# Impact of Chemical and Fish (Tilapia nilotica) manure Fertilization Methods on Growth, Anatomical Development, Mineral Nutrition, and Grain Quality of Triticum aestivum L. cv. Yecora Rojo

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## Abstract

# Background

High yields of *Triticum aestivum* L. cv. Yecora Rojo can be achieved sustainably when fertilization methods are designed to improve nutrient availability and reduce their potential ecological impact.

# Methods

This study evaluated the effects of fish manure, applied alone or in combination with chemical fertilizers, on growth, anatomy, mineral uptake, protein composition, and amino acid profile of wheat.

# Results

Obtained results demonstrated that fish manure significantly enhanced most morphological traits, including plant height, root length, fresh and dry weight of shoot and yield components (68.56 cm, 15.7 cm, 9.39, gm, 4.76 gm and 6.8 ton) respectively, compared with untreated plants (45.69 cm, 7.39 cm, 3.22 gm, 1.64 gm and 5 ton) respectively. Anatomical improvements compared with control plants included thicker stem diameter (+ 7.71%) and root diameter (+ 27.96%), expanded vascular tissues in stem (length + 38.04 and width 68.91%), thicker diameter in vascular cylinder in root (+ 18.27%) and increased mesophyll thickness in leaf (+ 9.11%), supporting improved physiological functioning. Mineral composition analysis revealed higher concentrations of macro- and micronutrients (N, P, K, Ca, Mg, Fe, Zn, Mn, Cu, Se, B) in treated plants. Nutritional quality indicators, crude protein, carbohydrate content, gluten, and both essential and non-essential amino acids, also increased under fish manure treatments. Correlation analysis showed strong positive associations between anatomical enhancement, nutrient enrichment, and protein quality traits, indicating an integrated response to organic fertilization.

# Conclusions

These findings confirm that fish manure is an efficient low-pollution fertilizer capable of improving wheat growth, productivity, and nutritional quality, aligning with sustainable agriculture strategies.


## 1. Introduction

Sustainable agriculture faces the ongoing challenge of increasing crop productivity while preserving soil health and minimizing environmental degradation. Achieving high yields without compromising ecosystem integrity requires the adoption of fertilization strategies that optimize nutrient availability and reduce ecological risks. Excessive or improper application of chemical fertilizers has long been associated with soil quality decline, such as reduced fertility and rising alkalinity, and contamination of water bodies [1–2] Consequently, integrating environmentally friendly fertilization approaches has become a central priority in modern crop production systems.

Biofertilizers and organic manures represent viable alternatives to synthetic fertilizers due to their beneficial effects on soil structure, nutrient cycling, and microbial activity. These inputs gradually release macro- and micronutrients, enhance soil biological processes, and contribute to long-term soil fertility [3–5]. Their use in sustainable farming is associated with improved plant growth, enhanced nutrient uptake, and increased productivity, while simultaneously minimizing environmental hazards [6]. Recent studies have further demonstrated that biofertilizer applications can promote yield, physiological attributes, and nutrient accumulation in various crops, including barley, spinach, tomato, maize, chili, wheat, and others [7–14].

Wheat (Triticum aestivum L.) remains a fundamental global staple crop, providing the main caloric and protein intake for billions of people [2, 15]. It is a major source of carbohydrates, proteins, dietary fiber, lipids, and essential minerals such as calcium, potassium, phosphorus, and magnesium, in addition to B-complex vitamins [16–18]. Wheat is cultivated more extensively than any other commercial crop, and its production plays a vital role in global food security and economic stability. Ensuring high and stable wheat yields is therefore imperative, particularly under increasing threats from climate variability, soil degradation, and biotic and abiotic stresses [2, 19–21].

Organic and biologically derived fertilizers, including those originating from fish wastes, are increasingly recognized for their potential to enhance crop growth while contributing to soil restoration. Fish manure and fish-derived fertilizers contain valuable nutrients, amino acids, carbohydrates, fatty acids, and minerals such as calcium and zinc, and have been shown to increase plant height, leaf development, stem diameter, chlorophyll content, and yield components across numerous crop species [11, 14, 22–24]. Fish sludge and effluents from aquaculture systems also provide considerable nitrogen, phosphorus, and organic matter, with reported N contents ranging between 27–70 g $kg^{-1}$ DM and C/N ratios of 6–15.5. [25]. Moreover, fish-based composts, liquid manures, and effluents have demonstrated significant enhancements in root and shoot growth, fruit yield, nutrient accumulation, and biochemical constituents in several crops including tomato, lettuce, mung bean, amaranthus, coriander, and cereals [9, 26–27].

Beyond physiological responses, organic and biofertilizer applications also induce beneficial anatomical modifications. Improvements such as increased stem diameter, enhanced parenchyma and sclerenchyma development, wider vascular bundles, thicker leaf blades, and expanded root tissues have been observed across several plant species treated with organic inputs [7, 27–29]. These structural adaptations often correlate with improved resource acquisition, photosynthetic capacity, and overall plant vigor.

Despite the growing interest in alternative fertilization strategies, limited information is available regarding the comparative performance of fish wastewater, an abundant yet underutilized by-product of aquaculture, against conventional chemical fertilizers in wheat cultivation. Fish wastewater is particularly promising due to its rich nutrient composition and potential to serve as a low-cost, eco-friendly fertilizer capable of supporting sustainable production systems. However, its effects on wheat growth, yield, yield components, and soil health remain insufficiently characterized.

Therefore, the present study aims to evaluate the impact of chemical fertilization and fish wastewater fertilization methods on wheat growth performance, productivity, morphological and yield traits, and soil quality indicators. By comparing these fertilization approaches, the study seeks to identify environmentally sustainable nutrient management practices capable of supporting high wheat productivity while mitigating environmental impacts and promoting long-term soil conservation.

## 2. Materials and Methods

## 2.1. Experimental Procedures

The present investigation was carried out at farm in Khoota, Hail region, during winter seasons 2024. Wheat (*T. sativum*) cultivar Yecora Rojo was used in this study. Seeds were obtained from Seed and Seedling Center- Ministry of Environment Water and Agriculture-Riyadh. The experiment was established in field experiment, used the statistical analysis by completely randomized design with three treatments fertilizer by fish manure alone, recommended fertilizer of chemical fertilizers + fish manure and control of fertilizers (recommended dose of fertilizers). In three replicated the aera for each replicate 2000 m2. The seeds rate (107–119 Kg/Hectar) were sown in the rows using a planter, plant seeds about 1 inch deep. Characteristics of different irrigated soils are shown in the following Table 1. The treatment of fish manure doses was carried out by irrigated from fish basins twice per week the same equilibrium of normal recommended irrigated water 25 Gallon per Minute/hectare. The treatment by fish manure fertilizer were applied at 25 days after sown by twice weekly and was continues to harvest stage. While the recommendation doses of chemical fertilizers treatment were added after 25 days from plant germination by rate 5 times to harvest stage.

Table 1
Pedological parameters of experimental soil.

| Parameters | Control | Sand with Fish manure | Sand with Fish manure+Chemicals |
|---|---|---|---|
| **Conductivity (us/cm)** | 0.85 | 1.23 | 1.65 |
| **Salinity (g/100g)** | 0.193 | 0.365 | 0.577 |
| **Saturation (mL)** | 65 | 85 | 100 |
| **pH** | 7.77 | 8,17 | 8.43 |
| **Phosphore [$P_2O_5$]/ppm** | 10 | 10 | 15 |
| **Carbon (%)** | 3,5 | 2,575 | 1.5 |
| **Clay (%)** | 16.687 | 25,419 | 16.687 |
| **Silt (%)** | 9.213 | 6,808 | 6.586 |
| **Soil texture** | Sandy loam | Sandy clay loam | Sandy loam |
| **Total Limestone (%)** | 3.5 | 4 | 7 |
| **Nitrogen** (mg/L) | 25 | 4 | 7 |

## 2.2. Morphological characters

Ten plants were taken from each replicate at 45days from sowing to determine morphological parameters which included: plant height (cm.), Number of Leaves, Root Length (cm), Root Fresh Wight (g), Root Dry Weight (g), Shoot Fresh Wight (g), Shoot Dry Weight (g). Moreover, the sample plants were oven -dried for 72 h at 70 oc to investigate the dry weight for shoot and root.

## 2.3. Yield and yield components

Harvesting parameters were determined by collected randomly chosen 10 plants from each plot to measurement the following yield components: Number of spikelets, Spikelet Length and weight of spikelet. In the end of trial harvesting took place on middle of May 2024, in age 135 days to calculate the grain yield for each treatment by Ton per hectare.

## 2.4. Anatomical Studies

Microscopical characters were examined through specimens of the median portion of the main stem and its corresponding leaf and root of wheat plant at 30 days for preparation of transverse sections. Specimens were killed and fixed for at least 48 h in FAA (10 mL formalin, 5 mL glacial acetic acid, 50 mL ethyl alcohol 95%-, and 35mL distilled water). The selected samples were then washed in 50% ethyl alcohol, dehydrated in a normal butyl alcohol series, embedded in paraffin wax with a melting point of 56°C, sectioned to a thickness of 20–25 µm, double-stained with safranine-light green, cleared in xylem, and mounted in Canda Balsam [30–31]. Six sections per treatment were prepared, observed, and photomicrographed with a microscope.

## 2.5. Chemical Analyses

Plant samples were oven-dried at 70°C for 24 h following the procedure of [32], then finely ground for elemental and biochemical analyses.

### 2.5.1. Sample Digestion

Wet digestion was performed on 0.2 g of dried plant material using sulfuric and perchloric acids [32]. Samples were mixed with 5 mL concentrated $H_2SO_4$ and heated for 10 min, followed by the addition of 0.5 mL $HClO_4$. Heating continued until the solution became clear.

### 2.5.2. Nitrogen and Crude Protein

Total nitrogen content was quantified using the modified micro-Kjeldahl method [32]. Crude protein was calculated by multiplying total nitrogen by 6.25 [33].

### 2.5.3. Phosphorus

Phosphorus concentrations were determined calorimetrically using the chlorostannous molybdophosphoric blue method in sulfuric acid, following [34].

### 2.5.4. Potassium

Potassium was measured using a flame photometer (CORNING M410, Halstead, England).

### 2.5.5. Total Carbohydrates and Sugars

Total carbohydrates were quantified using the phosphomolybdic acid method (Helrich 1990). A 2 g subsample was extracted with 10 mL of 80% ethanol and filtered. The alcohol-insoluble residue was hydrolyzed with 150 mL distilled water and 5 mL concentrated HCl for 30 min, cooled, and neutralized with $Na_2CO_3$. After filtration, the filtrate was condensed in a water bath (3–4 min), diluted, and refiltered for reducing sugar analysis.

For total sugars, 20 mL of the filtrate was re-hydrolyzed with 2 mL concentrated HCl for 30 min, cooled, neutralized, filtered, and brought to final volume. An aliquot (0.5 mL) was reacted with 1 mL Somogyi reagent, heated in a boiling water bath for 30 min, cooled, and mixed with 1 mL arsenomolybdate reagent. The volume was adjusted to 10 mL and absorbance was measured at 560 nm.

### 2.5.6. Mineral Elements (Ca, Mg, Fe, Zn, Mn, Cu, Se, B)

Elemental concentrations were determined using atomic absorption spectrophotometry with an air–acetylene flame (Pye Unicam SP-1900, Cambridge, UK).

### 2.5.7. Fat Content

Fat content was determined according to the standard analytical procedures described by [32].

### 2.5.8. Amino Acid Profiling (HPLC)

Amino acid identification and quantification were carried out using an ACQUITY UPLC system (Waters, Milford, MA, USA) equipped with a column oven, autosampler, binary pump, PDA detector (190–700 nm), and fluorescence detector. Chromatographic separation was performed on AccQ-Tag Ultra C18 (2.1 mm × 100 mm, 1.7 μm) and ZORBAX ODS C18 (4.6 mm × 250 mm, 5 μm) columns, following the method of [35].

### 2.5.9. Gluten Content

Wet and dry gluten contents were determined according to [36] standard methods by handwashing 25 g wheat flour dough to remove starch and soluble components.

### 2.6 Statistical Analysis

Data were analyzed using the COSTAT software package following the procedures for a Completely Randomized Design (CRD) described by [37]. Prior to conducting analysis of variance (ANOVA), the assumptions of normality and homogeneity of variances were verified. Normality was tested using the Shapiro–Wilk test [38], and homogeneity of error variances was assessed using Levene's test [39]. After confirming that the ANOVA assumptions were met, treatment means were compared using the Least Significant Difference (LSD) test at the 0.05 significance level. Post-hoc comparisons and graphical outputs were generated using R software.

## 3. Results

This research was performed to investigated to impact of using fish manure alone or combined with recommended doses of chemical fertilizer on wheat plants as tool or scientific application for clean agriculture with maximized yield, yield component and promotive vegetative growth. A General view of the results of this study revealed that irrigated by fish manure alone or combined with doses of chemical fertilizers achieved best results in most characters.

### 3.1. Morphological and Growth Characteristics

The results obtained at 45 days after sowing showed that the application of fish manure, either alone or in combination with chemical fertilizers, significantly improved all measured morphological traits of wheat (Triticum aestivum L. cv. Yecora Rojo) compared with the untreated control (Table 2). These improvements were consistent across plant height, root development, and shoot biomass, indicating a strong positive response of wheat seedlings to fish wastewater fertilization.

Plant height exhibited a clear and significant increase under all fertilization treatments. Wheat plants grown under the Fish + Chemical treatment reached the greatest mean height (82.8 cm), representing the highest growth response among treatments. Plants fertilized with fish manure alone also showed a substantial improvement (68.56 cm), corresponding to a 50% increase over the control (45.70 cm). These results highlight the capacity of fish manure to stimulate vegetative elongation and further suggest a synergistic effect when supplemented with chemical fertilizers. Root traits responded strongly to organic fertilization. The longest roots were recorded under fish manure alone (15.76 cm), achieving a remarkable 113% increase over control plants. This suggests that fish-derived organic nutrients may enhance root elongation by improving soil structure, moisture retention, or micronutrient availability. Although the Fish + Chemical treatment produced slightly shorter roots (13.17 cm), both fertilized treatments significantly exceeded the control (7.39 cm). Root fresh and dry weights followed a similar pattern. The Fish + Chemical treatment resulted in the greatest root biomass (6.07 g fresh weight; 0.85 g dry weight), while fish manure alone also produced significant increases in fresh weight (17% above the control) and a very large improvement in dry weight (135% above the control). These results imply that fertilization not only enhances root size but also promotes structural development and nutrient accumulation, contributing to stronger, more functional root systems. Shoot fresh and dry weights showed the most dramatic responses to fertilization. The Fish + Chemical treatment produced exceptionally high biomass (20.44 g fresh; 5.85 g dry), far surpassing both the control and fish manure alone. However, fish manure by itself also induced substantial increases, improving shoot biomass by approximately 190% compared with control plants. The magnitude of these increases suggests that organic nutrients from fish manure enhance photosynthetic activity, cell expansion, and overall vegetative vigor during early growth stages. In contrast to the substantial improvements observed in biomass-related parameters, no significant differences were detected in the number of leaves across treatments. This indicates that fish manure influences organ size and mass rather than the initiation of new leaves at early growth stages. Overall, early vegetative growth of wheat was strongly enhanced under both fish manure and combined Fish + Chemical fertilization. The promotive effects were most pronounced in shoot biomass, followed by root length and root dry weight. While the addition of chemical fertilizers further enhanced several parameters, particularly shoot and root mass, the improvements achieved with fish manure alone were comparable in many traits.

These findings confirm that fish wastewater acts as an effective organic fertilizer capable of providing essential nutrients in forms accessible to young plants. Its capacity to promote early growth makes it a promising input for sustainable wheat production. Given the similar performance between fish manure alone and the combined treatment for many morphological traits, the application of fish manure as a stand-alone organic fertilizer can be recommended to support clean and environmentally friendly agricultural practices.

Table 2
Means comparison of morphological characters of wheat plant cv. Yecora Rojo, aged 45 days, in the growing season of 2023/2024.

| Parameters\Treatments | Control | Fish manure | Fish+Chem |
|---|---|---|---|
| Plant height (cm) | $45.697 \pm 2.778^{c}$ | $68.561 \pm 5.532^{b}$ | $82.8 \pm 4.413^{a}$ |
| Root length (cm) | $7.394 \pm 1.037^{b}$ | $15.764 \pm 17.869^{a}$ | $13.167 \pm 0.834^{ab}$ |
| Root fresh weight (g) | $4.628 \pm 0.634^{b}$ | $5.421 \pm 0.49^{a}$ | $6.073 \pm 0.711^{a}$ |
| Root dry weight (g) | $0.267 \pm 0.059^{c}$ | $0.628 \pm 0.331^{b}$ | $0.851 \pm 0.356^{a}$ |
| Shoot fresh weight (g) | $3.228 \pm 0.504^{c}$ | $9.392 \pm 1.931^{b}$ | $20.444 \pm 3.756^{a}$ |
| Shoot dry weight (g) | $1.636 \pm 0.194^{c}$ | $4.768 \pm 1.162^{b}$ | $5.851 \pm 1.008^{a}$ |

*Means comparison based on the Duncan test was calculated between morphological traits (a, b, c). Averages followed by the same letters are not significantly different at $p < 0.05$ according to the LSD test.*

## 3.2. Yield and yield components

The results presented in Table 3 show that all fertilization treatments, including fish manure alone and fish manure combined with chemical fertilizers, produced substantial increases in yield and yield components compared with the untreated control. These findings are consistent with the promotive effects on vegetative growth observed earlier, indicating that improvements in early morphological traits were translated into enhanced reproductive performance and final grain yield. Both spikelet length and spikelet weight increased significantly under fertilization treatments. The highest values for both traits were recorded in the Fish + Chemical treatment, which produced a 195% increase in spikelet weight compared with the control. Fish manure alone also led to considerable enhancement, increasing spikelet weight by 66.9%. Similarly, spikelet length improved markedly, increasing by 90.11% under Fish + Chemical and by 77.15% under fish manure alone. These increases reflect enhanced assimilate allocation and spike development, likely driven by improved nutrient availability throughout the growth cycle. Despite these improvements, the number of spikelets per plant showed no significant differences among treatments. This suggests that fertilization influenced spikelet size and biomass rather than spikelet initiation, which is typically determined earlier in development and less responsive to nutrient availability at later stages. Seedling dry weight at maturity followed the same pattern as spikelet traits. The Fish + Chemical treatment resulted in the highest dry weight (6.65 g), significantly exceeding both the control and fish manure alone. However, plants treated with fish manure alone also exhibited a clear improvement over the control, indicating that organic fertilization supports stronger reproductive and structural biomass accumulation.

Grain yield per hectare showed substantial and statistically significant increases under both fertilization treatments. The Fish + Chemical treatment produced the highest yield, representing an 87.5% increase over the control, while fish manure alone resulted in a 70% increase. These improvements reflect the cumulative effect of enhanced plant height, root development, shoot biomass, and spikelet characteristics observed throughout the plant’s life cycle. These results confirm that both organic and combined fertilization strategies positively influenced yield components, with the most pronounced effects observed in spikelet weight and grain yield. Although the combined Fish + Chemical treatment achieved the highest absolute values, the performance of fish manure alone was comparably strong across most parameters. The absence of significant differences between the two fertilization treatments in several traits suggests that fish wastewater alone provides sufficient nutrients to sustain both vegetative vigor and high productivity.

Given these findings, the use of fish manure alone can be recommended as an effective, environmentally friendly fertilization strategy that enhances yield while reducing dependence on chemical fertilizers. This approach supports sustainable agricultural practices and provides a valuable pathway for integrating aquaculture by-products into crop production systems.

Table 3
Means comparison (LSD test) of yield and yield components characters of wheat plant cv. Yecora Rojo, aged 130 days, in the growing season of 2023/2024

| Parameters\Treatments | Control | Fish manure | Fish+Chem |
|---|---|---|---|
| Length of spikelets | $5.061 \pm 0.958^{b}$ | $8.964 \pm 0.784^{a}$ | $9.617 \pm 0.838^{a}$ |
| Weight of spikelets (g) | $1.236 \pm 0.466^{c}$ | $2.073 \pm 0.527^{b}$ | $3.667 \pm 0.6^{a}$ |
| Seedling dry weight (g) | $2.387 \pm 0.572^{b}$ | $2.81 \pm 0.495^{b}$ | $6.649 \pm 0.93^{a}$ |
| Yield | $5 \pm 0^{c}$ | $6.8 \pm 0^{b}$ | $7.5 \pm 0^{a}$ |

*Means comparison based on the Duncan test was calculated between morphological traits (a, b, c). Averages followed by the same letters are not significantly different at $p < 0.05$ according to the LSD test.*

## 3.3. Anatomical characters

Referring to the previous morphological and yield characters, it was observed that all treatments significant increase in most of the previous characters. This needs a further study on the internal structure of vegetative organs of wheat plants. Microscopical characters were examined through specimens of the median portion of the root and main stem and its corresponding leaf.

### 3.3.1. Anatomical Structure of the Stem

The data of histological measurements in cross section of the median portion of the main stem of wheat plant were presented in Table 4 likewise, microphotographs (Fig. 1: A,B and C). Microscopical examination of the median stem section of wheat plants revealed that both fertilization treatments induced notable anatomical modifications compared with the control (Table 4). These changes are consistent with the previously observed improvements in vegetative growth and yield, indicating that fish manure, alone or supplemented with chemical fertilizers, enhances the internal structural organization of the stem, supporting more efficient transport and mechanical strength.

Stem diameter increased under all fertilization treatments, reaching its maximum under Fish + Chemical (+ 20.72% over control), followed by fish manure alone (+ 7.71%). The enlargement of stem diameter suggests enhanced secondary growth and thicker supporting tissues. Both treatments also increased the diameter of the hollow pith cavity, with fish manure alone showing the highest increase (+ 24.95%). A larger pith cavity may improve stem flexibility and contribute to overall plant vigor.

A contrasting response was observed for stem wall thickness: fish manure alone led to a reduction of 37.36%, while the Fish + Chemical treatment increased wall thickness by 20.06% relative to the control. This indicates that the combined treatment promotes both thicker structural tissue and larger overall stem size, whereas fish manure alone tends to promote expansion of the pith cavity rather than wall thickening. Such differences may reflect distinct responses in lignification or tissue differentiation under organic vs. combined nutrient regimes.

Both treatments significantly increased the number of vascular bundles, with fish manure alone showing a 40.62% increase and Fish + Chemical achieving a 50% increase. An increased number of vascular bundles enhances the stem's capacity for water and nutrient transport and contributes to structural support. This anatomical enhancement aligns with the strong improvements observed in vegetative biomass and grain yield. The dimensions of large bundles (length and width) increased markedly under both fertilization regimes. Length increased by approximately 38–40%, while width increased by 69% (Fish) and 73% (Fish + Chem) relative to the control. Enlarged bundle dimensions reflect improved vascular development and more robust conducting tissues.

Phloem thickness also increased substantially, reaching + 18.3% under Fish and + 41% under Fish + Chem. This enhancement suggests improved assimilate translocation capacity, contributing to greater shoot biomass and grain production. Similarly, the diameter of metaxylem vessels increased by 33.83% (Fish) and 63.17% (Fish + Chem). Larger xylem vessels facilitate more efficient water transport, supporting higher rates of transpiration, photosynthesis, and growth.

Overall, the anatomical changes observed in stem tissues strongly support the promotive effect of fish manure and combined fertilization on plant performance. Both treatments enhanced vascular development, increased conducting tissue size, and improved stem structure. The Fish + Chemical treatment generally produced the largest anatomical enhancements; however, fish manure alone still induced substantial and functionally meaningful improvements. These structural modifications explain, at the tissue level, the earlier reported increases in biomass, spikelet weight, and grain yield. The strong performance of fish manure alone highlights its potential as a sustainable fertilization strategy capable of enhancing both internal stem anatomy and overall productivity.

Table 4
Anatomical Characters of stem wheat plant cv. Yecora Rojo, aged 45 days, in the growing season of 2023/2024.

| Histological Characters (Stem) | Control | Fish Manure | % Change vs Control | Fish + Chem | % Change vs Control |
|---|---|---|---|---|---|
| Stem diameter (µm) | 1895.50 | 2041.65 | + 7.71% | 2288.26 | + 20.72% |
| Stem wall thickness (µm) | 524.50 | 328.54 | −37.36% | 629.72 | + 20.06% |
| Diameter of pith cavity (µm) | 1371.00 | 1713.11 | + 24.95% | 1658.54 | + 20.98% |
| Number of vascular bundles | 32 | 45 | + 40.62% | 48 | + 50.00% |
| Large vascular bundle – length (µm) | 75.31 | 103.96 | + 38.04% | 105.32 | + 39.85% |
| Large vascular bundle – width (µm) | 48.15 | 81.33 | + 68.91% | 83.16 | + 72.71% |
| Phloem thickness (µm) | 20.60 | 24.37 | + 18.30% | 29.05 | + 41.02% |
| Metaxylem vessel diameter (µm) | 19.33 | 25.87 | + 33.83% | 31.54 | + 63.17% |

The microscopic examination of the median portion of the wheat main stem illustrates the promotive effects of fish manure, applied alone or in combination with chemical fertilization, compared with the control (Figures A, B and C). All fertilization treatments enhanced most anatomical characteristics of the stem (Table 3, Figures A, B and C). Stem diameter increased under both treatments, with Fish + Chemical showing the greatest enhancement (+ 20.72%) and fish manure alone also increasing diameter (+ 6.26%) relative to the control. Stem wall thickness responded differently: the combined treatment (Fish + Chemical) increased wall thickness by + 20.06%, whereas fish manure alone led to a reduction of − 37.36% compared with the control. The diameter of the hollow pith cavity was notably larger in fish manure-treated plants than in the combined treatment, suggesting that expansion of the pith cavity may contribute to more rapid early maturation.

The total number of vascular bundles per cross-section also increased, by + 40.62% in fish manure alone and + 50.0% in the Fish + Chemical treatment relative to control. Examination of the large vascular bundles revealed significant improvements in all measured histological traits. For the Fish + Chemical treatment, length and width of vascular bundles increased by + 39.85% and + 72.71%, phloem thickness increased by + 41.02%, and metaxylem vessel diameter increased by + 63.17% over the control. Fish manure alone also induced substantial improvements, though slightly lower than the combined treatment.
Overall, the enhancements in stem anatomical features under both fertilization regimes suggest improved water and nutrient transport capacity, which likely supports the observed increases in vegetative growth and grain yield. The most pronounced structural improvements were seen with Fish + Chemical treatment, while fish manure alone still produced considerable and functionally significant anatomical benefits.

### 3.3.2. Main Root Anatomy

Microscopic examination of transverse sections of the middle portion of the wheat main root revealed that both fertilization treatments, fish manure alone and fish manure combined with chemical fertilizers, substantially enhanced root anatomical traits compared with the control (Table 5, Fig. 2). These anatomical improvements align with the earlier observed enhancements in root length, root biomass, and overall plant growth. Root diameter increased under all fertilization treatments, with Fish + Chemical showing the largest increase (+ 43.88%) and fish manure alone increasing by + 27.96% relative to the control. Epidermis thickness showed only minor changes, increasing by 3.33% under fish manure and 4.40% under the combined treatment, indicating that structural modifications were primarily focused on internal tissues rather than the outer protective layer. The cortex thickness increased considerably under both treatments, particularly in the Fish + Chemical treatment (+ 69.73%), followed by fish manure alone (+ 35.57%). Similarly, pith thickness increased by + 32.54% and + 22.17% under Fish + Chemical and fish manure alone, respectively. These changes suggest enhanced capacity for nutrient storage and mechanical support within the root structure, which likely contributes to better anchorage and nutrient transport efficiency. The diameter of the vascular cylinder increased significantly with fertilization (+ 28.27% for fish manure and + 35.06% for Fish + Chemical), reflecting improved development of xylem and phloem tissues. Large vascular bundles also showed marked enhancements: phloem thickness increased by + 18.27% under fish manure and + 45.85% under Fish + Chemical, while metaxylem vessel diameter increased by + 20.01% and + 48.67%, respectively. These modifications indicate improved water and nutrient conduction capacity, which supports enhanced vegetative growth and grain production observed in earlier results.

This microscopic analysis demonstrates that both fertilization treatments promoted root development at the tissue level. The Fish + Chemical treatment generally produced the largest anatomical enhancements; however, fish manure alone still resulted in significant improvements, suggesting that fish wastewater provides sufficient nutrients to enhance root structure and function. The observed increases in cortex, pith, and vascular tissues likely contribute to greater nutrient and water uptake, providing a structural explanation for the earlier observed increases in root biomass and plant growth.

Table 5
Anatomical Characters of root wheat plant cv. Yecora Rojo, aged 45 days, in the growing season of 2023/2024.

| Histological Characters (Root) | Control | Fish Manure | % Change vs Control | Fish + Chem | % Change vs Control |
|---|---|---|---|---|---|
| Root diameter (µm) | 1000.22 | 1279.98 | + 27.96% | 1439.21 | + 43.88% |
| Epidermis thickness (µm) | 71.10 | 73.47 | + 3.33% | 74.23 | + 4.40% |
| Cortex thickness (µm) | 328.54 | 445.43 | + 35.57% | 557.65 | + 69.73% |
| Pith thickness (µm) | 152.05 | 185.76 | + 22.17% | 201.54 | + 32.54% |
| Vascular cylinder diameter (µm) | 448.53 | 575.32 | + 28.27% | 605.79 | + 35.06% |
| Phloem thickness (µm) | 65.56 | 77.54 | + 18.27% | 95.62 | + 45.85% |
| Metaxylem vessel diameter (µm) | 69.62 | 83.55 | + 20.01% | 103.51 | + 48.67% |

Microscopic examination of the median portion of the wheat main root grown for 45 days revealed that both fertilization treatments, fish manure alone and fish manure combined with chemical fertilizers, substantially enhanced root anatomical traits compared with the control (Table 5, Figs. 2A–C). Root diameter increased significantly under both treatments, by + 27.96% with fish manure alone and + 43.88% with Fish + Chemical fertilization relative to the control. This increase in root diameter was primarily associated with pronounced enhancements in cortex thickness (+ 35.57% and + 69.73%, respectively) and pith thickness (+ 22.17% and + 32.54%, respectively). The vascular cylinder diameter also increased, by + 28.27% under fish manure alone and + 35.06% under the combined treatment, reflecting improved internal structural development.

The thickness of phloem tissue was enhanced by + 18.27% with fish manure alone and + 45.85% with Fish + Chemical, while metaxylem vessel diameter increased by + 20.01% and + 48.67%, respectively. These modifications suggest enhanced water and nutrient transport capacity within the root system, supporting the observed improvements in root growth and overall plant performance. In contrast, epidermis thickness was only slightly affected by the treatments, indicating that structural changes were concentrated primarily in internal conductive and storage tissues.

Microscopic examination of cross-sections from the median portion of the leaf lamina in wheat plants demonstrated that both fertilization treatments, fish manure alone and fish manure combined with chemical fertilizers, significantly enhanced leaf anatomical traits compared with the control (Table 6, Fig. 3A,B and C). The thickness of the leaf blade increased by + 9.11% under fish manure alone and by + 14.65% under Fish + Chemical treatment. These enhancements were mainly attributed to the expansion of mesophyll tissue, which increased by + 12.79% and + 27.38%, respectively, compared with the control.

The dimensions of the main vascular bundle also showed notable improvements. Bundle length increased by + 8.71% under fish manure and + 14.83% under Fish + Chemical, while bundle width increased by + 31.34% and + 37.89%, respectively. Phloem tissue thickness increased by + 36.80% and + 14.73%, and metaxylem vessel diameter increased by + 8.69% and + 31.88% under fish manure and Fish + Chemical, respectively. These observations indicate that fertilization treatments promoted the development of both photosynthetic and vascular tissues in the leaf lamina. The Fish + Chemical treatment generally produced the greatest improvements, though fish manure alone also induced significant anatomical enhancements. These structural changes likely contribute to increased photosynthetic efficiency and improved translocation of water and nutrients, supporting the observed increases in vegetative growth and yield.

Table 6
Anatomical Characters of leaf wheat plant cv. Yecora Rojo, aged 45 days, in the growing season of 2023/2024.

| Histological Characters (Leaf) | Control | Fish Manure | % Change vs Control | Fish + Chem | % Change vs Control |
|---|---|---|---|---|---|
| Thickness of blade (µm) | 217.85 | 237.67 | + 9.11% | 249.77 | + 14.65% |
| Mesophyll thickness (µm) | 185.72 | 209.48 | + 12.79% | 236.57 | + 27.38% |
| Large vascular bundle – length (µm) | 109.76 | 119.33 | + 8.71% | 126.04 | + 14.83% |
| Large vascular bundle – width (µm) | 84.01 | 110.34 | + 31.34% | 115.84 | + 37.89% |
| Phloem thickness (µm) | 24.29 | 33.23 | + 36.80% | 27.87 | + 14.73% |
| Metaxylem vessel diameter (µm) | 34.50 | 37.50 | + 8.69% | 45.50 | + 31.88% |

## 3.4. Chemical Composition of seed

The chemical analysis of wheat revealed that both fertilization treatments, fish manure alone and fish manure combined with chemical fertilizers, substantially enhanced the nutrient content of plant tissues compared with the control (Table 7).

Concerning macronutrients (N, P, K, Ca, Mg), nitrogen content increased slightly under fish manure (+ 7.9%) and more strongly under Fish + Chemical fertilization (+ 11.5%) relative to the control, indicating improved protein synthesis potential. Phosphorus also increased progressively from 0.64% in the control to 0.68% in fish manure and 0.77% in Fish + Chemical treatment, reflecting enhanced energy metabolism and root activity. Potassium showed one of the most pronounced improvements, increasing by + 58.8% under fish manure and + 94.1% under Fish + Chemical treatment. This strong K enrichment suggests improved osmoregulation, photosynthesis, and stress tolerance. Calcium and magnesium followed similar trends, showing moderate but consistent improvements, with Ca increasing by + 29.4% and + 52.9%, and Mg by + 17.9% and + 21.4% under fish manure and Fish + Chemical fertilization, respectively. These increases indicate strengthened cell wall structure and chlorophyll biosynthesis.

On the other hand, fish manure notably enhanced micronutrient (Fe, Zn, Mn, Cu, Se, B) uptake, with iron increasing from 45.13 ppm in the control to 52.19 ppm (+ 15.65%), and zinc rising from 35.72 ppm to 41.35 ppm (+ 15.77%). Manganese and copper showed similar promotive patterns with increases of + 18.2% and + 27.9%, respectively. Selenium and boron, essential for antioxidant processes and reproductive development, also showed clear enhancements under fish manure alone.

Although Fish + Chemical treatment increased all micronutrients compared with the control, the magnitude of improvement was generally slightly lower than with fish manure alone for Fe, Zn, and Cu. This suggests that organic fertilization promotes micronutrient availability and assimilation more efficiently due to improved soil biological activity. Both treatments significantly improved the mineral profile of wheat shoots, demonstrating enhanced nutrient absorption and physiological functionality. Fish manure alone produced the highest enrichment in several micronutrients (Fe, Zn, Cu), highlighting its role in improving soil micronutrient bioavailability. The Fish + Chemical treatment produced the greatest improvements in macronutrients, especially N, P, and K, reflecting synergistic nutrient supply.

These increases in nutrient content support the previously observed improvements in morphological growth, anatomical development, and yield performance, confirming that enhanced mineral nutrition is a key mechanism behind the promotive effect of fish manure–based fertilization strategies.

Table 7
Chemical Analysis of wheat plant seed cv. Yecora Rojo in the growing season of 2023/2024.

| Treatment | N (%) | P (%) | K (%) | Ca (%) | Mg (%) | Fe (ppm) | Zn (ppm) | Mn (ppm) | Cu (ppm) | Se (ppm) | B (ppm) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Control** | 1.39 | 0.64 | 0.17 | 0.017 | 0.028 | 45.13 | 35.72 | 43.16 | 3.15 | 0.073 | 0.75 |
| **Fish manure** | 1.50 | 0.68 | 0.27 | 0.022 | 0.033 | 52.19 | 41.35 | 51.02 | 4.03 | 0.084 | 1.02 |
| **Fish + Chem** | 1.55 | 0.77 | 0.33 | 0.026 | 0.034 | 49.25 | 39.66 | 48.30 | 3.78 | 0.081 | 0.96 |

## 3.5 Biochemical Composition of seeds: Protein, Fat, Carbohydrates, and Gluten

The biochemical evaluation of wheat seeds revealed that both fertilization treatments—fish manure alone and fish manure combined with chemical fertilizers, substantially enhanced the nutritional quality of the plant tissues compared with the control (Table 8). Crude protein content increased from 8.68% in the control to 9.37% under fish manure (+ 7.96%) and reached its highest value (9.68%) under Fish + Chemical fertilization (+ 11.54%). This improvement reflects the enhanced nitrogen assimilation reported in the chemical analyses, confirming that both fertilization strategies significantly stimulate protein biosynthesis during early vegetative growth.

Fat percentage showed a modest but consistent increase, rising from 1.12% in the control to 1.28% under fish manure (+ 14.3%) and 1.33% under Fish + Chemical fertilizers (+ 18.8%). These increments suggest increased lipid metabolism and membrane formation—processes often associated with improved cell division and metabolic activity.

Carbohydrate content exhibited one of the strongest responses to the treatments, increasing from 71.59% in the control to 73.63% (+ 2.85%) with fish manure and 75.48% (+ 5.43%) with Fish + Chemical fertilizers. This enhancement indicates a substantial rise in photosynthetic efficiency and carbohydrate accumulation, likely attributed to improved nutrient availability (especially K, Mg, and micronutrients), which supports carbohydrate synthesis and translocation. Gluten percentage increased markedly under both treatments. Fish manure enhanced gluten content by 22.99% compared with the control (33.48% vs. 27.19%), while the Fish + Chemical treatment reached 33.15%, representing a 21.96% increase. These improvements highlight the positive effect of fish-based fertilization on grain quality traits linked to protein composition and gluten-forming fractions, suggesting a potential improvement in bread-making quality.

Each fertilization approaches significantly improved the biochemical composition of wheat seed, reinforcing the promotive effects observed in growth, anatomy, and mineral nutrition. Fish manure alone particularly enhanced gluten and overall nutrient density. But Fish + Chemical fertilization produced the strongest increases in protein, fat, and carbohydrate content. These enhancements collectively indicate that fish manure alone or combined with chemical fertilizers supports superior metabolic activity and contributes to improved nutritional and technological quality of wheat.

**Table (8)** Protein Analysis of wheat plant seeds cv. Yecora Rojo, aged 45 days, in the growing season of 2023/2024

| Treatment | Crude Protein | Fat (%) | Carbohydrate (%) | Gluten (%) |
|---|---|---|---|---|
| Control | 8.68 | 1.12 | 71.59 | 27.19 |
| Fish | 9.37 | 1.28 | 73.63 | 33.48 |
| Fish+Chem | 9.68 | 1.33 | 75.48 | 33.15 |

## 3.6. Biochemical Composition: Amino Acid Composition

The analysis of amino acid profiles (Table 9) revealed that both fertilization treatments, fish manure alone and the combined fish + chemical fertilization, altered the composition of several non-essential and essential amino acids in wheat shoots at 45 days.

## 3.6.1. Non-Essential Amino Acids

In general, fish manure and combined fertilization influenced distinct amino acids differently, reflecting variations in nitrogen assimilation and protein metabolism. Serine and Glycine: Serine increased slightly under both fish manure (5.02 g/100 g protein) and fish + chemical treatments (5.07 g/100 g), compared with the control (4.86 g). Glycine, however, decreased under both treatments relative to the control, with the largest decline in the fish manure treatment. These shifts suggest changes in photorespiration and carbon–nitrogen cycling pathways. Aspartic and Glutamic Acids: A substantial rise in aspartic acid was noted under the combined treatment (6.18 g) compared with the control (3.92 g). Glutamic acid, the most abundant amino acid, was further elevated by both treatments, reaching 33.95 g and 34.68 g in fish and combined treatments, respectively, compared with 31.73 g in the control. These increases reflect enhanced nitrogen assimilation since both amino acids serve as major nitrogen donors in plant metabolism. Alanine and Proline: Both alanine and proline showed declines under fertilized treatments relative to the control. Proline decreased sharply from 7.20 g (control) to 5.42 g (fish) and 3.51 g (Fish+Chem). Reduced proline levels may indicate lower stress conditions in fertilized plants, given that proline often accumulates under stress.

Cystine and Tyrosine: Fish manure notably increased cystine (4.57 g) by over fourfold compared with the control (1.09 g), while the combined treatment showed a modest rise (1.37 g). Tyrosine also increased in the fish manure treatment (4.58 g), suggesting enhanced sulfur-containing amino acid synthesis under organic fertilization. Arginine: Arginine increased moderately under fish manure (3.04 g) but declined under the combined treatment (2.16 g) relative to the control (2.75 g), reflecting treatment-specific effects on urea-cycle–related metabolism.

## 3.6.2. Essential Amino Acids Composition

The essential amino acid profiles showed notable enhancements under both treatments, surpassing the control in several important constituents. Valine, Leucine, Isoleucine (Branched-Chain Amino Acids): All three BCAAs increased significantly under both fertilization regimes. Valine more than doubled under fish manure (2.64 g) compared with control (1.13 g), while isoleucine and leucine showed similar upward trends. These amino acids are key determinants of protein nutritional quality. Lysine, often limiting in cereals, increased substantially under both treatments, particularly the combined fertilization (3.37 g vs. 1.22 g in control). This improvement highlights the nutritional enhancement contributed by the fertilization regimes. The two amino acids histidine and threonine showed modest increases under both treatments, indicating a general enhancement of total essential amino acid content. Phenylalanine and Methionine: Fish manure produced the largest increase in phenylalanine (5.10 g vs. 3.42 g in control), while combined fertilization maintained moderate levels (3.85 g). Methionine decreased in the fish manure treatment but increased slightly under the combined fertilizer (1.54 g), emphasizing differential impacts on sulfur-containing amino acids.

Table 9
Analysis of Non-Essential and Essential Amino Acids of wheat plant seed cv. Yecora Rojo, in the growing season of 2023/2024 .

| **Analysis of Non-Essential Amino Acids (g/100 g Protein)** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Treatment** | **Serine** | **Glycine** | **Aspartic Acid** | **Glutamic Acid** | **Alanine** | **Proline** | **Cystine** | **Tyrosine** | **Arginine** |
| **Control** | 4.86 | 5.39 | 3.92 | 31.73 | 7.33 | 7.20 | 1.09 | 3.36 | 2.75 |
| **Fish manure** | 5.02 | 4.11 | 4.67 | 33.95 | 6.37 | 5.42 | 4.57 | 4.58 | 3.04 |
| **Fish+Chem** | 5.07 | 4.23 | 6.18 | 34.68 | 5.46 | 3.51 | 1.37 | 3.22 | 2.16 |
| **Analysis of Essential Amino Acids (g/100 g Protein)** | | | | | | | | | |
| **Treatment** | **Valine** | **Histidine** | **Leucine** | **Threonine** | **Isoleucine** | **Phenylalanine** | **Methionine** | **Lysine** | |
| **Control** | 1.13 | 1.21 | 7.58 | 1.28 | 2.77 | 3.42 | 1.39 | 1.22 | |
| **Fish manure** | 2.64 | 1.58 | 9.41 | 1.56 | 3.54 | 5.10 | 0.93 | 2.81 | |
| **Fish+Chem** | 2.60 | 1.55 | 8.93 | 1.52 | 3.63 | 3.85 | 1.54 | 3.37 | |

## 3.7. Correlation Structure Among Anatomical, Nutritional, and Biochemical Traits

To estimate the interaction between measured quantitative traits we perform a correlation heatmap analysis (Fig. 4) which shows the pairwise correlations between all quantitative traits in this wheat experiment (root anatomy, mineral content, protein traits, and amino acids). The color scale presented indicates that Red mean strong positive correlation (close to + 1), Blue refer to negative correlation (close to − 1), White/light demonstrates that there is a weak or no correlation and Grey indicate missing, NA, or no comparable data. Because we experiment three treatments (Control, Fish, Fish+Chem), the correlation structure is strongly driven by treatment effects. The correlation heatmap (Fig. 3) revealed distinct clusters of traits that responded synchronously to fertilization. Morphological traits (PLH, RL, RFD, LT, LA) formed a strong positive correlation block, indicating coordinated growth stimulation under fish manure treatments. Root anatomical traits (cortex thickness, vascular cylinder diameter, meta-xylem diameter) grouped into a second correlation module, suggesting structural reinforcement of the root system. Mineral elements (N, P, K, Fe, Zn, Mn, Ca, Mg) exhibited high inter-correlation, reflecting improved nutrient assimilation capacity. Protein traits (crude protein, gluten) and amino acids formed the most homogeneous cluster, tightly associated with plant N content. This correlation structure demonstrates that fish manure alone or combined with chemical fertilizers induces a systemic enhancement in growth, nutrient uptake, and metabolic quality, with strong positive linkages between anatomical, nutritional, and biochemical traits.

## 4. Discussion

Enhancing crop productivity while reducing environmental impact is a global priority in sustainable agriculture. Organic amendments such as fish manure provide an eco-friendly alternative to synthetic fertilizers by supplying essential nutrients, improving soil biological activity, and reducing nutrient leaching. The present study demonstrates that applications of fish manure alone or with chemical fertilizers significantly improved wheat growth, anatomical structure, mineral nutrition, and nutritional quality. These results align with previous findings that highlight fish-based fertilizers as rich sources of nitrogen, phosphorus, potassium, micronutrients, amino acids, and lactic acid [24–25, 40]

## 4.1 Morphological response, yield, and yield components

Fish manure treatments consistently increased plant height, leaf number, root length, and biomass accumulation (fresh and dry). Yield components including spikelet number, spikelet length, spikelet weight, and total yield followed the same trend. The stimulatory effects are likely driven by enhanced nutrient availability, organic acids, and bioactive compounds present in fish effluents, which promote photosynthetic efficiency and metabolic activity [22, 41]. Similar positive responses to fish-based or organic fertilizers have been reported in wheat [6–7], maize [14], tomato [9], chili [13], amaranthus [12], and spinach [10]. These consistent results across species suggest that fish-derived nutrients provide reliable stimulatory effects on plant growth and yield formation. In our dataset, strong positive correlations were noted between plant height, number of leaves, and shoot biomass, as well as between root length and root biomass. Such correlations reflect the integrated nature of wheat growth: improved vegetative vigor supports greater assimilation and biomass partitioning to reproductive structures.

However, a few studies reported weak or non-significant growth responses to fish manure when nutrient concentrations were inadequate or when the crop had high nutrient demands [11]. This highlights the importance of optimized dosage and balanced nutrient supply.

## 4.2 Anatomical modifications and their functional significance

Anatomical analysis revealed substantial enhancements in stems, roots, and leaves under the fish manure treatments. Increased stem diameter resulted from thicker cortical tissues and larger vascular bundles, while root cross-sections exhibited thicker epidermis, cortex, and enlarged xylem cylinders. Leaf blades showed increased mesophyll thickness and improved vascular bundle development. These structural improvements support the enhanced growth observed. Thicker stems provide better mechanical support, allowing greater transport of water and nutrients. Enlarged root tissues increase absorptive capacity and storage potential, supporting plant vigor [26, 42]. The enlarged pith cavity observed particularly in fish-only treatments may reflect accelerated maturation, as pith collapse is associated with stem hollowing during reproductive development [43]. Comparable anatomical improvements under organic amendments were reported in wheat [7], tomato [9], mung bean [27], and red beet [29]. Enhanced mesophyll thickness is particularly critical, as it augments photosynthetic capacity and biomass accumulation [44–45].

## 4.3 Mineral nutrition: macronutrients and micronutrients

Fish manure significantly increased the concentration of essential macronutrients (N, P, K, Ca, Mg) and micronutrients (Fe, Zn, Mn, Cu, Se, B) in wheat shoots. This reflects the nutrient richness of fish-derived fertilizers, which typically contain amino acids, peptides, minerals, and organic acids that improve nutrient mobility and uptake [25]. The improved NPK status directly supports higher protein synthesis, osmotic regulation, enzymatic activity, and stress resilience. Higher Fe, Zn, and Mn contribute to enzyme activation, respiration, and antioxidant pathways, collectively supporting growth and yield formation.

## 4.4 Protein, carbohydrates, gluten, and amino acids: nutritional quality

Nutritional quality improved markedly with fish manure treatments. Crude protein, carbohydrate percentage, gluten, and many essential and non-essential amino acids increased substantially compared to the control. These improvements may be attributed to several interrelated factors. Firstly, the enhanced assimilation of nitrogen from organic sources plays a critical role in promoting plant growth and development. Additionally, an improved supply of micronutrients is instrumental in activating protein synthesis pathways, thereby facilitating various metabolic functions within the plant. Furthermore, increased photosynthesis is achieved through enhanced leaf anatomy, which optimizes light capture and energy conversion. Lastly, the organic acids and peptides provided naturally in fish waste contribute essential nutrients that support overall plant health and vigor, creating a synergistic effect that enhances growth performance. In fact, enhanced amino acids such as leucine, valine, lysine, serine, and glutamic acid reflect improved grain nutritional quality. Previous studies also reported similar improvements in rice, maize, and legumes when supplied with fish-based fertilizers [14, 27]

## 4.5 Correlation structure: linking anatomy, nutrition, and quality

The heat-map analysis revealed a coherent functional network wherein anatomical traits such as cortex thickness, mesophyll thickness, and root diameter, exhibited strong positive correlations with nutrient accumulation, particularly nitrogen, phosphorus, potassium, iron, and zinc. These nutrients, especially nitrogen, iron, zinc, and manganese, showed a robust correlation with protein, gluten, and essential amino acids, indicating that nutrient availability significantly enhances grain quality. Morphological traits like plant height and shoot weight were also found to correlate closely with both anatomical features and mineral nutrition, illustrating a coordinated interplay between structural and physiological improvements. These correlations suggest that anatomical reinforcement enhances the plant's capacity to acquire and transport nutrients, which in turn supports biochemical enhancements such as protein and amino acid synthesis. This integrated physiological loop effectively explains how fish manure not only promotes growth but also simultaneously improves nutritional quality.

## 5. Conclusions

The present study demonstrates that fertilization with fish manure applied alone or in combination with chemical fertilizers constitutes an effective and environmentally sound strategy for improving wheat growth, anatomical development, mineral nutrition, and biochemical quality. Across all morphological traits, fish manure consistently enhanced plant vigor, increasing shoot and root biomass, leaf development, and yield components. Anatomical observations further confirmed these improvements, revealing thicker stem and root structures, expanded cortex and pith tissues, and increased mesophyll thickness, all of which contribute to greater water and nutrient transport capacity and enhanced photosynthetic efficiency. Chemical and nutritional analyses showed that fish manure significantly improved the concentrations of essential macronutrients (N, P, K) and micronutrients (Fe, Zn, Mn, B, Se), while also increasing crude protein, carbohydrates, gluten content, and both essential and non-essential amino acids. These enhancements indicate a clear improvement in the nutritional quality of wheat shoots and highlight the capacity of fish manure to enrich plant tissues with physiologically important compounds. Correlation analysis revealed strong, positive linkages between growth variables, anatomical parameters, and nutritional traits. This integrated structure indicates that improvements in root and shoot anatomy facilitated higher nutrient uptake, which in turn supported greater protein formation, amino acid accumulation, and overall biochemical quality. The positive correlations between N%, protein, amino acids, and yield-related traits emphasize the coherent biological response of wheat to organic fertilization. However, our findings confirm that fish manure is not only a sustainable alternative to mineral fertilizers but also a potent enhancer of plant performance and nutritional value. When combined with moderate chemical fertilizers, its effects are maximized, suggesting an optimal integrated nutrient management strategy. This approach supports both agricultural productivity and environmental protection by reducing dependence on synthetic inputs. Future research should evaluate multi-season field performance and explore fish manure use across different wheat varieties and soil types to expand its application potential.

## Declarations

# Conflicts of Interest:

The authors declare no conflicts of interest.

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# Author Contribution

Conceptualization, Mamdouh Alshemmari and Mejdi Snoussi; methodology, Soumaya Arraouadi, Hafedh Hajlaoui, Yazeed Albulaihed, Ahmed Eisa Mahmoud Ghoniem, and Mejdi Snoussi; software, Hafedh Hajlaoui; validation, Mamdouh Alshemmari, Yazeed Albulaihed, and Mejdi Snoussi; writing—original draft preparation, Mamdouh Alshemmari, Soumaya Arraouadi, Hafedh Hajlaoui, Ahmed Eisa Mahmoud Ghoniem, Yazeed Albulaihed, and Mejdi Snoussi; writing—review and editing, Mamdouh Alshemmari, Soumaya Arraouadi, Hafedh Hajlaoui, Ahmed Eisa Mahmoud Ghoniem, and Mejdi Snoussi; supervision, Mamdouh Alshemmari; project administration, Mamdouh Alshemmari; funding acquisition,

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# Data Availability

The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding author.

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## Figures

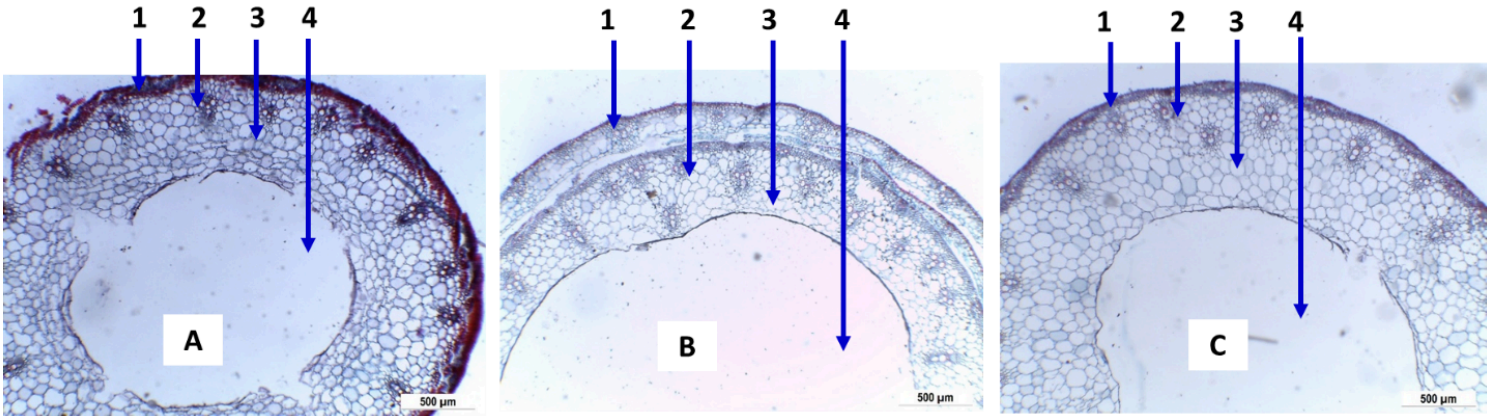


**Figure 1**

Transverse sections (x100) through the stem of wheat plants cv. Yecora Rojo, grown for 45 days in (A=Control, B=Fish manure, C= Fish + Chemical fertilization) treatments.

1 : Epidermis, 2:Vascular Bundle, 3: Stem wall, 4:Hollow Pith Cavity

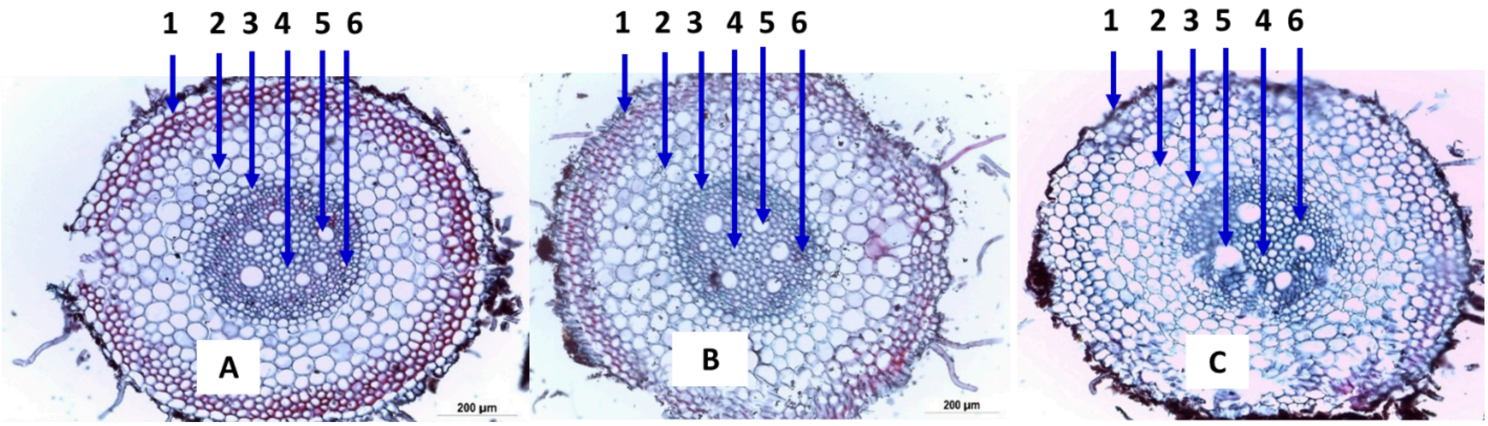


**Figure 2**

Transverse sections (x100) through the root of wheat plants cv. Yecora Rojo, grown for 45 days in (A=Control, B=Fish manure, C= Fish + Chemical fertilization) treatments.

1 : Epidermis, 2:Cortex, 3: Vascular Cylinder, 4: Pith, 5: Meta xylem, 6: phloem

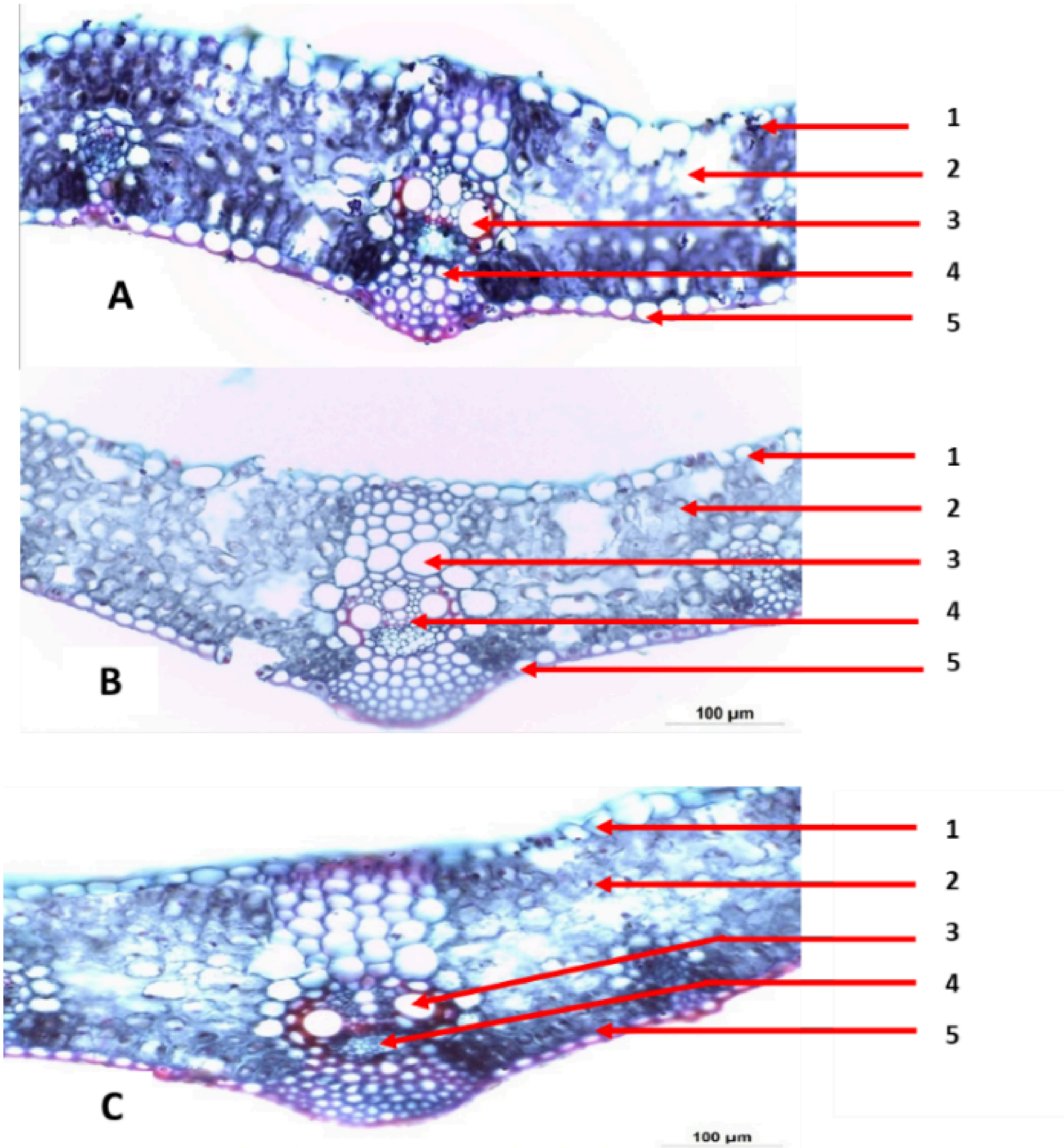


**Figure 3**

Transverse sections (x100) through the leaf of wheat plants cv. Yecora Rojo, grown for 45 days in (A=Control, B=Fish manure, C= Fish + Chemical fertilization) treatments.

1: Upper Epidermis, 2: Mesophyll Tissue, 3: Meta Xylem, 4: Phloem, 5: Lower Epidermis

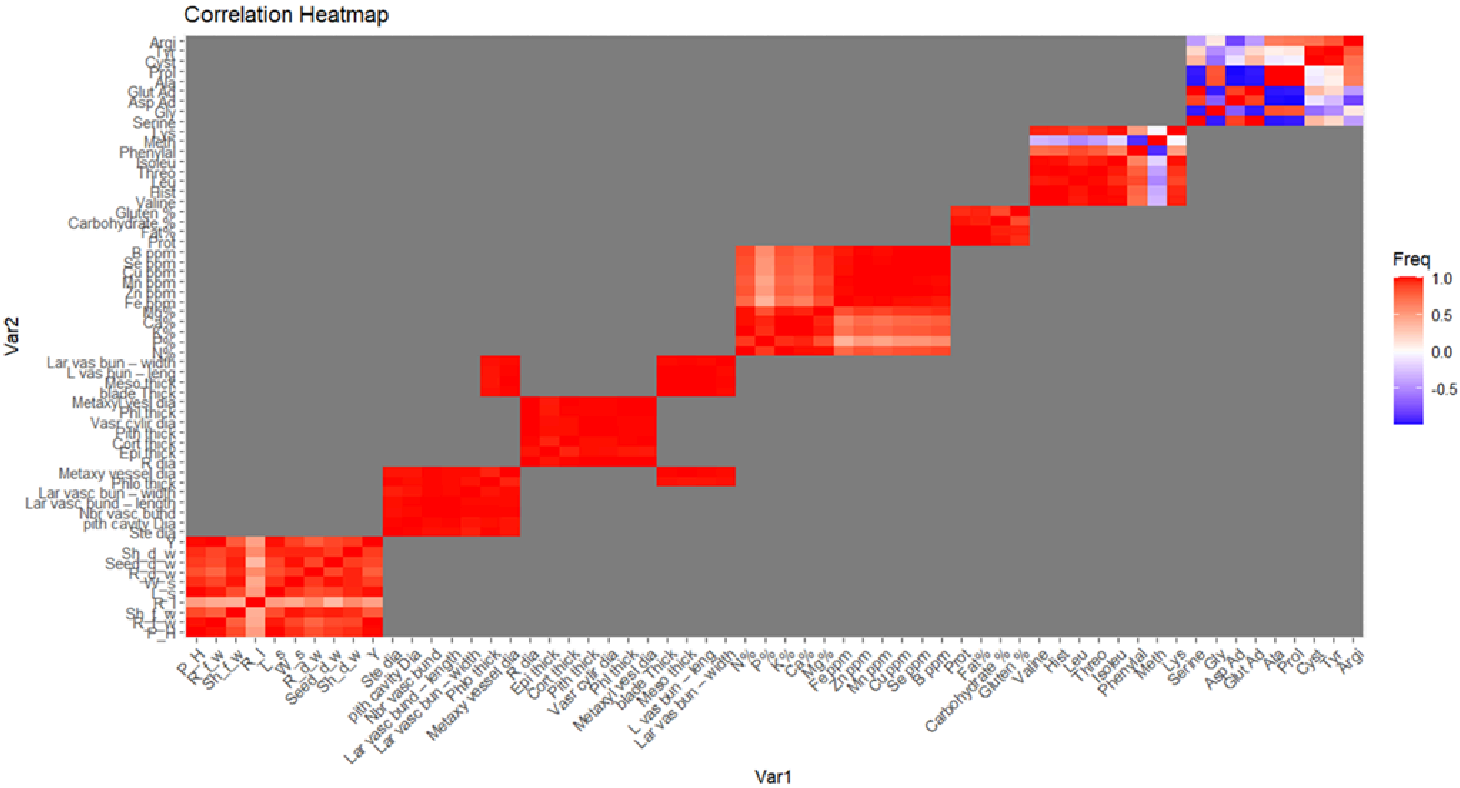


**Figure 4**

Correlation Heatmap Among Anatomical, Nutritional, and Biochemical Traits measured traits.

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