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Bio-Fermentation Improved the Nutritional Values of *Chromolena odorata* Utilization as Bali Cattle Feed Source

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Abstract: ***Objectives: The aims of this study were to analyze the effects of bio-fermentation of C/N30 ratio using different soluble rates of carbohydrate sources on in vitro physical quality, nutrition, anti-nutrient, digestibility, NH3 and VFA rumen fluid of Chromolaena odorata silage. Method: A Completely Randomized Design with 3 treatments and 6 replications was used in this study. The treatments were: (CO_{30}_GC): CO with C/N_{30} of liquid palm sugar + 10% rumen content; (CO_{30}_PTK): CO with C/N_{30} of palm pith flour or putak flour (Corypha gebanga) + 10% rumen content; (CO_{30}_JP): CO with C/N_{30} of rice straw flour + 10% rumen content. Providing liquid palm sugar as carbohydrate source increased the physical quality of Chromolaena odorata silage (color, odor, pH texture, presence of fungus) compared with the other treatments. Results: There were an increased ($P < 0.01$) in feed composition, dry matter digestibility, organic matter digestibility, pH, NH_3 concentration, VFA concentration, tannin content, but lower saponin and lower crude fiber compared with the other treatments when rice straw flour was added to the Chromolaena odorata. Conclusion: This study demonstrated that bio-fermentation during 21 days is better when adding rice straw flour as carbohydrate source with a C/N_{30} ratio to increase the utilization Chromolaena odorata as animal feed. However, if using other digestible carbohydrates sources then it is better bio-fermentation just before 21 days.***

Keywords: *Chromolena odorata* silage, C/N ration, Liquid palm sugar, Putak, Rice straw flour

1. Introduction

Low cattle production in East Nusa Tenggara (NTT) is hindered by a number of problems, including the lack of land due to degraded agricultural land, fluctuations of feed both quality and quantity coupled with increasing feed prices, and invasion of productive grazing land by plants or weeds such as *Chromolaena odorata* and others. Although, it is known as an invasive plant, *Chromolaena odorata* has the potential to be utilized as source of animal feeding. Previous studies reported that *Chromolaena odorata* possess high nutritional value especially protein about 13,2[1]or even reach 35% with its biomass production as much as 70 DM/Ha/year[2] and have protein score of 88,24%[3]. This protein score is higher than the score value of soy protein, corn, beans, rice and wheat, but lower than milk, eggs, cow's milk and beef[4]

Despite its high ecological potential and nutritional value, *Chromolaena odorata* has been reported to contain alkaloids, cyanogen glycosides, flavanoids (aurons, kalkon, flavones and flavonols), phytates, saponins) and other secondary metabolic compounds[5]which causes low edible to cattle in a fresh state. Therefore, the fresh raw materials of *Chromolaena odorata* need to be processed prior to be utilized in animal feeding.

The side effect of *Chromolaena odorata*, however can be eliminated by several treatments including physical or mechanical, chemical and or biological treatment. Bio-fermentation is an alternative biological way to reduce the negative effects of *Chromolaena odorata*, consequently increase its preference, and safe for animals[6]. It has been showed that bio-fermentation treatment decreased tannin content of *Chromolaena odorata* from 2.4% to 0.94% dry matter, reduced trypsin inhibitor from 32.99 mg/g to 25.47 mg/g while for ingestion of the ingredients (55.2% vs 53.13%) and organic content(61.7% vs 59.25%) were better than that of dried treatment. The ration of Carbon and Nitrogen (C/N) is an important factor that need to be considered when bio-fermentation treatments is applied to *Chromolaena odorata*, mainly to support the work of lactic acid bacteria in the process and to avoid the risk of decay and therefore increase the nutritional value of silage produced for animals. The objective of this study is to optimize rumen bio-fermentation to increase the nutritive value of *Chromolaena odorata* as Bali cattle feed.

2. Materials and Methods

2.1 Time and site of Study

The present study was conducted in May - October 2017. The *in vitro* study and nutrient contents of *Chromolaena odorata* was conducted at laboratory of chemistry and feed nutrition, Faculty of Animal Husbandry, Universitas Nusa Cendana, Kupang. The fermentative parameter was analyzed at laboratory of dairy feed, Faculty of Animal Husbandry IPB Bogor, Bioscience Laboratory of State Polytechnic of Jember-East Java and Laboratory of chemical

feed, Faculty of Animal Husbandry, University of Hasanudin.

2.2 Materials

Samples of *Chromolaena odorata* used in this study were collected from the area of Kupang, East Nusa Tenggara. Briefly, the harvested leaves of *Chromolaena odorata* obtained by pruning the plants at a height of about ± 40 cm from ground were then used as the treatments. Putak (*Corypha gebanga*), palm sugar and rice straw flour were obtained from the area of Kupang as well. The rumen contents were obtained from the abattoir of Kota Kupang.

2.2.1 Design of the study

A Completely Randomized Design with 3 treatments and 6 replicates was employedin this study. The treatments were as follows:

CO_{30}_GC: CO with C/N balance$_{(30)}$ palm sugar + 10% rumen content.

CO30_PTK: CO with C/N balance$_{(30)}$ palm pith flour + 10% rumen content.

CO30_JP: CO with C/N balance$_{(30)}$ rice straw flour + 10% rumen content.

Note:

$CO_{(30)}$: *Chromolaena odorata* with C/N_{30} balance; GC: Liquid Sugar; PTK: palm pith flour powder JP: rice straw flour

Palm pith flour or putak flour (*Corypha gebangan*), liquid palm sugar and rice straw were used as carbon sources to increase the C/N ratio in bio-fermentation. Those carbohydrate sources were chosen to represent an easy, moderate and difficult soluble carbohydrate, respectively. The amount of carbohydrate sources used was supplying the needs of Carbon in the media. The ratio C : Nused was $C/N_{(30)}$ as the better quality of silage produced than other treatments [6]The carbon content was calculated as followed previous study [7]as follows:

$$\text{Carbon (g)} = \frac{\text{Organic matter content (g)}}{\text{1.72 (constant)}}$$

While the nitrogen content was obtained by the formula:

$$\text{Nitrogen content} = \frac{\text{Gross Protein Content (g)}}{\text{6.25 (constant)}}$$

Calculation ratio C/N as follows:

$$\text{C/N} = \frac{\text{Carbon Content}}{\text{Nitrogen content}}$$

2.2.2 Parameters and measurement

Physical quality

Physical quality was measured using sensory test to determine the aroma, texture, color, pH, temperature, and presence of fungi. The physical quality determination used wasthe development of previous method [8] Briefly, the score determination was based on the following scores: Color: 1. Black; 2. Blackish brown; 3. Green brown and 4. Natural green; Odor: 1. Rotten; 2. slightly rotten; 3. slightly acidic and 4. Acid; Texture: 1. Soft (clump, watery and slimy); 2. slightly soft/slightly dense (some what clumped, there is mucus); 3. Solid (not clot, not slimy) and 4. Not clot, not slimy, crumbs; Mushroom existence: More (> 5% of total silage); 2. Enough (2-5% of total silage); 3. Less (< 2% of total silage) and 4. Not applicable; pH: 1 to 14; Temperature: 0^C.

Nutritional contents analysis (AOAC, 2005)

1) Air dry matter content determination

The air dry matter content was determined following procedures of proximate and AOAC. Briefly, the paper box is weighed (A g), then 200 g sample was weighed (B g) into a paper box.The sample was then placed into an oven with a temperature of 60-70^{0}C for ± 24 hours. Sample was then removed and aerated in the room for 1 hour and weighed (C g).

Calculation:

$$\text{KBU content} = \frac{\text{C - A}}{\text{B - A}} \times 100\%$$

where A = Paper weight (g); B = sample weight + paper (g) before 60-70 ^{0}C; C = sample weight + paper after oven 60-70 ^{0}C (g)

2) Dry Matter (DM) determination

The dry matter was determined following procedures of proximate and AOAC. Briefly Petri dish was placed in an oven of 105 ^{0}C for an hour, then the cup was taken and placed into desiccator for 1 hour then weighed (weight = A g). About 5 g of sample was weighed to petri dish (weight = Bg), then placed into an oven at 105 ^{0}C for 4 hours. The cup was taken and placed into desiccator for 1 hour and weighed (weight = C g)

Calculation:

$$\frac{\mathbf{C - A}}{\mathbf{B}} \mathbf{\times 100\ \%}$$

Where:A = weight of the stained cup is inserted into the oven; B = sample weight; C = cup weight after being removed from the oven

3) Crude protein determination

The crude protein was determined following Kjeldahl method. Sample (0.3 g) and paper were weighed and placed into Kjeldahl's flask. Paper was weighed again before 1.4 g catalyst and $5H_2SO_4$ concentrated added to the Kjeldahl flask and then destructed until the color of solution becomes green. For distillation process, 300 ml beaker glass was filled with 25 ml of H_2SO_4 0.1. N and 3 drops of mix indicator was added. 20 ml 40% NaOH is added to the beaker glass for distillation (as long as the purple color distillation remains). Distillation is complete if the solution in beaker glass has reached ± 100 ml. For titration process, the beaker glass containing distillation solution was titrated with NaOH 0.1 until the color turns clear.

Calculation:

$$\text{\% CP Content} = \frac{\text{(D - C) x n NaOH x 0.014 x 6.25}}{\text{B - A}} \times 100\%$$

Where, A = sample weight and paper; B = Paper weight; C = Volume of sample titration; D = volume of blank titration

2.3 Crude fiber measurement

The crude fiber content was determined following Kjeldahl method. About 0.1 g sample was weighed (weight = A g) and placed into a special beaker glass then 500 ml H_2SO_4 0.3 N and a boiling stone were added and boiled for 30 minutes. 25 ml NaOH 1.5 N was added before boiled for 25 minutes. 0.5 EDTA (Ethylene diaminetetraacetic acid) was added and boiled again for 5 minutes. Aquades was added during boiling process, and filtered with a filtration plate that filled with sand. Solution in the beaker glass was cleaned with hot aquadesto ensure all solution enters the filtration plate. 50 ml HCl 0.3 N was added to the cup and allowed to stand for 1 minute before suckedwith a vacuum pump. 10 ml of hot aquades and 1 ml of acetone were added to solution and then sucked. 49 ml of acetone was then added and allowed to stand for 1 minute before sucked until dry. Sample was then placed into the oven for 1.5 hours at temperature of 140 ^{0}C. Sample was then removed from the oven and placed into desiccator for 1 hour and weighed (weight = B g). Sample was then placed into the furnace (600 ^{0}C) for 3 hours before removed and placed into the desiccator for 1 hour and weighed (weight = C g).

Calculation:

$$\% CF = \frac{B - C}{A} \times 100\%$$

Secondary metabolite content measurement

The tannin content was determined using titrimetric methods as described by Atanassova and Christova-Bagdassarian [10].

Measurement Amino Acid

Measurement of amino acid content was carried out using the AOAC method[9]. Amino acids were analyzed using HPLC. The principle of this amino acid analysis is that the amino acids from the protein are liberated by hydrolysis with 6N HCl. Hydroxylate is dissolved with sodium citrate buffer, and each amino acid will be separated using HPLC before the hydrolysis process is carried out, protein extraction is carried out using the Kjeldahl method.

Nutrient digestibility measurement

The dry matter and nutrient digestibility were determined following the method of Tilley and Terry [11]. Briefly, McDougall's buffer solution was prepared by dissolving 49 g $NaNHCO_3$, $NaHPO_4$. 12 H_2O 46,50 g, NaCl 2,35 g and KCl 2,85 g in aquades until the volume reaches 1 liter. $MgCl_2$ 6% solution was made by weighing 6 g $MgCl_2$ and then placed into 100 ml volumetric flask and dissolved with 40 ml of aquades until homogeneous. About 4% $CaCl_2$ solution was then prepared in the same manner as the $MgCl_2$ solution preparation, but $CaCl_2$ was weighed 4 g. McDougall's buffer solution was then inserted into a stirredstorage flask and placed on a heater whichsupplied with CO_2 gas. Filtered rumen fluids were then placed in a thermos and transferred into a storage flask that already contains buffer solution. The ratio of the buffer solution to the rumen fluid was 4:1. The mixed buffer and rumen fluid conditions should be at pH 6.9 to 7.0 with a temperature of 38-39 ^{0}C. About 5 g of dry air sample was weighed and placed into the incubator 38-39 ^{0}C (± 1 day before the test). About 50 ml of mixed rumen fluid solution and buffer solution were sampled and placed fermenters tube containing sample and empty fermenter tube (blank) using dispenser and immediately closed with rubber plug coated with Bunsen valve. The CO_2 gas was attached to the sample determined. The fermenter tubes wereincubated at 39 ^{0}C for 48 hours and shakedevery 4 hours. After 48 hours incubation, the fermenter tubes were removed and immersed in ice water to stop microbial activity, then centrifuged at 2500 rpm for 15 minutes. HCl-pepsin solution was prepared by adding 2 g of pepsin into a 2000 ml Erlenmeyer, and HCl 0.1 N was added to 1 liter of volume.The supernatant was removed and the sample residue was added with 50 ml of HCl-pepsin solution, and then incubated again at 39 ^{0}C for 48 hours without Bunsen Valve cover and without CO_2. After 48 hours incubation, the tubes were removed and the liquid was filtered by using whatman paper number 41. The cup and residue were dried in 105 ^{0}C oven for 12 hours, then cooled in the desiccator and weighed. The plate containing the filter paper and the residue was then placed into a furnace at 550 ^{0}C until the color become white or gray, then cooled and weighed.

The values of DMD and OMD in-vitro were determined by using the following formula:

$$DMD = \frac{DM\ sample - (DM\ residue - blank)}{BK\ sample} \times 100\%$$

$$OMD = \frac{OM\ sample - (OM\ residue\text{-}OM\ stamp)}{OM\ samples} \times 100\%$$

Where: DM sample: initial sample weight x % DM sample; DM residue: (weight of plate + residue after heated in ovenat 105 ^{0}C) - (weight of plate + filter paper); DM sample: DM sample x % DM sample; OM residue: DM residue - {(weight of plate + residue after in furnace 550 ^{0}C) - (weight of plate + filter paper).

Silage pH measurement

The silage pH measurements were performed using electric pH meters (Hanna) at the time of physical quality assessment. The pH meter was calibrated with aquades into neutral pH (pH = 7) at room temperature (25 ^{0}C) prior to use. About 5 grams of silage was sampled and mixed 1: 4 with 20 mlaquades.

Silage temperature measurement

The silage temperature was measured using electric thermometer (Thermo One ALPHA1). The thermometer was plugged into silage for a while until the sound was heard and the temperature appeared in the monitor screen.

N-NH3 concentration measurement

N-Ammonia concentration was performed by Conway Microdiffusion (General Laboratory Procedure, 1966).Principally, the N-Ammonia content in supernatant of rumen fluid was expelled with Na_2CO_3 or NaOH. The expelled N-Ammonia diffuses in the H_3BO_3 solution (boric acid) and becomes NH_4^+. The anion of H_2BO_3- was titrated with H_2SO_4 or HCl. Briefly, 1 ml of rumen fluid supernatant was placed on one side of the Conway plate and 1 ml of saturated NaOH solution was placed in the other side.

Furthermore, 1 ml of boric acid solution was placed at the center of the Conway plate. Vaseline was applied to the lid of the Conway plate before it closed tightly. The supernatant and saturated NaOH solutionswere mixed evenly by shaking the cup. The N-Ammonia released from the reaction was then captured by boric acid indicated by changes of color (pink) after 24 hours.The N-ammonia concentration in rumen can be calculated by the formula:
N-Ammonia = (ml H_2SO_4 x N H_2SO_4 x 1000 ml) mM

VFA concentration measurement
Total VFA was analyzed by General Laboratory Procedure [12]. In brief, about 5 ml of the rumen fluid supernatant (filtrate) was placed into the Markham distillation tube, which was heated by boiling water in a distiller flask. The tube was immediately closed after adding 1 ml of 15% H_2SO_4 solution. The hot water vapor will push the VFA through the condensed cooling condenser and accommodated in an Erlenmeyer containing 5 ml of NaOH 0.5 N to reach a volume of about 100 to 300 ml. Furthermore, 2-3 drops of phenolphthalein were then titrated with HCl 0.5 N. The titration ends when the initial point of color change from pink becomes clear (colorless).

The VFA level was calculated by the formula:
VFA Total = (b - s) x N HCl x 1000/5 mM
Where: s = volume of sample titrant; b = volume of blank titrant; N = Normalities of HCl solution

Statistical analysis
The collected data were analyzed by ANOVA for Completely Randomized Design using SPSS version 23 according to the following statistical model:$Yij = \mu + \delta i + \in ij$; where Yij is the observed value, μ is the general mean, δI is the effect of the i treatment and ∈ij is the error term.

3. Results

Physical quality is one of the indicators to know the quality of *Chromolaenaodorata* silage produced. The physical qualities included color, odor/aroma, texture and the presence of fungus (Figure 1). The changes color of *Chromolaenaodorata* silages from green to brownish green indicated that the three different carbohydrate sources used as treatments occurred as a result of 21 days bio-fermentation process. The presence of this color change is probably caused by oxidation reactions catalyzed by the enzyme phenol oxidase or polyphenol oxidase. Both enzymes catalyze phenol into quinine which is polymerized into a brown melaniadin pigment[13]. Tannin color will turn dark when exposed to light or left in open air(Mulik, 2016), besides that there is also a high temperature during the process of ensilase.

The aroma of the *Chromolaena odorate* silage produced (Table 1), with liquid palm sugar as carbohydrate source (CO_{30}_GC) was acidic compared to the other two treatments were slightly acidic (CO_{30}_PTK and CO_{30}_JP, respectively). The good quality of silage produced acid compounds [14]. The difference between the aromas in the last two treatments indicates that there was inadequate ensilage during bio-fermentation process. This probably caused by the presence of tannin content in *Chromolaena odorata* thus inhibiting the work of bacteria, especially lactic acid bacteria. However, the texture of *Chromolaenaodorata*silage produced during 21 days bio-fermentation was solid, lumpy, not slimy and soft. This indicated that the *Chromolaena odorata* silage has a good texture (Sandi *et al.*, 2010).The presence of fungi, temperature and pH are those factors used to determine the quality of silage produced (Table 1). The percentage of fungus during bio-fermentation period was 1%, indicating the *Chromolaena odorata* silage produced still in the range of good quality as reported by previous study, which was 10% of good quality silage. However, the temperature yielded from the *Chromolaena odorate* silage ranged from 33.10 ^{0}C - 36.22 ^{0}C, which is above the maximum temperature for producing silage 30 ^{0}C [16]. This indicated that the temperature obtained in this study is above the maximum temperature for silage produced which in turn will affect the quality of *Chromolaena odorate* silage.

Low pH value of *Chromolaena odorata* silage gives an indication of good quality of the ensilage produced during bio-fermentation process. The average pH value in the present study ranged from 6.46 to 6.63. Treating the *Chromolaena odorata* silage with different sources of carbohydrate resulted in low pH ($P \leq 0,05$), indicating that the ensilage process was not perfectly good due to the pH yielded above the normal pH as expected . Previous study suggested four categories of pH ranging from excellent (pH 3.2 - 4.2); good (pH 4.2 - 4.5); medium (pH 4.5 - 4,8) and poor ($pH \geq 4.8$)[17]. Furthermore, recent study byHartati [18], have classified silage in several classifications: (1) lactic acid silage dominated by silage lactic acid bacteria with pH ranging from 3.7 to 4.2; (2) acetic acid silage mainly stable with high pH and but has amino acid deamination (3) butyric acid silage mostly the fermentation occupied by clostridia with pH between 5-6. The high pH value in this study was possibly caused by low ability of lactate acid bacteria or in other word the ability of lactate acid bacteria did not maximally performed to degrade carbohydrate and thereby lowering the pH of *Chromolaena odorata* silage as well as preserved the *Chromolaena odorata* silage.

4. Discussion

Nutritional Composition of Chromolaena odorata Silage

Duncan multiple-range test showed that the addition of different carbohydrate sources had significantly affect ($P<0.01$) the nutritional value of *Chromolaena odorata* silage. Treating *Choromolaena odorata* silage with rice straw flour (CO_{30}_JP) as carbohydrates source resulted in lower crude fiber content than the other two treatments (CO_{30}_GC) and (CO_{30}_PTK). The biological value of *Choromolaena odorata* silage treated with different carbohydrate sources (Table 2) was greatly determined by several factors such as dry matter, organic matter, crude protein, crude fiber and other nutrient contents. This may be due to the synchronization of the availability of carbon skeletons supplied by rice straw flour for the synthesis of microbial proteins, leading to an increase in the bio-fermentation process. The increase in organic matter content was also in line with the increase of crude protein in the treatment of rice straw flour (CO_{30}_JP), probably due to the availability of carbohydrates as an energy source used to

stimulate the microbes for bio-fermentation process thereby increased the crude protein content. This may also due to direct contribution by fermentative microbes and the breaking of the complex bonds of rice straw flour during the bio-fermentation process. High organic matter content in rice straw flour (CO_{30}_JP) may also due to the hydrogen contained metabolic compound such as tannin with higher tendency to bind to other organic matter such as cellulose and hemicellulose[19]. On the other hand, lower organic matter and crude proteins in *Chromolaeaodorata* silage treated with easily digestible carbohydrate sources (CO30_GC) and carbohydrate from palm pith flour(moderate carbohydrate) or (CO_{30}_PTK) may be due to the addition of the two carbohydrate sources that were peaked before 21 days of bio-fermentation.

The values of crude fat of *Chromolaea odorata* silage treated with rice straw flour (CO_{30}_JP) were higher (11.44%), followed by easily digested carbohydrate (CO_{30}_GC) and moderate powder carbohydrate (CO_{30}_PTK), 10.47 % and 7.58%, respectively. The high fat in the treatment (CO_{30}_JP) was probably caused by low water content of rice straw flour resulting in increased fat proportions. Increased fat content may be due to the fat content of *Chromolaena odorata* binding to a separate protein (lipoprotein) that can lead to increased fat content[20].

Addition of rice straw flour to *Chromolaena odorata* silage (CO_{30}_JP) had lowered the fiber content by 25.15% compared to the other treatments (CO_{30}_GC) of 29.59% and (CO_{30}_PTK) of 34.80 %, respectively. This perhaps due to the optimum bio-fermentation process resulted from addition of rice straw flour and therefore breaking down complex components of materials into simpler components that are so easy utilized by the microbes. In addition, the lower crude fiber content of *Chromolaena odorata* silage allows the microbes to better degrade it and being components that available for production purposes. This is in line with Winarno [21], stated that there are many components that are degraded into simpler components that are used for microbes during bio-fermentation process.

The availability of carbohydrates as a source of organic matter in *Chromolaenaodorata*is determined by BetN. Duncan multiple-range analysis revealed a significant difference between different carbohydrate sources (P 0,01) on BetN content of *Chromolaena odorata*. The highest BetN value (30.50%) was achieved in the treatment (CO_{30}_GC) followed by treatments (CO_{30}_JP) and (CO_{30}_PTK) 29.03% and 19.26%, resepctively. Higher value of BetN in the treatment (CO_{30}_GC) was probably due to the contribution of carbohydrates derived from the liquid sugar that causes the BetN content of this treatment to be higher than the other two treatments. On the other hand, lower content of BetN in the treatment (CO_{30}_PTK) was probably due to high fiber content resulted in low value of BetN.

Providing *Chromolaena odorata* silage with different carbohydrate sources resulted in ($P \leq 0.01$) higher neutral detergent fibre (NDF) content but lower Acid Detergent fibre (ADF) content ($P \geq 0.05$) (Table 2). The higher values of ADF and NDF in the *Chromolaena odorata* silage treated with rice straw flour probably due to the contribution of ADF and NDF components derived from both *Chromolaena odorata* and rice straw flour. ADF and NDF components are cell wall fractions that affect digestibility due to the high cell wall content in feedstuff , especially in fibrous forage. Therefore, in the formulation strategies of cattle and other herbivores, the existence of the ADF and NDF factions are urgently considered. The lower the NDF and ADF fractions, higher feed digestion Preston and RA Leng (1987) argued that ADF levels can also be used to estimate the energy supply (NE-maintenance, NE-gain, or Total Digestible Nutrient) of feedstuff. The regression equation between energy and the ADF of local feedstuff offered to cattle raised communal in a group which can supply the average that of 0.55 Mcal of Net Energy Maintenance, or Net Energy Gain of 0.31 Mcal or 58.74% of Total Digestible Nutrient. In addition, it was found that the value of net energy for maintenance (basic life/lactation) and for body weight gain as well as the undigested total nutrient (TDN) of local feed stuff offered to cattle kept in a group shed in Western Lombok had relatively equal compared to alfalfa with Acid Detergent Fiber levels of about 30-40%.

Tannin is a phenolic compound which can hinder rumen microbial activity by deactivating enzymes, can harm microbes, and can bind to any substance including binding and protecting proteins[23]However, the side effects of tannins can be reduced by several process including heating, drying, soaking, acid treatment, alkali, oxidizing, urea, polyvinyl-pyrrolidine (PVP), ferric salts [24]. Treating *Chromolaenaodorata* silage with different solubility levels of carbohydrate caused a highly significant effect on tannin (≤ 0,01) produced in this study (Table 2). Recent study hadreduced tannin content of *Chromolaena odorata* by 43% to 62% through fermentation treatment, boiling, water soak, and sun dried. Decrease in tannin content as reported by Mulik[8]was probably due to the chemical activity of enzymes produced by various fermentative microbes thus destroying the tannin-enzyme and protein-tannin bonds, thereby releasing the tannin and dissolving in the fermentation fluid solution. Furthermore, declinedtannin content may also be caused by microbial enzymes hydrolyzing some tannin, especially those dissolving into other compounds during the anaerobic fermentation process.

Saponins are type of glycoside found in many high-level plants, its specific character is water-soluble colloid and foaming after being shaken, has a bitter taste. Based on these properties, saponins compounds have a very wide range of uses, including detergents, foam formers in fire extinguishers, foam-formers in the shampoo industry and used in the pharmaceutical industry as well as in the field of photography. However, saponins in ruminant have the potential of defaunation agents that often used in manipulation the fermentation processes in the rumen. Oematan reported a decrease in the partial or total rumen protozoan population when saponins were added to the feed offered to animal[25]. In addition, saponins can hemolysis or destroy red blood cells [26]. Saponins are known to have an antimicrobial effect, inhibit fungi and protect plants from insect attack. Saponins can lower cholesterol, have antioxidant, antiviral, and anti-carcinogenic properties and rumen fermentation manipulators [27].

The protein quality is largely determined by the quality and balance of the amino acids contained in the feedstuff. Animal strongly requires some essential amino acids for growth, including argenine, histidine, isoleucine, luesin, lysine, valine, methionine, phenylalanine, threonine and tryptophan. Although amino acid is necessary important for ruminant animals, methionine is limited in *Chromolaena odorata* due to its content is very small (Table 3). Duncan multiplication test indicated that treated *Chromolaena odorata* silage with different carbohydrates sources had no significant effect ($P \geq 0,05$) on amino acid content, in other wordall treatments contributed the same effect on the amino acid content. However, this amino acid is very important in the transamination reaction, particularly the exchange of amino group of an amino acid to keto alpha, so that other amino acids and other alpha acids are formed with the help of transaminase enzyme [28].

Feedstuff quality is mostly determined by its digestibility. The aim of the *in vitro* method is mainly to assess the digestibility of both dry matter and organic matter are fermented or lost in the rumen or after the rumen of animals. The higher the digestibility of a feedstuff, the better its utilization by the animal, in other word it reflects how much it can be used by the animal. On average, the *in vitro* dry matter digestibility (DMD) and organic matter digestibility (OMD) of *Chromolaena odorata* silage treated with different carbohydrate sources (Table 4), were higher in the treatment of straw rice flour with the values of 48.49% and 44, 90%, respectively. In contrast, the lowest values were in the treatment of palm pith flour that is 38,37% and 35,39%, respectively. The ANOVA test indicated a significant effect ($P \leq 0,01$) differentcarbohydrate source treatment on both DMD and OMD. The DMD is usually closely related to the OMD therefore if the DMD increases then the OMD will also increase as well. The high values of DMD and OMD in the treatment of rice straw flour in this study was probably caused by the synchronization between carbohydrate and energy sources in forming the carbon frame work by microbes in degrading *Chromolaena odorata* silage. It was expected that carbon donations will arise from easily digestible carbohydrate (CO_{30}_GC) and moderate digestible carbohydrate (CO_{30}_PTK), however the results obtained were in contrast where DMD and OMD from rice straw flour (CO_{30}_JP) were the highest. This probably due to the time limit for synchronization of carbon frame formation by rumen microbes occurred during 21 days bio-fermentation just over the period for carbon skeleton formation by microbes and thereby resulting in dead of some microbes which lead to low microbial activity in degrading *Chromolaena odorata* silage. Another factor possibly causing high values of DMD and OMD was that low crude fiber content of rice straw flour (CO_{30}_JP) during bio-fermentation process, which led to releasing the lingo cellulosic allowing microbes to degrade *Chromolaena odorata* silage much better.The DMD and OMD values of *Chromolaena odorata* silage arising from bio-fermentation found in this study were lower (44,9% until 48,49%, respectively) than that of reported by Mulik which is the DMD and OM were 50,46% to 61,7% .[8]

In vitro rumen pH

Rumen pH is one of the important parameter indicating the condition where rumen microbial can perform normally during fermentation process in the rumen. On average, the pH obtained in this study ranged from 6.63 to 6.74 (Table 4). Duncan multiplication test indicated that treatment using different carbohydrate sources had a significant effect ($P < 0.01$) rumen pH, where rice straw raw flour treatment had more acid resulted in lower pH compared to other treatments. Higher rumen pH in both treatments (CO_{30}_GC) and (CO_{30}_PTK) is probably due to the increased of NH_3 production causing alkaline which resulted in the increase of rumen pH. However, the rumen pH obtained in this study still within the range of normal rumen pH in which support the growth of rumen bacteria, especially fiber bacteria that wasbetween 6.63 - 6.74. The rumen pH that supports microbial growth is between 5.7 and 7.3. In addition, the ideal pH to support growth and activity of rumen microbes especially cellulolytic bacteria is 5.6 to 7.3 [31, 32]. Whereas the optimum rumen pH is 6 to 7, and if dropped will resulted in decreased of cellulose digestibility.

In vitro Rumen Ammonia Concentration

Rumen NH_3 concentration is used determine feedstuff fermentationwhich is closely related to rumen microbial activity and population. On average, the NH_3 concentration obtained in this study was higher in the treatment (CO_{30}_JP), followed by treatment (CO_{30}_PTK) and the lowest values in the treatment (CO_{30}_GC) (Table 4). The ANOVA test revealed that treating *Chromolaena odorata* silage with different carbohydrate sources had highly significant effect ($P < 0.01$) on rumen NH_3 concentration. The lowest values of NH_3were observed in the easily digestible carbohydrate source (liquid sugar) and moderate carbohydrate than that of indigestible carbohydrate sources. This probably due to the process of breaking down protein into NH_3had just over the peak by rumen microbes and thereby some of NH_3had been used by rumen microbes which resulted in low NH_3concentration in the rumen. In contrast, treating *Chromolaenaodorata* silagewith hard-digested carbohydrates (rice straw flour) during 21 days of bio-fermentation process showed that more protein had broken down in the rumen compared to the other treatments. In addition, high NH_3 concentration may also due to the synchronization of energy requirements supplied by rice straw flour andhigh growth of microbes thus causing protein broken down in to NH_3. Another factor that contributed to high NH_3 concentrations in rumen fluid can be due to the inability of tannin to protect protein from rumen microbial degradation. NH_3 production is not only affected by tannin content but also influenced by the chemical properties of feed, and tannin at a certain level protects the protein from rumen microbial degradation, but in other conditions high and low tannin content can provide the same NH_3 production as found in the present study[30]. On average, the NH_3concentrations obtained in this study were ranged from 5.72 to 7.13 mM, indicating that all treatments were able to provide sufficient NH_3 for the growth of rumen microbes. The optimum concentrations of NH_3 supporting the growth of rumen microbes were 4 -12 mM [22].

In vitroVFA concentrations

The volatile fatty acid (VFA) concentration is used determine feedstuff fermentation which is closely related to rumen microbial activity and population. It can also be used to reflect the VFA production rate and its rate use (rate of VFA loss) in the body of the animal. Treating *Chromolaena odorata* silage with different source of carbohydrate in the present study had a highly significant effect on ($P< 0.01$) VFA concentrations (Table 4). On average, addition of rice straw flour to *Chromolaena odorata* silage resulted in higher VFA concentrations (90.99 mM; Table 4) compared to the other treatments. The VFA concentrations obtained in this study (90.99 mM) was within the range of VFA total which supports the growth of rumen microbial, compared to the other two treatments. Previous study stated that the normal range of total VFA that support the growth of rumen microbial were 80 - 160 mM [25]. Carbohydrates are the dominant components of feedstuff composed of cellulose, hemicellulose, starch, pectin and easily digestible carbohydrates with its main products of during digestion process in the rumen is VFA (acetate, propionate and butyrate). The product of carbohydrates digestion in the rumen is a monosaccharide, which is then fermented by rumen microbes into VFA, then transported by blood to the liver and to all body tissues for use as an energy source and for fat synthesis[31]. The butyric acid in the rumen epithel will be converted to β-hydroxybutyric acid and acetoacetate, then in the bloodstream in the form of keto bodies which will be used as energy source and body fat synthesis. Furthermore, the of fermentation products carbohydrate is in the form of VFA and NH_3, which is closely related to the synthesis of rumen microbial proteins which will then be distributed into the post-rumen and about 75% VFA absorbed by the animal as the source of amino acids for the animal and used as the main energy source . VFA production is a reflection of the number of bacteria, in synthesizing the protein. However that VFA is the result of polysaccharide hydrolysis to monosaccharide by rumen microbial enzyme which then fermented into VFA, CH_4 and CO_2. The VFA production consists of straight chain VFAs (acetate, propionate and butyrate) and branch-chain VFAs (iso butyrate, valerate and isovalerate)[32]. In addition, the VFA chains are used by rumen microbes as a source of carbon skeletons while ammonia is a source of nitrogen[33] for microbial growth.

5. Conclusion

Provision of different carbohydrate sources to *Chromolaena odorata* silage with C/N_{30} balance during 21 day bio-fermentation process affects the physical quality, feed composition, dry matter digestibility, organic matter digestibility, NH_3, VFA, pH, and lowering tannin content and saponin. Adding liquid sugar to *Chromolaena odorata* silage resulted in better physical quality (aroma, texture, color) than the other two treatments. In addition, adding rice straw flour to *Chromolaena odorata* silage as carbohydrate source had better feed composition, dry matter digestibility, organic matter digestibility, pH, NH_3 concentration, VFA concentration, tannin content, lower saponin and lower crude fiber compared with the other treatments. The authors recommended that if *Chromolaena odorata* is to be used as animal feed then adding rice straw flour as carbohydrate source with a C/N_{30} ratioduring 21 days bio-fermentation to increase its utilization. However, if using other digestible carbohydrates source then it is better before 21 days of bio-fermentation.

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Table 1: Physical quality of *Chromolaena odorata* during 21 days bio-fermentation

Variables	Treatments		
	CO_{30}_GC	**CO_{30}_PTK**	**CO_{30}_JP**
Color	Green	Green brownish	Green brownish
Smell	Acidic	Slightly acidic	Slightly acidic
Texture	Solid, clumped not slimy solid	Solid, clumped not slimy solid	Solid, clumped not slimy solid
Fungus existence	≤ 1%	≤ 1%	≤ 1%
Temperature	34,57[a]	36,22[a]	33,10[a]
pH	6,46[a]	6,49[b]	6,63[b]
P- values	0,05	0,05	0,05

Means within the same row with different superscripts differ significantly ($P < 0.05$). CO_{30}_GC : CO with $C/N_{(30)}$ ration liguid palm sugarontar + 10% rumen content; CO_{30}_PTK : CO with $C/N_{(30)}$ ration palm pith flour + 10% rumen content; CO_{30}_JP : CO with $C/N_{(30)}$ ration rice straw flour + 10% rumen content.

Table 2: Content of BK, BO, PK, SK, CHO, BetN, GE, EM, Ca, P, NDF, ADF, Tanin, Saponin silage *Chromolaena odorata* bio-fermentation results during 21 days of study.

Variables (%)	Tratments			P-values
	CO_{30}_GC	CO_{30}_PTK	CO_{30}_JP	
Dry matter *)	96,26^{c}	97,15^{b}	98,44^{a}	0,01
Organic matter *)	90,73^{b}	87,05^{c}	92,78^{a}	0,01
Crude Protein *)	19,13^{c}	25,42^{b}	27,48^{a}	0,01
Crude fat *)	10,47^{a}	7,58^{c}	11,44^{a}	0,01
Crude fiber *)	29,59^{b}	34,80^{a}	25,15^{c}	0,01
CHO *)	60,17^{a}	54,06^{b}	57,51^{ab}	0,05
BetN *)	30,58^{a}	19,26^{b}	29,03^{c}	0,01
Gross Energi, MJ/kg BK *)	18,947^{b}	18,097^{c}	19,763^{a}	0,01
Gross Energi, kkal/kgBK *)	4511,170^{b}	4308,758^{c}	4705,550^{a}	0,01
E. Metabolis, kkal/kgBK *)	3035.790^{b}	2471.738^{c}	3230.302a	0,01
Calsium **)	0,91^{a}	0,62^{c}	0,79^{b}	0,01
Pospor **)	0,32^{a}	0,24^{b}	0,35^{a}	0,01
NDF ***)	53,86^{c}	59,05^{b}	64,62^{a}	0,01
ADF ***)	41.93	45,99	45,47	-
Tanin ****)	2,35^{a}	1,95^{b}	2,04^{b}	0,01
Saponin *****)	0,39^{a}	0,58^{b}	0,38^{a}	0,01

Means within the same row with different superscripts differ significantly (P < 0.05)
Sources: *) Feed Chemicals Laboratory, FapetUndana, 2017; **) Nutrition Laboratory Animal Breeding Laboratory IPB, Bogor, 2017; ***) Laboratory Science and Technology Feed, IPB, Bogor, 2017; ****) Nutrition Laboratory Analysis and Feeding Livestock Fapet Unhas, Makasar, 2017; *****) Ciawi Livestock Research Institute, Bogor, 2017.

Table 3: Amino acid content *Chromolaenaodorate* during 21 days bio-fermentation

Variables (%)/ BK	Treatments			P- values
	CO_{30}_GC	CO_{30}_PTK	CO_{30}_JP	
Metionin*	0,0100^{a}	0,0095^{a}	0,0100^{a}	0,05
Sistein*	0,2445^{a}	0,3020^{a}	0,2200^{a}	0,05
Lisin*	1,1855^{a}	1,1415^{ab}	0,9490^{b}	0,05
Serin*	0,0055^{b}	0,0055^{b}	0,0040^{a}	0,05
Tiroksin*	0,0265^{a}	0,0465^{a}	0,0410^{a}	-
Treonin*	0,0150^{a}	0.0140^{a}	0.0090^{a}	-
Alanin*	0.0095^{a}	0.0075^{b}	0.0055^{c}	0,01
Fenilalanin*	0.0160^{a}	0.0170^{a}	0.0 90^{b}	0,01
Valin*	0,0470^{a}	0,0410^{a}	0,0205^{b}	0,01
Leusin-isoleusin*	0,0155^{b}	0,0170^{a}	0,0055^{c}	0,01
Glutamin*	0.0315^{a}	0.0265^{b}	0.0190^{c}	0,01
Aspartat*	0.0125^{a}	0.0175^{a}	0.0130^{a}	-
Prolin*	0.0690^{a}	0.0475^{b}	0.0215^{c}	0,01
TAAE**	1,289	1,540	1,003	-
TAANE**	0,3992	0,4530	0,3240	-

Means within the same row with different superscripts differ significantly (P < 0.05)
Sources: *) Biosain Laboratory Analysis of State Polytechnic of Jember, 2017; **) Calculated Essential and Non-Essential Amino Acid, 2017
TAAE = Total Essential Amino Acids; TAANE = Total Non-Essential Amino Acids.

Table 4: In vitro Digestibility Value of Dry and Organic Materials, NH_3, VFA, *Chromolaena odorata* of bio-fermentation results during 21 days of study

Treatments	Varaibles				
	DMD (%)	OMD (%)	NH_3(mM)	VFA (mM)	pH
CO_{30}_GC	45,24^{b}	40,93^{b}	6,08^{b}	77,01^{b}	6,74^{b}
CO_{30}_PTK	38,37^{c}	35,39^{c}	5,72^{b}	66,12^{c}	6,85^{a}
CO_{30}_JP	48,49^{a}	44,90^{a}	7,13^{a}	90,99^{a}	6,63^{c}
P-values	0,01	0,01	0,01	0,01	0,01

Information:
- NH_3 = ammonia; VFA = volatile fatty acid; DMD = dry matter digestibility; OMD = organic matter digestibility
- Result of Laboratory Analysis of Dairy Nutrition, Fapet IPB, 2017.
- Values with different superscripts in the same column differ very significantly in the P values listed under Duncan Multiple Duncan Test.

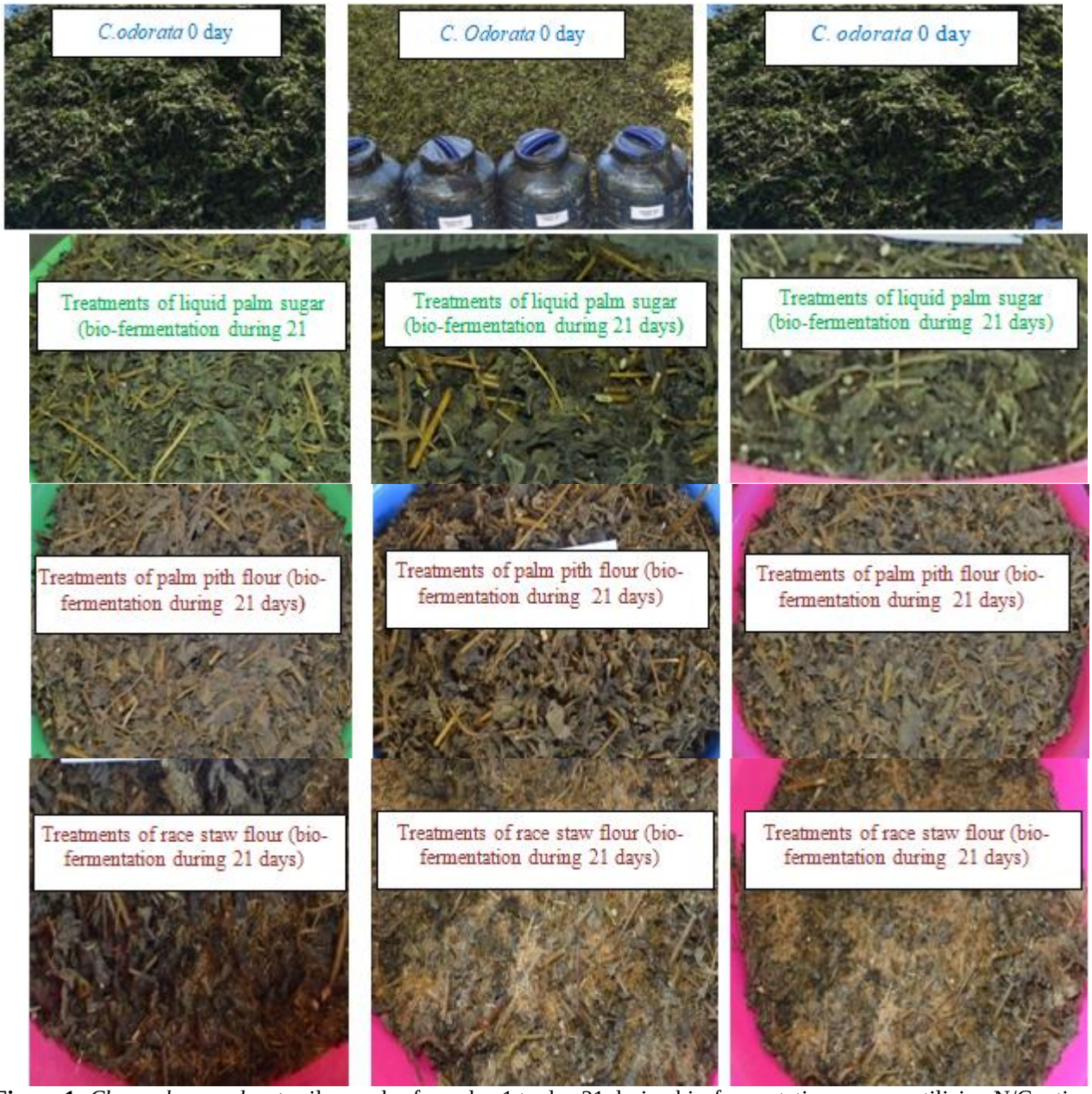

Figure 1: *Chromolaena odorata* silage color from day 1 to day 21 during bio-fermentation process utilizing N/C ration 30 with different carbohydrate sources