen (NPN) material to increase the nutrient value. NPN's readily accessible source at affordable prices is urea. Urea can be used by domestic cattle in protein formation. Urea's use of urea in ruminate nutrition is limited because its rapid hydrolysis produces nh3 in the rumen, surpassing the rate of carbohydrate in the rumen. Furthermore, rapid urea hydrolysis in the rumen can reduce the efficiency of nitrogen use and increase the excretion of nitrogen [4]. Moreover, nitrates are one of the potential sources of non-protein nitrogen for cattle containing the nitrogen element. Nitrates as effective methane inhibitors, acting as hydrogen acceptors in nitrate reduction to ammonia in the rumen, are one of the mechanisms used to reduce the metanogen rumen. When the reduction of incomplete microbial nitrates results in an accumulation of nitrants that can cause poisoning in livestock [5]. Therefore, the use of up risk nitrate as if it needs attention to the toxicity of ammonia in the rumen. Urea has been widely used in animal feed, but the hydrolysis of urea is fast so slow release technology is needed to inhibit the degradation of urea-nitrate in the rumen, one of which is by coating or coating techniques with biopolymer materials from starch. Starch is a natural polymer that is widely available from several types of plants and the product is quite easy to obtain on the market. Starch has been widely used in the food and pharmaceutical industries as a coating material [6]. Starch has potential as a urea-nitrate coating material because starch is not water soluble, but if the starch suspension is heated gelatinization will occur, the gelatination process causes the starch granules to expand and amylose is released. Changes occur during cooling and storage gelatinized starch is called retrogradation. During the retrogradation process, amylose bonds form again resulting in crystallization be strong.

2 Materials and Methods

The materials used in the present study were urea, nitrate, sago starch, cassava starch and rumen fluid. *In vitro* treatments were separated as a control group used only basal diet and experimental groups used basal diet supplemented with different urea and nitrates. Basal diet composed of forage and concentrate (40:60). The present experiment was carried out in two stages: the slow-release ureanitrate preparation stage and the *in vitro* analysis stage. The nutrient composition of the basal diet used showed in the Table 1.

Table 1. Nutrient composition of basal diet

Nutrien Composition (%DM)	Basal diet
Dry Matter	90.12
Ash	8.83
Crude Protein	14.93
Either Extract	2.69
Crude Fiber	15.19
NFE	48.48
TDN	59.73

2.1 Slow-release urea-nitrate preparation

The urea-nitrate was formed by the combination of urea and KNO3 salt granules. The preparation of slow-release urea-nitrate starch was following the Himmah. The urea-nitrate was formed by the combination of urea and nitrate granules, 6% w/v starch and RED gr mixed using a homogenizer then dried in powder form using a machine spray drying with an inlet temperature of 120ºC and an outlet temperature of 80ºC.

2.2 In vitro experiment

The present study was performed by 5x4 (treatments x bottles) experimental design (20 bottles in total) and was completed in two runs. Experimental treatments were: Control group that used a basal diet (T0), and experimental treatments that were used the basal diet + urea group (T1); basal diet + urea-nitrate (T2); basal diet + coating urea-nitrate with sago starch (T3); basal diet + coating urea-nitrate with cassava starch (T4). Control group were inserted with 0.75 g as fed basal diet consisted of elephant grass: concentrate 40:60 w/b, while the experimental groups basal diet (also 0.75 g as fed in each bottle) were added with 6.9 g/kg DM urea (T1), 12.2 g/kg DM urea-nitrate (T2), 18.9 g/kg DM coating urea-nitrate with cassava starch (T3), and 1.95 g/kg DM coating urea-nitrate with sago starch (T4). The in vitro experiment was performed following Theodorou *et al.* [7] protocol. Each experimental diet (T0, T1, T2, T3, and T4) was inserted in a 100 mL bottle. Each bottle was flowed with CO_2 and was added approximately 25 mL of rumen fluid and 50 mL of McDougall's solution that were flowed with CO_2 gas. Each bottle then immediately sealed with a rubber cap and aluminum then sealed using a crimper. All bottles then were directly putted into the thermostatic water bath at 39°C. Gas production of each bottle was measured at 2, 4, 6, 8, 10, 12, and 24 h. Methane gas sample was collected from the collected gas that previously transferred in the vacuum bottle. Each buffered rumen fluid in the bottle then transferred into 50 ml falcon and was centrifuged at 2500 rpm for 10 minutes. Several amount of buffered rumen fluid supernatant was taken to analyze ammonia (NH_3) microbial protein synthesis, and total volatile fatty acid (VFA). Digestibility and fermentability samples were carried out after buffered rumen fluid and precipitate residue was reincubated for 24 h after added with pepsi reagent. After another 24 h, precipitate residue was analyzed for its dry matter digestibility (DMD) and organic matter digestibility (OMD).

2.3 Data analysis

The research experiment used a randomized block design (RBD) with four replications. Data were analyzed using ANOVA and continued with Tukey's test. The observed variables are dry matter digestibility, organic matter digestibility, NH_3 production, total VFA, microbial protein synthesis values, total gas digestibility values, and methane gas production. The data obtained were statistically analyzed through ANOVA followed with Duncan test to observe significant differents among treatments using the SPSS 25 statistical software.

3 Results and Discussion

The results of in vitro fermentability analysis of rations with the addition of urea-nitrate coating using sago starch or cassava starch can be seen from the pH value, dry matter digestibility (DMD), organic matter digestibility (OMD), analysis of ammonia concentration (NH_3), concentration total volatile fatty acids (VFA) and microbial protein synthesis (MPS). The addition of urea-nitrate coating cassava starch (T4) can significantly reduce the ammonia concentration in the rumen. This is caused by cassava starch which has the ability to protect protein from rumen microbial degradation. The concentration of ammonia in the rumen is influenced by the amount of feed protein consumed by livestock which is then degraded in the rumen. Ammonia concentration can provide an idea of how high the solubility level of crude protein is during the fermentation process in the rumen [8]. The average NH_3 concentration in all treatments was in the range of 3.59 - 4.39 mM. Significant reduction in ammonia occurred in the T4 treatment with urea-nitrate protection using cassava starch. A low NH_3 concentration value can indicate the coating's ability to protect urea-nitrate and degrade protein using a slow release technique.

Table 2. Effect of treatment on rumen fermentability

Treatment		Parameter		
	pH	NH_3 (mM)	MPS (mg/10 mL)	VFA (mM)
7	“	“ DE	“	“
7	“	“ E	“	“
7	“	“ E	“	“
7	“	“ DE	“	“
7	“	“ D	“	“
3 *Value*				

T0 = control ration; T1 = T0 + urea; T2 = T0 + urea-nitrate; T3 = T0 + coating urea-nitrate with sago starch; T4 = T0 + coating urea-nitrate with sago starch
NH_3: ammonia concentration; VFA: volatile fatty acids; MPS: microbial protein synthesis.
Different superscripts in the same row with different letters showed significant differences (P<0.05).

Although urea-nitrate supplementation had no significant different compared to the control group, their MPS and VFA concentration still within the normal ranges. Although increased NH_3 concentration may affect in vitro rumen digestibility, no significant changes concerning in vitro rumen DM and OM digestibility by urea-nitrate and slow-release urea-nitrate supplementation. The composition of feed ingredients, the treatment given, the level of feeding can also affect digestibility and low microbial activity in the rumen, causing the ration used to have low digestibility [9].

Table 3. Results of measurements of dry matter digestibility and organic matter digestibility

Treatment	Parameter	
	DMD (%)	OMD (%)
T0	47.38 ± 6.50	45.68 ± 5.90
T1	50.88 ± 3.95	49.82 ± 4.00
T2	49.42 ± 3.15	48.24 ± 3.45
T3	49.04 ± 4.84	47.67 ± 4.84
T4	48.40 ± 3.44	47.27 ± 3.91
P-*Value*	0.581	0.388

T0 = control ration; T1 = T0 + urea; T2 = T0 + urea-nitrate; T3 = T0 + coating urea-nitrate with sago starch; T4 = T0 + coating urea-nitrate with sago starch. DMD: dry matter digestibility; OMD: organic matter digestibility.

High gas production suggests that fermentation on the rumen is also increasing (*nurussilmah*). The higher the methane yield, the more wasted energy that causes lack of feed efficiency. The percentage of methane gas production varies depending on various factors such as the breed and type of livestock, the organic material content in the feed, and environmental conditions of the rumen [10].

Table 4. Data on total gas production and methane gas

Treatment	Parameter	
	Total gas production (mL)	Methane gas (mL/L)
T0	105.08 ± 26.74	4.27 ± 1.35
T1	101.43 ± 30.47	4.31 ± 0.84
T2	96.73 ± 27.11	5.34 ± 0.87
T3	110.95 ± 28.24	4.86 ± 0.44
T4	89.82 ± 17.62	4.11 ± 0.86
P-*Value*	0.362	0.668

T0 = control ration; T1 = T0 + urea; T2 = T0 + urea-nitrate; T3 = T0 + coating urea-nitrate with sago starch; T4 = T0 + coating urea-nitrate with sago starch.

4 Conclusion

The utilization of sago starch and cassava starch as urea coating has no impact on fermentation, digestibility and methane gas production in the rumen in vitro. However, urea nitrate coating with cassava starch was proven to reduce NH3 concentrations.

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