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Nutrient Value in Leaves *Indigofera zollingeriana* Irradiated by Gamma Rays

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Abstract

Indigofera zollingeriana is a legume plant that has the potential to become an alternative feed ingredient for livestock protein sources. The study aimed to determine the effect of various doses of gamma irradiation on the nutritional content of indigofera plants. The benefit of this research is the genetic improvement of feed crops through gene mutation using nuclear technology for plant cultivation. The study used a randomized block design (RBD) for the experimental design in the field consisting of 5 treatment levels, namely the dose of gamma irradiation 0 Gy (Control), 50 Gy, 100 Gy, 150 Gy, and 200 Gy on *Indigofera zollingeriana* plant seeds. The planting plots consisted of three groups and each treatment was analyzed three replicates as samples. Data analysis conducted using analysis of variance using the SPSS application. Analysis of variance showed that the effect of gamma irradiation on the nutrient content of *Indigofera zollingeriana* leaves had a significant effect on crude fiber and crude fat but no significant effect on crude protein content. Based on the results of this study, the highest irradiation dose of 200 Gy showed the best nutrient content for livestock productivity

Keywords: *Indigofera zollingeriana*, Gamma Rays, Nutrient, Feed

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1. Introduction

Global climate change has caused a longer dry season, reducing the population of feed crops. This affects the availability of grass for feed, particularly for ruminants. Plants that are resistant to drought stress and high temperatures needed to mitigate the effects of drought and harsh climate change. *Indigofera zollingeriana is* one of the forages that has tolerance to environmental conditions such as drought, nutrient deficiency, and salinity. Indigofera was still able to survive and produce at severe levels of drought stress (field 25% capacity), although productivity decreased. Indigofera can withstand PEG stress at 20% concentration at the seedling stage showing that the plant is resistant to drought conditions [1]. Feeding management has a considerable influence on ruminant and poultry productivity, thus the amount of protein, fat, and minerals must be sufficient and balanced. Due to the low forage protein content of grass, farmers must rely on imported concentrates, which are rather expensive. Green concentrates from bean plants used to substitute imported protein sources. *Indigofera zollingeriana* is one form of legume that utilized as a feed plant [2].

Indigofera zollingeriana is a legume plant that has great potential to be an alternative feed ingredient for protein sources in beef cattle production. *Indigofera zollingeriana is* widely developed because it has a high biomass production with good benefits as a substitute for concentrates in dairy cattle rations [3]. Scientists reported that *Indigofera zollingeriana* legume has an average production of up to 63.57% of total fresh production [4]. A nursery technique that is widely developed today is gamma irradiation to increase diversity. Irradiation treatment on seeds affects plant growth and development because it can increase enzyme activity and induce genetic, biochemical, cytological, physiological, and morphological changes in cells and tissues. Irradiation is useful for increasing germination potential, growth, and improving adaptation to drought [5].

According to alfalfa plant tillers (Medicago sativa L.) demonstrated that gamma irradiation, particularly 300 Gy, was capable of producing higher or more tillers on the number of tillers generated [6]. This is a potential benefit of irradiation. These findings are consistent with the findings of those who discovered that gamma irradiation at 350 Gy can affect the genetic composition of plants and increase productivity [7]. The nutritional value of plants affected by genetic improvement, namely gene mutation by gamma irradiation. Gamma irradiation is not toxic to cattle and is believed to boost the nutritional value of *Indigofera zollingeriana*. Plant irradiation techniques that use gamma rays allow for genetic modifications that affect vegetative growth, adaptation, and are thought to affect plant nutritional content. This study aimed to determine the effect of several doses of Gamma-ray irradiation on the nutrient content of *Indigofera zollingeriana* and compare the value of nutrient content in *Indigofera zollingeriana* plants without Gamma irradiation. The purpose of this study was to determine the benefits of geneticimprovement of feed crops and its impact on plant productivity.

2. Materials and methods

2.1 Materials used

The materials used for the plant nursery were *Indigofera zollingeriana* plants and manure. Materials for laboratory analysis were Indigofera, selenium mix, concentrated H_2SO_4 (technical), distilled water, NaOH30 %, H_3BO_3 2 %, H_2SO_4 0.0142 N, and chloroform. The tools for laboratory analysis were analytical balance, porcelain cup, oven, desiccator, test tube clamp, 100 mL Kjedhal flask, fume cupboard, 100 mL measuring flask, 5 cc scale dropper pipette, distillator, spray flask, 100 mL Erlenmeyer, burette, 10 test tubes ml, electric heater, vacuum pump, tissue paper, and electric bath.

2.2 Research design

The research organized based on a completely randomized design (CRD) with five treatments and three replications, so there were 15 observation units. The treatments were arranged as follows P0: Without gamma irradiation, P1: Irradiation with a dose of 50 Gy, P2: Irradiation with a dose of100 Gy, P3: Irradiation with a dose of 150 Gy, P4: Irradiation with a dose of 200 Gy.

2.3 Procedure Methodology

This research was a continuation of research that previously studied productivity in indigofera, *Indigofera zollingeriana* comes from seeds that have been irradiated with gamma rays in the Isotope Laboratory BATAN, namely 50 Gy, 100 Gy, 150 Gy, and 200 Gy and then cultivated in the nursery and cultivation of indigofera plants in Moncongloe, Lappara, Maros Regency. Land preparation carried out by cleaning weeds or plant residues and tilling the soil to loosen the soil layer. The land processed using a shovel, hoe, and then made into beds with a planting distance of 1 x 1.5 meters using seeds that irradiated with various doses, and the plants grouped into three groups that were 5 months old after germinated. After that, maintenance was carried out for 2 months by paying attention to plant growth and spraying liquid organic fertilizer once a week at the recommended dose (10 ml/liter). This plant pruned in the initial stages by paying attention to pruning time, watering, applying fertilizer, and controlling weeds or nuisance plants around it. Plants harvested 60 days after initial pruning to analyze the nutritional content of the plant leaves. Analysis of the nutritional content tested included dry matter, organic matter, water content, ash content, crude protein, crude fiber, ether extract, and nitrogen free extract of Indigofera.

2.4 Statistical Analysis

The parameters of the research were dry matter content, organic matter, water content, ash content, crude protein, crude fiber, crude fat, and EMWN in Indigofera plants. The data obtained were processed using analysis of variance and using Office 2007 software and SPSS version 17. The analysis of variance for the content of the Indigofera zollingeriana based on the mathematical model design used.

$$Yij = \mu + \alpha i + \beta j + \varepsilon ij$$

Description:
Y: Observation or measurement value
μ: Average expected value
αi: influence of treatment
βj: The effect of blocks or groups or repetitions
ε: Effect of experimental error
i: Influence to i
j: Influence to j

3. Results and Discussions

Based on the data in Table 1, the dry matter content of Indigofera resulting from gamma-ray irradiation in the 200 Gy dose treatment gets the highest result of 24.79% while the lowest is 20.25% in the 150 Gy dose treatment. Apriliani [8] said that sorghum straw irradiated with a dose of 100 Gy obtained a result of 54.02% which was higher than non-irradiated straw of 40.98%, the value of sorghum straw irradiated with a dose of 50 Gy and 150 Gy irradiated sorghum straw was 46.26% and 52.45% respectively. Gamma irradiationat a dose of 200 Gy is more effective for increasing dry matter in Indigofera compared to indigofera irradiated with doses of 50 Gy, 100 Gy, and 150 Gy. According to Rahman et al [9] the effect of gamma irradiation is dependent on the dose of irradiation used. Irradiation exposure is one of the elements influencing the nutritional content of plant cell walls. This is consistent with belief that gamma irradiation treatment can increase plant quality and that variable doses of gamma irradiation can develop distinct plant kinds [10]. Gamma irradiation particularly alters the amino acid content of feed materials, increasing feed digestibility.

Based on the results of Duncan's test, the content of organic matter in indigofera plants that given a dose of gamma irradiation 50 Gy, 100 Gy, 150 Gy, and 200 Gy get avalue of about 88.60% - 90.43%. The highest organic matter content of indigofera plants resulting from gamma irradiation was at a dose of 200 Gy (P4) and the lowest was in the control treatment (P0) without dosing gamma irradiation. Apriliani [8] suggested that the sorghum straw that irradiated with a dose of 50 Gy and 100 Gy had the highest organic matter content of 87.95%, while non-irradiated sorghum straw had the smallest organic matter content of 87.71%. Sorghumstraw irradiated with a dose of 150 kGy tends to increase by 0.15% compared to non-irradiated sorghum straw. Teguh [10] stated that the dose of 100 Gy gamma irradiation was moreeffective in increasing the organic matter content of sorghum straw. It reported by that irradiation could increase the content of organic matter in rice straw, which means that gamma irradiation can change the fiber fraction of rice straw [8]. The rate of degradation of organic matter closely related to the degradation of dry matter because some dry matter consists of organic matter.

Table 1: Nutrient Content of *Indigofera zollingeriana* Plants at Various Doses of Gamma Irradiation.

Treatment	Nutrient content of *Indigofera zollingeriana*							
	Dry Matter	Organic Matter	Water Content	Ash Content	Crude Protein	Crude Fiber	Crude Fat	EMWN
P0	21.26±5.11^{a}	88.60±0.86^{a}	78.73±5.11^{a}	11.39±0.86^{a}	31.17±0.73^{a}	20.80±1.27^{b}	6.55±0.62^{a}	30.08±3.37^{a}
P1	20.85±0.62^{a}	87.62±0.50^{a}	79.14±0.60^{a}	12.38±0.50^{a}	31.13±0.27^{a}	23.43±1.52^{a}	7.01±0.48^{a}	26.04±0.97^{b}
P2	21.50±2.12^{a}	88.66±0.82^{a}	78.65±1.84^{a}	11.33±0.82^{a}	31.95±0.95^{a}	21.16±1.44^{b}	6.85±0.04^{a}	28.70±1.52^{a}
P3	20.25±2.63^{a}	89.19±0.94^{a}	79.74±2.63^{a}	10.80±0,94^{a}	31.13±1.23^{a}	19.80±0.86^{b}	5.50±0.92^{b}	32.68±2.43^{b}
P4	24.79±5.54^{b}	90.43±0.44^{b}	75.20±5.54^{b}	9.57±0.44^{b}	32.76±1.95^{a}	19.91±0.80^{b}	5.81±0.82^{b}	32.94±1.49^{b}

Note: Superscript ^{a} shows no significant difference to various doses of gamma ray irradiation, and ^{b} shows a real difference to various doses of gamma ray irradiation of *Indigofera zollingeriana* plants

Indigofera crude protein content of gamma irradiation results amounted to 31.17% - 32.76%. The research on Indigofera crude protein content in previous studies showed that the crude protein content in plants of the same age was 33.87% [11]. Besides, Sajimin et al [12] reported that the forage protein content of *Clitoria ternatea* M2 generation did not significantly affect the irradiation dose of 200 Gy. This result shows that gamma radiation treatment does not effect on protein content. The results in Table 1 show that the protein content in the 200 gray irradiation dose treatment is the highest value, while the lowest as the control treatment or without irradiation. This was because gamma-ray irradiation was able to induce nitrogen in proteins. This is consistent with the findings of who claim that gamma-ray-induced nitrogen can boost crude protein content if it penetrates the polypeptide chain generated by the cell's core genome [13]. Because the grass plants are still in the early generation stage, the ensuing irradiation effect has not yet considerably affected the physiological process.

Apriliani [8] stated that the protein content for sorghum straw irradiated with a dose of 100 kGy and 50 kGy tended to decrease by 0.72% and 0.43% compared to non-irradiated straw. This proves that gamma irradiation does not affect the content of dry matter, organic matter but affects the protein content of sorghum straw. The increase in crude protein attributed tothe reduction of some BK components, especially soluble carbohydrates. Soluble carbohydrates converted into protein during germination. The following is the reaction of respiration inplant cells. Handayati [14] claims that the plant nursery in the early generation (MV2) did not genetically obtain a stable mutant. This is consistent with the findings of Shawrang et al [15] who discovered that irradiating plants does not effect on their chemical makeup, such as crude protein. Irradiation can only diminish the amount of NDF and ADF in plants. The higher the irradiation dose, the greater the decrease in NDF and ADF, increasing dry matter and nutritional content deterioration. The composite crude fiber content of mutant two forage production reduced from 23.85% in control plants to 18.43% after 200 Gy radiation.

Shafifi [16] suggested that the interaction of irradiation dose with stem book did not significantly affect the crude protein content of plants with a coefficient of variation of 10.90% (50 Gy irradiation). This means that the interaction of gamma-ray treatment at the dose given is not optimal in changing the gene structure in plant hormones that affect the crude proteincontent. According to Shafifi [16] the optimal dose of irradiation treatment can reduce the protein content, leading to an increase in the crude protein content of the plant. This used to increase the nutritional value required by livestock. Crude fiber is a carbohydrate that generated mostly from plant cell walls and contains cellulose, hemicellulose, and lignin. The treatment is considerably different from the treatments P0, P2, P3, and P4 based on the findings of Duncan's test of Indigofera crude fiber content at various doses of irradiation (Table 1). The indigofera crude fiber content of gamma irradiation results was highest at a dose of 50 Gy (P1) and lowest at a dose of 200 Gy (P4). These findings show that the greatest irradiation dose in the offered therapy can lower crude fiber by 4.52% compared to other irradiation levels.

According to Teguh [10] the higher the dose of gamma irradiation, the more effective fiber degradation is, resulting in lower crude fiber content. Based on the results of this study, it is suspected that the higher the dose of gamma irradiation, the lower the crude fiber content, and when compared with the results of crude protein analysis, it is higher at the highest irradiation dose of 200 Gy. This is under the opinion of Wahyono [17] which states that the decrease in crude fiber content which is directly proportional to the increase in gamma irradiation dose is a representation of the high activity of cellulase enzymes. Increased fibrinolytic enzyme activity can result in a decrease incrude fiber content. The higher the irradiation dose, the lower the fiber content of the material. Crude fiber content correlates with ADF and NDF content of plant material. Shawrang et al [15] explained that ADF and NDF content in plants reduced by irradiation between 100-2000 kGy. The decrease in NDF and ADF content is caused by gamma irradiation where the irradiation can cut some lignohemicellulose and lignocellulose bonds [18].

Apriliani [8] stated that the NDF and ADF content decreased after irradiation and also changed the degradation of cellulose and hemicellulose into soluble materials. Duncan test results showed that the treatment without irradiation was significantly different from the dose of 150 Gy (P3) and 200 Gy (P4) but not significantly different from the dose of 50 Gy (P1) and 100 Gy (P2) (Table 1). Based on Table 1 Indigofera crude fat content of gamma irradiation results amounted to 7.01%-5.5%%. The results of the analysis of Indigofera crude fat content in previous studies showed that the crude fat content in plants of the same age was 7.02%. Indigofera crude fat content of gamma irradiation results was highest at a dose of 50 Gy (P1) while the lowest was at a dose of 150 Gy irradiation (P3). Furthermore, Sajimin et al. 2015 suggested that the fat content of *Clitoria ternatea* M2 at an irradiation dose of 200 Gy was 4.31% and 3.63%. Sari [19] observed that the variation in fatty acid concentration in microalgae *Nannochloropsis sp*. without and with radiation treatment may be attributed to alterations in enzymatic reactions that occur in the metabolism of the microalgae itself. Gamma-ray irradiation can generate oxidative stress in cells, which can decrease enzyme performance.

Results in Table 1. indicate the value of Extractable Material Without Nitrogen (EMWN) of gamma irradiated indigofera plants at doses of 50 Gy, 100 Gy, 150 Gy, and 200 Gy of 30.08% to 32.94%. Indigofera gamma irradiation results in the treatment dose of 200 Gy get the highest results of 32.94%. Barokah et al [20] suggested that this was the case. This can occur because in the calculation of EMWN content, there are several influencing factors, namely ash content, crude fiber, crude protein, and crude fat content. Ardiansyah et al [21] stated that EMWN can be said to be a soluble carbohydrate, as opposed to crude fiber which is an insoluble polysaccharide. Tilman et al. 1989 added that EMWN contains monosaccharides, disaccharides, trisaccharides, and polysaccharides, especially starch, which are easily soluble in acid and base solutions in crude fiber analysis and have high digestibility.

4. Conclusions

Gamma irradiation of Indigofera did not affect on crude protein content but did affect on crude fiber and crude fat content. When compared to lower doses, the content of Crude Protein, Crude Fiber, and Crude Fat at a dose of 200 Gy produced the best outcomes for cattle nutrition.

References

[1] M. Nadir, M.J. Anugrah, P.I. Khaerani. (2018). In *Salt salinity tolerance on nursery of Indigofera zollingeriana*, IOP Conference Series: Earth and Environmental Science, IOP Publishing: 2018; p 012027.

[2] I.K. Prayoga. (2018). The effect of differences of harvest age on productivity (fresh production, dry matter production, proportion of leaves and stems) of Forage Indigofera zollingeriana. Journal of Livestock Research and Innovation. 2(1), 1-7.

[3] N. Mayasari, & Ismiraj, M. R. (2019). Introduction to the Use of Indigofera Zollingeriana Legumes as a Partial Concentrate Substitute for Beef Cattle in the Putra Nusa Breeders Group, Kondangdjaja Village, Cijulang District, Pangandaran Regency. . Dharmarkrya. 8(2): 105.

[4] J. Sirait, K. Simanihuruk, R. Hutasoit. (2009). In *The potency of Indigofera sp. as goat feed: production, nutritive value and palatability*, Proceeding of International Seminar on Forage Based Feed Resources. Bandung, pp 3-7.

[5] N. Ikram, S. Dawar, Z. Abbas, M.J. Zaki. (2010). Effect of (60 cobalt) gamma rays on growth and root rot diseases in mungbean (Vigna radiata l.). Pakistan journal of botany. 42(3): 2165-2170.

[6] I. Prihantoro, A. Anandia, A. Aryanto, M. Setiana, P. Karti. (2019). The adaptation level of alfalfa (medicago sativa l.) that irradiated with gamma rays on a field scale. Pastura: Journal of Tropical Forage Science. 9(1), 1.

[7] M. Rejili, D. Telahigue, B. Lachiheb, A. Mrabet, A. Ferchichi. (2008). Impact of gamma radiation and salinity on growth and K+/Na+ balance in two populations of Medicago sativa (L.) cultivar Gabès. Progress in Natural Science. 18(9): 1095-1105.

[8] W. Apriliani. (2017). *Degradabilitas In Sacco Jerami Sorgum (Sorghum Bicolor (L .) Moench)* Gamma Irradiation Results of the Chemistry Study Program 2017 M / 1438 H.

[9] H. Rahman, S. Hafid, R. Rahadi, A. Aka, A. Anas, S. Baena. (2015). In *The nutrients potential of agricultural waste as feed of ruminants in Southeast Sulawesi*, Proc. Int. Seminar Improving Tropical Animal Production for Food Security. pp 180-4.

[10] W. Teguh & F. Firsoni. (2016). The Changes of Nutrient Composition and In Vitro Evaluation on Gamma Irradiated Sweet Sorghum Bagasse. Scientific Journal of Isotope and Radiation Applications. 12(1): 69-78.

[11] A. L. Munasirah. (2019). The effect of different pruning times on the dry matter, crude protein and crude fat content of indigofera plants (Indigofera zollingeriana). The thesis is not published. Faculty of Animal Husbandry. Hasanuddin University, Makassar

[12] D. Sajimin, A. Fanindi, N. Purwantari. (2015). In *The Effect of Gamma Ray Radiation on the Production and Quality of Seeds of the Telang Flower Forage Plant (Clitoria ternatea* M2*) in Bogor*, Proceedings of the National Seminar on Animal Husbandry and Veterinary Technology, pp 635-642.L.

[13] Alfariatna, F. Kusmiyati, S. Anwar. (2018). Physiological characteristics and estimation of heritability of m1 shallot (Allium ascalonium L.). plants resulting from gamma ray irradiation. Journal of Agro Complex. 2(1): 19-28.

[14] W. Handayati. (2013). Advancement of mutation breeding on ornamental plants in Indonesia. Scientific Journal of Isotope and Radiation Applications. 9(1): 67-80.

[15] P. Shawrang, A. Majdabadi, A.A. Sadeghi. (2012). Changes in cell wall compositions and degradation kinetics of electron beam-irradiated sugarcane bagasse. Turkish Journal of Veterinary & Animal Sciences. 36(5): 527-532.

[16] M.I. Shafifi. (2020). Increasing Crude Protein Content and Agronomic Characteristics of Elephant Grass Plants (Pennisetum Purpureum Schumach.) Through Gamma Ray Irradiation on Mv1 and Mv2. Faculty of Science and Technology UIN Syarif Hidayatullah, Jakarta.

[17] T. Wahyono, N. Nurhasni. (2018). NATIONAL SEMINAR Theme: Isotopes and Radiation Support National Independence.

[18] T. Wahyono, N. Lelananingtyas, S. Sihono. (2017). Effects of Gamma Irradiation on Ruminal Degradation of Samurai 1 Sweet Sorghum Bagasse. Atom Indonesia. 43(1): 35-39.

[19] I.P. Sari. (2016). Effect Gamma Rays Irradiation To Nannochloropsis Sp . On The Biomass and Total Lipid Content.

[20] Y. Barokah, A. Ali, E. Erwan. (2017). Nutrition of Palm Frond Silage added with Indigofera Biomass (Indigofera zollingeriana):(The Nutrient Content Of Oil Palm Frond Silage added with Indigofera zollingeriana). Jurnal Ilmiah Ilmu-Ilmu Peternakan. 20(2): 59-68.

[21] A. Ardiansyah, D. Dahlia, H. Hartinah, I. Ibrahim, W. Wahidah. (2018). Improvement of the nutritive quality of Sargassum powder through Aspergillus niger, Saccharomyces cerevisiae, and Lactobacillus spp. fermentations.