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Virgin Coconut Oil and Folic Acid Alone or Combined Modulate Growth Hormone Expression in Rotenone-Induced Stunted Zebrafish Larvae

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Abstract
Stunting is a global health problem associated with impaired linear growth and decreased growth hormone (GH) expression, often linked to oxidative stress. Both Virgin Coconut Oil (VCO) and folic acid possess antioxidant properties that may restore growth pathways. The present study sought to evaluate the impact of VCO and folic acid, administered alone or in combination, on GH expression within a rotenone-based zebrafish larvae stunting model. This investigation employed a true experimental design employing post-test only controlled groups, utilizing zebrafish larvae (*Danio rerio*) as the biological model. The study subjects were categorized into five distinct groups: normal control, negative control, VCO at 6.25% concentration, folic acid at 70 µM, and a combined treatment group. Quantification of GH expression was performed at 9 days post-fertilization (dpf) via RT-qPCR methodology. Statistical evaluation employed one-way ANOVA followed by Tukey's HSD post hoc test. Intergroup analysis revealed statistically significant variation in GH expression levels ($p < 0.001$). The negative control showed the lowest expression. Within the treatment arms, VCO showed the highest increase in GH expression (0.9122 ± 0.09704), followed by folic acid (0.5186 ± 0.24155), while the combination showed minimal improvement (0.0563 ± 0.01577). VCO and folic acid individually improved GH expression. However, their combination did not provide additional benefits, indicating a lack of synergistic effect, which may be associated with dose-dependent interactions in GH regulation.

Keywords: folic acid, growth hormone, stunting, VCO

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Minyak Kelapa Murni dan Asam Folat Secara Tunggal Maupun Kombinasi Memodulasi Ekspresi *Growth Hormone* pada Larva *Zebrafish* Model *Stunting* yang Diinduksi Rotenon

Abstrak
Stunting merupakan masalah kesehatan global yang berkaitan dengan gangguan pertumbuhan linier yang berhubungan dengan penurunan ekspresi *growth hormone* (GH) dan sering dikaitkan dengan stres oksidatif. *Virgin Coconut Oil* (VCO) dan asam folat memiliki sifat antioksidan yang berpotensi memulihkan jalur pertumbuhan. Studi ini dirancang untuk mengevaluasi dampak pemberian VCO dan asam folat, baik secara individual maupun dalam bentuk kombinasi, terhadap ekspresi GH pada larva zebrafish yang mengalami stunting akibat diberikan rotenon. Eksperimental laboratorium dengan pendekatan *post-test only controlled group*, digunakan untuk desain studi ini, juga dengan memanfaatkan larva *zebrafish* (*Danio rerio*) sebagai hewan model. Subjek penelitian dibagi ke dalam lima kelompok perlakuan, meliputi kelompok kontrol negatif, kontrol positif, perlakuan VCO (6,25%), perlakuan asam folat (70 µM), serta kelompok yang menerima kombinasi kedua zat tersebut. Pengukuran ekspresi GH dilaksanakan pada hari ke-9 setelah fertilisasi dengan menggunakan metode RT-qPCR. Data yang diperoleh kemudian dianalisis menggunakan uji one-way ANOVA dan dilanjutkan dengan uji post hoc Tukey's HSDTerdapat perbedaan yang signifikan pada ekspresi GH antar kelompok($p < 0,001$). Kelompok kontrol positif menunjukkan ekspresi terendah. Di antara kelompok perlakuan, VCO menunjukkan peningkatan ekspresi GH tertinggi (0,9122 ± 0,09704), diikuti oleh asam folat (0,5186 ± 0,24155), sedangkan kombinasi keduanya menunjukkan peningkatan yang minimal (0,0563 ± 0,01577). VCO maupun asam folat yang diberikan secara terpisah terbukti mampu meningkatkan ekspresi GH. Meskipun demikian, kombinasi keduanya tidak memberikan manfaat tambahan, yang mengindikasikan kemungkinan tidak adanya efek sinergis dan berkaitan dengan interaksi yang bergantung pada dosis dalam regulasi GH.

Kata Kunci: asam folat, growth hormone, stunting, VCO

1. Introduction

Stunting is defined as a state of chronic growth failure in children, arising from sustained nutritional insufficiency and recurrent infectious episodes, and is clinically identified when a child's height-for-age measurement falls more than two standard deviations below the WHO-established reference median.1 The 2024 WHO data revealed that 150.2 million children aged under five were affected by stunting.[2]

Chronic malnutrition and recurrent infections can trigger excessive reactive oxygen species (ROS) accumulation, driving the onset of oxidative stress and chronic inflammation that disrupt cellular homeostasis.[3] These conditions may impair growth hormone (GH) signaling, thereby disrupting the growth hormone–insulin-like growth factor-1 (GH–IGF-1) axis, which plays a central role in linear growth regulation. GH, as an upstream regulator in this axis, stimulates hepatic and peripheral production of insulin-like growth factor-1 (IGF-1), which subsequently mediates cell proliferation, protein synthesis, and skeletal growth. Disruption of GH signaling may therefore reduce downstream IGF-1 activity and impair activation of growth-related pathways.[4] Therefore, evaluation of GH expression remains relevant because it reflects upstream endocrine regulation associated with growth impairment under oxidative stress conditions.

Beyond its physical manifestations, stunting arising from impaired growth carries enduring consequences for long-term health trajectories and quality of life, necessitating the exploration of alternative therapeutic strategies. In this regard, Virgin Coconut Oil (VCO) has emerged as a noteworthy candidate, valued for its distinctive medium-chain fatty acid composition, particularly its lauric acid content, and its established antioxidant and anti-inflammatory activities.[5] VCO is capable of inhibiting ROS, which contribute to oxidative stress and increased production of pro-inflammatory cytokines that are known to suppress GH expression and reduce activity of growth signaling pathways. By reducing ROS, GH expression may increase, consequently activating anabolic pathways that mediate bone elongation.[6]

In addition, folic acid plays an essential role in one-carbon metabolism. Through its involvement in the folate cycle, folic acid contributes to the formation of nicotinamide adenine dinucleotide phosphate (NADPH), which is required for the regeneration of reduced glutathione (GSH) from its oxidized form (GSSG).[7] This mechanism helps suppress the generation of ROS and maintain cellular redox homeostasis.[8] Furthermore, the reduction in ROS levels leads to decreased oxidative stress and inflammation, both of which are known to inhibit the activity of the GH–Insulin-like Growth Factor 1 (IGF-1) axis.[4]

VCO is recognized for its potent antioxidant activity due to its phenolic compounds and medium-chain fatty acids, which are capable of enhancing antioxidant enzyme activity and inhibiting the generation of ROS. These medium-chain fatty acid components can integrate into phospholipid membranes, thereby stabilizing membrane structure and inhibiting lipid peroxidation. This process results in lipophilic antioxidant activity that acts directly within the cellular lipid phase to suppress ROS accumulation and reduce oxidative stress in tissue.[9]

Similarly, folic acid has been shown to enhance systemic antioxidant status. By supporting NADPH production and glutathione (GSH) regeneration through one-carbon metabolism, this activity generates hydrophilic antioxidants that function within the cytoplasmic fluid phase, thereby contributing to the systemic reduction of ROS.[7]

When VCO and folic acid are combined, their respective antioxidant effects are expected to complement one another, thereby enhancing the cellular capacity to neutralize ROS and inhibit the development of chronic inflammation and oxidative stress more effectively. The selection of intervention dosages in this study was based on previous experimental findings. Earlier studies demonstrated that VCO at a concentration of 6.25% showed the most optimal biological effect in zebrafish models.[10] While folic acid at 70 µM was reported to produce significant improvements in growth-related parameters.[11] Rotenone, as one of the environmental toxicants, exerts its effects by blocking Complex I function within the mitochondrial Electron Transport Chain. This inhibition disrupts normal electron flow, leading to electron accumulation and leakage within the mitochondrial matrix. The leaked electrons subsequently react with oxygen, generating Superoxide ($O_2^{\bullet-}$), the primary form of ROS. Elevated ROS levels induce oxidative stress and activate inflammatory pathways, leading to the secretion of pro-inflammatory cytokines that exacerbate chronic.[3] These conditions may impair growth hormone (GH) signaling, thereby disrupting the GH–IGF-1 Axis, which plays a central role in linear growth regulation.[4]

A study has shown that rotenone can be utilized as an induction agent to establish a stunting model in Zebrafish larvae. Administration of rotenone at a concentration of 12.5 ppb from 2 hours post-fertilization (hpf) to 3 days post-fertilization (dpf) was shown to inhibit larval body length growth compared with both the control group and other treatment groups. These

findings further strengthen the evidence that rotenone is capable of inducing stunting conditions in zebrafish and may serve as a reliable experimental model for evaluating various therapeutic interventions.[12]

This investigation aimed to examine the influence of GH expression following the administration of VCO, folic acid, and their combination in a zebrafish larvae-based stunting model induced by rotenone. These agents were expected to attenuate stunting-related impairments and provide a scientific foundation for natural therapeutic development. However, existing interventions tend to concentrate predominantly on nutritional supplementation or pharmaceutical approaches, studies evaluating the mechanistic impact of VCO and folic acid on GH regulatory pathways *in vivo* using biological stunting models remain limited. Therefore, this research offers novel perspectives on a natural and more attainable therapeutic approach that addresses stunting mechanisms associated with oxidative stress, thus advancing scientific knowledge in the exploration of alternative interventions aimed at promoting growth.

2. Materials and Method

This study was designed as a laboratory experimental investigation adopting a post-test only controlled group framework, with Danio rerio specimens maintained in aquaria at the Reproduction Laboratory, Faculty of Fisheries and Marine Sciences, Universitas Brawijaya. Developmental monitoring spanned from embryonic stages through to larval stages. Subjects were randomly assigned to treatment groups and subsequently benchmarked against both the normal and negative control groups following the administration of treatments based on predetermined parameters.A total of 450 healthy zebrafish (Danio rerio) embryos at 0–2 hours post-fertilization (hpf). Healthy embryos were identified under an optilab microscope based on the presence of a clear and transparent chorion, spherical morphology, and the absence of fungal infection. The selected embryos were allocated into five experimental groups using manual randomization, with 30 embryos per well and three biological replicates per group. These groups consisted ofnormal control group (Only administered embryonic medium), negative control group (Only administered 12.5 ppb rotenone without VCO or folic acid intervention, Treatment 1 (Administered VCO intervention at a concentration of 6.25%), , Treatment 2(Administered folic acid intervention at a concentration of 70 µM), and Treatment 3 (Administered a combination intervention of VCO at 6.25% concentration and folic acid at 70 µM). . All experimental procedures were conducted under controlled laboratory conditions, with treatment administration initiated at 2 hpf and continued until 72 hpf, daily media replacement, and scheduled observations performed at 3, 6, and 9 dpf to ensure research reproducibility, By employing this controlled experimental design, meaningful comparisons across groups were guaranteed and dependable reproducibility in subsequent research was made possible.

2.1. Materials

The VCO utilized in this study was VCO Palm 7, a commercially available product manufactured by KWT Vigur Asri (Cemoro Kandang, Malang, Indonesia), derived from fresh coconut meat through cold-press method that excludes heat treatment and chemical additives, yielding a product naturally abundant in MCFAs.The folic acid employed was Sigma F7876, provided in the form of a pale yellow crystalline powder with stability under both elevated temperatures and humid conditions. All preparation and treatment procedures involving VCO and folic acid were performed using Iwaki-brand laboratory equipment, including 6-well culture plates, micropipettes, 48-well culture plates, and glass beakers, designated for larval maintenance and compound administration. Molecular analysis via Reverse Transcription quantitative Polymerase Chain Reaction (RT-qPCR) was carried out using RNA extraction kits from Geneaid Biotech, a CFX Opus 96 real-time PCR instrument, PCR tubes supplied by Gunster Biotech, and sterile single-use consumables. At every stage of the experimental workflow, suitable protective gear, consisting of gloves and face masks, was consistently worn to preserve sterility and uphold laboratory safety standards.

2.2. Methods

2.2.1. Formulation of VCO Concentrations

The VCO concentration applied in this investigation was selected on the basis of prior experimental evidence indicating that 6.25% represented the most biologically effective dose.[10] To formulate the VCO working solution, 4 mL of VCO was combined with 80 µL of DMSO, after which distilled water was added incrementally until a total volume of 64 mL was reached. The resulting mixture was then processed through sonication to achieve uniform dispersion, yielding a homogeneous 6.25% VCO solution ready for administration.[10]

2.2.2. Formulation of Folic Acid Concentrations

The folic acid concentration adopted in this study was established by reference to prior investigations reporting 70 µM as the dose yielding the most optimal biological response.[11] A stock solution of 5 mM (5000

μM) was first formulated by dissolving 22.07 mg of folic acid powder alongside 7 mg of Na_2CO_3 in distilled water, brought to a final volume of 10 mL. From this stock, 0.896 mL was withdrawn and subsequently diluted with distilled water to a total volume of 64 mL, thereby attaining the target working concentration of 70 μM.[11]

2.2.3. Formulation of Combined VCO and Folic Acid Concentrations

Referring to previously established optimal doses, the combination solution was formulated using 6.25% VCO and 70 μM folic acid.[10,11] Formulation began with the addition of 0.896 mL folic acid solution into 4 mL of VCO, followed by gradual introduction of distilled water until the total volume reached 64 mL. To ensure a homogeneous mixture, the solution was then subjected to sonication prior to use.

2.2.4. Rotenone- Induced Stunting Model

Stunting in zebrafish larvae was induced through the administration of rotenone. Within this study, the negative control group was constituted by zebrafish larvae subjected solely to rotenone exposure, functioning as the basis for establishing a stunting model, serving as the pathological reference for comparison with treatment groups. Rotenone was solubilizedin DMSO to yield a working concentration of 12.5 ppb.[13] The negative control was subjected to this rotenone concentration, whereas the normal control group received no rotenone, VCO, or folic acid and was maintained solely in embryonic medium for the study's duration. An equivalent rotenone concentration was applied uniformly across all treatment groups. Treatment 1 was designated to receive rotenone in conjunction with VCO, Treatment 2 was administered rotenone alongside folic acid, and Treatment 3 was given rotenone in combination with both VCO and folic acid simultaneously. The success of the rotenone-induced stunting model was evaluated by measuring the body length of zebrafish larvae at 3 dpf. The induction was considered successful if the body length of larvae in the negative control group showed a significant difference compared to that of larvae in the normal control group.

2.2.5. Preparation of Embryonic Medium

Embryonic medium for zebrafish was formulated from four ionic compounds: potassium chloride (KCl), magnesium sulfate ($MgSO_4$), sodium chloride (NaCl), and calcium chloride ($CaCl_2$), as its constituent components. Before application, the concentrated stock was diluted using distilled water at a stock-to-water ratio of 1:9 before being applied to the experimental wells.[13]

2.2.6. Experimental Animal Model

This study received ethical clearance from the Research and Innovation Ethics Committee of Universitas Brawijaya, under approval number 490/EC/KEPK-S2/12/2025. The experimental animal model adopted in this investigation was zebrafish larvae (Danio rerio), with embryos at 2 hpf designated as the research subject, and larval development monitored throughout 0 to 9 dpf. The selection of zebrafish was attributed to their close physiological resemblance to humans, along with several advantages that render them particularly suitable for stunting investigation, encompassing their rapid and optically transparent embryonic development, as well as the feasibility of manipulating both dietary and environmental conditions.[14] With regard to developmental equivalence, in terms of developmental equivalence, a 3 dpf zebrafish larva approximates the maturation level of a human at birth, and by 6 dpf, the larva reaches a stage comparable to early childhood around age two, and larvae reaching 9 dpf reflect a developmental stage akin to that of a human child aged approximately 8 years.[15]

2.2.7. Measurement of Growth Hormone (GH) Expressions RT-qPCR

GH expression analysis was carried out on larvae at 9 dpf, with quantification achieved through the application of RT-qPCR. Following the harvesting of zebrafish larvae, total RNA extraction was performed with the RNA Mini Kit Tissue (Genoid), after which the isolated RNA underwent reverse transcription into cDNA through the use of ReverTra Ace qPCR RT Master Mix with gDNA Remover (Toyobo).

The base sequences of the primers employed are provided in Table 1, and the corresponding RT-qPCR protocol is detailed in Table 2.

Amplification was executed on a real-time PCR instrument (CFX Opus 96), with each reaction

Table 1. Primer base sequence of Growth Hormone

Gene	Forward Primer	Reverse Primer
GH	5'-GCA TCA GCG TGC TCA TCA A-3'	5'-CCC TAC GGT CAG GTA GAA ATC C -3'
β-Actin	5'-CGA GCA GGA GAT GGG AAC-3'	5'-CAA CGG AAA CGC TCA TTG C-3'

Table 2. RT-qPCR protocol

Step	GH
Pre-denaturation (time/temperature)	2'/95°C
Denaturation (time/temperature)	5"/95°C
Annealing (time/temperature)	30"/60°C
Cycles	40 *cycles*

Note:
' : Minutes
" : Seconds

consisting of the following components: 7.4 µL of nuclease-free water, 10 µL of SensiFAST SYBR, 0.8 µL of forward primer, 1 µL of cDNA template, and 0.8 µL of reverse primer.

2.2.8. Statistical Data Analysis

Statistical processing was conducted using SPSS software version 27. Group comparisons were carried out by means via one-way ANOVA with Tukey's HSD as the post hoc procedure, with $p < 0.05$ considered statistically significant. ΔCt values served as the basis for data analysis, while determined through the $2^{-\Delta\Delta Ct}$ approach and presented as fold change values in graphical form.

3. Result

GH expression was quantified across all experimental groups in zebrafish larvae at 9 dpf following euthanasia, using RT-qPCR as the detection method. The bar chart of RT-qPCR results of GH expression across all experimental groups are presented in Figure 1. Analytical procedures were based on ΔCt values. Shapiro-Wilk normality testing confirmed that the ΔCt distribution did not deviate significantly from normality ($p = 0.992$), and Levene's homogeneity of variance test verified data homogeneity ($p = 0.179$). The ΔCt dataset thus satisfied the prerequisite assumptions for parametric statistical testing.

One-way ANOVA applied to the GH ΔCt values demonstrated statistically significant differences across the experimental groups ($p < 0.001$). To facilitate visual interpretation, relative gene expression data were transformed and presented as fold change values ($2^{-\Delta\Delta Ct}$) in Figure 2, derived from the same ΔCt dataset used in the primary statistical analysis. Among all experimental groups, the highest mean fold change was recorded in the normal control group (1.0175 ± 0.23136), whereas the negative control group showed the lowest mean fold change (0.0533 ± 0.02859). This finding is likely attributed to the fact that the normal control group was the only group not exposed to rotenone induction, receiving only embryonic medium treatment. Therefore, this group represented normal physiological conditions and maintained the highest gene expression level among all groups. Within the treatment groups, the most pronounced GH upregulation was recorded in the VCO only group (T1), yielding a mean fold change of 0.9122 ± 0.09704. Nevertheless, the magnitude of elevation in T1 exhibited only a marginal difference relative to that of the T2 group, which recorded a mean fold change of 0.5186 ± 0.24155. The T3 group, in contrast, displayed a comparatively lower elevation than both T1 and

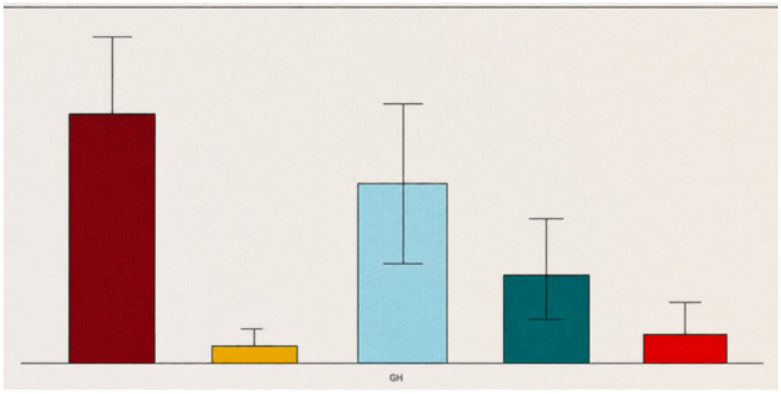

Figure 1. Bar chart of RT-qPCR results of GH expression

Note:
: Normal Control
: Negative Control
: VCO 6,25%
: Folic Acid 70 µM
: Combination of VCO 6,25% and Folic Acid

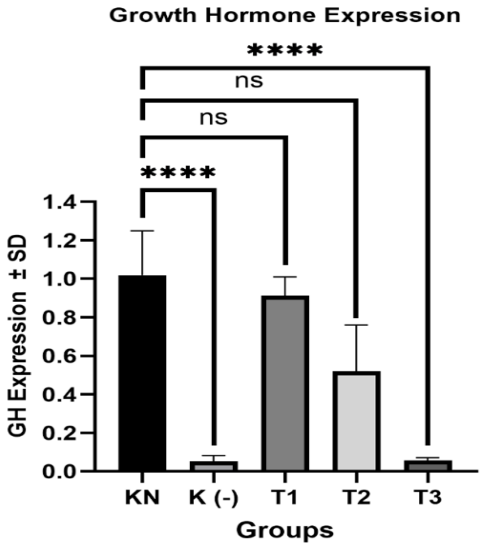

Figure 2. The effect of virgin coconut oil, folic acid, and their combination on GH expression in zebrafish larvae stunting model.

Note:
KN : Normal Control
K (-) : Negative Control
T1 : VCO 6,25%
T2 : Folic Acid 70 µM
T3 : Combination of VCO 6,25% and Folic Acid 70 µM
ns : Not significant
**** : p ≤ 0,0001

T2, with a mean fold change of 0.0563 ± 0.01577. Collectively, GH expression was upregulated upon individual administration of either VCO or folic acid. Nonetheless, the concurrent administration of both compounds (T3) failed to produce a greater elevation in GH expression in comparison to their separate application.

Post hoc analysis using the Tukey HSD test disclosed statistically significant disparities in GH ΔCt values across multiple group comparisons. The negative control differed significantly from the normal control, T1, and T2 groups. A significant difference was also identified between the normal control and T3 ($p < 0.001$), as well as between T1 and T3 ($p < 0.001$) and between T2 and T3 ($p < 0.001$). In contrast, GH expression in T1 and T2 did not differ significantly from the normal control ($p = 0.997$ and $p = 0.148$), and no significant difference was observed between T1 and T2 themselves ($p = 0.242$).

4. Discussion

The present results confirm that both VCO or folic acid is capable of restoring GH expression suppressed by rotenone exposure in zebrafish larvae. The markedly lower mean fold change observed in the negative control group may be attributed to rotenone-induced mitochondrial dysfunction as a strong blocker of mitochondrial complex I in the electron transport chain, rotenone compromises ATP synthesis while concurrently triggering an excessive accumulation of ROS. The resultant perturbation of the oxidant-antioxidant equilibrium sets the stage for the onset of oxidative stress and chronic inflammation, which collectively drive the downregulation of GH expression, a phenomenon clearly manifested in the negative control group. Conversely, the absence of rotenone exposure in the normal control group allowed physiological homeostasis to be preserved, accounting for the highest mean fold change recorded in that group. These findings collectively highlight the detrimental impact of rotenone on cellular growth and development, as reflected by the marked attenuation of GH expression.[16] MCFAs, which constitute the predominant lipid fraction of VCO, are rapidly transported into mitochondria and undergo β-oxidation, serving as efficient substrates for energy metabolism and contributing to ATP production through oxidative pathways.[17] ATP availability reflects cellular energy state and serves a pivotal function in maintaining metabolic homeostasis by regulating biochemical reactions and energy balance.[18] Adequate energy status is tightly regulated by the hypothalamus, which integrates peripheral metabolic signals and coordinates neuroendocrine responses related to energy homeostasis. Adequate energy status is tightly regulated by the hypothalamus, which integrates peripheral metabolic signals and coordinates neuroendocrine responses related to energy homeostasis. Therefore, improved energy availability may contribute to the modulation of GHRH activity through neuroendocrine mechanisms linked to energy homeostasis. Increased GHRH

activity subsequently stimulates the anterior pituitary to enhance GH secretion, as GHRH functions as the primary hypothalamic regulator of GH release (established endocrine physiology).[19]

In addition, VCO may enhance GH levels by reducing ROS. MCFAs, which are the major components of VCO, can integrate into phospholipid membranes, thereby stabilizing membrane structure and inhibiting lipid peroxidation. This process contributes to lipophilic antioxidant activity, which acts directly within the lipid phase of cells to suppress ROS accumulation and reduce oxidative stress in tissues.[9] The reduction of oxidative stress plays a critical role in maintaining the function of the GH axis, as oxidative stress and chronic inflammation are known to suppress GH secretion and impair tissue sensitivity to IGF-1, ultimately leading to impaired linear growth.[4]

Furthermore, the consumption of medium-chain triglycerides (MCTs), the major components of VCO, has been reported to increase circulating levels of acyl-ghrelin, a hormone that stimulates GH secretion, thereby potentially enhancing the activity of the GH axis.[20]

A notable upregulation of GH expression was also discerned in the group subjected to folic acid treatment alone (T2), with the magnitude of this elevation being statistically significant when contrasted against the negative control group. Such an observation is thought to stem from the indispensable contribution of folic acid to one-carbon metabolism. Through its involvement in the folate cycle, the biosynthesis of NADPH, a compound indispensable for the regeneration of GSH, is contingent upon the availability of folic acid. This activity contributes to the formation of a hydrophilic antioxidant system that functions within the cytoplasmic fluid phase. This mechanism helps suppress the generation of ROS and maintain cellular redox homeostasis.[7,8]

Consequently, the reduction in ROS leads to decreased oxidative stress and inflammatory processes, both of which are recognized to inhibit the activity of the GH axis.[4]

Despite the capacity of VCO and folic acid to individually restore GH expression toward levels more reminiscent of normal physiological conditions, their concurrent administration at respective optimal doses nonetheless failed to elicit a superior augmentation of GH expression beyond that achievable through either agent administered in isolation.

The combined administration at optimal doses not only failed to produce a greater effect than single-agent treatment,but also did not demonstrate any improvement relative to the stunted group. These observations suggest that the GH response may be contingent upon the dose ratio rather than being inherently synergistic, given that GH activation is also governed by the broader metabolic context and the relative abundance of available substrates. Findings reported in the literature highlight that when nutrient stimulation is delivered in excess or in an unbalanced manner, compensatory feedback mechanisms are activated, attenuating the sensitivity of the relevant pathways and preventing a proportional amplification of the anabolic response as doses are increased. Such a observations are in agreement with the hormesis principle, or biphasic dose–response relationship, whereby physiological outcomes peak within a specific concentration range and subsequently decline once cellular regulatory thresholds are exceeded. It follows, therefore, that the simultaneous delivery of two agents, each at their individually optimal dose, does not inherently translate into an enhanced biological outcome, as the GH response is fundamentally determined by the overall balance of nutrient availability rather than by the simple sum of the absolute quantities of each individual compound.[21]

This study is subject to several limitations that should be recognized. First, it was conducted under controlled laboratory conditions, including treatment administration initiated at 2 hpf and continued until 72 hpf, daily media replacement, and scheduled observations performed at 3, 6, and 9 dpf, which may not fully represent the variability of biological conditions occurring in natural settings. Second, the optimal doses of each agent incorporated in this study were drawn from previously established findings; consequently, the combination dose was determined exclusively by summing the two individual optimal doses, without any dedicated investigation into alternative dose ratios or variations in combinatorial dosing strategies.

Subsequent investigations are encouraged to overcome these limitations through the systematic examination of dose variations and combinatorial ratios of VCO and folic acid, with the aim of identifying whether their interaction yields synergistic, additive, or antagonistic outcomes on GH axis activity. A comprehensive dose-response investigation incorporating varied combinatorial agent ratios would yield more profound mechanistic resolution into the dose-ratio dependency underlying the observed GH reaction patterns. Applying this framework would strengthen scientific understanding of the dose-ratio dynamics and the complex relationship between both agents in the context of GH axis modulation, ultimately reinforcing the scientific foundation upon which more efficacious combination strategies may be established.

5. Conclusion

This study demonstrates that VCO and folic acid, when administered individually, are capable of restoring GH expression suppressed by rotenone-induced stunting in zebrafish larvae. Among the treatments, VCO showed the most pronounced effect, suggesting its strong potential in modulating growth-related pathways, likely through its role in improving energy metabolism and reducing oxidative stress. Folic acid also contributed to increased GH expression, supporting its function in maintaining redox homeostasis through one-carbon metabolism. However, the concurrent use of both agents did not produce a superior outcome relative to either monotherapy, indicating that the interaction between these agents may not be synergistic and could be influenced by dose ratio and metabolic balance. The present results underscore the promise of VCO and folic acid as naturally derived growth-promoting agents through modulation of GH-related signaling under conditions of metabolic stress. The present investigation furnishes important experimental data corroborating the function of nutritional-based strategies in restoring growth-associated molecular pathways, thereby contributing to a broader understanding of stunting pathophysiology. In light of the fact that stunting persists as a predominant global public health challenge, these findings may act as a scientific groundwork for the future formulation of accessible nutritional interventions aimed at preventing or mitigating growth impairment. Additional research elucidating dose refinement and mechanistic pathways is needed to strengthen their potential relevance for stunting management.

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Conflict of Interest

The authors declare no conflicts of interest.

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