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Nutrient analysis of fermented chicken feather fertilizer waste using *Bacillus subtilis* on the growth of green spinach (*Amaranthus tricolor*)

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ABSTRACT

Introduction: Farmers generally provide NPK chemical fertilizers (Nitrogen, Phosphorus, Potassium) to provide nutrients for green spinach plants. However, the use of NPK chemical fertilizers harms the environment. Nowadays, chicken feathers are known as beneficial for fertilizing plants, similar to NPK chemical fertilizers. Fermentation of chicken feathers by *Bacillus sp.* can improve the quality of protein in chicken feather fertilizers which can be used as a supplement in renewing soil conditions. Thus, this study is aimed to evaluate the nutrient component of fermented chicken feather fertilizer waste using Bacillus subtilis on the growth of green spinach (*Amaranthus tricolor*).

Methods: This was a cross-sectional study. In this study, we used a total sampling technique. In this study, three groups got different chicken father fertilizers, and two groups of standard and did not get the chicken father fertilizer. Data from the research will be statistically analyzed descriptively. The descriptive data will provide in the table.

Results: In the first treatment group, the value of nitrogen (N) was 2.439, phosphorus (P) was 2.225%, and potassium (K) was 2.408%. In the second treatment group, the nitrogen (N) value obtained 2.866%, the phosphorus (P) value was 2.174%, and the potassium (K) value was 2.477%. In the third treatment group, the nitrogen (N) value was 3.291%, the phosphorus value was 3.082 %, and the Potassium (K) value was 3.135%.

Conclusion: According to the results, the higher the chicken feather fermented fertilizer dose, the higher the NPK content. This is certainly good for the growth of spinach.

Keywords: Chicken feathers, *Bacillus subtilis,* green spinach, fertilizer.
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INTRODUCTION

The availability of nutrients is one factor affecting the productivity of vegetable crops. Green spinach plants, in their development, require several macronutrients such as Nitrogen (N), Phosphorus (P), and Potassium (K), as well as micronutrients such as calcium (Ca) and magnesium (Mg).[1] Farmers generally provide chemical fertilizers NPK (Nitrogen, Phosphorus, Potassium) to provide nutrients for green spinach plants. However, NPK chemical fertilizer can negatively impact the environment. In several incidents, using chemical fertilizers in the long term harmed soil conditions. Soil becomes hard and less able to store water capacity, making soil pH conditions acidic, ultimately reducing plant productivity.[2]

Chicken feather waste still has the potential to be utilized because it has a very high protein nutritional content. Besides that, chicken feathers processed into feather meals have a total nitrogen (N) content of 14.2%.[3] Barone et al. reported that keratin could form complexes with high molecular weight without chemical modification, making it interesting to be used as a new material in various fields.[4] Chicken feathers also contain nitrogen nutrients, the keratin content contained in chicken feather waste can be converted into amino acids, which can be used as animal feed.[4–6]

Several studies have reported that many microorganisms could degrade these wastes by secreting keratinolytic and proteolytic keratinase enzymes such as *Actinomycetes* and fungi.[7] Fungi and bacteria can grow with nutrients such as collagen.[8] Bacteria isolated from various environments, especially places with abundant keratin, can degrade keratin. Bacteria have great potential for widespread use as they grow widely because they grow fast, and their enzymes maintain activity under different conditions. Keratin-degrading microorganisms include *Bacillus* sp.[9]. *Bacillus subtillis* is a bacteria that can degrade keratin. These bacteria can degrade the keratin found in chicken feathers because of the keratolytic enzymes they produce. Fermentation of chicken feathers by *Bacillus sp.* can improve digestibility and affect protein quality in chicken feather flour.[7] However, publications on the nutritional characteristics of fermented chicken feathers by *B. subtillis* and the effect of its use as plant fertilizer, especially red spinach, have yet to be widely available. Thus, this study aimed to evaluate the utilization of fermented chicken fur waste flour bacillus subtilis on the growth of green spinach (*Amaranthus tricolor*).

METHODS

Study Design and Sample Criteria

This is a cross-sectional study. In this study, we used a total sampling technique. The inclusion criteria in this study was the chicken feather waste taken from the RPA owned by Mr. Zaenal, located on Bratang Street. The exclusion criteria were the dirty chicken feather, even though it has been cleaned, and the incomplete dried chicken feather.

Materials

The materials used in this study consisted of two parts, namely (1) the ingredients used in the manufacture of fermented chicken feather flour, including chicken feathers, *Bacillus subtillis* bacteria, Nutrient Agar (NA), Nutrient Broth (NB), and distilled water. (2) materials needed to plant red spinach plants include: ground red spinach (*Amaranthus tricolor*) seeds.

Data Collection Procedures

The tools that will be used in this research include a grinding machine, plastic container, bucket, plastic clip, polybag, petri dish, ose needle, bunsen, syringe, Laminar Air Flow (LAF), autoclave, incubator, analytical scale, tube reactions, microplates, books, stationery and cameras.

1. Bacterial rejuvenation
   Bacterial isolates of B. subtilis will be obtained from online sales still in agar slant (slanted NA media). Before fermentation, bacteria were cultured or propagated using Nutrient Agar (NA) media which had been sterilized in an autoclave at 121°C for 30 minutes. Bacterial isolates were taken using a sterile tube and then scratched on the surface of the agar medium and incubated for 24 hours at 37°C. Cultures rejuvenated for 24 hours were handled using a clean loop and then inserted into the dilution medium to be diluted to 10-6. After being incubated during the bacterial log phase, the bacterial suspension can be used for testing.
2. Making chicken feather fertilizer
   Chicken feather waste was taken from the RPA owned by Mr. Zaenal, located on—Bratang Street. Chicken feathers are washed with running water until clean and dried in the sun to dry completely. Dried chicken feathers are ground using a grinding machine to produce chicken feathers into flour form.
3. Fermentation of chicken feather flour
   Before fermentation, 100 grams of chicken feather flour were sterilized at 121°C for 30 minutes to remove associated microorganisms or prevent contaminants caused by other organisms. After cooling, the chicken feather flour was added with 10 ml (10%) of *B. subtillis* bacterial inoculum in Erlenmayer at pH 8.5. The inoculated chicken feather flour was incubated at 55°C for 72 hours. Fermented chicken feather flour was analyzed for nitrogen, phosphorus and potassium content before being applied as fertilizer for red spinach.
4. Nitrogen (N) Analysis
   The N content will be determined according to the SNI 19-7030-2004 method. A total of 1 g of unfermented and unfermented chicken feather samples were weighed carefully, put into a digestion tube, added concentrated H2SO4 and added, with a ± 2 g selen/catalyst mixture. The mixture is heated or digested to 350° C. for ±. 1.5-2 hours. Then distillate by adding 20 mL of 40% NaOH, tamping the distillate in 25 ml of boric acid. Distillation was terminated when the distillate volume in the reservoir reached 50-75 mL. The distillate was titrated with a standard acid solution, namely 0.050 N H2SO4 or 1 N HCL until the endpoint was a color change from green to pink.
5. Analysis of phosphorus (P) content
   Carefully weigh 2 grams of the sample and put it in a shaker bottle. Add 10 mL of 25% HCL solution using a 10 mL pipette. Shake for 30 minutes, let stand for 1 x 24 hours, then filter using filter paper and tamping the solution/filtrate. Pipette 0.5 mL of solution/filtrate into a test tube, add 2 mL of distilled water (5x dilution) and shake with a vortex until homogeneous. Then pipette the key and 1 mL long P series into a test tube. Add 5 mL of each mixed reagent, shake with a vortex until homogeneous and then measure with a spectrophotometer at a wavelength of 693 nm with the P standard series as a comparison.
6. K content analysis
   Accurately weigh 2 g of the sample and put it in a shaken bottle; add 10 ml of 25% HCl solution using a 10 ml volume pipette. Shake for 30 minutes, let stand for 1 x 24 hours, then filter using filter paper and tamping the solution/filtrate. Pipette 0.5 ml of solution/filtrate and add 9.5 ml of distilled water (20x dilution). Shake with a vortex until homogeneous, then measure with a flame photometer/flame photometer with K series for comparison.
7. Seeds of red amaranth (*Amaranthus tricolor*)
   A total of 4 polybags were prepared before seeding with a soil dose of 1 Kg per polybag and let stand for 1 week so that air circulation in the soil runs well. The spinach seeds used were of good quality seeds with the red arrow stamp brand. The seeds are ordered online. Seeing the expiration date was still 2 years, as many as 5 seeds per polybag were sprinkled evenly on the soil and then piled up with soil to a thickness of 2-3 cm and then watered slowly.
8. Transplanting planting
   Before transplanting, two weeks before, fill the polybag with soil and leave it for a week to expose the ground to wind and heat so that the air circulation in the soil runs smoothly. After the red spinach seeds were around 1 week old or had 3-4 leaves, they were ready to be transplanted into polybags. The red spinach seeds were planted by making a planting hole of approximately 2-3 cm. then backfilled with thin soil, then polybags were arranged randomly according to the table below:
9. Watering
   Watering was done every day, namely in the afternoon, while watering with fertilizer was done weekly in the afternoon, according to the treatment. The watering volume for each fermented chicken feather flour fertilizer was injected using a syringe on the plant at a dose of 2.5 ml per plant from 10 ml of chicken feather

Table 1. **Completely Randomized Design (CRD) on polybag**

Fertilizer Treatment with Dosage	Repetition			
	1	2	3	4
P (-)	P (-) 1	P (-) 2	P (-) 3	P (-) 4
P (+) 0,03 g	P (+) 1	P (+) 2	P (+) 3	P (+) 4
P1 (0,025 g)	P1 1	P1 2	P1 3	P1 4
P2 (0,0375 g)	P2 1	P2 2	P2 3	P2 4
P3 (0,0625 g)	P3 1	P3 2	P3 3	P3 4

Table 2. **Nutrient analysis of the fermented chicken feather flour analysis**

Groups	N (%)	P (%)	K (%)
P (-)	0.131	0.089	0.116
P (+)	5.814	5.233	5.529
P1	2.439	2.225	2.408
P2	2.866	2.174	2.477
P3	3.291	3.082	3.135

flour fertilizer, with the number of plants per treatment being 4 plants.

Dosage of Chicken Feather Flour x number of plants per treatment x amount of fertilization x volume of *B. subtillis* bacteria inoculum

P1 = 0.025 x 4 plants x 4 fertilization x 10 mL inoculant volume

P2 = 0.0375 x 4 plants x 4 fertilization x 10 mL inoculant volume

P3 = 0.0625 x 4 plants x 4 fertilization x 10 mL inoculant volume

Data Analysis

Data from the research on plant height, number of leaves and yields will be statistically analyzed descriptively. The descriptive data will provide in the table.

RESULTS

Based on the data in Table 2, fermented chicken feather flour using *Bacillus subtilis* bacteria can be used as a substitute for NPK chemical fertilizers because the appropriate composition can affect the quality of green spinach plants as the chemical fertilizers. In the first treatment group, the value of nitrogen (N) was 2.439, phosphorus (P) was 2.225%, and potassium (K) was 2.408%. In the second treatment group, the nitrogen (N) value obtained 2.866%, the phosphorus (P) value was 2.174%, and the potassium (K) value was 2.477%. In the third treatment group, the nitrogen (N) value was 3.291%, the phosphorus value was 3.082 %, and the Potassium(K) value was 3.135%. According to our findings, we can see a high concentration of NPK in group 3. It was in line with the enhancement of chicken feather flour dosage.

DISCUSSION

The chicken feather is a good source of keratin proteins and amino acids, and it also has the potential to be renewable. Due to their high keratin protein content, chicken feathers, typically considered poultry waste, will be a potential subcritical water (SCW) liquid fertilizer. An ecologically friendly approach with the right SCW therapy treatment might be promoted. This SCW product could be a fluid fertilizer source to cultivate *G. sulphuraria* and plant growth. A study reported that liquid fertilizer from chicken feather waste had a similar effect to commercial fertilizer, even though commercial fertilizer had a higher rate of height, length, width, and number of leaves in spinach plants. They found that the size of the spinach was 16.5 cm, the distance was 9.6 cm, the width was 5.4, and the number of leave was 9. Moreover, the spinach plant that fertilizes with commercial fertilizer had a height of 18.2 cm, a length of 10.1 cm, a width of 7.0 cm, and the leaves were 12. The analysis of the macronutrient concentration in the SCW organic liquid fertilizer showed that total nitrogen concentration (34,200 mg/L) and total phosphorus concentration (1,380 mg/L) were low than commercial fertilizers, which were 250,000 mg/L and 232,000 mg/L for total nitrogen and phosphorus, respectively.[10] A similar study that evaluated the fermented chicken feathers using *Bacillus subtilis* to improve the quality of nutrition as a fish feed material reported that the processing fermentation with 10 ml inoculum *B. subtil* gave the best results in the highest keratinase activity (273.33 U/ml) increased the protein content of chicken feather meals (74.16 to 85.20%), but decreased of lipid content (2.44 to 1.42%) and carbohydrate content (7.86 to 2.05%).[11] In our study, we found that in the first treatment group, the value of nitrogen (N) was 2.439, phosphorus (P) was 2.225%, and potassium (K) was 2.408%. In the second treatment group, the nitrogen (N) value obtained 2.866%, the phosphorus (P) value was 2.174%, and the potassium (K) value was 2.477%. In the third treatment group, the nitrogen (N) value was 3.291%, the phosphorus value was 3.082 %, and the Potassium(K) value was 3.135%. According to our findings, we can see a high concentration of NPK in group 3. It was in line with the enhancement of chicken feather flour dosage. In our study, we used *Bacillus subtilis* bacteria for the fermentation.

In addition, Hamri et al. explained that the results showed that applying different fermentation methods using *Bacillus subtilis* bacteria only had a different effect on the parameters of fat content. In contrast, protein and crude fiber levels did not significantly impact.[12] While 0.5% of *Bacillus subtilis* did not affect this parameter, a treatment that involved inoculation with 2% of the bacteria accelerated the decline in the C/N ratio. However, during the middle and late stages of composting, adding 0.5% Bacillus subtilis encouraged the transformation of humic substance precursors into humic substances. In certain cases, inoculants with two or more different species of microbes may not have the expected impact on the composting process.[13]

The limitations of this study are that the research is conducted only in one place, we do not analyze chicken father waste in specific types of chicken, and we could not compare with other microorganisms.

CONCLUSION

According to the results, the higher the chicken feather fermented fertilizer dose, the higher the NPK content. This is certainly good for the growth of spinach.

DISCLOSURE

Ethical Consideration

The institution has approved this research.

Author Contribution

All of the authors contributed equally to this research.

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Conflict of interest

Based on the research that has been done, no significant obstacles were found, both research and financial process constraints.

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