







OPTIMIZATION OF THE GROWTH AND NUTRITIONAL QUALITY OF LESSER MEALWORM (*Alphitobius diaperinus*) USING THE PROTEIN CONTENT OF THE MEDIUM

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Supporting Information



ABSTRACT: This study aims to examine the performance and nutritional quality of lesser mealworms (*Alphitobius diaperinus*) reared with different protein levels in the culture medium. The research method used was a completely randomized design (CRD) with 4 treatments and 5 replications. The treatments involved providing different protein levels in the culture medium: A (13%), B (15%), C (17%), and D (19%). The variables observed were larval consumption (g/500 g medium), larval production (g/500 g medium), larval body weight gain (g/500 g medium), chitin content (%), crude protein (%DM), nitrogen retention (%), crude fat (%DM), crude fat digestibility (%), and metabolizable energy (kcal/kg) of lesser mealworms. The result of this study showed that differences in protein levels in the culture medium had a very significant effect ($P < 0.01$) on larval consumption, larval production, larval body weight gain, chitin content, crude protein, nitrogen retention, crude fat, crude fat digestibility, and metabolizable energy of lesser mealworms. This study concluded that 19% protein culture medium was the optimal treatment for lesser mealworm rearing, yielding larval consumption of 181.87 g/500 g medium, larval production of 122.52 g/500 g medium, larval body weight gain of 107.51 g/500 g medium, chitin content of 21.17%, crude protein of 46.77% (DM), crude fat of 25.69% (DM), nitrogen retention of 78.72%, crude fat digestibility of 79.55%, and metabolizable energy of 2994.25 kcal/kg. The findings indicate that smaller mealworms hold considerable potential as an alternative source of animal protein for poultry feed.

Keywords: Feedstuff, Lesser Mealworm, Nutritional Quality, Performance, Protein Medium.

INTRODUCTION

The increase in demand for high-quality poultry products has driven the development of the poultry industry in Indonesia. To achieve optimal results, nutritious poultry feed that is rich in protein resources is essential. Feed ingredients that are increasingly being used today are those derived from larvae. Many studies have shown that larvae are highly effective in poultry feed. For example, replacing up to 12% of soybean meal with *Hermetia illucens* larvae improves growth performance, blood hematology, intestinal morphometry, and the meat quality of broiler chickens (Sajjad et al., 2024). Kaddour et al. (2025) found the moderate supplementation with *T. molitor* can enhance feed efficiency while maintaining egg quality, positioning insect proteins as a viable pathway toward more sustainable and resilient poultry production. Another study reported that yellow mealworms (*Tenebrio molitor*) can be used in quail feed up to 12% to reduce cholesterol and egg yolk fat while maintaining eggshell thickness and egg white protein content (Nuraini et al., 2023). Larvae other than *Hermetia illucens*, and *Tenebrio molitor* that can be used as feed additives in poultry feed include lesser mealworms.

The lesser mealworm is a larval stage of the beetle *Alphitobius diaperinus*, which belongs to the family Tenebrionidae and also includes other species such as the superworm and yellow mealworm. *Alphitobius diaperinus* larvae are morphologically similar to yellow mealworms (*Tenebrio* spp.), characterized by three pairs of legs and a segmented body that tapers at the tip of the larva (Tilak et al., 2023). These larvae have a strong, yellowish-brown exoskeleton (Sammarco et al., 2023). Lesser mealworms are rich in nutrients, containing 47.8% crude protein and 23.7% crude fat in dry matter (Soetemans et al., 2020). Lesser mealworm larvae contain amino acids (mg/100 g protein), including arginine 500, histidine 380, leucine 610, lysine 620, isoleucine 400, phenylalanine 460, methionine 190, threonine 390, valine 510, alanine 700, aspartic acid 790, glycine 400, glutamate 1050, serine 310, proline 619, cysteine 210, and tyrosine 710 (Kurečka et al., 2021).

The nutritional composition of larvae depends on the media consumed by the larva (Scala et al., 2020). Lesser mealworm culture media can be derived from industrial or agricultural byproducts such as soybean meal, rice bran, and chicken manure. According to Molese et al. (2023), tofu residue can be used as a protein-rich feed ingredient with a crude protein content of 28.36%, 5.52% fat, 7.06% crude fiber, and 45.44% NFE. Energy gross of tofu residue is 4858,16

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kcal/kg. *Alphitobius diaperinus* thrives in poultry houses (poultry manure) and grain silos (Crippen et al., 2022). The nutritional content of layer chicken manure includes crude protein 13.12%, crude fiber 18.34%, crude fat 3.10%, ash 0.13%, and NFE 62.61% (Puteri et al., 2022).

The research findings of Amran et al. (2024) reported that concentrate, dried tofu residue, and rice bran are culture media for producing lesser mealworms, with the selected media being concentrate and tofu residue. To date, the protein requirements of the culture medium for producing lesser mealworms remain unknown. Generally, lesser mealworms live in chicken manure, which contains 13% crude protein (Puteri et al., 2022). Additionally, Soetemans et al. (2020) states that lesser mealworms living on a medium with 18.6% crude protein have higher larval production and nutrient content; specifically 106% larval production, 47.8% crude protein content, and 23.7% crude fat content in dry matter. Tofu residue, rice bran, and layer chicken manure complement each other as culture media. However, there has been no research on the protein requirements of culture media for lesser mealworms. Culture medium protein will influence the growth, chitin content, crude protein, crude fat, nitrogen retention, crude fat digestibility, and metabolizable energy of lesser mealworms. Based on the above explanation, research was needed to determine the influence of culture medium protein level and the optimal level to improve the nutritional quality and metabolizable energy of lesser mealworms.

MATERIALS AND METHODS

Materials

The material used in this study was culture media consisting of dried tofu residue, rice bran, and dried laying chicken manure. There were 320 parent beetles (*Alphitobius diaperinus*) per biopon. Broiler chickens of the Lohman MB 202 strain, 6 weeks old (body weight ± 1500 g), totaling 24 chickens, were used for measuring nitrogen retention, crude fat digestibility, and metabolizable energy. Chemical reagents were used for analyzing protein, fat, and chitin content. The equipment used in this study included an analytical scale, biopon (plastic breeding containers) measuring 40×30×10 cm, nets to cover the biopon, egg trays as hiding places and egg-laying sites for beetles, a blender as a media grinder, a 2-mesh sieve, a metabolizable cage measuring 60×30×20 cm for measuring nitrogen retention, crude fat digestibility, and metabolizable energy, an oven, and a set of equipment for analyzing protein, fat, and chitin content, as well as a bomb calorimeter. Lesser mealworms (*Alphitobius diaperinus*) cultivation (Figure 1).



Parent beetles *Alphitobius diaperinus*



Dried tofu residue



Rice bran



Dried laying chicken manure



Media production



Biopon set-up



Lesser Mealworm



Lesser mealworms in a single biopon



Harvesting of lesser mealworms

Figure 1 - Lesser mealworm (*Alphitobius diaperinus*) cultivation.

Method

This study used a completely randomized design (CRD) according to [Steel and Torrie \(1995\)](#), consisting of 4 treatments and 5 replicates. The treatments were the protein levels in the culture medium, consisting of: A = 13%; B = 15%; C = 17%; D = 19%.

Lesser mealworm cultivation

Lesser mealworm cultivation is carried out according to the research by [Amran et al. \(2024\)](#). The culture medium consists of tofu residue, rice bran, and laying chicken manure. Tofu pulp and laying hen feces are dried in the sun. Once dry, the media is blended until smooth. Next, each medium is filtered through a 2-mesh sieve and weighed to 500 grams for each treatment. Then, 320 adult lesser mealworms were introduced into a medium per biopon. Egg trays were placed as hiding spots and egg-laying sites for the beetles. Unripe papaya was cut into 2×2×1 cm pieces and placed at 9 points on the medium. The papaya is replaced once every three days. After 10 days, the beetles are removed from the medium by sieving. During cultivation, biopons are placed in a location that is not exposed to direct sunlight, with good air ventilation and an average room temperature and humidity of 27°C and 71%, respectively. The larvae are reared for 18 days after the beetles are removed from the medium. The larvae are harvested according to [Nuraini et al. \(2022\)](#) by sieving with a 2-mesh sieve to separate the larvae from the medium, then weighed to obtain the fresh weight. Thereafter, the larvae are steamed for 10 minutes to kill them, then drained and dried in an oven (50°C, 24 hours). The dried larvae were weighed to determine their dry weight. The variables observed were larval consumption, larval production ([Maha et al., 2021](#)), larval body weight gain ([Fitri, 2022](#)), chitin content (No, 1987), crude protein, crude fat ([AOAC, 2016](#)), nitrogen retention, crude digestibility, and metabolizable energy ([Sibbald and Wolynetz, 1985](#)).

Measurements of nitrogen retention, crude fat digestibility, and metabolizable energy were conducted using 24 broilers with a weight range of \pm 1500 grams (20 broilers were used for the lesser mealworm treatment with protein levels in the culture medium, and 4 broilers were used for endogenous N), which were placed in individual metabolic cages. The broilers were first fasted for 24 hours, then force-fed 15 grams of the treatment feed. The excreta were then collected for 36 hours and sprayed with 0.3 N H₂SO₄ every 5 hours, after which the nitrogen, crude fat, and gross energy content of the excreta were analyzed.

RESULTS AND DISCUSSION

The average performance of lesser mealworms is shown in Table 1.

Table 1 - Performance of lesser mealworms.

Treatment (protein medium)	Larval consumption (g/500 g medium)**	Larval production (g/500 g medium)**	Larval body weight gain (g/500 g medium)**
A (13%)	110.30 ^d	72.54 ^b	42.47 ^d
B (15%)	124.53 ^c	80.11 ^b	57.17 ^c
C (17%)	176.14 ^b	115.94 ^a	91.21 ^b
D (19%)	181.87 ^a	122.52 ^a	107.51 ^a
SE	1.82	4.38	2.91

**Significantly different ($P < 0.01$), a,b,c,d Means within a column with different superscripts differ significantly ($P < 0.01$), SE = Standard Error.

Larval consumption

Table 1 shows that the highest consumption of larvae from lesser mealworms was found in treatment D (19% protein medium), which was 181.87 g/500 g medium, and the lowest was found in treatment A (13% protein medium), which was 110.30 g/500 g medium. The low average consumption of lesser mealworm larvae in treatment A (110.30 g/500 g medium) was due to the consumption of larvae in treatment A (13% protein medium), which originated from 100% chicken manure. This phenomenon is because the manure contains ammonia, which reduces the palatability of the lesser mealworms, leading to decreased larval consumption. Ammonia increases media moisture and pH, creating an unfavorable environment for the growth of lesser mealworms. Media with a strong odor can reduce feed palatability, making the feed unappealing to lesser mealworms ([Fernández et al., 2020](#)). [Crippen et al. \(2022\)](#) stated that high ammonia concentrations can be toxic and cause physiological stress in lesser mealworms, although lesser mealworms can utilize ammonia for metabolism; excessive levels can disrupt the nervous system and feeding behavior.

The average consumption rate of lesser mealworm larvae in treatment D (19% protein medium) was 181.87 g/500 g medium. This was due to the medium mixture consisting of 47% tofu residue, 38% rice bran, and 15% chicken manure. The preferred medium texture for lesser mealworm habitats is dry, loose, not too dense, and not wet. Media such as dry tofu residue and concentrates like rice bran have proven optimal for lower mealworm growth and production, as their

textures are easy to dig into, do not clump, and maintain sufficient moisture without becoming soft or watery. The fine, loose, and non-clumping texture of dried tofu residue makes it easier for lesser mealworms to consume and digest the culture medium, thereby enhancing palatability and increasing larval consumption. This assertion is supported by [Amran et al. \(2024\)](#), who stated that the use of dried tofu residue and concentrates can promote high larval consumption in lesser mealworm. Larval consumption of the selected lesser mealworm larvae was observed in treatment D (19% protein medium), amounting to 181.87 g/500 g medium. The research findings of [Safitri and Widiyaningrum \(2024\)](#) indicated that the average larval consumption of lesser mealworm larvae for 50 individuals was 1.138 grams. Metabolizable energy factors also influence the consumption rate of lesser mealworm larvae. Lower metabolizable energy in the culture medium leads to greater consumption of mealworm larvae, as fewer mealworms require it.

Larval production

Table 1 shows that the highest larval production from lesser mealworms was found in treatment D (19% protein medium), which was 122.52 g/500 g medium, and the lowest was found in treatment A (13% protein medium), which was 72.54 g/500 g medium. The low larval production from lesser mealworms in treatment A, which was 72.54 g/500 g medium, was due to the low ratio energy-protein of the culture medium, which was 182.42:1 in treatment A. One of the important components in the culture medium for lesser mealworms is energy and protein, which play a crucial role in cell synthesis and tissue formation. Low protein content in the culture medium can reduce mealworm growth, resulting in lower larval production. This aligns with the research by [Amran et al. \(2024\)](#), which found that the low live weight of lesser mealworms is influenced by the low protein content of their culture medium. [Scott et al. \(1992\)](#) also emphasized that the balance of nutrients, particularly protein and energy, significantly influences the rate of body weight gain in larvae. The protein-to-energy ratio is also an important factor in lesser mealworm production. According to [Schøn et al. \(2025\)](#), a higher protein-to-carbohydrate ratio accelerates larval growth and development by increasing protein deposition.

Treatment C achieved the highest larval production from lesser mealworms, with 115.94 g/500 g medium, followed by treatment D with 122.52 g/500 g medium. This was due to the high energy-protein ratio of the culture medium in treatments C and D, which were 233.15 : 1 and 227.81 : 1, respectively. The high protein content of the lesser mealworm culture medium can influence larval production because energy and protein play a crucial role in the growth and development of the lesser mealworm's body. Protein is the primary source of amino acids used by lesser mealworms to build and repair their body tissues, as well as to support various important metabolizable functions. Culture media with high protein content provide lesser mealworms with sufficient nutrients to support their optimal growth. This conclusion is supported by [Kotsou et al. \(2021\)](#), who showed that high protein content in lesser mealworm feed can increase growth and survival rates as well as shorten development time.

Larval body weight gain

Table 1 shows that the highest body weight gain of lesser mealworm larvae was found in treatment D (19% protein medium), which was 107.51 g/500 g medium, while the lowest body weight gain of lesser mealworm larvae was found in treatment A (13% protein medium), which was 42.47 g/500 g medium. The lower average body weight gain of lesser mealworm larvae in treatment A (42.47 g/500 g medium) was due to the medium's lower protein content (13%). The low protein content of the culture medium fails to meet the protein requirements for lesser mealworm growth, resulting in low body weight gain of lesser mealworm larvae. Low protein content in the medium can disrupt metabolizable processes, leading to suboptimal conversion of nutrients into energy and building materials for the body, thereby reducing growth and body weight gain of lesser mealworm larvae. Research shows that culture media with low nutrient content reduce metabolism in lesser mealworms, resulting in lower fresh weights and lengths than media with high nutrient content, such as dried tofu residue or concentrate. This aligns with the findings of [Amran et al. \(2024\)](#), where the low live weight of lesser mealworm was influenced by the low protein content of the culture medium.

The highest average body weight gain of lesser mealworm larvae in treatment D (19% protein content) was 107.51 g/500 g medium. This is due to the high crude protein content of the medium in this treatment, with crude protein content in treatment D at 19%. Crude protein content influences the magnitude of body weight gain in lesser mealworm larvae, as they are selective in the feed they consume. Protein plays a crucial role in metabolic processes, the growth of new tissues, and the repair of damaged tissues. The higher the protein content in the medium, the more nutrients are available to support the growth and development of lesser mealworm, resulting in a significant increase in their body weight. [Scott et al. \(1992\)](#) also emphasized that the balance of nutrients, particularly protein and energy, significantly influences the rate of body weight gain in larvae. The high body weight gain in larvae is also due to their high consumption. High larval consumption provides sufficient nutrient intake, particularly protein and energy, which are the primary factors in accelerating the growth and body weight gain of lesser mealworm larvae.

Digestibility analysis

The average nutritional content and nutritional digestibility of lesser mealworms are shown in Table 2.

Table 2 - Nutritional content and nutritional digestibility of lesser mealworms.

Treatment (protein medium)	Chitin (%)**	Crude Protein (%DM)**	Nitrogen Retention (%)**	Crude Fat (%DM)**	Crude Fat Digestibility (%)**	Metabolizable Energy (kcal/kg)**
A (13%)	9.35 ^d	23.35 ^c	30.33 ^c	17.70 ^c	71.62 ^d	2378.85 ^d
B (15%)	13.58 ^c	25.63 ^c	52.08 ^b	20.98 ^b	74.12 ^c	2561.18 ^c
C (17%)	18.22 ^b	37.26 ^b	62.75 ^b	22.59 ^b	76.78 ^b	2762.94 ^b
D (19%)	21.17 ^a	46.77 ^a	78.72 ^a	25.69 ^a	79.55 ^a	2994.25 ^a
SE	0.60	2.66	5.01	0.49	0.71	7.78

Note: **: Significantly different (P<0.01); a,b,c,d: Means within a column with different superscripts differ significantly (P<0.01); DM = Dry Matter; SE =Standard Error.

Chitin content

Table 2 shows that the highest chitin content in lesser mealworm was observed in treatment D (19% protein medium; 21.17%) and the lowest in treatment A (13% protein medium; 9.35%). The low chitin content of lesser mealworm in treatment A (9.35%) is related to the low larval production of lesser mealworm in treatment A. Lesser mealworms with low larval production have low live weight, which leads to reduced skin production. This is because chitin is located in the outer skin of the lesser mealworm. [Iber et al. \(2022\)](#) stated that chitin is the main component of the exoskeleton or shell of crustaceans and insects, as well as the cell walls of yeast, algae, and fungi. The chitin content of the lesser mealworm in treatment D was higher than in other treatments, at 21.17%. This is related to the high larval production in treatment D. Lesser mealworm living on a high-protein medium can meet their protein needs, resulting in fewer mealworm with higher live weight and larger body size, thereby increasing chitin content. This aligns with the findings of [Alhidayat et al. \(2025\)](#), who reported that lesser mealworm culture media with high crude protein content produce lesser mealworms with larger body weights, thereby increasing chitin content. The chitin content of lesser mealworm is high in treatment D, but its administration in poultry feed certain limits (not in high concentrations because of chitin), will not interfere with poultry productivity, as can be seen from the research of [Wandira et al. \(2016\)](#), which found no difference in body weight gain between ducks not given chitin and those given 1%, 2%, and 3% chitin in the ration. Another opinion by [Sedgh-Gooya et al. \(2022\)](#) states that 5% chitin in dry matter does not harm broilers. Regarding the effect of chitin on nitrogen retention, [Khempaka et al. \(2011\)](#) reported that feeding shrimp meal at 20% with a chitin content of 3.8% resulted in nitrogen retention of 50.89%.

Crude protein

Table 2 shows that the highest crude protein content of lesser mealworms was found in treatment D (medium protein 19%) at 46.77% and the lowest was found in treatment A (medium protein 13%) at 23.35%. The crude protein content of lesser mealworms in treatments A and B is low, at 23.35% and 25.63%, respectively, due to the low crude protein content in the culture media, which are 13% and 15%, respectively. If lesser mealworms lack protein in the culture media, their bodies will experience limitations in metabolism. The enzymes formed from protein will also decrease in number, so that the mealworm's metabolism cannot function optimally. Digestive enzymes function to break down food into smaller nutrients that can be absorbed. Protein deficiency disrupts enzyme synthesis, which ultimately affects nutrient absorption and crude protein production in the mealworm's body. This finding is supported by [Nuraini et al. \(2022\)](#), who found that mealworm protein content is influenced by the nutrient composition of the culture medium.

The high crude protein content of lesser mealworms in treatment D (46.77%) is related to the high crude protein content of the culture medium in treatment D (19%). The crude protein content in the culture medium directly affects the crude protein content of the mealworms. If the culture medium contains a high amount of crude protein, then the mealworms raised in that medium will also exhibit a high crude protein content, and vice versa. Protein functions in tissue formation through protein synthesis within the lesser mealworm's body. According to [Fuso et al. \(2021\)](#), the protein level achieved by larvae is greatly influenced by the amount of protein and fiber available in their food substrate.

The crude protein content of the selected lesser mealworms was found in treatment D (culture medium protein 19%) at 46.77%. Other studies indicate that lesser mealworms have a protein content of 48% when fed a 18.6% crude protein culture medium ([Soetemans et al., 2020](#)). The difference in crude protein content is due to the difference in the culture medium used, which consisted of wheat flour mixed with 15% rapeseed flour and beer waste.

Nitrogen retention

Table 2 shows that the highest nitrogen retention in lesser mealworms was found in treatment D (19% protein medium) at 78.72%, while the lowest was found in treatment A (13% protein medium) at 30.33%. The low protein intake in treatment A (3.11 g/head) influences the lower nitrogen retention in lesser mealworms (30.33%). The low protein consumption is due to the low crude protein content of lesser mealworms in treatment A, at 23.35%. The culture

medium's low protein content of 13% contributes to the low crude protein content of lesser mealworms. If the culture medium has low protein content, the lesser mealworm's body will also have a limited amount of available amino acids. As a result, the lesser mealworm's body has difficulty maintaining or storing nitrogen for metabolic needs, leading to low nitrogen retention. This phenomenon can be seen from the research by [Alhidayat et al. \(2025\)](#), which found that yellow mealworms living in a culture medium with crude protein of 22% had lower nitrogen retention of 72.27% compared to a culture medium with crude protein of 26%, which had nitrogen retention of 80.89%.

Lesser mealworms in treatment D exhibited a high nitrogen retention of 78.72%. This was influenced by the high protein intake in treatment D, which was 6.25 g/head. The high protein intake was due to the high crude protein content of lesser mealworms in treatment D, which was 46.77%. The high crude protein content of lesser mealworms in this treatment was due to the high protein content of the culture medium, which was 19%. Lesser mealworms with adequate protein intake have better nitrogen balance between intake and excretion. This aligns with the opinion of [Ren et al. \(2025\)](#) that to achieve nitrogen balance in insects, there needs to be a balance between food intake, metabolic conversion, and excretion. Digested protein provides nitrogen used for tissue synthesis. High nitrogen retention indicates that lesser mealworms can utilize most of the nitrogen intake.

The high nitrogen retention in treatment D reflects the satisfactory protein quality of the lesser mealworms. This aligns with the research by [Alhidayat et al. \(2025\)](#), which found that lesser mealworms with high nitrogen retention also have high protein quality. The high protein quality in treatment D is also reflected in the amino acid composition of the lesser mealworms. The results of amino acid content of lesser mealworms in treatment D contain several amino acids, namely lysine 0.39%, methionine 0.56%, threonine 1.31%, tryptophan 0.47%, arginine 1.11%, glutamic acid 4.07%, alanine 3.32%, aspartic acid 2.45%, glycine 2.31%, histidine 1.08%, isoleucine 1.73%, cysteine 1.20%, leucine 2.70%, valine 2.26%, phenylalanine 1.93%, proline 2.32%, serine 1.44%, and tyrosine 2.07%.

The highest nitrogen retention in lesser mealworms in this study was observed in treatment D (19% protein medium), at 78.72%. This result is lower than the nitrogen retention of yellow mealworms (*Tenebrio molitor*), which was 80.89% ([Alhidayat et al., 2025](#)). The nitrogen retention of lesser mealworms in treatment D was lower than that of superworms (*Zophobas morio*), which was 80.36% ([Nuraini et al., 2025](#)).

Crude fat

Table 2 shows that the highest crude fat content was found in treatment D (culture medium protein 19%) at 25.69%, and the lowest crude fat content was found in treatment A (culture medium protein 13%) at 17.70%. The low crude fat content of the lesser mealworms in treatment A (17.70%) is related to the low crude fat content of the culture medium in treatment A (3.1%), which affects fat metabolism as an energy reserve for the lesser mealworms. In treatment A, the culture medium used was 100% chicken feces. Chicken feces can also have toxic properties due to their ammonia content, which inhibits mealworm growth, resulting in low fat storage in the mealworm's bodies. According to [Rumpold and Schlüter \(2013\)](#), although chicken manure can be used as a physical medium for mealworm growth, its nutrient content is low, which increases physiological stress on the mealworms, causing available energy to be primarily used for survival rather than fat storage.

The high crude fat content of larvae in treatment D (crude protein 19%) at 25.69% is due to the high protein content of the medium. The high protein content of the culture medium is not entirely used for body tissue synthesis; the larvae undergo deamination, where the amino groups of amino acids are released, and the remaining carbon is utilized in energy metabolism pathways. If the energy produced from this metabolism is not immediately utilized, the excess energy is converted into energy reserves in the form of fat through the process of lipogenesis. This process leads to an increase in crude fat content in the mealworm's body. Additionally, the high protein content promotes faster growth, causing the mealworms to require more energy reserves for the subsequent metamorphosis phase, which are stored in the form of body fat. Research conducted by [Kim et al. \(2019\)](#) shows that media with high protein content produce mealworms with higher crude fat content compared to media with low protein content. Thus, it can be concluded that the higher the protein content in the culture medium, the greater the potential for increased crude fat content in the mealworm's body.

The crude fat content of lesser mealworms is greatly influenced by the feed medium; lesser mealworms grown on high-quality medium tend to have high crude fat content. The fat content of the culture medium greatly influences the growth of lesser mealworms. According to [Suwannayod et al. \(2025\)](#), fat-rich culture media significantly increase the fat content in larval bodies.

The crude fat content of the selected mealworms was 25.69% in treatment D, which used a mixture of tofu pulp, feces, and rice bran (crude protein 19%) as the medium. Another study stated that media using industrial waste produced lesser mealworms with a crude fat content of 26% ([Soetemans et al., 2020](#)).

Crude fat digestibility

Table 2 shows that the highest crude fat digestibility was found in treatment D (culture medium protein 19%) at 79.55%, and the lowest crude fat digestibility was found in treatment A (culture medium protein 13%) at 71.62%. The low

crude fat digestibility of lesser mealworms in treatment A (71.62%) is attributed to the low crude fat content of lesser mealworms in treatment A (17.70%). The low crude fat content of lesser mealworms (*Alphitobius diaperinus*) may be one of the causes of low fat digestibility when they are used as feed for poultry. This is because the amount of fat substrate available in the feed significantly determines the activity and efficiency of lipid-digesting enzymes in poultry, such as pancreatic lipase. When the fat content in the ration is very low, lipase activity in the digestive tract tends to decrease because the stimulus for enzyme secretion is reduced. This condition prevents some of the available fat fractions from being optimally digested and absorbed. The crude fat had the highest digestibility in treatment D, at 79.55%. This was due to the high fat content of the culture medium (25.69%). The high fat content led to increased fat digestibility because more fat substrate was absorbed. In addition to the high fat content of the mealworms, the medium's protein content also affected their fat digestibility. According to Miguéns-Gómez et al. (2020), when the protein requirements of the larvae are met, energy and fat metabolism occur more efficiently, resulting in larvae with tissue structure and fat composition that are easier to digest by the poultry digestive system. It is further explained that lesser mealworms reared on a protein-rich medium tend to produce softer body tissue with a fat composition that is more easily digested by the poultry digestive system.

Metabolizable Energy

Table 4 shows that the highest metabolizable energy (ME) was found in treatment D (crude protein 19%) at 2994.25 kcal/kg, while the lowest metabolizable energy (ME) was found in treatment A (crude protein 13%) at 2378.85 kcal/kg. The low metabolizable energy (ME) of the lesser mealworms in treatment A, which is 2378.85 kcal/kg, is due to the low protein content (CP 13.12%) and fat content (Fat 3.1%) of the culture medium in treatment A. The nutritional content of the culture medium also affects the growth and energy metabolism of the lesser mealworms. Culture media with low fat content will inhibit caterpillar growth, resulting in low metabolizable energy content in caged caterpillars because fat is the highest source of energy in insects, so a decrease in fat is directly proportional to a decrease in metabolizable energy (Schiavone et al., 2017). The highest metabolizable energy content in lesser mealworms was observed in treatment D (crude protein 19%), with 2994.25 kcal/kg. This indicates that the culture medium has high nutritional quality. High-quality culture media enhance the feed conversion efficiency of lesser mealworms. This means that lesser mealworms can convert feed into body tissue with higher energy content, such as fat and protein. High-quality media rich in protein, fat, and energy increases the metabolizable energy content of lesser mealworm bodies, due to higher growth and nutrient absorption rates. This aligns with the research by Fowles and Nansen (2020), which found that protein-rich media also promote optimal growth and metabolism in larvae. Adequate protein supports enzyme synthesis, the formation of body tissues, and the conversion of nutrients into energy reserves.

CONCLUSION

This study concludes that crude protein at 19% is the optimal protein content for the selected lesser mealworms culture medium, resulting in lesser mealworm with a larval consumption rate of 181.87 g/500 g medium, larval production of 122.52 g/500 g medium, larval body weight gain of 107.51 g/500 g medium, chitin content of 21.17%, crude protein content of 46.77% (dry matter), nitrogen retention of 78.72%, crude fat content of 25.69% (dry matter), crude fat digestibility of 79.55%, and metabolizable energy of 2994.25 kcal/kg. This result is due to the availability of adequate protein media at 19%, which efficiently support the synthesis of body tissue protein and lipogenesis, producing larvae with greater body mass and a rich nutritional profile, making them a highly valuable alternative feed source for poultry.

DECLARATIONS

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Availability of data and materials

The raw data, study data, and materials are accessible upon request.

Ethical consideration

Before publication in this journal, all authors have reviewed ethical concerns, including data fabrication, duplicate publication and submission, redundancy, plagiarism, consent to publish, and misconduct.

Authors' contribution

Nuraini designed the research, coordinated the research and data analysis, drafted and edited the publication manuscript, and provided final approval. Mirzah and Yuliaty Shafan Nur assist with laboratory and data analysis, and with editing publication manuscripts. Abdul Azim, Dinah Fathinah, and Rezki Wahyudi conducted the cultivation of lesser mealworms, analyzed the data, and drafted the manuscript.

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Competing interests

The authors declare no competing interests in this research and publication.

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