

Research Article



Medium for Cultivation *Tenebrio molitor* Larvae and its Effect as an Alternative Animal Protein Source in the Diet on Performance of Broiler

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Abstract | *Tenebrio molitor* larvae are an alternative feed option for protein sources. This research includes 2 phases. Phase 1. Effect of different medium compositions on the production and nutritional content of *Tenebrio molitor* larvae. Phase 2. Effect of *Tenebrio molitor* larvae in the diet on broiler performance. Phase 1 used a completely randomized experimental design (CRD) with six treatments and three replications. The treatment variations were treatments A (100% Concentrate), B (100% Chicken Manure), C (50% Concentrate + 50% Rice Bran), D (50% Concentrate + 50% Chicken Manure), E (50% Concentrate + 50% Tofu Dregs) and F (50% Concentrate + 50% Coconut Dregs). The variables observed were crude protein content (%), fat content (%), and larval production (g). The research method in phase 2 used a completely randomized design (CRD) consisting of 5 treatments and four replications. The treatments were 0%, 2.5%, 5%, 7.5%, and 10% of *Tenebrio molitor* larvae in the diet. The variables observed were feed consumption, average daily weight gain, feed conversion, carcass percentage, abdominal fat percentage, and taste of meat carcass. Phase 1 demonstrated that different substrate compositions had significant effects ($P < 0.01$) on crude protein, fat, and larvae production. The conclusion of phase 1 was that treatment E (50% concentrate + 50% Tofu dregs) was an effective treatment and obtained protein content (71.13%), fat content (17.60%), and production of larvae (105.12 g). Phase 2 showed that the use of 12% *Tenebrio molitor* larvae (substituted 100% fish meal) could maintain the performance of broilers.

Keywords | Medium fermentation, Natura, *Tenebrio molitor* larvae, Nutrient content, Broiler performance

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INTRODUCTION

Broilers are purebred chickens that can proliferate to produce meat relatively quickly (5-7 weeks). Broilers have an essential role as a source of animal protein from livestock. Their maintenance is influenced by several factors so that the livestock produced is of good quality. One of the factors that can improve the quality of broiler livestock is the animal feed provided.

The supply of conventional feed ingredients still depends

on imported fish meals, causing high prices. Indonesia has abundant fishery resources. However, another problem related to local Indonesian fish meal production is that the quality does not meet the quality requirements of fish meals for animal feed ingredients. For this reason, efforts have been made to find alternative feed ingredients for protein sources such as *Tenebrio molitor* larvae.

The cultivation of *Tenebrio molitor* larvae is one of the community's most promising potential businesses. *Tenebrio molitor* larvae cultivation is easy to do and requires little

cost and has promising business opportunities because the demand for *Tenebrio molitor* larvae is increasing. According to [Dabbou et al. \(2019\)](#), birds, fish, and poultry can be fed *Tenebrio molitor* larvae, and the *Tenebrio molitor* larvae farming business improves quantity and quality. Economically, *Tenebrio molitor* larvae have value and benefits because they can be cultivated and traded as a source of poultry, fish, and reptile feed. According to [Mariod \(2020\)](#), the nutritional content of *Tenebrio molitor* larvae includes 53% crude protein, 28% crude fat, 6% crude fiber, and 5% moisture, along with complete and high amino acid contents (arginine 12.95%, alanine 25.68%, proline 16.94%, lysine 10.65% and, taurine 17.53%), fatty acids (linoleic 0.70% and linolenic 2, 24%) and minerals (calcium 55.65%, sodium 13.71%, potassium 10.00%, and magnesium 3.50%). According to [Nuraini et al. \(2021\)](#), *Tenebrio molitor* larvae have crude protein 59.97%, glutamic acid 6.86%, aspartic acid 4.80%, leucine 4.49%, lysine 4.75%, tyrosine 3.04%, valine 3.83%, glycine 3.40%, and methionine 0.43%.

The protein content of *Tenebrio molitor* larvae depends on the nutritional content of the culture medium. The culture media commonly used are concentrates. According to [Hartingsih dan Sari \(2014\)](#), farmers still use concentrate media to fatten *Tenebrio molitor* larvae, but agricultural waste is recommended to reduce costs. The concentrate can be combined with less expensive agricultural waste. Therefore, this study used a combination of concentrate and scrap in chicken manure, rice bran, tofu dregs, and coconut dregs.

The chicken concentrate is semifinished chicken feed that contains animal and plant proteins and is low in carbohydrates and fiber. According to [Pratiwi et al. \(2018\)](#), chicken concentrate's nutritional content is 35% crude protein, 8.3% fat, 3% crude fiber, 10% calcium, 1.1% phosphorus, and ME 3300 kcal/kg.

Chicken manure is used as a medium for *Tenebrio molitor* larvae. According to [Helda \(2019\)](#), chicken manure contains proteins ranging from 9.65-11.62%, dry matter 91.75-94.04%, fat approximately 3.67-6.16%, metabolic energy 1100 kcal/kg, and 14.13-17.89% crude fiber. According to [Azizah et al. \(2019\)](#), the use of chicken manure media at a dose of 25 mg/larvae/day can increase the body length of *Tenebrio molitor* larvae by 0.861 cm.

Rice bran, a by-product of rice milling, can also be used as a medium for *Tenebrio molitor* larvae. According to [Setiawan \(2017\)](#), rice bran has a nutritional content of dry matter 86.5%, ash 8.7%, crude protein 10.8%, crude fiber 11.5%, fat 5.1%, extract material without nitrogen (BETN) 50.4%, 0.2% calcium and 2.5% phosphorus.

Tofu dregs can also be used as a medium for *Tenebrio molitor*

larvae to grow. Tofu dregs are waste from the tofu processing industry used as animal feed. The nutritional content of tofu waste is 25.91% protein, 2.71% fat, and 5.97% ash ([Raharjo et al., 2016](#)).

According to research by [Hapsari et al. \(2017\)](#), a rearing medium consisting of a mixture of 50% tofu dregs and 50% rice bran is better used to maintain *Tenebrio molitor* larvae. It can increase the percentage of larvae and reduce mortality. According to [Purnamasari et al. \(2020\)](#), using a mixture of concentrate and tofu waste (1:1) can increase the growth and production of *Tenebrio molitor* larvae.

Coconut dregs are used as a medium for *Tenebrio molitor* larvae. Coconut dregs are waste from coconut that has separated from coconut milk. To date, coconut dregs are thrown away and are not typically used for anything. According to [Irya \(2018\)](#), the nutritional content of coconut pulp is 5.81% crude protein, 20.84% crude fiber, 24.59% crude fat, 0.05% Ca, and 0.02% P. According to [Elyana \(2011\)](#), to increase the digestibility of coconut dregs, they must be processed through fermentation.

Culture media derived from agricultural products and animal husbandry waste are of low quality (low protein content), especially coconut dregs. Media made of coconut dregs, tofu dregs, and chicken manure have high water content, so they quickly rot, inconveniencing farmers when cultivating *Tenebrio molitor* larvae. Efforts have been undertaken to improve the quality of the media from which the waste originates (i.e., increase the protein content and reduce the odor) by fermentation. To reduce the odor caused by manure waste, [Purnamasari et al. \(2020\)](#) added EM4 to livestock drinking water and sprayed EM4 in the area around the cage to reduce odors and kill pathogenic bacteria in the feces. According to [Nuraini et al. \(2015\)](#) and [Sharma et al. \(2020\)](#), fermentation is a natural way of improving vitamins, essential amino acids, anti-nutrients, proteins, food appearance, flavors, and enhanced aroma.

Fermentation uses microorganisms with Natura Organic Decomposer, which contains many enzymes, including protease, cellulase, xylanase, beta-glucanase, pectinase, amylase, lipase, and phytase enzymes, and contain probiotic *Bacillus* sp. 5.5 x cfu/g, *Lactobacillus* sp. 4.7 x cfu/g, *Acetobacter* sp. 5.9 x cfu/g, *Streptomyces* sp. 4.4 x cfu/g, *Aspergillus* sp. 3.9 x propagule/g, *Saccharomyces* sp. 5.3 x propagule/g, and *Trichoderma* sp. 3.6 x propagule/g ([Natura BioResearch, 2013](#)).

The results of the fermentation study with Natura Organic Decomposer showed an increase in the protein content and quality of the waste material. According to [Burhan \(2016\)](#), using Natura Organic Decomposer at a dose of 3%

with an incubation period of 7 days is more efficient for improving the protein quality of pineapple skin, increasing the crude protein by 33.72%.

After fermenting media for the growth of *Tenebrio molitor* larvae, Liu Changqi et al. (2020) stated that the different types of feed given showed differences in growth, yield, fresh weight, and length of *Tenebrio molitor* larvae. *Tenebrio molitor* larvae with teak leaf media fermented with microorganisms in EM4 experienced tremendous growth because the feed with EM4 fermentation had a high protein content, resulting in high protein production in *Tenebrio molitor* larvae.

The use of *Tenebrio molitor* larvae as a substitute for fish meal in broiler rations is not expected to have adverse effects on broiler carcass performance. Therefore, the present study evaluated feeding TML as a substitute for broiler production performance and meat quality.

MATERIAL AND METHODS

PHASE 1. EFFECTS OF DIFFERENT MEDIUM COMPOSITIONS ON THE PRODUCTION AND NUTRITIONAL CONTENT OF TENEBRIO MOLITOR

The materials used in this study were rearing media which consisted of concentrate Bravo 311 (from PT Cahroen Phokphan), chicken manure, rice bran, tofu drugs, and coconut pulp. Microorganisms used are microorganisms contained in Natura Organic Decomposers from poultry shop. *Tenebrio molitor* larvae are obtained from *Tenebrio molitor* or Hong Kong caterpillar farms. The media concentrate obtained from poultry shop, chicken manure obtained from chicken farms, tofu pulp obtained from tofu manufacture, and coconut pulp obtained from coconut milk sales around the Unand campus. Chemicals to analyze protein and fat content. This research uses tools such as analytical balance, laminar flow, oven, autoclave, a set of equipment to analyze crude protein and crude fat.

FERMENTATION MEDIA

The media were prepared as follows:

A = 100% concentrate

B = 100% chicken manure

C = 50% concentrate + 50% fermented rice bran

D = 50% concentrate + 50% fermented chicken manure

E = 50% concentrate + 50% fermented tofu waste

F = 50% concentrate + 50% fermented coconut waste

Rice bran and concentrate were prepared. Chicken manure, tofu waste, and coconut waste were dried in the sun. Each treatment contained 500 grams of culture medium. In the C, D, E, and F treatments added 200 ml of distilled water. Then, 2% Natura Organic Decomposer was mixed into media C, D, E, and F and stirred evenly. After that,

the media were covered with plastic, tied, and incubated for four days. After four days, the media were aerated for 24 hours in a shady, open place.

The fermentation medium was used from P3 to P6. The fermentation media used were P3=50% concentrate + 50% rice bran, P4=50% concentrate + 50% chicken manure, P5=50% concentrate + 50% tofu drugs, P6=50% concentrate + 50% coconut pulp. Dry media used as much as 1000 g. Each dry media mixture was added 800 ml of water and then homogenized. Furthermore, the media was sterilized using an autoclave (121°C) for \pm 15 minutes, then cooled down to room temperature (25-27°C). After that, it was inoculated with 1% Natura Organic Decomposer and incubated for four days. Then the fermented products are dried and ground. The fermented product is ready to be used as a medium for rearing *Tenebrio molitor* larvae. The procedure for fermenting media can be seen in Figure 5. The nutritional content of the rearing media before and after fermentation with Natura Organic Decomposers can be seen in Table 3.

TENEBRIO MOLITOR CARE AND CULTIVATION

One hundred grams of *Tenebrio molitor* larvae aged ten days was placed in the media, and fresh young papaya with a size of 2 x 2 x 0.5 cm was added to the drinking water and placed at 9 points above the media. Young papaya that had been dried was taken and replaced with a new papaya every two days. On the 15th day, the skin of the *Tenebrio molitor* larvae changed, and the skin was split from the media. *Tenebrio molitor* larvae were kept until the age of 30 days. *Tenebrio molitor* larvae were harvested on the 30th day after the eggs hatched. *Tenebrio molitor* larvae were harvested by sieving to separate the *Tenebrio molitor* larvae from the media and then weighed to obtain the fresh weight. After that, the *Tenebrio molitor* larvae were boiled to kill the larvae and dried in the sun for one day (until dry). The dried *Tenebrio molitor* larvae were weighed to obtain their dry weight.

VARIABLES

The parameters measured were crude protein content (%), crude fat content (%), and production of *Tenebrio molitor* larvae (g).

DATA ANALYSIS

The data obtained were analyzed by one-way analysis of variance in a completely randomized design (CRD). The Duncan multiple range tests determined significant differences between treatments, with $P < 0.05$ considered significantly different.

PHASE 2. EFFECT OF THE APPLICATION OF TENEBRIO MOLITOR LARVAE IN THE DIET ON BROILER

PERFORMANCE

BIRDS AND HOUSING

Ethics study of experimental animals guideline, according to the law number 18 of the Republic of Indonesia (2009) about Animal livestock and animal husbandry. The livestock used were 100 broiler chickens aged two days strain Arbor Acres (AA) CP-707 without sex separation produced by PT. Charoen Pokphand Jaya Farm Medan. The experimental cage used in this study was a 20-box cage with a wire floor. Five chickens per cage unit occupied each box (1 m x 1 m x 1 m). The equipment used included a place to eat (feeder), a drink (drinker), digital scales to weigh the chickens and rations, and black plastic sheets to accommodate chicken feces. For heating and lighting in chickens, a 60-watt incandescent lamp is paired until the chickens are two weeks old.

RATIONS

The ration used in this study was made using feed ingredients in corn meal, soybean meal, fish meal, *Tenebrio molitor* larvae, rice bran, coconut oil, and top mix. The feed ingredients, the content of food substances, and the metabolic energy of the ration components (as feed) are listed in Table 1.

EXPERIMENTAL DESIGN

A experimental method used with a completely randomized design (CRD) of five treatments rations with different *Tenebrio molitor* meal levels and four replications. The treatments were feed containing *Tenebrio molitor* at 0%, 3%, 6%, 9%, and 12%. We prepared iso proteins and iso energy with a crude protein content of 22% and metabolic energy of 3200 kcal/kg. For tropical countries, metabolic energy can be reduced by 200 cal/kg so that it becomes 3000 kcal/kg as recommended by Scott et al. (1982). The composition of the research diet is shown in Table 2. The content of food substances (%) and the study's metabolic energy (kcal/kg) are shown in Table 3.

RESEARCH IMPLEMENTATION

The ingredients for the ration used consisted of: ground corn, fish meal, soybean meal, TML, rice bran, coconut oil, and top mix. The material is weighed according to each ration and then stirred evenly. The cages were cleaned, chalked, and sprayed with disinfectant, as well as the feed and drinking areas were cleaned and sterilized using hot water. Each cage unit is provided with a 60-watt incandescent lamp, a feed, and a drinking place.

The treatment started when the broiler was one week old until five weeks. At 2-7 days old, broilers were given Bravo 311 rations. At the age of 8 days, research treatment was given. The rations are given three times a day, in the morning (7.00 am), noon, and evening (5.00 pm). Drinking wa-

ter is given continuously (*ad libitum*). Every morning, the cage mats in the form of newspapers are replaced until they are two weeks old. After that, the plastic mats are cleaned of dirt, and the eating and drinking places are cleaned every day. The rest of the rations were collected and weighed daily. The bodyweight weight every week. After the chickens were five weeks old, one chicken was taken from each cage unit close to the average weight for slaughter to obtain live weight and carcass percentage.

OBSERVED VARIABLES

Broiler performance: Feed consumption (g/head/day) was calculated based on the weekly consumption rates. The initial ration minus the remaining is divided by duration (seven days). Average daily weight gain (g/head/day) was calculated: the initial weights minus the final weight are then divided by seven days to gain weight per head per day. Feed conversions were calculated by comparing feed intake per chick per day divided by weight gain per head per day.

Broiler Carcass: Live weights or final weights (g/head) were estimated at five weeks. Broiler from each trial unit was weighed for their life weight (g/head). The percentage of the carcass was presented as the ratio between carcass weight and live weight times 100%. The ratio between abdominal fat weight with live weight multiplied by 100% was abdominal fat.

DATA ANALYSIS

All data obtained were processed statistically by analysis of variance. The difference between the treatments was tested with the multiple range test (DMRT) according to Steel and Torrie (1995).

RESULTS

PHASE 1. EFFECTS OF TREATMENTS ON CRUDE PROTEIN, CRUDE FIBER, NITROGEN RETENTION, AND FIBER DIGESTIBILITY

EFFECT OF MEDIUM ON THE CRUDE PROTEIN CONTENT OF *TENEBRIO MOLITOR* LARVAE

The medium type (Table 5) had a highly significant impact ($P < 0.01$) on the crude protein content of *Tenebrio molitor* larvae. The crude protein of *Tenebrio molitor* larvae was high in treatment E (50% C + 50% TD), at 71.13%, and low in treatment B (100% CM), at 31.40%.

EFFECT OF TREATMENT ON THE CRUDE FAT CONTENT OF *TENEBRIO MOLITOR* LARVAE

The effect of the medium type had a highly significant impact ($P < 0.01$) on the crude fat content of *Tenebrio molitor* larvae. The lowest crude fat of *Tenebrio molitor* larvae was found in treatment B (100% CM), at 6.02%, and the

Table 1: Feed ingredients, nutrient contents (%), and metabolic energy levels (kcal/kg) of feed ingredients that make up the rations (as feed) ^a.

Feed Ingredients	Crude Protein (%)	Fat (%)	Crude Fiber (%)	Ca (%)	P available (%)	ME. (Kcal) ^b
Ground corn	9.58	2.66	3.50	0.38	0.19	3300
Soybean meal	43.76	2.49	4.50	0.63	0.36	2240
Fish flour	64,00	2.85	3.45	3.10	1.89	2540
<i>T. molitor</i> larvae	62.35	14.96	6.18	0.19	0.82	3362 ^a
Rice bran	12.34	5.09	14.50	0.69	0.26	1630
Coconut oil	-	10.00 ^b	-	-	-	8600
Bone flour	-	-	-	24.00	12.00	-
Top mix	-	-	-	0.06 ^c	-	-

Information: a: Nuraini et al. (2021); b: Scott et al. (1982); c: Product Packaging Labels of PT. Medion

Table 2: Research ration composition (%)

Ingredients Feed	Treatment				
	A	B	C	D	E
Ground corn	55.00	55.00	55.00	55.00	55.00
Soybean meal	18.25	18.25	18.50	18.50	18.50
Fish flour	12.00	9.00	6.00	3.00	0.00
<i>Tenebrio molitor</i> larvae	0.00	3.00	6.00	9.00	12.00
Rice bran	9.25	9.25	9.25	9.50	9.75
Coconut oil	3.75	3.50	3.25	3.00	2.75
Bone flour	1.25	1.50	1.75	2.00	2.00
Top mix	0.50	0.50	0.50	0.50	0.50
Total	100.00	100.00	100.00	100.00	100.00

Table 3: Nutrient contents (%) and metabolic energy levels (kcal/kg) of research rations.

Nutrient content (%) and metabolic energy (kcal/kg)	Treatment				
	A	B	C	D	E
Protein	21.34	21.30	21.34	21.30	21.33
Fat	6.37	6.42	6.47	6.52	6.57
Fiber	5.66	5.73	5.77	5.84	5.88
Ca	1.03	1.01	1.00	0.99	0.98
P available	0.55	0.57	0.59	0.58	0.60
ME	2902.50	2901.57	2902.17	2901.25	2901.85

Description: Calculated based on Tables 1 and 2

Table 4: Nutrient content of medium before and after fermentation

Treatment	Crude Protein (%DM)		Fat (%DM)	
	Before	After	Before	After
P1	23.94	-	8.13	-
P2	12.16	-	4.04	-
P3	16.72	19.97	6.77	4.34
P4	17.87	22.92	7.03	5.82
P5	26.81	39.41	9.50	7.42
P6	13.68	18.57	16.49	7.69

Table 5: Contents of crude protein, crude fat, and production of *Tenebrio molitor* larvae in different media

Treatment (Medium)	Crude Protein (% DM)	Crude Fat (%DM)	Production <i>Tenebrio molitor</i> (g)
A (100% C)	48.31 ^b	16.15 ^b	97.47 ^b
B (100% CM)	31.40 ^f	6.02 ^d	11.21 ^f
C (50% C + 50% RB) Fermentation	43.68 ^d	13.24 ^c	74.88 ^d
D (50% C + 50% CM) Fermentation	45.08 ^c	15.11 ^b	88.65 ^c
E (50% C+ 50% TD) Fermentation	71.13 ^a	17.60 ^b	105.12 ^a
F(50% C + 50% CD) Fermentation	40.26 ^e	26.20 ^a	67.96 ^e
Standard Error	0.47	1.67	0.74

Description: * = very significantly different effect (P<0.01)

C= Concentrate; CM = Chicken Manure; RB = Rice Bran; TD = Tofu Dregs; CD= Coconut Dregs

Table 6: Amino acid contents of *Tenebrio molitor* larvae

No	Amino Acid Profile	%
1	Aspartic Acid	4.80
2	Threonine	2.04
3	Serine	2.46
4	Glutamate	6.86
5	Glycine	3.40
6	Valine	3.83
7	Methionine	0.43
8	Ileucine	2.69
9	Leucine	4.49
10	Tyrosine	3.04
11	Phenylalanine	2.21
12	Histidine	1.59
13	Lysine	4.75
14	Arginine	2.91

Source: Nuraini et al. (2021)

Table 7: Feed consumption, average daily weight gain, and feed conversion of broilers (age five weeks) affected by the use of TML in the feed ^{ns}

Treatment	Feed Consumption (g/head/day) ^{ns}	Average Daily Weight Gain (g/head/day) ^{ns}	Feed Conversion ^{ns}
A (0% TML)	60.00±0.21 10.468.980.45 ^a	35.38±0.42	1.69±0.02
B (3% TML)	59.06±0.21	35.56±0.25	1.66±0.04
C (6% TML)	59.19±0.25	36.00±0.24	1.64±0.03
D (9% TML)	60.04±0.61	35.50±0.21	1.69±0.03
E (12% TML)	59.88±0.24	36.56±1.01	1.64±0.03

Information: ns= nonsignificant

Table 8: Percentage of the carcass, percentage of abdominal fat, and carcass meat taste of broilers (age five weeks) fed TML in the diet ^{ns}

Treatment	Percentage of the Carcass	Abdominal Fat Percentage	Taste of Carcass Meat
A (0% TML)	71.21±0.02	1.86±0.04	3.06±0.04 ^b
B (3% TML)	71.95±0.01	1.85±0.01	3.19±0.01 ^b
C (6% TML)	72.33 ±0.04	1.91±0.02	3.91±0.02 ^b
D (9% TML)	71.99±0.03	1.95±0.03	4.15±0.03 ^b
E (12% TML)	72.44±0.04	1.93±0.04	5.43±0.04 ^a

Information: Different superscripts in the same column indicate that the treatment affected was highly significant (P<0.01).

highest was found in treatment F (50% concentrate + 50% Coconut Dregs), at 26.20%.

EFFECT OF TREATMENT ON THE PRODUCTION OF *TENEBRIO MOLITOR* LARVAE

The treatment had a significant effect ($P < 0.05$) on the production of *Tenebrio molitor* larvae. The production of *Tenebrio molitor* larvae in treatment E (50% C + 50% TD) was high, at 105.12 g. Treatment B's lowest production was found (100% CM) at 11.21 g.

PHASE 2. EFFECT OF *TENEBRIO MOLITOR* LARVAE UTILIZATION ON THE PERFORMANCE OF BROILERS

The influence of *Tenebrio molitor* larvae in the diet on feed consumption, average daily weight gain, and feed conversion are shown in Table 7. The statistical analysis results showed that the use of *Tenebrio molitor* larvae in the diet was not significantly affected ($P > 0.05$) by the feed consumption, average daily weight gain, or feed conversion of broilers.

EFFECT OF THE TREATMENT ON FEED CONSUMPTION

The inclusion of TML in the diet was not significantly affected ($P > 0.05$) by the feed consumption of broilers. Feed consumption in the 0% TML treatment was 60.00 g/hen/day, and increasing utilization of TML until 12% (substituted 100% fish meal) resulted in similar feed consumption (59.88 g/hen/day).

EFFECT OF THE TREATMENT ON AVERAGE DAILY WEIGHT GAIN

TMl in the Diet had no significant influence ($P > 0.05$). In the control treatment (0% TML), the average daily weight gain was 35.38 g/hen/day and increasing the utilization of TML to 12% resulted in a similar effect on the average daily weight gain (36.56 g/hen/day).

EFFECT OF THE TREATMENT ON FEED CONVERSION

The feed conversion ratio of broilers was not significantly affected ($P > 0.05$) by the inclusion of TML in the diet. The feed conversion with the 0% TML treatment (1.69) was not different from that with the 12% TML treatment (1.64).

The influence of *Tenebrio molitor* larvae in the diet on the carcass percentage and abdominal fat rate is shown in Table 8. The *Tenebrio molitor* larvae in the broiler diet were not affected ($P > 0.05$) by the carcass percentage, abdominal fat percentage, or taste of the broiler meat.

EFFECT OF THE TREATMENT ON CARCASS PERCENTAGE

The carcass percentage of broilers was not significantly affected ($P > 0.05$) by supplementation with TML in the diet. The carcass percentage in the 0% TML treatment

(71.21%) was similar to that in the 12% TML (72.44%).

EFFECT OF THE TREATMENT ON FAT ABDOMINAL PERCENTAGE

The abdominal fat percentage of broilers was not significantly affected ($P > 0.05$) by inclusion with TML in the diet. The abdominal fat rate in the control treatment (1.86%) was similar to that in the 12% TML (1.93%).

EFFECT OF THE TREATMENT ON THE TASTE OF THE CARCASS MEAT

The taste of carcass meat of broilers was highly significantly affected ($P > 0.05$) by supplementation with TML in the Diet. The taste of broiler meat with the control treatment (3.06) was lower than in the 12% TML group (5.43).

DISCUSSION

PHASE 1. EFFECTS OF THE TREATMENT ON CRUDE PROTEIN, CRUDE FIBER, NITROGEN RETENTION, AND FIBER DIGESTIBILITY

EFFECT OF TREATMENT ON THE CRUDE PROTEIN CONTENT OF *TENEBRIO MOLITOR* LARVAE

The crude protein content of *Tenebrio molitor* larvae in treatment E (50% concentrate + 50% Tofu dreg fermented media) was 71.13%, which was higher than in the other treatments due to the high protein content in the medium. After fermentation with the microorganisms in Natura Organic Decomposer, the crude protein content of treatment E medium increased by 28.41% (from 23.51% before fermentation to 30.19% after fermentation). The increase in crude protein in the concentrate and tofu dreg medium fermented with the Natura microorganisms was related to the number of microorganisms that grew more with a whole colony of 12×10^{15} CFU/g compared to other treatments. Gold et al. (2020) found that the metabolism of microorganisms in larval media could convert proteins and various nutrients (organic matter) to increase the nutritional value of the medium.

The addition of microorganisms through the fermentation process can also increase the protein value in a growth medium. The number of microorganisms significantly affects the increase in protein in the ever-increasing larval media. In addition, the increase in crude protein in the media is due to protease enzymes that can remodel proteins into amino acids. Yiin Wong et al. (2020) stated that microbes reuse amino acids to form body proteins, thus donating crude protein to the substrate by 30-40%. Some of the microorganisms found in Natura can produce protease enzymes, namely, *Bacillus* sp. (Morikawa, 2006), *Lactobacillus* sp. (Putranto, 2007), *Saccharomyces* sp. (Ahmad, 2005), and *Aspergillus* sp. (Preetha, 2012).

According to [Natura Bioresearch \(2013\)](#), Natura Organic Decomposer contains several enzymes: protease, cellulase, xylanase, beta-glucanase, pectinase, amylase, lipase, and phytase. In addition, the Natura Organic Decomposer contains several microorganisms, namely, *Bacillus* sp. 5.5×10^8 cfu/g, *Lactobacillus* sp. 4.7×10^8 cfu/g, *Acetobacter* sp. 5.9×10^8 cfu/g, *Streptomyces* sp. 4.4×10^8 cfu/g, *Aspergillus* sp. 3.9×10^8 propagules/g, *Saccharomyces* sp. 5.3×10^8 propagules/g, *Trichoderma* sp. 3.6×10^8 propagules/g, and *Bifidobacterium* sp. 2.1×10^8 cfu/g. In addition, the number of microorganisms that grow and develop during the fermentation process affects the crude protein in the media. Treatment B had the lowest crude protein from *Tenebrio molitor* larvae. This result was related to the protein concentration in the medium (CP 31.40%) being lower than the others because chicken manure and unfermented media were used in this treatment. The crude protein from *Tenebrio molitor* larvae from concentrate and tofu dreg culture media fermented with microorganisms in Natura was 71.13% DM. This result was higher than that of [Ooninx et al. \(2015\)](#), who obtained 50.58% crude protein from *Tenebrio molitor* larvae using 60% spent grains+20% beer yeast+20% cookie. [Zhao et al. \(2016\)](#) reported using sorghum and sorghum brain medium to obtain 51.5% DM crude protein from *Tenebrio molitor* larvae.

EFFECT OF TREATMENT ON THE CRUDE FAT CONTENT OF *TENEBRIO MOLITOR* LARVAE

The crude fat content of *Tenebrio molitor* larvae in treatment F (Concentrate + coconut pulp fermented medium) was high, at 26.20%, which was related to the concentrate + coconut pulp fermented medium having a high-fat content of 16.50% compared to fat in other media treatments. According to [Aldi et al. \(2018\)](#), different media can affect the fat content of larvae. The higher the fat content of the medium, the higher the fat content of the larvae.

The crude fat of *Tenebrio molitor* larvae in treatment B (chicken manure media) was low, at 6.02%, due to the lower fat content in this medium. The fat content of the chicken manure medium in the P2 treatment was 5%. According to [Lestari et al. \(2020\)](#), the nutrient content in the medium significantly affects the nutritional content of larvae because it can provide sufficient nutrients for larval growth, among which are the protein and fat contents in the larvae.

PRODUCTION OF *TENEBRIO MOLITOR* LARVAE

The *Tenebrio molitor* larvae weight in treatment E (50% Concentrate+50% tofu dregs medium fermented with Natura microorganisms) was highest, at 105.12 g. The increase of *Tenebrio molitor* larvae weight was due to the higher crude protein content of the fermentation medium, which was 30.19% compared to other treatments. The high

protein content of the fermentation medium can meet the protein needs of *Tenebrio molitor* larvae, resulting in the production of *Tenebrio molitor* larvae with a higher weight. According to [Made \(2020\)](#), the activity of microorganisms in a fermentation medium can increase growth. According to [Kinasih \(2018\)](#), the nutritional needs of *Tenebrio molitor* larvae are obtained from materials rich in protein and carbohydrates to produce good larval development.

Before fermentation, the crude protein content of the concentrate and tofu waste medium ranged from 26.81% to 39.41%, and several Natura microorganisms grew and developed with full colony sizes of 6×10^{15} CFU/g; as a result, the enzyme activity also increased. Natura is a commercial product that contains many enzymes, including protease enzymes and many microorganisms, including protease enzyme producers. The protease enzyme-producing microorganisms present in Natura are *Bacillus* sp. ([Morikawa, 2006](#)), *Lactobacillus* sp. ([Putranto, 2007](#)), *Saccharomyces* sp. ([Ahmad, 2005](#)), and *Aspergillus* sp. ([Preetha, 2012](#)). Protease enzymes can remodel proteins into amino acids. Then, microbes reused amino acids to form body proteins, thus donating crude protein to the substrate because microorganisms contain high protein levels, namely, 30-40% ([Krishna and Devi, 2005](#)). Supported by research by [Raharjo and Arief \(2016\)](#), the use of tofu dregs and chicken manure fermented with microorganisms in EM4 can increase the protein content of the medium to increase the growth of higher *Tenebrio molitor* larvae.

The production of *Tenebrio molitor* larvae in the treatment B medium was low, at 11.21 g. This result is related to the crude protein in the P2 treatment (chicken manure medium was also standard, at 12.16%).

The media were then analyzed to determine their amino acid, calcium, phosphorus, and energy metabolism profile. The amino acid contents of the selected fermentation medium (concentrated and tofu dreg mixture fermented with microorganisms in Natura organic decomposers) are shown in [Table 5](#).

The nutritional content of *Tenebrio molitor* larvae with cultivar medium 50% concentrate + 50% tofu dregs fermented with microorganisms in Natura Organic Decomposer included crude protein 71.13% DM, fat 17.60% DM, fiber 6.18% DM, calcium 0.19%, phosphorus 0.82%, and metabolic energy 3362.98 kcal/kg ([Nuraini et al., 2021](#)).

PHASE 2. EFFECT OF *TENEBRIO MOLITOR* LARVAE ON THE PERFORMANCE OF BROILERS

The feed consumption levels in treatments B, C, D, and E using TML in the diet were similar to those in treatment A (0% TML). It is shown that 12% TML is palatable as

a substitute for 100% fish meal in the broiler diet because treatment E with 12% TML (0% fish meal) has the same feed consumption as treatment A (100% fish meal). TML, as a source of animal protein, has high amino acid contents, especially glutamic acid, lysine, tyrosine, and glycine (Table 5), and could replace fish meals. The feed consumption of broilers with TML in this study ranged from 70-71.88 g/hen/day. This result research was lower than that reported by Aljubori et al. (2017), with a feed consumption of 72.96 g/hen/day when broilers were fed 10% fermented canola, but higher than that reported by Djulardi et al. (2018), with a feed consumption of 68.91 g/bird/day when broilers were fed 20% palm oil sludge fermented with *Lentinus edodes*.

TML up to a level of 12%, which substituted 100% fish meal, had the same quality as treatment A (100% fish meal), with the same average daily weight gain. To achieve maximum body weight gain, paying attention to feed quality is necessary. The feed must contain nutrients in sufficient and balanced conditions to support optimal growth. The nutrient content and feed intake affect body weight. The quality of the diet must be considered to obtain a maximum body weight gain (Chiang et al., 2010; Jahan et al., 2006). Similar daily weight gains and carcass percentages of the 12% and 0% TML groups resulted from similar feed consumption. The nutrients consumed and digested were also not different, especially protein and energy. In treatment E, 10% TML was used instead of 100% fish meal, and the nutrient content in the ration and the feed consumption did not affect the body weight gain.

In the present study, the average daily weight gain (43.56 g/hen/day) was lower than that observed by Aljubori et al. (2017) (46.25 g/bird/day) in broilers fed 10% fermented canola oil and higher than that reported by Djulardi et al. (2018), which was 42.34 g/bird/day in broilers fed 20% palm oil sludge fermented with *Lentinus edodes* for five weeks. The carcass percentage in the present study was 72.44%. This value is not different from Nuraini et al. (2013), who found that the carcass percentage of broilers was 72.69% when fed 16% fermented product in the diet. The feed conversion ratios for all TML treatments were similar. The feed conversion is the ratio between feed intake and the meat produced. The equal feed conversion ratios between the 0% TML (1.65) and 12% TML treatments (1.65) demonstrated that up to 10% TML as a substitute for 100% fish meal did not significantly affect feed consumption average daily weight gain. The feed conversion ratio in the present study was higher than that presented by Djulardi et al. (2018), who found a feed conversion of 1.63 in broilers fed 20% fermented palm oil sludge and higher than that presented by Aljubori et al. (2017), who found a feed conversion of 1.58 in broilers fed 10%

fermented canola oil. This value is lower than Nuraini et al. (2013), who found a feed conversion ratio of 1.87. The size of the feed conversion is determined by the amount of feed consumption and the body weight gain because the feed conversion is determined from feed consumption divided by body weight gain. In Rasyaf's (2008) opinion, feed modification compares the amount of feed consumption in one week with the increase in the body weight of chickens achieved that week.

The abdominal fat percentage of broiler strain CP-707 obtained in treatment E (12% TML) for five weeks of the study was 1.93%. This value was also lower than the percentage of abdominal fat in broilers aged five weeks reported by Massolo (2016), namely, by 2.15%. Lu et al. (2007) reported that broilers reared in a warmer environment showed abdominal fat weight loss. Haro (2005) reported that the body fat content of broiler chickens reached 13-14.5% of live weight, while the percentage of abdominal fat in the body of chickens reached 2-3% of live weight. Abdominal fat accumulation in broiler chickens can reduce energy consumption (Rosa et al., 2007).

The highest carcass meat tastes in broilers in treatment E using 12% TML was 5.43, which is related to the high glutamic acid content in TML, at 6.86%. The glutamate content in TML is an essential ingredient for flavoring food because it causes a savory taste. The presence of glutamate in TML can impart a delicious flavor to food, almost the same as meat (Nuraini et al., 2021; Widyastuti et al., 2015). According to Renee (2015), Jajić et al. (2020) mealworm larvae have the highest glutamic amino acid content, at 6.44 % DM.

CONCLUSION

This research concludes that 50% concentrate + 50% fermented tofu dregs constituted the medium that could increase the crude protein level and the production of *Tenebrio molitor* larvae. Inclusion of up to 12% *Tenebrio molitor* larvae could substitute 100% fish meal and not negatively affect the performance of broilers.

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The authors declare that they have no competing interests.

NOVELTY STATEMENT

Previous research has never been done on improving *Tenebrio molitor* caterpillar nutrient and its application in broiler feed. This study found a suitable alternative feed for substitution fish meal and ration formulation with 12% Tmc in the diet (substituted 100% fish meal) of broiler.

AUTHORS' CONTRIBUTIONS

Nuraini contributed to designing the experiment (fermentation), analyzing data, and writing this article. Yuliaty Shafan Nur checked Fermentations. Ade Djulardi was responsible for analyzing data. Robi Amizar was responsible for checking data. Yesi Chwenta Sari analyzed data. All authors confirmed the final revised form of the article for publication in this journal.

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