



Liquid feather protein hydrolysate as a potential fertilizer to increase growth and yield of patchouli (*Pogostemon cablin* Benth) and mung bean (*Vigna radiata*)

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Abstracts

Purpose Protein hydrolysates (PHs) have attracted much interest in recent years owing to the beneficial effects on plant growth. Feather, one of the most abundant wastes generated from the poultry industry, is rich in proteins and amino acids. It can be utilized to generate value-added bioproducts such as liquid feather protein hydrolysate (FPH). This research aims to evaluate the effect of FPH as fertilizer on plant growth.

Methods Hydrothermal treatment (HTT) at a temperature of 160–180 °C and a holding time of 30 min was employed to convert feathers into a liquid feather-derived protein hydrolysate (FPH) containing nitrogen and amino acids. To evaluate the effect of FPH produced from the HTT process on plant growth, FPH (0.5–5 mL/L) and its combination with 50% recommended dose of chemical fertilizers were applied to patchouli and mung bean plants.

Results Results showed that the combination of FPH and 50% dose chemical fertilizer on patchouli yielded a statistically significant increase in leaf area, dry weight, and chlorophyll content in comparison to the control, suggesting that the application of FPH along with the inorganic fertilizer can increase fertilizer use efficiency. Combined treatment of inorganic fertilizer and FPH on mung bean also showed a relatively higher yield per plant compared with control.

Conclusion Hydrothermal treatment (HTT) can be a useful method for nutrient recovery from animal residuals such as feather. The application of FPH obtained through HTT could improve crop productivity and reduce chemical fertilizer consumption.

Keywords Protein hydrolysate · Feather waste · Hydrothermal treatment (HTT) · Mung bean · Patchouli

Introduction

The rapid growth of intensive poultry production has generated a large number of poultry by-products that creates a serious environmental problem (Jayathilakan et al. 2012). These by-products are potential sources of pathogenic

microorganisms which can cause disease spread when not managed properly (Shih 1992). However, they are mainly organic materials and are convertible to useful resources (Brandelli et al. 2015). Therefore, waste management becomes an important consideration for sustainability of poultry production.

The poultry by-products include feathers, poultry litters, mortalities, and processing wastes. Feathers are generated in large quantities as they account for around 8% of chicken body weight and consist of 90% crude protein (Onifade et al. 1998). Due to high-protein content in keratin, at a commercial level, feathers are used as animal feed. Although feather meal has the potential to be the primary protein source, it has poor digestibility and limited nutrient bioavailability (Papadopoulos et al. 1985). Moreover, the increasing concerns over diseases such as spongiform encephalopathy and avian influenza hinder the utilization of feathers in animal

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feed (Gwyther et al. 2011). Currently, researchers have been searching for new applications of feathers while developing processing methods.

Feathers are rich in nitrogen (up to 15% total N), and therefore, they could be a renewable source of N fertilizer. Moreover, feather is a proteinaceous material which can be a source of mixtures of amino acids and peptides (Taskin et al. 2012). However, feather is limitedly utilized for agricultural sector due to highly recalcitrant nature of feathers. William and Nelson (1992) studied the use of a protein-degrading bacterium (*Bacillus licheniformis*) to enhance the plant growth by increasing the biodegradability of the feather. However, as N release from feathers to soil was not sufficient, the *Bacillus*-treated feather did not improve the plant growth. Raw feathers contain more than 90% keratin, a structural fibrous protein that extremely insoluble and difficult to degrade by most proteolytic enzymes (Onifade et al. 1998). Therefore, proper pretreatment is required to improve the N availability of feather for plant uptake.

In general, pretreatment technologies to improve the characteristics of feather can be classified into biochemical and thermochemical processes. The biochemical process using specific enzyme or microorganism has been widely studied, because it offers lower cost and eco-friendlier route than thermochemical process. However, the biochemical process is characterized by slow reaction rates and high sensitivity to a particular contaminant. Thermochemical processing has several advantages relative to biochemical processing, including faster reaction rates and robustness to eliminate possible pathogen microorganism.

Among thermochemical methods, hydrothermal treatment (HTT) has been widely known to convert waste materials into fuels, fertilizers, biomaterials, and chemicals. The HTT is thermal conversion process in water under an autogenous pressure and relatively moderate temperatures (150–350 °C) that involves decarboxylation, hydrolysis, condensation, and dehydration reactions (Zaini et al. 2017). To date, the use of the HTT for nutrient recovery has been widely investigated (Novianti et al. 2016; Perera et al. 2015; Reza et al. 2016; Sun et al. 2013). The hydrothermal treatment was demonstrated to be able to produce a high yield of amino acid from various kinds of biomass wastes (Cheng et al. 2008). Mixtures of peptides and amino acids obtained from protein sources, known as protein hydrolysates (PHs), have been demonstrated to be able to promote plant growth (Colla et al. 2015). This suggests an opportunity for the development of PH which aimed to improve crop productivity and eliminate the problem of waste disposal.

The HTT of feather can produce a mixture of water-soluble amino acids, referred to liquid feather protein hydrolysate (FPH), which potential to be used as liquid fertilizer (Nurdiawati et al. 2018). Compared to solid fertilizer, the most significant advantage of liquid fertilizer is in its convenience

and versatility of use. Moreover, it can be applied through a drip irrigation system which has been widely used in agricultural practices. Drip irrigation has been considered as the most efficient irrigation method as it offers higher efficiency in terms of water and nutrient application as well as better crop yield and lower pollutants emitted to the environment compared to the conventional method (Lu et al. 2016).

In the application of the treated feather as fertilizer, most of the previous works have been focused only on product analysis and nutrient release characteristics, but little is known about its effects and further applications on the plant growth. Gurav and Jadhav (2013) demonstrated the effect of feather hydrolysate obtained through biological method on banana cultivation. In this study, the positive effect of feather hydrolysate on the productivity and product quality was reported. In the other study, Joardar and Rahman (2018) reported the influence of composted feather on the growth of *Ipomoea aquatica*. The result also showed a positive effect of composted feather on the growth and yield of *Ipomoea aquatica*. The plant growth experiment on these previous works employed treated feather obtained from a biological method. Depending on the type of process (biological, chemical, or thermochemical), the effects of treated feather on the plant growth may also vary. Therefore, it is worth studying the effect of treated feather obtained from thermochemical process on the plant growth.

Colla et al. (2015) listed state-of-the-art in the research of PHs effects on horticultural crops. Study of different type of PHs, crops, application modes, and experimental condition were reported. Several studies reported the positive effect of PHs in increasing nutrient uptake (Ertani et al. 2009) and improving crop tolerances to biotic and abiotic stresses (Ertani et al. 2013). The foliar feeding of plants with animal based-PH showed a good effect on the growth of kiwifruit, lily, papaya, and passion fruit (Morales-Payan & Stall 2003; Morales-Payan & Stall 2004; Lucini et al. 2015). On the contrary, another study reported adverse effects of animal-derived PHs such as phytotoxicity and plant growth depression (Lisiecka et al. 2011). The nature of different raw materials and different hydrolytic processes can affect the properties of hydrolysates and their efficacy as biostimulant. It can be concluded that there are still some gaps in understanding the efficacy of protein hydrolysates in plants (Colla et al. 2015). Therefore, more studies are needed to understand the effect of PHs obtained from various feedstocks and different methods.

According to our previous studies, FPH produced by the HTT contains various amino acids and a considerable amount of N compounds (Nurdiawati et al. 2018). The FPH exhibited moderate N mineralization of up to 50% of the total N, during the 30-d incubation study which showed the



Table 1 Properties of feedstocks: feather and poultry litter

Property	Unit	Feather	Poultry litter
C	%	46.01	17.41
H	%	6.62	2.55
N	%	12.96	1.47
Ash	%	5.20	57.90
C:N ratio		3.6	11.8
P	g/kg	0.93	12.50
K	g/kg	1.50	2.38
Ca	g/kg	1.00	20.17
Na	g/kg	1.32	0.79
Mg	g/kg	0.04	0.02
S	g/kg	23.60	3.74
Mn	g/kg	ND	0.64
Fe	g/kg	0.50	17.86
Cu	g/kg	ND	0.22
Zn	g/kg	ND	0.31

potential use of FPH as fertilizer (Nurdiawati et al. 2019). In the present study, feathers which were converted into liquid FPH through the pilot-scale HTT were applied to patchouli and mung bean plants. This study aims to evaluate the effect of the application of sole FPHs, chemical/inorganic fertilizers, and their combinations on the growth and yield of patchouli and mung bean by conducting plant growth tests.

Materials and Methods

Materials

Chicken feathers and poultry litters used for FPH production were collected from a broiler farm in Bandung City, Indonesia. The samples were received in dry condition. Table 1 summarizes the property of raw feather and poultry litter.

Commercial liquid organic fertilizer was used as a comparison for evaluating FPH performances. POC Nasa (NASA Ltd., Indonesia) is an organic liquid fertilizer derived from animal and plant residue which contains macroelements, microelements, and several growth hormones. Based on fertilizer label, it contains 0.12% N, 0.03% P₂O₅, 0.31% K, 0.15% Na, 0.12% S, 0.29% Cl, 16.9 ppm Mg, 2.5 ppm Mn, 12.9 ppm Fe, Cu < 0.03 ppm, 4.7 ppm Zn, 60.4 ppm Ca, 60.8 ppm B, Co < 0.05 ppm, 6.4 ppm Al, 0.1 ppm Se, 0.1 ppm As, Cr < 0.06 ppm, Mo < 0.2 ppm, V < 0.04 ppm, and 0.72% protein, and contains organic acids (0.01% humic acids), auxin, gibberellin, and cytokinin. The pH is 7.5 which is almost neutral.

The inorganic fertilizers used as a benchmark in this study were N–P₂O₅–K₂O fertilizer in the form of urea

[(CO)NH₂]₂, superphosphate [Ca(H₂PO₄)], and potassium chloride [KCl]. The normal dose (250 kg/ha, 30 kg/ha, 30 kg/ha of urea, superphosphate, and potassium chloride, respectively, for patchouli and 50 kg/ha of each inorganic fertilizer for mung bean) refers to the dose recommended by the previous study and Ministry of Agriculture of Indonesia (Wahyuni et al. 2009).

Preparation of liquid products

HTT process was conducted using a 250 L pilot-scale unit comprising a reactor, an oil-filled heater, a boiler, and a direct-contact steam condenser. The experimental scheme is presented in Fig. 1. In the pilot-scale productions, the reactor was run with 10 kg of feathers and 30 kg of water (biomass:water ratio = 1:3). HTT was conducted at two different operating conditions (160 °C, 0.6 MPa and 180 °C, 0.9 MPa) to produce liquid products with varying concentrations of nutrient. The HTT-160 liquid product was applied to the mung bean, while the HTT-180 liquid product was applied to patchouli. Saturated steam generated by a boiler was fed to the reactor to bring the system to achieve target operating condition. When the HTT temperature has been achieved, the process was maintained for 30 min. After the reaction was completed, the steam was discharged until the pressure of the reactor reaches atmospheric pressure. The obtained slurry product was collected and then filtered to separate the liquid product from solid residue. HTT of poultry litter was also prepared as described for HTT of feathers at a different batch. The liquid product from HTT of poultry litter was then mixed with liquid product from HTT of feathers in 1:5 ratio to enrich the product with potassium and other micronutrients. In this study, FPH denotes for the final mixture of the liquid product from HTT of feather and poultry litter. Table 2 summarizes the property of FPH.

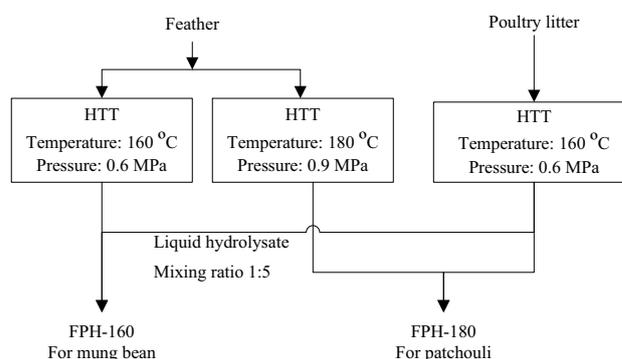
**Fig. 1** The experimental scheme of FPH production

Table 2 Main characteristics of the produced hydrolysates

Property	Unit	HTT-160-feather	HTT-180-feather	HTT-160-poultry litter	FPH-160	FPH-180
pH					6	6.8
EC	dS/m				7.2	11.2
TOC	ppm	72,539	135,673	27,433	65,021	117,633
TN	ppm	7074	15,734	2000	6228	13,445
C:N ratio		10.3	8.6	13.7	10.4	8.7
Total Ca	ppm	153.8	53.4	327.5	182.8	99.1
Total S	ppm	566.5	1142.2	357.5	531.7	1011.4
Total K	ppm	476.6	400.2	560.0	490.5	426.8
Total Mg	ppm	57.6	56.8	99.0	64.5	63.8
Total P	ppm	94.1	124.2	56.0	87.8	112.8
Total Na	ppm	204.7	65.4	177.5	200.2	84.1
Total Fe	ppm	42.9	231.6	42.0	42.8	200.0
Total Zn	ppm	ND	ND	ND	ND	ND
Total Cu	ppm	ND	ND	ND	ND	ND
Total Mn	ppm	ND	ND	ND	ND	ND

HTT denotes for hydrothermal treatment product followed by treatment temperature and feedstock. FPH refers to the final mixture of litter and feather hydrolysates in a 1:5 mixing ratio

Physicochemical analyses of feather protein hydrolysates (FPH)

The total organic carbon (TOC) and total N of the liquid FPH were determined with a TOC-L/TN analyzer (Shimadzu, Japan). The ultimate composition (C, H, N, and ash) of solid raw materials were analyzed using Vario Micro Cube Elemental Analyzer (Elementar, Germany). Micronutrients' content was determined using ICPE-9000 (Inductively Coupled Plasma Emission Spectrometer) (Shimadzu, Japan). Chemical decomposition using a mixture of concentrated acids is required prior to ICPE analysis to dissolve the matrices of organic as well as inorganic samples (Novianti et al. 2015). This acid digestion method is described in detail elsewhere (Nurdiawati et al. 2015).

Test plant

- Patchouli (*Pogostemon cablin*) was selected as the test plant in the experiment. It is a leafy herb planted for its essential oil. Patchouli oil is an important commodity in the fragrance industry and oil trading world. Patchouli crop requires high doses of nitrogen and potassium. It has been reported that the productivity of patchouli increases with the application of N fertilizers (Singh et al. 2002).
- Mung bean (*Vigna radiata*), a legume plant which produces edible seeds, was also selected as the test plant in the experiment. The growth of mung bean can be categorized into two stages: vegetative (from germination to appearance of first flower) and generative (from flowering, pod-filling, until pod maturity stage). In contrast to

patchouli, mung bean is a nitrogen-fixing plant, so fertilization with nitrogen is unnecessary (Murakami et al. 1991). Although nitrogen fertilization is not required, application of nitrogen may encourage early growth, faster establishment, and maximize yields.

Site, experimental layout, and treatments

The field experiment was carried out at Ciparanje Experimental Station, Faculty of Agriculture, Padjadjaran University (06°54'58.1"S, 107°46'18.1"E) from May to August 2017 (mung bean) and September 2017 to February 2018 (patchouli). The experimental site has a tropical climate, inceptisol type of soil, and type C of rainfall according to Schmidt and Fergusson Classification (Schmidt & Fergusson 1951). The physicochemical properties of soil at a depth of 0–30 cm were measured. Soil texture was clay having the following characteristics: sand (5%), silt (35%), clay (60%), pH (5.81), EC (1.5 dS/m), C-organic (1.19%), total N (0.15%), available P (P_2O_5) (93.07 mg/100 g), exchangeable K (K_2O) (66.01 mg/100 g), and ion-exchange capacity (25.06 cmol/kg).

The experiment was arranged in a randomized block design with 16 treatments. There were two replicates of each treatment arranged within two blocks. The details of 16 treatments are listed in Table 3.

Experimental setup: patchouli

Polybag (40 × 50 cm) containing 10 kg of soil in each polybag were used. To prevent waterlogged soil, the polybags

Table 3 Details of 16 treatments

No.	Treatment	No.	Treatment
1	Without fertilizer (control)	9	50% of recommended dose of NPK + 0.5 mL/L of FPH
2	0.5 mL/L of FPH	10	50% of recommended dose of NPK + 1 mL/L of FPH
3	1 mL/L of FPH	11	50% of recommended dose of NPK + 2 mL/L of FPH
4	2 mL/L of FPH	12	50% of recommended dose of NPK + 3 mL/L of FPH
5	3 mL/L of FPH	13	50% of recommended dose of NPK + 4 mL/L of FPH
6	4 mL/L of FPH	14	50% of recommended dose of NPK + 5 mL/L of FPH
7	5 mL/L of FPH	15	2 mL/L of POC NASA
8	50% of recommended dose of NPK	16	100% of NPK

have several drainage holes at the bottom. Patchouli seedlings were obtained from cutting method. Cuttings are planted in the polybag at 5 cm depth, one plant per pot. The pots were installed in a completely randomized design within two replication blocks, five plants per treatment per block. Blocking was used to account for the effects of nuisance factors and to reduce the error term used in conducting the test for the significance of the treatment effect. The block 1 represents the first replicate, and block 2 represents the second replicate, where each block contains all the treatments. The blocks were located 50 cm apart and consist of 16 treatments and total of 80 plants/block. The planting spacing used was 50 × 40 cm. The FPH/POC NASA/inorganic fertilizer treatments were applied five times (soil application) every 2 weeks. Plants (patchouli and mung bean) were irrigated manually once a day with applied water or by natural rainfall to adequately moist the soil. The FPH applied to patchouli is the one obtained from HTT at the temperature of 180 °C which has a higher nitrogen content.

Experimental setup: mung bean

Mung bean plots were single rows, 2.5 m long with a space of 25 cm between plants and 50 cm between plots in each block. The blocks were located 1 m apart. The mung beans were thinned to ten single plants per plot at 3 weeks after sowing. The 16 fertilizer treatment plots are shown in Table 3.

The FPH/POC NASA fertilizer treatments were applied two times (soil application) at 21 days after sowing (DAS) and 35 DAS. The FPH applied to mung bean is the one obtained from HTT at the temperature of 160 °C which contain less nitrogen. Inorganic fertilizers were applied only once before sowing the seeds.

Agronomic parameters

The following parameters were measured:

1. average plant height (cm): the distance between the ground surface to the tip of the stem was measured.

2. average number of leaves: total leaves of the plants from each pot/plot were counted.
3. average leaf area (cm²): gravimetric method was used for leaf area measurement at the harvest time.
4. average dry weight (g): after harvesting, the samples were oven-dried at 80 °C to constant weight in an electric oven. When it has reached room temperature, the sample was weighed using an electric balance.
5. SPAD value (index of relative chlorophyll content): observed on the leaf at the fourth node (for mung bean) and random leaf in each pot (for patchouli). A chlorophyll meter (SPAD-502, Minolta Corp. Ltd, Osaka, Japan) was used to take dimensionless SPAD values.

For mung bean, additional agronomic parameters (generative stage) were measured:

1. Average number of pods per plant: observed on a sample of five plants per plot.
2. Average pod length (cm): observed on a sample of 30 pods per plot.
3. Yield per plant (g): observed on a sample of three plants per plot.
4. Average hundred seed weight (g): randomly selected for each treatment and weighed with three replications.

Generative growth parameters (number of pods and pod length) were measured at 64 DAS.

Statistical analysis

All data were statistically analyzed using the SPSS software package 21.0 (SPSS Inc., Chicago, IL). Data were analyzed using two-way analysis of variance (ANOVA) with treatment as the fixed effect and block as the random effect. Means were compared using Duncan's multiple range test. For the effect of FPH application to be statistically significant at a 95% confidence level, the P value should be less than or equal to 0.05 (Fegade et al. 2013).

Table 4 Significance of the effects of treatments and block on growth parameters of patchouli and mung bean using ANOVA

Source	Patchouli					Mung bean						
	Height	Number of leaves	Chlorophyll	Leaf area	Dry weight	Height	Number of leaves	Chlorophyll	Number of pods	Pod Length	Yield	Weight 100 seeds
Treatment	1.58 ^{ns}	1.74 ^{ns}	11.68**	4.22**	2.59*	3.51*	2.98 ^{ns}	8.49*	1.34 ^{ns}	3.17*	3.81*	3.22*
Block	2.18 ^{ns}	0.94 ^{ns}	0.78 ^{ns}	2.29 ^{ns}	0.02 ^{ns}	0.01 ^{ns}	22.09**	6.45 ^{ns}	10.08**	1.63 ^{ns}	0.9 ^{ns}	0.27 ^{ns}

Numbers are F values (Fegade et al. 2013)

Superscript letters and stars indicate the level of significance (ns = not significant, * = $P < 0.05$, ** = $P < 0.01$)

Results and Discussion

Effect of FPH application on growth of patchouli

Table 4 summarizes the F-statistic and P value of the effects of treatments and block on growth parameters of patchouli and mung bean using ANOVA. P value is used to determine statistical significance (Fegade et al. 2003). Larger F-statistics with treatment relative to indicate that treatment had a greater net effect than block. The ANOVA test showed that there were significant differences in SPAD value ($P < 0.01$), leaf area ($P < 0.01$), and dry weight ($P < 0.05$) among the treatments, but no significant difference in height and number of leaves among the treatments. Moreover, there are no

differences among the mean responses of the blocks for all the growth parameters in patchouli.

The height, number of leaves per plant, leaf area, and SPAD value representing chlorophyll content at different dosages of FPH are shown in Table 5. Plant height is one of the parameters of growth performance (Joardar & Rahman 2018). It can be seen from the table that additional FPH from 0.5 to 5 mL/L did not significantly increase the height of patchouli. A similar result was also observed for T15 treatment (2 mL/L POC Nasa). The highest height (18.0 cm) was recorded at T11 treatment (2 mL/L FPH and 50% NPK). However, all treatments including T16 (100% NPK) showed no statistically significant effect of treatments on the height.

The number of leaves in plants tends to increase along with the application of FPH in the soil. The combination of FPH and 50% inorganic fertilizer can increase the number

Table 5 Mean values \pm standard errors for plant height, number of leaves, leaf area, and SPAD value of patchouli plants

Treatment	Height (cm)	Number of leaves	Leaf area (cm ²)	Chlorophyll (SPAD value)
T1	11.3 \pm 1.5a	106 \pm 18a	18.7 \pm 0.9ab	22.9 \pm 1.9a
T2	11.7 \pm 0.5a	134 \pm 10a	19.6 \pm 3.7ab	25.9 \pm 4.3a
T3	12.3 \pm 0.1a	99 \pm 2a	14.8 \pm 1.3a	25.9 \pm 1.7a
T4	13.9 \pm 1.2a	139 \pm 9a	18.1 \pm 1.6ab	22.2 \pm 2.0a
T5	11.3 \pm 1.8a	127 \pm 2a	20.5 \pm 3.2ab	25.5 \pm 3.0a
T6	11.2 \pm 1.7a	107 \pm 3a	18.2 \pm 5.5ab	26.7 \pm 1.1a
T7	10.4 \pm 1.4a	136 \pm 15a	18.8 \pm 1.4ab	23.6 \pm 3.5a
T8	14.5 \pm 0.7a	149 \pm 66a	24.5 \pm 4.4abc	43.7 \pm 6.2b
T9	12.6 \pm 0.3a	154 \pm 25a	26.9 \pm 2.4bc	44.5 \pm 5.9b
T10	13.1 \pm 2.8a	205 \pm 28a	33.6 \pm 2.9c	41.8 \pm 4.7b
T11	18.0 \pm 3.1a	168 \pm 37a	27.8 \pm 1.0bc	41.1 \pm 2.7b
T12	14.3 \pm 0.6a	172 \pm 27a	26.3 \pm 4.3abc	50.4 \pm 1.1b
T13	14.4 \pm 1.8a	203 \pm 21a	33.4 \pm 6.2c	43.9 \pm 3.6b
T14	14.4 \pm 3.2a	209 \pm 48a	33.4 \pm 3.9c	51.9 \pm 0.9b
T15	11.9 \pm 0.4a	128 \pm 3a	17.9 \pm 3.1ab	23.9 \pm 0.6a
T16	17.4 \pm 2.6a	175 \pm 1a	35.8 \pm 4.2c	48.3 \pm 2.1b

Values are the means of two replications and five plants per replicate

Different letters show the significant differences according to Duncan's test ($P = 0.05$)

of leaves to nearly twice of the total leaves in control. The highest number of leaves per plant was found in T14 treatment (FPH 5 mL/L and 50% inorganic fertilizer), yet it is not significantly different if compared with control.

The leaf area is also presented in Table 5. Additional of FPH only from 0.5 to 5 mL/L did not significantly increase the leaf area. Combined treatment yielded on a larger leaf area which significantly higher than the FPH only and the control. The largest leaf area was recorded in T16 treatment (100% NPK). However, it can be seen that combined treatment, especially T10, T13, and T14, can give a similar response with T16 statistically.

The chlorophyll content will increase in proportion to the amount of nitrogen present in the leaf (Konica Minolta 2009). A similar response with the other parameters was observed where the sole application of FPH in the range of concentration tested did not significantly improve the plant growth. Additional of 50% NPK can boost the chlorophyll content as presented in T8 treatment. Moreover, the combination of FPH (5 mL/L) and reduced dosage inorganic fertilizer can further increase the chlorophyll content which gave the maximum value of chlorophyll content compared to all the treatments.

Effect of FPHs and inorganic fertilizers and their combinations on dry weight of patchouli is presented in Fig. 2. The sole application of FPH showed similar response with control in dry weight. The dry weight was increased when the combinations of FPH and inorganic fertilizer were applied. The maximum dry weight was obtained at 1 mL/L FPH and

50% inorganic fertilizer treatment (T10) which almost produce similar yield with T14 and T16.

Patchouli crop requires high doses of nitrogen. In this study, the concentration of FPH tested is based on the common dose of liquid organic commercial fertilizer (2–5 mL/L). It should be noted that, within that range, the nutrient content given to the plant is almost 20 times lower compared to 100% NPK (Table 8). It is possible that, due to much lower nutrient content in FPH, the sole application of FPH did not significantly improve the plant growth parameter. However, when FPH is combined with 50% NPK, it resulted in a better performance in height, number of leaves, SPAD value, leaf area, and weight compared with blank (T1), FPH only (T1–T7), and 50% NPK only (T8). This indicates that combined treatment can increase fertilizer use efficiency which results in reduced inorganic fertilizer consumption.

Treatment of poultry feather waste by HTT can significantly reduce the volume of solid waste of up to 90% reduction (at 180 °C, holding times 30 min). Therefore, HTT of poultry feather could eliminate waste disposal issue as well as enables nutrient recovery from feather. Using thermal conversion, fast degradation of feather and pathogen inactivation can be expected. In the FPH, soluble protein, amino acids, and other simple nitrogenated compounds are water-soluble which can be quickly absorbed by the plants. Moreover, amino acids have a specific benefit for promoting plant growth as it can cause a fast release of inorganic N into soil (Jones & Kielland 2012). The high content of organic carbon

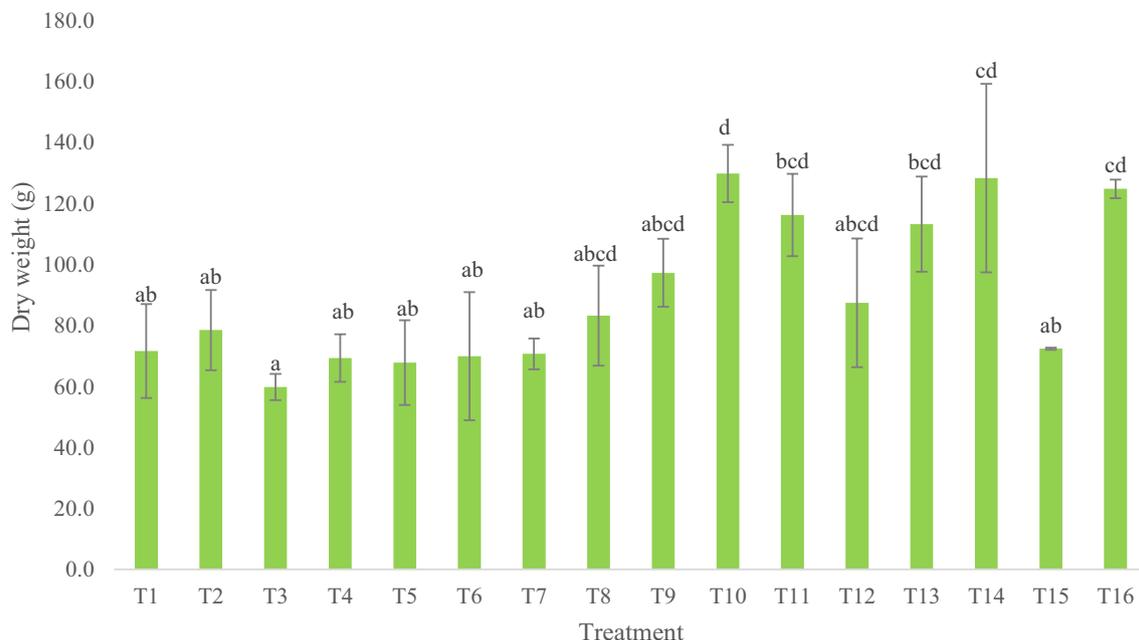


Fig. 2 Effect of fertilizer treatments on dry weight of patchouli. Error bars indicate standard error of two replications and three plants per replicate. Different letters show significant differences according to Duncan's test ($P=0.05$)

(TOC) content in the FPH (Table 2) indicated that application of FPH could be an option to enrich soil organic matter.

Effect of FPH application on vegetative growth of mung bean

The ANOVA test revealed that there were significant differences in height, SPAD value representing chlorophyll content, pod length, yield, and weight of hundred seeds of mung bean among the treatments (Table 4). However, we found a significant effect of block on number of leaves and number of pods of mung bean. In contrast with patchouli experimental design, instead of using polybag, we directly planted the mung bean on the field. Therefore, it is possible that high environmental variation, e.g., soils, resulted in the insignificance effect of treatments on the number of leaves and number of pods.

Table 6 shows the effects of FPH application on vegetative growth parameters including plant height, number of trifoliolate leaves, and chlorophyll content at 50 DAS. Our data indicated that application of FPH, within tested concentrations, did not significantly affect the growth of mung bean as evidenced by the height and number of trifoliolate leaves when compared with control. In the case of combination treatments (T9–T14), there were also no significant differences in the plant height. T6 and T10 treatments showed the lowest plant height. Those two treatments also showed the lowest value in the other growth parameters. According to

Table 6 Mean values \pm standard errors for plant height, number of trifoliolate leaves, and SPAD value of mung bean at 50 DAS

Treatments	Height (cm)	Number of trifoliolate leaves	Chlorophyll (SPAD value)
T1	23.1 \pm 0.3abc	7.9 \pm 0.1a	45.35 \pm 0.03a
T2	28.4 \pm 1.0c	8.7 \pm 1.3a	50.46 \pm 3.04abc
T3	27.3 \pm 0.8bc	7.4 \pm 0.8a	47.99 \pm 0.75abc
T4	24.6 \pm 1.8abc	7.8 \pm 0.4a	48.31 \pm 2.31abc
T5	23.9 \pm 0.5abc	7.2 \pm 1.0a	50.62 \pm 2.76abc
T6	20.1 \pm 0.2a	5.8 \pm 0.4a	45.58 \pm 0.28a
T7	22.5 \pm 2.4abc	7.6 \pm 0.2a	47.46 \pm 1.36abc
T8	23.5 \pm 0.8abc	6.6 \pm 0.6a	46.76 \pm 3.90abc
T9	25.9 \pm 0.0abc	7.5 \pm 0.5a	50.67 \pm 3.89abc
T10	20.3 \pm 0.4a	6.1 \pm 0.1a	51.15 \pm 0.23abc
T11	23.3 \pm 0.9abc	8.1 \pm 0.7a	50.81 \pm 2.51abc
T12	25.6 \pm 0.1abc	8.7 \pm 0.5a	52.46 \pm 0.72bc
T13	21.4 \pm 2.7ab	7.4 \pm 1.2a	50.89 \pm 4.07abc
T14	23.7 \pm 1.4abc	7.1 \pm 0.3a	53.22 \pm 1.98c
T15	23.9 \pm 0.1abc	7.6 \pm 0.6a	48.51 \pm 1.05abc
T16	24.8 \pm 0.2abc	8.4 \pm 0.0a	46.80 \pm 0.20abc

Values are the means of two replications and five plants per replicate. Different letters show significant differences according to Duncan's test ($P=0.05$)

visual observations, several plants at those two plots suffered from diseases which were indicated by relatively smaller plant size and the presence of necrotic spots. Therefore, T6 and T10 are not included in the trend discussion.

According to our results, the highest height (28.4 cm) was recorded at T2 treatment (0.5 mL/L FPH) which is the lowest concentration of FPH treatment. A study reported an increase in tomato plant height after the application of animal enzymatic PH in the greenhouse experiment of tomato plant (Parrado et al. 2008). A study by Ertani et al. (2009) revealed that animal-derived PHs could show a weak phytohormone-like activity including auxin and gibberellins. Auxin was identified as a plant growth hormone because of its ability to promote cell and elongation rate, and stimulate root growth and fruit development (Zerony & Hall 1980; Parrado et al. 2008). On the other hand, gibberellins, like auxins, stimulate cell elongation rate, leaf growth, flowering, and fruit set (Phillipson 1985; Parrado et al. 2008). Since FPH is a by-product of animal origin, it does not present any concentration of the phytohormones. However, the application of 0.5 and 1 mL/L of FPH led to an increase of up to 22.9% in the plant height when compared to the control which might indicate weak auxin-like activity and gibberellin-like activity. At higher concentration of FPH, the increase in height was not observed. This phenomenon seems related to the higher content of salts, and intermediate compound contains at a higher concentration of FPH. HTT at a high temperature form a certain organic acids, soluble salts, ions which can cause the possibility of phytotoxicity effect caused by FPH (Nurdiawati et al. 2015a). Plant growth depression in terms of height reduction may indicate the possibility of phytotoxicity effect caused by FPH. However, the potential phytotoxicity and possible phytohormone-like effect of the HTT products needs further investigations.

The highest number of leaves was found in 100% of the recommended dose of NPK treatment (T16). However, there were no significant differences in the number of trifoliolate leaves between control and all treated groups. In this study, no significant differences between the control (T1) and 100% NPK (T16) in height and number of leaves either in patchouli or mung bean. Based on the literature study on the effect of inorganic fertilizer on mung bean, we found that the application of 100% NPK did not always give a statistically significant difference in height and the number of leaves. In one study, significantly higher plant height and number of leaves per plant of mung bean recorded in 100% of the optimum dose of inorganic fertilizer treatment compared to the control/unfertilized (Armin et al. 2016). In contrast, some studies reported no significant effect between 100% of NPK treatment and control in height and number of leaves of mung bean (Bandani et al. 2014; Barus et al. 2014). The difference is presumably due to different adaptability to the environment. Gardner et al. (1991) explained that the growth

of crops is influenced by internal factors (genetic trait) as well as external factors (environmental factors, such as climate, soil, and biotic factors).

Application of FPHs from 0 to 5 mL/L tends to increase the SPAD index. However, there is no clear-cut trend between the dosage and the effect observed in this study. It can be due to small increment differences among concentrations tested. The SPAD value is highly correlated with leaf N and chlorophyll content. An increase in chlorophyll content would enhance photosynthesis. The highest increase in SPAD value was noted in T12 and T14 treatments (+15.7 to +17.3%). SPAD values of these two treatments was statistically significant compared to control. Based on our previous study, FPH may contain various amino acids (Nurdiawati et al. 2018). Amino acids are the starting point for the synthesis of many metabolites which are important in the chlorophyll synthesis (Shekari and Javanmardi, 2017). Therefore, the increase in chlorophyll content can be enhanced by the presence of amino acids. This result is consistent with the other studies that reported an increase in nitrogen assimilation due to the applications of protein hydrolysates (Ertani et al. 2009; Ertani et al. 2013; Baglieri et al. 2014; Calvo et al. 2014). In addition, it has been demonstrated that plants are able to take up N from the other N sources in the forms of amino acids and peptides which may undergo different assimilation pathways (Jones et al. 2005; Kielland 1994; Ertani et al. 2009). The positive effects of PH application on leaf chlorophyll content have also been observed on other crops such as banana and lettuce (Gurav & Jadhav 2013; Lucini et al. 2015).

The SPAD value and other vegetative parameters tended to be higher in combined treatments of inorganic fertilizer and FPH than FPH alone or sole use of inorganic fertilizer. The commercial fertilizer treatments (T8 and T16) showed no significant increase in vegetative parameters (leaf area and SPAD values) in patchouli and SPAD value in mung bean. However, we found a tendency that T16 gave a higher leaf area and SPAD values in patchouli. The small differences of SPAD value in mung bean (T8 and T16) can be due to small increment in real N added (Table 8). Moreover, it might be because generally mung bean does not need nitrogen fertilizer; thereby, it is less sensitive to additional nitrogen fertilizer.

Effect of FPHs application on generative growth of mung bean

Table 7 lists the effects of FPH application on the generative growth including the number of pods, pod length, yield per plant, and weight of hundred seeds. There was no significant difference in the number of pods among all the treatments. The highest mean pod numbers (26 pods) was observed in plots treated with 50% NPK and 2 mL/L FPH (T11). On the contrary, the control and some treated groups showed significant differences in the pod length. The highest mean pod lengths were recorded in plots treated with 5 mL/L of FPH (T7) and 50% NPK and 4 mL/L FPH (T13) with an increase of up to 15.6%. The shortest pod length was recorded in the control plot. The visual observation of pods collected in the first harvest can be seen in Fig. 3.

Table 7 Mean values \pm standard errors for number of pods, pod length, yield per plant, and hundred seeds weight of mung bean

Treatments	Number of pods per plant	Pod Length (cm)	Yield per plant (g)	Hundred seeds weight (g)
T1	19 \pm 5a	9.6 \pm 0.3a	10.92 \pm 1.49ab	6.76 \pm 0.25ab
T2	26 \pm 6a	10.4 \pm 0.5abc	10.97 \pm 1.27ab	6.51 \pm 0.47ab
T3	18 \pm 7a	10.6 \pm 0.1abc	13.01 \pm 2.13ab	7.26 \pm 0.58a
T4	18 \pm 2a	10.9 \pm 0.2bc	12.69 \pm 2.77ab	6.97 \pm 0.05ab
T5	17 \pm 4a	10.2 \pm 0.0abc	10.36 \pm 1.49ab	6.63 \pm 0.36ab
T6	13 \pm 1a	9.8 \pm 0.7ab	8.61 \pm 2.15a	5.69 \pm 0.65b
T7	16 \pm 1a	11.0 \pm 0.7bc	12.15 \pm 1.44ab	7.42 \pm 0.20a
T8	14 \pm 1a	10.1 \pm 0.0abc	11.03 \pm 1.88ab	7.04 \pm 0.35a
T9	19 \pm 3a	10.3 \pm 0.6abc	10.2 \pm 0.69ab	6.42 \pm 0.39ab
T10	14 \pm 3a	10.2 \pm 0.1abc	9.76 \pm 0.55ab	6.70 \pm 0.25ab
T11	26 \pm 8a	10.6 \pm 0.2abc	15.00 \pm 2.00b	7.25 \pm 0.08a
T12	20 \pm 3a	10.5 \pm 0.4abc	11.74 \pm 1.09ab	6.90 \pm 0.43ab
T13	18 \pm 5a	11.1 \pm 0.7c	11.08 \pm 1.59ab	6.77 \pm 0.36ab
T14	17 \pm 1a	10.5 \pm 0.2abc	11.08 \pm 1.2ab	6.71 \pm 0.26ab
T15	17 \pm 2a	10.5 \pm 0.1abc	11.69 \pm 1.91ab	7.14 \pm 0.07a
T16	19 \pm 1a	10.5 \pm 0.0abc	11.52 \pm 1.16ab	7.32 \pm 0.60a

Values are the means of two replications and three plants per replicate

Different letters show significant differences according to Duncan's test (P=0.05)



Fig. 3 Visual observations of pods of mung bean obtained at the first harvesting: T1 vs. T7, T16 vs. T7, and T8 vs. T7 (from left to right)

Our data indicated that the yield per plant and hundred seeds weight was not significantly affected by the treatments. However, there was a tendency that the combined application of reduced inorganic fertilizer and 2 mL/L of FPH (T11) could boost reproductivity performance as evidenced by the increase of yield per plant (+37.7%) and hundred seeds weight (+7.3%). Compared to the application of inorganic fertilizer alone, the combined treatments showed a higher yield (Table 6). Another study also reported the positive effect of the combination of organic and inorganic fertilizer in improving the yield of peanut (Argaw 2017). The result inferred that integrated the application of organic and inorganic fertilizers could enhance the nitrogen assimilation process due to their synergistic effects of increasing nutrient use efficiencies.

The highest hundred seeds weight was observed in plots treated with 5 mL/L of FPH (T7). The inorganic treatments (T8 and T16) and commercial liquid fertilizer treatment (T15) also showed an increase in hundred seeds weight compared to control. It indicated that the root application of FPH could enhance fruit development as good as inorganic fertilizer and commercial liquid fertilizer.

Application of higher concentrations of FPH gave better results in the generative parameters, even though the effect is not statistically significant. The data indicated that the application of 5 mL/L FPH yielded relatively longer pods, heavier seeds, and higher yields compared to the control. This might be because higher concentration of FPHs can provide higher nutrients to the soil to be absorbed by the plant. At this dosage, FPH gave comparable results to the commercial liquid fertilizer and inorganic fertilizer.

Table 2 presents that FPH contains considerable amounts of secondary nutrients such as calcium, magnesium, and sulfur, as well as micronutrient such as iron. Micronutrients are also essential for promoting plant growth due to their role in plant nutrition and improving the soil productivity. Zain et al. (2015) reported that there is a significant increase in wheat yield by applying micronutrients (Zain et al. 2015). It is expected that micronutrients in FPH may also play a vital role in improving mung bean growth and productivity.

In general, our study showed that the combined treatment of organic and inorganic fertilizer is better than sole FPH

application on increasing patchouli and mung bean yields. Different nitrogen sources can highly affect the yield potential due to the difference in nutrient absorption behavior (Ahmad et al. 2008). An increase in parameters observed in combined treatments might be again due to their synergistic effects of increasing nutrient use efficiencies. Moreover, additional organic sources, such as FPH, may enhance soil physical and chemical properties which then results in higher crop yield (Myint et al. 2010). The application of FPH also may allow for a reduction in inorganic fertilizers without significantly affecting yield and quality.

Conclusion

In summary, liquid products from HTT of poultry residues, such as feather, can be a promising source of organic nutrients. The result showed that the combination of FPH and 50% dose inorganic fertilizer on patchouli yielded a statistically significant increase in leaf area, dry weight, and chlorophyll content in comparison to the control. Moreover, the combined treatment showed similar response with 100% inorganic fertilizer, suggesting that the application of FPH along with the inorganic fertilizer can increase nutrient use efficiency. The application of 5 mL/L FPH on mung bean yielded relatively longer pods, heavier seeds, and higher yields, compared to the control (with no statistical difference). The conversion of feather into FPH will provide both profitability and sustainability of poultry industry, and could be of practical interest in reducing the use of chemical fertilizers.

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Appendix

See Table 8.

Table 8 Actual N added (in mg) per application

Treatment	Added N for each application (mg)	
	Patchouli	Mung bean
T1	0	0
T2	13	3
T3	25	6
T4	50	12
T5	75	18
T6	100	24
T7	125	30
T8	1400	46
T9	1413	49
T10	1425	52
T11	1450	58
T12	1475	64
T13	1500	70
T14	1525	76
T15	60	6
T16	2800	93

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