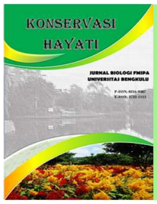

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Growth of Female Mice (*Mus musculus*) Fed with Mung Beans (*Vigna radiata*) Based Feed

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ABSTRACT

Laboratory mice are widely used in biomedical research due to their genetic similarities to humans. The dietary needs of mice in Indonesia. However, they are frequently not satisfied by commercially available feed. This indicates the necessity for locally obtained, customized feed formulations, like those that include mung beans, which are high in vital nutrients and promote sustainable agriculture. This study aims to compare the effect of various mung bean-based feeds on the weight gain of female mice and their feed conversion value. Four feed formulations with varying concentrations of mung beans (85%, 75%, 65%, and 55%) were compared to commercial Broiler II feed, using 20 female mice over two months. The study involved daily feeding, weighing consumed feed, and mice's body weight to determine the Feed Conversion Ratio (FCR), and the Kruskal-Wallis test for statistical analysis. The results show no statistical difference between treatments, which means all treatments are comparable to the control groups in promoting mice growth in all the parameters observed. Nevertheless, a certain trend was observed where the higher the mung bean content, the more similar the results to the control group. Thus, treatments MB85 containing 85% mung beans might have the greatest potential to promote mice growth.

Keywords: *Feed conversion, Female mice, Growth, Mung beans*

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INTRODUCTION

Laboratory mice, *Mus musculus*, serve as one of the most prevalent model organisms in biomedical research (Canales & Walz, 2019; Gurumurthy & Lloyd, 2019) and genetic testing (Peters *et al*., 2007) due to their biological and genetic similarities to humans (Jena & Chawla, 2021; Rydell-Törmänen & Johnson, 2018). Breeding and farming conditions for these animals are meticulously regulated to ensure consistency in experimental results. These environments are designed to minimize stress and maintain health, with controlled temperature, humidity, and light cycles, alongside social enrichment opportunities (National

Research Council, 2011). However, these conditions can vary across different research facilities, potentially impacting the reproducibility of scientific studies (Jaric *et al*., 2024).

Mice need specific nutritional requirements for their growth, reproduction, and well-being (Knapka, 1999). A balanced diet for laboratory mice typically includes a mix of proteins, carbohydrates, fats, vitamins, and minerals, all of which might be specially formulated for specific research purposes (National Research Council, 1995; Wang & Liao, 2012). Adequate nutrition is crucial not only for the physiological development of mice but also for the validity of experimental outcomes, as malnutrition can lead to altered physiological states that may confound research data (Wainwright, 2001). Currently in Indonesia, the feed provided to laboratory mice is typically commercial feed or common livestock (Mutiarahmi *et al*., 2021). While this feed meets basic nutritional standards, it is not tailored specifically for mice. We could not find any papers that investigated the reason why this happened. However, availability and price might be the determining factors (Ningtias *et al*., 2020; Zainullah *et al*., 2022). Therefore, a new feed formulation constructed from locally sourced ingredients might be required to provide better alternatives.

Mung beans, *Vigna radiata*, are crucial as feed additives for animals (Akpapunam, 1996), for example in broilers (Al-Homidan *et al*., 2020; Creswell, 1981), turkeys (Milby, 1945), cattle (Sengupta, 2018), pigs (Lee *et al*., 2017), lambs (Tsega *et al*., 2024), and even tilapia fish (Soliman *et al*., 2018). This legume is rich in protein and iron (Dahiya *et al*., 2015). Mung beans also have high levels of vitamins A and D, calcium, and phosphorus (Akpapunam, 1996). Additionally, mung bean plants also fit so well in sustainable agriculture because of their ability to use atmospheric nitrogen (Shahrajabian *et al*., 2019), therefore lessening the need for chemical fertilizer, decreasing water pollution risk, and making them a great choice for farmer (Gebska *et al*., 2020).

Water is an important factor for mung beans, as they are quite sensitive to drought (Ranawake *et al*., 2012), just like their close relative from the same family, *Phaseolus vulgaris* (Pratiwi & Nafira, 2021). Apart from nutritional value, Indonesia has reached a surplus on mung bean production and could even export 49.14 thousand tons of mung beans worth US$ 52.48 million in 2020 (Ningsih *et al*., 2022), meaning there are spare that could be allocated for other use, such as mice feed.

The mung beans contain 22.9% protein, 1.2% fat, 61.8% carbohydrates, 4.4% fiber, and 3.5% ash (Sathe, 1996), and are high in phosphorus and potassium (Akpapunam, 1996). This means that mung beans contain higher protein than the commercially available feed BR (Broiler) II, which only consists of 19-21% crude protein, although BR II contains more fat (5%) and fiber (5%) than mung beans (Nggena *et al*., 2019). The high protein content is an important factor for female mice, as natural-ingredient diets containing about 18% crude protein are needed for gestating/lactating mice to last through several pregnancies (National Research Council, 1995). Thus, it is necessary to investigate the effect of mung bean-based feed on mice growth. The feed conversion value should also be calculated to estimate the efficiency of the new feed formulation. Therefore, this study aimed to compare the effect of various mung bean-based feeds on the weight gain of female mice, and their feed conversion value.

METHODS

This research was carried out from January to April 2024 at the Zoology Laboratory, Institut Teknologi Sumatera. This research was conducted in 3 major steps: preparations, treatments and data collection, and data analysis. We used a Complete Randomized Design with 5 treatments, each with 4 repetitions.

1. Preparations

This research used 4 combinations of feed formulation with various concentrations of mung beans. These formulations will be compared to commercial feed Broiler (BR) II commonly used for mice feed. Details of each feed formulation composition can be seen in Table 1.

Table 1. Components of each Feed Formulation Treatment

Group	Component(s)
Control	Commercial Feed BR (broiler) II
MB85	Mung beans 85% + Bran 10% + Tapioca Flour 5%
MB75	Mung beans 75% + Bran 20% + Tapioca Flour 5%
MB65	Mung beans 65% + Bran 30% + Tapioca Flour 5%
MB55	Mung beans 55% + Bran 40% + Tapioca Flour 5%

Each feed formulation was prepared by hand to ensure the recipe was easily adaptable should the experiment show promising results. Mung beans were cleaned, mashed, and then mixed with bran according to the formulations in Table 1. Tapioca flour and water were added to compact the feed into a dough. After that, the dough was shaped into pellets and put in the oven for 1 hour at 70°C. The finished pellets should be about 2 cm in size with a moderate texture, not too hard or soft.

About 20 female mice (*Mus musculus*) about 2-3 months old and weighing about 20-30 grams were obtained from the Laboratory of Lampung Veterinary Center. Female mice are chosen because the mother's nutrition intake could affect the reproductive ability of the offspring (Meikle & Westberg, 2001). Before proceeding with the treatments, the mice were acclimated for one week. According to BMKG data from Radin Inten II Meteorology Station at the time of the research, the temperature range was 26°C - 29.2°C and the humidity range was 74% - 93% (BMKG, 2024). Each mouse was housed in 33cm x 22cm x 15cm plastic boxes that provided their living needs. Commercial feed and water were given ad libitum. After acclimatization, the mice are weighed to get the initial body weight.

2. Treatments and Data Collections

Treatments and data collection were done every day for two months. Feed was given daily and withdrawn after the mice had finished eating. Each mouse was weighed before and after feeding to determine how much feed they consumed. Weight data was obtained by placing mice in a closed basket on a digital scale with 0.1-gram accuracy, in which the scale was tared with the basket before measurements. No additional feed was given outside of their feeding time.

3. Data Analysis

Body weight data was used to get the Feed Conversion Ratio (FCR) for every treatment. The calculation (Listyasari *et al*., 2022) was as follows:

$$FCR = \frac{F}{Wt - Wo}$$

F is for total feed consumed in this period, Wt means mice weight at the end of the period, and Wo means mice weight at the start of the period. A smaller FCR is better. This study used the Kruskal-Wallis test to compare between treatment groups.

RESULT AND DISCUSSION

Figure 1 illustrates the weekly fluctuations of the mice's body weight over an 8-weeks. All treatment groups exhibited a general upward trend in body weight, except for Week 4, during which a decline was observed across all groups. The Kruskal-Wallis test was used to determine the statistical significance of differences between the treatment groups. The test results available in Table 2 indicated no significant differences, suggesting that the observed weight loss in Week 4 was likely attributable to external factors rather than the treatments administered. The ideal temperature range for mice activity is 20°C - 26°C and above 26°C for maintenance and resting behaviors (National Research Council, 2011). The average temperature recorded from Radin Inten II Meteorology Station is 26°C - 29.2°C (BMKG, 2024), which should have invoked more resting behavior in these mice and increased their weight. Based on this observation, the mice displayed elevated levels of activity. This observation may indicate that the mice have adapted to the local temperature and can engage in their daily activities without significant impediments. The increased activity may necessitate a greater expenditure of energy, which could result in a reduction in weight gain.

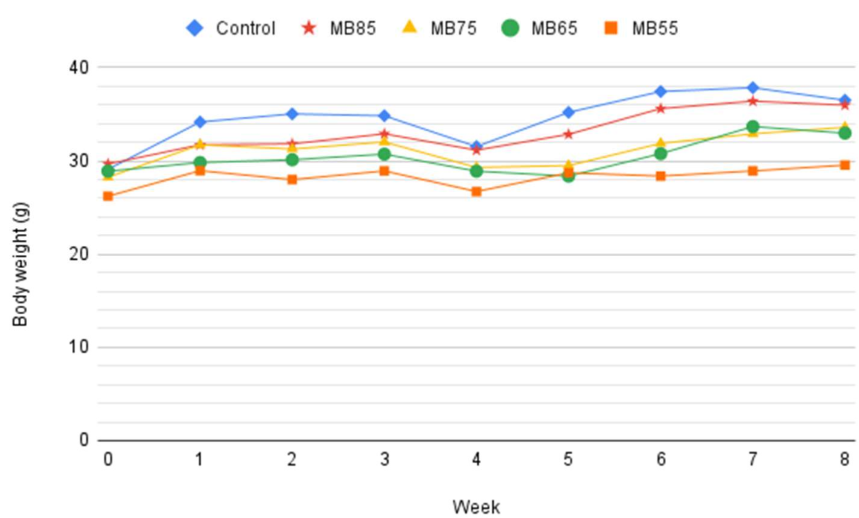

Figure 1. Weekly body weight over the 8 weeks of treatments

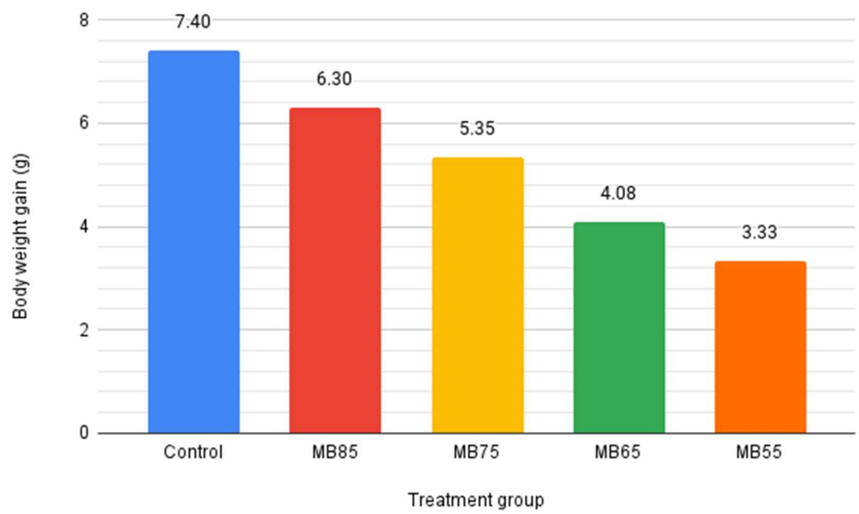

Figure 2. Average weight gain after 8 weeks of treatments

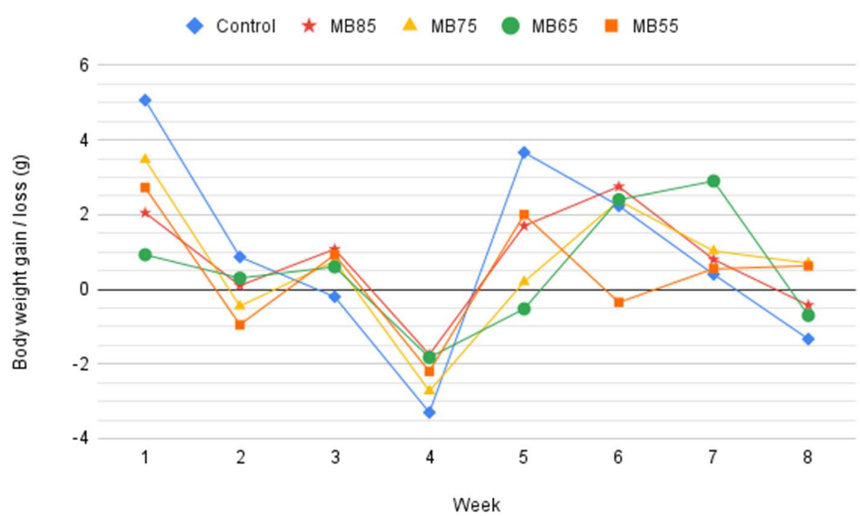

Figure 3. Weekly weight gain/loss over the 8 weeks of treatments

Based on the body weight gain data over the 8 weeks of treatments, the new feed gave the same results as the commercial feed. In Figure 2, we could see an illustration of the body weight gain of mice after an 8-week treatment period. The Kruskal-Wallis test for this parameter also revealed no significant differences (see Table 2), indicating that the administration of mung bean feed resulted in comparable weight gain to that observed with commercial feed. Notably, a trend suggesting that higher concentrations of mung beans yielded results that were increasingly, similar to the control group. As demonstrated in Figure 3, the fluctuations in weight gain and loss were relatively the same. All treatment groups exhibited a downward trend in body weight gain until Week 4, followed by a dramatic increase and subsequent stabilization. The Kruskal-Wallis test results shown in Table 2 confirmed the absence of significant differences between the treatments, further supporting the conclusion that mung bean feed produces weight gain outcomes equivalent to those of commercial feed.

From the results shown in Figures 1, 2, and 3, it could be concluded that mung bean treatments gave statistically the same results as the commercial feed. The notable difference or variation that could be seen might be caused by other factors. This research was conducted during the rainy season, a temporal period in which the ambient noise caused by rainfall could potentially incite anxiety in mice and increase their activity, running around inside their cage in search of a "safe" zone (Peng *et al*., 2023). An elevated estrogen level related to their reproductive phase could be another contributing factor (Morgan & Pfaff, 2002). The smells coming from stressed mice could also affect other mice (Zalaquett & Thiessen, 1991). Wright *et al*. (2022) found that the heritability of body weight declined with age under all diets, except the 40% calorie restriction diet. They also found that room temperatures and the amount of muscle and fat in a mouse's body were the biggest influences on energy balance. A study published in the journal Obesity by Yang *et al*. (2014) also discussed how age, sex, and diet are some of the strongest factors influencing body weight in C57BL/6 mice. Corrigan *et al*. (2020) used a big-data approach to understand metabolic rate and response to obesity in laboratory mice and found that body composition, ambient temperature, and institutional site of experimentation significantly influence energy balance and body weight. Circadian timing of feeding could also contribute to mice weight-fed with a high-fat diet (Arble *et al*., 2009).

The mice also seem to be able to accept the new mung bean-based feed. This can be seen from Figure 4 which shows a graph of feed consumption per mouse per day. The average feed consumption of each treatment shows results that are not significantly different. When viewed from the weekly data in Figure 5, the trend of mice feed consumption also shows a similar trend between treatments. Feed consumption increased in Week 2, then stabilized until the end of the 8 weeks. There are no significant differences between treatments (see Table 2). This means the mice can accept and eat the new feed formulation, just like the commercial feed.

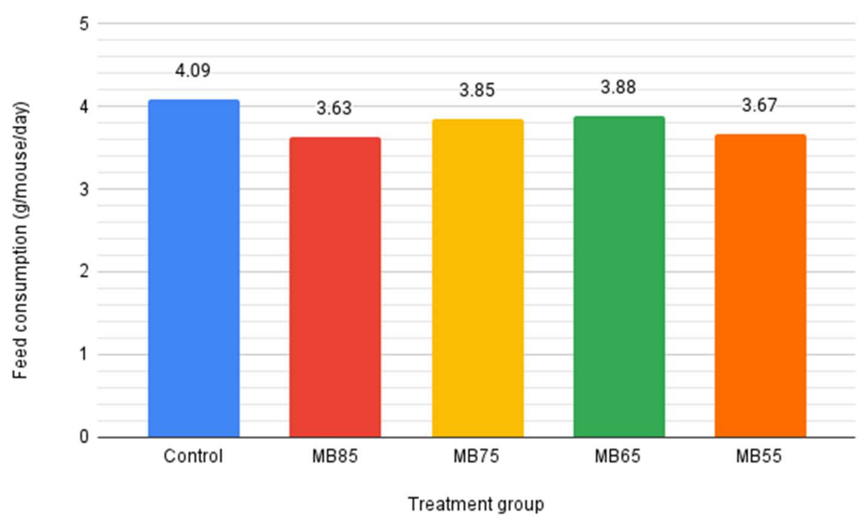

Figure 4. Average feed consumption per mouse per day

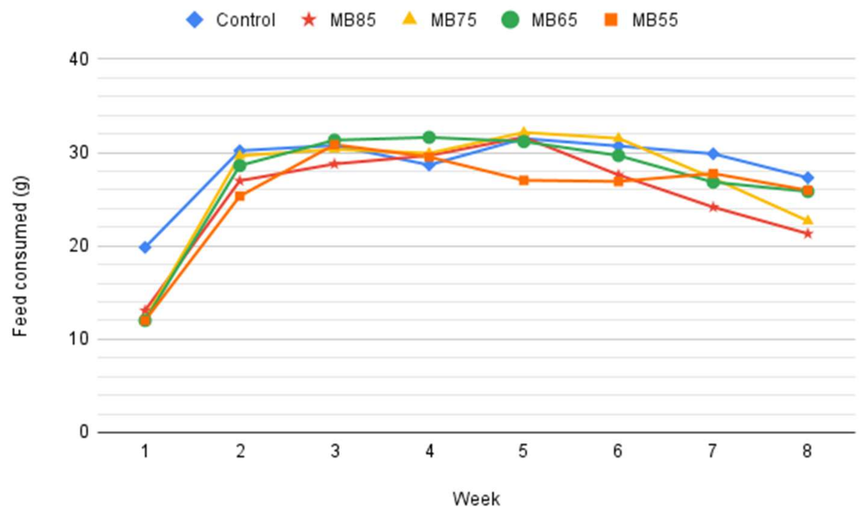

Figure 5. Weekly feed consumption over the 8-weeks of treatments

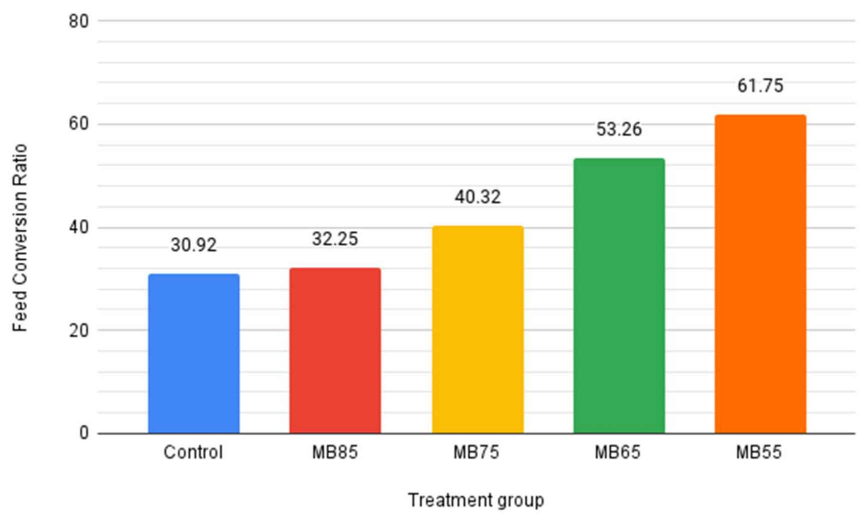

Figure 6. Feed Conversion Ratio of each treatment

The data on body weight gain and feed consumption were analyzed to calculate the Feed Conversion Ratio (FCR), a critical metric for assessing feed efficiency. A lower FCR indicates a more efficient feed in promoting body weight gain in mice. Figure 6 shows a trend where higher concentrations of mung beans in the feed correlate with improved FCR values. Although the Kruskal-Wallis test results shown in Table 2 did not reveal statistically significant differences between all treatment groups, this observed trend might suggest that increasing the proportion of mung beans in the diet may enhance feed efficiency, making it a promising alternative to commercial feed formulations.

Table 2. Kruskal Wallis Test Results

Parameter	Group	Mean Rank	Test Statistic (H)	Critical Chi at df=4	p-value*
Body weight after 8 weeks	Control	560.33	5.65526315789474	9.48733	0.226415
	MB85	702.25			
	MB75	400.00			
	MB65	306.25			
	MB55	110.25			
Average weight gain after 8 weeks	Control	533.33	2.00789473684211	9.48733	0.734307
	MB85	529.00			
	MB75	420.25			
	MB65	225.00			
	MB55	256.00			
Average feed consumption per mouse per day	Control	560.33	3.50789473684211	9.48733	0.476679
	MB85	196.00			
	MB75	462.25			
	MB65	552.25			
	MB55	240.25			
Feed Conversion Ratio (FCR)	Control	147.00	2.88157894736843	9.48733	0.577834
	MB85	342.25			
	MB75	484.00			
	MB65	729.00			
	MB55	289.00			

* *Test results are considered significant if the p-value is lower than 0.05 OR if the Test Statistic (H) is higher than the Critical Chi value at df=4*

Mung beans have been a subject of interest as a feed for animals for a considerable amount of time. Legumes, including mung beans, have great potential as a nutrient-rich feed supplement in poultry farming, improving feed efficiency and supporting sustainable agriculture (Nalluri & Karri, 2021). Feeds containing mung beans can effectively replace soybean meal without compromising the growth performance of broilers, showing that mung beans can be a viable alternative to traditional feed ingredients (Murwani, 2012). In Rodentia, the legumes have shown promising results in improving weight in malnourished rats (Rezaldi, 2021). The feed quality could be further enhanced by subjecting it to a boiling and sprouting process before giving them as feeds for rats and hamsters to increase soluble fiber content, hypocholesterolemia potential, and liver-protective properties of mung beans (Liyanage *et al*., 2018; Lopes *et al*., 2018). Additional fermentation processes might even increase the quality of mung bean proteins in Wistar rats (Lawal *et al*., 2022). Mung bean protein also has antioxidant properties and can regulate intestinal flora, thus improving overall health and nutrient absorption in animals (Zhang *et al*., 2022). Looking at the result of this research, mung beans show promising potential as an alternative feed source locally available for lab mice in Indonesia. However, we suggest further research is needed for a comprehensive understanding.

CONCLUSION

This study found no statistical difference between the treatments and the control group. Treatment MB85 showed the closest result to the control group based on two months of weight gain data and FCR value. Additional research is needed to explore other alternatives or formulations to find the best feed formulation for mice growth.

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