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Nutritional Quality of Amofer (Ammonia Fermentation) Corn Straw Using EM4 and M21 Decomposer

(Kualitas Nutrisi Jerami Jagung Amofer (Amonia Fermentasi) Menggunakan Dekomposer EM4 dan M21)

Restuti Fitria[1*], Subhanul Arafat Chayatul Luthfi[1], Novita Hindratiningrum[1]

[1]Animal Husbandry Departement, Nahdlatul Ulama University of Purwokerto, Jl. Sultan Agung, No. 42 Purwokerto Selatan, 53144

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*Corresponding author
restutifitria@gmail.com

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ABSTRACT

This study aimed to identify the best starter levels of commercial cultures and incubation time on crude protein (CP), crude fiber (CF), extract ether (EE), and nitrogen-free extract (NFE) of amofer corn straw. This research used a completely randomized design with ten treatments and four replications. The treatments were the additions of EM4 (EM) and M21 decomposer (MD) at different levels (control/without amofer; 0.04 % EM; 0.06 % EM; 0.04 % MD; and 0.06 % MD) and incubation times (4 weeks and 2 weeks). The obtained data were analyzed by analysis of variance (ANOVA), then continued with the Duncan Multiple Range Test (DMRT). The result showed that the use of commercial starters with different fermentation times could increase the CP and NFE levels while reducing the EE and CF of amofer corn straw. The most effective treatment was the addition of 0,04 % EM and 2 weeks incubation which contained CP of 5.9415 %, CF of 37.8545 %, EE of 4.5183 %, and NFE of 32.4245 %.

Kata Kunci:
Amofer
EM4
Jerami jagung
M21 decomposer

ABSTRAK

Penelitian ini bertujuan untuk mengidentifikasi level kultur starter komersial dan waktu ikubasi terbaik terhadap protein kasar (CP), serat kasar (CF), ekstrak eter (EE), dan ekstrak bebas nitrogen (NFE) jerami jagung amofer. Penelitian ini menggunakan rancangan acak lengkap dengan 10 perlakuan dan 4 ulangan. Perlakuan yang diberikan adalah penambahan EM4 (EM) dan M21 decomposer (MD) pada kadar yang berbeda yaitu: kontrol/tanpa amofer; 0,04 % EM; 0,06 % EM; 0,04 % MD; dan 0,06 % MD) serta waktu inkubasi 4 minggu dan 2 minggu. Data yang diperoleh dianalisis dengan menggunakan analisis varians (ANOVA) yang dilanjutkan dengan uji jarak berganda duncan (DMRT). Hasil penelitian menunjukkan bahwa penggunaan starter komersial dengan lama fermentasi berbeda dapat meningkatkan kadar CP dan NFE sekaligus menurunkan EE dan CF jerami jagung amofer. Perlakuan yang paling efektif adalah penambahan EM 0,04 % dan inkubasi 2 minggu dengan kandungan CP sebesar 5.9415 %, CF sebesar 37.8545 %, EE sebesar 4.5183 %, dan NFE sebesar 32.4245 %.

1. Introduction

As the main feed of ruminant cattle, fodder is highly affected by seasons that also hinder sufficient supply for cattle. An alternative solution is utilizing agricultural waste, such as from corn plantations. The dried corn area reaches 2,76 million ha in 2022 (Badan Pusat Statistik, 2023) and the by-product (stalks and leaves) can be utilized as feed for ruminant cattle. Corn straw can make up 10.8 ton/ha dry harvest and contain 6.38 % crude protein, 30.19 % crude fiber, 2.81 % extract ether, 51,69 % Nitrogen-free extract, 8.94 % ash, and 53.12 % TDN (Bahar, 2016).

Some obstacles in harnessing corn straw potentials as cattle feed are the low digestibility and low protein content. Corn straw is low-digestible because the plant tissue has undergone lignification that made the cellulose and hemicellulose bound with lignin difficult to absorb (Amin, Hasan, Yanuarianto, Iqbal, & Karda, 2019). Efforts to increase the nutritional value and digestibility of corn straw may include chemical treatment (ammoniated), biological treatment (fermentation), or both (amofer).

Ammoniated commonly uses urea as the N source to soften the corn straw fibers through the swelling process. Meanwhile, fermentation technology uses microorganisms through the addition of microbial starters. The combination of both ammonia and fermentation technologies is called amofer. The fermentation process wil be more effective when combined with ammonia because of the nitrogen supply. The addition of a starter in making amofer can increase crude protein (Prasetyo, Fitria, & Hindratiningrum, 2022) and reduce crude fiber (Hindratiningrum, Primanndini, & Kristiawan, 2021). In addition, the fermentation process can also increase crude fat content by 0.5 – 1.5 % (Suningsih, Ibrahim, Liandris, & Yulianti, 2019) and increase the content of nitrogen-free extract (Amrullah, Liman, & Erwanto, 2015).

The process of making feed fermentation requires the addition of a starter so that the fermentation process can take place more quickly and optimally. The most readily available starter for farmers is the commercial starter. EM4 (effective microorganism 4) is one of the commercial starters often used in making feed fermentation. Another commercial starter that is easy to find is M21 decomposer. However, the use of M21 decomposer in feed fermentation has not been widely studied.

Based on this, the use of M21 decomposer to make amofer corn straw still needs to be further evaluated. Fitriani, Hindratiningrum, & Fitria (2022) reported that the addition of M21 decomposer at the level of 0,04% in the manufacture of amofer corn straw was able to reduce pH from 5,120 (control) to 4,025. Hindratiningrum, Fitria, & Santosa (2022) also reported that adding M21 decomposer at 0,04 % produced the best dry matter digestibility and organic matter digestibility of 31,68 % and 30,13 %. However, it takes further investigation to figure out the best level of commercial starters and incubation time in producing the most effective amofer corn straw, so this study tried to identify the impact of these variables on the level of crude protein, crude fiber, extract ether, and nitrogen-free extract. In addition, the use of M21 decomposer and EM4 in the production of amofer corn straw was also compared in this study.

2. Materials and Methods

2.1. Materials

The materials used were corn straw, EM 4 (containing microorganisms, e.g., *Lactobacillus* sp., *Actinomycetes*, *Streptomyces*, and yeast), M21 decomposer (containing microbes, e.g., *Pseudomonas*, *Actinomycetes*, *Lactobacillus*, *Acetobacter*, *Trichoderma*, and *Rhizobium*), N content in urea, molasses, and water. The analysis of the nutrient content of corn straw was subjected to the proximate analysis (AOAC, 2005).

2.2. Sample Preparation

Corn straw was chopped using a chopper. M21 decomposer and EM4 were each developed by adding the amount according to the treatment, namely 10 ml (0.04 %) and 15 ml (0.06 %), then each added 250 ml molasses in 25 literes of water. The dose of use was 120 ml per kg DM. Corn straw with no starter or urea (without amofer) used as a control.

2.3. Ammonia Fermentation

Ammonia fermentation was carried out by weighing 1 kg DM of chopped corn straw and adding urea 4%. The first treatment was corn straw without amofer (control), the second treatment was corn straw amofer with 0.04 % M21 decomposer solution, the third treatment was corn straw amofer with 0.06 % M21 decomposer solution, the fourth treatment was corn straw amofer with 0.04 % EM4 solution, and the fifth treatment was corn straw amofer with 0.06 % EM4 solution. Each solution of M21 decomposer or EM4 and urea was then poured

on the corn straw until evenly distributed. After each was mixed, it was placed in a plastic bag. Fermentation lasts for 2 weeks and 4 weeks.

2.4. Experimental Design

This study used a completely randomized design with ten treatments and four replications. The treatments were D_0T_1= control/without amofer and 4 weeks incubation; D_0T_2 = control/without amofer and 2 weeks incubation; D_1T_1 = 0.04 % EM and 4 weeks incubation; D_1T_2 = 0,04 % EM and 2 weeks incubation; D_2T_1 = 0,06 % EM and 4 weeks incubation; D_2T_2 = 0,06 % EM and 4 weeks incubation; D_3T_1 = 0,04 % MD and 4 weeks incubation; D_3T_2 = 0,04 % MD and 2 weeks incubation; D_4T_1 = 0,06 % MD and 4 weeks incubation; D_4T_2 = 0,06 % MD and 2 weeks incubation.

2.5. Variables and Data Analysis

The measured variables in this study were crude protein (CP), crude fiber (CF), extract ether (EE), and nitrogen-free extract (NFE). The data were analyzed using analysis of variance (ANOVA) and then continued with the Duncan Multiple Range Test (DMRT) (Steel & Torrie, 1993).

3. Result and Discussion

3.1. The Effect of Starter Levels and incubation times on Crude Protein and Crude Fiber

The average of CP and CF based on the analysis of variance (ANOVA) were shown in Table 1. The data showed that the treatment has significant effect ($P<0.05$) on CP and CF.

Tabel 1. The average level of crude protein and crude fiber of amofer corn straw

Treatments	Average ± SD	
	Crude protein (%)	Crude fiber (%)
D_0T_1	4.5475 ± 0.007^{i}	38.8233 ± 0.061^{e}
D_0T_2	5.7993 ± 0.026^{h}	38.0588 ± 0.174^{g}
D_1T_1	5.8080 ± 0.006^{h}	38.9878 ± 0.044^{d}
D_1T_2	5.9415 ± 0.015^{g}	37.8545 ± 0.094^{h}
D_2T_1	6.5063 ± 0.014^{a}	40.8900 ± 0.091^{a}
D_2T_2	6.3035 ± 0.014^{c}	38.6718 ± 0.083^{f}
D_3T_1	6.4537 ± 0.006^{b}	40.4125 ± 0.04^{b}
D_3T_2	6.0493 ± 0.017^{f}	40.4085 ± 0.114^{b}
D_4T_1	6.0795 ± 0.01^{e}	40.3275 ± 0.064^{b}
D_4T_2	6.1440 ± 0.013^{d}	39.5230 ± 0.086^{c}

Note: The numbers followed by the different superscript letters within column were significantly different at DMRT 5 %. D_0 = control; D_1 = 0.04 % EM; D_2 = 0.06 % EM; D_3 = 0.04 % MD; D_4 = 0.06 % MD; T_1 = 4 weeks; T_2 = 2 weeks.

Using a commercial starter to making amofer corn straw could increase the CP level of amofer corn straw because the microorganism in EM or MD become the source of protein. It was in line with Rahmadina & Febriani (2017) that protein is a macromolecule that builds microorganism cells. According to Prasetyo et al. (2022) M21 decomposer is a formula of detritivore organisms as decomposers of organic matter and contains complete nutrients and microorganisms such as *Actynomycetes*, *Pseudomonas*, *Lactobacillus*, *Trichoderma*, *Acetobacter*, and *Rhizobium*). EM4 is a mixed culture in a brown liquid medium containing several microorganisms such as *Lactobacillus*, *Actinomycetes*, *Streptomyces*, and yeast.

The higher CP levels in the corn straw amofer compared to the CP level in the D_0 may also be due to the addition of urea. Adding urea to corn straw amofer contributed to the increased CP level because urea contained 46 % of the nitrogen which is equal to 287 % of crude protein (Beig et al., 2020), so incorporating urea helps improve CP level through nitrogen absorption in the urea (Suryani, Hernaman, & Ningsih, 2017).

The highest CP was 6,5063 % observed in treatment D_2T_1 (4 weeks). Additionally, the result showed that the longer the incubation time, the higher the CP level of amofer corn straw. This finding was confirmed by Ginting & Pase (2018) that crude protein level increases with incubation time. Meanwhile, the decreased CP level in treatment D_4T_1 (4 weeks incubation) may be due to the diminishing chance of optimum growth and fermentation for the microorganisms in the starter. The increased CP in treatment D_4T_2 (2 weeks) showed that this incubation time allowed a more optimum growth and fermentation for the microorganisms in the starter compared with another the 2 weeks incubation. Furthermore, Larangahen, Bagau, Imbar, & Liwe (2017) also reported that the crude protein levels of shoe banana peel silage decreased with longer incubation times.

Using a commercial starter to making amofer corn straw also could increase the CF level of amofer corn straw. The increased CF in amofer treatment may be attributed to the high lignin, which is a component in crude fiber in the corn straw that hinders the microorganism from digesting (Fitria, Hindratiningrum, & Santosa, 2021). Therefore, high lignin results in high CF in the amofer corn straw.

The lowest CF observed in treatment D_1T_2 (37.8545 %) indicated that using 0.04% starter EM could lower the CF level in the corn straw. In other words, 0.04 % EM could optimize the growth and development of microorganisms that contribute to CF digestion in the corn straw. According to Krismiyanto, Sutama, & Wahyuni (2014) one of the microorganisms that help decrease CF in corn straw is *Lactobacillus* sp. through fermentation by cellulolytic bacteria. The other microorganisms contained in EM4 starter and contributed to CF level is *Actinomycetes* which is a bacterium that produces cellulase enzyme that can degrade cellulose that is a component of crude fiber (Saini, Aggarwal, Sharma, & Yadav, 2015).

The highest CF was 40.8900 % obtained in treatment D_2T_1 (4 weeks). The analysis result showed that the longer the incubation time the higher the CF level of amofer corn straw. This finding contradicted Mangalisu, Armayanti, Arief, & Wulandari (2022) that the longer the incubation time, the longer it takes for the bacteria to optimally degrade the complex compound of the crude fiber including lignin, thus decreasing the CF. The results of this study are also not in line with the opinion of Setiyawan & Thiasari (2016) who state that the longer incubation time in the fermentation process will reduce the crude fiber level as a result of microbial activity. According to Hatma, Tampoebolon, & Prasetiyono (2018), microorganisms in the starter used for fermentation in the second week are still able to reduce the crude fiber level because nutritional needs are still met and the fermentation process is optimal. However, in the third week, the microorganisms began to decline due to not fulfilling nutritional needs so the fermentation process is not optimal.

Treatment D_1T_2 (0.04 % EM + 2 weeks fermentation) was the most effective in producing CP of 5.9415 % and CF of 37.8545 % in the amofer corn straw because this treatment has increased CP by 1.394 % and decreased CF by 0.9688 %. This result was due to microorganisms in the starter of 0.04 % incubated for 2 weeks could grow and develop most optimally to increase CP and lower CF compared to other treatments.

The type of microorganisms used, the availability of nutrient sources for microorganisms, and the length of fermentation are factors that can affect the fermentation process (Hatma et al., 2018). The CP level in the treatment using EM starter at the level 0.04 % with an incubation time of 4 weeks and 2 weeks was lower than the treatment with MD starter at the same level and the same incubation time. This could be due to the different types of microbes found in EM and MD starters. It is suspected that the content of microorganisms in MD starter is more capable of increasing the CP level of corn straw amofer at the incubation time of 4 weeks. Pseudomonas contained in MD starter can decompose proteins and other organic matter into CO_2, ammonia gas, and other simpler compounds (Rahmadian et al., 2018). Trichoderma contained in MD starter could also increase crude protein levels because it could form mycelium with ammonia and carbon substrate so that it could increase protein levels in line with the increase in incubation time in the biodegradation process (Jaelani, Widaningsih, & Mindarto, 2015).

3.2. The Effect of Starter Levels and Incubation Time on Extract Ether and Nitrogen-Free Extract

The analysis data results show that increasing the starter level during amofer process could decrease the EE level of the corn straw due to microorganisms in the starter (EM or MD). The average EE and NFE in Table 2 show that the treatment had a significant effect ($P<0.05$) on EE and NFE levels.

Trichoderma is one of the microorganisms that play a vital role in decreasing EE in corn straw. According to Daning & Karunia (2018), *Trichoderma* could produce lipase enzymes during fermentation. It allows Trichoderma to utilize the substrate fat as an energy source, thus decreasing the EE of the corn straw. The lowest EE (4.1478 %) produced in treatment D_4T_2 was probably due to *Trichoderma* contained in the 0.06 % addition of MD starter to the amofer. The decrease of EE in amofer corn straw could be due to the degradation of triglyceride complex binds into more simple binding like fatty acid and alcohol (Pratiwi, Fathul, & Muhtarudin, 2015). Most of these fatty acids will evaporate, thus decreasing the EE.

EE levels in corn straw amofer using EM starter both 0.04 % and 0.06 % were lower at 4 weeks fermentation than 2 weeks fermentation. The decrease in EE levels can be caused by the

many complex bonds of triglycerides into simpler bonds such as fatty acids and alcohol which are more volatile (Pratiwi et al., 2015). In contrast, the EE levels in corn straw amofer using MD both 0.04 % and 0.06 % were higher at four weeks of fermentation than at two weeks of fermentation. This shows that the use of MD starter could increase the activity of microorganisms in producing fatty acids along with the increased fermentation time, thus increasing EE levels. This is consistent with the opinion of Pratiwi et al. (2015) that bacterial activity that produces high levels of fatty acids causes an increase in EE levels.

Tabel 2. Extract ether dan nitrogen-free extract of amofer corn straw

Treatments	Average ± SD	
	Extract ether (%)	NFE (%)
D_0T_1	4.8480 ± 0.008^{c}	33.3568 ± 0.052^{c}
D_0T_2	5.9600 ± 0.027^{a}	29.1408 ± 0.133^{i}
D_1T_1	4.3105 ± 0.005^{g}	32.7258 ± 0.037^{d}
D_1T_2	4.5183 ± 0.011^{e}	32.4245 ± 0.081^{e}
D_2T_1	4.4365 ± 0.01^{f}	31.8343 ± 0.071^{g}
D_2T_2	5.0318 ± 0.011^{b}	33.8875 ± 0.073^{a}
D_3T_1	4.7225 ± 0.005^{d}	32.2658 ± 0.032^{f}
D_3T_2	4.5023 ± 0.013^{e}	30.8030 ± 0.087^{h}
D_4T_1	4.2540 ± 0.007^{h}	33.7698 ± 0.054^{b}
D_4T_2	4.1478 ± 0.009^{i}	33.7130 ± 0.073^{b}

Note: The numbers followed by the different superscript letters within column were significantly different at DMRT 5 %. D_0 = control; D_1 = 0.04 % EM; D_2 = 0.06 % EM; D_3 = 0.04 % MD; D_4 = 0.06 % MD; T_1 = 4 weeks; T_2 = 2 weeks; NFE = nitrogen-free extract.

The addition of commercial starters during the corn straw amofer process could increase the NFE level of corn straw amofer (Tabel 2). The increased NFE is a beneficial nutritional aspect because it shows that higher organic matter would help ease digestion and produce energy. Increasing the NFE level in corn straw is due to microorganisms contained in the added starters. The result showed that the highest addition of the starter (0.06 %) produced the highest NFE compared to the 0.04 % addition.

The highest NFE was observed in D_2T_2 (33.8875 %), and the lowest was in D_0T_2 (29.1408 %). This result showed that the longer the incubation time, the higher the NFE in amofer corn straw because the longer time allows for structural carbohydrates to be degraded into the soluble matter, hence, increasing NFE. The increase in NFE level at the 4 weeks of fermentation is also thought to be due to an increase in the number of microorganisms, especially *Lactobacillus*. This is in line with Pratiwi et al. (2015) who state that increasing the number of lactic acid bacteria will increase NFE levels.

The most effective treatment to increase EE and NFE of amofer corn straw was obtained from treatment D_2T_2 (0.06 % EM with two weeks of fermentation) which produced 5.0318 % of EE and 33.8875 % of NFE. It was because these treatments can improve EE of 0.1838 % compared to non-amofer corn straw with four weeks of fermentation and increasing NFE of 0.5307 % - 4.7467 % compared to non-amofer corn straw fermented for four weeks dan two weeks. The increased EE and NFE produced in treatment D_2T_2 may be because the environment created by the combined addition of 0.06 % EM starter (EM4) and two weeks of fermentation allows microorganisms to grow the most optimum in increasing EE and NFE compared to the other treatments.

Trichoderma is one of the microorganisms that affect NFE levels by degrading structural carbohydrates, especially hemicellulose into soluble matter (Daning & Karunia, 2018). In addition to *Trichoderma*, *Lactobacillus* in the starter can increase the NFE of corn straw. *Lactobacillus* sp. produce celulase enzym, namely β-glucosidase. Cellulase enzyme consists of endoglucanase and exoglucanase that work synergetically to produce cellobiose and β-glucosidase which mainly produce glucose (Lokapirnasari, Widodo, & Koestanti, 2018).

4. Conclusion

The most effective treatment to increase CP levels and reduce CF levels was the treatment with the addition of 0.04 % EM4 with 2 weeks of fermentation which was able to produce CP levels of 5.9415 %, CF levels of 37,8545 %, EE levels of 4,5183 %, and NFE levels of 32,4245 %.

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