Article

Growth of Tea Nursery Plants as Influenced by Different Rates of Protein Hydrolysate Derived from Chicken Feathers

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Abstract: The conversion of chicken feathers, generated annually worldwide on a large scale as a by-product of the poultry industry into value-added products, has economic and environmental benefits. Protein hydrolysate produced from feathers has attracted significant attention in agriculture as a potential plant growth stimulant. Therefore, a study was established with the aim to produce and characterize chicken feather protein hydrolysate (CFPH) and investigate the effects of this product on the early growth of nursery tea plants. Alkaline hydrolysis was used to produce CFPH with the yield of 165 mg amino acids per gram of feathers. Then, the produced CFPH was applied on nursery tea plants as a soil drench at different doses (0.5, 1, 2, 3, and 4 g L⁻¹) in 2-week intervals until the 10th application. Commercially available fish protein hydrolysate (FPH) was included as a treatment to compare the effects with CFPH. The treatments were arranged in a completely randomized block design with three replications. CFPH and FPH significantly improved the shoot and root growth parameters. Plant height (+98%), leaf number (+61%), shoot dry biomass (+128%), root length (+94%), root surface area (+15%), and root dry biomass (+152%) were significantly increased by the application of CFPH (2 g L⁻¹ dose) compared to control. Although the highest CFPH dosage (4 g L⁻¹) showed a reduction in growth parameters, the values obtained were similar or higher than the untreated control plants. The chlorophyll content (*a*, *b*, and total) was enhanced by the CFPH dosage of 1 g L⁻¹, whereas the highest photosynthetic rate was recorded in the CFPH 3 g L⁻¹ treatment. The application of protein hydrolysates (PH) did not positively influence stomatal conductance and intercellular CO₂ concentration. Leaf nitrogen, phosphorous, manganese, and copper were positively affected by the CFPH application. The effect of CFPH on growth parameters was more pronounced than FPH. Our findings reveal that CFPH produced by alkaline hydrolysis could be used as a growth booster in raising vigorous tea nursery plants, which are most suitable for field planting and subsequently higher yields.

Keywords: chicken feather; alkaline hydrolysis; protein hydrolysate; growth promoter; tea nursery plants

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1. Introduction

In recent years, the production of poultry meat has snowballed to cater to the growing demand. Poultry meat production reached 112.9 million metric tons in 2018, contributing 33% of the world meat production [1]. Feathers comprise 5–7% of the total live body weight of a mature chicken [2] and every week, nearly 3000 tons of feather waste is generated globally [3]. Feather waste consists of bloodstains and may have a considerably large load of viruses and other pathogenic microorganisms [4]. In addition, their improper disposal causes severe damage to the environment and human health.

Feathers are made up of over 91% keratin, a structural and fibrous protein with a high degree of cross-linking of peptide chain by forming disulphide bonds [4]. Keratin is highly insoluble, mechanically stable, and resistant to degradation by most proteolytic

enzymes [5]. Since the natural degradation of feathers takes more than 2 years [6], leftover feathers are disposed of through landfilling or burning. Nevertheless, these methods are not eco-friendly. Feathers are utilized to produce feather meal, a potential animal feed. However, avian disease outbreaks and potential risks of disease transmissions limited the recycling process [7]. Feather waste is rich in nitrogen that is up to 15% of its total weight and is a good protein and amino acids source. The production of protein hydrolysates (PH) from agro-industrial by-products is a sustainable remedy for waste disposal and for the addition of economic value [8]. In the recent past, the isolation of PH from the by-products in slaughterhouses has been re-used due to their functional and bioactive properties [5].

PH are produced from both plant and animal protein sources using partial hydrolysis [9], which consists of a mixture of peptides, oligopeptides, and amino acids. However, they may have carbohydrates, small quantities of mineral elements, phenols, and other compounds [10] that have shown promising influence on plant growth performances [11]. PH are used in broad areas, including medicine, nutrition, food, and agriculture, due to their nutritional and functional/bioactive properties [12]. These are available in the markets as liquid extracts or soluble powder and granule forms and can be applied as root or foliar applications [11]. Many studies have reported that PH with low molecular weight peptides and free amino acids showed more pronounced effects due to the easy absorption by all plant tissues [13,14]. In addition, a significant growth promotion was observed when it is applied at low rates irrespective of the type of crop [15–17]. PH directly influence the plant by stimulation of carbon and nitrogen metabolism, regulation of nitrogen assimilation [18,19], and interference with hormonal activities through bioactive peptides [11,20]. In addition, they indirectly affect growth and plant nutrition [18]. The foliar and root application of PH increases the nutrient uptake and nutrient use efficiency [11,21,22]. Enhanced nutrient uptake links with root architecture modification result in more available micronutrients, due to complexation and chelation as well as higher microbial activity [23].

Compared to vegetal-derived PH, animal-derived PH have higher nitrogen content (9–16%) and are released gradually [24]. Furthermore, the use of animal-derived PH as a biostimulant in agriculture is a cost-effective approach, since most of the raw materials used are collected from wastes. Animal-derived PH have been employed for more than 50 years due to their capacity to stimulate plant growth and ameliorate the effects of environmental stresses [25]. Despite the positive effects on plants, studies have reported adverse effects of commercial PH derived from animal sources, which mainly cause phytotoxicity and plant growth retardation when applied as foliar [14,26,27]. This negative impact on growth is associated with a higher concentration of free amino acids [28] and salts [20] in the products. In addition, there is a limitation on the usage of animal-derived PH on food safety due to the ban on its use on edible parts of crops under organic farming (European Regulation no. 354/2014). Nevertheless, the evaluation of safety and fertilizer efficacy revealed that it could be applied in conventional and organic farming with no potential risk to the human health and the ecosystem [29].

The application of PH for a short period increased the root dry weight of maize plants compared to the untreated plants [21]. Furthermore, Nardi et al. [30] reported that exerted short-term effects on root growth were consistent and comparable with humic substances, and finally resulted in enhanced shoot growth over long periods. Moreover, an increase in root dry weight might result in a greater survival rate of transplanted plants, which leads to higher yields. Furthermore, the application of chicken feather protein hydrolysate (CFPH) enhanced the germination and secondary root development of Bengal gram seedlings [31]. In another study, wheat seedlings treated with CFPH increased root and shoot lengths, fresh and dry mass, and the photosynthetic pigment content [32]. Although the beneficial effects of CFPH on the early growth of annual crops has been reported, the information available on the early growth of woody perennials is limited. Tea [*Camellia sinensis* (L.) O. Kuntze] is a woody perennial commercial crop widely cultivated in tropical and subtropical regions of the world. The proper management of tea plants in the nursery stage is the first and foremost step in successful tea cultivation in the field. The growth performance of tea

nursery plants in response to CFPH has not been explored. Therefore, this study aimed to produce and characterize the CFPH and identify the optimum rate that would exert the biostimulant action on the early growth of tea nursery plants.

2. Materials and Methods

2.1. Preparation of Chicken Feather

Chicken feathers were collected from a broiler farm located in Selangor, Malaysia. The collected feathers were thoroughly washed with tap water to remove blood and other foreign materials. Feathers were re-washed with distilled water and oven-dried at 75 °C for 3 days. Oven-dried samples were cut into small pieces and ground until they turned into thin feather wool.

2.2. Production of Chicken Feather Protein Hydrolysate

The hydrolysis of chicken feathers was carried out with a modification of a method used by Taskin et al. [33] and Genç and Atici [32]. Briefly, 5 g of feather wool was weighed into a shaking bottle, and 100 mL of 2 N KOH was added. A hydrolysis process was conducted by gentle shaking the mixture in an orbital shaker for 3 days. Then, the pH of the extract was adjusted to a pH of 7 using 10 N H₃PO₄ and it was filtered using Whatman filter paper to remove the non-hydrolysable particles. The filtrate was collected and oven-dried at 70 °C until a constant weight was obtained. This oven dried sample was referred to as CFPH.

2.3. Analysis of Chicken Feather and CFPH

Total carbon, nitrogen, and sulphur contents were determined using a TruMac CNS analyzer (LECO, Saint Joseph, MI, USA). Both the chicken feather and CFPH were digested using an acid digestion and the macro and micronutrients were determined by the inductively coupled plasma—optical emission spectrometry (ICP-OES) (Optima 8300, PerkinElmer, Waltham, MA, USA). Table 1 summarizes the nutrient composition of both the raw chicken feather and CFPH.

Table 1. Chemical composition of the raw chicken feather and CFPH.

Elements	Chicken Feather	CFPH
Total C (%)	47.09	9.42
Total N (%)	13.54	3.06
C/N ratio	2.81	2.31
P (%)	0.12	17.13
K (%)	0.29	25.41
Ca (%)	0.31	0.074
Mg (%)	0.03	0.007
S (%)	1.61	0.44
Zn (mg kg ^{−1})	0.02	<0.01
Mn (mg kg ^{−1})	<0.01	<0.01
Cu (mg kg ^{−1})	<0.01	<0.01
Fe (mg kg ^{−1})	0.04	<0.01

2.4. Amino Acid Profile

The amino acid composition of CFPH was determined by high-performance liquid chromatography (Waters Alliance e2695) with a fluorescence detector using the HPLC column (AccQ tag/3.9 × 150 mm). The amino acid amount was determined by establishing an external standard calibration curve for individual amino acids.

2.5. Plant Materials and Treatment Application

This experiment was carried out at University Putra Malaysia located in Serdang, in a polyethylene greenhouse under 70% shading. Herein, 8-week-old uniform size healthy

tea (*Camellia sinensis*) plants propagated by single-node cuttings were selected for this experiment. These plants were planted in poly bags containing a mixture of topsoil and sand (ratio 2:1). Each experimental unit consisted of five plants. The treatments were arranged in a complete randomized block design with three replicates with a total of 21 experimental units. The treatments were the control, different doses of CFP (0.5, 1, 2, 3, and 4 g L⁻¹), and fish protein hydrolysate (FP) at the recommended dosage (10 mL L⁻¹), which was given by the manufacturer. The pH of the solution was adjusted to 4.5–5.5 before the application in order to ensure an optimum pH for the growth of tea plants. The applied PH volume was 20 mL plant⁻¹, and the control plants received the same amount of water. The PH were applied as a soil drench at 2-week intervals following the fertilization. The FP was used as a positive control to compare the effects. Fertilization was conducted by dissolving the fertilizer mixture in water and applied at fortnight intervals alternatively to the PH application.

2.6. Plant Growth Measurements

Plant height was measured from the apical bud of the stem to the ground surface, and the number of leaves was counted. Plant fresh weight was measured immediately after harvesting, and the average dry weight was determined after an oven-dried condition at 70 °C for 72 h.

2.7. Root Growth Measurements

Roots were carefully washed to remove soil, and the fresh root weight was taken. Roots were fully spread on a transparent tray and scanned at 400 dpi with a scanner (Epson Perfection V700). Root positioning and scanning were conducted with Regent's positioning system. The images acquired by WinRHIZO were identified by colored lines and processed using an image analysis software (WinRHIZO Pro 2000, Regent Instruments Inc., Quebec City, QC, Canada).

2.8. Leaf Gas Exchange

The photosynthetic rate, stomatal conductance, intercellular CO₂ concentration, and transpiration rate were measured using an infrared gas analyzer (Li-6400XT Portable Photosynthesis System, Li-Cor, Lincoln, NE, USA). The Li-6400XT is an open system, which measures photosynthesis and transpiration based on the differences in CO₂ and H₂O in the in-chamber and pre-chamber conditions. Measurements were taken from the tip of the fourth leaf. The chamber airflow and CO₂ concentrations were 300 µmol s⁻¹ and 400 ppm, respectively. Measurements were recorded at the same time of the day (09.00–11.00) to minimize the changes in the readings due to environmental factors.

2.9. Chlorophyll Content

Chlorophyll *a*, *b*, and total chlorophyll were determined based on the method described by Coombs et al. [34]. Briefly, four-leaf discs of 1 cm² were taken from the fourth leaf using a cork borer. The samples were brought to the lab and kept in a vial together with 80% (*v/v*) acetone in the dark for 48–60 h at room temperature. After the pigments were totally absorbed into the acetone solution, each sample was measured for absorbance at 647 and 664 nm using a UV-visible spectrophotometer (UV-1700 PharmaSpec, Shimadzu, Japan).

2.10. Leaf Nutrient Analysis

Leaf samples were oven-dried at 60 °C until a constant weight. Dried leaves were ground using a leaf grinder to a particle size of 1 mm. The total nitrogen content of the leaf samples was determined by the Kjeldahl method [35], following acid digestion in sulphuric acid in the presence of a catalyst (Kjeldahl tablets). Regarding the other macro and micronutrient analyses, 0.2 g of finely ground leaf samples were subjected to dry-washing in a muffle furnace at 550 °C for 8–10 h. Thereafter, the ash was moistened with a few drops of distilled water, followed by 2 mL of con HCl and 10 mL of 20% HNO₃. The ash

was dissolved by placing it in a water bath for 1 h. The sample was filtered and transferred to a 50 mL volumetric flask and made to volume with deionized water. Phosphorous was determined by the vanadate yellow color method, using a UV-visible spectrophotometer (UV-1700 PharmaSpec) at 425 nm wavelength. Potassium, Ca, Mg, and micronutrients were measured using an atomic absorption spectrometer (Analyst 400, PerkinElmer, USA).

2.11. Statistical Analysis

All of the data were subjected to the one-way analysis of variance (ANOVA) using the statistical analysis system (SAS) 9.4 and expressed as mean values at $\pm$ standard error. The least significant difference (LSD) at a 5% confidence level was set to compare the treatment means.

3. Results

3.1. Production and Characterization of CFPH

The amino acid composition of the CFPH produced from alkaline hydrolysis of feathers is summarized in Table 2.

Table 2. Amino acid composition of chicken feather protein hydrolysate.

Amino Acids	Amino Acids (%)	Chicken Feather (mg g ⁻¹)
Alanine	6.21	10.27
Arginine	6.61	10.94
Aspartic acid	8.03	13.29
Cysteine	0.79	1.30
Glutamic acid	13.36	22.11
Glycine	7.28	12.04
Histidine	ND	0.00
Isoleucine	6.28	10.40
Leucine	9.42	15.59
Lysine	2.73	4.53
Methionine	0.75	1.25
Phenylalanine	4.77	7.90
Proline	11.75	19.45
Serine	8.11	13.43
Threonine	2.42	3.71
Tyrosine	2.56	4.24
Valine	9.06	14.99
Hydroxyproline	ND	0.00
Total	100	165.50

ND: Not detected.

The production of chicken feather protein hydrolysate using chemical hydrolysis is a faster and simpler process with higher yields. The alkaline hydrolysis adopted in this study, without any heating treatment, yielded a higher concentration of amino acids (165.5 mg g⁻¹ of feathers). Amino acid profiling revealed the presence of 16 amino acids in the CFPH including higher amounts of essential amino acids (valine, leucine, and isoleucine) and non-essential amino acids (serine and glycine). Both the glutamic acid and proline alone contribute 25% of the total amino acid composition. Minute quantities of cysteine and methionine were detected, while histidine and hydroxyproline were absent.

3.2. Shoot Growth

In this experiment, PH derived from CFPH and FPH showed a positive influence on plant height, leaf number, and shoot fresh and dry weights (Figure 1, Table 3). CFPH applied at the rate of 2 g L⁻¹ significantly increased all of the shoot growth parameters compared to the FPH and control treatments. The response of tea plants treated with CFPH 1 g L⁻¹ was comparable with the CFPH 2 g L⁻¹ dosage, except for plant height.

Figure 1. The influence of different treatments: (A) Control; (B) CFPD 0.5 g L⁻¹; (C) CFPD 1 g L⁻¹; (D) CFPD 2 g L⁻¹; (E) CFPD 3 g L⁻¹; (F) CFPD 4 g L⁻¹, and (G) FPD 10 mL L⁻¹ on the growth of tea nursery plants.

Table 3. Effect of animal origin PH on plant height, leaf number, and shoot fresh and dry weights.

Treatment	Plant Height (cm)	Leaf Number (n plant ⁻¹)	Shoot Fresh Weight (g plant ⁻¹)	Shoot Dry Weight (g plant ⁻¹)
Control	22.7 ± 1.4 ^e	7.0 ± 0.6 ^d	6.6 ± 0.4 ^e	2.13 ± 0.17 ^e
CFPD 0.5 g L ⁻¹	27.2 ± 1.0 ^d	8.0 ± 0.6 ^{cd}	8.5 ± 0.5 ^d	2.76 ± 0.23 ^{de}
CFPD 1 g L ⁻¹	39.6 ± 0.7 ^b	10.0 ± 0.6 ^{ab}	12.5 ± 0.6 ^{ab}	4.23 ± 0.43 ^{ab}
CFPD 2 g L ⁻¹	45.0 ± 1.1 ^a	11.3 ± 0.3 ^a	13.7 ± 0.7 ^a	4.87 ± 0.27 ^a
CFPD 3 g L ⁻¹	37.7 ± 0.6 ^{bc}	8.6 ± 0.3 ^{bc}	12.2 ± 0.6 ^{abc}	4.03 ± 0.32 ^{bc}
CFPD 4 g L ⁻¹	34.3 ± 1.3 ^c	8.6 ± 0.3 ^{bc}	10.6 ± 0.5 ^c	2.73 ± 0.06 ^{de}
FPD 10 mL L ⁻¹	37.0 ± 1.5 ^{bc}	8.6 ± 0.3 ^{bc}	11.5 ± 0.5 ^{bc}	3.31 ± 0.13 ^{cd}

The same letters within each column are not significantly different at the $p < 0.05$ level.

Increasing the dosage of CFPD significantly enhanced the shoot growth parameters up to 2 g L⁻¹, in which it showed growth inhibition. The highest CFPD dosage of 4 g L⁻¹ improved all of the shoot growth parameters, except for the shoot dry biomass compared to the control plants. All of the applied doses of CFPD increased the plant height and shoot fresh weight. Tea plants positively responded to the FPD application and the exerted effects were comparable with 1 and 3 g L⁻¹ CFPD dosages.

3.3. Root Growth

A well-developed root system is considered an essential requirement for the successful field planting of tea. The applied animal-based protein hydrolysates positively influenced the root growth parameters of tea nursery plants (Figure 2).

Figure 2. Effect of animal origin PH on root length (A), root surface area (B), root fresh weight (C), and root dry weight (D). The small bar shows the standard error. The columns with the same letters are not significantly different at the $p < 0.05$ level.

Soil drenching of CFPH had positive effects on root length, surface area, as well as root fresh and dry weights, especially when applied at the rate of 2 g L^{-1} compared to the control plants. The influence of CFPH 3 g L^{-1} dosage on root length and surface area were comparable with CFPH 2 g L^{-1} , whereas root fresh and dry weights were significantly lower. No differences were found for root length, surface area, and root dry weight between 1 and 3 g L^{-1} doses of CFPH treatments. The plants treated with the lowest (0.5 g L^{-1}) and highest (4 g L^{-1}) doses of CFPH showed a similar response on all the root growth parameters. The increasing concentration of CFPH showed a similar trend observed in shoot growth, where maximum values were observed at the 2 g L^{-1} dosage. The positive action of FPH on root growth was observed and the exerted effects were comparable with both the 1 and 3 g L^{-1} rates of CFPH.

3.4. Chlorophyll Content

The application of PH significantly increased the leaf chlorophyll *a*, chlorophyll *b*, and total chlorophyll contents (Figure 3). The highest values were recorded in the CFPH 1 g L^{-1} treatment. There were no significant differences observed between the 1 and 3 g L^{-1} dosages. Although both animal origin PH improved the leaf chlorophyll content, the positive effects of CFPH were more pronounced than FPH.

Figure 3. Effect of animal origin PH on leaf chlorophyll *a*, chlorophyll *b*, and total chlorophyll contents. The small bar shows the standard error. The columns with the same letters are not significantly different at the $p < 0.05$ level.

3.5. Leaf Gas Exchange

Table 4 shows the influence of PH application on leaf gas exchange. The photosynthetic and transpiration rates were enhanced by 9.65 and 26.5%, respectively with the CFPH 3 g L⁻¹ treatment compared to the control. Both the CFPH and FPH positively enhanced the photosynthetic rate of nursery plants. However, the transpiration rate was significantly higher in the CFPH 3 g L⁻¹ treatment than FPH.

Table 4. Effects of animal origin PH on leaf gas exchange.

Treatment	Pn	Gs	Ci	Tr
Control	10.88 ± 0.21 ^b	0.22 ± 0.02 ^a	290 ± 12 ^a	6.17 ± 0.36 ^{bc}
CFPH 0.5 g L ⁻¹	11.15 ± 0.20 ^{ab}	0.26 ± 0.01 ^a	292 ± 10 ^a	6.99 ± 0.13 ^{ab}
CFPH 1 g L ⁻¹	10.59 ± 0.20 ^b	0.21 ± 0.03 ^a	283 ± 07 ^a	5.78 ± 0.09 ^c
CFPH 2 g L ⁻¹	10.47 ± 0.26 ^b	0.23 ± 0.04 ^a	289 ± 04 ^a	6.54 ± 0.46 ^{bc}
CFPH 3 g L ⁻¹	11.93 ± 0.42 ^a	0.25 ± 0.02 ^a	287 ± 03 ^a	7.81 ± 0.18 ^a
CFPH 4 g L ⁻¹	10.65 ± 0.24 ^b	0.26 ± 0.01 ^a	295 ± 07 ^a	7.03 ± 0.44 ^{ab}
FPH 10 mL L ⁻¹	11.20 ± 0.35 ^{ab}	0.24 ± 0.02 ^a	289 ± 03 ^a	6.77 ± 0.23 ^b

Pn: Photosynthetic rate ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$); Gs: Stomatal conductance ($\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$); Ci: Inter-cellular CO₂ concentration ($\mu\text{mol CO}_2 \text{ mol}^{-1}$); Tr: Transpiration rate ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$). The same letters within each column are not significantly different at the $p < 0.05$ level.

3.6. Leaf Nutrients

Table 5 shows the leaf macro and micronutrient contents of tea plants at the end of the experiment. Increasing the rates of CFPH had raised the leaf N content. Plants that received CFPH ($\geq 2 \text{ g L}^{-1}$) and FPH showed a significantly higher N content than the control and other CFPH treatments. Compared with the control treatment, the leaf N concentration was 18.2 and 15.6% higher in the CFPH 3 g L⁻¹ and FPH treatments. Moreover, the leaf P

content showed a similar result, such as N, where significantly higher values were observed in the treatments of CFPH ($\geq 2 \text{ g L}^{-1}$) and FPH, highlighting the biostimulant action of animal-origin PH. The leaf P concentration was 19.5 and 16.3% higher in the CFPH 2 g L^{-1} and FPH treatments, respectively than the control. Significantly higher Mn and Cu contents were observed in FPH and higher concentrations of CFPH applied treatments. Chicken feather protein hydrolysate applied at the rate of 3 g L^{-1} increased Mn by 26%, while the CFPH 4 g L^{-1} dose increased the Cu content by 21% compared to the control treatment. Other macro and micronutrients were not significantly affected by the PH application.

Table 5. Effects of animal origin PH on leaf nutrient content.

	N (g kg^{-1})	P (g kg^{-1})	Mn (mg kg^{-1})	Cu (mg kg^{-1})
Control	38.4 ± 0.4^b	2.20 ± 0.07^b	49.7 ± 0.6^d	6.23 ± 0.36^c
CFPH 0.5 g L^{-1}	37.7 ± 1.8^b	2.15 ± 0.03^b	54.0 ± 1.4^{cd}	6.31 ± 0.51^{bc}
CFPH 1 g L^{-1}	38.9 ± 1.2^b	2.18 ± 0.07^b	59.6 ± 1.2^{bc}	7.38 ± 0.54^{abc}
CFPH 2 g L^{-1}	43.7 ± 1.9^a	2.63 ± 0.19^a	64.3 ± 3.3^{ab}	7.95 ± 0.53^a
CFPH 3 g L^{-1}	45.4 ± 0.7^a	2.53 ± 0.12^a	67.2 ± 1.3^a	7.56 ± 0.16^{ab}
CFPH 4 g L^{-1}	44.0 ± 1.1^a	2.63 ± 0.04^a	66.8 ± 2.7^a	8.36 ± 0.17^a
FPH 10 mL L^{-1}	44.4 ± 0.7^a	2.56 ± 0.03^a	65.5 ± 1.1^a	7.85 ± 0.55^a

The same letters within each column are not significantly different at the $p < 0.05$ level.

4. Discussion

4.1. Production and Characterization of CFPH

Protein hydrolysates manufactured from by-products or waste materials generated from agriculture industries have been widely used as plant biostimulants. The chemical properties of PH are primarily determined by the used production process and protein source [11]. The adopted production process is a crucial factor which determines the amino acid composition and yield of CFPH. The production of CFPH involves physical, chemical, and biological treatments. In chemical treatments, feathers are hydrolyzed by acid or alkali, in which the keratin structure is altered by rupturing the disulphide and peptide bonds [36]. A recent past combination of physical, chemical, and biological methods was adopted to enhance the feather degradation rate and subsequently higher protein recovery [37]. However, detrimental effects of some animal-origin PH on the plants were reported due to the higher concentration of free amino acids [28]. In this experiment, alkaline hydrolysis was used to produce CFPH since the process is straightforward with many benefits, unlike acid hydrolysis, which requires special equipment to provide the higher temperature and high pressure [38]. The total amino acid content and amino acid composition were more similar to the results obtained by Genç and Atici [32]. The total amino acid obtained in this process was 16.65%, which is higher than the microwave alkali treatment (6.94%) by Lee et al. [33] and the hydrothermal treatment (13.6%) by Nurdawati et al. [4]. In this study, the produced CFPH contains a higher amount of glutamic acid and proline. Glutamic acid plays a vital role in seed germination, root architecture, pollen germination, and pollen tube growth, while under stress conditions, it takes part in wound response, pathogen resistance, and adaptation to abiotic stress [38]. Proline protects plants from different abiotic stresses and accelerates the recovery of stressed plants [39]. Furthermore, short-chain peptides and certain amino acids, such as phenylalanine, have been reported to increase the production of endogenous auxin by functioning as signaling molecules, resulting in favorable effects on roots and improved vegetative growth [20,40]. The presence of small quantities of cysteine (0.79%) and threonine (2.42%) in CFPH may have resulted due to the aggressive reaction of alkali that ended up in the loss of these amino acids [41]. The peptides and amino acids present in the PH might be directly occupied by plants through the roots and leaf, reaching the target tissues and potentially acting as plant biostimulants [11].

4.2. Shoot and Root Growth

Animal-origin PH have been widely used in sustainable agriculture for many decades due to their potentiality to enhance plant growth and minimize the impact of environmental stresses. The research explored on CFPH as a potential biostimulant on crop growth has been limited compared to the other animal-derived PH. To the best of our knowledge, the influence of CFPH on the growth of young tea plants has not been previously reported.

In most of the plant experiments, the assessment of plant height, leaf number, and fresh and dry weights can be used as crucial growth indicators reflecting the growth and development of plants. In this experiment, all of the measured parameters related to shoot growth in tea plants were significantly affected by CFPH. Our results are in agreement with the findings of Joardar and Rahman [42], where the treated poultry feather waste enhanced the plant height, leaf number, and plant fresh weight of *Ipomoea aquatica*. However, contradictory results were reported in mung bean and patchouli plants in response to liquid feather protein hydrolysates, where the plant height and leaf number showed no significant differences with the untreated control, but the plant dry weight was significantly improved [43]. The effect of FPH on the shoot growth of tea was less pronounced than CFPH since the recommended dosage may not be optimum for the growth of nursery tea.

In this study, CFPH significantly modified the root system by increasing the root length, surface area, and root biomass compared to the untreated control. In another study, the root application of chicken feather hydrolysate produced by feather degrading bacterium significantly increased the root length and root fresh and dry weights in chickpea [31]. The possible mechanism involved in the induced root development could be related to the auxin-like activity of chicken feather hydrolysates [12]. Cristiano et al. [44] reported that root drenching of animal-derived PH positively influenced the root growth of snapdragon plants compared to the foliar application. The application of PH for a short period increased the root dry weight of maize plants compared to the untreated plants [22]. This increase in root dry weight may lead to a higher success rate in field planting, which subsequently resulted in better yields [21,45].

The usage of animal origin PH at higher concentrations may cause plant growth depression in terms of shoot and root growth due to phytotoxicity. Interestingly, the present study revealed that the growth performance of tea plants, which received the highest CFPH dosage (4 g L^{-1}) was comparably better than the untreated plants. These results clearly indicate the positive response of tea plants to higher concentrations, as well as the repeated applications of animal-derived PH, which were reported to be toxic to other fruit crops [14,27]. Genç and Atici [32] observed that a low concentration of CFPH (0.75 g L^{-1}) significantly enhanced the root length, shoot length, and dry weight of wheat seedlings. Gousterova et al. [46] found that the usage of CFPH at low concentrations exerted a positive effect on seed germination and the growth of ryegrass. Consistent with our findings, previous studies have demonstrated that the beneficial effects of PH on plants could be seen at low concentrations [20,47].

4.3. Chlorophyll Content

In addition to changes in shoot and root growth parameters, the CFPH evaluated in this experiment enhanced the chlorophyll content (*a*, *b*, and total). Similar results have been reported by Genç and Atici [32] in wheat seedlings in response to the CFPH application, where the 0.75 g L^{-1} dosage was recorded as the highest value. Several authors have found that the animal-derived PH enhanced the chlorophyll content in beans, corn, soybeans, and tomatoes [14,48,49]. The mechanisms behind the enhancement of chlorophyll content by PH are largely unknown [49]. However, the produced CFPH in this study (Table 1) has a higher amount of glutamate (13.36%), which is needed for the formation of precursor 5-aminolevulinate, which is involved in the biosynthesis of chlorophyll [50].

4.4. Leaf Gas Exchange

It is well-known that the PH improve the photosynthetic rate and ultimately lead to the higher yield with better quality [11,51]. Enhancement of physiological parameters, such as the photosynthetic and transpiration rates in tea plants, agrees with the findings by Xu and Mou [52] for lettuce and Cristiano et al. [44] for potted snapdragon, assuring the higher carbon assimilation. In another study, chicken feather hydrolysate increased the photosynthetic rate in bananas [53]. A high photosynthetic rate of shoots resulted in the higher root activity by supplying an adequate amount of photosynthates to the roots [54]. However, the positive response of CFPH on stomatal conductance and intercellular CO₂ concentration was not observed in this experiment.

4.5. Leaf Nutrients

Our results suggested that tea plants treated with CFPH and FPH were capable of maintaining a better nutritional status, revealed as higher N, P, Mn, and Cu concentrations in the leaf. No statistical differences were found on the leaf nutrient contents of N, P, Mn, and Cu among the three dosages (2, 3, and 4 g L⁻¹) of CFPH. The plants treated with CFPH 4 g L⁻¹ showed higher nutrient contents in leaves despite the reduction in growth parameters. In agreement with our findings, the soil application of animal-derived protein hydrolysates significantly enhanced the leaf N content by 17% in potted snapdragon [44]. In a most recent study, root drenching of animal-origin protein hydrolysates improved the leaf N and P contents of Petunia plant by 38 and 47%, respectively [55]. The root length and surface area are the key criteria determining the uptake of nutrients and water [56]. In this research, the application of CFPH and FPH positively influenced the root growth of tea, which could facilitate the increased uptake and accumulation of nutrients in plant tissues.

5. Conclusions

This study was conducted for the first time to elucidate the positive effects of CFPH on the growth promotion of tea nursery plants. In this experiment, six doses of CFPH (0, 0.5, 1, 2, 3, and 4 g L⁻¹) were applied to assess the growth of tea nursery plants. The results clearly showed that the CFPH applied at 2 g L⁻¹ significantly enhanced all of the shoot growth and root growth parameters. The chlorophyll content of plants treated with CFPH 1 g L⁻¹ showed significantly higher values, whereas the photosynthetic rate was enhanced by the CFPH 3 g L⁻¹ treatment. Moreover, the highest dosage of CFPH (4 g L⁻¹) showed a positive response on plants' growth and nutrient content compared to the untreated plants. Both of the animal derived PH applications increased the concentrations of N, P, Mn, and Cu in the leaves. The FPH positively enhanced the growth of nursery plants, but the exerted positive effect was less pronounced than CFPH. Based on the findings, soil drenching of CFPH at the rate of 2 g L⁻¹ could be used as the optimum dose for the successful raising of tea nursery plants. Therefore, the conversion of chicken feathers into CFPH not only improves the growth performance of nursery tea plants, but also eliminates a serious environmental pollution.

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