Labelling of corn as forage for ruminants using isotope $^{15}$N

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Abstract. In vitro and in vivo testing for ruminant feed efficiency can be done by utilizing the stable isotope Nitrogen-15 ($^{15}$N) as a tracer. Feed can be traced by labeling the forage using isotope $^{15}$N. Feed crops are labeled using an isotope $^{15}$N-enriched fertilizer. The critical thing to note is to know the content of isotopes $^{15}$N in the part of forage feed plants that have been labeled. This research aims to know the effect of urea fertilizer on the percent of atom excess $^{15}$N on corn. Corn are labeled using urea enriched with isotopes $^{15}$N in the form of urea fertilizer (10% excess atom $^{15}$N) with different doses (0 - 200% recommended urea dose). As a control used corn plants given urea fertilizer is not labeled $^{15}$N. The results showed that corn forage feed was successfully labeled and correlated with the dose of fertilizer. The range of atom excess $^{15}$N was 4.28 – 6.99% in corn forage. Biomass production showed no significant difference between the dose of fertilization and control, but neither protein content. Based on data, the corn forage can be used for further testing.

1. Introduction

The animal protein derived from ruminants needs to be increased along with the increasing human population. Ruminant livestock can be divided into two groups, namely large ruminant livestock such as cattle and buffalo and small ruminant livestock groups such as goats and sheep [1]. One of the main factors that need to be considered to increase the productivity of ruminants is the availability of quality feed. Feed is anything eaten by livestock, can be fully or partially digested and does not interfere with livestock health. Generally, ruminant feed is divided into forages, concentrates, and supplements. Forage is the main feed ingredient. Types of forage that can be given include grass, legumes, agricultural waste, and agro-industry. Forage feed given is generally as much as 10-12% of body weight [2].

Animal feed research is still being developed, focusing on finding quality forage, concentrate formulas or supplements, and probiotics. The research was carried out by testing the nutritional quality of the feed, followed by in vitro and in vivo testing and ending with field testing [3,4]. Nuclear technology in the form of isotopes can help determine the efficiency of forage by ruminants. Isotopes can label animal feed so that information can be obtained which feed is converted into microbial protein or feed that is distributed into meat and milk. Knowledge of digesta passage kinetics in ruminants is essential to predict nutrient supply to the animal in relation to optimal animal performance, environmental pollution and animal health [5,6]. Isotopes that can be utilized are nitrogen-15($^{15}$N), carbon-13 ($^{13}$C), and phosphorus-32 ($^{32}$P) [7].
In this study, we used corn as a forage feed labeled with a stable isotope of $^{15}$N in the form of urea (CO$_{15}$N$_2$H$_4$). Corn is a cereal plant as a source of carbohydrates cultivated as ruminant animal feed, especially in marginal and dry areas in Indonesia. The nutritional content of corn consists of 5.54 – 7.43 % crude protein, 23.13 – 28.77 % crude fiber, 6.10 – 7.43 % ash, and 52.86 – 61.80 % IVDMD [8].

The most important thing for research on feed formulations using isotopes is the availability of labeled feed with the right isotope content. Therefore, this study aimed to analyze the content of the labeled $^{15}$N isotope in corn plants given different doses of urea fertilization. It is expected that from this study, appropriate labeling techniques will be obtained, so that corn labeled with $^{15}$N can be used as forage in research on ruminant feed formulations both in vitro and in vivo.

2. Methods

2.1. Location and time
This research was conducted from September to December 2020. Corn was planted in a greenhouse and analyzed for $^{15}$N isotopes at the IRMS Laboratory - Center Isotope and Radiation Application (CIRA) - BATAN, South Jakarta.

2.2. Corn planting
Latosol soil was placed in a bucket of 8 kg. Planting is carried out by placing 5 cm deep, each planted as much as three corn seeds per bucket, in the ground. Plants were fertilized using $^{15}$N isotope enriched urea (10% atom excess $^{15}$N) with different doses (0, 50 and 100% recommended urea dose). Urea fertilization was applied twice or in stages, namely 1/3 when the plant is seven days after planting (DAP), and 2/3 is given when the plant is 72 days old. Plant maintenance is done by watering and weeding. Harvesting of maize plants was carried out at 72 DAP. Harvesting is done by pulling out the entire plant or stover. Parameters observed were biomass stover, protein by Kjeldahl method [9], and $^{15}$N content using infrared mass spectrometry (IRMS) [10].

2.3. Data analysis
Data were analyzed using Analysis of variance (ANOVA) test with the help of SPSS application. The data were tabulated and tested according to the diversity analysis to see the effect of treatment. Further tests using Duncan's were carried out to know the difference between treatment group.

3. Results and discussion
Treatment of $^{15}$N fertilizer doses (0-200%) produced biomass in fresh and dry matter of corn stover, which was different and not comparable to doses (Figure 1). This result shows that corn requires fertilizer at a specific dose, and if it is excessive, it will impact its growth. Supposedly, the higher the nitrogen content in the soil, the more nitrogen available to plants will increase and cause plant growth to be more accelerated and ultimately result in higher biomass weight [11]. The statistical analysis showed no significant effect of dose treatment on biomass of corn stover (p≥0.05).

One of the organic compounds contained in biomass is protein. The content of corn protein was 10.57 to 14.47% after 72 DAP (Figure 2). The highest content occurred at a dose of 200% urea fertilizer. Statistical results showed an effect of urea fertilization dose on protein content of corn stover (p≤0.05). The addition of nitrogen affects forage yields and chemical composition if all other nutrients are available in the soil under optimum conditions [12]. This condition illustrates that plants utilize the high nitrogen available from urea fertilization to form plant body nitrogen which will later become plant protein. The complete function of nitrogen for plants is to increase growth, promote healthy leaf growth, broad plant leaves with a greener color, increase protein levels in the plant body, improve the quality of leaf-producing plants and increase microorganisms in the soil [11].
Most of the $^{15}$N isotopes used will be labeled in the form of protein compounds. The test results showed that the corn forage feed was successfully labeled with % atomic excess $^{15}$N of 4.28 – 6.99% (Figure 3). The % atom excess was equal to the dose of fertilizer. While in control, the $^{15}$N content is equal to the natural abundance in nature, which is 0.366% $^{15}$N atoms or 0% excess $^{15}$N atoms. Statistical results showed that there was an effect of urea fertilization dose on the protein content of corn forage ($p \leq 0.05$)

Based on these data, forage corn can be used for further testing, either in vitro or in vivo. The $^{15}$N isotope-labeled feed, when utilized by rumen microbes, will be converted into organic or inorganic nitrogen compounds. Nitrogen organic compounds can be structural or functional proteins (enzymes) [13]. With the traced isotopes in microbial cells, accurate analysis of microbial protein synthesis can be carried out due to the utilization of forage feeds. When tested in vivo, labeled forage feed can be used to determine the effectiveness of an experiment by looking at its distribution in meat and milk or feces and urine. Research conducted by Cheng et al. [14] stated that the $^{15}$N isotope content in milk, urine, and feces were all affected by the $^{15}$N isotope content of the feed.
4. Conclusions
According to the study, it can be concluded that corn stover has been successfully labeled with the \textsuperscript{15}N isotope and can be used for in vitro and in vivo feed formulation testing.

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