Butyric acid and ammonia content of Napier grass silage at different cutting age and level of additive

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Abstract

The aim of the study was to determine the effect of cutting age and different level of additive on butyric acid and ammonia content of Napier grass (Pennisetum purpureum) silage. Silage was made from Napier grass with different cutting ages, namely 40, 60 and 80 days (P4, P6 and P8) as factor I and different level of additive, namely 0; 2.5; 5 and 7.5% w/w DM of fine bran (D0, D1, D2 and D3) as factor II. The study was designed in a completely randomized design (CRD) with 3x4 factorial pattern with 3 replications. Data were analyzed statistically by analysis of variance at 5% confidence level, followed by Duncan's multiple range test. The results showed that different cutting age had no significant effect ($P>0.05$) on butyric acid content, while level of additive had significant effect ($P<0.05$) on butyric acid content, and there was no interaction between cutting age and level of additive on butyric acid content of silage. Different cutting age and level of additive and had significant effect ($P<0.05$) on ammonia content, but there was no interaction ($P>0.05$) between cutting age and level of additive on ammonia content of silage. It was concluded that increasing level of cutting age decreased ammonia content, increasing level of additive decreased butyric acid and ammonia content of Napier grass silage; cutting age and additive level did not synergize in reducing butyric acid and ammonia content of silage.

Introduction

The older the plant will cause the proportion of non-structural carbohydrates to decrease, conversely the proportion of structural carbohydrates to increase [1]. The low proportion of non-structural carbohydrates is not beneficial in making silage because the content of soluble carbohydrates which are constituents of non-structural carbohydrates is very limited for lactic acid-forming bacteria to produce lactic acid. If the production of lactic acid is low, then low pH in silage making becomes difficult to achieve and causes poor quality silage due to undesirable reactions in silage making, such as the formation of butyric acid as a result of fermentation by Clostridia bacteria. Therefore, it is necessary to give additives in the form of fine bran to increase soluble carbohydrate content.

Napier grass is commonly used as silage in the tropics [2], because its high production and is favored by livestock [3]; however, Napier grass harvested at more than 80 days has a low percentage of soluble carbohydrates in fresh forage [4]. Low soluble carbohydrates in old grass can be overcome by adding additives as a source of soluble carbohydrates such as fine brand to stimulate the formation of lactic acid so that it can produce good quality silage.

Butyrate silage is formed when the silage has been fermented by Clostridia bacteria [5, 6]. The acidity of butyrate silage ranges from 5-6 and contains lactic acid and soluble carbohydrates in low concentrations [1]. The extensive breakdown of amino acid caused by Clostridia bacteria in butyrate silage in turn increases ammonia content which can exceed 200 g/kg total N.

The objective of the research was to examine the effect of different cutting age and level of additive on butyric acid and ammonia content of Napier grass silage. The benefit of the research is to determine optimum cutting age and addition of additives to produce good quality of Napier grass silage.

Materials and Methods

The study was conducted at Faculty of Animal and Agriculture Science, Diponegoro University, Semarang.

Materials

Materials used in the study were Napier grass (Pennisetum purpureum) at 40, 60 and 80 days of cutting age and fine bran as an additive. Equipment used include a silo made of a cylindrical plastic container, and equipment for analysis of butyric acid and ammonia content of silage.

Methods

Making silage includes preparing Napier grass at the cutting age of 40, 60 and 80 days; wither Napier grass until the moisture content reaches + 65%, mix Napier grass with fine bran at level 0; 2.5; 5 and 7.5% (w/w) DM of Napier grass evenly, then put into a silo and compacted so that the density was + 650 kg/m3. Ensilage was carried out for 3 weeks (21 days).
Determination of butyric acid content was carried out using gas chromatography technique which was carried out at Biochemistry Laboratory, Faculty of Agricultural Technology, Gadjah Mada University, Yogyakarta. Determination of ammonia was carried out using microdiffusion technique according to Conway [7]. The study was conducted in a completely randomized design (CRD) with a factorial pattern of 3 x 4, 3 replications. Factor I was cutting age of Napier grass, namely 40, 60 and 80 days (P4, P6 and P8). Factor II was additive level, namely addition of fine bran by 0; 2.5; 5 and 7.5% w/w DM (D0, D1, D2 and D3). Data were analyzed by analysis of variance at 5% level followed by Duncan's multiple range test [8].

Results and Discussion

Butyric acid and ammonia content of Napier grass silage was summarized in Table 1.

Table 1. Butyric Acid and Ammonia Content of Napier Grass Silage

Variable	Additive	Cutting Age			Mean	Significance		
	Level	P4	P6	P8		P	D	PxD
Butyric acid (g/kg DM)	D0	3,59	4,10	4,48	4,06[p]	ns	*	ns
	D1	4,61	2,19	4,23	3,67[pq]			
	D2	3,08	2,19	2,19	2,48[q]			
	D3	3,21	1,29	1,17	1,89[r]			
	Mean	3,62	2,44	3,01				
Ammonia (g/kg DM)	D0	40,65	39,56	39,87	40,03[p]	*	*	ns
	D1	40,18	37,52	38,93	38,88[q]			
	D2	39,56	36,73	37,04	37,78[r]			
	D3	38,14	36,89	37,52	37,52[r]			
	Mean	39,63[a]	37,67[c]	38,34[b]				

a,b,c Different superscript on the same line showed differences (P<0.05)
p,q,r Different superscript on the same column showed differences (P<0.05)
* Significance (P<0.05)
ns Non significance

Butiric Acid Content of Napier Grass Silage

Butyric acid content of silage showed an increase with increasing of Napier grass cutting age. Results of analysis of variance showed that cutting age had no effect on butyric acid content, but additive level had a significant effect (P<0.05) in reducing the butyric acid content of Napier silage. There was no interaction between cutting age and additive level on butyric acid content of Napier silage.

Butyric acid can be formed when silage is fermented by Clostridia bacteria. The presence of butyric acid showed that in this study there was a fermentation by Clostridia bacteria, but the production of butyric acid tended to decrease with increasing cutting age of Napier grass as indicated by the mean value of each cutting age, which was 3.62; 2.44 and 3.01 g/kg DM. The growth of Clostridia bacteria is thought to occur at the beginning of ensilage, when the acidity of the silage was still high, considering that Clostridia bacteria grow well at pH 5 - 6. Acidity of butyrate silage ranged from 5 - 6 and contained lactic acid and soluble carbohydrates in low concentrations [1].

The decrease in butyric acid content occurred along with the increase of additive level (P<0.05), which was seen from the mean value of the increasing of each additive level, which was 4.06; 3.67; 2.48 and 1.89 g/kg DM. Decreasing in butyric acid content between additive level of 0% was not significant when compared to the additive level of 2.5% (D0 vs D1), but there was significance (P<0.05) when compared to additive level of 5% and 7.5% (D0 vs D2 and D3). 2.5% additive level treatment was not significant compared to 5% additive level treatment (D1 vs D2), but significant (P<0.05) when compared to 7.5% additive level (D1 vs D3). Butyric acid content at 7.5% additive level was lower (P<0.05) when compared to 5% additive level (D3 vs D2).

Decreasing in butyric acid content along with the increasing in additive levels was thought to be due to an increasing in nitrogen-free extract as a provider of soluble carbohydrate. The content of nitrogen-free extract from fine bran as an additive in silage making was 51.58% DM; in addition, fine bran also contains 9.81% protein in DM which was used as a source of N by lactic acid bacteria. Soluble carbohydrate plays a role in the formation of lactic acid and acetic acid in ensilage. Increasing in soluble carbohydrate from fine bran resulted an increasing in lactic acid and acetic acid content along with the increasing in additive level which in turn lowered pH of silage. Decreasing pH affects the suppression of Clostridia growth. Suppressed growth of Clostridia also resulted in low butyric acid which was a product of fermentation by Clostridia bacteria.

Butyric acid was formed when silage fermented by Clostridia bacteria [5, 6]. Butyric acid content of silage ranged from 1.17 to 4.48 g/kg DM. The lowest butyric acid content of silage was achieved at P8D3 treatment, followed by P6D3, P6D1, P6D2 and P8D2 treatment combination (1.17; 1.29; 2.19; 2.19 and 2.19 g/kg DM).

Lactate silage generally contains small amounts of acetic acid and very small amounts of propionic and butyric acids [1]. Based on the data obtained, it was suspected that there was fermentation by Clostridia bacteria, although the activity was relatively low. However, butyric acid content of silage was still acceptable because the highest butyric acid content (4.48 g/kg DM) was below 0.1% (equivalent to 10 g/kg DM) according to Breirem and Ulvesli cited McIlroy [3].

Ammonia Content of Napier Grass Silage

Results of analysis of variance showed that cutting age had significant effect ($P<0.05$) on the decreasing of ammonia content of silage, as well as the additive level. However, there is no interaction between cutting age and additive level on ammonia content.

Based on the results of Duncan's multiple range test, it was able to be seen that there was a decreasing in ammonia content of Napier grass silage along with the increasing of cutting age, which was able to be seen from the mean value of each cutting age, namely 39.63; 37.67 and 38.34 g NH3/kg DM (Table 1). There was a decreasing ($P<0.05$) in ammonia content at 60 and 80 days of cutting age compared to 40 days of cutting age (P6 and P8 vs P4). Ammonia content at P6 was lower ($P<0.05$) than P8. The presence of ammonia in the silage was thought to be due to the extensive breakdown of amino acids by Clostridia bacteria at the beginning of ensilage which resulted in accumulation of ammonia which was not utilized by bacteria as a source of N for the proliferation process. This was able to happen considering that the acidity of silage at the beginning of ensilage was still quite high which allowed Clostridia bacteria to grow. Results of the study which showed a decreasing in ammonia content with increasing of cutting age was in accordance with the results of research by Adogla-Bessa and Owen [9] and Snyman and Joubert [10]. Ammonia content of silage made from whole-crop wheat forage based on the plant growth phase from heading, mealy ripe, medium and hard dough phases was 60, 170, 87 and 41 g NH3/kg DM, respectively [9]. These data showed that at first there was an increaseing followed by a decreasing of ammonia content along with the growth phase of Napier grass. Snyman and Joubert [10] reported the results of their research which stated that ammonia content of forage sorghum silage decreased along with the increasing in the maturity phase of the plant.

Decreasing of ammonia content occurred along with the increasing of additive levels, i.e. 40.03; 38.88; 37.78 and 37.52 g NH3/kg DM. Ammonia content at 2.5; 5 and 7.5% additive level was lower ($P<0.05$) than 0% additive level (D1, D2 and D3 vs D0). Ammonia content at 5% and 7.5% additive level was lower ($P<0.05$) than 2.5% additive level (D2 and D3 vs. D1). However, there was no significant decrease of ammonia content between 5% and 7.5% % additive level (D2 vs D3). Ammonia content of sorghum silage made without addition of chemical additives in pipe, bloom and ripe phases was 345, 230 and 229 g/kg N, respectively [10]. Increasing additive level caused a decreasing in ammonia content of silage. Silage additive in the form of fine bran as an energy provider was thought to play a role in stimulating fermentation by homofermentative and heterofermentative bacteria to produce lactic acid and acetic acid which took a role in lowering pH and suppressed the growth of Clostridia bacteria. The research of Vanbelle and Bertini cited by Xiccato et al. [11] stated that ammonia concentration expressed the occurrence of protein degradation. Extensive amino acid breakdown caused by Clostridia bacteria to butyric acid increased ammonia content [1].

Table 1 showed that cutting age and additive level had the same effect on ammonia content and pH of silage. Increasing of cutting age affected the decreasing in ammonia content and pH of silage. Increasing additive level also affected the decreasing in ammonia content and pH of silage. This situation showed that the lower the pH achieved in silage making resulted in a lower ammonia content. Low pH in silage making was proven to be able to suppress the growth of Clostridia bacteria which was reflected by the lower ammonia content as a product of extensive amino acid breakdown with the lower of pH of silage.

Ammonia content of Napier grass silage ranged from 36.73 to 40.65 g NH3/kg DM. The lowest ammonia content of silage was achieved in P6D2 treatment combination which was not significant with P6D3 (36.73 vs 36.89 g NH3/kg DM). The highest ammonia content was 40.65 g NH3/kg DM which was achieved in the P4D0 treatment combination. Ammonia content of the research was still acceptable. Acceptable ammonia content in silage did not exceed 110 g/kg [12].

Conclusion

It was concluded that increasing level of additive decreased butyric acid and ammonia content of Napier grass silage. Increasing level of cutting age decreased ammonia content. Additive level and cutting age did not synergize in reducing butyric acid and ammonia content of Napier grass silage.

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