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Improving performance of *Tenebrio molitor*: The effects of media composition and thickness on chitin content, protein quality, and fat digestibility

Peningkatan performa Tenebrio molitor: Pengaruh komposisi dan ketebalan media terhadap kandungan kitin, kualitas protein, dan kecernaan lemak

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ABSTRACT

This study investigated the potential of *Tenebrio molitor* (mealworm) larvae as a sustainable protein source for animal feed. The effects of growth media composition and thickness on performance, chitin content, protein quality, and fat digestibility were analyzed. The research used an experimental Completely Randomised Design in a 3x3 factorial arrangement with three replications to examine the following factor A media compositions: A1 (50% Commercial ration + 50% Tofu dregs), A2 (50% Commercial ration + 50% Palm kernel meal), A3 (50% Commercial ration + 50% Rice bran) and Factor B media thicknesses: B1 (1 cm), B2 (2.5 cm), and B3 (4 cm). The parameters evaluated were performance metrics, chitin concentrations, crude protein, nitrogen retention, and fat digestibility. Variance analysis demonstrated a highly significant interaction between composition and thickness across all parameters (P<0.01). The ideal formulation was a combination of commercial feed combined and tofu dregs at a thickness of 4 cm producing the following of exceptional outcomes: consumption of 291.40 g per 1000 larvae, body weight gain of 80.79 g per 1000 larvae, media production of 90.13 g per 500 g, chitin content of 14.34%, crude protein of 55.64%, nitrogen retention of 77.74%, and digestibility of crude fat of 89.28%. Therefore, it can be concluded that the combination of 50% commercial feed and 50% tofu waste with a medium thickness of 4 cm was the most effective treatment for improving the performance, chitin content, protein quality, nitrogen retention, and fat digestibility of *Tenebrio molitor* larvae.

ABSTRAK

Studi ini mempelajari larva Tenebrio molitor (ulat tepung) sebagai sumber protein berkelanjutan untuk pakan ternak dengan menganalisis pengaruh komposisi dan ketebalan media pertumbuhan terhadap kinerja, kandungan kitin, kualitas protein, dan daya cerna lemak. Penelitian ini menggunakan Rancangan Acak Lengkap pola faktorial 3x3 dengan tiga ulangan untuk menguji komposisi media Faktor A: A1 (50% Ransum komersial + 50% Ampas tahu), A2 (50% Ransum komersial + 50% Bungkil inti sawit), A3 (50% Ransum komersial + 50% Dedak padi), dan Faktor B: ketebalan media: B1 (1 cm), B2 (2,5 cm), dan B3 (4 cm). Parameter yang dievaluasi adalah performa, kandungan kitin, protein kasar, retensi nitrogen, dan daya cerna lemak. Analisis varian menunjukkan ada interaksi yang sangat signifikan antara komposisi dan ketebalan media pada semua parameter (P<0,01). Kesimpulan dari penelitian ini adalah pakan komersial

Kata kunci:
Komposisi ketebalan media
Kecernaan lemak
Performa
Kualitas protein
Tenebrio molitor.

yang dikombinasikan dengan ampas tahu dengan ketebalan 4 cm menghasilkan konsumsi 291,40 g/1000 larva, peningkatan berat badan 80,79 g per 1000 larva, produksi larva 90,13 g/500 g media, kandungan kitin 14,34%, protein kasar 55,64%, retensi nitrogen 77,74%, dan daya cerna lemak kasar 89,28%. Dapat disimpulkan bahwa kombinasi media 50% ransum komersial + 50% ampas tahu dengan ketebalan 4 cm merupakan perlakuan terbaik karena memberikan pengaruh paling optimal terhadap performa, kandungan kitin, kualitas protein, retensi nitrogen, dan daya cerna lemak larva Tenebrio molitor.

INTRODUCTION

Feed is a crucial input in poultry farming, yet feed costs account for 60-70% of total production costs. This is primarily due to the continued use of imported raw materials, such as fishmeal. Fishmeal is the main source of animal protein in poultry feed. One way to reduce these costs is to use alternative ingredients. *Tenebrio molitor* larvae are a potential substitute for fishmeal.

Tenebrio molitor larvae contain 43.60-53.40% crude protein, 13.84-30% crude fat, 2.7-13.36% ash, 6-10% crude fiber, 0.17-2% Ca, 0.80-2% P, and 3351-3400 kcal/kg of metabolic energy (Iding et al., 2020; Nuraini et al., 2022a; López-Gámez et al., 2024). They also contain various amino acids, including glutamic acid, lysine, leucine, alanine, tyrosine, aspartic acid, valine, glycine, and methionine (Nuraini et al., 2022a; Nuraini et al., 2023). The unsaturated fatty acids range from 22 to 37%, including linoleic acid (omega-6), oleic acid (omega-9), and palmitic acid (Dabbou et al., 2020; Nuraini et al., 2023).

Tenebrio molitor larvae have great potential as a source of animal protein. However, this nutrient content is greatly influenced by the growth medium used. However, this is greatly influenced by the growth medium used. The growth medium serves as both feed and a production site, thus affecting growth and nutritional content (Nuraini et al., 2024). Commercial rations are generally used by *Tenebrio molitor* larval cultivators (Nuraini et al., 2022b). The use of commercial rations, however, due to their complete nutritional content, can be expensive for cultivation.

Using commercial rations as a growth medium requires combining them with other ingredients to reduce cultivation costs. Possible ingredients include tofu dregs, palm kernel meal, and rice bran. Tofu dregs, a byproduct of tofu production, contain 28.00% crude protein, 2.71% fat, 7.06% crude fiber, and 2,500 kcal/kg of metabolizable energy (Nguru et al., 2024). Palm kernel meal, a byproduct of palm oil processing, provides 16.00% crude protein, 8.45% fat, 16.89% crude fiber, and 2,682 kcal/kg (Bárcena et al., 2022). Rice bran, a byproduct of rice milling, provides 9.00% crude protein, 7.40% fat, 12.95% crude fiber, and 2255 kcal/kg (Rosani et al., 2024).

Tenebrio molitor larvae reared on tofu dregs medium produced larvae containing 49.69% crude protein (Nuraini et al., 2025). The use of rice bran as a growth medium has also been reported by Hapsari et al. (2018), who found that a 50% rice bran and 50% tofu dregs mixture provided the best results for enhancing the growth and development of *Tenebrio molitor* larvae. The use of palm kernel meal as a growth medium for *Tenebrio molitor* larvae has not been studied, but its use in maggot larvae has produced larvae with a protein content of 48.67-50.58% (Bokau et al., 2018).

Another factor to consider in cultivating *Tenebrio molitor* larvae is the thickness of the media used (Ghaly et al., 2009; Nuraini et al., 2022b). Media thickness affects the larvae's mobility. Deruytter et al. (2022) found that media that is too thin (less than 2 cm) restricts movement, hindering *Tenebrio molitor* larvae from consuming the media.

Based on the above description, this study was conducted to determine the effect of media composition and thickness on the performance, nutritional content, and quality of *Tenebrio molitor* larvae.

MATERIALS AND METHODS

Research Materials

The materials used in this study were dry growth media consisting of commercial ration Bravo 311 vivo, palm kernel meal, and bran

obtained from poultry shops, and tofu dregs obtained from a tofu factory in Payakumbuh that had been dried (moisture content 14%). Tenebrio molitor larvae were obtained from a farmer specialising in *Tenebrio molitor* larvae farming located in Padang Harapan, Mungka District, Lima Puluh Kota Regency, Payakumbuh City. Other materials include chemicals for chitin analysis, crude protein, nitrogen retention, and crude fat digestibility.

The tools used in this study were an analytical balance, 30 cm x 20 cm x 10 cm-sized biopons (baskets), 60 cm x 30 cm x 10 cm-sized cages, and a set of tools for analyzing chitin, crude protein, nitrogen retention, and crude fat digestibility.

Research Methodology

Research implementation

Previously, the tofu dregs were dried (moisture content 14%), and the palm kernel meal and rice bran were ground. A growth medium composition (according to treatment) weighing 250 g per treatment and biopon measuring 30 cm × 20 cm × 10 cm was prepared. To get depths 1 cm, 2,5 cm, and 4 cm, the biopon is segmented by partitions based on the treatment thickness. *Tenebrio molitor* larvae were weighed to obtain their initial body weight, then 10-day-old Tenebrio molitor larvae were placed into a biopon containing 1,000 worms (2 g) of growing medium. Fresh young papaya pieces measuring 2 x 1 x 0.5 cm were placed at 9 points above the culture medium.

The dried papaya pieces were removed and replaced with fresh ones every 2 days. On the 15th day, *Tenebrio molitor* larvae molt, and every 3 days, the medium surface is fanned and lifted. *Tenebrio molitor* larvae are raised until they are 30 days old. The temperature is recorded daily in the morning, afternoon, and evening until the *Tenebrio molitor* larvae are harvested. The variables observed were chitin content, crude protein, nitrogen retention, and crude fat digestibility.

Animal and ethic

All experimental protocols were sanctioned by the Research Ethics Committee of the Faculty of Medicine, Universitas Andalas, Indonesia (No. 393/UN.16.2/KEP-FK/2025). All investigations were executed in accordance with the International Guiding Principles for Animal Research and Welfare.

Measurements of nitrogen retention and crude fat digestibility were conducted using 30 six-week-old broiler chickens weighing about 1500 g, consisting of 27 broiler chickens for treatment diets and 3 broiler chickens for endogenous nitrogen determination. The chickens were fasted for 24 hours and placed in metabolic cages equipped with drinking water and a plastic sheet for fecal collection. The *Tenebrio molitor* larvae are finely ground into flour, then weighed at 15 g and mixed with water to form a paste. After the 24-hour fasting period, the broilers were force-fed 15 g of mealworm paste. This procedure was conducted in accordance with standard ethical guidelines for animal research, minimizing distress to the broilers. Feces were collected over 36 hours, sprayed with 0.3 N H2SO4, and air-dried at room temperature for approximately 5 hours. After 5 hours, the feces were oven-dried at 60°C for 24 hours, then weighed. The excreta were ground and analyzed for nitrogen content.

Nitrogen retention (%) was calculated using the crude fat digestibility of mealworms was tested on broilers according to Sibbald et al. (1985), with modifications. The broiler chickens used for fat digestibility testing were the same as those used for nitrogen retention testing (without endogenous measurement). The excreta were ground and analyzed for crude fat content using the Soxhlet method (AOAC, 2016).

$$\text{N Retention} = \frac{\text{N consumption (g/head)} - (\text{N excreta (g/head)} - \text{N endogenous (g/head)})}{\text{N consumption (g/head)}} \times 100$$

N consumption: dry matter of the consumed feed × nitrogen (%) of the feed.
N excreta: dry matter excreted × nitrogen (%) of the excreta.
N endogenous: dry matter endogenous excreta ×nitrogen (%) of the endogenous excreta

$$\text{Crude fat digestibility} = \frac{(FC \times CFF) - (AEE \times CFE)}{FC \times CFF} \times 100\%$$

FC = Feed consumption (g); CFF = Crude fat in feed (%); AEE = Amount of excreta excreted (g); CFE = Crude fat in excreta (%)

Research Design

This study used an experimental method with an experimental design of a Completely randomized design (CRD) with a 3x3 factorial pattern and 3 replications. The treatments are as follows:

Factor A is the composition of the growth medium:

A1= 50% Commercial ration + 50% Tofu dregs
A2= 50% Commercial ration + 50% Palm kernel meal
A3= 50% Commercial ration+ 50% Rice bran

Factor B is the thickness of the growth medium:

B1 = Media thickness 1 cm
B2 = Media thickness 2.5 cm
B3 = Media thickness 4 cm

The nutritional content (%DM) and gross energy (kcal/kg) of the *Tenebrio molitor* larvae growth medium after mixing can be seen in the table 1.

Table1. Nutritional content of the *Tenebrio molitor* larvae growth medium

Nutritional content	A	B	C
	(50% CR + 50% TD)	(50% CR+ 50% PKM)	(50% CR + 50% RB)
Crude protein (%)	25.00	19.00	16.00
Crude fat(%)	3.86	6.73	6.30
Crude fibe(%)	6.03	10.95	8.98
GE (Kkal/kg)	4000.00	4130.00	3825.00

Note: CR = Commercial ration, TD = Tofu dregs, PKM = Palm kernel meal, RB = Rice bran, GE = Gross energy

Data analysis

The obtained data were statistically analyzed using a Completely randomized design (CRD) Analysis of variance (ANOVA) in a 3 × 3 factorial pattern with three replications. Differences between treatments were tested using Duncan's multiple range test (DMRT) (SPSS v22.0) software from IBM. All results are presented as mean values, with $P<0.05$ as the cut-off for statistical significance.

RESULTS AND DISCUSSION

Feed Consumption of *Tenebrio molitor* Larvae

The average feed consumption of *Tenebrio molitor* larvae influenced by the composition and thickness of the media can be shown in Table 2.

The highest consumption of *Tenebrio molitor* larvae was observed in treatment A1B3 (50% Commercial ration + 50% Tofu dregs with a media thickness of 4 cm), at 291.40 g, and the lowest in treatment A3B1 (50% Commercial ration + 50% Rice bran with a media thickness of 1 cm), at 222.17 g. The results of the variance analysis show a highly significant interaction ($P<0.01$) between the composition and thickness of the medium, which affects the consumption of *Tenebrio molitor* larvae. The DMRT test results show that the consumption of *Tenebrio molitor* larvae in treatment A1B3 with a medium thickness of 4cm is significantly higher ($P<0.01$) than in other treatments.

High consumption of *Tenebrio molitor* larvae in treatment A1B3 (50% Commercial ration

Table 2. The average feed consumption of *Tenebrio molitor* larvae

Factor A (Media composition)	Factor B (Media thickness)			Mean	SEM
	B1(1cm)	B2(2.5cm)	B3(4cm)		
A1 (50% CR + 50% TD)	254.53^{c}	262.97^{b}	291.40^{a}	269.40	11.15
A2 (50% CR + 50%PKM)	244.00^{d}	255.10^{c}	264.83^{b}	254.64	6.02
A3 (50% CR + 50% RB)	222.17^{e}	245.60^{d}	255.93^{c}	241.23	9.99
Mean	240.23	254.56	270.72	255.17	9.05

Note: ^{abcde}Different superscripts in the same row indicate a highly significant effect ($P<0.01$). CR = Commercial ration, TD = Tofu dregs, PKM = Palm kernel meal, RB = Rice bran

+ 50% Tofu dregs with a media thickness of 4 cm) because *Tenebrio molitor* larvae prefer tofu dregs, and their movement to the bottom of the media is smoother. *Tenebrio molitor* larvae prefer tofu dregs as a ration because they have a distinctive aroma of fresh soybeans that remains strong even after becoming dregs, which enhances the sensory response in *Tenebrio molitor* larvae. Dried tofu dregs have a finer, softer texture than high-fiber bran and hard palm kernel meal. In line with the research findings of Nuraini et al. (2025), the higher feed consumption and weight gain observed in the media containing tofu dregs indicate that tofu dregs are a suitable feed substrate for *Tenebrio molitor* larvae, as the nutritional quality and palatability of the substrate can influence larval growth and feed utilization. The fine texture of tofu dregs also makes it easier for the worms to chew and swallow, thus increasing mechanical palatability (consumption mechanism).

The lowest consumption of *Tenebrio molitor* larvae occurred in treatment A3B1 (50% Commercial ration + 50% Rice bran with a media thickness of 1 cm). The low larval consumption in this treatment is due to the thin media thickness, which can easily stress the larvae, and to the coarse, sharp texture of the bran, which, due to its high crude fiber content, results in low palatability for *Tenebrio molitor* larvae. Despite its high crude fiber content, palm kernel cake is still better than rice bran because it provides more crude protein and metabolizable energy, supporting growth performance and nutrient utilization efficiency more effectively. This finding is consistent with Serra et al. (2026), who reported that certain feed ingredients, such as rice bran, may reduce larval performance due to their unbalanced nutrient composition.

Body Weight Gain of *Tenebrio molitor* Larvae

The average body weight gain of *Tenebrio molitor* larvae influenced by the composition and thickness of the media can be seen in Table 3.

The highest mean weight gain of *Tenebrio molitor* larvae was observed in treatment A1B3 (50% Commercial ration + 50% Tofu dregs at a media depth of 4 cm), with a value of 80.79 g, and the lowest in treatment A3B1 (50% Commercial ration + 50% Rice bran at a media depth of 1 cm). The results of the variance analysis showed a highly significant interaction ($P<0.01$) between the composition and thickness of the medium, which affected the weight gain of *Tenebrio molitor* larvae. The DMRT test results indicate that the weight gain of *Tenebrio molitor* larvae in treatment A1B3 (50% RK + 50% AT at a media thickness of 4cm) is significantly higher ($P<0.01$) than in other treatments.

The highest weight gain in *Tenebrio molitor* larvae was observed in treatment A1B3 (50% Tofu dregs + 50% Wheat bran media at a depth of 4 cm), with a gain of 80.79 g (0.09 g/larva). This occurred because tofu dregs are high in protein (28%) and energy (2,500 kcal), which supports optimal growth of *Tenebrio molitor* larvae. The high weight gain in treatment A1B3 was due to high ration consumption (90.73 g). The weight gain of *Tenebrio molitor* larvae is highly influenced by larval consumption, as the ration provides the essential energy and nutrients needed for tissue formation. The more optimal the consumption, the higher the potential for daily weight gain. The weight gain in the treatment with a media thickness of 4 cm is considered a thickness that allows for a greater volume of ration per unit area, meaning the worms are freer to roam and eat without high competition.

Table 3. The average body weight gain of *Tenebrio molitor* larvae

Factor A (Media composition)	Factor B (Media thickness)			Mean	SEM
	B1(1cm)	B2(2.5cm)	B3(4cm)		
A1 (50% CR + 50% TD)	68.27^{c}	71.27^{b}	80.79^{a}	73.44	3.78
A2 (50% CR + 50% PKM)	65.20^{d}	68.60^{c}	71.52^{b}	68.44	1.83
A3 (50% CR + 50% RB)	58.70^{e}	65.17^{d}	68.18^{c}	64.02	2.80
Mean	64.06	68.34	73.50	68.63	2.80

Note: ^{abcde}Different superscripts in the same row indicate a highly significant effect ($P<0.01$). CR = Commercial ration, TD = Tofu dregs, PKM = Palm kernel meal, RB = Rice bran

The treatment with the lowest weight gain was A3B1 (50% Commercial ration + 50% Rice bran media at a media thickness of 1 cm), with a gain of 58.70 g (0.06 g/animal). This is because rice bran contains 11–15% crude fiber, which is high enough for *Tenebrio molitor* larvae to require a low-fiber ration for digestive efficiency. The low weight gain is also due to the thin media in treatment A3B1. At a media thickness of 1 cm, the amount of ration is too thin to meet the nutritional needs of the larvae during their growth phase. This increased competition among *Tenebrio molitor* larvae, and most worms did not receive optimal nutrition.

Production of *Tenebrio molitor* Larvae

The average production of *Tenebrio molitor* larvae influenced by the composition and thickness of the media is shown in Table 4.

Table 4. The average production of *Tenebrio molitor* larvae

Factor A (Media composition)	Factor B (Media thickness)			Mean	SEM
	B1(1cm)	B2(2.5cm)	B3(4cm)		
A1 (50% CR + 50% TD)	76.80^{c}	79.83^{b}	90.13^{a}	82.26	4.04
A2 (50% CR + 50% PKM)	73.93^{d}	77.00^{c}	80.12^{b}	77.02	1.78
A3 (50% CR + 50% RB)	67.80^{e}	73.57^{d}	77.31^{c}	72.89	2.77
Mean	72.84	76.80	82.52	77.39	2.86

Note: ^{abcde}Different superscripts in the same row indicate a highly significant effect (P<0.01). CR = Commercial ration, TD = Tofu dregs, PKM = Palm kernel meal, RB = Rice bran

Mean production of *Tenebrio molitor* larvae (g/1000 larvae) influenced by the composition and thickness of the growth medium. The highest mean production of *Tenebrio molitor* larvae was found in treatment A1B3 (50% Commercial ration + 50% Tofu dregs at a medium thickness of 4 cm) at 90.13 g/1000 individuals, and the lowest in treatment A3B1 (50% Commercial ration + 50% Rice bran at a medium thickness of 1 cm) at 67.80 g/1000 larvae. The results of the analysis of variance show a highly significant interaction (P<0.01) between media composition and media thickness, which affects the production of *Tenebrio molitor* larvae. The DMRT test results indicate that the production of *Tenebrio molitor* larvae in treatment A1B3 (50% CR + 50% TD) at a media thickness of 4cm) is significantly higher (P<0.01) than in other treatments.

The high production of *Tenebrio molitor* larvae obtained in treatment A1B3 was 90.13 g/1000 larvae. This is due to the nutritional content of tofu dregs, which is highly effective in meeting nutritional requirements and promoting rapid weight gain in *Tenebrio molitor* larvae. The availability of protein and energy in tofu dregs is believed to trigger the high production of *Tenebrio molitor* larvae. A thick growth medium allows *Tenebrio molitor* larvae to move freely to the bottom of the medium, leading to increased rationing activity and high ration consumption. According to Amran et al. (2021), the fresh production of larvae is influenced by the crude protein in the medium. According to Putri et al. (2024), media that provide sufficient space for movement allow larvae to move more actively, thereby increasing growth.

The lowest production of *Tenebrio molitor* larvae was obtained in treatment A3B1, which was 67.80 g/1000 individuals. The low production of *Tenebrio molitor* larvae is due to the rice bran medium, which contains high levels of crude fiber. According to Purnamasari et al. (2018), the low fresh production of *Tenebrio molitor* larvae on a rice bran medium is due to its high crude fiber content, which inhibits digestion and the absorption of essential nutrients, thereby slowing larval growth.

Chitin Content of *Tenebrio molitor* Larvae

The average chitin content of *Tenebrio molitor* larvae influenced by the composition and thickness of the media can be seen in Table 5.

The highest chitin content in *Tenebrio molitor* larvae is found in treatment A1B3 (50% Commercial ration + 50% Tofu dregs with a media thickness of 4 cm), which is 14.34%, and the lowest chitin content in *Tenebrio*

Table 5. The average chitin content of *Tenebrio molitor* larvae (%)

Factor A (Media composition)	Factor B (Media thickness)			Mean	SEM
	B1 (1 cm)	B2 (2.5 cm)	B3 (4 cm)		
A1(50% CR+50% TD)	8.63^{c}	11.58^{b}	14.34^{a}	11.52	1.65
A2(50% CR+50% PKM)	8.00^{c}	9.10^{c}	12.13^{b}	9.74	1.24
A3(50% CR+50% RB)	5.57^{d}	5.78^{d}	7.51^{c}	6.29	0.62
Mean	7.40	8.82	11.33		1.17

Note: ^{abcd}Different superscripts in the same row indicate a highly significant effect (P<0.01). CR = Commercial ration, TD = Tofu dregs, PKM = Palm kernel meal, RB = Rice bran

molitor larvae is found in treatment A3B1 (50% Commercial ration + 50% Rice bran with a media thickness of 1 cm), which is 5.57%. The results of the analysis of variance showed that there was an interaction between the composition and thickness of the growth medium, which had a highly significant (P<0.01) and different effect on the chitin content of mealworms. The DMRT test results show that the chitin content of *Tenebrio molitor* larvae in treatment A1B3 is significantly higher (P<0.01) than in other treatments.

The high chitin content of *Tenebrio molitor* larvae obtained in treatment A1B3 (50% Commercial ration + 50% Tofu dregs with a thickness of 4 cm), which is 14.34%, is due to the high production of *Tenebrio molitor* larvae from that treatment, which is 90.13 g/500 g of media. The high larval production is because the *Tenebrio molitor* larvae were given a growth medium containing high crude protein, which is 25%, with a media thickness of 4 cm. *Tenebrio molitor* larvae that live on a growth medium with high protein and a thick growth medium can meet their protein needs, resulting in worms with high live weight and larger body size, leading to more exoskeleton formation. The more exoskeletons that form, the higher the chitin content will be, as the exoskeleton is the outer body component of the mealworm. This aligns with the opinion of Kaya et al. (2015), who stated that insect exoskeletons are the primary source of chitin and that the amount of exoskeleton formed is influenced by the insect's body growth. According to Morales et al. (2022), the process of exoskeleton formation and insect growth is influenced by nutrient availability and media depth.

The low chitin content in treatment A3B1 (50% Commercial ration + 50% Rice bran with a thickness of 1 cm), which is 5.57%, and A3B2 (50% Commercial ration + 50% Rice bran with a thickness of 2.5 cm), which is 5.78%, is due to the low larval production in treatment A3B1, which is 67.80 g/500 g of media, and treatment A3B2, which is 73.57 g/500 g of media. The low chitin content in *Tenebrio molitor* larvae under both treatments was also caused by the low protein content in the growth medium, which did not meet the protein needs of the *Tenebrio molitor* larvae. The thin growth medium also made *Tenebrio molitor* larvae more easily stressed, resulting in low body weight and small worm size, which in turn led to reduced exoskeleton formation and lower chitin content. According to Van Broekhoven et al. (2015), a low protein content in the ration inhibits growth and slows exoskeleton formation in Tenebrionidae larvae, resulting in lower body weight. According to Nuraini et al. (2025), media with low protein content results in suboptimal skin replacement in *Tenebrio molitor* caterpillars, leading to low chitin content in the resulting *Tenebrio molitor* larvae.

The chitin content of *Tenebrio molitor* larvae in treatment A1B3 (50% Commercial ration + 50% TD media with a thickness of 4 cm) was 14.34%. The chitin content of *Tenebrio molitor* larvae in this study was higher than that obtained by Nuraini et al. (2025), which showed that the chitin content of *Tenebrio molitor* caterpillars was 8.08%. The results of this study were also higher than the chitin content of German caterpillar larvae (*Zophobas morio*) and mealworm larvae (*Alphitobius diaperinus*). Research by Nuraini et al. (2022a) shows that the chitin content of German mealworms is 12.54%. Research by Soetemans et al. (2020) shows that the chitin content of mealworms is 6.2%.

Crude Protein Content of *Tenebrio molitor* Larvae

The average crude protein content of *Tenebrio molitor* larvae influenced by the composition and thickness of the media can be seen in Table 6.

The highest crude protein content was found in treatment A1B3 (50% Commercial ration + 50% Tofu dregs media with a media thickness of 4 cm), which was 55.64%, and the lowest crude protein content of *Tenebrio molitor* larvae was found in treatment A3B1 (50% commercial ration + 50% Rice bran media with a media thickness of 1 cm), which was 46.80%. The analysis of variance showed an interaction between media composition and growth medium thickness, with a highly significant effect on the crude protein content of *Tenebrio molitor* larvae ($P<0.01$). The DMRT test results indicate that the crude protein content of the *Tenebrio molitor* larvae in treatment A1B3 is significantly higher ($P<0.01$) than in other treatments.

Table 6. The average crude protein content of *Tenebrio molitor* larvae (%)

Factor A (Media composition)	Factor B (Media thickness)			Mean	SEM
	B1 (1 cm)	B2 (2.5 cm)	B3 (4 cm)		
A1(50% CR+50% TD)	50.23^{c}	53.37^{b}	55.64^{a}	53.08	1.57
A2(50% CR+50% PKM)	49.11^{c}	50.18^{c}	53.94^{b}	51.08	1.46
A3(50% CR+50% RB)	46.80^{d}	47.33^{d}	49.31^{c}	47.81	0.76
Mean	48.71	50.29	52.96		1.27

Note: ^{abcd}Different superscripts in the same row indicate a highly significant effect ($P<0.01$). CR = Commercial ration, TD = Tofu dregs, PKM = Palm kernel meal, RB = Rice bran

The high crude protein content obtained in treatment A1B3 (50% Commercial ration + 50% Tofu dregs with a thickness of 4 cm), which is 55.64%, is due to the high protein content of the growth medium, which is 25% with a growth medium thickness of 4 cm. The high protein content of the growth medium can increase protein availability, enabling mealworms to meet their protein needs and resulting in higher crude protein content. In addition, the thickness of the growth medium also affects the crude protein of *Tenebrio molitor* larvae. A growth medium with a thickness of 4 cm provides ample space for mealworms to move actively to the bottom of the medium in search of food, and there is no competition among the worms, so the protein needs of the *Tenebrio molitor* larvae can be met. The thicker the growth medium, the more nutrients available and the higher the crude protein content of the *Tenebrio molitor* larvae produced. According to Nuraini et al. (2022b), the protein content of caterpillars is influenced by the nutritional content of the growth medium. According to Kröncke et al. (2023), optimal medium thickness enables more even nutrient distribution, creates a stable microenvironment, and reduces competition among individuals.

The low crude protein content obtained in treatments A3B1 (46.80%) and A3B2 (47.33%) is due to the low protein content of the growth medium (16%) with thicknesses of 1 cm and 2.5 cm, respectively. The low protein content of the growth medium cannot provide enough protein for the needs of the *Tenebrio molitor* larvae, so the crude protein content of the mealworm will also be low. This indicates that the lower the protein content of the growth medium, the lower the crude protein content of the mealworm produced. Additionally, the thinness of the growth medium also affects the crude protein content of *Tenebrio molitor* larvae. Thin growth media provide limited nutrients and a narrow space, leading to increased competition for nutrients and hindering growth, resulting in caterpillars with low crude protein content. According to Hapsari et al. (2018), the quality and quantity of rations affect the metabolism and growth of caterpillars, including their body protein content. According to Pascacio et al. (2023), limited space in thin media can hinder caterpillar movement and rationing behavior, resulting in slower caterpillar growth and lower crude protein content.

The crude protein content in treatment A1B3 (50% Commercial ration + 50% Tofu dregs media with a thickness of 4 cm) was 55.64%. The results of this study were higher than those reported by Nuraini et al. (2025), who found that the crude protein content of *Tenebrio molitor* larvae fed a 50% Commercial ration + 50% Tofu dregs media was 24.50%.

Nitrogen Retention of *Tenebrio molitor* Larvae

The average nitrogen retention of *Tenebrio molitor* larvae influenced by the composition and thickness of the media can be seen in Table 7.

The highest nitrogen retention in *Tenebrio molitor* larvae was found in treatment A1B3 (50% Commercial ration + 50% Tofu dregs media with a thickness of 4 cm), which was 77.74% DM, and the lowest nitrogen retention in *Tenebrio molitor* larvae was found in treatment A3B1 (50% Commercial ration + 50% Rice bran media with a media thickness of 1 cm), which was 66.41% DM. The results of the analysis of variance show that the interaction between the composition and thickness of the growth medium significantly affects nitrogen retention in *Tenebrio molitor* larvae ($P<0.01$). The DMRT test results show that the nitrogen retention of *Tenebrio molitor* larvae in treatment A1B3 is significantly higher ($P<0.01$) than in other treatments.

The high nitrogen retention obtained in treatment A1B3 (77.74%) is related to the high protein consumption in that treatment (8.34 g/head). High protein consumption is caused by the high crude protein content of *Tenebrio molitor* larvae in treatment A1B3 (55.64%). The high crude protein content of mealworms is due to the high crude protein content of the growth medium (25%) with a thick growth medium (4 cm), as it can provide sufficient protein to meet the needs of mealworms and give them ample space to move, making it easier for the worms to eat, resulting in high protein consumption. The higher the protein content of the growth medium and the more space available, the higher the consumption of *Tenebrio molitor* larvae protein and nitrogen absorption, leading to increased nitrogen retention. According to Nuraini et al. (2022a), the crude protein content of mealworms depends on the crude protein content of the growth media; higher crude protein in the media increases protein consumption and consequently increases nitrogen retention in mealworms.

The low nitrogen retention observed in treatment A3B1 (66.41% DM) is associated with the low protein intake in that treatment (6.97 g/head). Low protein intake is due to the low crude protein content of mealworms in treatment A3B1 (46.80%). The low crude protein content of *Tenebrio molitor* larvae is due to the low protein content of the growth medium in treatment A3B1 (16%) and the thin growth medium (1 cm), which are unable to provide sufficient protein for mealworms to consume and absorb. The thin medium can also hinder the movement of *Tenebrio molitor* larvae, reducing their food consumption. Consequently, the amount of crude protein used and remaining in the mealworm's body is also low, resulting in low nitrogen retention. According to Nuraini et al. (2017), protein quality and protein digestibility affect nitrogen retention; if protein quality is low or an amino acid is lacking, nitrogen retention will also be low.

The best nitrogen retention for *Tenebrio molitor* larvae in this study was achieved with treatment A1B3 (50% Commercial ration +

Table 7. Nitrogen retention of *Tenebrio molitor* larvae (%)

Factor A (Media composition)	Factor B (Media thickness)			Mean	SEM
	B1 (1 cm)	B2 (2.5 cm)	B3 (4 cm)		
A1(50% CR+50% TD	71.00^{c}	75.14^{b}	77.74^{a}	74.63	1.96
A2(50% CR+50% PKM)	68.96^{cd}	69.31^{cd}	74.09^{b}	70.79	1.66
A3(50% CR+50% RB)	66.41^{e}	68.12^{de}	69.50^{cd}	68.01	0.89
Mean	68.79	70.85	73.78		1.45

Note: ^{abcde}Different superscripts in the same row indicate a highly significant effect ($P<0.01$). CR = Commercial ration, TD = Tofu dregs, PKM = Palm kernel meal, RB = Rice bran

50% Tofu dregs medium, 4 cm thick), which was 77.74%. This research result is higher than that reported by Nuraini et al. (2025), which showed that the nitrogen retention of *Tenebrio molitor* larvae fed a medium containing 50% Commercial ration + 50% Tofu dregs was 75.19%.

Crude Fat Digestibility of *Tenebrio molitor* Larvae

The average crude fat digestibility of *Tenebrio molitor* larvae influenced by the composition and thickness of the media can be seen in Table 8.

The highest crude fat digestibility was found in treatment A1B3 (growth media composition 50% Commercial ration + 50% Tofu dregs with a growth media thickness of 4 cm), which was 89.28%, and the lowest crude fat content was found in treatment A3B1 (growth media composition 50% Commercial ration + 50% DP with a growth media thickness of 1 cm), which was 68.40%. The results of the variance analysis showed that there was an interaction between the type of media and the thickness of the growth media, which had a highly significant ($P<0.01$) different effect on the crude fat digestibility of *Tenebrio molitor* larvae. The DMRT test results show that the crude fat digestibility of *Tenebrio molitor* larva in treatment A1B3 is significantly higher ($P<0.01$) than in other treatments.

The high digestibility of crude fat obtained in treatment A1B3, which was 89.97%, is due to the high crude fat content of *Tenebrio molitor* larvae in treatment A1B3, which was 31.14%. The high crude fat content of *Tenebrio molitor* larvae is also related to the high protein content of the growth medium, as a medium with high protein can provide sufficient energy and nutrients for *Tenebrio molitor* larvae growth, resulting in worms with a soft body texture and high fat accumulation. Excess energy during the growth process will be stored in the *Tenebrio molitor* larvae's body in the form of fat. The higher the protein content in the growth medium, the more energy available for metabolism, thus promoting the formation of coarse fat in the larva's body. This aligns with the opinion of Grau-Bové et al. (2020) that when the protein needs of insects are met, energy and fat metabolism proceed efficiently, producing tissue structures and fat compositions that are more easily digested by the poultry digestive system. In addition, the thickness of the 4 cm growth medium affects the digestibility of crude fat, as a thicker medium provides more space for *Tenebrio molitor* larvae to engage in optimal rationing, thereby accelerating growth rates and allowing for maximum accumulation of crude fat.

The low digestibility of crude fat in treatment A3B1 (50% Commercial ration + 50% Rice bran) at 68.40% is related to the low crude fat content of *Tenebrio molitor* larvae, which is 20.16%. The low crude fat content is also caused by the low protein content of the growth medium. A medium with low protein provides limited nutrients and energy, making it unable to meet mealworm growth needs optimally, resulting in slow growth, a rough body texture, and a low crude fat content. Therefore, the low crude fat content in the larvae's body will result in a low crude fat digestibility rate. Additionally, the 1 cm media thickness also acts as a limiting factor, as thin media restricts larvae movement and increases environmental stress, thereby hindering metabolic efficiency and nutrient absorption, which in turn reduces fat accumulation in the larvae's body and lowers the digestibility of crude fat.

Table 8. The average crude fat digestibility (%dm) of *Tenebrio molitor* larvae

Factor A (Media composition)	Factor B (Media thickness)			Mean	SEM
	B1 (1 cm)	B2 (2.5 cm)	B3 (4 cm)		
A1 (50% CR+50% TD)	75.02^{d}	85.82^{b}	89.28^{a}	83.73	4.29
A2 (50% CR+50% PKM)	71.15^{e}	80.05^{c}	87.76^{a}	79.66	4.80
A3 (50% CR+50% RB)	68.40^{f}	73.56^{d}	80.41^{c}	74.12	3.48
Mean	71.53	79.81	85.82		4.19

Note: ^{abcdef}Different superscripts in the same row indicate a highly significant effect ($P<0.01$). CR = Commercial ration, TD = Tofu dregs, PKM = Palm kernel meal, RB = Rice bran

The digestibility of crude fat in selected mealworms was highest in treatment A1B3 (50% Commercial ration + 50% Tofu dregs, 4 cm thick), at 89,28%. This research result is lower than that of Nuraini et al. (2025), who obtained a crude fat digestibility of 90.41% for *Tenebrio molitor* larvae fed a medium of 50% Commercial ration and 50% Tofu dregs.

CONCLUSIONS

This study concludes that a mixed media composition of 50% commercial ration and 50% tofu dregs with a thickness of 4 cm is the selected growth medium for mealworms, resulting in a ration consumption, body weight gain, production, chitin content, a crude protein content of 55.64% DM, a nitrogen retention protein quality, and crude fat digestibility.

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