

Effect of Fish Protein Hydrolysate Concentrations on the Growth and Quality of Hydroponic Lettuce (*Lactuca sativa* L.)

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Abstract

The study investigated the effects of fish protein hydrolysate (FPH), derived from fish processing by-products, on the growth, yield, and quality of lettuce (*Lactuca sativa* L.) grown in a static hydroponic system. Four nutrient treatments were evaluated: a control using a commercial inorganic Snap solution (T1) and three FPH-based solutions at concentrations of 300ppm (T2), 600ppm (T3), and 900ppm (T4). Results showed that the 600ppm FPH treatment (T3) significantly enhanced plant growth and physiological performance compared with the control. Lettuce under T3 achieved the greatest root length (21.4cm), canopy diameter (27.6cm), SPAD value (38.5), and fresh yield (438.2 g plant⁻¹), representing a 22-30% increase over T1. Quality attributes also improved under moderate FPH application. Vitamin C content increased to 10.5 mg 100 g⁻¹ in T3 compared with 7.5 mg 100 g⁻¹ in the control, while nitrate concentration decreased markedly from 398.5 to 242.1 mg 100 g⁻¹. Soluble solids content rose from 3.7 to 4.2°Brix, indicating improved sweetness and flavor. No *Escherichia coli* or *Salmonella* spp. contamination was detected in any treatment, confirming product safety. Overall, the application of 600ppm FPH provided an optimal balance between yield, nutritional quality, and microbial safety. These findings demonstrate that fish protein hydrolysate offers a cost-effective and sustainable nutrient alternative for hydroponic lettuce production, promoting circular resource utilization and environmentally friendly agriculture.

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Keywords

Fish protein hydrolysate, nutrient concentration, hydroponic lettuce, growth, quality

Introduction

The increasing demand for safe food products free from chemical residues has accelerated the development of modern vegetable production systems. Among these, hydroponic cultivation has gained widespread adoption owing to its remarkable advantages. In

hydroponics, plants are grown without soil and are instead supplied with a nutrient-enriched water solution containing the essential elements required for optimal growth. This cultivation approach offers several benefits, including precise nutrient management, efficient water utilization, reduced pest incidence, and the elimination of soil-borne chemical residues (Resh, 2022; Rajendran *et al.*, 2024). Consequently, hydroponics is regarded as one of the key technologies driving the advancement of smart and urban agriculture.

Despite these advantages, a major limitation of hydroponic systems lies in their heavy reliance on synthetic inorganic nutrient solutions, which substantially contribute to production costs. These solutions are typically formulated from commercial chemical fertilizers whose raw materials are expensive and, in many cases, must be imported to ensure consistent crop performance (Heintze *et al.*, 2024; Park & Williams, 2024). Such dependency imposes economic constraints, particularly on small-scale growers and regions with limited access to affordable and locally produced nutrient inputs (Heintze *et al.*, 2024). Moreover, the continuous use of chemical compounds in vegetable cultivation raises concerns about food safety and long-term environmental sustainability if they are not properly managed (Goddek *et al.*, 2015). Therefore, the identification and utilization of eco-friendly, cost-effective, and locally available organic nutrient sources for hydroponic production have received increasing attention from researchers and agribusinesses.

Vietnam's seafood processing industry, a vital economic sector, generates a considerable number of fish by-products, including heads, bones, skin, and viscera, which account for approximately 40-60% of the total raw material mass. Without appropriate management, these by-products can lead to serious environmental pollution and resource wastage (Ghaly *et al.*, 2013). Recent studies have demonstrated that enzymatic or mild acid hydrolysis of fish by-products can produce fish protein hydrolysate (FPH), a nutrient-rich product containing amino acids, short-chain peptides, and essential minerals beneficial to plant growth (Madende & Hayes,

2020; Igor *et al.*, 2024). FPH has been recognized as a natural biostimulant with the potential to partially replace or supplement chemical nutrient solutions in hydroponic systems. Several studies have reported that FPH application can enhance plant growth, yield, and quality, while improving the antioxidant content and reducing environmental impacts (Ahmed *et al.*, 2021; Hassan *et al.*, 2024; Nuzhyna *et al.*, 2024).

However, most studies on protein hydrolysates as plant biostimulants have been conducted under soil-based or substrate cultivation conditions, while their application in hydroponic systems, particularly under recirculating nutrient regimes, remains less explored (Colla *et al.*, 2017). In hydroponic production, nutrient dynamics and root–nutrient interactions differ fundamentally from those in soil or inert substrates, which may lead to distinct plant responses to organic-derived inputs such as FPH (Savvas & Gruda, 2018). Moreover, plant responses to protein hydrolysates are strongly influenced by the application rate, crop species, and cultivation system, underscoring the need for system-specific and dose-dependent evaluations (Colla *et al.*, 2015).

Therefore, this study aimed to evaluate the effects of different concentrations of FPH on the growth and quality of lettuce (*Lactuca sativa* L.) cultivated in a dynamic hydroponic system. Lettuce is widely used in studies on nutrient management and biostimulant application (Gómez & Gómez, 2022; Choi *et al.*, 2024; Monterisi *et al.*, 2024), making it a suitable model crop for assessing the effectiveness of FPH in hydroponic cultivation. The results are expected to provide a scientific basis for optimizing FPH application rates in hydroponic systems and to support the valorization of fishery by-products within circular and environmentally sustainable agricultural production.

Materials and Methods

Materials

The curly lettuce (*Lactuca sativa* L. var. *crispa*) variety used in this study was Lion VA.099, supplied by the Viet A Seed Company (Vietnam). This variety has a growth duration of approximately 35-40 days.

The organic nutrient solution was prepared from fish processing by-products, including heads, bones, and viscera, through a controlled hydrolysis process. Briefly, 1L of molasses was dissolved in 1L of clean water, and 1L of the resulting solution was subsequently mixed with 0.9L of the microbial preparation TVHBIO. After 4h of incubation, the mixture was added to containers containing the fish by-products. The contents were stirred once daily to ensure uniform fermentation. After 20 days of hydrolysis, the liquid fraction was filtered to obtain the nutrient solution, which was subjected to chemical analysis for total N, total P₂O₅, total K₂O, and total CaO following TCVN 8518:2018, TCVN 5815:2018, TCVN 8562:2010, and TCVN 9284:2018, respectively. The primary nutrient composition of the resulting FPH solution was: N = 0.87%, P₂O₅ = 2.34%, K₂O = 1.44%, and Ca = 5.58%.

SNAP, a commercially available inorganic hydroponic nutrient solution widely used in vegetable production, was used as the control treatment and supplied by the Gia Vien Hydroponics One Member Limited Liability Company (Vietnam). The solution was prepared according to the manufacturer's instructions at a dilution ratio of 1:200 by mixing 50mL of nutrient solution A and 50mL of nutrient solution B with 20L of clean water. Nutrient solution A contained 37.51 g L⁻¹ nitrate nitrogen (NO₃⁻-N), 43.54 g L⁻¹ calcium (Ca), 39.77 g L⁻¹ potassium oxide (K₂O), and 0.98 g L⁻¹ iron (Fe-EDTA). Nutrient solution B contained 10.50 g L⁻¹ phosphorus pentoxide (P₂O₅), 2.00 g L⁻¹ ammonium nitrogen (NH₄⁺-N), 6.68 g L⁻¹ sulphur (S), 5.00 g L⁻¹ magnesium (Mg), and micronutrients including manganese (Mn, 394ppm), boron (B, 140ppm), zinc (Zn, 100ppm), copper (Cu, 24ppm), and molybdenum (Mo, 20ppm).

Experimental design

The experiment was conducted in a completely randomized design (CRD) with four treatments and three replications: T1 (Control): SNAP nutrient solution (applied according to the manufacturer's instructions), T2: FPH solution at 300ppm, T3: FPH solution at 600ppm, and T4: FPH solution at 900ppm.

Lettuce was cultivated in a recirculating (dynamic) hydroponic system. Plants were grown on cultivation channels measuring 3.6m in length and 0.10m in width, with a spacing of 0.05m between adjacent channels. Each treatment consisted of four channels per replication. Lettuce seedlings were transplanted into foam plugs at a spacing of 15cm, with one plant per plug, corresponding to 98 plants per experimental unit.

The pH of the nutrient solutions was regularly monitored and maintained at stable levels throughout the experiment. Upon nutrient solution supplementation, the pH ranged from 7.5 to 8.0 and was subsequently adjusted using citric acid. The pH was measured weekly to ensure stability of the nutrient solution.

An automatic irrigation system was programmed as follows: from 06:00 to 18:00, the pump operated every 60 minutes for 5 minutes per cycle; from 18:00 to 06:00, the pump operated every 120 minutes for 5 minutes per cycle. The nutrient solution was stored in an 80-L reservoir, with a flow rate of 1 L min⁻¹ supplied to the plants.

Sampling parameters

Root length (cm) and SPAD values were recorded weekly from three randomly selected plants per plot. Root length was measured from the bottom of the foam plug to the tip of the longest root. SPAD readings were obtained using a SPAD-502 chlorophyll meter (Konica Minolta Sensing Inc., Osaka, Japan) on three fully expanded leaves per plant.

At harvest (28 days after transplanting), five representative plants from each plot were sampled to determine the plant height (cm), number of leaves per plant, canopy diameter (cm), leaf length (cm), leaf width (cm), and individual yield (g plant⁻¹). Plant height was measured from the base of the plant to the tip of the tallest leaf. Canopy diameter was measured at the widest part of the canopy. Leaf length was measured from the petiole base to the leaf tip, and leaf width was measured at the widest point perpendicular to the midrib.

For quality analysis, composite samples were prepared by homogenizing plant materials

from each replication. The soluble solids content ($^{\circ}$ Brix) was measured using a digital refractometer (Milwaukee MA885, Milwaukee Instruments, USA). The fiber content (%), vitamin C ($\text{mg } 100 \text{ g}^{-1}$), nitrate content (NO_3^- , $\text{mg } 100 \text{ g}^{-1}$), and microbial safety (presence of *Escherichia coli* and *Salmonella* spp.) were determined according to the following Vietnamese standards: TCVN 4329:2007, TCVN 6427-2:1998, TCVN 8160-1:2016, TCVN 7924-3:2017, and TCVN 10780-1:2017, respectively.

Statistical analysis

Data were analyzed using one-way analysis of variance (ANOVA) at a significance level of $P < 0.05$. When significant differences were detected, treatment means were compared using Tukey's honest significant difference (HSD) test. Statistical analyses were performed using Microsoft Excel 2019 (Microsoft Corporation, USA) and Minitab statistical software (version 20; Minitab LLC, State College, PA, USA).

Results and Discussion

pH value of the nutrient solutions

The pH of the nutrient solution is a critical parameter that directly affects the solubility and uptake of mineral nutrients by plants. According to Resh (2022), the optimal pH range for hydroponically grown lettuce lies between 5.5 and 6.5, within which micronutrients such as Fe, Mn, and Zn remain in their most plant-available forms. When the pH exceeds 7.0, the solubility of these elements declines markedly, potentially leading to nutrient deficiencies and impaired plant growth.

As shown in **Table 1**, at 7 days after transplanting (DAT), all the treatments exhibited an initial pH of 6.0, corresponding to the ideal starting condition for hydroponic systems. Between 14 and 21 DAT, however, pH levels in the treatments supplemented with FPH (T2, T3, and T4) increased more rapidly than in the control (T1). Specifically, at 21 DAT, the pH values in T2, T3, and T4 reached 7.0, 7.1, and 7.3, respectively, while the control remained at 6.6. This increase could be attributed to the

decomposition of organic compounds in the FPH, which may have reduced H^+ ion concentrations and consequently elevated the pH (Olle *et al.*, 2021). Additionally, pH shifts in hydroponic systems are influenced by root ion exchange, where NH_4^+ uptake lowers pH and NO_3^- uptake increases it (Bugbee, 2004).

By 28 DAT, the pH values in all the treatments showed a decreasing trend, ranging from 6.0 to 6.3. This suggests that the system's biological buffering capacity gradually restored the pH to the optimal range after the active decomposition phase. These results are consistent with Savvas & Gruda (2018), who noted that although pH fluctuations commonly occur during hydroponic cultivation, the system tends to stabilize when organic nutrients are properly managed.

Despite these fluctuations, the pH values across the treatments remained within the acceptable range for lettuce growth, particularly during the vegetative and biomass accumulation stages (21-28 DAT). This indicates that the nutrient solutions derived from fish by-products can serve as a viable organic alternative in hydroponic systems, provided that the pH is periodically monitored and adjusted as needed.

Effects of nutrient concentrations on root length of lettuce

Root length is a vital indicator of root system development, reflecting the plant's capacity for water and nutrient absorption. Vigorous root growth forms the foundation for shoot development and overall productivity. As presented in **Table 2**, root length increased progressively with plant age across all the treatments; however, the extent of increase varied significantly.

At 7 DAT, T3 exhibited the greatest root length (3.7cm), approximately 54% higher than the control, and the difference was statistically significant ($P < 0.05$). Treatments T2 and T4 also showed higher root lengths than the control. A similar trend was observed at 14 DAT, where T3 maintained the highest value (8.4cm), markedly exceeding the control (5.8cm). This demonstrates a pronounced root-promoting effect during the seedling stage, a critical phase

Table 1. Changes in pH of nutrient solutions

Treatment	7 DAT	14 DAT	21 DAT	28 DAT
T1	6.0	6.2	6.6	6.3
T2	6.0	6.5	7.0	6.0
T3	6.0	6.6	7.1	6.1
T4	6.0	6.7	7.3	6.1

Table 2. Effects of nutrient concentrations on root length of lettuce (Unit: cm)

Treatment	7 DAT	14 DAT	21 DAT	28 DAT
T1	2.4 ^c	5.8 ^c	8.0 ^b	11.1 ^{bc}
T2	3.0 ^b	6.9 ^b	9.3 ^{ab}	12.0 ^b
T3	3.7 ^a	8.4 ^a	10.7 ^a	13.9 ^a
T4	3.1 ^b	7.4 ^b	8.0 ^b	10.8 ^c

Note: Values within a column followed by the different letter indicate significant differences at 0.05 level by Tukey's HSD test. DAT, days after transplanting.

in hydroponic systems where roots act as the primary interface with the nutrient solution (Xu & Mou, 2017).

At 21 DAT, T3 continued to produce the longest roots (10.7cm), significantly greater than those of the control and T4 (8.0cm). Despite its higher nutrient concentration, T4 resulted in shorter roots, suggesting that excessive nutrient levels may inhibit root elongation due to osmotic stress or ionic imbalance, as also reported by Bugbee (2004).

This pattern persisted until the end of the experiment (28 DAT): T3 achieved the greatest root length (13.9cm), followed by T2 (12.0cm), T1 (11.1cm), and T4 (10.8cm). These results indicate that an appropriate concentration of FPH stimulates both early root initiation and sustained root development, while conventional inorganic solutions promote only moderate growth. The findings align with Khan *et al.* (2019), who reported that amino acids in fish extracts can activate root meristem enzymes and stimulate the synthesis of auxins and cytokinins—key hormones for root cell division and elongation. The superior performance of T3 may therefore be attributed to its balanced composition of organic nitrogen, micronutrients, and bioactive compounds, which collectively enhanced root growth.

Effects of nutrient concentrations on SPAD value of lettuce

The SPAD index reflects the leaf chlorophyll content and serves as an important indicator of photosynthetic capacity, biomass accumulation, and nitrogen use efficiency (Xiong *et al.*, 2015; Mendoza-Tafolla *et al.*, 2019). At 7 DAT, although the SPAD values varied only slightly among the treatments (35.07-35.53), significant differences were still observed, with T3 recording the highest value, which was significantly higher than those of T4 and T1 (**Table 3**). By 14 DAT, T3 maintained the highest SPAD value (39.88), significantly exceeding all the other treatments ($P < 0.05$). This suggests enhanced chlorophyll synthesis resulting from the uptake of amino acids and readily available nutrients in the FPH, consistent with Ahmed *et al.* (2021).

At 21 DAT, corresponding to the phase of active photosynthesis and rapid biomass accumulation, T3 maintained the highest SPAD value (45.54), significantly higher than the control (44.04) and T4 (44.21). Although T4 exhibited a slight increase, its value was not significantly different from the control, implying that an excessive nutrient concentration does not further enhance photosynthetic efficiency.

At 28 DAT, T3 still exhibited the highest SPAD value (48.63), approximately 6.2% higher

Table 3. Effects of nutrient concentrations on the SPAD of lettuce

Treatment	7 DAT	14 DAT	21 DAT	28 DAT
T1	35.07 ^c	39.19 ^b	44.04 ^b	45.8 ^c
T2	35.39 ^{ab}	39.31 ^b	44.96 ^{ab}	47.16 ^b
T3	35.53 ^a	39.88 ^a	45.54 ^a	48.63 ^a
T4	35.23 ^{bc}	38.97 ^b	44.21 ^b	46.22 ^{bc}

Note: Values within a column followed by a different letter indicate significant differences at the 0.05 level by Tukey's HSD test. DAT, days after transplanting.

than the control, followed by T2 (47.16). These results demonstrate that FPH effectively supplies organic nitrogen and trace elements that promote chlorophyll biosynthesis. Xu & Mou (2017) reported that short peptides and free amino acids in fish extracts are rapidly assimilated into chlorophyll and enzyme synthesis pathways. The relatively lower SPAD in T4 may have been associated with nutrient stress or ionic accumulation, which can disrupt physiological balance (Bugbee, 2004).

Effects of nutrient concentrations on the growth parameters of lettuce at harvest

The nutrient concentration significantly affected lettuce vegetative growth at harvest (Table 4). Among the treatments, T3 consistently produced the best growth performance. The tallest plants were recorded in T3 (23.2cm), significantly higher than T1 and T2 (22.3 and 22.4cm, respectively), and markedly greater than T4 (20.6cm). This supports the conclusions of Bugbee (2004) that balanced nutrient composition enhances cell division and elongation in hydroponic crops.

T3 also produced the highest number of leaves (14.8 leaves plant⁻¹), while the other treatments ranged from 12.8 to 13.2 leaves. Similar results were reported by Alkaabi *et al.* (2025), who found that organic nutrient supplementation improved vegetative growth compared with inorganic fertilizers. Canopy diameter followed the same trend, with T3 exhibiting the widest canopy (19.0cm), significantly greater than those of T1 (17.2cm), T2 (17.4cm), and T4 (15.9cm).

Regarding leaf morphology, plants in T3 achieved the greatest leaf length (18.3cm) and

width (13.0cm), while T4 showed the smallest values (16.7cm and 11.4cm, respectively). Xu & Mou (2017) similarly noted that amino acid-rich organic nutrient solutions promote balanced vertical and horizontal growth, particularly during vigorous development (15-25 DAT).

These results indicate that the nutrient concentration applied in T3 provided optimal conditions for lettuce growth, resulting in superior plant height, canopy expansion, and leaf development. The reduced growth in T4 likely reflected osmotic stress or ionic imbalance caused by excessive nutrient concentration. The high organic matter content in the concentrated FPH may have been insufficiently mineralized, leading to localized hypoxia or increased osmotic pressure in the root zone (Savvas & Gruda, 2018).

Effects of nutrient concentrations on the individual yield of lettuce

The application of FPH significantly affected individual lettuce yield compared with the control (Figure 1). The highest yield was obtained in the 600ppm FPH treatment (T3), reaching 223.1 g plant⁻¹ and significantly exceeding all the other treatments ($P < 0.05$). The 300-ppm treatment (T2) resulted in a moderate yield increase (199.9 g plant⁻¹); whereas the 900ppm treatment (T4) produced a lower yield (180.2 g plant⁻¹) that was not significantly different from the control (170.0 g plant⁻¹). Overall, lettuce yield exhibited a clear dose-dependent response to FPH application, with moderate concentrations enhancing biomass accumulation, while higher concentrations provided no additional benefit or constrained yield.

The superior yield performance observed at the optimal FPH concentration (T3) was consistent with improved root development,

Table 4. Effects of nutrient concentrations on the growth parameters of lettuce at harvest

Treatment	Plant height (cm)	Number of leaves	Canopy diameter (cm)	Leaf length (cm)	Leaf width (cm)
T1	22.3 ^b	12.8 ^b	17.2 ^b	17.3 ^b	11.4 ^c
T2	22.4 ^b	12.9 ^b	17.4 ^b	17.3 ^b	12.0 ^b
T3	23.2 ^a	14.8 ^a	19.0 ^a	18.3 ^a	13.0 ^a
T4	20.6 ^c	13.2 ^b	15.9 ^c	16.7 ^c	11.4 ^c

Note: Values within a column followed by a different letter indicate significant differences at the 0.05 level by Tukey's HSD test.

higher SPAD values, and enhanced vegetative growth throughout the cultivation period, which together contributed to greater biomass accumulation at harvest. In contrast, the reduced yield under excessive FPH application (T4) coincided with limited root elongation, a lower chlorophyll status, and suppressed vegetative growth, ultimately constraining yield formation. Similar dose-dependent yield responses have been reported in hydroponic lettuce, where optimal application rates of protein hydrolysate-based biostimulants promoted yield, whereas excessive inputs resulted in diminished performance (Gómez & Gómez, 2022; Choi *et al.*, 2024; Monterisi *et al.*, 2024).

Compared with the inorganic SNAP nutrient solution, which supplies nutrients predominantly in readily available mineral forms, FPH provides nutrients mainly in organic forms, including amino acids and peptides. The observed differences in yield responses suggest that FPH may influence plant performance not only through nutrient supply but also through biostimulant-related effects, whereas SNAP primarily functions as a conventional mineral nutrient source.

Effects of nutrient concentrations on lettuce quality

Dietary fiber is a key determinant of lettuce texture, nutritional value, and consumer preference. In this study, the fiber content varied considerably among the treatments, ranging from 1.53% to 3.14% (Table 5). The control treatment (T1) exhibited the highest fiber concentration (3.14%), while all the FPH-supplemented treatments showed significantly lower values (1.53-1.80%). The reduction in fiber content under the FPH treatments suggests the formation

of softer and more tender leaf tissues, which may enhance sensory quality and palatability. However, an excessive decrease in fiber could potentially reduce leaf firmness and shorten postharvest shelf life. Similar reductions in fiber content in organically fertilized lettuce were reported by Xu & Mou (2017), who attributed this effect to improved water retention and increased metabolic activity within leaf mesophyll tissues.

Vitamin C is an important antioxidant compound contributing to both the nutritional quality and oxidative stress tolerance of leafy vegetables. Application of FPH significantly enhanced vitamin C accumulation compared with the control. The highest vitamin C concentration (10.5 mg 100 g⁻¹) was observed in T3, followed by T2 and T4 (both 8.6 mg 100 g⁻¹), while the control exhibited the lowest value (7.5 mg 100 g⁻¹). The elevated vitamin C content at the moderate FPH concentration could be attributed to enhanced nutrient bioavailability and activation of enzymatic systems associated with antioxidant biosynthesis, particularly those linked to nitrogen and micronutrient metabolism. This observation is consistent with previous studies that have indicated that biostimulants such as protein hydrolysates and seaweed extracts can increase the synthesis of antioxidants, including ascorbic acid, in leafy vegetables (Xu & Mou, 2017; Madende & Hayes, 2020; Choi *et al.*, 2022).

Nitrate accumulation in lettuce is a major concern due to its implications for food safety and human health. In the present experiment, the nitrate content decreased with increasing FPH concentrations. The highest nitrate accumulation was recorded in T2 (644.2 mg 100 g⁻¹), while the lowest value was observed in T4 (165.2 mg 100

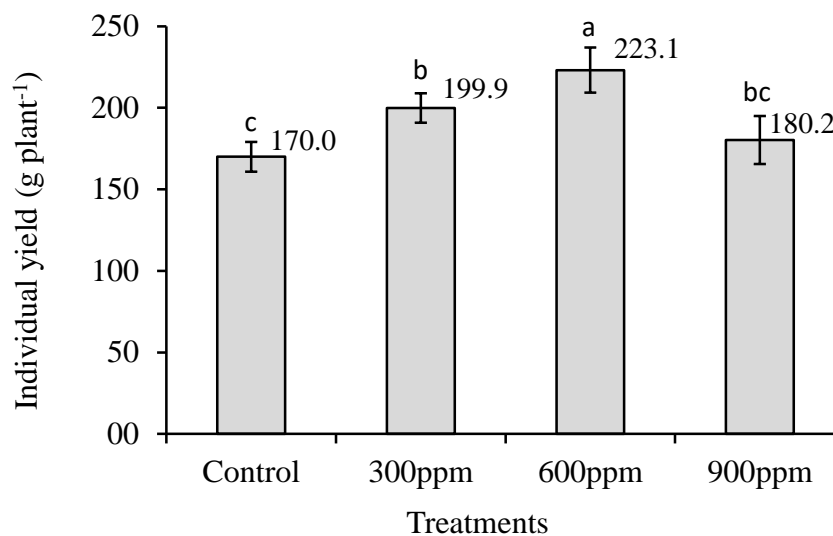


Figure 1. Effect of nutrient concentrations on the individual yield of lettuce

Table 5. Effect of nutrient concentrations on lettuce quality

Treatment	Fiber content (%)	Vitamin C content (mg 100g ⁻¹)	NO ₃ ⁻ content (mg 100g ⁻¹)	Brix	<i>E.coli</i>	<i>Salmonella</i>
T1	3.14	7.5	398.5	3.7	nd	nd
T2	1.53	8.6	644.2	3.9	nd	nd
T3	1.57	10.5	242.1	4.2	nd	nd
T4	1.80	8.6	165.2	4.1	nd	nd

Note: nd: not detected

g⁻¹), with the control showing an intermediate level (398.5 mg 100 g⁻¹). These results indicate that higher FPH concentrations may promote more efficient nitrogen assimilation by enhancing the activity of nitrate reductase enzymes and improving the conversion of nitrate to organic nitrogen compounds. Matallana *et al.* (2010) similarly reported that the use of microbially active organic fertilizers improves nitrate metabolism, thereby reducing residual nitrate in edible tissues.

Soluble solids content (°Brix), an indicator of sugar accumulation and overall flavor quality, also increased with FPH application, ranging from 3.7 in the control to 4.2 in T3. The highest °Brix, observed in the 600ppm FPH treatment, suggests improved carbohydrate synthesis and translocation, likely due to enhanced photosynthetic capacity and nutrient uptake efficiency. Lettuce grown under moderate to high FPH concentrations (T3 and T4) exhibited a

sweeter taste and higher flavor intensity, consistent with previous reports showing that organic nutrient sources can enhance sugar metabolism and sensory attributes (Choi *et al.*, 2022).

Microbial safety is a critical aspect of hydroponic vegetable production, particularly when organic inputs are used. In the current study, *E. coli* and *Salmonella* spp. were not detected in any treatment, indicating that the use of FPH as an organic nutrient source did not compromise product hygiene. These results confirm that FPH-based nutrient solutions, when managed under appropriate cultivation and sanitation conditions, are microbiologically safe for hydroponic lettuce production.

Relationships among growth, physiology, and yield

To further interpret the observed growth and physiological responses, correlation and multivariate analyses were conducted to identify

the interrelationships among the key traits. The Pearson correlation matrix (**Table 6**) revealed significant positive relationships among all the growth, physiological, and yield parameters of the hydroponically grown lettuce. Root length exhibited strong correlations with the SPAD value ($r = 0.80^{**}$), canopy diameter ($r = 0.82^{**}$), plant height ($r = 0.72^{**}$), number of leaves ($r = 0.67^{**}$), and individual yield ($r = 0.83^{**}$), underscoring its fundamental role in nutrient uptake and subsequent shoot growth and productivity. Similar interrelationships among the root traits, chlorophyll status, and biomass-related parameters have been reported in hydroponically grown lettuce, highlighting the central contribution of root development to yield performance (Rosli *et al.*, 2023).

Canopy diameter also showed a high correlation with plant height ($r = 0.87^{**}$) and moderate associations with the SPAD value ($r = 0.69^{**}$), number of leaves ($r = 0.59^{**}$), and individual yield ($r = 0.68^{**}$), indicating that canopy expansion is a key determinant of light interception and photosynthetic capacity. Consistent relationships between canopy-related traits and biomass accumulation have been observed under controlled hydroponic lettuce production (Dai *et al.*, 2024).

The SPAD values were positively correlated with plant height ($r = 0.59^{**}$), number of leaves ($r = 0.62^{**}$), and individual yield ($r = 0.69^{**}$), suggesting that a higher chlorophyll status is associated with enhanced biomass accumulation. The number of leaves also exhibited a moderate positive correlation with individual yield ($r = 0.58^{**}$), reflecting the contribution of photosynthetically active leaves to dry matter production. Although plant height showed the weakest correlation with individual yield ($r = 0.55^{**}$), it remained an important indicator of overall growth vigor.

The hierarchical cluster dendrogram (**Figure 2**) illustrates the relationships among the morphological, physiological, and yield traits based on their similarity coefficients. Two major clusters were identified. Root length and individual yield formed the closest cluster, indicating a tight association between root development and productivity, consistent with

the correlation analysis (**Table 6**). The inclusion of SPAD value within this cluster further suggests a close linkage among chlorophyll status, root vigor, and yield formation. The second cluster comprised canopy diameter, plant height, and number of leaves—traits primarily associated with aboveground morphology and light capture—indicating coordinated shoot expansion and photosynthetic efficiency in response to FPH application. Collectively, these clusters delineate two synergistic functional groups: (1) root–physiological traits governing nutrient acquisition and metabolic efficiency; and (2) shoot–morphological traits influencing canopy architecture and photosynthetic capacity.

Principal component analysis (PCA) further clarified the structure of variation among traits. The first two principal components (PC1 and PC2) accounted for 83.9% of the total variance, with PC1 explaining 73.6% (eigenvalue = 4.4176) and PC2 accounting for 10.2% (eigenvalue = 0.6149) (**Table 7**).

The PCA score plot and biplot (**Figure 3**) clarified treatment distribution and trait associations. Lettuce plants treated with 600ppm FPH (T3) were positioned on the positive side of PC1 and closely associated with yield-related traits, whereas the control (T1) and 300ppm treatment (T2) clustered on the negative side, indicating comparatively lower performance. The 900-ppm treatment (T4) was separated mainly along PC2, suggesting distinct physiological responses likely associated with nutrient oversupply or osmotic stress (**Figure 3a**).

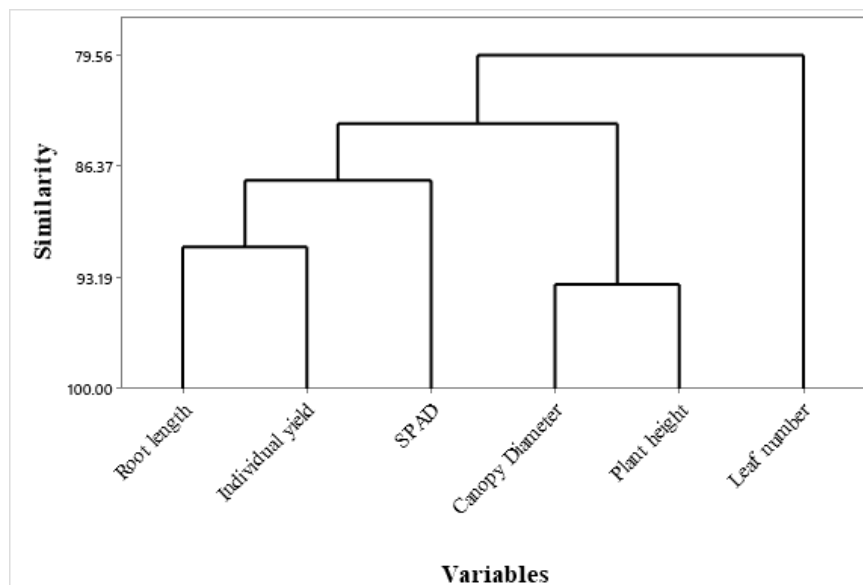
In the biplot (**Figure 3b**), the yield, SPAD value, root length, and canopy diameter vectors were strongly aligned along PC1, confirming their close interdependence and representing the main axis of growth and productivity. Conversely, the number of leaves and plant height contributed less to PC1 but showed moderate associations with the other traits, indicating supportive yet secondary roles in determining overall yield potential.

Taken together, the Pearson correlation, cluster, and PCA analyses demonstrate that strong interactions among root length, SPAD value, canopy diameter, and yield jointly determined productivity in hydroponic lettuce under FPH-based nutrient management.

Table 6. Pearson correlation coefficients among growth, physiology parameters, and yield

	Root length	SPAD	Canopy diameter	Plant height	Number of leaves	Yield
Root length	1.00					
SPAD	0.80**	1.00				
Canopy diameter	0.82**	0.69**	1.00			
Plant height	0.72**	0.59**	0.87**	1.00		
Number of leaves	0.67**	0.62**	0.59**	0.49**	1.00	
Yield	0.83**	0.69**	0.68**	0.55**	0.58**	1.00

Note: **, Correlation is significant at the 0.01 level (2-tailed)

**Figure 2.** Cluster dendrogram of the lettuce parameters**Table 7.** Summary of the principal component analysis showing the eigenvalues and percentage of variance explained by each component

Principal component	Eigenvalue	Percent of variance (%)	Cumulative percentage (%)
PC1	4.4176	73.6	73.6
PC2	0.6149	10.2	83.9
PC3	0.4392	7.3	91.2
PC4	0.3063	5.1	96.3
PC5	0.1191	2.0	98.3
PC6	0.1029	1.7	100

Conclusions

This study demonstrated that nutrient solutions derived from fish by-products can effectively replace or supplement conventional inorganic fertilizers in hydroponic lettuce production. Among the tested treatments, the application of fish protein hydrolysate (FPH) at 600ppm (T3)

provided the most favorable outcomes in terms of growth, physiological performance, yield, and quality. Lettuce grown under this treatment exhibited the longest roots, highest SPAD values, and greatest canopy development and yield, indicating enhanced nutrient absorption and photosynthetic efficiency.

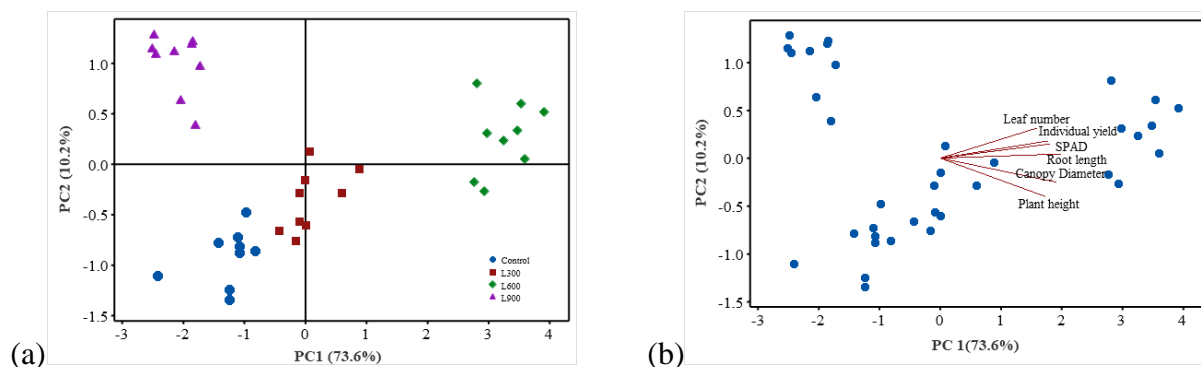


Figure 3. Score plot (a) and biplot (b) of the principal component analysis (PCA) showing the relationships among growth, physiological performance, and yield traits of lettuce

Moderate FPH concentrations improved the vitamin C and soluble solids contents while significantly reducing nitrate accumulation, thereby enhancing both the nutritional and safety attributes of the lettuce. The absence of *E. coli* and *Salmonella* spp. confirmed that FPH use did not compromise product hygiene under controlled hydroponic conditions.

Correlation and PCA analyses highlighted strong positive associations among root growth, chlorophyll content, and yield, underscoring the importance of balanced root–shoot development in optimizing hydroponic performance. Overall, the results provide a scientific foundation for utilizing fishery by-products as a sustainable nutrient source, contributing to circular agriculture and environmentally friendly vegetable production.

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