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Use of fermentable carbohydrate in efforts to improve in vitro rument fermentation products

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Abstract Global warming increases as the gas concentration rises into the atmosphere such as N-NH_3, CO_2, CH_4, carbon (c), Florine, Chlorine, and Bromine. One method used to overcome this problem is to use additive feed ingredients containing carbohydrates *fermentable*. This study aims to examine the use of carbohydrates *fermentable* in ammonia of rice straw in an effort to improve rumen fermentation products *in vitro* to the concentration of N-NH_3 and total VFA concentration. The experiment was carried out using 1000 g of dry ingredients of rice straw, 2%carbohydrates *fermentable*, 4% urea, and 10% water. Carbohydrate *Fermentablebalance* in ammoniated rice straw with 40: 60 concentrate and divided into four treatments, namely R_0: rice straw without carbohydrate *fermentable*, R_1 : onggok, R_2 : cassava, R_3: bran and R_4 soun waste and five replications. Data were analyzed using Honest Real Difference Test (BNJ) variance. The results showed that the highest VFA concentration was found in group R_4 (47.5 mM) and the lowest in group R_1(15.0 mM). The highest N-NH_3 concentration was found in group R_2 (9.8 mM) and the lowest in group R_4 (6.0). This study concluded that absorption of VFA in the rumen was much better in group R_1 and absorption of N-$NH_{3\ was}$ much better in group R_2.

1. Introduction

Global warming is one of the most important problems because around 60 - 70 percent of NH_3 comes from ammonia to the atmosphere which causes depletion of the ozone layer so that it can threaten life in the world, due to global warming various adverse effects arise which are problems environment [1]. One way that is used to reduce high gas N-NH_3 and CO_2 are wasted free to air is to use feed additive ingredients containing a source of carbohydrate *fermentable* in the process of ammoniation are the easily fermented height that can bind gas N-NH_3 and CO_2 wasted into the atmosphere [1,2]. Ammonia is a process of preserving feed ingredients by using spontaneous work of lactic acid fermentation under conditions *anaerobic*. Epiphytic lactic acid bacteria (LAB) carbohydrates *fermentable* into lactic acid and a small portion is converted to acetic acid [3].

Addition of additives in the form of carbohydrates *fermentable* such as onggok, cassava, bran, and soun waste are used to accelerate the ammonia process and decrease pH so that acidic conditions can be obtained, and as an energy source for the growth of lactic acid-forming bacteria that can use excess NH_3 when ammonia with urea then the function of urea is to destroy the lignin, cellulose and silica bonding tissue which causes increased ammonia digestibility of rice straw due to loosening of lignocellulose and lignohemellulose bonds and increasing non-protein nitrogen (NBP), protein and non-protein nitrogen (NPN) are needed for the synthesis of rumen microbial proteins [2–6]. Rumen microbes will hydrolyze non-protein proteins and nitrogen (NPN) into peptides and amino acids that are easily degraded to ammonia (NH_3). This easily fermented ammonia is used by rumen microbes as the main nitrogen source for microbial protein synthesis.

2. Methods
Study was conducted at the Laboratory of Animal Food Nutrition Science (ITMT) of the General Sudirman University in Purwokerto from July to September 2015. Proximate analysis: VFA and N-NH_3 at the Animal Food Nutrition Science Laboratory (ITMT) Jenderal Sudirman University Purwokerto. The variables measured in the study are rumen fermentation products which include: Total VFA concentration and N-NH concentration$_3$. The design used is Complete Random Design (CRD) according to the method [7]. This study consisted of 5 treatments and 4 replications. Each treatment was calculated based on dry matter in proportion to ammoniated rice straw which was added *fermentable* carbohydrate with 40:60 concentrates, namely R_0 control; R_1 onggok; R_2 cassava; R_3 bran; R_4 soun waste. The treatment of each sample can be tested as follows:

1) 1000 g BK JP A + 4 % Urea + 10 % Air + No f*ermentable carbohydrates*
2) 1000 g BK JP A + 4 % Urea + 10 % Air + 2 % Onggok
3) 1000 g BK JP A + 4 % Urea + 10 % Air + 2 % Gaplek
4) 1000 g BK JP A + 4 % Urea + 10 % Air + 2 % Dedak
5) 1000 g BK JP A + 4 % Urea + 10 % Air + 2 % Soun waste

2.1. Making ammonia straw flour the
Experimental preparation for making ammoniated rice straw flour with the following stages (1) Preparation of the tested material and tools used (2) ammonia material as for the work order: After the 21-day saoked time is finished then the bags of ammonia material opened and aerated under the sun for 2 hours to reduce the water content and ammonia level, then the ammonia material was weighed for each treatment as much as 1 g then the ammonia material was put into the oven at 50^0 C for 24 hours to remove the water content. After drying, the ammoniated material is blended or smoothed to flour after it is filtered to get smoother results.

2.2. Manufacture of ammoniated straw flour
Making McDougl's solution pH 6.8 was composed of 19.8 g $NaHCO_3$, 14 g Na_2HPO_4, 7 H_2O. (2985 g, Na_2HPO_4 $2H_2O$), 1.14 g KCl 0.9433 g $MgSO_4$ 7 H_2O and 0.08 g CaCl. The ingredients are dissolved with distilled water in Erlemmeyer flask, up to 2 liters, pH is monitored to 6.8, and to determine the pH by means of CO_2 gas until the pH reaches 6.8.

2.3. Mathematical model
The mathematical model can be described as follows:

$$Y_{ij} = \mu + \tau_i + \varepsilon_{ij}$$

Description :

Y_{ij} = The variable measured from the treatment that gets carbohydrates *fermentable* at the end of the test
μ = Middle value of treatment variable
τ_i = Effect of treatment ofcarbohydrates *fermentable* to-i
ε_{ij} = Error trial
i = Number of treatment (1,2,3,4, 5)
j = Number of replications (1,2,3, 4).

1. Regarding total VFA levels can be calculated as follows:

$$\text{Total VFA} = ((Y - Z) \times N\,HCl \times (1000/5))\ mM$$

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Information :

Y: ml HCl used for blank titration

Z: ml HCl used for titillation of distillates (samples)

2. Calculation of $N\text{-}NH_3$ levels can be calculated by the formula:

Levels of $N\text{-}NH_3$ = (ml titran x N H_2SO_4 x (1000/1)) mM.

Table 1. Nutrient composition ofcarbohydrates *fermentable* used.

No	Bahan Pakan	R_0 JPA*	$R_{1\ Onggok}$**	$R_{2gaplek}$***	R_{3dedak}****	$R_{4soun\ waste}$*****
1	Crude protein	8.9	1.9	3.34	12.5	50.82
2	Dry Materials	52.47	9.28	7.48	9.10	-
3	Crude fat	-	0.3	1.16	8.20	4.93
4	Rough Fiber	-	8.9	5.50	8.00	2.32
5	Ash	15.3	2.4	2.34	-	-
6	TDN	54.17	8.3	-	64.30	-
7	Organic materials	84.7	-	-	-	-
8	NDF	89.8	-	-	-	-
9	ADF	15.0	-	-	-	-
10	BETN	-	-	-	50.9	91.41

Source : * R_0 JPA [8] ; **R_1 Onggok [9]; *** R_2 Gaplek[10];****R_3 Dedak[4] and ***** R_4 Soun waste [11].

3. Results and discussion

3.1. Production of volatile fatty acid (vfa) rumen

VFA is the end product of carbohydrate feed metabolism in the rumen which is the main energy source for ruminants and also rumen microbes. In addition, VFA is also a carbon framework for the formation of microbial proteins. The average VFA production in various treatments is shown in Table 2. The results of the variance analysis showed that the treatment had a very significant effect ($P < 0.05$) on VFA production.

Table 2. Average production of VFA, N-aNH_3, in various treatments

Data	NH_3 (mM)	VFA (Mm)
R_0	9.5[a]	37.5 [ab]
R_1	8.3[a]	15.0[b]
R_2	9.8[a]	27.5[b]
R_3	9.5[b]	40.0 [ab]
R_4	6.0[c]	47.5 [a]

Superscript: Numbers followed by letters that are not the same in the column the same shows the test results that are significantly different ($P < 0.05$).

Table 3. Average results of measurements ofcarbohydrates *fermentable* in ammoniation of rice straw with concentrations to total VFA concentrations in each treatment.

Treatments	Replication				Total	Average	Standard Deviation
	1	*2*	*3*	*4*			
R_0	40	40	40	30	150	37.5	5.0
R_1	20	10	10	20	60	15.0	5.0
R_2	30	20	30	30	110	27.5	5.0
R_3	40	50	40	30	160	40.0	5.8
R_4	50	50	40	50	190	47.5	8.2
Total					670	33.5	SD of sample =12.7

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The results of the real honest difference test showed that VFA production in R_4 glassy waste (47.5) was higher (P <0.05) than R_0 and R_3, but the same (P> 0.05) with R_1 and R_2. The high total VFA production in the treatment of R_4 soun waste (47.5) compared to VFA produced from R_0 and R_3 reflects the amount of carbohydrate ration material that is easily fermented by rumen microbes for microbial protein formation or in other words the addition of soun waste as a source the initial energy used by rumen microbes to ferment structural carbohydrates such as cellulose and hemicellulose. However, when viewed from R1 and R2 containing additives onggok and cassava, it tends to be higher than R4. This causes the activity of microorganisms in R1 and R2 to be more optimal so that the utilization of VFA in both is better than R4. Soun waste may contain chemicals or preservatives that interfere with the activity of rumen microorganisms so that their activities are not optimal which ultimately disrupts the digestion process of cellulose and hemicellulose and other nutrients. [8] This is different from rice bran which has lower fermentability, which tends to cause slower energy availability. Aside from being the main energy source for landlords, VFA is also an energy source for rumen microorganisms and their high and low levels are highly dependent on carbohydrate fermentability in the substrate or feed.

Good VFA levels for optimum rumen microbial growth are around 80–160 mM (Toha Sutardi, 1979). In this study, total VFA production ranged from 15.0. mM and 47.5 mM or was in the range below normal to support rumen microbial growth [9]. Several factors that influence the concentration of VFA include microbial utilization, absorption, and fermentability of carbohydrates [6].

3.2. N-NH_3 concentration

The average concentration of NH_3 on various additives in rice straw ammonia is shown in Table 2. The results of the variance analysis showed that the treatment had a very significant effect (P <0.01) on the concentration of N-NH_3 (Table). The production of N-NH_3 at R4 is the lowest (P <0.01) compared to R0, R1, R2, and R3. Between R0, R1 and R2 showed no significant difference (P> 0.05).

Table 4. Average results of measurement of carbohydrate use *fermentable* in ammoniation of rice straw with concentrations to N-NH concentrations$_3$ in each treatment.

Treatments	Replications				Total	Average	Standard Deviation
	1	*2*	*3*	*4*			
R0	10	9	10	9	38	9.5	0.6
R1	10	9	9	10	38	9.5	0.6
R2	9	10	10	10	39	9.8	0.5
R3	9	8	8	8	33	8.3	0.5
R4	6	6	6	6	24	6.0	0.0
Total					6172	38.6	SD sample = 1.5

Real honest difference test showed that the production of N-NH_3 in R_2 (cassava) was highest (P <0.05) compared to R_0 (Ammoniated Straw without Carbohydrates *Fermentable*), R_1 (onggok), R_3 (rice bran) and R_4 (soun waste) but between R_1 (onggok) and R_4 (soun waste) were not significantly different (P> 0.05) presented in table 2. Increased ammoniated rice straw added with cassava (R_2) caused an increase in N-NH concentration$_3$. This occurs because of the increase in NPN contained in ammoniated rice straw and all NPNs degraded by microbes in the rumen into concentrations of N-NH_3 and CO_2. This is consistent with the opinion which states that N-concentration increases NH_3. The rumen is affected by an increase in urea (NPN). In addition, this increase occurs because of the activity of microorganisms

4. Conclusion

Based on the results of the study it can be concluded that:

The use of carbohydrates *fermentable* in rice straw in the ammoniation process with the balance of ammoniated rice straw: 40: 60 percent concentrate has not been able to influence the value of N-NH_3

and Soun Waste is a carbohydrate source *fermentable* tends to increase the total rumen VFA concentration *in vitro*

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