TESTING FEED OF SUGAR CANE PULP AMMONIATION WITH UREA AND AMMONIUM SULFATE ADMINISTRATION BY MEASURING TOTAL VFA CONCENTRATION AND BACTERIA AND PROTOZOA POPULATION OF SHEEP RUMEN FLUID

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Abstract

The objectives of this research was to study the effect feeding amoniation sugar cane pulp with supplement urea and amonium sulphate by measurement concentration volatile fatty acid (vfa) and bacterial and protozoa population of sumedang local sheep rumen liquor. The experimental method arranged in split-split plot design 2x3x3, first factor is resource of amonia, second factor, is amonia concentration, and threeth factor is water concentration was replicated three time. The test result revealed the significant effect (P<0,05) from resource of amonia on bacterial and protozoa population but not significant on concentration volatile fatty acid (VFA). Amonia concentration revealed the significant (P<0,05) on bacterial population and concentration volatile fatty acid (vfa)but not significant on population protozoa. Water concentration revealed the significant (P<0,05) on bacterial population and concentration volatile fatty acid (vfa)but not significant on population protozoa. There are interaction between resource and concentration of amonia on all parameter. Also, there are interaction between concentration of amonia and water and resource of amonia on all parameter.

Key word: amoniation sugar cane pulp, urea, amonium sulphate, concentration volatile fatty acid (vfa), bacterial and protozoa population, local sheep, rumen liquor

Introduction

Providing quality feedstuffs, both in the number of lots, cheap, and not compete with humans are the main target in an attempt to reach the level of livestock production and maximum profit. Businesses that have been done to obtain the feed material is agricultural waste utilization.Until now, the forage provided by farmers in Indonesia in general still rely forage from outside the farming business, which is derived from rice field, fields, road, irrigation canal embankments, and other fields with low quality, as well as a small part of was one of sugar cane pulp waste agricultural industry with huge potential as livestock feed, for production of many throughout the year. When compared with other components derived from sugarcane, sugar cane pulp is the largest component. According Oediyono (1985) sugar cane pulp content ranges from 24 to 36%, temporal by Mochtar and Anand (1986) ranged from 30 to 35%. If the production of milled sugar cane production for the whole region in Indonesia is 19,818,210.4 tons (P3GI, 1997), the sugar cane pulp produced is 4,708,370, 5 tons.

Sugar cane pulp consist largely of crude fiber as the cell wall structure that can be utilized by livestock as a source of energy. But if you want to use as feed maximally, necessary to be processed first, because in addition to sugar cane pulp containing low protein, also contains lignin and silica content is high, this is the limit of digestibility.Lignin can not be destroyed by rumen microbes. This condition is an inhibiting factor in the use of sugar cane pulp as feed. Ruminants sugar cane pulp only able to consume less than 2% of body weight. Efforts to increase utilization of sugar cane pulp is generally done by increasing kecernaannya that is through breaking the bond between the lignin to other cell wall fraction.

One of the processing of agricultural wastes like rice straw for example is to use a chemical substance that is alkaline (alkali) such as ammonia (NH3), Na OH and Ca (OH) 2 (Jackson, 1977) Of the three chemical substances, the treatment with ammonia provide more advantages because in addition to improving the digestibility also increased the levels of nitrogen (Sundstol and Owen, 1984). This fact can be introduced into processing sugar cane pulp because it has properties similar to rice straw.

Sources of ammonia are cheap and easily available in the market is urea, so far the most commonly used are urea while there are other sources of ammonia are also many in the market is ammonium sulfate. In common practice, especially in some Asian countries, in the process of ammoniation found the two techniques. First, that is by mixing a solution of ammonia or urea directly to the substrate (ammonia release method). Second, the method of air-tight container. In Indonesia an airtight container method started being introduced by Abdel Komar (1984). But so far the observation of existing container systems in Indonesia shows a weakness that is the hydrolysis of urea into ammonia does not run fast.

The effectiveness of treatment with ammonia is determined partly by the dose of ammonia and water content of the substrate in this case sugar cane sugar cane pulp. Some researchers say that the optimum dose is 2.5 to 5% ammonia. While the increase in water content followed by elevated levels of nitrogen and the digestibility of sugar cane pulp. But this still needs to be proved in order to obtain levels of ammonia, which is really the optimum because different sources of ammonia are used .

The digestive process is very complex in sheep because the sheep, including animals that have a ruminant stomach that is plural rumen, reticulum, omasum, and abomasum. Digestion in sheep occurs in mechanical (mastication in the mouth), hydrolytic (digestive enzymes), and Fermentative (rumen). On the hydrolytic digestion, food substances that form polymers having hydrolysis into monomers, whereas at monomers Fermentative digestion was soon experiencing further catabolism, such as: protein into ammonia (NH3) and carbohydrates into volatile fatty acid (VFA), which consists of propionate, acetate, and butyrate (Church and Pond, 1988).

In terms of their feed requirements, sheep as a ruminant is different from other herbivores. The difference lies in (1) the ability to utilize coarse fibrous feed as a source of higher energy, (2) The protein requirement can be met from the crude protein includes non-protein nitrogen (NPN), (3) able to synthesize vitamin B complex (Preston and Leng , 1987). Mechanisms like this happen in the first three of

the four stomachs of ruminant stomach plural because of the role of microorganisms that live in that place.

Large rumen role are bacteria, protozoa, and fungi. Bacteria are the most species, the number reached 10 billion per ml of rumen fluid (Yokohama, and Johnson, 1988). There is extensive interaction between microorganism in the rumen, the form of interaction can be a mutual dependence on the substrate, the substrate competition of mutual benefit or harm in the form of relationships (Yokohama and Johnson, 1988). Forms of interaction between rumen divided into three namely the interaction of bacteria with bacteria, the bacteria with protozoa, and fungi by protozoa (Preston and Leng, 1987). Protozoa and bacteria compete in the utilization of feedstuffs. Protozoa also use bacteria as a source of protein for his life, so the number of bacteria will be reduced by half or more (Yokohama, and Johnson, 1988).

All interactions will affect the balance of rumen microbial population and rumen fermentation process is in progress. The ideal comparison between the populations of protozoa and bacteria in the rumen is approximately 10^6-10^{10} cells per ml of rumen fluid .. Improved rumen microbial activity, livestock due to the increase rumen microbial population. Rumen microbial activity can be seen from the rumen pH, total bacteria and protozoa rumen, digestibility of dry matter and organic including dio it flew fatty acid production (VFA) and ammonia content of rumen fluid (Preston and Leng, 1987). The final result of fermentation of feed components by rumen microbes that can be utilized by livestock is a fatty acid fly (VFA), microbial protein, and vitamin B complex. While the end product that can not be exploited is in the form of CH4, CO2, and nitrate, which is excreted through eructation from the rumen (Owens and Goetsch, 1988).

Sugar cane pulp ammoniation would increase levels of nitrogen, but the measurement of total nitrogen can not describe the amount of nitrogen dissolved in the sugar cane pulp. Often due to chemical reactions during the ammoniation process, nitrogen is fixed firmly attached to the cell some hay and go wasted with feces. While the dissolved nitrogen is important because it will be used directly by rumen microbes to metabolism and in turn will determine the effectiveness of the digestibility of food (INRA, 1978). Based on the measurement of volatile fatty acid (VFA) and the population of bacteria and protozoa are the two sides are closely related variables in describing a conducive atmosphere in the rumen that can are manifestation in the use of feed materials such as sugar cane pulp.

Seeing the problem, then the sugar cane pulp can be used optimally if it is done before processing. The approach adopted is sugar cane pulp ammoniation process by using two different sources of ammonia is urea and ammonium sulfate.

MATERIALS AND METHODS

This research was carried out by basic experimental design was Randomized Design community group (RDB) 2 x 3 x 3, split split plot with three factors: the first factor as the main plot are two different sources of ammonia, both as a subplot factor was the concentration of ammonia ie different equalization 3, 5, and 7% ammonia, and the third factor as children are the difference plots of different water content of 20, 30, and 40%.

Ammoniation method

Ammoniation method used is the method of release of ammonia "wet method" that is by mixing a solution of ammonia in sugar cane pulp. Sugar cane pulp

is then inserted into a plastic bag and tied tightly. Old ammoniation process is carried out 14 days according to Komar (1984). After reaching the specified time the plastic bag is opened, the result of ammoniation then further aerated for roasted ground and tested in vitro.

The conduct of in vitro procedures using rumen fluid (Tilley and Terry method, 1963).

Variables measured:

1. Concentration Total Fatty Acids Rumen Fluid

   Measurement of the concentration determined by steam distillation refers to the (General Laboratory Procedures, 1966). First of all rumen fluid supernatant were 5 ml and 1 ml of 15% H2SO4 solution in a tube is inserted into the distiller. Hot water vapor will urge the VFA through the cooling tube, condensed and deposited in a erlenmeyer containing 5 ml of 0.5 N NaOH until a certain volume. Next add 2 drops of indicator PP (phenolphtalein) for further titrated with HCl 0.5 N. Titration end at the starting point changes colors, from pink to clear (no color). Blank titration was also performed on 5 ml of NaOH as a comparison. Total VFA concentration was calculated using the formula:

   Total VFA = (b - s) x N HCl x 1000 ml

   Where: b = blank titrant volumes

   s = volume of titrant sample

   N = normality of HCl solution

2. Counting the population of bacteria and protozoa rumen fluid

   The number of rumen fluid bacteria

   The method used to calculate the number of bacteria is a method of rumen fluid dilution and staining by formalsalyne 10% solution, then observed and counted using a hemocytometer (Ogimoto and Imai, 1981)

   The number of protozoa rumen fluid

   The method used to calculate the number of protozoa rumen fluid dilution method and staining by the FMS solution and then observed and counted by using haemocytometer (Ogimoto and Imai, 1981)

   Data Analysis

   Differences between treatments were tested sta (tistically by Sidik Variety, while the differences among the treatments performed by Least Significant Difference test (LSD = Least Significant Difference) (Gaspersz, 1991).

RESULTS AND DISCUSSION

Effect of Ammonia Source On Total VFA Concentration of Rumen Fluid

The average total VFA concentration measurements sheep rumen fluid from two sources ie Ammonia and Urea Ammonium Sulphate (ZA) on sugar cane pulp ammoniation process after 48 hours in vitro can be seen from Table 1. follows.

Table 1. The average concentration of total VFA Sheep Rumen Fluid in Different Sources of Ammonia

Ammonia Sources	Total VFA Concentration (mM/L)
Urea	49.60 a

ZA	51.32 a

Description: The same letter within a column indicate no significant difference (P> 0.05)

VFA (Volatile Fatty Acid) or fatty acid fly is one of the fermented products of feed material by rumen microorganisms. The number of total VFA was influenced by the chemical composition of ration and rumen ecological conditions. From the above data that the ZA gives better value than urea, it gives the sense that ZA provides chemical and ecological composition more conducive to rumen microorganisms.

Ammonia Sources Statistical analysis did not provide tangible effect on levels of total VFA concentrations (P> 0.05).

Effect of Ammonia Concentration on Total VFA Concentration of Rumen Fluid

The average total VFA concentration measurements sheep rumen fluid after 48 hours in vitro can be seen from Table 2. The table below shows that the highest total VFA concentration was obtained at the ammonia content of 7% followed by 3% and 5%.

Table 2. The average concentration of total VFA Sheep Rumen Fluid in Different Levels of Ammonia

Ammonia Concentration (%)	KonsentrasiVFA total (mM / Lt)
3	48.68 a
5	49.17 a
7	7 53.52 a

Description: The same letter within a column indicate no significant difference (P> 0.05)

Results of statistical analysis in Appendix 1. showed that the levels of ammonia did not affect significantly (P> 0.05) on total VFA concentration but provides significant interaction (P <0.01) between the source of ammonia and ammonia levels mean change in value of total VFA concentration did not stand alone but is influenced by interaction between sources and levels of ammonia amonianya. Thus, based on research results above shows that there are differences in total VFA concentration in ammonia content and related ammonia source.

Effect of Water on Total VFA Concentration of Rumen Fluid

The average measured concentrations of total VFA of rumen fluid from sheep after 48 hours in vitro at different water content in the process of ammoniation can be seen from Table 3. The table below shows that the highest total VFA concentration was obtained at 40% moisture content followed by the water content of 20% and 30%.

Table 3. The average concentration of total VFA Sheep Rumen Fluid in Different Water Levels

Water Content (%)	Total VFA concentration (mM / Lt)
20	47.82 a
30	46.06 a

40		57.50 b

Description: The same letter within a column indicate no significant difference (P> 0.05)

Statistical analysis showed that water content in sugar cane pulp ammoniation process provides significant effect on total VFA concentration (P <0.05), also showed significant interaction between water content both with the source of N and the ammonia content (P <0.01 .) The results further test the least significant difference (LSD = Least Significant Difference), indicating that there was significant difference (P <0.001) between the water content of 40% with water content of 20% and 30%. Although no difference between the levels of 20% and 30% water content.

Results like these indicate that in the process of N fixation in the ammoniation require sufficient moisture to penetrate the cells possessed sugar cane pulp is relatively more coarse than the straw that require maximum water content of 30%.

Most of the rumen microbes (mainly bacteria) can not utilize amino acids langsungklarena do not have the transportation system to transport amino acids into the cell. Approximately 82% of rumen microbes using the N-ammonia to revamp some amino acids into ammonia required (Sutardi, 1977).

During processing, the 30 to 60% of ammonia used is absorbed (fixated) into a network that will increase forage crude protein content in forages. The existence of nitrogen fixation by some of the ammonia absorbed by the moisture of the forage tissue. Ammonia is absorbed will bind to the acetyl group of forage and form the ammonium acetate salt. Which can be directly used by microorganisms in the rumen. Fixed nitrogen that can survive in forage tissue crops despite the heated ones.

Effect of Ammonia Source on Population of Rumen Fluid Bacteria and Protozoa

The result of counting the population of bacteria and protozoa, rumen fluid of sheep on different sources of urea and ammonia are presented in Table ZA. 4.

Table 4. The average total population of Bacteria and Protozoa of Sheep Rumen Fluid in Different Sources of Ammonia

Ammonia Sources	bacteria population (x109)	Protozoa Population (x106)
Urea	3.13 a	3.83 a
ZA	1.31 a	3.10 b

Description: The same letter within a column indicate no significant difference (P> 0.05)

Statistical analysis of the population of bacteria and protozoa suggests that the source of ammonia to give real impact on the populations of bacteria and protozoa (P <0.05) but further test the least significant differences evident only in protozoa population (P <0.05). Based on the above results indicate that urea provides a good source of ammonia to the growth of bacteria but also against protozoa, another case

with ZA which provide a good source of ammonia to bacterial population but slightly suppress the growth of protozoa. This is partly due to ZA contain sulfur which is very helpful in the synthesis of amino acids Methionine which is indispensable for bacterial growth. 1 / 3 part).±According Sutardi (1997), the transfer of methionine and amino acid (AA) branched out into the large rumen (

Effect of Ammonia Concentration on the Population of Rumen Fluid Bacteria and Protozoa

The result of counting the population of bacteria and protozoa in sheep rumen fluid ammonia concentration differences are presented in Table. 5.
Table 5. The average population of bacteria and Protozoa Sheep Rumen Liquid Ammonia at Different Levels

Table 5. The average population of bacteria and Protozoa Sheep Rumen Liqiud at Different Ammonia Levels

Ammonia concentration (%)	bacteria population (x109)	Protozoa population (x106)
3	3 3.73 a	3.51 a
5	5 0.88 b	3.15 a
7	7 2.05 c	3.74 b

Description: The same letter within a column indicate no significant difference (P> 0.05)

Results of statistical analysis the influence of ammonia on the population levels of bacteria and protozoa showed significant differences (P <0.01) both on the population of bacteria and protozoa population. Based on the data above shows that the highest bacterial growth was obtained at 3% ammonia concentration and protozoa population with the highest ammonia content of 7%. Interpretation of these results can be very diverse because of the interaction of bacteria and protozoa can change at any time. Growth of protozoa can not be released from its food supply is from bacteria. The above results can be assumed that the best result obtained at the ammonia content of 7% because the population of bacteria remained relatively high despite high protozoan. While the ammonia content of 5% could be interpreted that the population of bacteria began depressed because protozoa are very dominating.

Effect of Water Content on Population of Rumen Fluid Bacterial and Protozoa

Table 6. The Average Population Of Bacteria and Protozoa Sheep Rumen Ammonia At Different Levels of Water Content

Water Content (%)	bacteria population (x109)	Protozoa population (x106)
3	20 1.18 a	3.66 a
5	30 2.72 b	3.65 a
7	40 2.76 b	3.09 a

Description: The same letter within a column indicate no significant difference (P> 0.05)

Statistical analysis of water content effect on the population of bacteria and protozoa showed significant differences (P <0.05) against the population of bacteria and no significant (P> 0.05) on protozoan population. The data above show that the highest bacterial population obtained by the water content of 40% and the lowest protozoa population was obtained at 40% moisture content. Based on these results can interpreted that the water content of 40% to provide an atmosphere conducive to bacterial growth and still provide a good atmosphere also against protozoa.

During processing, the 30 to 60% of ammonia used is absorbed (fixated) into a network that will increase forage crude protein content in forages. The existence of nitrogen fixation by some of the ammonia absorbed by the moisture of the forage tissue. Ammonia is absorbed will bind to the acetyl group of forage and form the ammonium acetate salt.

Conclusions and Suggestions

Conclusion

1. Sources of urea and ammonia that ZA does not show significant effect on total VFA concentration edge effect was significantly (P <0.05) to the population of bacteria and protozoa.
2. Levels of ammonia to give the effect was significantly (P <0.01) against the population of bacteria and protozoa population, but not to the levels of total VFA.
3. The water content gives the effect was significantly (P <0.05) against all the variables namely total VFA concentration and the population of bacteria and protozoa.
4. There is interaction between the source of ammonia and ammonia levels of all variables, there is interaction between water content, ammonia, and the source of ammonia to the population of bacteria and protozoa but not on levels of total VFA.

Suggestion

1. Ammonium Sulfate (ZA) could be an alternative source of ammonia is a better addition to the price is relatively the same provide better quality views of the parameters measured.
2. Ammonia levels between 5% and 7% is ammonia levels are good for sugar cane pulp ammoniation process.
3. The water content of between 30 and 40% is the optimal water content for sugar cane pulp ammoniation process

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