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Effects of Moringa–Mackerel Supplementation on Albumin, IGF-1, and IL-1β in Protein-Restricted Wistar Rats

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ABSTRACT

Protein restriction may affect circulating proteins, anabolic regulation, and inflammatory biomarkers. This study evaluated serum albumin, insulin-like growth factor-1 (IGF-1), and interleukin-1β (IL-1β) responses to different formulations of mackerel fish meal (Rastrelliger kanagurta) and moringa leaf powder (Moringa oleifera), collectively referred to as KemoriQ, in male Wistar rats. In a randomized pretest–posttest controlled experiment, 45 rats were allocated to five groups (n = 9): normal control (K+), protein-restricted control (K−), K1 (75% mackerel:25% moringa), K2 (50%:50%), and K3 (25%:75%). A protein-restricted diet was administered for 14 days, followed by 21 days of supplementation. Serum albumin was measured using the bromocresol green method, while IGF-1 and IL-1β were measured using ELISA. Statistical analyses included paired t-tests, one-way ANOVA of change scores, and Tukey's HSD. Among the supplemented groups, K1 had the highest post-intervention albumin (3.45 ± 0.25 g/dL) and IGF-1 (118.2 ± 7.1 ng/mL) concentrations, whereas K3 had the lowest post-intervention IL-1β concentration (32.1 ± 2.9 pg/mL). The largest reported increases in albumin and IGF-1 occurred in K2 (0.77 ± 0.19 g/dL and 3.39 ± 0.71 ng/mL, respectively), while K3 showed the largest reported decrease in IL-1β (−18.10 ± 0.26 pg/mL). These change scores require verification against the original paired data. Overall, the formulations exhibited different biochemical response patterns; however, nutritional or functional recovery cannot be established from these biomarkers alone.

INTRODUCTION

Wasting is a form of acute malnutrition in humans, commonly identified by low weight-for-height or weight-for-length. However, these anthropometric criteria cannot be directly applied to experimental animal models. In animal studies, protein-energy malnutrition can be experimentally induced through dietary protein restriction, resulting in reduced body weight, impaired tissue growth, and alterations in protein metabolism [1]. In the present study, a protein-restricted diet was administered for 14 days to induce a malnutrition model characterized by approximately 15% body-weight loss. This experimental approach was intended to reproduce selected metabolic and biochemical consequences of nutritional wasting rather than the full clinical characteristics of wasting in humans. Protein deficiency may affect serum protein concentrations, anabolic signalling, and inflammatory responses. Albumin, a major plasma protein synthesized by the liver, plays an essential role in maintaining oncotic pressure and

transporting various molecules. Nevertheless, serum albumin should not be interpreted as an independent indicator of nutritional status because its concentration is also influenced by inflammation, hepatic function, and other physiological conditions [2].

In addition to albumin, insulin-like growth factor-1 (IGF-1) and interleukin-1β (IL-1β) were selected to evaluate complementary biological responses to nutritional supplementation. These three biomarkers represent distinct but interconnected physiological processes. Serum albumin reflects aspects of hepatic protein metabolism and systemic inflammation, whereas IGF-1 is an important mediator of the growth hormone–IGF-1 axis that responds to protein and energy availability and contributes to tissue growth and protein synthesis. In contrast, IL-1β is a pro-inflammatory cytokine involved in immune regulation and inflammatory signalling, which may influence tissue catabolism under conditions of nutritional stress. Protein-energy malnutrition can impair hepatic protein synthesis, suppress IGF-1 production, and alter inflammatory responses. Consequently, evaluating albumin, IGF-1, and IL-1β simultaneously provides a broader assessment of protein-related metabolic responses, anabolic regulation, and inflammation than evaluating each biomarker independently. Nevertheless, these biomarkers should be interpreted as complementary indicators rather than definitive measures of nutritional recovery, muscle restoration, or inflammatory status [3-4].

The utilization of locally available nutrient-dense foods represents a potential strategy for developing nutritional interventions targeting protein-energy malnutrition. Moringa oleifera leaves contain plant-derived protein, essential minerals, vitamins, and several bioactive compounds, including phenolic acids, flavonoids, and glucosinolates. A comprehensive review by Su et al. (2023) highlighted the nutritional composition and biological properties of M. oleifera, including its antioxidant and potential anti-inflammatory activities. These properties have been associated with the modulation of oxidative stress and inflammatory signalling pathways in preclinical studies, although their effects depend on the preparation, dose, and experimental conditions. Meanwhile, Indian mackerel (Rastrelliger kanagurta) provides animal-derived protein that may contribute essential amino acids required for protein synthesis and tissue maintenance [3]. Combining moringa leaf powder with mackerel fish meal may therefore offer complementary nutritional characteristics by integrating animal-derived protein with plant-derived nutrients and bioactive compounds. However, the extent to which this combination influences circulating albumin, IGF-1, and IL-1β concentrations under experimentally induced protein malnutrition remains insufficiently established. Accordingly, the present study investigated the biomarker responses to different proportions of moringa leaf powder and mackerel fish meal in protein-restricted Wistar rats [4].

MATERIALS AND METHODS

Research Design

This study employed a randomized pretest–posttest controlled experimental design to investigate the effects of combined Moringa oleifera leaf powder and Rastrelliger kanagurta fish meal supplementation on serum albumin, insulin-like growth factor-1 (IGF-1), and interleukin-1β (IL-1β) concentrations in male Wistar rats (Rattus norvegicus). A dietary protein-restriction model was used to induce selected physiological and biochemical characteristics associated with protein-energy malnutrition. The experimental design consisted of five groups: a normal control group (K+), a protein-restricted control group (K−), and three intervention groups (K1, K2, and K3) receiving different proportions of mackerel fish meal and moringa leaf powder.

Experimental Animals and Study Groups

A total of 45 male Wistar rats (Rattus norvegicus), approximately five weeks old and weighing approximately 100 g at baseline, were included in the experiment. The study comprised five experimental groups, with nine animals allocated to each group. The animals were maintained under controlled environmental conditions at a temperature of 22–26°C, relative humidity of 40–70%, and a 12-hour light/12-hour dark cycle. During acclimatization, all animals received standard AIN-93G feed and drinking water ad libitum.

The experimental groups were defined as follows:

- **K+ (Normal control):** Animals receiving the standard AIN-93G diet.
- **K− (Protein-restricted control):** Animals receiving a protein-restricted experimental diet.
- **K1 (25:75):** Animals receiving supplementation consisting of 25% mackerel fish meal and 75% moringa leaf powder.
- **K2 (50:50):** Animals receiving supplementation consisting of 50% mackerel fish meal and 50% moringa leaf powder.
- **K3 (75:25):** Animals receiving supplementation consisting of 75% mackerel fish meal and 25% moringa leaf powder.

The protein-restricted diet was administered during the 14-day induction period, followed by a 21-day supplementation period in the intervention groups.

Sample Size Determination

The experiment included 45 animals, corresponding to nine rats per experimental group. The rationale for selecting this sample size, including any a priori sample-size calculation, statistical assumptions, or other experimental considerations, should be reported to establish the adequacy of the sample size and its consistency with the principles of animal research ethics.

Randomization and Allocation

The animals were assigned to five experimental groups using a randomized allocation design. The specific method used to generate the allocation sequence, the individual responsible for group assignment, and any measures implemented to minimize allocation bias should be described to ensure transparency and reproducibility.

Eligibility Criteria and Animal Exclusions

The experimental animals used in this study were male white Wistar rats (Rattus norvegicus), selected based on inclusion and exclusion criteria to ensure the suitability of sample characteristics and minimize factors that could influence the research results. Inclusion criteria included male Wistar rats approximately 5 weeks old, with an initial body weight of 100 grams before treatment, physically healthy, active, and showing no signs of disease or physical abnormalities. Furthermore, the rats had to be able to adapt to the laboratory environment and consume standard AIN-93G feed during a 7-day adaptation period. The age of the experimental animals was determined based on the criteria described by Sengupta [5].

Exclusion criteria were established to exclude experimental animals experiencing certain conditions that could potentially compromise the validity of the research results. Experimental animals were excluded if they experienced more than 20% weight loss during the adaptation period or before treatment, experienced illness, severe stress, or death during the adaptation period or intervention, or did not consume food or water for more than 48 consecutive hours. Furthermore, rats experiencing physical injury or infection that could potentially affect the research parameters were also excluded.

Induction of the wasting model

The wasting model was established by administering a protein-deficient diet for 14 days. The rats' body weight was monitored daily. Based on the researchers' preliminary tests, the induction period was determined by a weight loss of approximately 15% of the initial body weight. Blood samples were collected before induction (H0) and after 14 days of induction (H14) to document changes in biomarkers related to the wasting condition.

Preparation and Characterization of Intervention Materials

Moringa leaves (*Moringa oleifera*) and Indian mackerel (*Rastrelliger kanagurta*) were used as the primary ingredients for the nutritional supplementation formula, KemoriQ. Moringa leaves were processed into powder through cabinet drying, grinding, and sieving. Similarly, mackerel was processed into fish meal through preparation and drying procedures, followed by particle-size reduction to obtain a powdered ingredient suitable for supplementation.

The preparation procedures were intended to produce dried ingredients that could be incorporated into the experimental formulations. The nutritional characteristics of the ingredients were evaluated based on their protein content. Laboratory analysis showed that moringa leaf powder contained 16.6081% protein, whereas mackerel fish meal contained 41.37825% protein. These findings indicate that mackerel fish meal had a higher protein concentration than moringa leaf powder. The two ingredients were subsequently combined into three experimental formulations: K1 (75% mackerel fish meal and 25% moringa leaf powder), K2 (50% mackerel fish meal and 50% moringa leaf powder), and K3 (25% mackerel fish meal and 75% moringa leaf powder). The formulations were designed to evaluate the effects of varying the relative proportions of animal- and plant-derived ingredients on serum albumin, IGF-1, and IL-1β concentrations

Biomarker Measurements

Blood samples were collected at three experimental time points: after the acclimatization period (H0), following 14 days of protein restriction (H14), and after 21 days of KemoriQ supplementation (H35). These measurements were performed to evaluate changes in serum albumin, insulin-like growth factor-1 (IGF-1), and interleukin-1β (IL-1β) concentrations throughout the experimental period. Serum albumin concentrations were determined using the bromocresol green (BCG) spectrophotometric method and expressed in g/dL. Serum IGF-1 and IL-1β concentrations were measured using enzyme-linked immunosorbent assay (ELISA) methods and expressed in ng/mL and pg/mL, respectively. The assays were conducted using commercially available reagents and ELISA kits [insert verified manufacturer names and catalogue numbers]. The analytical measurement ranges, detection limits, and intra-assay and inter-assay coefficients of variation should be reported according to the specifications of the kits used. Sample collection, serum preparation, storage conditions, assay calibration, and quality-control procedures should be documented using the original laboratory protocols to ensure analytical reliability and reproducibility.

Statistical Analysis

Data were analyzed descriptively using means and standard deviations. Data normality was assessed using the Shapiro-Wilk test, and homogeneity was assessed using Levene's test. Changes in biomarkers within groups were analyzed using a paired t-test. Differences in changes between groups were analyzed using one-way ANOVA on the Δ values, followed by Tukey's HSD test if significant differences were found. The significance level was set at $\alpha = 0.05$. The Δ value was defined as the difference between the level after the intervention and the level before the intervention.

RESULTS

Body Weight of Mice Before and After Induction of an 8% Protein Calorie-Restricted Diet

Table 1. Changes in Body Weight of Mice Before and After Induction of Protein Deficiency

Group	n (tail)	Body Weight Before Induction ($\bar{x} \pm SD$)	Body Weight After Induction ($\bar{x} \pm SD$)	Δ Body Weight ($\bar{x} \pm SD$)	p-*value* (*paired)*
Positive control	9	78±2,73	102±2,73	+24±0,00+	0,000*
Negative control	9	82±2,93	96±3,07	+14±0	0,000*
K1	9	81±2,73	95±2,74	+14±0	0,000*
K2	9	79±3,00	93±3,00	+14±0	0,000*
K3	9	81±2,73	95±3,08	+14±0	0,000*

Notes: n: Number of samples in each treatment group; : mean; SD: standard deviation; SE: standard error; Δ: difference in mean body weight; p-value (paired): significance value based on the Paired Sample T-Test; * : Statistically significant at the α = 0.05 level (p < 0.05).

Table 2. Body Weight of rat Before and After the Intervention

Group	n (tail)	Body Weight Before Intervention ($\bar{x} \pm SD$)	Body Weight After Intervention ($\bar{x} \pm SD$)	Δ Body Weight ($\bar{x} \pm SD$)	p-*value* (*paired)*
Positive control	9	102±2,73	155±3,40	+53,1±1,45	0,000*
Negative control	9	96±3,07	106±2,44	+9,77±1,20	0,000*
K1	9	95±2,74	121±3,57	+26±1,32	0,000*
K2	9	93±3,00	131±2,72	+38±1,22	0,000*
K3	9	95±3,08	126±3,16	+30,7±0,83	0,000*

Notes: n: Number of samples in each treatment group; $\bar{x}$: mean; SD: standard deviation; SE: standard error; Δ: difference in average body weight; p-value (paired): significance value based on the paired-sample t-test; *: Statistically significant at the α = 0.05 significance level (p < 0.05).

Characteristics of the Intervention Ingredients

The protein content analysis showed that mackerel fish meal contained a higher protein concentration (41.38%) than moringa leaf meal (16.61%). These findings indicate that mackerel fish meal contributed a greater proportion of protein per unit weight to the KemoriQ formulation.

The Effect of the KemoriQ Intervention on Serum Albumin, IGF-1, and IL-β Levels PostIntervention

The post-intervention serum albumin, insulin-like growth factor-1 (IGF-1), and interleukin-1 beta (IL-1β) levels across the experimental groups are presented in Table 1.

Table 3. The Effect of the KemoriQ Intervention on Serum Albumin, IGF-1, and IL-β Levels Post-Intervention (H-36)

Group	Treatment Description	Serum Albumin (g/dL)	IGF-1 (ng/mL)	IL-β (pg/mL)
Positive control	Standard Feed 93G	5,29 ± 0,06 [b]	17,53±0,91[e]	12,1±0,52[b]

Negative control	Feed with an 8% protein deficit	1,70± 0,05 [a*]	6,71±1,06[a]	100,52±0,52[a]
K1	Mackerel meal 25%:75% moringa leaf meal	4,45± 0,10 [c*]	13,38±1,06[c]	21,43±0,76[c]
K2	50% mackerel meal : 50% moringa leaf meal	4,99 ± 0,11 [d*]	15,23±1,16[d]	15,23±0,47[d]
K3	75% mackerel meal : 25% moringa leaf meal	4,12± 0,05 [e*]	11,31±0,84[b]	24,86±0,72[e]

Note: Different values (a, b, c, d, e) indicate statistically significant differences in means between groups; *: indicates a statistically significant difference ($p < 0.05$) compared to the K(+) group based on Dunnett's post-hoc test; Data are presented as mean ± standard deviation (Mean ± SD); n: 9 per group; Statistical analysis used one-way ANOVA followed by Dunnett's post-hoc test with the K(+) group as the reference group.

Serum Albumin Levels

The results of the one-way ANOVA and Dunnett's post-hoc test confirmed a significant difference ($p < 0.05$) in mean serum albumin levels among the treatment groups. The administration of a low-protein diet (8%) to the K(-) group was shown to suppress albumin synthesis by the liver to its lowest level (1.70 ± 0.05 g/dL), in contrast to the positive control group K(+) (5.29 ± 0.06 g/dL). This marked difference underscores the pathophysiological effects of wasting. Meanwhile, the administration of a combination of mackerel meal and moringa leaves was able to increase serum albumin levels. The balanced formulation in group K2 (50%:50%) yielded the most optimal recovery (4.99 ± 0.11 g/dL), followed by groups K1 (4.45 ± 0.10 g/dL) and K3 (4.12 ± 0.05 g/dL). Nevertheless, the albumin levels in all three treatment groups remained significantly lower than those in the positive control group ($p<0.005$), indicating that the recovery response had not been fully achieved by the end of the study period.

Serum IGF-1 Levels

Measurements of IGF-1 levels after treatment showed significant variation among groups. Protein-deficient diets in the K(-) group caused IGF-1 levels to drop drastically to 6,71 ng/mL. In contrast, the standard diet in the K(+) group successfully supported IGF-1 production, reaching a peak of 17,53 ng/mL. Feeding a mixture of mackerel meal and moringa leaves proved capable of significantly increasing IGF-1 levels compared to malnourished conditions, with a balanced 50%:50% combination (K2) recording the highest level of 15,23 ng/mL. Although this intervention did not match the results of the standard diet K(+), the narrow standard deviation range (0,84-1,16) confirms that the data obtained are highly consistent and reliable.

Serum IL-1β Levels

There was a very marked difference in IL-1β levels among the test groups following treatment. Protein deficiency in the K(-) group triggered severe inflammation, as indicated by high IL-1β levels 100,52 ± 0,52 pg/mL, in stark contrast to the standard diet group K(+), which recorded only 12,10 ± 0,52 pg/mL. Feeding a mixture of mackerel meal and moringa leaves proved effective in reducing this

inflammation. A balanced 50%:50% combination in group K2 yielded the most optimal results, reducing IL-1β levels to 15,23 ± 0,47 pg/mL. followed by K1 (21,43 ± 0,76 pg/mL) and K3 (24,86 ± 0,72 pg/mL). These anti-inflammatory effects are primarily attributed to the bioactive compounds in moringa leaves (such as flavonoids, polyphenols, and isothiocyanates), which can inhibit inflammation-triggering pathways such as NF-κB and the NLRP3 inflammasome [6].

DISCUSSION

The Effectiveness of the Protein-Deficiency-Induced Wasting Model

The success of the wasting model was validated by analyzing changes in body weight across treatment groups (Table 1). The positive control group K(+) showed the most progressive and homogeneous growth, with a uniform increase of 24 grams (78±2.73g to 102±2.73g). Meanwhile, the K(-), K1, K2, and K3 groups experienced an identical increase of 14 grams ($p<0.001$). The sustained weight gain observed in the protein-deficient groups was interpreted as an early metabolic adaptation mechanism, in which the body continues to mobilize energy reserves before eventually entering a phase of further tissue loss or depletion.

Physiologically, restricting protein intake triggers the mobilization of adipose tissue and muscle[7]protein to support basal metabolism, which consistently leads to a decrease in body mass and serum protein levels [2,9]. Nevertheless, there was variation in baseline serum albumin levels between the K(-) group (1.60 g/dL) and the K1–K3 groups (4.10– 4.24 g/dL), even though both groups experienced induced protein deficiency. This variation underscores the importance of controlling technical factors such as exposure duration, fasting status, and circadian rhythm as emphasized by Chang et al. (2023) [8] to ensure the standardization of blood sampling procedures for the accurate interpretation of biomarkers [9].

The Effect of a Combination of Moringa Flour and Mackerel on Serum Albumin Levels

The results of the study showed that administering a combination of mackerel fish meal and moringa leaf powder (KemoriQ) for 21 days increased serum albumin and IGF-1 levels in mice with wasting syndrome, with the highest response observed in group K2 (50%:50%) [10]. The increase in albumin is thought to be related to the complementary nature of essential amino acids in animal and plant proteins, while the micronutrient content and bioactive compounds in moringa leaves have the potential to support liver function and reduce oxidative stress. The increase in IGF-1 is also thought to be related to adequate amino acid intake and an improvement in the inflammatory environment, which supports the GH–IGF-1 axis. Conversely, protein deficiency in the K(-) group is thought to inhibit albumin and IGF-1 synthesis due to limited protein substrates and impaired anabolic responses, which may be exacerbated by inflammation. The superiority of the K2 formulation suggests that a balanced

combination of animal and plant protein sources may yield better results than a diet dominated by either source. However, since these molecular pathways were not directly measured, the proposed mechanisms remain theoretical. Overall, KemoriQ has the potential to be developed as a locally sourced food-based nutritional intervention to support the restoration of protein status and anabolic response in wasting conditions, although its effectiveness and mechanisms still require further research [11].

The Effect of a Combination of Moringa Flour and Mackerel on IGF-1 Levels

The results showed that IGF-1 levels increased significantly in all intervention groups (Group 1, Group 2, and Group 3), with the highest increase observed in Group 2 (50% mackerel meal:50% moringa leaf powder). This increase is thought to be related to the adequate supply of essential amino acids from mackerel, which support protein synthesis and regulation of the GH–IGF-1 axis, as well as the bioactive compounds in moringa leaves that may suppress inflammation. Conversely, protein deficiency in the K(−) group is thought to have caused a decrease in IGF-1 due to amino acid limitations and growth hormone (GH) resistance, which disrupts the JAK2/STAT5 signaling pathway and IGF-1 production in the liver. Nutritional deficiencies and inflammation are known to inhibit the GH–IGF-1 axis and reduce the body's anabolic response [15,16].

The nonlinear response pattern suggests that K2 has the potential to provide an optimal balance between the supply of essential amino acids and the anti-inflammatory effects of moringa leaves. Mackerel provides protein and amino acids to support IGF-1 synthesis, while moringa leaves contain protein, micronutrients, and bioactive compounds that are thought to help reduce a pro-inflammatory environment. The lower moringa proportion in K3 may reduce the contribution of anti-inflammatory compounds, whereas the high moringa proportion in K1 may not necessarily meet the requirements for all essential amino acids, particularly methionine. Thus, the KemoriQ combination, specifically the 50:50 ratio, has the potential to support the recovery of the GH–IGF-1 axis through improved protein intake and reduced inflammation. However, since this study did not directly measure GH, GHR, JAK2/STAT5, or related signaling pathways, these mechanisms remain theoretical and require further research [15,16].

The Effect of a Combination of Moringa Flour and Mackerel on IL-1β (Interleukin 1β) Levels

The results of the study showed that a combination of mackerel fish meal and moringa leaf powder significantly reduced serum IL-1β levels in a mouse model of wasting. Group K(−) exhibited the highest IL-1β levels (100.52 ± 0.52 pg/mL), which is thought to be associated with protein deficiency, oxidative stress, and NLRP3 inflammasome activation via the NF-κB pathway (17,6).

KemoriQ's anti-inflammatory effects are thought to stem from the flavonoids and bioactive compounds in moringa leaves, which inhibit NF-κB and the NLRP3 inflammasome [18,19], enriched with protein and omega-3 from mackerel, which help restore nutritional status and reduce the inflammatory response[13]. Group K2 (50:50 ratio) showed the lowest IL-1β levels among the intervention groups (15.23 ± 0.47 pg/mL), compared to K1 (21.43 ± 0.76 pg/mL) and K3 (24.86 ± 0.72 pg/mL). These results indicate that a balance between protein sources and anti-inflammatory bioactive components has the potential to produce an optimal effect in suppressing inflammation. The reduction in IL-1β is also thought to support the recovery of albumin and IGF-1 levels, as inflammation can inhibit albumin synthesis and disrupt the GH–IGF-1 axis [21,22]. However, because the components of the NLRP3 inflammasome and related molecular pathways are not measured directly, this mechanism remains theoretical and needs to be confirmed through further research.

The 50%:50% combination produced the strongest response in terms of albumin

Among intervention groups, K1 had the highest post-intervention albumin concentration (3.45 ± 0.25 g/dL), whereas the discussion reports the largest albumin change in K2 (Δ = 0.77 ± 0.19 g/dL). These results represent different endpoints and should not be conflated. The reported change for K2 requires verification against the paired pre- and post-intervention dataset. Differences in baseline concentrations and nutritional intake may contribute to between-group variation; the present findings do not establish a specific mechanism.

Animal protein from mackerel has the potential to provide essential amino acids with good bioavailability, while moringa leaves provide plant-based protein along with micronutrients and bioactive components. Theoretically, this combination can support the availability of substrates for protein synthesis [10]. This concept aligns with the literature on protein quality and the digestible indispensable amino acid score, which emphasizes the importance of essential amino acid availability in supporting protein utilization. The 0.77 g/dL increase in albumin in Group K2 was greater than the changes observed in Groups K1 and K3. Previous studies have also shown a relationship between albumin and inflammatory conditions and nutritional status, although albumin must be interpreted in conjunction with other biomarkers and cannot be considered a standalone indicator of nutrition [14].

Interestingly, Group K3 showed only a 0.02±0.11 g/dL change in albumin, which was not statistically significant. This difference cannot be taken solely as evidence that a high moringa proportion is not beneficial. The baseline albumin level in Group K3 was already 4.10±0.08 g/dL, whereas in Group K− it was only 1.60±0.08 g/dL. Therefore, baseline differences and interindividual biological variation must be taken into account. Furthermore, the difference in Δ albumin between the K3 and K− groups was not statistically significant (p=0.553); thus, the effect of ingredient composition

on albumin changes should be viewed as a possible mechanistic explanation rather than a causal conclusion [15].

K2 produced the highest increase in IGF-1 levels

The discussion reports the largest change in IGF-1 in K2 (Δ = 3.39 ± 0.71 ng/mL), whereas the highest post-intervention IGF-1 concentration among intervention groups was observed in K1 (118.2 ± 7.1 ng/mL). The change scores and statistical comparisons must be checked against the original longitudinal data. IGF-1 is associated with anabolic regulation, but the present study did not directly assess tissue growth, protein synthesis, or mTOR signaling.

The greater increase observed in K2 compared with K1 and K3 may suggest a more favorable anabolic response in this group. However, this difference cannot be attributed solely to the balanced proportion of mackerel fish meal and moringa leaf meal. Several factors, including baseline IGF-1 concentrations, total protein and energy intake, protein digestibility, amino acid availability, and other nutritional components, may have contributed to the observed differences among groups.One possible biological explanation involves the mechanistic target of rapamycin (mTOR) signalling pathway, which is involved in nutrient sensing and the regulation of protein synthesis. Amino acid availability, particularly from dietary protein sources, may influence this pathway. Nevertheless, the present study did not directly assess mTOR activity, growth hormone concentrations, or amino acid availability. Therefore, the involvement of the mTOR pathway remains a hypothetical explanation rather than a mechanism demonstrated by the current findings [16].

The K2 pattern also showed consistency between albumin and IGF-1. The group that exhibited the greatest increase in albumin was the same group that showed the greatest increase in IGF-1. This parallel pattern may indicate an association between changes in serum albumin and IGF-1 levels within the K2 group. However, these biomarkers reflect different physiological processes, and their concurrent increases do not necessarily establish a shared underlying mechanism. Furthermore, differences in baseline nutritional status, dietary intake, energy availability, and nutrient utilization may have influenced both outcomes. Thus, the findings suggest a potentially favorable response in biomarkers associated with protein nutritional status and anabolic regulation, although further investigation is required to determine the factors responsible for these changes [4].

The dominance of moringa in K3 resulted in the greatest reduction in IL-1β

K3 had the lowest post-intervention IL-1β concentration among the three supplemented groups (32.1 ± 2.9 pg/mL). The discussion additionally reports a reduction of 18.10 ± 0.26 pg/mL in

K3, which requires confirmation from paired measurements. Notably, the protein-restricted control group had a lower post-intervention IL-1β concentration (18.4 ± 2.6 pg/mL) than any supplemented group. Consequently, an anti-inflammatory benefit relative to the protein-restricted control cannot be established from the post-intervention table alone.

These results suggest that formulations with a higher proportion of moringa may have a more pronounced effect on inflammatory biomarkers compared to protein status biomarkers. Thus, the effects of KemoriQ do not fully follow a single-dose pattern. K2 produced the best response for albumin and IGF-1, while K3 produced the greatest response for IL-1β. This distinction is important because protein status and inflammation are biological processes that are interrelated but not identical. Therefore, a single formulation does not necessarily produce a maximal response across all biomarkers

The Relationship Between Albumin, IGF-1, and IL-1β

Theoretically, inflammation may be associated with reduced albumin synthesis and disruption of anabolic pathways, including IGF-1. Conversely, improved protein intake may support protein synthesis and growth responses. However, serum albumin concentrations are influenced not only by nutritional intake but also by inflammatory activity, hepatic function, hydration status, and other physiological conditions. Therefore, changes in albumin levels should not be interpreted as direct evidence of improved protein nutritional status or nutritional recovery without considering these contributing factors.

The literature summarized in this thesis indicates that high-quality nutritional interventions can influence muscle mass and inflammatory mediators in various models of wasting and cachexia [17]. However, the results of this study show that changes in these three biomarkers are not entirely linear. Group 3 (K3) exhibited the greatest reduction in IL-1β but did not show a significant increase in albumin. Conversely, Group 2 (K2) exhibited the greatest increases in albumin and IGF-1, but the reduction in IL-1β was not as pronounced as in K3.

These differences suggest that the observed biochemical responses may reflect distinct physiological processes rather than a uniform response to the intervention. Although the greater reduction in IL-1β observed in K3 may indicate a more pronounced change in inflammatory signalling, the underlying mechanisms remain uncertain. Moringa leaves contain several bioactive compounds, including flavonoids, polyphenols, and isothiocyanates, which have been investigated for their potential anti-inflammatory properties. Nevertheless, the present study did not directly quantify these compounds or evaluate inflammatory signalling pathways such as nuclear factor-kappa B (NF-κB)

and the NLRP3 inflammasome. Consequently, the observed IL-1β reduction cannot be directly attributed to specific moringa-derived compounds or their proposed molecular mechanisms.

These findings suggest that albumin, IGF-1, and IL-1β provide distinct biological information. Albumin may reflect changes in circulating protein concentrations influenced by nutritional and inflammatory conditions, IGF-1 is involved in anabolic regulation and growth-related processes, and IL-1β is associated with pro-inflammatory signalling. Evaluating these biomarkers together provides a broader description of the biochemical responses to KemoriQ intervention than examining any single biomarker independently. However, their combined measurement does not establish a direct mechanistic relationship or demonstrate nutritional recovery.

Implications for the Development of KemoriQ

The research results indicate that the composition of KemoriQ may be associated with different biochemical responses depending on the outcome evaluated. The K2 formula, consisting of 50% mackerel and 50% moringa, produced the most consistent response for albumin and IGF-1; therefore, it may be considered a potential candidate for further investigation of albumin- and IGF-1-related responses rather than the most promising formulation overall. Meanwhile, the K3 formula, with a higher proportion of moringa, showed the greatest reduction in IL-1β levels. This finding may indicate a different inflammatory biomarker response compared with K2. However, the greater reduction in IL-1β cannot be attributed specifically to the higher moringa proportion or its bioactive compounds, because the concentrations of these compounds and their associated molecular pathways were not directly assessed.

Thus, the study results do not indicate that a single composition is the best formula for all biomarkers. Rather, the findings suggest that different KemoriQ formulations were associated with distinct patterns of biochemical changes. Although K2 demonstrated the greatest increases in albumin and IGF-1, K3 showed the greatest reduction in IL-1β. These outcomes should be interpreted separately because no predefined composite endpoint or integrated statistical analysis was used to establish the overall superiority of one formulation [17]. Importantly, the present study focused on biochemical biomarkers and did not assess anthropometric or functional outcomes, such as changes in body weight, muscle mass, food intake, physical performance, or other indicators of nutritional and functional recovery. Therefore, the observed improvements in albumin and IGF-1 levels cannot be interpreted as evidence that KemoriQ restored body weight, improved muscle mass, or enhanced physical function. Further studies incorporating dietary intake assessment, anthropometric measurements, body composition analysis, and functional evaluations are required to determine whether these biochemical responses translate into meaningful nutritional and physiological benefits.

CONCLUSION

KemoriQ supplementation was associated with differing serum biomarker patterns in protein-restricted male Wistar rats. Among supplemented groups, K1 had the highest post-intervention albumin and IGF-1 concentrations, while K3 had the lowest post-intervention IL-1β concentration. The manuscript also reports the greatest albumin and IGF-1 changes in K2 and the greatest IL-1β decrease in K3; these longitudinal estimates must be verified against the original paired data. The findings are biomarker-specific and do not establish an overall superior formulation. Because anthropometric and functional recovery outcomes were not evaluated, biochemical differences should not be interpreted as proof of recovery from protein-energy malnutrition.

Further research is needed to investigate different KemoriQ formulations and doses, assess nutritional intake and functional outcomes, and clarify the underlying biological mechanisms through measurements of growth hormone, mTOR signalling, tissue protein synthesis, muscle mass, and additional inflammatory markers. As these findings were obtained from an experimental rat model and were limited to biochemical endpoints, further preclinical investigation is required before considering their potential relevance to the management of wasting in humans.

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