Maliza *et al.*
*Food Production, Processing and Nutrition* (2024) 6:97
https://doi.org/10.1186/s43014-024-00269-x

Food Production, Processing and Nutrition

RESEARCH **Open Access**

# Lima bean (*Phaseolus lunatus* L.) powder ameliorates pituitary-liver-axis regulation and anti-inflammatory activity in malnourished rats

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**Abstract**

The endocrine system is critical for adapting to malnutrition, which can disrupt the pituitary-liver axis and induce inflammation, leading to health complications. Lima beans (*Phaseolus lunatus* L.), known for their high protein content and anti-inflammatory properties, present a potential nutritional intervention. This study investigated the effects of lima bean powder on pituitary-liver axis regulation and anti-inflammatory activity in malnourished rats. Rats were divided into four groups: Normal (N), Malnourished (MAL), MAL treated with 25% lima bean flour (MAL + 25% LB), and MAL treated with 50% lima bean flour (MAL + 50% LB) for 6 weeks. Proximate composition was determined to analysed its major nutrients and metabolites in the methanol extract were analysed through LC–MS/MS. Parameters such as weight gain, serum albumin, total protein levels, Growth Hormone (GH), Insulin-like Growth Factor 1 (IGF1), and liver inflammation markers were measured. Bioactive compounds such as L(-)-pipecolinic acid, choline, trigonelline, L-phenylalanine, and oleamide were identified, highlighting the nutritional and therapeutic potential of lima beans. Compared to the N group, the MAL group showed significant decreases in body weight gain, serum albumin, and total protein levels. However, both MAL + 25% LB and MAL + 50% LB groups demonstrated significant improvements in these parameters, approximating the levels observed in the N group. Lima bean supplementation appeared to regulate GH at both the cellular and mRNA levels, positively impacting the pituitary-liver axis. Additionally, the study revealed reduced liver inflammation in the MAL + 25% LB and MAL + 50% LB groups, suggesting the anti-inflammatory properties of lima beans. These findings indicate that lima bean flour supplementation can ameliorate disruptions in the pituitary-liver axis and reduce inflammation in malnourished rats.

**Keywords** Lima Bean, Malnutrition, Growth Hormone (GH), Insulin Growth Factor 1 (IGF1), Pituitary Gland, Inflammatory, Wistar Rats

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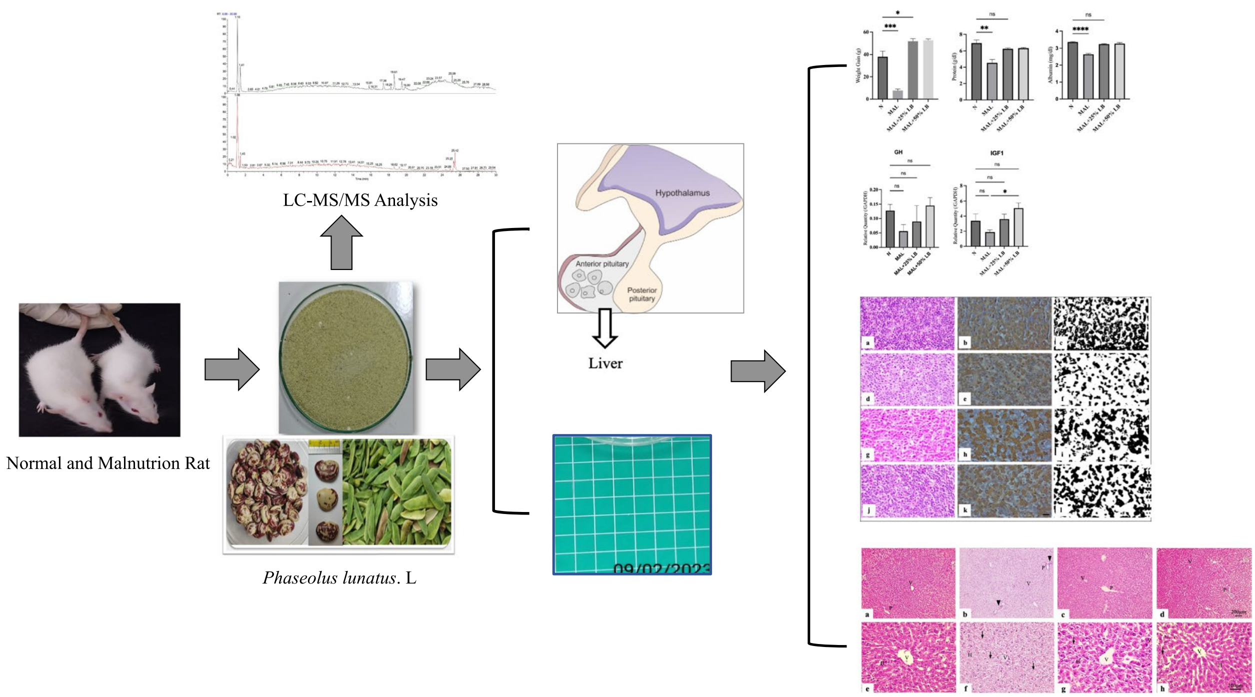
## Graphical Abstract




## Introduction

The lima bean (*Phaseolus lunatus* L.) is a food source that various cultures traditionally consume due to its abundant nutritional content. Lima beans are packed with essential nutrients, such as dietary fibers, proteins, vitamins, and minerals, significantly contributing to overall health and wellness (Campos-vega et al., 2010). With a protein content varying between 14.24% and 24.92%, moisture content at 4.30%, crude fat at 2.62%, crude fiber at 18.00%, ash content at 2.13%, and carbohydrate content at 56.14%, lima beans exhibit the capacity to function as a substantial provider of essential nutrients (Ibeabuchi et al., 2019; Jayalaxmi et al., 2016). They are also rich in essential amino acids (FAO, 2018). In addition to their impressive macronutrient composition, lima beans are considered health promoting pulse due to their low glycemic index resulting from slow-release carbohydrates (Bello-Pérez et al., 2007). They also have antioxidant and anti-inflammatory properties, which are key to preventing and managing various diseases (Johnson & De Mejia, 2016; Tamayo et al., 2018).

Malnutrition, a worldwide health concern, can seriously impair this axis, leading to various health problems (World Health Organization, 2020). Malnutrition can impact the production of growth hormone (GH), which stimulates growth and development in children and sustains muscle and bone mass in adults. A decrease in GH production can result in stunted growth in children and loss of muscle and bone mass in adults (Fazeli et al., 2014). GH triggers the production of insulin-like growth factor 1 (IGF1) in the liver. Moreover, GH deficiency and the resulting reduction in IGF1 can lead to several developmental problems, primarily affecting growth, puberty, muscle and bone and development. Undernutrition can cause a reduction in the number of GH Receptors (GHRs) in the liver (Fazeli, 2014). This decrease in GHR can lead to GH resistance, which can further intensify the decrease in IGF1 levels and add to the negative effects of malnutrition on growth and development (Yakar et al., 1999). Malnutrition significantly impairs cognitive function metabolic health, learning, mental development, and endurance, increasing susceptibility to illnesses and the risk of mortality (Berardino et al., 2020; Braga et al., 2014; Forgie et al., 2020; Maliza et al., 2023; Woldehanna et al., 2017). Chronic inflammation, a common consequence of malnutrition, can lead to various health issues, including heart disease and cancer (Fatyga et al., 2020; Furman et al., 2019). Previous studies have shown that dietary interventions can regulate the body's inflammatory response (Calder et al., 2017).

Prior investigations have documented the impact of lima beans given to rats and mice, revealing notable effects on hematological, biochemical, and enzyme activities (Soetan., 2018; Nweze et al., 2023; Oboh & Omofoma., 2008). However, research exploring the efficacy of lima bean flour on malnourished rats has not yet been reported. Lima beans have the potential to address malnutrition problems worldwide due to their high nutrient density, cost-effectiveness, and ease of cultivation. However, further research is needed to fully realize this potential. Investigating the bioavailability of nutrients and the health effects of regular consumption is essential. Addressing these aspects will enhance the effectiveness of lima beans as a global nutritional intervention and help alleviate malnutrition on a broader scale. Given the widespread prevalence of malnutrition, finding measures to mitigate its effects is crucial. With their abundant nutrient content and potential health benefits, lima beans could offer an affordable and accessible solution to this global health issue. This study aims to explore how lima bean (*Phaseolus lunatus* L.) powder affects the regulation of the pituitary-liver axis and its anti-inflammatory activity in malnourished rats, providing valuable insights into its therapeutic potential.

## Materials and methods

### Production of *Lima* Bean Flour

Lima beans was collected from Alahan Panjang, Solok, West Sumatra Indonesia (-1.075245,100.784014). Lima beans were initially cleaned and then soaked in distilled water at a ratio of 1:5 for 12 h at room temperature. After soaking, they were boiled in distilled water at a 2:1 water-to-grain ratio for 60 min. Following this preliminary cooking stage, the lima beans were oven-dried at 60 °C with air circulation until they reached a constant weight. After cooling, the lima beans are ground using a grinding machine and sieved using a 20 mesh sieve to produce flour, which is then stored in polyethylene bags at 8 °C (Jayalaxmi et al., 2016).

### Proximate Composition of the *Lima* Bean Flour

The methods used for chemical analysis adhered to the guidelines set out by the Association of Official Analytical Chemists (AOAC) and included considerations for moisture (AOAC 925.10), ash (AOAC 923.03), crude protein (AOAC 979.09), crude fiber (AOAC 962.09), fat (AOAC 920.39), and carbohydrates calculated by difference (100—sum of moisture, ash, crude protein, crude fiber, and fat). The results were obtained in triplicate from unhulled lima bean seed flour studies.

### LC–MS/MS Analysis of the *Lima* Bean Flour Extracts

The sample solution was prepared by dissolving 500 mg of lima bean flour in 5 ml of methanol and subjected to ultrasonication for 30 min at room temperature. After ultrasonication, the solution was filtered through a 0.22 µm PTFE membrane filter. A 5 µL aliquot of the filtrate was injected into the LC–MS/MS system. For quality control (QC), a solution was prepared by mixing 20 µL of each sample analyzed, from which a 5 µL aliquot was subsequently injected into the LC–MS/MS system. Metabolite separation and identification were performed using a UHPLC Vanquish Tandem Q Exactive Plus Orbitrap HRMS from Thermo Scientific (Germany). Metabolites were identified employing LC–MS/MS. The analysis involved two mobile phases, 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B), with a flow rate of 0.2 mL/min. A gradient elution system was used for the mobile phase composition, which was set as follows: 0–1 min at 5% B, 1–25 min from 5 to 95% B, 25–28 min at 95% B, and 28–30 min back to 25% B. An Accucore C18 column (100 × 2.1 mm, 1.5 µm from ThermoScientific) was used for the separation. The MS ionization source was ESI (+) coupled with a Q-Orbitrap mass analyzer, covering a m/z range of 100 to 1500, with ionization energies of 18, 35, and 53 eV. Data from the LC–MS/MS analysis were processed using Compound Discoverer 3.2 software (Thermo Scientific, Germany) and compared with an in-house database to tentatively identify the metabolites present in the lima bean flour.

### Animals and experimental design

Young male Wistar rats (4–5 weeks old, 55-60 g) were procured from "Rumah Tikus" (West Sumatra, Indonesia). The Wistar rats were maintained in a room with regulated temperature (22 ± 1 °C) and subjected to a 12-h light/12-h dark cycle, with lights on from 07.00 h to 19.00 h. The Wistar rats were provided ad libitum access to standard food and water. The National Academy of Sciences, USA's Guide for the Care and Use of Laboratory Animals and the Guidelines for Animal Experimentation were followed when handling the animals.

Eight male Wistar rats were assigned to a group (N group) receiving a standard diet containing 20% protein for 12 weeks. Simultaneously, 24 Wistar rats were fed a diet containing 11% protein, creating a nutritional deficit over six weeks (MAL). At the start of the seventh week, these malnourished rats were randomly divided into three groups (MAL, MAL + 25% LB, and MAL + 50% LB). In the MAL group, eight rats continued the low-protein (11%) diet for another six weeks (Solang and Adriani., 202; Mentang et al., 2023). In the MAL + 25% LB group, eight rats were fed a diet with the same low protein content but with 25% of the feed substituted with lima bean flour. Similarly, in the MAL + 50% LB group, eight rats were fed a diet in which 50% of the meal was replaced with lima bean flour while maintaining the same low protein content. Throughout the study, all rats had ad libitum access to food and water.

#### Body Weight Measurement

During the 12-week treatment period, the body weight of rats in each treatment group was assessed and documented on a weekly basis: N, MAL, MAL + 25%, and MAL + 50% LB cohorts. The mean body weight following the 12-week treatment period within the N, MAL, MAL + 25%, and MAL + 50% LB groups was 232.33 g, 69 g, 146.66 g, and 157 g, respectively. The calculation of the rats' body weight discrepancy was conducted by subtracting the weight recorded at the conclusion of the 12-week period from the weight measured in the sixth week subsequent to the malnutrition intervention. This methodology was employed to ascertain the weight variance and subsequent gain in grams after the administration of lima bean flour.

#### Serum Biochemical Analyses

We collected whole blood (5 cc) samples via cardiac puncture and placed them in ethylene diamine tetraacetic acid (EDTA)-embedded sample bottles at the end of the experiment. These samples were then incubated for 30 min before centrifugation at 800 × g for 10 min to isolate the serum. We proceeded to analyze the total protein and serum albumin levels. Total protein and serum albumin were quantified using a DiaSys Kit (Germany) following the manufacturer's instructions for the colorimetric estimation of total protein and albumin.

#### Histological Analyses and Immunohistochemistry

The animals were euthanized using ketamine as an anesthetic at the end of the experimental study. The pituitary gland and liver samples were immediately extracted, preserved in 10% formal saline for 72 h, dehydrated using a series of alcohol solutions of increasing concentration and clarified with two rounds of xylene before being embedded in paraffin wax. A microtome was used to generate serial transverse sections with a thickness of 4–5 microns. These sections were stained with hematoxylin and eosin (H&E).

The process of immunohistochemistry involves the use of an antibody for rat GH (Maliza et al., 2017). Rabbits were inoculated with an oligopeptide matching rat GH amino acid 330–341 (YVLGNPLTQGIN) and connected to keyhole limpet hemocyanin to form the GH antibody. Following overnight incubation with GH primary antibodies (at a 1:10,000 dilution) in phosphate-buffered saline at room temperature, the sections were exposed to a biotin-conjugated secondary antibody (Vector Laboratories, Burlingame, CA, USA). The sections were then stained with 3,3'-diaminobenzidine, which served as a substrate, using an avidin–biotin-peroxidase reagent (Vectastain ABC Kit, Vector Laboratories, USA) as part of the ABC procedure.

#### Reverse transcription (RT)-PCR

We extracted total RNA from the pituitary gland and liver tissue using an RNeasy Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. As outlined in our previous report (Maliza et al., 2016), we carried out quantitative real-time PCR. We utilized a PrimeScript First Strand cDNA Synthesis Kit (Takara Bio, Otsu, Japan) for cDNA synthesis. Quantitative real-time PCR was conducted using an ABI PRISM 7900HT (Applied Biosystems, CA, USA) with gene-specific primers and SYBR Premix Ex Taq (Takara Bio) containing SYBR Green I. The primers used to amplify the fragments are listed in Table 1. For normalization, GAPDH was also quantified. We calculated relative gene expression by comparing the cycle times for each target PCR. The cycle threshold values were transformed into relative gene expression levels using the $2^{-(\triangle Ct\ sample-\ \triangle Ct\ control)}$ method.

#### Statistical analysis

We conducted statistical comparisons using one-way analysis of variance (ANOVA) with the assistance of GraphPad PRISM 9 software. If the ANOVA revealed significant differences, we then applied Tukey's post hoc test to compare the mean values of the treatment groups with those of the control group. Statistical significance was denoted using the following symbols: **** for p.0001, *** for p.001, ** for p.01, * for p.05, and ns for no significant correlation. Unless stated otherwise, the results from the analyses are presented as the mean value with the standard error of the mean (SEM).

## Results and Discussion

### Proximate Composition of the *Lima* Bean Flour

The nutritional composition of the lima bean flour is presented in Table 2. Lima beans, also called butter beans,

**Table 1** List of primers used for the real-time polymerase chain reaction

| Genes | Primer sequence (5'-3') |
|---|---|
| Growth Hormone (GH) | Forward: CAAGAGGCTGGTGCTTTACC |
| | Reverse: AATGTAGGCACGCTCGAACT |
| Insulin-like growth factor 1 (IGF 1) | Forward: GCTGAAGCCGTTCATTTAGC |
| | Reverse: GAGGAGGCCAAATTCAACAA |
| Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) | Forward: GTCTTCACCACCATGGAGAA |
| | Reverse: ATGGCATGGACTGTGGTCAT |

**Table 2** The nutritional composition of lima bean flour

| Component | Value % |
|---|---|
| Moisture | 7.465 ± 0.388 |
| Ash | 8.51 ± 0.601 |
| Protein | 38.57 ± 3.76 |
| Fat | 4.66 ± 0.84 |
| Carbohydrate | 40.65 ± 1.72 |
| Crude Fibre | 1.335 ± 0.33 |

All data are presented in the form of Means ± SD

are a noteworthy food source with substantial nutritional benefits (El-Adawy, 2002). They are recognized for their high protein and carbohydrate content, significant mineral content, and comparatively low-fat content, which positions them as an optimal food source for addressing malnutrition (Adeleke & Odedeji, 2010; FAO, 2018). The fat content in Lima beans flour varies depending on factors such as the variety of beans, processing methods, and whether the beans are dehulled. The previous studies in the general indicate that Lima beans flour has a low-fat content, typically ranging from 0.62% to 4.67%, similar with our data 4.66% (Ogechukwu & Ikechukwu., 2017; Yellavila et al., 2015; Ibeabuchi et al., 2019). The substantial protein content in lima beans (38.57 ± 3.76 g/100 g) was particularly significant. Proteins, which are essential for the growth, repair, and maintenance of body tissues, can lead to protein-energy malnutrition if deficient, a critical health concern, particularly in developing nations (Schoenfeld et al., 2013; Young & Pellett, 1994). Thus, incorporating lima beans into the diet can greatly assist in fulfilling daily protein needs and fighting protein-energy malnutrition (Adeleke & Odedeji, 2010). Carbohydrates, the body's primary energy source, are also abundant in lima beans (40.65 ± 1.72 g/100 g). Sufficient carbohydrate consumption is crucial for maintaining energy equilibrium, especially for malnourished individuals needing to replenish their energy stocks (FAO, 2018; WHO, 2003).

Despite the somewhat low crude fiber content of 1.335 ± 0.33 g/100 g, lima beans play a significant role in increasing overall dietary fiber intake and are essential for addressing malnutrition (Anderson et al., 2009; Slavin, 2005). Research has shown that combining fiber with adequate protein can promote growth and improve body composition. The consumption of dietary fiber, especially when paired with a high-protein diet, can promote gut health, enhance nutrient absorption, lower systemic inflammation, and enhance lipid profiles, all of which are crucial for overall health, growth and development (Hermes et al., 2009; Ni et al., 2022; Xie et al., 2015). The ash content of the lima beans (8.51 ± 0.601 g/100 g) signifies a considerable mineral content. Minerals play a vital role in various bodily functions, including bone health, fluid balance, and metabolic processes (FAO, 2018; WHO, 2004). Therefore, ensuring sufficient mineral intake is a key aspect of addressing malnutrition. However, nutrient bioavailability in lima beans, like in other legumes, can be influenced by certain factors. These include cooking methods and the addition of antinutritional elements such as phytic acid and tannins (El-Adawy, 2002; Hefnawy, 2011). Proper processing methods, such as soaking and boiling, can help diminish these antinutritional factors and enhance nutrient bioavailability (Hotz & Gibson, 2007; Reddy et al., 1982). Lima beans can be important for addressing malnutrition by providing vital proteins, carbohydrates, fiber, and minerals.

### Metabolites identified in *lima* bean flour extracts

The data in Table 3 reveal the presence of various metabolites, including amino acids, peptides, alkaloids, andty acids, identified in the methanol extract of lima bean flour. The compound with the highest peak area was L(-)-pipecolinic acid, followed by choline and trigonelline. L(-)-pipecolinic acid has the highest peak area and accounts for about 34.40% of the total peak area. This indicates that this compound is the dominant component in the sample. L(-)-Pipecolinic acid, also known as L-Pipecolic acid, it is primarily known as a metabolite derived from lysine, one of the essential amino acids. As the L-enantiomer of pipecolic acid, it plays roles in various biological processes and is often studied for its involvement in metabolic disorders in humans. L(-)-Pipecolinic acid, choline, trigonelline, and oleamide influence malnutrition activities by modulating metabolic processes, inflammatory responses, and appetite regulation. Their roles in enhancing metabolic health and managing nutritional deficiencies highlight their potential in addressing malnutrition-related challenges. Previous research indicates that trigonelline has anti-diabetic and anti-inflammatory effects (Li et al., 2019) and antioxidant activity, offers potential health benefits (Yadav & Baquer, 2014). Choline plays a major role in improving malnutrition by promoting healthy liver function and aiding crucial physiological functions (Chawla et., 1989; Zeisel, et al., 2003; Zeisel & Blusztajn., 1994). The significance of its involvement in gut microbiota balance and metabolic activities highlights its critical role in the treatment and prevention of malnutrition (Romano et al., 2015). Additionally, Oleamide is a bioactive compound with relatively long retention times, was also identified with significant peak areas. These organic compounds, belonging to the amide class, are involved in cellular signaling and have potential biological activities, including anti-inflammatory, immunomodulatory, and antioxidant activities (Carrillo et al., 2012; Rahimi et al., 2019) and oleamide regulates feeding

**Table 3** Metabolites identified in lima bean flour extracts

| Compound Number | RT [min] | Molecular Formula | Area [max] | Compounds | Percentage Area (%) |
|---|---|---|---|---|---|
| 1 | 1.12 | C6 H11 N O2 | 5,075,984,987 | L(-)-Pipecolinic acid | 34.39 |
| 2 | 1.045 | C5 H13 N O | 1,838,195,128 | Choline | 12.45 |
| 3 | 1.086 | C7 H7 N O2 | 1,834,691,271 | Trigonelline | 12.43 |
| 4 | 25.424 | C18 H35 N O | 1,085,980,488 | Oleamide | 7.35 |
| 5 | 1.085 | C5 H11 N O2 | 1,073,743,444 | Betaine | 7.27 |
| 6 | 1.424 | C6 H11 N O2 | 732,771,694.6 | D-(+)-Pipecolinic acid | 4.96 |
| 7 | 1.576 | C6 H13 N O2 | 427,500,108.9 | L-Isoleucine | 2.89 |
| 8 | 1.063 | C5 H9 N O4 | 389,403,705.7 | L-Glutamic acid | 2.63 |
| 9 | 1.115 | C6 H8 O7 | 322,285,540.1 | Citric acid | 2.18 |
| 10 | 1.042 | C4 H8 N2 O3 | 182,332,276.4 | Asparagine | 1.23 |
| 11 | 24.888 | C16 H33 N O | 176,020,433.7 | Hexadecanamide | 1.19 |
| 12 | 2.502 | C9 H11 N O2 | 169,183,722.7 | L-Phenylalanine | 1.14 |
| 13 | 1.422 | C6 H8 O7 | 166,212,078.1 | Citric acid | 1.12 |
| 14 | 1.171 | C6 H13 N O2 | 150,126,680.9 | L-(+)-Leucine | 1.01 |
| 15 | 1.024 | C6 H14 N4 O2 | 119,805,814.8 | DL-Arginine | 0.81 |
| 16 | 1.023 | C6 H9 N3 O2 | 106,149,555.4 | DL-Histidine | 0.71 |
| 17 | 1.08 | C5 H9 N O2 | 101,628,572.1 | L-Proline | 0.68 |
| 18 | 5.107 | C11 H12 N2 O2 | 99,285,158.81 | D-(+)-Tryptophan | 0.67 |
| 19 | 1.119 | C4 H6 O5 | 79,383,505.46 | DL-Malic acid | 0.53 |
| 20 | 1.07 | C5 H7 N O3 | 73,982,756.43 | L-Pyroglutamic acid | 0.50 |
| 21 | 1.047 | C4 H9 N O2 | 72,823,967.54 | gamma-Aminobutyric acid | 0.49 |
| 22 | 1.033 | C3 H10 N O4 P | 65,326,442.18 | N-methylethanolamine phosphate | 0.44 |
| 23 | 17.691 | C26 H48 N O7 P | 64,869,094.91 | LysoPC(18:3(9Z,12Z,15Z)) | 0.43 |
| 24 | 27.593 | C18 H37 N O | 52,191,344.89 | Stearamide | 0.35 |
| 25 | 5.228 | C11 H20 N2 O5 | 51,328,742.36 | L-gamma-Glutamyl-L-leucine | 0.34 |
| 26 | 1.042 | C9 H15 N3 O2 | 48,847,596.11 | hercynine | 0.33 |
| 27 | 26.424 | C20 H27 N | 46,350,302.21 | Alverine | 0.31 |
| 28 | 1.495 | C9 H11 N O3 | 41,063,297.27 | DL-Tyrosine | 0.27 |
| 29 | 1.048 | C4 H9 N O3 | 37,376,181.06 | L-(-)-Threonine | 0.25 |
| 30 | 1.098 | C7 H14 N2 O3 | 37,000,072.44 | L-Theanine | 0.25 |
| 31 | 0.986 | C10 H16 N2 O5 | 35,165,408.55 | 5,6-Dihydrothymidine | 0.23 |

behavior through cannabinoid and serotonin receptors (Cheer et al., 1999; Thomas et al., 1997). Bioactive compounds such as betaine and gamma-aminobutyric acid (GABA) suggest that lima bean extract may have osmoregulatory, neurotransmitter, and neuroprotective effects (Craig, 2004). Various essential amino acids, such as L-isoleucine, L-glutamic acid, L-phenylalanine, and L-leucine, indicate that lima beans are a good source of protein, supporting growth, tissue repair, and immune function (Wu G, 2016). The presence of various metabolites in the methanol extract of lima bean flour, indicating its potential significance in biological processes and suggesting that lima beans are a rich source of diverse, biologically active compounds including amino acids and bioactive peptides.

### Analysis of weight gain, albumin, and total protein

Figure 1a shows the differences in body weight gain of the rats at week 12 compared to week 6, which varied significantly across the groups. The normal group (N) gained 38.00 g, while the malnourished group (MAL) gained a markedly lower weight of 7.667 g. Interestingly, the malnourished groups treated with 25% and 50% lima bean flour (MAL + 25% LB and MAL + 50% LB) showed substantial weight gain, with 51.67 g and 52.67 g, respectively, exceeding those of the normal group. The observed decrease in weight gain in the MAL group is consistent with previous studies indicating that malnutrition can lead to stunted growth and weight gain due to insufficient intake of necessary nutrients (Scrimshaw, 2003). This could be due to the body's prioritization of

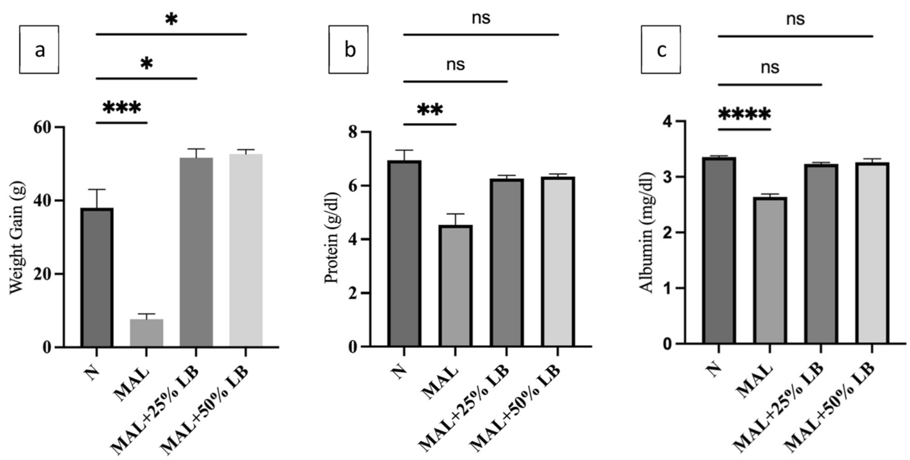



**Fig. 1** Weight Gain (**A**), Albumin (**B**), and Total Protein Level (**C**) in Normal (N), Malnourished (MAL) and Treated with 25% (MAL + 25% LB) and 50% (MAL + 50% LB) Lima Bean Flour. Data represent (Mean ± SEM, n = 4) ***p.001, **p.01, *p.05, and ns = no significant vs. control in each group

maintaining essential bodily function overgrowth during nutrient scarcity (Olson dkk., 2020). However, the substantial weight gain observed in the MAL + 25% LB and MAL + 50% LB groups suggested that the intake of lima bean flour may have contributed to improved weight gain in malnourished rats.

Albumin has a crucial function in addressing malnutrition through enhancing nutrient absorption and sustaining plasma colloid osmotic pressure (Mobarhan, 1988). Enhancing enteral nutrition tolerance in severely malnourished individuals can be achieved through albumin infusion to combat malabsorption issues. Moreover, albumin serves as a reliable marker of nutritional status, notwithstanding its susceptibility to variations due to inflammation and protein consumption (Cabrerizo et al., 2015). Therefore, ensuring sufficient protein intake and managing inflammation are vital components in the efficient treatment of malnutrition (Don & Kaysen., 2004). Albumin production is heavily reliant on the availability of dietary protein and energy (Levitt & Levitt, 2016). As shown in Fig. 1b, the normal group (N) exhibited an albumin level of 3.353 ml/dl. However, the serum albumin concentration in the malnourished group (MAL) decreased to 2.637 ml/dl. Interestingly, the MAL + 25% LB and MAL + 50% LB groups had serum albumin levels of 3.233 ml/dl and 3.260 ml/dl, respectively, resembling the levels found in the normal group. Lima beans, a rich protein source, are vital for albumin production (Adebo et al., 2023).

As presented in Fig. 1c, the total protein level varied across the groups. The normal group (N) had a total protein level of 6.937 g/dl. In contrast, the malnourished group (MAL) exhibited a reduced total protein level of 4.530 g/dl. This finding aligns with previous studies that have shown that malnutrition can lead to hypoproteinaemia, a condition marked by unusually low levels of proteins in the blood (Golden, 1991; Scrimshaw, 2003). This may result from inadequate dietary protein intake, hindered protein absorption, or enhanced protein catabolism, which are often linked to malnutrition. Interestingly, the malnourished groups treated with 25% and 50% lima bean flour presented total protein levels of 6.250 g/dl and 6.323 g/dl, respectively, indicating a recovery toward the levels observed in the normal group.

Lima beans are rich in dietary proteins, essential amino acids, and dietary fiber, which are important for growth and weight gain (Campos-vega et al., 2010; Schoenfeld et al., 2013; Young & Pellett, 1994). Furthermore, the essential amino acids in lima beans, such as leucine, have been shown to stimulate the mammalian target of the rapamycin (mTOR) pathway, a key regulator of cell growth and proliferation (Kimball & Jefferson, 2006). The high protein content in lima beans may contribute to increased muscle mass, while dietary fiber aids in maintaining healthy gut microbiota, which plays a crucial role in nutrient absorption and overall growth (Cani et al., 2007; Koh et al., 2016). Enhanced nutrient absorption might have aided in maintaining serum albumin levels by ensuring the availability of essential precursors for albumin synthesis.

### Analysis of the mRNA expression of GH and IGF1

Significant variations were observed in the average levels of GH mRNA expression in the anterior pituitary gland across the groups (Fig. 2). The normal group (N) had a relative quantity score of 0.1276. On the other hand, the malnourished group (MAL) demonstrated a substantial decrease in GH mRNA expression, as reflected by

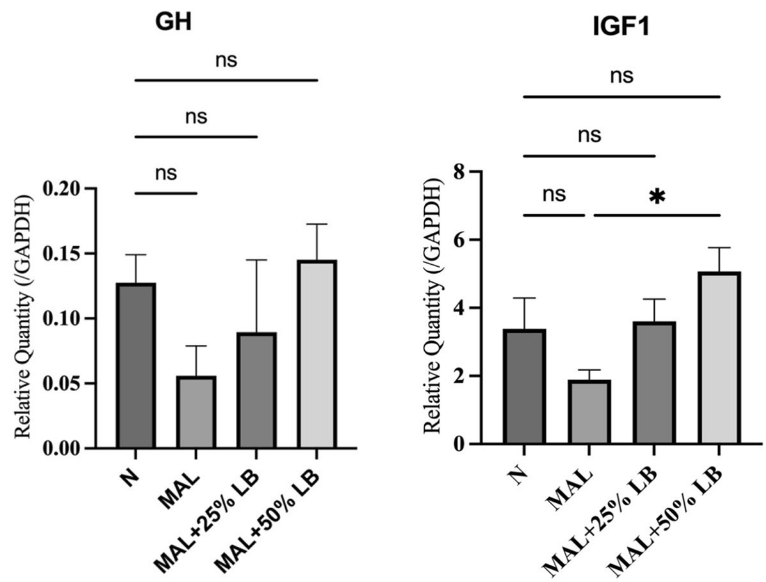



**Fig. 2** Expressions of mRNA GH and IGF1 in Normal (N), Malnourished (MAL), and treated with 25% (MAL + 25% LB) and 50% (MAL + 50% LB) Lima Bean Flour. Data represent (Mean ± SEM, n = 3) *p.05, and ns = no significant vs. control in each group

a relative quantity score of 0.05590. Interestingly, the malnourished groups treated with 25% and 50% lima bean flour showed an increase in GH mRNA expression, with relative quantity scores of 0.08944 and 0.1452, respectively.

There was a variation in the average levels of IGF1 mRNA expression in the liver tissue among the groups (Fig. 2). The normal group (N) had a relative quantity score of 3.384. In contrast, the malnourished group (MAL) displayed a significant decrease in IGF1 mRNA expression, with a relative quantity score of 1.889. Interestingly, the malnourished groups that received 25% and 50% lima bean flour treatments showed an increase in the IGF1 mRNA expression level, with relative quantity scores of 3.601 and 5.071, respectively. Malnutrition can profoundly affect the pituitary liver axis, leading to significant alterations in the growth and development of an organism. The GH-IGF1 axis promotes growth and maintains metabolic homeostasis. Malnutrition can lead to reduced GH secretion, which can result in lower levels of circulating IGF1, a hormone primarily responsible for the growth-promoting effects of GH. A decrease in GH secretion is considered adaptive, as it reduces the metabolic rate and conserves energy during insufficient nutrient intake (Rogol et al., 2000).

Moreover, malnutrition can also impact the GH receptor (GHR). Studies have shown that malnutrition can decrease GHR expression in the liver, resulting in GH resistance. This means that even if GH is present, it may be unable to exert its effects efficiently because of the reduced number of receptors (Fazeli, 2014).

Furthermore, a decrease in IGF1 levels due to malnutrition can have significant effects on various body tissues. IGF1 plays a critical role in cell proliferation, differentiation, and survival. Consequently, decreasing IGF1 levels can lead to impaired growth and development (Yakar et al., 1999). Malnutrition can significantly impact the GH-IGF1 axis by reducing GH secretion and decreasing GHR expression, leading to decreased IGF1 levels. These changes can have profound effects on growth and metabolic processes.

Treatment of malnourished rats with lima bean flour significantly increased GH and IGF1 mRNA expression, suggesting a beneficial effect of lima bean nutrients on GH and IGF1 synthesis. Lima beans are known to be rich in proteins and essential amino acids (Palupi et al., 2022). The essential amino acid of lima bean activates the mTOR pathway and can increase the transcription of the GH and IGF1 genes, leading to increased protein synthesis and cell growth (Norton & Layman., 2006). In particular, branched-chain amino acids (BCAAs) have been shown to stimulate the release of insulin, which can increase the expression of IGF1. Insulin and IGF1 have similar structures and functions and play critical roles in cell growth and metabolism (Doi et al., 2003).

#### Pituitary gland histopathology and GH immunohistochemistry.

Figure 3 shows that the histology of the anterior pituitary gland in malnourished rats did not show significant changes on routine HE staining other than slightly paler cytoplasmic staining of the endocrine glands. This histological phenomenon might be due to changes in the

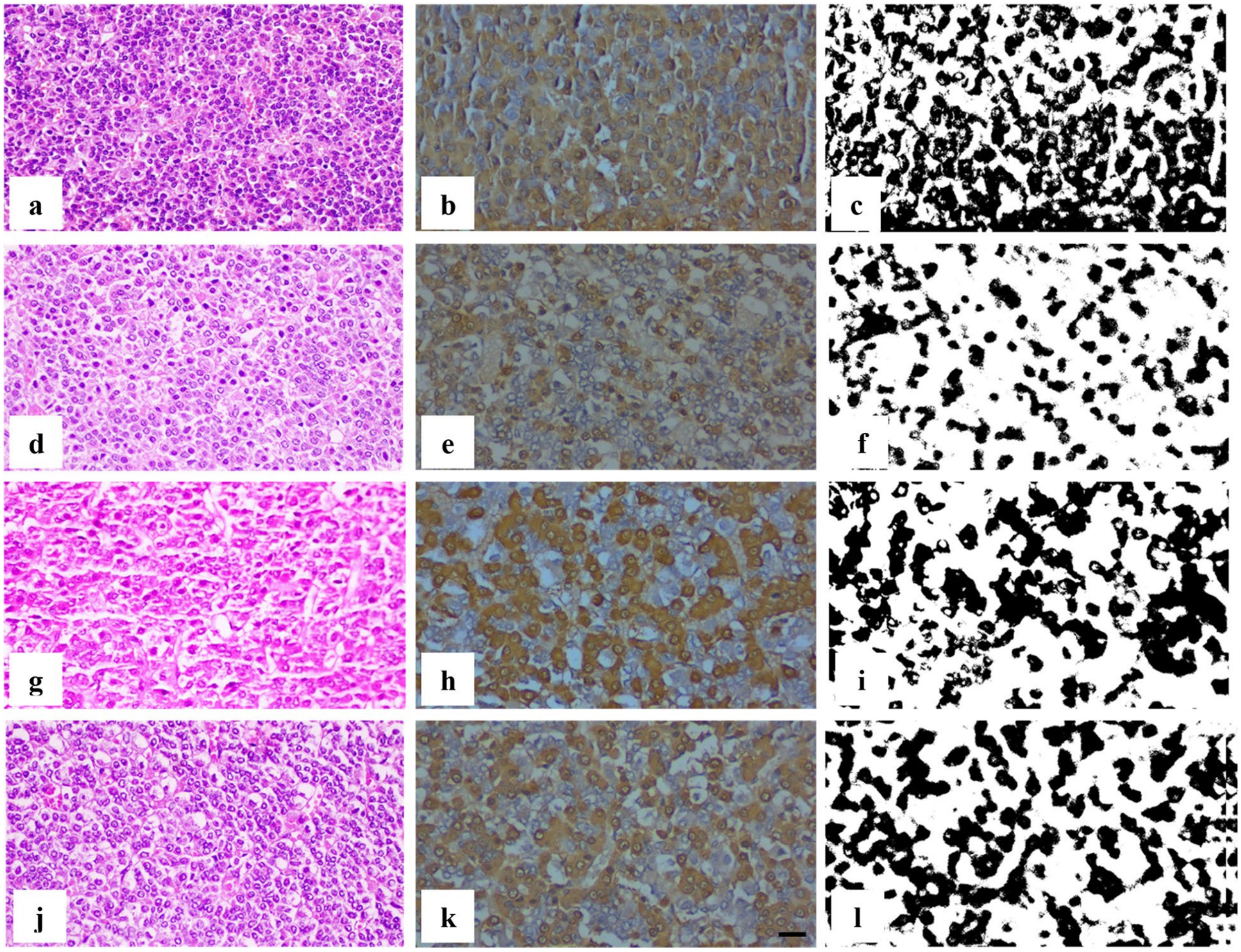



**Fig. 3** Photomicrograph of the anterior lobe of pituitary tissues showing histology on routine Hematoxylin–Eosin staining (a, d, g, j), Immunohistochemistry of GH hormone (b, e, h, k), and GH-stained fraction area (c, f, i, l). Normal rats (N; a, b, c), Malnourished rats (MAL; d, e,f ), and 25% (MAL + 25% LB; g, h,i) and 50% (MAL + 50% LB; j, k l) Lima Bean Flour Treatment, Hematoxylin eosin staining shows pale stained cytoplasm on the malnourished animal, and Immunohistochemistry shows reduction of GH expression on the malnourished rats. The treatment of Lima Bean Flour ameliorates GH staining on the pituitary gland

cytoplasmic content of cells, especially cytoplasmic proteins, including hormones. GH immunohistochemistry correlated with histological staining, revealing reduced GH expression in malnourished rats.

The percentage of somatotroph cells (GH cells) in the anterior pituitary gland varied between the groups (Figs. 3 & 4). In the normal group (N), the percentage was 42.20%. However, in the malnourished group (MAL), the percentage significantly decreased to 24.03%. In contrast, the percentage of somatotroph cells increased in the malnourished groups treated with 25% and 50% lima bean flour. The percentage in the MAL + 25% LB group was 38.38%, whereas that in the MAL + 50% LB group was 44.09%. The observed decrease in the percentage of somatotroph cells in the MAL group aligns with previous research suggesting that malnutrition can impact the population of these cells, potentially due to energy conservation and altered hormone regulation (Roelfsema et al., 2016; Luque et al., 2007). The increase in the percentage of somatotroph cells in the MAL + 25% LB and MAL + 50% LB groups suggested that the intake of lima bean flour may counteract the effects of malnutrition on the population of these cells, possibly through the provision of essential amino acids that stimulate GH secretion (Isidori et al., 1981; Norton & Layman, 2006). Arginine is known to stimulate the release of GH-releasing hormone (GHRH), which stimulates GH secretion (Albaroth et al., 1988). Leucine is recognized for its ability to trigger the mTOR pathway, a signaling pathway that modulates protein synthesis in response to nutrients, growth factors, and cellular energy. EAAs induce a conformational change in mTORC1, which permits linear growth. The activation of the mTOR pathway promotes protein, lipid, and nucleotide synthesis and subsequently

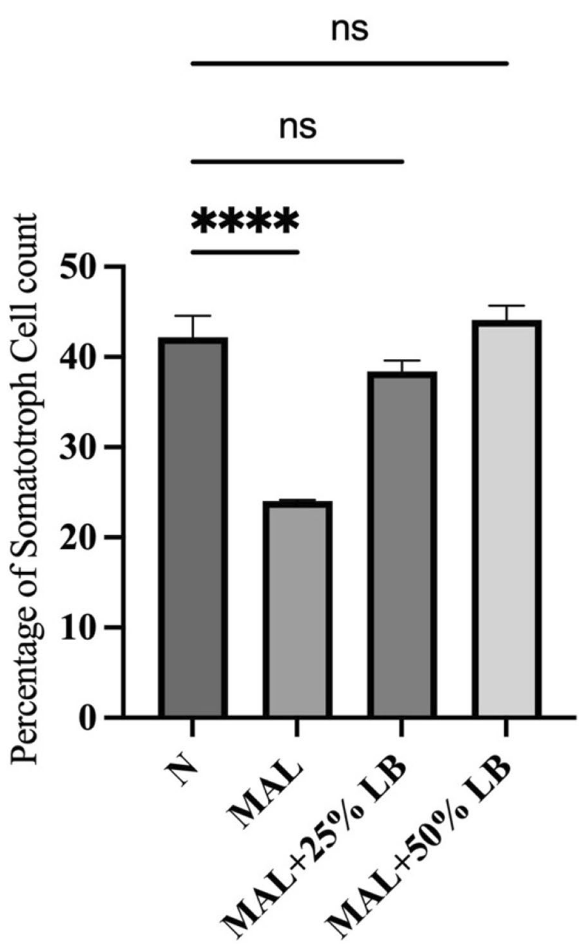



**Fig. 4** Rat somatotroph cell percentage distribution in the anterior lobe of the pituitary gland in Normal (N), Malnourished (MAL) and treated with 25% (MAL + 25% LB) and 50% (MAL + 50% LB) Lima Bean Flour. Data represent (Mean ± SEM, n = 4) ****p.0001, ***p.001, **p.01, *p.05, and ns = no significant vs. control in each group

enhances GH secretion (Kimball et al., 2006; Aylett et al., 2016; Laplante & Sabatini, 2012). The greater percentage of somatotroph cells in the MAL + 50% LB group than in the MAL + 25% LB group suggested a dose-dependent effect of the lima bean flour treatment. This could be due to a greater intake of essential nutrients in the 50% group, further supporting the importance of nutrition in maintaining the population of somatotroph cells.

## Liver histopathology

The histological assessment of the effect of lima beans on the liver of malnourished experimental animals revealed histological differences between the control and treatment groups (Fig. 5). In the N group, the liver parenchyma consisted of hepatocytes arranged orderly in the trabeculae and separated by sinusoids, with no signs of steatosis, degeneration, hepatocyte necrosis, fibrosis, or inflammation. In the MAL group, liver tissue showed signs of severe and diffuse steatosis. Hepatic cells with clear vacuolated cytoplasm, loss of hepatocyte eosinophilic granularity, degenerating cells, necrosis, and mild fibrosis in the periportal area were observed. The histological appearance of liver cells with a clear cytoplasm indicates nonalcoholic fatty liver disease (NAFLD) steatosis, which is consistent with decreased glycogen in the liver and the accumulation of hepatic triglycerides due to reduced hepatic phospholipids, as well as mitochondrial damage. Compared with those in the MAL group, liver histology improved in the MAL + 25% LB and MAL + 50% LB groups, which was characterized by reduced steatosis of cells. The histological appearance of the experimental animals given 50% lima beans showed histological improvement approaching that of the N group, with reduced steatosis and liver cell damage.

As evaluated by HE staining, the necrosis, steatosis, and fibrosis cell scores in liver tissue differed significantly between the groups. There was no necrosis in the control group (N). The MAL group, on the other hand, had a significant increase in necrosis, with a score of 1.75. Interestingly, compared to the MAL group, the malnourished animals fed 25% and 50% lima bean flour demonstrated a reduction in necrosis, with scores of 0.50 and 0.2500, respectively (Fig. 6a). The steatosis cell scores varied substantially between the groups, with the N group showing no steatosis, as indicated by a score of 0.00. The MAL group, on the other hand, had a significant increase in steatosis, with a score of 3.00. Intriguingly, compared to the MAL group, the malnourished groups fed 25% and 50% lima bean flour showed a reduction in steatosis, with scores of 1.00 and 0.17, respectively (Fig. 6b). The fibrosis cell scores revealed significant differences between the groups. The N group, MAL + 25% LB group, and MAL + 50% LB group showed no evidence of fibrosis, with scores of 0.00. On the other hand, the malnourished group (MAL) had a slight increase in fibrosis, with a score of 0.50 (Fig. 6c).

The increase in necrosis cell scores in the MAL group compared to the N group is consistent with previous studies indicating that malnutrition can induce cellular damage and necrosis in liver tissue (Das et al., 2008). As a metabolically active organ, the liver is particularly vulnerable to nutrient deficiencies, leading to cell death. Lima beans are a good source of essential amino acids for cell repair and regeneration (Adebo et al., 2015). The decrease in necrosis scores in the MAL + 25% LB and MAL + 50% LB groups suggested that lima bean flour treatment may have a protective effect and mitigate some of the liver damage caused by malnutrition. Malnutrition can lead to hepatic steatosis, which is characterized by fat accumulation in liver cells (Cave et al., 2007). This could be

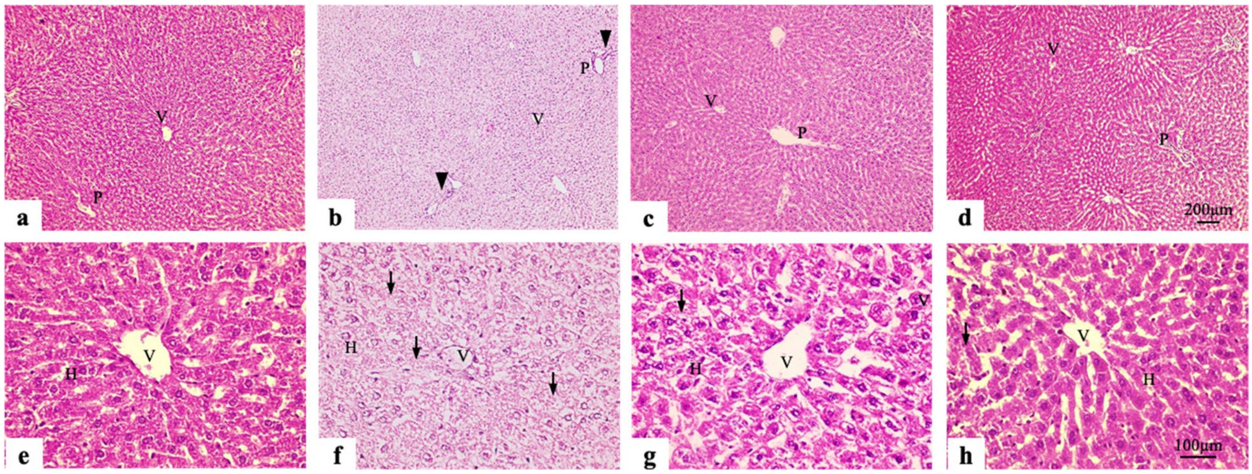



**Fig. 5** Photomicrograph of liver tissues of Normal (N; a, e), Malnourished Rats (MAL; b, f) and 25% (MAL + 25% LB; c, g) and 50% (MAL + 50% LB; d, h) Lima Bean Flour treatment showing hepatocyte (H), central vein (v), portal area (p), Malnourished animal showed hepatocytes necrosis (↓), as well as periportal fibrosis, (▼)

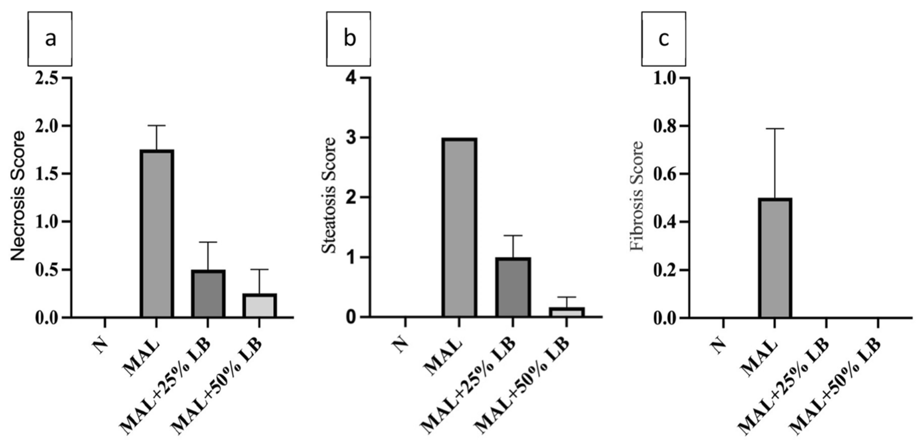



**Fig. 6** Necrosis score (a), Steatosis score (B) and Fibrosis score (C) of Normal (N), Malnourished (MAL) and treated with 25% (MAL + 25% LB) and 50% (MAL + 50% LB) Lima Bean Flour

due to the body's attempt to store energy in the form of fat in response to a state of nutrient deficiency. The lower steatosis scores in the MAL + 25% LB and MAL + 50% LB groups suggest that the intake of lima bean flour may have mitigated the development of hepatic steatosis.

Furthermore, malnutrition can contribute to liver fibrosis, a condition characterized by the excessive accumulation of extracellular matrix proteins, including collagen (Mak et al., 2017; Tsuchida & Friedman, 2017). Our study revealed an increase in the fibrosis cell score in the MAL group compared to the N group. This may be due to prolonged liver injury caused by nutrient deficiency (Schuppan & Kim, 2013).

Interestingly, the MAL + 25% LB and MAL + 50% LB groups did not show signs of fibrosis, suggesting that lima bean flour treatment may have ameliorated the development of liver fibrosis. The increase in necrosis cell scores in the MAL group compared to the N group indicates that malnutrition can induce cellular damage and necrosis in liver tissue (Das et al., 2008). As a metabolically active organ, the liver is particularly vulnerable to nutrient deficiencies, leading to cell death. Lima beans are a good source of essential amino acids for cell repair and regeneration (Adebo et al., 2023). The decrease in necrosis scores in the MAL + 25% LB and MAL + 50% LB groups suggested that lima bean flour treatment may have a protective effect and mitigate some of the liver damage caused by malnutrition. Malnutrition can lead to hepatic steatosis, which is characterized by fat accumulation in liver cells (Cave et al., 2007). This could be due to the body's attempt to store energy in the form of fat in response to a state of nutrient deficiency. The lower steatosis scores in the MAL + 25% LB and MAL + 50% LB groups suggest that the intake of lima bean flour may have mitigated the development of hepatic steatosis.

Furthermore, malnutrition can contribute to liver fibrosis, a condition characterized by the excessive accumulation of extracellular matrix proteins, including collagen (Mak et al., 2017; Tsuchida & Friedman, 2017). Our study revealed an increase in the fibrosis cell score in the MAL group compared to the N group. This may be due to prolonged liver injury caused by nutrient deficiency (Schuppan & Kim, 2013). Interestingly, the malnourished animals in the 25% and 50% lima bean flour (MAL + 25% LB and MAL + 50% LB) groups did not show signs of fibrosis, suggesting that lima bean flour treatment may have ameliorated the development of liver fibrosis. The protective effects of lima beans may be attributed to their ability to modulate hepatic fibrogenesis, possibly through the inhibition of hepatic stellate cell activation, a key event in liver fibrosis.

Like many other legumes, Lima beans are abundant in dietary fiber and antioxidants. The dietary fiber they contain is fermented by the gut microbiota, which produces short-chain fatty acids (SCFAs), including butyrate, acetate, and propionate. Research has shown that these SCFAs can reduce inflammation by inhibiting the production of proinflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) (Koh et al., 2016). Antioxidants, on the other hand, can counteract oxidative stress, a key driver of inflammation. Lima beans are rich in antioxidants, including flavonoids and phenolic acids, which have been shown to have anti-inflammatory effects (Tello, 2018; Agostini et al., 2015, Maliza et al., 2023). Lima beans may help prevent the progression of liver injury and damage by promoting liver cell regeneration.

## Conclusion

This research provides evidence that lima bean (*Phaseolus lunatus* L.) powder can potentially ameliorate the negative effects of malnutrition and inflammation on the pituitary liver axis in rats. Our findings demonstrate that lima bean supplementation can significantly improve malnutrition markers, including body weight, serum albumin, and total protein levels, which are closer to those observed in the normal group. This suggests that lima beans, which are rich in protein and essential nutrients, can help restore the body's protein balance disrupted by malnutrition. Furthermore, our study showed that lima bean supplementation can regulate GH at both the cellular and mRNA expression levels, positively impacting the pituitary liver axis. This indicates that lima beans might be crucial for stimulating growth and development, particularly under malnourished conditions. Moreover, our research revealed that lima bean powder can ameliorate liver inflammation, indicating its potential as an anti-inflammatory agent.

### Abbreviations

| | |
|---|---|
| GH | Growth hormone |
| IGF1 | Insulin-like growth factor 1 |
| MAL | Malnutrition |
| GHR | Growth hormone receptor |
| HE | Hematoxylin and eosin |
| EAA | Essential amino acid |
| mTOR | Mammalian target of rapamycin |
| mTORC1 | Mammalian target of rapamycin complex 1 |
| NAFLD | Nonalcoholic fatty liver disease |

### Acknowledgements

Universitas Andalas, Universitas Indonesia, and Padjadjaran University, through the Indonesia Collaboration Research Grant (RKI), have provided funding for this research (RKI; Contract No. 8/UN16.19/PT.01.03/KO-RKI Scheme A (HOST) Fiscal Year 2023). The authors would like to express their gratitude to Dr.-Ing Uyung Gatot S. Dinata, MT and LPPM UNAND for facilitating this grant. Furthermore, we would like to thank Prof. Takashi Yashiro (Jichi Medical University) and Prof. Ken Fujiwara (Kanagawa University) for providing the GH primary antibody.

### Author contributions

R. Maliza, R. Syaidah, B. Arya and A. Tofrizal were involved in the development of the study's concept, experimental design, data analysis, and manuscript preparation. P. Santoso, R. Maliza, A M. Rosdianto, and R. Lesmana conducted the experiments, investigations, and measurements. R. Syaidah and B. Arya managed the project administration. The authors have all accepted the article's final version.

### Funding

Financial support for this research was provided by the Indonesia Collaboration Research Grant (RKI) under contract number 8/UN16.19/PT.01.03/KO-RK, Scheme A (HOST) for fiscal year 2023.

### Availability of data and materials

The data that support the findings of this study are available from the corresponding author upon reasonable request.

## Declarations

### Ethics approval and consent to participate

The experimental methodologies used in this study received approval from Andalas University's Institutional Committee for Research Ethics and Conduct (No. 985/UN. 16.2/KEP-FK/2022). Any concerns regarding misconduct, plagiarism, fabrication, or redundancy relating to the manuscript have been thoroughly addressed and resolved.

### Consent for publication

Not applicable.

### Competing interests

All authors declare no conflicts of interest.

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Received: 6 March 2024 Accepted: 15 August 2024
Published online: 05 December 2024

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