



Phaseolus lunatus L. supplementation improved testicular structure and estradiol synthesis in protein-restricted rats

[La suplementación con *Phaseolus lunatus* L. mejoró la estructura testicular y la síntesis de estradiol en ratas con restricción proteica]

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Abstract

Context: Protein deficiency remains a critical concern in developing countries, with implications in many physiological aspects, including male fertility. Lima beans (*Phaseolus lunatus*; PL), a legume rich in protein and commonly consumed in Indonesia, may serve as a traditional plant-based alternative to support male reproductive health.

Aims: To investigate the potential benefits of *P. lunatus* supplementation on testicular histology and hormonal profiles in protein-restricted rats.

Methods: Male rats were fed a 12% protein-restricted diet for six weeks, followed by supplementation with 25% (PL25) or 50% (PL50) *P. lunatus* powder for another six weeks. Testicular structure was examined histologically under a microscope. In addition, serum levels of testosterone and estradiol, as well as the activity of androgen receptors (AR) and estrogen receptor- α (ER- α), were measured.

Results: Protein restriction led to reduced testicular size, disrupted seminiferous tubule integrity, decreased sperm count, and a selective impairment of estradiol synthesis, accompanied by downregulation of AR and ER- α expression. Supplementation with PL50 significantly reversed these effects, improving testicular architecture, estradiol production, and receptor activity.

Conclusions: *P. lunatus* supplementation shows potential in mitigating testicular damage and dysfunction induced by protein deficiency, possibly by restoring estrogen signaling and spermatogenesis. Further studies are required to elucidate the underlying mechanisms by which *P. lunatus* exerts its protective effects on the male reproductive system.

Keywords: genitalia; gonadal hormones; malnutrition; plant lectin; steroid receptors.

Resumen

Contexto: La deficiencia de proteínas sigue siendo una preocupación crítica en los países en desarrollo, con implicaciones en muchos aspectos fisiológicos, incluyendo la fertilidad masculina. El frijol de Lima (*Phaseolus lunatus*; PL), una leguminosa rica en proteínas y de consumo común en Indonesia, puede servir como una alternativa vegetal tradicional para favorecer la salud reproductiva masculina.

Objetivos: Investigar los posibles beneficios de la suplementación con *P. lunatus* en la histología testicular y los perfiles hormonales de ratas con restricción proteica.

Métodos: Se alimentó a ratas macho con una dieta restringida en proteínas al 12% durante seis semanas, seguida de una suplementación con polvo de *P. lunatus* al 25% (PL25) o al 50% (PL50) durante seis semanas más. La estructura testicular se examinó histológicamente al microscopio. Además, se midieron los niveles séricos de testosterona y estradiol, así como la actividad de los receptores de andrógenos (RA) y del receptor de estrógeno- α (RE- α).

Resultados: La restricción proteica provocó una reducción del tamaño testicular, alteración de la integridad de los túbulos seminíferos, disminución del recuento de espermatozoides y un deterioro selectivo de la síntesis de estradiol, acompañado de una regulación negativa de la expresión de RA y RE- α . La suplementación con PL50 revirtió significativamente estos efectos, mejorando la arquitectura testicular, la producción de estradiol y la actividad del receptor.

Conclusiones: La suplementación con *P. lunatus* muestra potencial para mitigar el daño y la disfunción testicular inducidos por la deficiencia proteica, posiblemente mediante la restauración de la señalización estrogénica y la espermatogénesis. Se requieren más estudios para dilucidar los mecanismos subyacentes por los cuales *P. lunatus* ejerce sus efectos protectores sobre el sistema reproductor masculino.

Palabras Clave: desnutrición; genitales; hormonas gonadales; lectina vegetal; receptores de esteroides.

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INTRODUCTION

Protein is one of the essential macronutrients required to maintain the function and health of the body. The adequate presence of protein in the body affects various physiological processes, including muscle development, immune system function, enzyme production, and reproductive system function (de Moraes Oliveira et al., 2021; LaPelusa and Kaushik, 2024). The critical function of the reproductive system relies on the availability of amino acids and other nutrients in protein-rich foods (Gao, 2020). In males, protein is needed for sperm formation as well as for the synthesis and regulation of hormones involved in male reproductive function (Zambrano et al., 2005). Inadequate protein intake may affect the structure and function of male reproductive organs, such as the testes, and disrupt hormonal balance, leading to reduced sperm count and quality (Braga et al., 2012).

Although the importance of protein in the diet has been widely recognized, the fact that protein deficiency remains a global health issue cannot be dismissed. In Indonesia, malnutrition is still a huge burden affecting a large population, despite the government's efforts to tackle this problem. The 2022 nutritional status survey conducted by the Indonesian Ministry of Health reported that 21.6% of Indonesian children under five are categorized as malnourished, with a deficiency in protein intake identified as one of the contributing factors (Kemenkes, 2023). Protein undernutrition in children may have lifelong effects on their health and well-being, including potential consequences for future fertility (Ghosh, 2016). These concerns are particularly urgent, as Indonesia ranks among those with the highest prevalence of infertility and the greatest burden of disability-adjusted life years (DALYs) linked to reproductive health disorders (Feng et al., 2025).

Despite Indonesia being known as a country rich in food resources, the major cause of protein deficiency in Indonesia is associated with poverty and poor protein quality. The long-standing tradition of consuming a rice-based diet is one of the main factors. Indonesia has the highest per capita rice consumption in the world, with the average of more than 120 kg per person annually (FAOSTAT, 2023). While rice provides energy, it lacks specific essential amino acids crucial for protein synthesis (Yang et al., 2022). Another overlapping factor is Indonesia's economic condition. Protein-rich foods, especially those derived from animal sources, are expensive and not accessible to everyone. Low-income families may struggle to afford an adequate amount of animal-based protein in

their daily diet. Alternatively, plant-based protein is widely accepted as part of the diet in Indonesia.

Lima beans, scientifically known as *Phaseolus lunatus* L., are a type of legume that belong to the *Fabaceae* family. In Indonesia, it is widely known as "kacang kapri" or "kacang polong" and has long been part of Indonesian cuisine. It is an excellent source of plant-based protein, containing about 5-7 grams of protein per 100 grams (Chel-Guerrero et al., 2011). It also contains other essential nutrients such as dietary fiber, vitamins, and minerals (Betancur et al., 2001). The presence of these beans in Indonesian cuisine may offer an affordable alternative for a plant-based protein source diet.

Other legumes, such as *M. pruriens*, which also belongs to the *Fabaceae* family and is native to Africa, have already been proven to be a promising nutritional supplement for male fertility in both rats and humans (Shukla et al., 2009; Singh et al., 2013). However, there is a lack of scientific evidence regarding *P. lunatus*'s potential to reverse the effects of protein deficiency on male reproductive function. This gap is particularly relevant in the Indonesian context, where plant-based protein alternatives are widely consumed due to economic and cultural factors. While *P. lunatus* is known to contain essential amino acids and micronutrients, its specific role in supporting the recovery of reproductive organs following protein undernutrition remains unexplored.

Therefore, the present study was designed to evaluate the effects of *P. lunatus* supplementation on testicular architecture and reproductive hormone levels in protein-restricted male rats. We hypothesized that *P. lunatus* supplementation would mitigate the adverse effects of protein deficiency by improving testicular morphology and restoring hormonal balance, thereby supporting male reproductive health.

MATERIAL AND METHODS

Diets

P. lunatus were collected from Solok regency, West Sumatra, Indonesia (-1.075245,100.784014). The cleaned beans were soaked in a 1:5 ratio of distilled water for 12 hours at room temperature and then boiled for 60 minutes with a 2:1 water-to-grain ratio. The *P. lunatus* were then dried in an oven with air circulation at 60°C until constant weight and sieved into flour.

Animals

This research was conducted at Universitas Andalas, Faculty of Medicine, Indonesia. All procedures for

this research have been approved by the Health Research Ethics Committee, Faculty of Medicine, Universitas Andalas, Padang, Number 985/UN.16.2/KEP-FK/2022. Sixteen male Wistar rats, aged 3-4 weeks, were obtained from “Rumah Tikus” (West Sumatra, Indonesia) and acclimated for 7 days before treatment. During the acclimation period, rats were given water and fed *ad libitum*, and were placed in a well-ventilated cage. The room temperature is approximately 22°C, with a 12-hour light/dark cycle and direct sunlight from 7:00 a.m. to 7:00 p.m. Cages and areas for eating and drinking were cleaned three times a week. Food and drink were given at daily intervals according to the treatment group. The rats were conditioned to a protein-restricted diet by feeding a low 12% protein diet for six weeks, followed by 25% (PL25) and 50% (PL50) *P. lunatus* powder for the next six weeks. Negative control (N) was given a standard diet containing 20% protein for 12 weeks, while positive control was given a low 12% protein-restricted (PR) diet for 12 weeks. The rat’s weight was recorded every 3 days. All the animals were slaughtered, and the testes were excised, dissected, weighed, and collected for further experiments. All experiments were conducted using three samples per group (n = 3), unless otherwise specified. Each measurement represents a biological replicate. The treatments of the animals were in accordance with the Guidelines for Animal Experimentation, which is based on the Guide for the Care and Use of Laboratory Animals of the National Academy of Sciences, USA.

Histological assessment procedure

After the testes were excised and weighed, it was immediately fixed in 10% formalin solution. Serial sections (5 µm thick) were made and stained by hematoxylin-eosin (H & E). The stained sections were assessed microscopically by taking pictures using an Olympus CX 33 microscope, a 3.1 MB Sony Exmor camera, and the Betaview program. Semi-quantitative histological assessment was scored from H&E staining based on Johnsen's scoring system (Johnsen, 1970), based on germ cell maturation within each

seminiferous tubule cross-section. One indicated no seminiferous epithelium, and 10 indicated full spermatogenesis, as assessed at 100× magnification by randomly examining at least 10 seminiferous tubules per sample. A score was assigned for each tubule by quantifying the cells encountered within the seminiferous tubules. A score of 10 indicated maximum spermatogenesis, whereas a score of 1 indicated complete absence of germ cells. The number of tubules was multiplied by the score and then divided by the number of evaluated tubules, giving the final score.

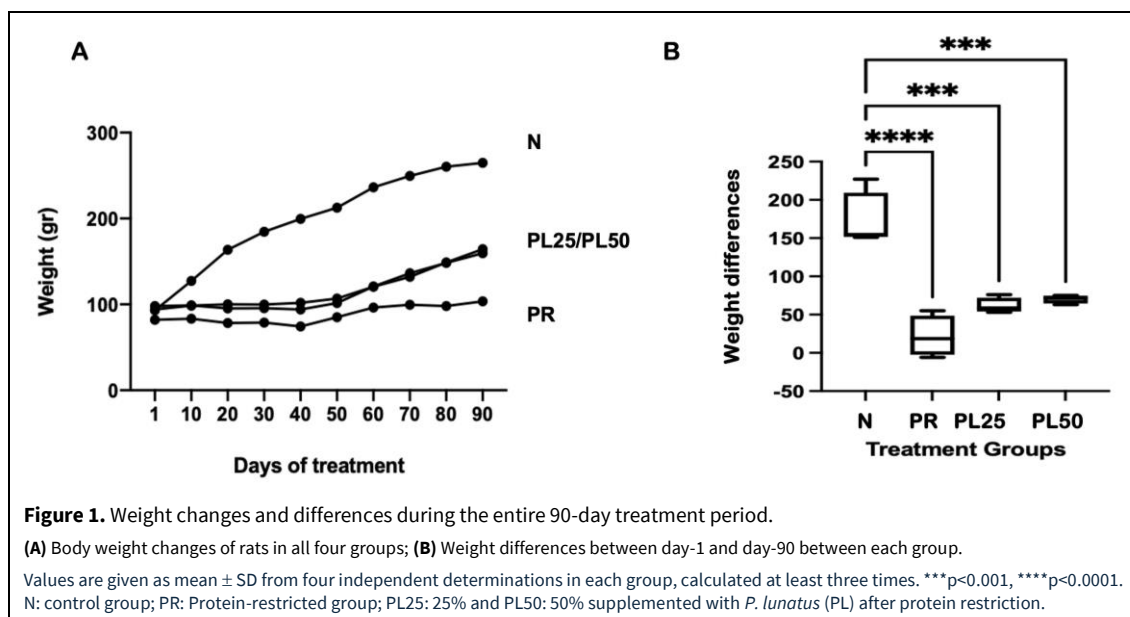
The density, diameter, and thickness of tubules were also calculated. The density of the seminiferous tubules was determined by counting the number of tubules with visible lumens per unit area. The diameter of the tubules perpendicular to the long axis (µm) on cross-sectional sections and the thickness of the tubule walls perpendicularly from the basement membrane of the seminiferous tubules to the limit of cells that are still viable in the lumen of the tubules were measured with the help of the Betaview program. All assessments were carried out on five different fields of view and reported as the mean number or mean value (µm).

RNA isolation, cDNA synthesis, and quantitative real-time PCR (qPCR)

Total RNAs from testis tissues were isolated using RNeasy Mini Kit (Qiagen, Hilden, Germany), according to the manufacturer’s instructions. Quantitative real-time PCR (ABI PRISM 7900HT; Applied Biosystems, CA, USA) was performed using gene-specific primers and SYBR Premix Ex Taq (Takara Bio) containing SYBR Green I. cDNA was synthesized by a PrimeScript First Strand cDNA Synthesis Kit (Takara Bio, Otsu, Japan). Relative gene expression was calculated by comparing the cycle times for each target PCR. Cycle threshold values were converted to relative gene expression levels by using the $2^{-(\Delta C_t \text{ sample} - \Delta C_t \text{ control})}$ method. GAPDH was quantified for normalization. The primers used for qPCR are listed in Table 1.

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

Genes	Primer sequence (5'-3')	Length	Product size
Rat ER-α	Forward: CCAGGGAAGCTACTGTTTGC	21	134 bp
	Reverse: GATGTAGCCAGCAGCATGT	23	
Rat AR	Forward: CTACTCTTCAGCATTATTCAG	22	178 bp
	Reverse: CAATAGGCTGCACAGAATCC	20	



Enzyme-linked immunosorbent assays (ELISA)

Serum and testes' testosterone and estradiol levels were determined using a testosterone (BioAssay Tech Lab) and Estradiol (BioAssay Tech Lab) ELISA kit according to the manufacturer's instructions.

Statistical analysis

Statistical analysis was performed using Prism 10 for Mac. The data are presented as the means $\pm$ standard deviation (SD) for independent or group experiments repeated at least three times. One-way ANOVA was used to analyze the data. Normality and Homogeneity are analyzed by Shapiro-Wilk test. When significant differences were detected, Tukey's post hoc test was applied for multiple comparisons. Differences between groups were statistically significant at a p -value of less than 0.05.

RESULTS

Body and testis weight

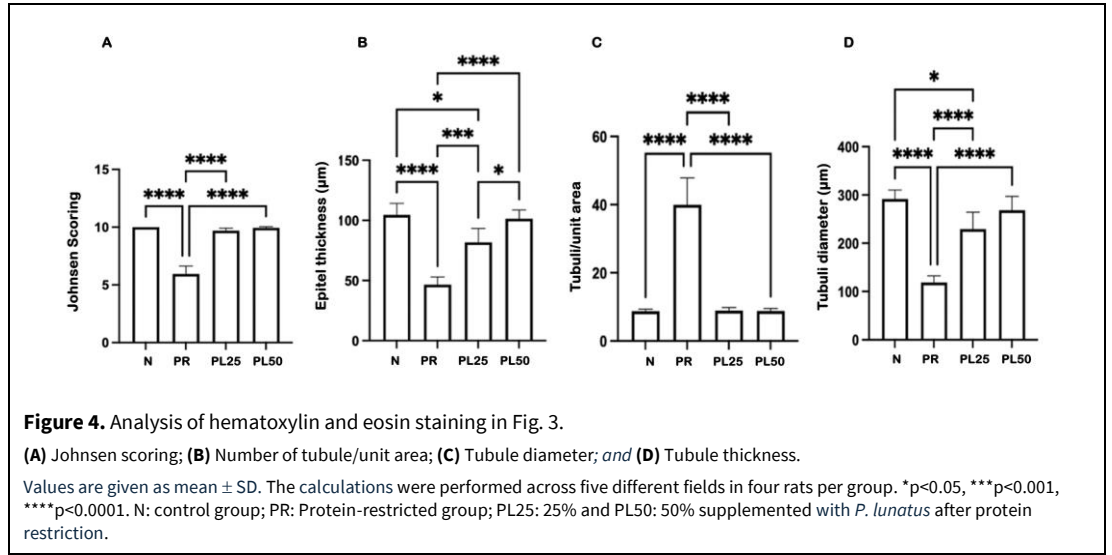
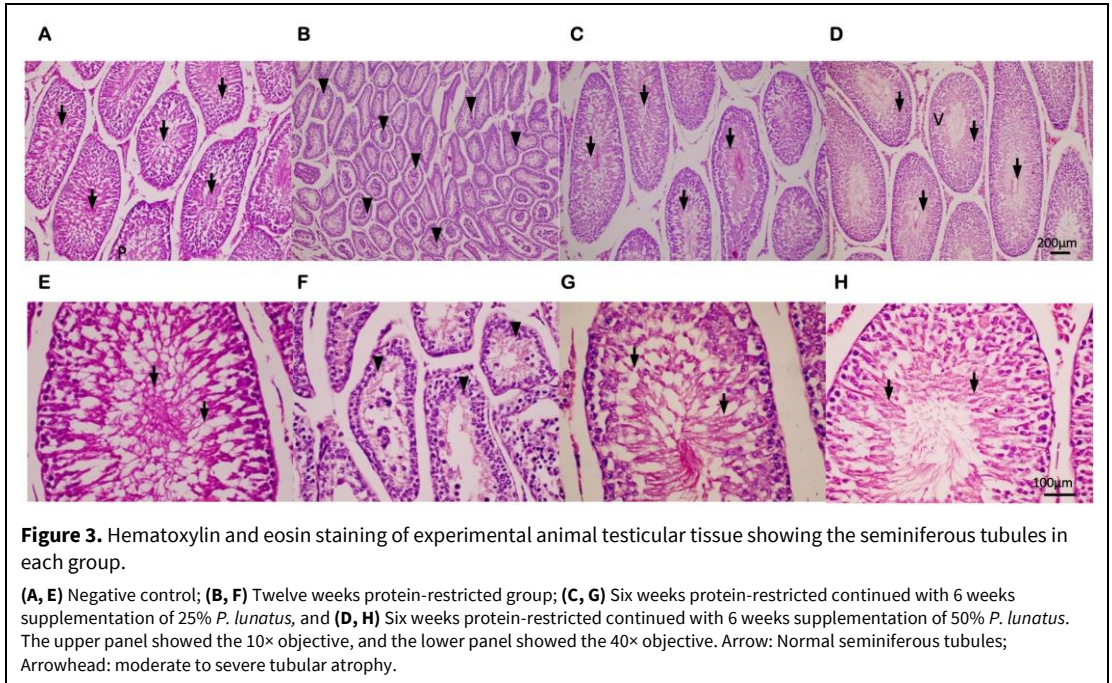
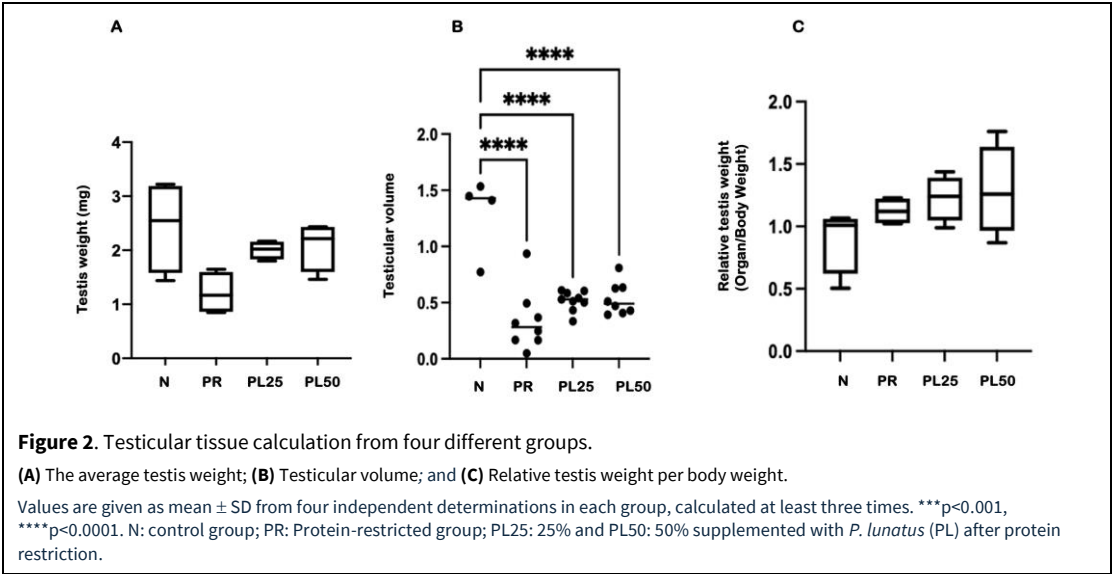
The changes in body weight during the 12-week experiment are shown on Fig. 1. The initial body weight in all groups ranged from 82 to 98 grams. During the 90-day experiments, the body weight of rats in the normal groups increased in a linear pattern, with changes almost tripling from an average of 93 mg on day 1 to 272.5 mg on day 90. On the other hand, the protein restriction diet group gained weight at a much slower pace and only gained 29.2% of their body weight by day 90. The addition of 25% and 50% *P. lunatus* powder can slightly increase the body weight of PR animals to 68% and 80% (Fig. 1B), although these differences are not significant.

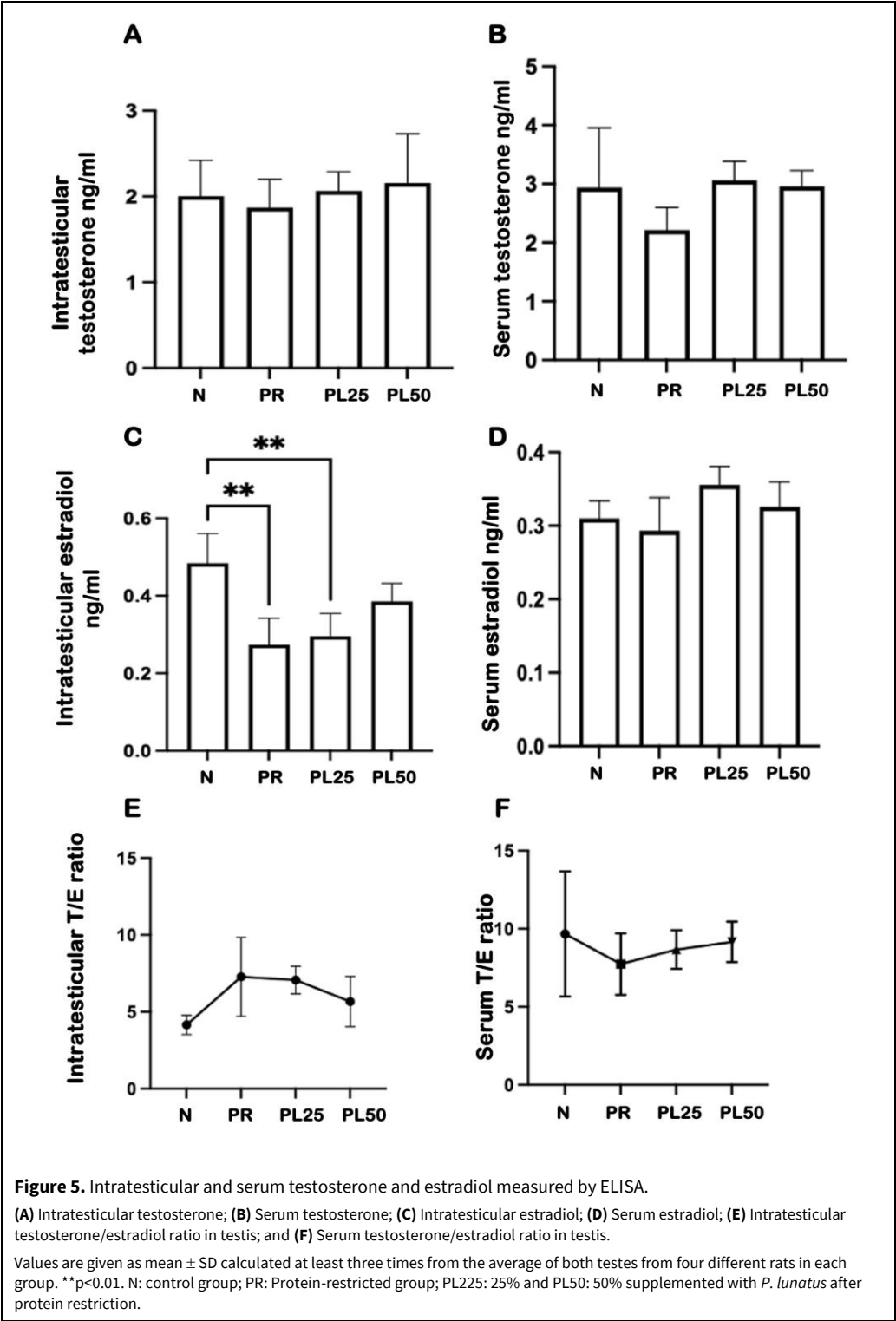
To see whether supplementation of *P. lunatus* powder affected the male reproductive system, we calculated the absolute testis weight, relative testis weights, and testicular volumes in the various groups. The data is presented in Fig. 2. The absolute testis weight in the PR group was much smaller than that of the normal group. Although supplementation of *P. lunatus* did not significantly affect the body weight, addition of 25% and 50% PL diet could significantly increase the testis weight compared to the PR group (Fig. 2A). Fifty percent of PL diet (PL50) increased the testis weight more than PL25. Although this supplementation did not affect the testicular volume (Fig. 2B), *P. lunatus* increased the relative testis weight (Fig. 2C).

Histological assessment

The microscopic structure of the testis was observed after being stained with standard hematoxylin-eosin staining (Fig. 3). The histological feature in the normal group showed normal seminiferous tubules characterized by uniform size, open tubule lumen containing many spermatozoa cells (Fig. 3A and E). On the other hand, the PR group showed moderate to severe tubular atrophy, characterized by thinning of the germinal epithelium and decreased tubular diameter, with reduced to absent spermatozoa cells (Fig. 3B and F). This morphology improved with supplementation of PL25 (Fig. 3C and G) and PL50 (Fig. 3D and H), showing histological features closer to normal.

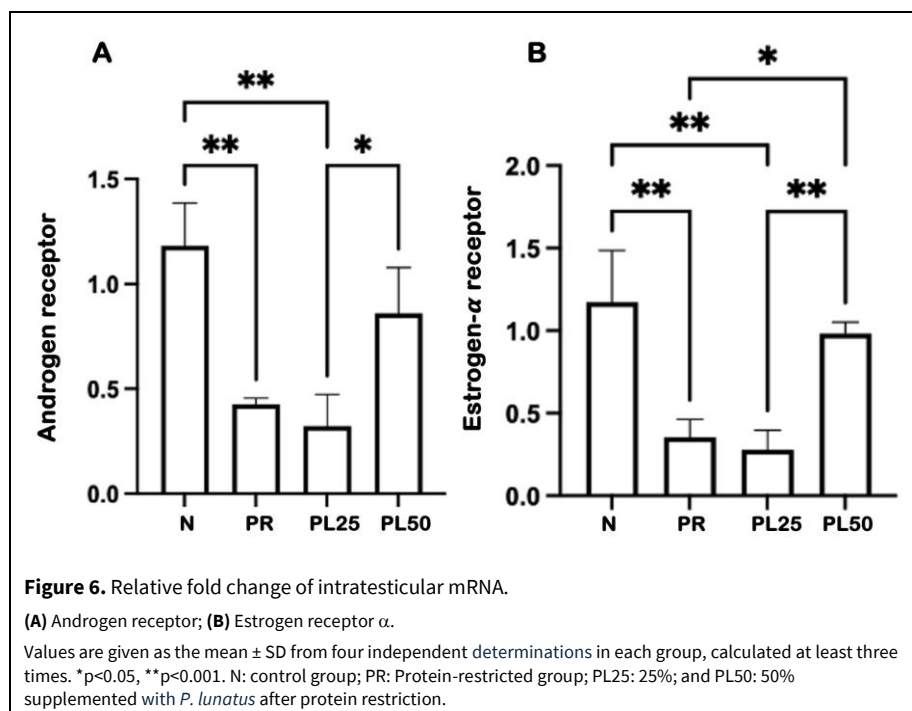
A pathologist further analyzed the histological staining to see the level of microscopic destruction in testis of all groups by Jhonsen scoring as well as the density, diameter and wall thickness of seminiferous





tubule (Fig. 4). The Jhonsen scoring is decreased in protein restriction group to 4.475 that indicate no sperm cells or spermatid, but few to numerous spermatocytes. The supplementation of PL25 and PL50 improved the score to 9.7 and 9.95, respectively (Fig. 4A). The changes were also observed in the density of tubules/field from 8 tubules/unit area in the normal

group, increased to 39.98 tubules/unit area in malnutrition. The density of tubules significantly improved back to 8.95 and 8.85 in PL25 and PL50 (Fig. 4B). Supplementation of PL25 and PL50 also increased the tubule diameter (Fig. 4C) and tubule wall thickness (Fig. 4D).



Serum and intratesticular testosterone and estradiol

We evaluated the serum and intratesticular testosterone and estradiol levels in all groups (Liao et al., 2022). Results showed that protein restriction did not affect the synthesis of serum and intratesticular testosterone (Fig. 5A and 5B) and serum estradiol (Fig. 5D), but affected the synthesis of intratesticular estradiol (Fig. 5C). Addition of PL25 did not increase the synthesis of intratesticular estradiol, but PL50 slightly increased the synthesis of testicular estradiol. We calculated the T/E ratio, and the result showed that the PR condition increased the T/E ratio in the testis (Fig. 5E) but reduced it in serum (Fig. 5F). Although not statistically significant, supplementation with PL25 and PL50 tended to restore the T/E ratio toward normal values.

Androgen receptor and estrogen receptor- α

As the male reproductive function is regulated by the binding of hormones to receptors, the expression of mRNA AR and ER in the testis was calculated (Fig. 6). The relative fold change of mRNA AR and ER was significantly decreased in the PR group. Supplementation with PL50 improved the expression almost to normal levels.

DISCUSSION

Insufficiency of daily protein intake contributes to difficulty in weight gain, as protein is a crucial macronutrient required for the maintenance of muscle tissue mass (Lewicka et al., 2017). A prolonged situation of this condition may cause disturbances in nor-

mal body weight. Our study demonstrated that 12 weeks of protein restriction (PR) resulted in reduced body weight and testicular weight compared to the control group. These results are in line with previous experiments that showed the impact of low protein diet on body weight (Crean et al., 2023) and testicular development (Hanai and Esashi, 2007). Reduced body weight on a low-protein diet is associated with metabolic disturbances, including increased endogenous protein catabolism to maintain critical organ function (Campbell et al., 2002; Du et al., 2000; White et al., 2000). This means that to handle protein-restricted conditions, the body prioritizes vital organs, including the testis, at the expense of skeletal muscle mass. Moreover, the body weight can also be used to determine the reproductive potential, as it may influence fertility (Leisegang and Dutta, 2021).

Although the absolute testicular weight was reduced, the relative testis weight in the PR group was slightly higher. Both absolute and relative organ weights are frequently used to estimate the proportion of the organ and physiological adaptation. As rats in PR groups were much smaller, their lower overall body weight may have resulted in a proportionally higher allocation of available nutrients to reproductive organs. This suggests a body prioritization of testicular maintenance under nutritional stress conditions. This finding is in accordance with previous studies that showed that the relative testis weight was significantly higher on a low protein diet (Crean et al., 2023; Hanai and Esashi, 2012). Furthermore, the testis is among the organs less directly influenced by

overall body mass in terms of structural integrity (Lanning et al., 2002).

P. lunatus, like other legumes, is a nutrient-rich food that contains high levels of protein, complex carbohydrates, and dietary fiber. They have specific roots that contain rhizobium bacteria, which makes them rich in protein (Gilbert et al., 2011). It is also a great source of essential amino acids, which are critical for protein synthesis and physiological maintenance (Bennetau-Pelissero, 2019). Despite its nutritional value, *P. lunatus* supplementation for six weeks following an initial period of protein restriction led to only a modest improvement in body weight. This limited response may be attributed to the relatively short duration of supplementation or differences in the bioavailability and metabolic utilization of its plant-derived proteins. Different proteins from food has distinct pathways in managing body weight. It depends on several factors, such as protein content, amino acid composition, protein digestion ability, and digestion kinetics (Gilbert et al., 2011). While *P. lunatus* is a healthy, rich-protein food choice (Chel-Guerrero et al., 2011), further studies are still needed to evaluate the specific metabolic pathways by which *P. lunatus* influences weight restoration in protein-deficient states.

Although *P. lunatus* supplementation did not alter body weight under protein-restricted conditions, it significantly affected the testicular weight, especially in the PL50 group. The testis is among the organs that are not directly influenced by body weight (Lanning et al., 2002), which may explain this selective recovery. This finding is corroborated by the microscopic appearance of the testes, which showed that the PR- diet caused moderate to severe tubular atrophy, characterized by thinning of the germinal epithelium and a decrease in tubular diameter. These alterations were accompanied by a marked reduction or absence of spermatozoa within the seminiferous tubules, which is consistent with the findings of previous studies on diet-induced testicular degeneration (Crean et al., 2023; Widdowson et al., 1964). Our results demonstrate that *P. lunatus* supplementation mitigated structural damage and restored testicular morphology to a state comparable to that of the control. These structural improvements suggest a recovery of the spermatogenic process, particularly at the higher dose (PL50).

Besides its role in spermatogenesis, the testis is also important in the synthesis of sex hormones, namely testosterone and estradiol. The synthesis of these hormones is regulated by the hypothalamic-pituitary-gonadal axis (Boggio et al., 2013). A previous experiment by Kraemer et al. (2006) indicated that environmental factors, such as physical exercise and diet,

might affect the expression of androgens in the testis. In our study, although protein restriction disrupted testicular architecture and impaired spermatogenesis, it did not significantly affect testosterone levels in either serum or testicular tissue. However, intratesticular estradiol levels were markedly reduced in the PR group, suggesting selective impairment of the local estrogen synthesis pathway.

Testosterone is a main male sex hormone and is produced by the interstitial cells of Leydig in the testis (McQuaid and Tanrikut, 2014). As steroid hormones are synthesized from cholesterol (Eacker et al., 2008), their production is more sensitive to lipid metabolism than to protein availability in diet (Murillo-Ortiz et al., 2017), so it can probably be suggested that the testosterone synthesis in the testis was not significantly affected in our protein-restricted rats. However, this study is contradicted by a previous experiment that demonstrated a decrease in testosterone concentration in male rats treated with 8% low-protein diet (Herbert, 1980). The difference may be due to variations in protein concentration, as our study used a 12% protein diet, which may not have been sufficiently low to impair steroidogenesis. Similarly, Hanai and Esashi (2012) reported that testosterone levels were only reduced in low-protein-fed animals when combined with environmental stressors, such as altered light exposure.

Estradiol in the testis is mainly synthesized by the germinal cells (Carreau et al., 2012), with some additional contributions from Leydig and Sertoli cells (Carreau et al., 1999). Although estradiol is a steroid hormone, its synthesis is, to some extent, dependent on testosterone, following its binding to the androgen receptor in the presence of aromatase (Roselli et al., 1997; Schulster et al., 2016). This binding will trigger different molecular pathways, including Sertoli cells and germ cells proliferation and maturation, as well as testosterone conversion to estradiol (Russell and Grossmann, 2019; Wang et al., 2022). In our study, reduced AR expression in protein-restricted rats coincided with impaired spermatogenesis and decreased intratesticular estradiol levels. These findings suggest that diminished AR activity may have disrupted testosterone aromatization, contributing to the observed hormonal imbalance and testicular dysfunction.

P. lunatus supplementation restored AR expression in a dose-dependent manner. While PL25 improved testicular histology and spermatogenesis, it still did not improve the synthesis of estradiol. This may be attributed to insufficient AR expression in the lower-dose group, suggesting that spermatogenic recovery at this dose may occur via AR-independent pathways. In contrast, PL50 not only improved testicular histology to almost normal, but also increased ER- α

expression, indicating a more restoration of testicular function. Although estradiol alone does not initiate steroidogenesis (Akingbemi et al., 2003), the upregulation of ER- α by PL50 suggests enhanced sensitivity to hormonal signaling. *P. lunatus* is rich in amino acids such as arginine and leucine, which are known to influence mTOR and PI3K/Akt pathways (Bennetau-Pelissero, 2019; Wu et al., 2009). These signaling cascades are implicated in receptor expression regulation and may underline the observed recovery of AR and ER- α levels in protein-restricted rats.

Testosterone, as the main hormone in the male reproductive system, is responsible not only for supporting spermatogenesis, the development of male reproductive tissues, and regulation of libido, but also for other organs, such as maintenance of muscle mass and bone density (Walker, 2010). The absence or dysfunction of hormone-receptor binding will lead to male infertility and impaired testicular function (Carreau et al., 2012; Chang et al., 2013; Smith and Walker, 2014). Although the low protein diet did not significantly affect the testosterone synthesis in this study, the reduction in AR expression may have impaired downstream androgen signaling, contributing to reduced spermatogenesis (Fig. 4A) and estradiol synthesis (Fig. 5). Supplementation with *P. lunatus*, particularly at higher doses, restored AR and ER- α expression, improved testicular architecture, and partially normalized hormonal profiles. These findings suggest that *P. lunatus* supports reproductive recovery in protein-deficient states, possibly through amino acid-mediated modulation of hormone receptor signaling. Future studies should further investigate the molecular pathways involved and evaluate broader systemic effects of *P. lunatus* in reproductive endocrinology.

While our current findings provide important morphological and hormonal insights, several limitations must be acknowledged. First, the study did not assess key fertility parameters such as sperm motility, viability, or sperm count, which are crucial for determining functional reproductive outcomes. Second, the short duration of *P. lunatus* supplementation (6 weeks) may have limited physiological effects, particularly on body weight. Longer-term studies could better assess the restoration of metabolic and endocrine function. Despite these limitations, our study offers novel evidence for the role of *P. lunatus* in testicular recovery under protein-restricted conditions and establishes a foundation for more mechanistically detailed investigations.

CONCLUSION

Phaseolus lunatus supplementation can partially restore testicular structure, hormonal balance, and

AR/ER- α expression in male rats subjected to protein-restricted diets. While the intervention had limited effects on body weight, it significantly improved testicular weight, histoarchitecture, and the expression of androgen and estrogen receptor- α in a dose-dependent manner. These findings suggest a potential role for *P. lunatus* in supporting reproductive recovery under nutritional stress, likely mediated by its rich amino acid profile. Given the global prevalence of undernutrition and subfertility, these results may have implications for developing affordable, plant-based dietary interventions to support male reproductive health. Future research should investigate the mechanisms underlying these effects and the translational potential of *P. lunatus* in clinical settings, particularly among populations at risk for dietary protein deficiency.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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AUTHOR CONTRIBUTION:

Contribution	Syaidah R	Maliza R	Tofrizal	Rosdianto AM	Ilham K
Concepts or ideas	x	x		x	
Design		x	x		x
Definition of intellectual content		x			
Literature search	x	x			
Experimental studies		x	x		
Data acquisition	x	x	x		x
Data analysis	x	x			
Statistical analysis	x				
Manuscript preparation	x				
Manuscript editing	x	x	x	x	x
Manuscript review	x	x	x	x	x

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