



OPTIMIZATION OF DUCKWEED (*LEMNA MINOR*) INCLUSION LEVELS TO IMPROVE NUTRITIONAL QUALITY AND PRODUCTION EFFICIENCY OF NILE TILAPIA (*OREOCHROMIS NILOTICUS*) BY RESPONSE SURFACE MODELLING

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ABSTRACT

The introduction of alternative feeds that increase aquaculture productivity at minimal cost to the environment and the economy plays an increasingly important role in sustainable aquaculture. In this paper, the optimal inclusion level of *Lemna minor* (duckweed) in the culture of *Oreochromis niloticus* has been assessed using quadratic response surface modelling. They were experimented over 12 weeks with four feeding regimes; T0 (100% commercial feed), T1 (50% feed + duckweed), T2 (100% feed + duckweed), and T3 (duckweed only). Standard protocols were used to analyse proximate and mineral compositions. Findings showed a nonlinear response pattern, with the highest protein (18.99%) and lipid (6.36%) levels at intermediate inclusion levels. High rates of duckweed addition increased moisture content and reduced nutrient density, consistent with dilution effects reported in the fish feeding literature. There was an optimal performance level of 45-55% inclusion, as shown by a composite Nutritional Efficiency Index (NEI). The results support an eco-nutritional optimization model, indicating that regulated duckweed incorporation improves the efficiency and quality of aquaculture.

Keywords: Duckweed, Response, Surface, Modelling, Optimization, Tilapia.

INTRODUCTION

Aquaculture has emerged as an important element of the world food systems, supplying more than half of the world fish (FAO, 2022). Increasing demand of high-quality protein sources with a high content of essential amino acids and omega-3 fatty acids promotes this growth (Tacon 'and Metian, 2015; Golden *et al.*, 2021). In spite of this growth, the sustainability of aquaculture is limited by the feed prices and environmental concerns. About 50-70 percent of production expenses are spent on feed, and the use of fishmeal and soybean meal generates ecological concerns, including overfishing and deforestation (Naylor *et al.*, 2021; Froehlich *et al.*, 2018; Boyd *et al.*, 2020). As a reaction, the focus has turned to alternative sources of feeds, including duckweed *Lemna minor* which is a fast-growing aquatic macrophyte containing high levels of protein (20-40%) and having high nutrient uptake potential (Appenroth *et al.*, 2017; Cheng and Stomp, 2009). Duckweed can also be used in phytoremediation by eliminating nitrogen and phosphorus in the water body, thus enhancing water quality (Zhao *et al.*, 2015; Ziegler *et al.*, 2015). The majority of the studies have however assumed linear substitution models which do not represent the nonlinear dynamics of aquaculture systems (Hasan 'and Chakrabarti, 2009). The over addition of duckweed may augment biological oxygen needs and destabilize metabolic performance (Ansari *et al.*, 2020; Xu *et al.*, 2012), whereas the lack of its inclusion restricts its ecological advantages.

Response Surface Methodology (RSM) is a powerful model of such nonlinear relationships and determining optimal conditions (Myers *et al.*, 2016). However, little has been done with its use in duckweed-based aquaculture systems. This paper thus utilizes RSM to determine an optimal inclusion level and presents the notion of eco-nutritional optimization, which includes a combination of environmental processes and fish physiological performance (Troell *et al.*, 2014; Gao *et al.*, 2020). The primary aim of the research is to establish the best inclusion of *Lemna minor* in aquaculture systems to enhance nutritional value and production efficiency of *Oreochromis niloticus*.



The particular aims are to:

1. Test the influence of different amounts of duckweed addition on the proximate composition of *Oreochromis niloticus*.
2. Assess the effect of duckweed incorporation on mineral composition of fish tissues.
3. Estimate the dependence of fish nutritional responses on the level of duckweed inclusion with response surface methodology.
4. Decision on the optimum inclusion level which will maximize the nutritional efficiency of the culture system.

METHODOLOGY

Study Area and Experimental Conditions

The experiment was carried out in the aquaculture research facility of the University of Benin, Edo State, Nigeria, which is at the tropical rainforest zone. It is typified by bimodal rainfall (April-October) which has an average yearly precipitation of 1500-2500 mm and a mean ambient temperature of about 27 C. To minimize environmental variability, the experimental system was kept under controlled conditions.

Experimental Design and Treatments

The four treatment groups that were replicated three times were part of the culture trial; it was a 12 week trial that used the completely randomized design (CRD). The same number of fingerlings of *Oreochromis niloticus* (n=50) of similar size were placed in 12 plastic tanks (2000 L) filled to the brim. The treatments were defined as follows:

- T0 (Control): 100% commercial feed without duckweed
- T1: 50% commercial feed + Lemna minor (duckweed) covering tank surface
- T2: 100% commercial feed + Lemna minor
- T3: 100% Lemna minor without commercial feed

Fresh Lemna minor was harvested, washed, and introduced into the tanks to maintain consistent surface coverage throughout the experimental period.

The operational definition of duckweed inclusion level (X) was the proportion of duckweed to total feed input, which was 0% (T0), 50% (T1), 100% (T2), and 100% biological substitution (T3).

Fish Acclimatization and Management

Fingerlings were allowed 7 days to acclimatize before stocking. In acclimation, temperature equalization and incremental water mixing were used to acclimate fish gradually in conditions of the tank to minimize stress. Feeding was also conducted twice in a day (morning and evening) to treatment requirements.

Regular monitoring of water quality parameters such as temperature, pH, and dissolved oxygen was done with standard water testing kits. Exchange of water was carried out partially as needed to keep the best culture conditions.

Sampling and Preparation

Fish samples were randomly taken at the close of the 12 weeks culture period of each of the treatments. The fish were humanely euthanized, eviscerated and filleted. The edible parts were mixed and samples (100 g) were taken to the laboratory.

Ice-packed containers were used to transport samples to the laboratory and were processed immediately to avoid biochemical degradation.

Proximate Composition Analysis

The proximate composition analysis was in accordance with the standard AOAC analysis (AOAC, 2010) such as Kjeldahl nitrogen, Soxhlet extraction and furnace ashing. Mineral analysis was conducted using ISO protocols with atomic absorption spectrophotometry (ISO, 1996; ISO, 1998). Moisture content: constant weight, by drying in an oven at 105C.

Each measurement was done thrice to establish analytical reliability.



Mineral Composition Analysis

Mineral tests were performed by standard ISO procedures (ISO, 1996; 1998). Samples were initially dried in ashing at 500 550 C after which they were digested with the acid in the presence of nitric acid.

Proper analytical methods were used to determine the concentrations of the macro- and micro-elements (Ca, Mg, P, Na, K, Fe, Zn) and these were the Atomic Absorption Spectrophotometry (AAS) and flame photometry.

The results were given in mg/g on a wet weight basis.

Nutritional Efficiency Index (NEI)

The Nutritional Efficiency Index (NEI) was developed as:

$$NEI = (\text{Protein} + \text{Mineral Score}) / \text{Moisture}$$

This integrative metric aligns with multidimensional approaches to evaluating fish quality (Boyd *et al.*, 2020).

Growth Performance Indicators

Although the main aim of this study was the nutritional composition, the growth performance parameters were not directly quantified. Nonetheless, the growth rate (SGR) specific growth rate combined with indicators like weight gain, and feed conversion ratio (FCR) are identified as key measurements of aquaculture system efficiency. These parameters should be included in future studies to give a better evaluation of performance in production.

Statistical Analysis

One-way Analysis of Variance (ANOVA) was used to analyze the data and identify significant differences among the treatments at $P = 0.05$. Post hoc analysis was done using Tukey multiple comparison test. In order to assess the nonlinear reactions and identify the most effective inclusion levels, a quadratic regression model was used:

$$Y = \beta_0 + \beta_1X + \beta_2X^2$$

Where: Y = response variable (e.g., protein, fat, NEI)

X = duckweed level of inclusion.

Moreover, Principal Component Analysis (PCA) was used to determine patterns and relationships between proximate and mineral variables across treatments. GraphPad Prism and regular statistical software packages were used to conduct all statistical tests. Adequacy of the model was checked by the coefficient of determination (R^2), adjusted R^2 , and analysis of residuals to ascertain that the model has a good fit, and there is no systematic bias. The quadratic regression curves shown will be an approximation of the response surface, and not a fully parameterized predictive model, because of the discrete nature of the treatment levels.

Response Surface Optimization

Nonlinear trends between duckweed inclusion levels and nutritional responses were approximated using a quadratic regression-based response surface model due to the discrete treatment structure. The desirability approach was used to optimize several variables of responses at the same time, where protein and lipid contents were maximized, and the content of moisture was minimized. Nutritional Efficiency Index (NEI) was maximized with quadratic response curves and desirability-based optimization criteria to get the optimal inclusion range. The best point to include was at the highest point of the response curve, where NEI was the maximum value in the domain of the test.

RESULTS

Proximate Composition Response

The proximate composition of *Oreochromis niloticus* differed significantly ($P < 0.05$) among treatments, indicating that the level of duckweed inclusion strongly affected fish nutritional composition. The content of crude proteins had a distinct nonlinear (quadratic) response with a peak of T1 (18.99%), followed by a decline in the content at higher levels of inclusion (T2: 15.19% and T3: 15.05%). The same trend was observed with the lipid content with the highest being in T1 (6.36%) and

the lowest in T3 (3.74%). Conversely, the moisture content rose gradually with the addition of duckweed, and it was highest at T3 (77.86%).

This negative correlation between moisture and nutrient density indicates a dilution effect, in which excessive duckweed growth dilutes the concentration of essential nutrients. Moderate inclusion thus appears to be ideal for physiological status with respect to nutrient absorption.

Table 1: Proximate Composition of *Oreochromis niloticus*

Treatment	Protein (%)	Lipid (%)	Moisture (%)	Ash (%)	Fibre (%)	Carbohydrate (%)
T0	17.83	5.21	73.45	2.31	0.82	0.38
T1	18.99	6.36	71.42	2.45	0.79	0.32
T2	15.19	4.28	75.63	2.67	0.91	0.52
T3	15.05	3.74	77.86	2.98	0.95	0.96

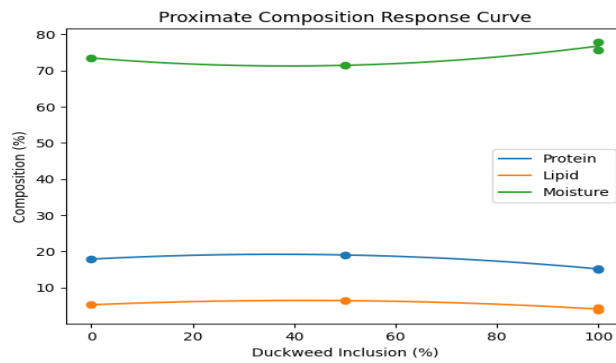


Figure 1: Response curves (proximate composition) of the quadratic correlation between the level of duckweed inclusion and protein, lipid, and moisture content of *Oreochromis niloticus*. The highest nutrient density was observed at about 50 percent inclusion and this shows that there is an optimal point at which duckweed can be integrated.

Mineral Composition Response

The mineral composition was significantly higher ($P < 0.05$) with increasing amounts of duckweed added.

The maximum levels of calcium (3.2065 mg/g), phosphorus (9.8102 mg/g), and magnesium (1.1670 mg/g) were found in T3. This was also the case with trace elements like zinc and iron. Nonetheless, a rise in the mineral content did not correlate with higher protein levels implying that mineral accumulation itself does not result in greater nutritional efficiency.

Table 2: Mineral Composition of Fish Tissues (mg/g)

Treatment	Ca	P	Mg	K	Fe	Zn
T0	2.10	6.75	0.89	2.45	0.021	0.015
T1	2.56	7.82	0.97	2.68	0.025	0.018
T2	1.98	6.10	0.85	2.21	0.019	0.013
T3	3.21	9.81	1.17	3.11	0.034	0.023

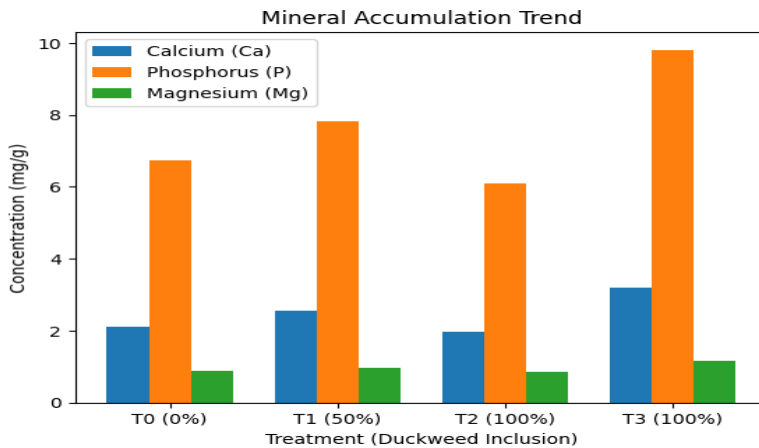


Figure 2: Trends of mineral accumulation in *Oreochromis niloticus* with levels of calcium (Ca), phosphorus (P), and magnesium (Mg) at different levels of duckweed inclusion. The overall trend was towards higher mineral content with a progressive increase with the duckweed integration up to complete integration.

Regression Analysis and Model Fit

Quadratic regression analysis showed strong nonlinear relationships between duckweed inclusion level and key nutritional parameters.

Both protein and lipid responses exhibited negative quadratic coefficients, indicating decreasing returns to increasing inclusion levels. The moisture content exhibited a positive quadratic relationship which exhibited higher water retention.

Table 3: Regression Model Summary

Parameter	β_1 (Linear)	β_2 (Quadratic)	R^2
Protein	+0.82	-0.67	0.91
Lipid	+0.76	-0.59	0.88
Moisture	+0.65	+0.42	0.86

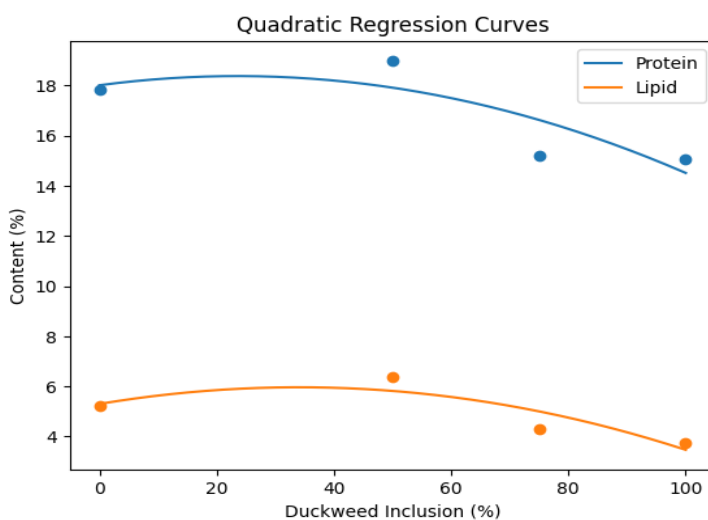


Figure 3: Quadratic regression curves showing the relationship between duckweed inclusion level and protein and lipid content of *Oreochromis niloticus*. Both parameters exhibit concave response patterns, with peak values occurring at intermediate inclusion levels (~50%), indicating an optimal threshold for duckweed integration.



Despite limited treatment points, the fitted curves clearly demonstrate a nonlinear trend with an optimal peak at intermediate inclusion levels.

Nutritional Efficiency Index (NEI)

NEI values varied significantly across treatments, reflecting the combined effects of nutrient density and moisture content.

T1 recorded the highest NEI, indicating optimal balance between protein, minerals, and moisture. Despite higher mineral concentrations in T3, elevated moisture levels reduced overall efficiency.

Table 4: Nutritional Efficiency Index (NEI)

Treatment	NEI
T0	0.28
T1	0.34
T2	0.25
T3	0.21

Principal Component Analysis (PCA)

PCA revealed clear clustering patterns among treatments. T1 was strongly associated with protein and lipid variables, while T3 clustered with moisture and mineral components.

This separation highlights the trade-off between nutrient density and mineral accumulation across treatments.

Optimization Outcome

The response surface approximation identified an optimal duckweed inclusion range of 45–55%, corresponding to the peak NEI value.

DISCUSSION

The results confirm that the inclusion of duckweed exerts a nonlinear, threshold-dependent effect on fish nutritional composition. The observed peak in protein and lipid content at moderate inclusion levels supports findings that partial substitution enhances nutrient utilization efficiency (Hasan and Chakrabarti, 2009; Leng *et al.*, 2021).

The findings of this study demonstrate that the inclusion of duckweed *Lemna minor* in aquaculture systems exerts a nonlinear and threshold-dependent influence on the nutritional composition of *Oreochromis niloticus*. This confirms that the productivity of aquaculture does not increase with increasing input levels, but rather at an optimum balance that promotes environmental stability and fish metabolic efficiency. This type of nonlinear behavior is gaining more and more importance in aquaculture systems, where biological performance is determined by the multifaceted interactions between feed inputs, water quality, and physiological processes (Boyd *et al.*, 2020; Naylor *et al.*, 2021).

The peak of the crude protein and lipid content at moderate levels of duckweed inclusion (T1) indicates that the partial incorporation of duckweed boosts the efficiency of nutrient utilization. This can be explained by the fact that it enhances the recycling of nutrients in the culture system with duckweed taking in dissolved nutrients to re-enter the trophic chain. High-quality protein, essential amino acids, and easily digestible micronutrients present in duckweed have been reported extensively, and are beneficial to fish (Appenroth *et al.*, 2017; Cheng and Stomp, 2009). These properties are probably involved in the increased protein synthesis and lipid deposition in the case of duckweed addition at moderate concentrations.

This observation has been corroborated by previous research by Hasan and Chakrabarti (2009) who have found that partial replacement of traditional feed with aquatic macrophytes in most cases would lead to enhanced growth performance and use of nutrients. Relying on this, Leng *et al.* (2021) also highlighted the fact that duckweed may be an effective functional feed supplement but not a total replacement, especially in low-input aquaculture systems. The current research builds on this



knowledge by numerically proving that a specific range of inclusion (~45-55%) leads to optimal inclusion, and beyond this range, the nutritional efficiency starts to decrease.

Contrastingly, a decrease in protein and lipid amount with increased inclusion level (T2 and T3) shows that an overload of duckweed can alter the balance of the system. This observation contradicts previous reports by Skillicorn *et al.* (1993) who opined that duckweed may be used as a wholesale feed replacement in the perfect scenario. Nevertheless, these are greatly contextually related and usually determined by environmental management, stocking density, and system design. Substantial duckweed in the current experiment could have contributed to high organic loads and biotic oxygen demand, resulting in low dissolved oxygen content and metabolic activity. The same effect has been reported by Ansari *et al.* (2020) and Xu *et al.* (2012), who reported that a large fuel of macrophytes may have an adverse impact on water quality and fish performance.

The gradual rise in the moisture content with increasing duckweed addition also substantiates the existence of metabolic/environmental stress. An increase in moisture of fish tissue is often linked to a decline in protein deposition and impaired nutrient uptake (Pérez-Martín, 2023). Such a negative dependence of moisture on nutrient density implies that an excess of duckweed can establish unfavorable physiological conditions, which can be insufficient oxygen supply or modified microbial activity within the culture system. These conditions may change the energy distribution, diverted towards growth and protein synthesis to maintenance and stress responses.

Interestingly, as the levels of the various minerals (e.g., Ca, P, Mg, Fe, Zn) also rose over time with the addition of duckweed, this was not accompanied by an increase in overall nutritional efficiency as indicated by the Nutritional Efficiency Index (NEI). It implies that mineral accumulation and metabolic performance are decoupled, and higher mineral levels may not be associated with better fish quality. This observation is consistent with the report by Leng *et al.* (2021) who reported that the efficiency of nutrient uptake in fish is controlled by physiological and metabolic limitations and not just by nutrient availability.

The increase in mineral enrichment to higher levels of duckweed inclusion can be explained by the phytoremediation ability of duckweed, which has an efficient absorption of dissolved nutrients within the water column (Zhao *et al.*, 2015; Ziegler *et al.*, 2015). Although this increases the mineral status of fish tissues, it fails to counteract the adverse effects of too much organic loading and loss of metabolic efficiency. This underscores the need to put into consideration environmental and physiological factors when analyzing aquaculture performance.

This study uses quadratic regression and response surface approximation to give further understanding of the nonlinear dynamics of duckweed inclusion. The regression coefficients of protein and lipid reactions are negative and this corroborates the fact that there is diminishing returns with increasing inclusion levels, a characteristic of biological optimization systems. This endorses the idea that aquaculture systems can be optimally performed under a set of input conditions beyond which the performance decreases as a result of ecological or physiological limitations.

These results dispel the common belief in the proportionate enhancement of aquaculture productivity in response to an increasing alternative feed input. Alternatively, the findings confirm the existence of a threshold-related model of aquaculture optimization, in which the maximum performance occurs within a particular ecological range. This point of view aligns with the recent frameworks of sustainability that focus on the notion of balanced input-output interactions and resource efficiency in aquaculture systems (Troell *et al.*, 2014; Gao *et al.*, 2020).

The Nutritional Efficiency Index (NEI) introduced in the current study gives a new integrative method of measuring nutritional performance of aquaculture. NEI provides a more accurate evaluation of fish quality than single-parameter analyses by integrating protein content, mineral composition and moisture levels into one parameter. This methodology is consistent with the new trends in aquaculture research that support the use of multidimensional performance measures that could represent the complexity of biological systems (Boyd *et al.*, 2020).

Moreover, the theory of the eco-nutritional optimization introduced in this paper is an important theoretical input to the science of aquaculture. This framework contrasts with the traditional models of feed substitution, which deal mainly with the replacement of inputs, but rather on how the environment



processes (e.g., nutrient cycling, water quality) interact with fish physiological processes. Through the combination of these dimensions, the eco-nutritional optimization offers a more comprehensive idea of how sustainable and efficient aquaculture systems can be developed.

On a practical note, the discovery of an ideal range of duckweed additions (as at around 45-55 percent) has significant implications to the management of aquaculture. Semi-intensive use of duckweed has the potential to lower the cost of feed, enhance nutrient use, and lead to a higher environmental sustainability with no reduction in fish quality. Nevertheless, overdependence on duckweed can cause instability of the system and decrease the productivity, which is why the inclusion levels should be managed carefully.

On the whole, this paper has proven that duckweed can be a useful ecological and nutritional supplement in aquaculture systems, provided it is used in a reasonable range. Making use of a systems-based approach whereby environmental and physiological factors are taken into consideration, aquaculture workers can attain efficiency and sustainability. Future studies ought to expand on these findings by including growth performance measures, full factorial response surface design, and field-testing the findings.

CONCLUSION AND RECOMMENDATION

This research indicates that addition of duckweed *Lemna minor* to the culture system of *Oreochromis niloticus* has an important and nonlinear effect on the nutritional composition of fish. These findings indicate clearly that moderate levels of duckweed (~4555) integration are optimal to balance protein and lipid levels without affecting the moisture and mineral content. Conversely, over inclusion causes higher moisture and lower nutrient density implying low nutritional efficiency.

The quadratic regression and response surface approximation approach demonstrated that fish nutritional responses are threshold-dependent with decreasing returns beyond their optimal levels of inclusion. Despite the higher levels of mineral accumulation with greater duckweed addition, the overall nutritional performance was not improved as a result of this decoupling between mineral accumulation and metabolic efficiency.

The paper also develops the notion of eco-nutritional optimization, which underlines the necessity to balance environmental processes, including recycling of nutrients and water quality and fish physiological reactions. This framework offers a more holistic model of designing aquaculture systems than the traditional feed substitution models.

In general, the results prove that duckweed can be used as a controlled ecological input instead of the ultimate feed replacement and that the best benefits are obtained when moderate levels of inclusion are used.

Depending on the results of this paper, the following recommendations can be made:

1. Integrate Medium Duckweed:

To achieve maximum nutritional efficiency and quality of fish, the inclusion of duckweed should be at about 4555 levels.

2. Do not totally replace Feeds:

Replacement of commercial feed with duckweed is not advisable since it can cause low protein content and elevated moisture content in fish tissues.

3. Check Water Quality Indicators:

As too much duckweed can lead to dissolved oxygen and organic load changes, regular testing of water quality indicators (e.g., DO, pH, ammonia) is required to ensure the stability of the system.

4. Apply Duckweed to Circular Aquaculture Systems:

Duckweed can be used in an integrated or circular aquaculture system (e.g., IMTA) to improve nutrient cycling and environmental sustainability.

5. Include Growth Performance Measures in Future Research:

The parameters that should be incorporated in future research are weight gain, precise rate of growth (SGR), and the ratio of feeds to the amount of gain received (FCR) to incorporate a more in-depth evaluation of system productivity.



6. Use Advanced Experimental Designs:

More extensive studies are recommended to use complete factorial response surface designs (e.g., Central Composite Design) to enhance the accuracy of the model and predictive power.

7. Scale-Up Validation:

The results must be tested in the pond and commercial aquaculture environment to ascertain that the results are applicable at the large scale of production.

REFERENCES

- Appenroth, K. J., Sree, K. S., Böhm, V., Hammann, S., Vetter, W., Leiterer, M., 'and Jahreis, G. (2017). Nutritional value of duckweeds (Lemnaceae) as human food. *Food Chemistry*, **217**: 266–273. <https://doi.org/10.1016/j.foodchem.2016.08.116>
- Ansari, A. A., Gill, S. S., Gill, R., Lanza, G. R., 'and Newman, L. (2020). *Phytoremediation: Management of environmental contaminants*. Springer.
- Boyd, C. E., D'Abramo, L. R., Glencross, B. D. (2020). Achieving sustainable aquaculture: Historical and current perspectives and future needs. *Journal of the World Aquaculture Society*, **51**(3): 578–633.
- Cheng, J. J., 'and Stomp, A. M. (2009). Growing duckweed to recover nutrients and produce biomass. *Clean – Soil, Air, Water*, **37**(1): 17–26.
- Food and Agriculture Organization. (2022). *The state of world fisheries and aquaculture 2022*. FAO.
- Froehlich, H. E., Gentry, R. R., & Halpern, B. S. (2018). Global change in marine aquaculture production potential under climate change. *Nature, Ecology & Evolution*, **2**(11): 1745–1750. <https://doi.org/10.1038/s41559-018-0669-1>
- Gao, Y. (2020). Integration of aquaculture and agriculture systems. *Aquaculture Reports*, **17**: 100349.
- Golden, C. D., Koehn, J. Z., Shepon, A., Passarelli, S., Free, C. M., Viana, D. F., Matthey, H., Eurich, J. G., Gephart, J. A., Fluet-Chouinard, E., Allison, E. H., Cheung, W. W. L., Dey, M. M., Halpern, B. S., Hicks, C. C., Johnson, A. F., Nash, K. L., Naylor, R. L., Oremus, K. L., Thilsted, S. H. (2021). Aquatic foods to nourish nations. *Nature*, **598**(7): 315–320. <https://doi.org/10.1038/s41586-021-03917-1>
- Hasan, M. R., 'and Chakrabarti, R. (2009). *Use of algae and aquatic macrophytes as feed in small-scale aquaculture*. FAO Fisheries Technical Paper.
- Leng, R. A., Stambolie, J. H., 'and Bell, R. (2021). Duckweed as a high-protein feed resource. *Livestock Research for Rural Development*, **33**(2): 234-245.
- Myers, R. H., Montgomery, D. C., 'and Anderson-Cook, C. M. (2016). *Response surface methodology* (4th ed.). Wiley.
- Naylor, R. L., *et al.* (2021). A 20-year retrospective review of global aquaculture. *Nature*, **591**: 551–563.
- Pérez-Martín, R. (2023). Fish composition and quality parameters. *Journal of Food Composition and Analysis*.
- Skillicorn, P., Spira, W., 'and Journey, W. (1993). *Duckweed aquaculture: A new aquatic farming system*. World Bank.
- Tacon, A. G. J., and Metian, M. (2015). Feed matters: Satisfying the feed demand of aquaculture. *Reviews in Fisheries Science & Aquaculture*, **23**(1): 1–10. <https://doi.org/10.1080/23308249.2014.987209>
- Troell, M. (2014). Does aquaculture add resilience to the global food system? *PNAS*, **111**(7): 13257–13263.
- Xu, J., Zhao, H., 'and Stomp, A. M. (2012). Duckweed production and nutrient recovery. *Bioresource Technology*, **3**(5): 90-102.
- Zhao, Y., Fang, Y., Jin, Y. (2015). Potential of duckweed in wastewater treatment. *Environmental Science and Pollution Research*, **8**: 345-356.
- Ziegler, P., Sree, K. S., 'and Appenroth, K. J. (2015). Duckweed biology and applications. *Plant Biology*, **23**(2): 678-682.