Polish Journal of Veterinary Sciences Vol. 28, No. 4 (2025), 567–574

DOI 10.24425/pjvs.2025.156084

Original article

Comparative assessment of tempe from germinated and non-germinated soybeans to address protein-energy malnutrition using rat experimental model

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Abstract

Protein-energy malnutrition (PEM) is a major global health concern, especially in resource-limited settings, leading to stunted growth, weakened immune systems and increased mortality. This study aimed to evaluate the efficacy of Tempe protein isolates from germinated (GTI) and non-germinated (NGTI) soybeans as alternatives to animal protein (casein) in recovering from PEM using a rat model. The rats were divided into five groups: the control group (20% casein diet), protein-malnourished (PM) group (3% casein diet), and three intervention groups (supplemented with GTI, NGTI or casein). After a 3-week PEM induction phase, the rats were re-fed with their respective diets for 3 weeks.

The NGTI group showed significantly better growth recovery compared to the GTI and PM groups, demonstrating higher body weight gain, feed intake and skeletal development. While GTI showed some recovery, NGTI outperformed GTI in terms of nitrogen retention, protein digestibility, and net protein utilization, suggesting that NGTI provides superior protein bioavailability. Furthermore, NGTI rats exhibited improvements in hematological indices (e.g., hemoglobin and hematocrit) and biochemical markers (e.g., serum urea, creatinine), indicating better overall health recovery.

The casein group, which served as the animal protein reference, showed the best growth and nitrogen utilization, yet NGTI demonstrated comparable performance in growth recovery and protein bioavailability, highlighting its potential as an alternative to animal protein. The results suggest that NGTI can serve as a sustainable and effective alternative protein source for rehabilitation from PEM, with potential applications in both human and veterinary nutrition, particularly for companion animals or those recovering from illness or malnutrition.

Keywords: bioavailability, protein isolates, protein energy malnutrition, soybean, tempe

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Introduction

Protein-energy malnutrition (PEM) remains a significant global public health concern, particularly prevalent in low- and middle-income countries, adversely affecting millions of individuals (Das et al. 2020). PEM results in impaired growth, weakened immune function, and heightened risk of mortality, especially among children (Michaelsen et al. 2009, Branca et al. 2015). In Pakistan, India and Bangladesh, the prevalence of all kinds of malnutrition has exceeded the threshold levels of 10% underweight, 15% wasting, and 30% stunting. Pakistan, in particular, faces severe malnutrition challenges, with current reports indicating stunting in approximately 40% of children under five, wasting in 17.7%, and underweight conditions in 28.9%, emphasizing the urgent necessity for effective nutritional interventions (National Nutrition Survey Pakistan 2018).

Soybean (Glycine max) has gained recognition as a nutritionally dense dietary resource due to its high protein content, balanced essential amino acid profile, and economic affordability (Rizzo and Baroni 2018). Soybean proteins offer a sustainable alternative to animal-derived proteins, particularly beneficial in regions where resources are limited (Chatterjee et al. 2018). Nevertheless, soybeans naturally contain anti-nutritional factors such as phytates and trypsin inhibitors, which can inhibit nutrient absorption and digestibility (de Camargo et al. 2019). Using processing techniques such as fermentation is crucial to mitigate these factors and enhance protein quality and availability (Handa et al. 2017, Samtiya et al. 2020).

Fermentation, particularly in producing Tempe, a traditional fermented soybean food, significantly improves the protein attributes of soybeans. The fermentation process effectively reduces anti-nutritional factors, enhances protein digestibility and boosts protein bioavailability, thereby converting complex nutrients into simpler forms more readily absorbed by the body (Astawan et al. 2020). While fermented soy products such as Tempe have demonstrated nutritional and protein-specific benefits, comparative evaluations focusing explicitly on Tempe protein from germinated versus non-germinated soybeans remain limited. Germination is another traditional processing method that has been shown to further decrease anti-nutritional compounds and enhance protein bioavailability, potentially improving the restorative effectiveness of Tempe protein (Nkhata et al. 2018). This study aims to assess Tempe protein's restorative potential derived from germinated and non-germinated soybeans in addressing PEM in humans and animals.

This research involves a bio-efficacy trial using albino rats to evaluate parameters specifically related to protein yield and quality, bone growth metrics, and liver and kidney function serum biomarkers. The findings from this study aim to provide scientifically robust evidence supporting the incorporation of soybean-based Tempe protein into targeted nutritional intervention programs for effectively combating PEM in Pakistan and similar global contexts.

Materials and Methods

Experimental Animals

Twenty-five weaned albino rats (4-5 weeks old, weighing 70-110 g, both sexes) were procured and housed in the Animal Room at the University of Veterinary and Animal Sciences, Lahore. The rats underwent one-week acclimatization with free access to standard laboratory chow and water. Ethical approval for animal experimentation was obtained from the Institutional Animal Ethics Committee (No. DR/166). Following the conclusion of the trial, rats that had undergone an overnight fasting period would be subjected to decapitation. Humane euthanasia was performed using isoflurane anaesthesia in accordance with standard ethical protocols. Subsequently, their bodies were left to desiccate until a consistent weight was achieved, accomplished by placing them in a hot air oven set at a temperature of 105°C. The desiccated remains were pulverized, and an estimation of their nitrogen content was conducted.

Experimental design

The bio-efficacy trial was conducted over six weeks and comprised two phases:

Phase 1: Protein malnutrition (weeks 0-3)

All rats were divided into five groups (five rats per group). The control group received a standard 20% casein diet, while the remaining four groups received a low-protein diet with 3% casein to induce PEM.

Phase 2: Protein intervention (weeks 4-6)

Post-malnutrition, each group received the following dietary treatments:

Control group: Continued on 20% casein diet.

PM group: Continued on 3% casein diet.

PM + GTI group: Received 20% protein from germinated Tempe protein isolates.

PM + NGTI group: Received 20% protein from non-germinated Tempe protein isolates.

PM + Casein group: Received 20% casein to assess recovery from PEM.

Diet preparation and modifications

Tempe was prepared using standardized fermentation procedures. Soybeans were soaked, dehulled, and cooked. For germinated Tempe preparation, soybeans were germinated for 24 hours prior to fermentation. Both germinated and non-germinated soybeans were inoculated with Rhizopus oligosporus and fermented at 31°C for 40 hours. Fermented products were dried, ground, and turned into protein isolates to be formulated into diets, ensuring a protein content of 20%. GTI and NGTI replaced casein in a standard AIN93G diet. The standard AIN93G diet, originally proposed by Reeves et al. (1993), was modified primarily by reducing the protein level to 3% casein to induce protein malnutrition.

Feed intake, body weight gain and bone growth estimation

Daily feed intake was calculated by subtracting feed spills from the total feed offered (Hameed et al. 2022). Body weights were recorded weekly using an electronic balance, and body weight gain was computed as the difference between the weights at the end of the malnutrition and intervention phases (Bhagya et al. 2006). Post-euthanasia, femur lengths were also measured using hand-held vernier calipers to evaluate skeletal growth across experimental phases.

Nutritional attributes assessment

Feed, feces, urine, and dried carcasses were analyzed for nitrogen content using the AACC (2000) method as described in the literature (Ingbian et al. 2007). The following parameters were calculated:

Protein Efficiency Ratio (PER) = Weight Gain / Protein Intake

Net Protein Ratio (NPR) = Weight Gain + Weight Loss of Control / Protein Intake

Relative Net Protein Ratio (RNPR) = NPR of test protein / NPR of casein × 100

True Digestibility (TD) = [(Ni − (Nf − Nef)) / Ni] × 100

Biological Value (BV) = [(Ni − (Nf − Nef) − (Nu − Neu)) / (Ni − (Nf − Nef))] × 100

Net Protein Utilization (NPU) = [(Ni − (Nf − Nef) − (Nu − Neu)) / Ni] × 100

Where: Ni = nitrogen intake, Nf = fecal nitrogen, Nef = endogenous fecal nitrogen, Nu = urinary nitrogen, Neu = endogenous urinary nitrogen.

Complete blood count (CBC) and serum biomarker analysis

Whole blood was collected in EDTA-coated tubes and analyzed using an automated hematology analyzer. The parameters assessed included: Hemoglobin (Hb), Hematocrit (HCT), Red Blood Cell (RBC) count, Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC) and White Blood Cell (WBC count.

For serum biomarker evaluation, blood samples were obtained by cardiac puncture under anaesthesia. Serum was analyzed for:

Serum proteins: The estimation of serum total protein albumin was conducted following the established procedures described by Al-Gaby (Song et al. 2019).

Liver function and kidney function: The sera of rats were subjected to liver function tests using enzymatic assessment, specifically alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) (Li et al. 2020). In addition, the GLDH-method was employed to analyze the serum urea levels for kidney functioning tests, while the estimation of creatinine was conducted using the Jaffe-method with the use of commercially available kits (Othman et al. 2020).

Lipid profile: Cholesterol, triglycerides, LDL-C (Li et al. 2020)

Statistical analysis

Data were analyzed using the Statistical Package for Social Sciences (SPSS version 26). One-way ANOVA and repeated measures ANOVA were applied, followed by Tukey's post hoc test. A completely randomized design was used, and differences were considered significant at $P<0.05$. Results were expressed as mean ± standard deviation (SD).

Results

Growth performance and feed efficiency of experimental rats fed formulated diets

Growth trends, feed intake and skeletal development of rats across dietary groups are shown in Fig. 1 and Fig. 2. Feed intake differed significantly among groups ($p<0.001$), with the PM group consuming the least. Post hoc tests confirmed significantly higher intake in Control, NGTI, GTI, and Casein groups compared to PM, with no significant differences among the well-nourished groups. Control and Casein groups showed the highest weight gains, reflecting adequate protein support. NGTI rats exhibited moderate recovery after protein restoration, while GTI rats showed partial catch-up growth, higher than PM but lower than Control and Casein.

Final body weights positively correlated with linear and skeletal development (Fig. 2). Control rats reached

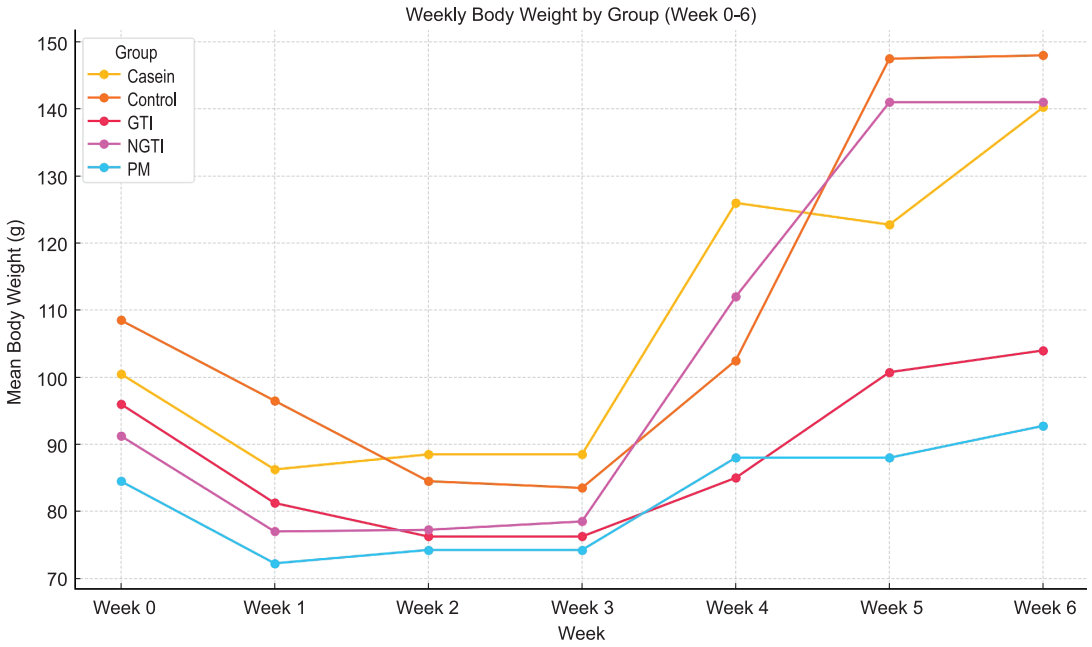

Fig. 1. Weekly body weight trends for all five dietary rat groups over the 7-week period.

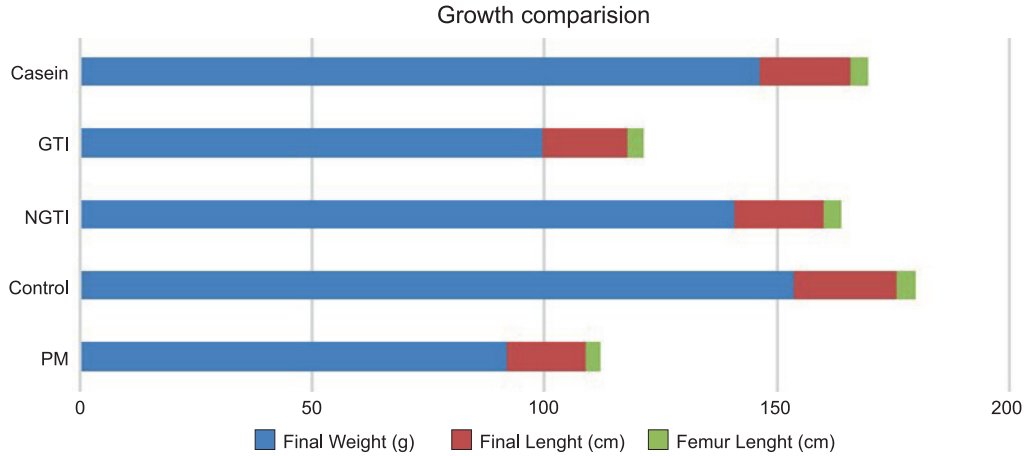

Fig. 2. Overall growth comparison of all rat groups.

the greatest body length (22.17 cm) and femur length (3.99 cm), followed by Casein. NGTI showed modest recovery, while PM rats had the shortest length (16.97 cm) and femur (3.22 cm), reflecting poor skeletal growth. BMI was highest in NGTI and Casein (0.385 g/cm²), suggesting better tissue accretion. Overall, protein restoration improved outcomes across all groups. GTI rats demonstrated better catch-up growth than PM and NGTI but did not match Control or Casein, underscoring the importance of timely and sufficient protein repletion.

Nutritional quality and relative organ weights of experimental rats fed formulated diets

The nutritional quality and relative organ weights of rats fed formulated and control diets are summarized in Table 1. Weight gain ranged from 15.00 g in the PM group to 64.67 g in the Control group, with the PM group showing significantly lower gains ($p \leq 0.05$), consistent with expected outcomes of protein deficiency. The Control and Casein groups exhibited the highest gains, highlighting effective nutritional repletion. Feed intake followed a similar pattern: Control rats consumed the most (946.50 g), and PM the least (269.00 g). Increased intake among protein-restored groups may reflect improved palatability and nutrient density. Despite comparable intake, the PM group had a significantly lower feed efficiency ratio (0.06), indicating inefficient feed conversion under protein deprivation.

Nitrogen retention (NR) was lowest in PM (0.46 g) and highest in Control (9.54 g), confirming enhanced assimilation with complete proteins. NPR and RNPR values were also calculated, and casein showed the

Table 1. Nutritional quality and organ weights of experimental rats.

Parameters	PM (n=5)	Control (n=5)	NGTI (n=5)	GTI (n=5)	Casein (n=5)	P-Value
Weight gained (g)	15.00±0.47^{c}	64.67±2.05^{a}	41.67±1.32^{b}	28.67±0.91^{d}	64.33±2.03^{a}	<0.001
Food intake (g)	269.00±8.51^{c}	946.50±29.93^{a}	836.50±26.45^{b}	817.60±26.32^{b}	929.40±29.38^{a}	<0.001
Feed efficiency ratio	0.06±0.0019^{c}	0.07±0.0022^{a}	0.05±0.0016^{d}	0.04±0.0013^{e}	0.07±0.0022^{a}	<0.001
Nitrogen retention (g)	0.46±0.015^{e}	9.54±0.30^{a}	5.58±0.18^{c}	4.96±0.16^{d}	9.13±0.29^{b}	<0.001
True digestibility (%)	59.80±1.89^{e}	89.98±2.85^{a}	71.90±2.27^{c}	67.21±2.13^{d}	91.3±2.89^{a}	<0.001
Net protein utilization (%)	35.65±1.13^{e}	80.21±2.54^{a}	52.92±1.67^{c}	47.80±1.51^{d}	77.7±2.45^{a}	<0.001
Biological value (%)	59.50±1.88^{e}	90.60±2.87^{a}	75.50±2.39^{c}	66.70±2.11^{d}	90.00±2.85^{a}	<0.001
Protein efficiency ratio	1.86±0.06^{a}	0.34±0.01^{b}	0.25±0.01^{c}	0.18±0.01^{d}	0.35±0.01^{b}	<0.001
Liver weight (g)	7.06±0.48^{a}	7.08±0.45^{a}	5.35±0.15^{b}	5.62±1.23^{b}	5.24±1.45^{b}	< 0.001
Kidney weight (g)	1.49±0.08^{b}	1.73±0.12^{a}	1.49±0.05^{b}	1.35±0.23^{b}	1.54±0.10^{ab}	0.002
Heart weight (g)	0.60±0.03^{bc}	0.76±0.05^{a}	0.63±0.08^{b}	0.53±0.47^{c}	0.48±0.17^{d}	< 0.001

Note: Values are presented as group means ± standard deviation. Different superscript letters within a row indicate statistically significant differences between groups based on one-way ANOVA followed by Tukey's post hoc test (P≤0.05). Groups sharing the same superscript are not significantly different.

Table 2. Haematological parameters of experimental rats fed formulated diets.

Parameter	PM (n=5)	Control (n=5)	NGTI (n=5)	GTI (n=5)	Casein (n=5)	p-value
WBC (×10³ mm^{-3})	8.80±0.38	6.20±0.15	3.00±0.21	4.20±0.45	5.40±0.20	0.220
RBC (×10³ mm^{-3})	5.14±0.23	6.45±0.17	6.38±0.15	5.45±0.22	5.81±0.16	0.510
HGB (g/dl)	8.70^{b}±0.15^{b}	13.00±0.16^{a}	12.90±0.19^{a}	10.30±0.12^{a}	12.00±0.20^{a}	0.034
HCT (%)	38.00±0.87	41.80±1.37	42.60±1.34	33.00±1.31	38.30±1.61	0.781
MCV (fl)	73.90^{b}±0.67^{a}	64.40±1.11	66.80±0.94	60.60±1.52	65.90±1.05^{b}	0.021
MCH (pg)	22.40±0.58^{a}	20.00±0.58^{b}	20.20±0.29^{b}	18.90±0.34^{b}	19.30±0.31^{b}	0.013
MCHC (g/dl)	30.30±0.30	31.10±0.47	30.30±0.56	31.20±0.36	31.30±0.30	0.399

Note: Values are means ± standard deviation. Different superscript letters within a row indicate statistically significant differences between groups based on one-way ANOVA followed by Tukey's post hoc test (P≤0.05). Groups sharing the same superscript are not significantly different.

highest efficiency (set at 100%), while NGTI and GTI groups reached 82% and 65%, respectively. PM rats, when compared to the control and casein groups, showed markedly reduced values for true digestibility, net protein utilization (NPU), and biological value (BV), indicating metabolic inefficiency. Casein and Control diets supported the most efficient nitrogen use, while NGTI and GTI performed moderately, likely due to plant protein limitations. The PM group showed an anomalously high protein efficiency ratio (PER = 1.86), a known artifact in low protein intake contexts. Other groups had PER values between 0.18 and 0.35, reflecting balanced protein quality.

Hematological properties of experimental rats fed formulated diets

Hematological parameters of rats fed various diets are summarized in Table 2, providing insight into physiological and immune responses to dietary treatments. WBC counts ranged from 3.10 × 10³/mm³ (Casein) to 8.80 × 10³/mm³ (PM), with no significant group differences (p>0.05). Elevated WBC in PM may reflect immune stress due to protein deficiency. RBC values showed no significant variation (p>0.05), ranging from 5.14 × 10⁶/mm³ (PM) to 7.12 × 10⁶/mm³ (NGTI), suggesting erythropoiesis remained broadly stable. Hemoglobin (Hb) was significantly lower in PM (8.70 g/dl) versus Control (13.00 g/dl, p=0.037) and NGTI (13.40 g/dl, p=0.033), indicating compromised hemoglobin synthesis under protein restriction. GTI and Casein showed moderate but non-significant improvement. Hematocrit (HCT) ranged from 26.70% to 47.40% with no significant differences (p=0.781). MCHC also showed no group differences (p=0.399), though Control and Casein groups had slightly higher values. In contrast, MCV and MCH differed significantly among groups (p=0.021 and p=0.013). PM rats had the highest MCV (73.90 fl), significantly higher than Control (p=0.013) and Casein (p=0.049), suggesting macrocytic changes due to impaired erythrocyte maturation.

Table 3. Biochemical parameters of rats fed on different diets.

Parameter	PM (n=5)	Control (n=5)	NGTI (n=5)	GTI (n=5)	Casein (n=5)	p-value
ALT (U/L)	42.46±2.44[a]	50.30±2.22[a]	56.97±6.96[a]	43.43±3.30[a]	44.20±8.93[a]	0.041
ALP (U/L)	386.55±1.84[a]	203.50±5.37[d]	242.67±5.49[c]	300.00±15.00[b]	232.67±9.37[c]	<0.001
AST (U/L)	50.02±0.97[a]	72.40±3.10[b]	89.40±7.20[c]	67.30±2.70[d]	66.30±5.60[d]	<0.001
AST:ALT Ratio	1.16±0.51	1.44±0.00	1.57±0.00	1.55±0.00	1.50±0.00	0.238
Albumin (g/dL)	2.89±0.00[a]	3.43±0.07[b]	3.26±0.08[b]	3.17±0.20[b]	3.23±0.17[b]	0.055
Total Protein (g/dL)	5.17±0.00[a]	6.66±0.76[b]	6.32±0.07[b]	5.95±0.27[b]	6.93±0.38[b]	0.002
Urea (mg/dL)	82.10±4.17[a]	31.60±6.90[c]	42.53±3.47[b]	44.40±5.44[b]	42.57±13.77[b]	<0.001
Creatinine (mg/dL)	1.44±1.15	0.29±0.24	0.37±0.13	0.29±0.06	0.27±0.09	0.088
Cholesterol (mg/dL)	54.09±2.71[a]	66.30±6.11[b]	53.50±0.71[a]	55.00±5.29[a]	42.00±2.83[c]	<0.001
LDLc (mg/dL)	4.22±1.61[a]	10.20±1.90[c]	7.10±0.80[b]	7.80±2.16[b]	13.50±2.95[d]	0.002
Triglycerides (mg/dL)	45.01±1.86[a]	63.50±4.95[b]	47.00±12.17[a]	39.50±6.36[a]	59.50±5.29[b]	0.008

Note: Values are presented as mean±standard deviation. Different superscript letters within a row indicate statistically significant differences between groups based on one-way ANOVA followed by Tukey's post hoc test (P≤0.05). Groups sharing the same superscript are not significantly different.

Overall, protein malnutrition adversely affected erythrocyte indices and hemoglobin status, while Control and Casein diets preserved hematological stability. GTI and NGTI showed intermediate recovery.

Biochemical parameters of experimental rats fed on formulated diets

Biochemical markers including AST, ALT, ALP, total protein, albumin, urea, creatinine, and lipid profiles were analyzed to assess liver, kidney, and metabolic function across diet groups (Table 3). AST ranged from 49.2 to 89.4 U/L, with the highest levels in the NGTI group, suggesting elevated liver stress following protein refeeding. ALT values (42.46-56.97 U/L) showed a similar trend, with NGTI again highest, while PM and GTI groups had the lowest. ALP was markedly elevated in the PM group (386.55 U/L), indicating impaired liver or bone function due to prolonged protein deficiency. In contrast, Control rats had the lowest ALP, with GTI and Casein groups showing partial normalization. Total protein and albumin were lowest in PM rats (5.17 and 2.89 g/dL), confirming malnutrition. Control rats had the highest values (6.66 and 3.43 g/dL), while GTI and Casein groups showed intermediate recovery. Urea levels were highest in PM (82.1 mg/dL), indicating renal stress, and lowest in Control (31.60 mg/dL). GTI and NGTI had intermediate levels, suggesting partial renal recovery. Creatinine levels remained largely comparable, with a slight rise in PM rats. Lipid profiles showed the Control group with highest cholesterol (66.3 mg/dL) and LDLc (10.2 mg/dL). The casein group showed lower cholesterol but higher LDLc, possibly due to differential protein source effects. Triglycerides followed a similar trend: Control rats had the highest levels, while PM rats had the lowest, reflecting suppressed lipid synthesis under malnutrition.

In summary, the PM group exhibited the most pronounced disruptions in liver and kidney markers, while the Control group maintained optimal values. Protein restoration via GTI and Casein diets supported partial biochemical recovery, underscoring the importance of timely nutritional intervention.

Discussion

Growth and nutritional recovery

Results showed greater weight gain, feed intake, and skeletal growth in the NGTI group compared to GTI, suggesting non-germinated Tempe protein is more bioavailable for nutritional recovery. Previous studies confirm that non-germinated soy protein is more effective than germinated soy due to a complete amino acid profile and reduced proteolysis during germination (Kohli and Singha 2024). Notably, the protein efficiency ratio (PER) for NGTI and GTI was below the FAO/WHO-recommended value (2.7) for ideal recovery foods, indicating that even though NGTI supported better growth, its PER still fell short (Adejuwon et al. 2021). In PEM, reduced absorption and catabolism lower BV. Refeeding with digestible, high-quality protein such as casein or NGTI restores BV to near-normal, supporting faster growth (Teixeira et al. 2022).

Nitrogen utilization and protein digestibility

This study identifies key findings, including superior nitrogen retention (NR), true digestibility (TD) and net protein utilisation (NPU) in the NGTI group compared to GTI. Enhanced nitrogen assimilation was linked to better protein bioavailability in NGTI (Marín-García

et al. 2022). TD reflects the proportion of ingested protein absorbed, NR represents retained nitrogen after losses, and NPU combines both to assess protein use efficiency (Adejuwon et al. 2021). These findings align with Adhikari et al. (2022), who reported that protein quality influences nitrogen metabolism and protein turnover. Soy protein has also been shown to improve protein retention and gastric efficiency over other plant proteins (Qin et al. 2022). NGTI's superior protein efficiency is further supported by Sabrina et al. (2022), who noted Tempe protein enhances nutrient absorption and reverses malnutrition. Nutritional rehabilitation is marked by recovery of organ weights (heart, liver, kidney), which are suppressed during PEM due to impaired cellular maintenance. Protein refeeding restores organ mass by promoting cellular regeneration, enzyme activity and metabolic balance (Osundahunsi et al. 2003).

Hematological and biochemical parameters

During hematological recovery, the NGTI group showed better hemoglobin (Hb) and hematocrit (HCT) levels than the GTI group. This supports earlier findings that protein supplementation corrects malnutrition-induced anemia (Augustin et al. 2024). Higher Hb in NGTI-fed rats reflects more effective hemoglobin synthesis and red blood cell maturation, likely due to superior protein bioavailability. The NGTI group also showed improved red cell indices – MCH, MCHC and MCV – which are key indicators of erythropoietic health. Low values suggest anemia, while elevated values may reflect recovery (YP et al. 2018).

Anderson (2008) found soy-based protein improved liver function during recovery. Lower urea and creatinine in NGTI and casein groups suggest better renal clearance and reversal of PEM-related dysfunction (Rusul et al. 2014). Serum liver enzymes (AST, ALT, ALP) further indicated liver recovery. NGTI-fed rats showed balanced AST and ALT levels, suggesting less hepatic stress and better regeneration. This agrees with Xiao and Hendry (2022), who reported that soy diets support liver regeneration and reduce hepatic lipid buildup. Since AST and ALT correlate with tissue injury (Botros and Sikaris 2013), normalization of these levels reflects NGTI's hepatoprotective potential.

Conclusion

The findings of this study underscore the effectiveness of non-germinated Tempe protein isolates (NGTI) in promoting nutritional recovery from protein-energy malnutrition (PEM). NGTI performed very well compared to germinated Tempe protein isolates (GTI) across multiple recovery parameters, including growth, nitrogen utilization, hematological indices and biochemical markers. However, the casein and control groups outperformed in many parameters, indicating them as a high-quality protein source. These results highlight the potential of NGTI as a sustainable, complementary and cost-effective dietary intervention for addressing protein-energy malnutrition, but not a true replacement of casein. The study also reinforces the importance of non-germinated Tempe protein as an optimal choice for improving protein digestibility and bioavailability, making it a valuable resource for combating malnutrition in resource-limited settings for the human population as well as in the formulation of recovery diets for animals recovering from illness or undernutrition. Despite fruitful results, this study has several limitations that need to be addressed in further studies. The sample size was small and the intervention period relatively short, limiting our ability to understand long-term physiological adaptations. The restricted sample size was determined in accordance with institutional ethical guidelines to minimize animal use, which, while ensuring humane practice, inherently limited the strength of statistical inference, so the findings should be regarded as suggestive and exploratory. Furthermore, species-specific differences restrict direct translational applicability to humans and companion animals. Future studies comprising larger cohorts should conduct human and veterinary clinical trials to evaluate long-term metabolic, immunological and cognitive recovery outcomes following NGTI intervention. Such studies should also explore NGTI's impact on gut microbiota composition and inflammatory pathways.

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