Effects of Carbon:Nitrogen Ratio on Quality of *Chromolaena odorata* Silage

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ABSTARCT

The main problem arises in silage making using protein source forages is decomposing process due to buffer activity of proteins that causing higher pH than that recommended values for a good ensilage process. Until recently, researches in silage processing merely focused on levels of carbohydrate source as additive, and lack of efforts to examine the efficacy of nitrogen:carbon (C/N) ratio in ensilage process. The present study was designed to test the effects of increasing carbon:nitrogen ratio on quality of *Chromolaena odorata* (an alternative protein source forage) silage. Four treatments with four replicates were tested namely **CoN**= Chromolaeana without additional carbon source (C/N ratio of 14,9); **CN20**= Chromolaeana + additional carbon source for a C/N ratio of 20; **CN25** = Chromolaeana + additional carbon source for a C/N ratio of 25; **CN30** = Chromolaeana + additional carbon source for a C/N ratio of 30. The carbon source used was *Corypha gebanga* meal. Variabel measured were organoleptic profiles, proportion of rotten fraction, nutrient composition, and *invitro* digestibility. Analysis of variance for completely randomized designed was employed in statistical analysis. The results showed that increased C/N ratio by up to 30 significantly improved silage organoleptic profiles, organic matter and crude protein content, yet reduced crude fiber content. Dry matter (DMD) and organic matter digestibility (OMD) indexes were significantly improved by up to 15% and 14% for DMD and OMD respectively. It can be concluded that increasing C/N ratio by up to 30 in ensilage process of protein source forage such as *Chromolaena odorata* will improve organoleptic profiles, nutrient content and digestibility of the silage.

Key words: *Chromolaena odorata,* C/N ratio, Silage, Protein source, Nutrient content.

INTRODUCTION

High decomposing activity is the main problem observed in silage making using high nitrogen forages. This is due to high buffer activity of proteins (amino acids) that cause higher pH in bio-fermented materials that always far above the ideal pH (> 5) recommended for good silage . Decomposing event significantly reduce nutrient content and quality of the silage since decomposer microbes dominate biochemical reaction during ensilage process. It is one of the reason why silage of nitrogen source forages are less popular than grass or other high carbon sources. Until recently, researches in silage processing were more focused on testing levels of carbohydrates source as additive, but lack of efforts to examine the efficacy of carbon:nitroen (C/N) ratio in ensilage process.

The optimum C/N varies for among fermentation and biofermentation processes. For biogas production, the optimum C/N ratio is 30 (Shuler dan Kargi, 1992), whereas the optimum C/N ratio for composting processes in organic fetrilozer production is 15-25 (Jimenez dan Garcia, 1997). There should be a certain optimum ratio C/N for silage making since predominant lactic acid bacteria (BAL) definitely requires the right C/N ratio for their maximum growth. The present study was designed to test diffent level of C/N ratio in silage making using *Chromolaena odorata* (an alternative protein source forage) on the quality of silage.

MATERIALS AND METHODS

The main material used was *Chromoalena odorata* (C/N ratio of 14,9). Freshly harvested *C. odorata* were chopped to a length of 2-4 cm and wilted for 24 hours. Prior to silage making, sample of wilted material dan carbon source feedstuff samples were taken and oven dried at 105^{O}C for 24 hours to determine dry matter content. Further analysis was performed to obtain organic matter and nitrogen content for calculation of C/N ratio. Carbon content was calculated using formulated of Jimenez dan Garcia (1992). There were four C/N ratios formulated as treatments. The treatments were **C/N_{ori}** = chromolaena with no carbon addition, **C/N_{20}** = C/N ratio of 20, **C/N_{25}** = C/N ratio of 25, and **C/N_{30}** = C/N ratio of 30. Four replicates were applied for each treatment. All treatments were given 10% of the weight of silage material rumen content as source enzymes and microbes. Palm (*Corypha gebanga*) pith meal was used as carbon source (C/N ratio of 119,5). Sixteen water containers (capacity of 10 L) were used as siloes in ensilage process. Each container was manually filled with 5 kg mixture of wilted chromolaena-palm pith meal-rumen content. After filling, the containers were air-tied sealed and stored at room temperature to undergo bio-fermentation process for 21 days.

After the bio-fermentation, the containers were opened and measurement taken immediately for pH and temperature. Approximately 2 kg of the silage from each container were taken for further organoleptic assessments (colour, odor, and texture). Ten panelists were asked to give score. Organoleptic assessment indicators of Mulik *et al* (2016) were used in this study. After organoleptic review, 200 g of samples from each container was taken for chemical analysis (dry matter, organic matter, crude protein, crude lipid, and crude fiber). All chemical analysis followed protocol of AOAC (2005).

Collected data were then analyzed using General Linear Model for completely randomized experimental design. The treatment differences were detected using P value of 0,05. SPSS 21 software were used in the statistical analysis.

RESULTS AND DISCUSSION

Organoleptic and physical variables

Increasing C/N ratio have positive effect on pH, odor and color of chromolaena silage, whereas texture and proportion of rotten materials in the silage were not affected (Table 1). Lower pH (5.7) in the higher C/N ratio treatment (C/N30) suggested that additional carbon from palm pith meal provided extra carbon for microbes during ensilage process of chromolaena material hence suppressed the pH. This extra carbon likely to facilitate dissimilatory reactions that bacteria use to produce energy, through various reduction-oxidation reactions. Heterotroph microbes can use the pair of electron acceptor and donor to run a redox reaction that produces sufficient energy for cell growth (Shuler and Kargi, 1992). Generally, lactic acid bacteria are the predominant microbes during ensilage process (Yahaya *et al,* 2004) with lactic acid as the end product (Stanbury and Whitaker, 1984).

Since carbon availability is an essential element for low C/N ratio forages, including *C. odorata,* extra carbon tends to support microbial activities in the silage. Therefore, temperature of C/N diets with C/N ration of 20-30 tended to be warmer than C/N_{ori} treatment (Table 1).

Increasing C/N ratio significantly improved texture and odor of the chromolane silage. It appears that merely small proportion (<2%) of silage was invested by fungus (Table 1). Small proportion (<2%) of rotten materials across all treatments indicated that (1) the silage was well-filled and -sealed to allow no oxygen in the silos to be used by decomposing microbes to shift biochemical reactions toward decomposition process, and (2) various secondary metabolic compounds in chromolaena (Ikhimioya *et al*, 2007) might act as anti-fungal agents.

Table 1. Organoleptic and physical variables scores for Chromolaena silage with carbon/nitrogen (C/N) ratio of 14.9 (C/N_{ori}), 20 (C/N_{20}), 25 (C/N_{25}), and 30 (C/N_{30})

Treatment	Variable					
	pH	Temperature (°C)	Odor	Color	Texture	Rotten (%)
C/N_{ori}	6.8^{b}	32.4^{a}	2.0^{a}	1.5^{a}	2.5	3
C/N_{20}	6.3^{ab}	34.2^{b}	2.5^{ab}	2.0^{ab}	2.5	3
C/N_{25}	6.2^{a}	34.9^{b}	3.0^{b}	2.5^{b}	3.0	3
C/N_{30}	5.7^{a}	35.1^{b}	3.5^{c}	3.0^{b}	3.0	3
SEM	0.001	0.012	0.002	0.001	0.001	0.001
P-value	<0.025	<0.040	<0.031	<0.029	<0.073	<0.716

**Different superscript in the same column shows significant different*

Nutrient content and digestibility coefficients

Organic matter, crude protein, crude fiber content, and digestibility of dry matter and organic matter of the chromolaena silage were significantly improved when the C/N ratio was increased (Tabel 2).

Table 2. Nutrient content of Chromolaena silage with carbon/nitrogen (C/N) ratio of 14.9 (C/N_{ori}), 20 (C/N_{20}), 25 (C/N_{25}), and 30 (C/N_{30})

Treatment	Nutrient content (g/kg dry matter)					Digestibility (%)	
	Dry matter (g/kg)	Organic matter	Crude protein	Crude Fat	Crude Fiber	Dry matter	Organic matter
C/N_{ori}	282	911^{b}	194a	61	147^{c}	61.1	67.0
C/N_{20}	276	906^{b}	222^{ab}	67	133^{b}	64	68.3
C/N_{25}	295	897^{a}	240^{b}	58	125^{b}	68	74.9
C/N_{30}	306	886^{a}	258^{b}	57	109^{a}	70.4	76.6
SEM	0.132	0.121	0.163	0.035	0.07	0.056	0.042
P-value	<0.063	<0.034	<0.001	<0.137	<0.001	<0.001	<0.001

**Different superscript in the same column shows significant different*

Reduction in organic matter in the present study is expected since additional carbon was likely to stimulated the growth of microbes hence utilizing some organic matter in the silage. In the contrary, crude protein content showed an increase trend. This could be attributed to the accumulation of microbial protein in the silage and might release additional proteins from protein-bound compounds such as tannins and anti-trypsin in chromolaena. Crude fiber content in the silage was also significantly reduced by up to 26% by incremental C/N ratio.

This reduction most probably the effect of chemical breakdown of complex carbohydrates by rumen microbial enzymes and rumen microbes plus action of complex fermenting microbes in the silo during ensilage process (Weinberg *et al*, 2007). An increase in crude protein and reduction in crude fiber can be used to explain the significant improvement in digestibility coefficient of dry matter and organic matter.

CONCLUSION

It is concluded that C/N ratio of 30 is suggested to be used in silage making for low C/N ratio forages such as *Chromolaena odorata* for a better quality of silage

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DAFTAR PUSTAKA

AOAC. 2005. Official Methods of Analysis. 17th Ed. AOAC International. Washington.

Ikhimioya, I., Bamikole, M. A. and Omoregie, A. U., Ikhatua, U. J. 2007. Compositional evaluation of some dry season shrub and tree foliages in a transitionally vegetated zone of Nigeria. *Livestock Research for Rural Development 19 (3): 1-9*

Jimenez, E.L. and Gacia, C.V. 1997. Relationship between organic carbon dan total organic matter in municipal solid waste and refuse composts. *Bioresour Technology, 41:25-272.*

Bo, J., Pinghe Y, Yibong, M and Ling, Z.O. 2005. *Production of Lactic Acid and Fungal Biomassa by Rhizopus Fungi from Food Processing Waste Streams,* Jurnal Ind. Microbiol. Biotechnol. 32 : 678 – 686.

Santoso, B., B.Tj. Hariadi, H. Manik and H. Abubakar. 2009. Kualitas Rumput unggul tropika hasil ensilase dengan aditif bakteri asam laktat dari ekstrak rumput terfermentasi. *Media Peternakan*. 32: 138-145.

Shuler, M.L.and Kargi F. 1992. *Bioprocess Engineering Basic Concepts*, Prentice-Hall International Inc., New Jersey.

Stanbury, P.F. and Whitaker, A. 1984. *Principles of Fermentation Technology*, Pergamon Press, New York.

Weinberg, Z.G., Shatz, O., Chen, Y., Yosef, E., Nikbahat, M., Ben-Ghedalia, D. and Miron J. 2007. Effect of lactic acid bacteria inoculants on *in vitro* digestibility of wheat and corn silages. *J. Dairy Sci*. 90: 4754-4762.

Yahaya, M.S., Goto, M., Yimiti, W., Smerjai, B. and Kuwamoto,Y. 2004. Evaluation of fermentation quality of a tropical and temperate forage crops ensiled with additives of fermented juice of epiphytic lactic acid bacteria (FJLB). *Asian-Aust. J. Anim. Sci*. 17: 942-946.