The effect of fermentation with different times of corn husk which has obtained ammoniation treatment in the production of VFA-NH3 by in vitro digestibility

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Abstract. The objective of the research is to examine the effect of the difference time of fermentation process using Aspergillus niger to corn husk that has been ammoniated on fermentability parameter in vitro which included the production of VFA and NH3. The aimed of this research was to determine the proper processing technique for corn husk in an effort to improve its quality as a complete feed ingredient for beef cattle. The experimental design used was completely randomized design with 3 treatment (different time of fermentation : 0; 1 and 2 weeks) and 4 replication. Corn husk which has been ammoniated by 6% ammonia with the temperature of 60 °C for 3 days, added 5% of Aspergillus niger as starter, than fermentation with different time. Parameter observed were fermentability parameter in vitro including the production of VFA and NH3. Data was analyzed using analysis of variance and if there was significant effect continued by Duncan’s test. The results indicate that the longer the fermentation process, the production of VFA and NH3 increased (P<0.05). The conclusion obtained is that the fermentation process using starter of A. niger 5% to 2 weeks can increase the production of VFA, NH3. The best production of VFA and NH3 occurs in 2 weeks, namely: 122.42 ± 4.35 and 5.99 ± 0.53 mm.

1. Introduction
Effort to increase the availability of feed and an effort for feed cost efficiency can be done by finding new feed sources that have not been used or not commonly used by farmers (unconventional feed ingredients). Some types of agricultural and plantation waste that are not commonly used as food are found in several regions such as corn husk, corncob, soybean peel, cassava peel, cacao peel, peanut skin and coffee peel, but in some other areas has already used these ingredients as animal feed [1].

One of the remaining food crops and plantations that has considerable potential are corn cob and corn husk. The potential of cob as feed has been widely studied, among others, by Tampoebolon [2,3,4], while the corn husk is still not widely studied. The area of harvested corn in Central Java Province in 2015 was 542.804 ha, with corn seed yield of 3,212,391 tons and corn husk waste was approximately 432,387 tons [5]. Basymeleh [6], reported that the corn waste consisted of 50% stems, 20% leaves, 20% cobs, 10% husks. Most of the waste has not been utilized. The corn husk is the outer husk of corn which wraps the corn and usually thrown away. The use of corn husk as a feed has an obstacle because the corn husk has low crude protein content (2.8%) and high crude fibre (36.52%) [7]. Therefore, in its utilization as the feed ingredient, the corn husk needs to be improved its quality, one of them uses ammonia-fermentation processing technology (Amofer).
One of the important functions of ammoniation is able to stretch and or to break lignocellulose and hemicellulose bonds while microbial starter can reduce crude fibre level while increasing digestibility and crude protein ingredient. The fermentation process, among others, aims to produce a product (feed ingredient) that has better nutritional and biological availability [8]. The use of ammonia-fermentation technology can increase the crude protein content of corn husk, reduce the crude fibre content and increase the digestibility of corn husk so that it can be used as a good alternative as ruminant animal feed.

Volatile fatty acid (VFA) is the main energy source for the ruminant’s body needs, especially beef cattle [9, 10]. The optimum production of VFA from a digestion of feed ingredient is needed to support the productivity of livestock. Tillman et al. [11], states that the increased digestibility of feed ingredient will increase VFA production. The feed protein is transformed by bacterial proteolytic enzymes and rumen protozoa to be peptides, amino acid and further transformed to be ammonia (NH3) [11, 12]. Ammonia production which is high enough from a degradation of feed ingredient will be able to supply the nitrogen needs of rumen microbe which will eventually be utilized by cattle itself [13]. This research aims to examine the effect of the ripening time of fermentation process toward amoniated corn husk on fermentability parameter in vitro including the production of VFA (volatile fatty acid) and NH3.

2. Materials and methods

The material used were corn husk, Aspergillus niger (A. niger), mineral solution for A. niger growth according to “American Association of Textile Chemist and Colorist Mineral Salt Iron” (3 g (NH4)2NO3, 2.5 g KH2PO4, 2 g K2HPO4, 0.2 g MgSO4.7 H2O, 0.1 g Fe SO4.7 H2O), aquades, vinegar (CH3 COOH) and rice, and one chemical kit for testing in vitro parameter including the production of VFA and NH3. The research equipment used including fermenter, autoclave, thermometer, blender, weight with the capacity of 2 kg with accuracy limit of 10 g, analytical weight with the capacity of 120 g with accuracy of 0.0001 g, universal pH indicator, and one unit of equipment for fermentability testing in vitro includes the production of VFA and NH3.

The research is divided into 3 phases. The research activity of phase I started with high-temperature ammoniation (60°C) of corn husk using 5% of ammonia level with 3 days of curing period. The research activity of phase II is to carry out the fermentation process using the Aspergillus niger as starter with level of 5%. The experimental design used was completely randomized design with 3 treatment (different time of fermentation: 0; 1 and 2 weeks) with 4 replication. The research activity of phase III is to conduct laboratory analysis of fermentability parameter in vitro including the production of VFA (volatile fatty acids) and NH3. The analysis in the test of in vitro was carried out according to the Haris method [14].

Data from the observation of each parameter was analyzed using analysis of variance (F Test) and if there was a real effect followed by the DMRT test (Duncan’s Multiple range test) according to Steel and Torrie [15].

3. Results and discussion

3.1. Production of Volatyl Fatty Acid (VFA)

The data in Table 1. shows that the time of fermentation have significant effect to VFA production (P <0.05). Volatile Fatty Acid production increased with increasing time of fermentation. The increase in VFA production is mainly due to an increase in organic matter content, mostly in the form of carbohydrate as the main source of VFA. The average VFA production of corn husk the treatment is ranged from 81.67±0.82 to 122.42±4.35 mM, with median value of 101.25 mM. The production of VFA is sufficient to support the maximum rumen microbial protein synthesis. Optimal production of VFA is to support the maximum rumen microbial protein synthesis, according to Sutardi et al. [13] ranges from 80 - 160 mM. The results of the analysis of digestibility of organic material in this research respectively are 37.39% for T0, 44.80% for T1 and 60.10% for T3. The high
digestibility of organic material in fermented corn husk shows that the fermentability of the material is high as well. The more fermentable a feed ingredient, the higher the production of VFA [11]. The optimum production of VFA from a digestion of feed ingredient is really needed to support the livestock productivity.

Table 1. VFA and ammonia production from corn husk fermentation

| Parameter | In vitro | T₀ (0 week) | T₁ (1 week) | T₂ (2 weeks) |
|-----------|----------|-------------|-------------|--------------|
| VFA (mM)  |          | 81.67 ± 0.82<sup>a</sup> | 99.67 ± 6.22<sup>b</sup> | 122.42 ± 4.35<sup>a</sup> |
| NH₃ (mM)  |          | 3.98 ± 0.07<sup>b</sup> | 4.11 ± 0.16<sup>b</sup> | 5.99 ± 0.53<sup>a</sup> |

<sup>a,b,c</sup> Different superscript in the same row shows a significant difference (P <0.05)

3.2. Production of NH₃
The results of the variance analysis in table 1. show that the treatment have significant effect (P <0.05) to the production of NH₃. The improvement of production of NH₃ in vitro along with the increasing time of fermentation. The factors that determine the production of rumen NH₃, among others, are the nitrogen or crude protein content of the fibrous feed ingredient, and its degradability. The protein degradation is also reflected in the digestibility of organic material of the feed ingredient because protein is a component of the organic material. The results of the analysis of crude protein level in the treatment group of T₀, T₁ and T₂ are: 3.94; 5.49 and 8.59%. This increase in NH₃ production is evidenced by the increased digestibility of organic material for longer time of fermentation (2 weeks). The average NH₃ production of corn husk the treatment is ranged from 3.98±0.07 to 5.99±0.53 mM, with an average median value of 4.70 mM. The average production of NH₃ is sufficient to support the rumen microbial protein synthesis. According to Sutardi <i>et al.</i> [13], the concentration of optimal N\(\text{NH}_3\) to support the rumen microbial protein synthesis is 3.5 - 7.14 mM.

4. Conclusion
Based on the results of the research, it can be concluded that the treatment of time of fermentation process using <i>A. niger</i> 5% to 2 weeks can increase the production of VFA and NH₃.

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References
[1] Anggraeny Y N, U Umriyah, and N H Krishna 2008 The potential for corn waste is ready to be released as a forage for beef cattle. <i>Proceedings of the National Workshop on the Development of the Corn-Cow Integration System</i> (Pontianak: Puslitbangnak) 149-155.
[2] Tampoebolon and Surono 2012 <i>Effect of Ammoniation and Fermentation of Corn Cobs Waste on Proximate Content, Dry Matter Digestion, Organic Matter Digestion and In vitro Fermentation Products</i>. Competitive Grant Research Report (Semarang : Diponegoro University)
[3] Tampoebolon B.I.M. and BWHE Prasetiyono 2014. <i>Quality Complete Production of Rations Using “Amofer” Corn Cob and Protein By Pass Supplement for Beef Fattening</i>. Competitive Research Report The First Year (Semarang : Diponegoro University)
[4] Tampoebolon B.I.M. and BWHE Prasetiyono 2014. <i>Quality Complete Production of Rations Using “Amofer” Corn Cob and Protein By Pass Supplement for Beef Fattening</i>. Competitive Research Report The Second Year II (Semarang : Diponegoro University)
[5] Central Java Central Bureau of Statistics 2012 Central Java in Numbers.
[6] Basymeleh S 2009 Effect of type of forage and storage time on the physical properties of wafers Essay (Bogor: Bogor Agricultural Institute)

[7] Anggraeny Y N, U Umiiyasih and D Pamungkas 2005 Effect of multinutrient supplementation on the performance of beef cattle that obtain corn straw basalt feed Proceedings of the National Seminar on Animal Husbandry and Veterinary Technology (Bogor: Center for Animal Husbandry Research and Development) 147 – 152.

[8] Tampoebolon B I M 1997. Selection and Characterization of Cellulase Enzymes of Buffalo Rumen Cellulolytic Microbial Isolates. Postgraduate (Yogyakarta: Master Program of Gadjah Mada University)

[9] Sutardi, T 1980 Foundation for Nutrition. Faculty of Animal Husbandry (Bogor: Bogor Agricultural Institute) (Not published).

[10] Soewardi B 1974. Nutrition Ruminants. Department of Animal Feed Science, Faculty of Animal Husbandry (Bogor: Bogor Agricultural Institute) (Not published).

[11] Tillman A.D., H. Hartadi, S. Reksohadiprodjo, S. Prawirokusumo and S. Lebdosoekiyo. 1998. Basic of Animal Feed Science. 5th print. (Yogyakarta : Gadjah Mada University Press)

[12] Hungate, R.E. 1966. The Rumen and Its Microbes (London : Academic Press).

[13] Sutardi T, NA Sigit and T Toharmat 1983 Standardization of Protein Quality of Ruminant Animal Feed Based on Metabolic Parameters by Rumen Microbial. (Jakarta: Research Report of the Science and Technology Development Project of the Director General of Higher Education)

[14] Harris L E 1970 Nutrition Research Techniques for Domestic and Wild Animal Vol 1 Animal Science Department (Logan: Utah State University)

[15] Steel, R.G.D. and J.H. Torrie. 1991. Principles and Procedures of Statistics (Jakarta : Gramedia Pustaka Utama)