Running title: Biological Performance of Laying Quails

**Biological Performance of Laying Quails Substituted with *Alphitobius diaperinus* Larvae Meal in Their Feed**

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**Novelty Statement**

*Alphitobius diaperinus* larvae are insect pests on poultry farms, but on the other hand, these larvae potentially are a source of protein for poultry because of their high protein content with complete essential amino acids. This study proves that the substitution of *A. diaperinus* larvae meal as a substitute for fish meal in egg-laying quail feed does not reduce body weight gain, feed consumption, and eggshell thickness in quail. We found that *A. diaperinus* larvae meal has the potential to be further developed as an alternative protein source for poultry and opens up opportunities for cultivating *A. diaperinus* so that its availability is sustainable.

**Abstract**

*Alphitobius diaperinus* is one of the insect pests that have the potential to be a source of protein in poultry feed. The high protein content of *A.* *diaperinus* larvae makes this insect potentially an alternative protein source in the quail feed component in the *grower* phase. The purpose of this study was to analyze the production performance of laying quails in the *grower* phase substituted with flour of *A. diaperinus* larvae. The study was an experimental design using Complete Randomized Design with 4 treatments and 5 repeats. The treatment given was the feeding of *A. diaperinus* larval flour in the feed replacing the components of fish meal, with percentages of 0, 5, 10, and 15%. The results showed that the substitution of *A. diaperinus* larval flour did not significantly affect (p>0.05) daily feed consumption, egg weight, and shell thickness produced, but significantly (p<0.05) body weight gain. Feeding 10% and 15% larval flour resulted in no significant weight gain (Tukey test; p<0.05), but both were higher than controls. Overall, the substitution of *A. diaperinus* flour up to 15% had no adverse effect on feed consumption, growth, egg weight, and shell thickness. Thus, *A. diaperinus* larval flour can be an alternative to fish meal in quail feed and open up opportunities to develop *A. diaperinus* larval cultivation for sustainable use.

**Keywords:** *Alphitobius diaperinus*, feed consumption, weight gain, biological performance.

**Introduction**

*Alphitobius. Diaperinus* is an insect member of the family Tenebrionidae (order: Coleoptera). These insects are known as pests in chicken farms by consuming chicken feed and litter as a source of nutrition. Economically, these insects are very detrimental to poultry farmers in almost the entire world (Renault & Colinet, 2021). In addition to poultry houses, *A. diaperinus* species are also found in various post-harvest grain storage warehouses. Adult fleas and their larvae are able to damage cage buildings because of their ability to gnaw through the walls and ceilings of wooden buildings, lay eggs, and hide larvae during pupation (Del Valle et al., 2016; Lyons et al., 2017; Dzik & Mituniewicz, 2020).

Behind its nature as a pest, *A. diaperinus* larvae are known to have high protein content. In Indonesia, *A. diaperinus* larvae are often used as feed for domestic birds as well as several types of reptiles and ornamental fish. Until now, the use of animal protein source feed ingredients in poultry feed still depends on fish meal and soybean meal. Therefore, the search for alternative protein sources to replace conventional proteins still continues to be a concern for the researcher. Veldkamp & Bosch (2015) state that insects are a potential source of animal protein. Khalifah et al. (2023) said there are seven species of insects, namely house crickets (*Acheta domesticus*), field crickets (*Grylloides assimilis*), banded crickets (*Gryllodes sigillatus*), yellow mealworm (*Tenebrio molitor*), lesser mealworm (*Alphitobius diaperinus*), black soldier fly (*Hermetia illucens*), and house flies (*Musca domestica*) which can be used as a source of nutrition for poultry and fish. In addition to high protein content (Makkar et al., 2014), insects are able to act as bio converters for organic waste which is a breeding medium. Thus, ecologically insects have the potential to be cultivated and help overcome environmental problems (Mutafela & Richard, 2015).

*A. diaperinus* larvae are one of the insects included in the list of the European Union Commission Regulation (EU 2017/893), which is a list of insect species that are safe to use as a source of feed protein for livestock and fish (Gasco et al., 2018). These insects can be produced on a large scale, harvested by the time the larvae enter instar three, and potentially used as a source of protein in animal feed (Adámková et al., 2016; Janssen et al., 2017; Rumbos et al., 2019).. In addition to containing protein and fat, *A. diaperinus* also contains minerals such as Zn, Mn, Mg, Fe, Ca, and P (Roncolini et al., 2020). The protein content of *A. diaperinus* larval flour was recorded between 49% - 65% (Vrabec et al., 2015; Brandon et al., 2018; Urbanek et al., 2020), with low chitin with a fat content of 13.4 – 24.3%. Insect larvae are also low in chitin and have a high digestibility rate (De Marco et al., 2015; Józefiak et al., 2016; Kurečka et al., 2021). Janssen et al. (2017) stated that the most appropriate nutritional composition of insects for their use as feed is when insects are still in the larval stage of instar three. At this stage, the chitin content of *A. diaperinus* is much lower than the larvae of *Tenebrio molitor*, and *Hermetia illucens.*

One of the birds that require high protein in their feed is quails (*Coturnix coturnix japonica*) in the grower phase. (Emadinia et al., 2020) referring to The National Research Council (NRC) 1994 recommends protein levels in quail feed production periods between 20-24%, while energy levels are at least 2900 Kcal/kg. This study aimed to analyze the biological performance of grower phase laying quails substituted with *A. diaperinus* larval flour in their feed. The next implication was expected to change public perception, from harmful pest insects to beneficial insects, and open opportunities for sustainable cultivation in order to provide poultry feed protein to replace fish meal.

**Materials and Methods**

The study was conducted for approximately 60 days in the Biological Laboratory, Universitas Negeri Semarang. The study was designed by using the Complete Randomized Design (RAL) method with four treatments and five repeats. A total of 100 female quails aged 15 days (grower phase) were used in this study. Each unit consists of five quails placed in a *battery* cage with a unit size of 35×20×37 cm. The treatment given was feeding with the substitution of *A. diaperinus* larval flour by 0%, 5%, 10%, and 15% in feed. Referring to Emadinia et al. (2020) who used the NRC-1994 standard, the nutrition of grower phase quail feed was formulated to contain at least 20% protein and a minimum energy content of 2900 Kcal/kg. Table 1 is the formulation of the composition of the treatment feed, while the calculation of the nutritional value of feed based on the NRC-1994 table for each treatment can be seen in Table 2.

Table 1. The composition of the grower phase quail treatment feed.

|  |  |
| --- | --- |
| Ingredient (%) | Experimental diets |
| P0 | P1 | P2 | P3 |
| Yellow Corn | 52 | 52 | 51 | 51 |
| Rice bran | 10 | 10 | 11 | 11 |
| Soybean meal | 13 | 13 | 13 | 13 |
| Fish meal | 15 | 10 | 5 | 0 |
| *Lesser mealworm*  powder | 0 | 5 | 10 | 15 |
| Coconut oil cake | 8 | 8 | 8 | 8 |
| Vitamins and minerals (Premix) | 2 | 2 | 2 |  2 |
| Total (%) | 100 | 100 | 100 | 100 |

Notes:

P0 = Control treatment (without *A. diaperinus* larval flour)

P1 = Feed treatment with 5% larval flour *A. diaperinus*

P2 = Feed treatment with 10% larval flour *A. diaperinus*

P3 = Feed treatment with 15% larval flour *A. diaperinus*

Table 2. Calculation of nutritional value in each treatment feed\*

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Nutritional Content | P0 | P1 | P2 | P3 |
| Protein (%) | 20.50 | 20.58 | 20.71 | 21.00 |
| Crude Fiber | 7.67 | 7.00 | 6.84 | 6.67 |
| Fat (%) | 7.52 | 8.01 | 8.50 | 9.01 |
| Ca (%) | 3.73 | 3.44 | 3.17 | 2.88 |
| P (%) | 0.76 | 0.7 | 0.63 | 0.57 |
| ME (Kcal)  | 3002.27 | 3014.48 | 3006.68 | 3108.88 |

 \*The nutritional content of each ingredient refers to the NRC table (1994).

The biological performance parameters measured include measurements of feed consumption, weight gain, egg weight, and shell thickness. Observation and data collection were carried out for five weeks.

**Palatability measurements.** The average feed consumed per head per day was assumed to be an indicator of feed palatability. In this study, feed was given as much as 30 g/head/day, given 2 times a day at 06.30 am and 15.00 pm, and drinking water was carried out ad libitum. The rest of the feed was taken and weighed daily in the morning before replacing it with new feed. Feed consumption was calculated from the difference in the amount of feed given with leftover feed, divided by the length of the maintenance period multiplied by the number of quails per experimental unit.

**Weight Gain.** The body weight of quails was carried out at the beginning of treatment and once every week for four weeks, using digital scales. Weighing was carried out in the morning before the quails were given feed. The average weight gain per head for four weeks was obtained from the difference between quail body weight at the end of the week and body weight at the beginning of the week, using the formula:

*Gain in weight = Final Body Weight - Initial Body Weight in a specified period*

**Egg Weight Measurement.** Egg samples were taken from the results of nesting days 5-7 after laying eggs for the first time. From each cage, five egg samples were taken. Each was weighed and calculated for the average weight of eggs per head per cage.

**Shell Thickness Measurement.** All egg samples were broken and removed from the eggshells (centipedes). Each shell was carried out three times thickness measurements in different parts, namely at the blunt end, at the pointed end, and at the equator. Measurements were made using a digital caliper. The average measurement result was representative of the thickness of the shell of each egg.

**Data Analysis.** All data were analyzed by using ANOVA's one-way, and further tests used Tukey-test.

**Result**

Table 3 presents the measurement result of four biological performance parameters, namely daily feed consumption, weight gain, egg weight, and shell thickness.

Table 4. Biological Parameters of laying quails after treatment

|  |  |
| --- | --- |
| Treatment | Biological Parameter |
| Feed Consumption (g/head/day)ns | Overall Weight Gain (g/head) | Egg Weight (g/egg)**ns** | Shell Thickness (mm)**ns** |
| P0 | 25,17±0,58 | 146,08 ± 2,72a | 11.10 ± 0.97 | 0.18 ± 0.06 |
| P1 | 25,58±0,25 | 151,35 ± 3,43a | 10.74 ± 0.82 | 0.16 ±0.02 |
| P2 | 25,17±0,50 | 165,27 ± 2,66b | 11.37 ± 0.86 | 0.18 ±0.03 |
| P3 | 25,52±0,16 | 169,97 ± 3.22b | 11.22 ± 0.56 | 0.15± 0.04 |

Notes:

ns = non-significant

Different superscript within the same row is significantly different at p<0.05.

P0 = Control treatment (without *A. diaperinus* larval flour)

P1 = Feed with 5% larval flour *A. diaperinus*

P2 = Feed with 10% larval flour *A. diaperinus*

P3 = Feed with 15% larval flour *A. diaperinus*

**Feed Consumption.** The results of ANOVA statistical analysis showed that the substitution of larval flour at the level of 5% ̶ 15% in the feed had no real effect (p>0.05) on the average feed consumed. The average feed consumption of grower phase quails in this study ranged from 25.17-25.58 g/head/day.

**Body Weight Gain**. The results of ANOVA analysis showed the difference in flour substitution of *A. diaperinus* larvae in the feed had a real effect (p<0.05) on the weight gain of the grower phase laying quail. The highest weight gain was found in the P3 treatment, which reached 169.97 g (Table 4). The difference test using the Tukey test showed that P0 and P1 were not significantly different (p-value > 0.05), but they were significantly different (p<0.05) when compared to P2 and P3 treatments. These data indicate that substitutions of 10% and 15% of *A. diaperinus* flour had a better growth effect than the control group.

**Egg Weight.** The effect of the substitution of *A. diaperinus* flour in feed on quail egg weight in the first week of egg-laying is presented in Table 4. The results of the statistical analysis of egg weight data did not have an effect (P ≥ 0.05), which means that egg weights in all treatments were not different when compared to the control group. The average egg weight in the control group was 11.10 g/egg, while the other three treatments were 10.74 in a row; 11.37; and 11.22 g/egg. Thus, the substitution of *A. diaperinus* flour to 15% in this study did not affect the size of the egg weight.

**Eggshell thickness.** Table 4 shows the average thickness of quail eggshells in each treatment. Compared to the control group, the results of statistical analysis showed that the substitution of *A. diaperinus* flour did not affect the thickness of the shell (P ≥ 0.05). The average shell thickness in the control group was 0.18 mm, while the treatment group ranged from 0.15- 0.18 mm.

**Discussion**

The average consumption of the quail grower phase was still in the normal range, not much different from the research of Li et al. (2023) which recorded an average daily feed consumption of laying quail of 25.19 g/head/day. The absence of the effect of treatment on feed consumption in this study indicated that feed containing *A. diaperinus* flour does not change the palatability of feed for laying quails. Physically, *A. diaperinus* flour appears drier than fish meal, bright brown in color, and does not emit a pungent odor. According to research by Janssen et al. (2017), *A. diaperinus* larval flour contains little *chitin* (0.304%), and this figure is lower than chitin in *T. molitor* larvae (0.624%) and *H. illuciens* (0.529%) of dry weight. *Chitin* is a polymer of N-acetyl-D-glucosamine that serves as the main structural component of the exoskeleton of Insects, Crustaceans, and some fungi. *Chitin* includes polysaccharides that cannot be digested in the body of animals (Tabata et al., 2017). *A. diaperinus* larvae contain complete essential amino acids with an essential amino acid index of 71.8% (Kurečka et al., 2021).

Palatability is one of the factors that greatly determines the level of feed consumption where feed palatability is influenced by taste, smell, and color as physical and chemical factors of feed (Sadarman et al., 2022). Metabolic needs and satiety can also affect feed palatability (Mc.Crickerd & Forde, 2016). The level of feed palatability can be used as a benchmark in assessing the efficiency of feed consumption. One indication that feed is palatable is if birds show eating behavior that is always no leftover or leaves little residue as seen in this study.

In this study, the *grower* phase quail weight gain was calculated based on the average weight gain at the end of each week with a maintenance period of four weeks. The total weight gain in the control group of 6.08g/head/day was not much different from the control group in grower phase quail studies, as seen in the study of Khan et al (2022) which recorded a total body weight gain for four weeks of 159.06 g/head; recorded 5.47g/head/day or equivalent to 153.16g/head; and Reda et al. (2020) recorded 5.91g/head/day or equivalent to 165.48g/head. Previous studies using larvae flour *Hermetia illucens* also produced the same trend, namely increasing the growth of laying quails (Widjastuti et al., 2014). Better growth is thought to be due to feed containing *A. diaperinus* larval flour successfully increasing feed efficiency compared to control feed (without the addition of *A. diaperinus* larval flour). Good feed efficiency is due to the high digestibility rate of *A. diaperinus* larval flour. (Bosch et al., 2014) reported that the digestibility of *T. molitor* and *A. diaperinus* larval proteins is about 91%. In addition, the protein content reaches 62% of dry matter, as well as the low chitin content, lower than the larvae of *Tenebrio molitor*, and *Hermetia illucens*(Janssen et al., 2017). Feed digestibility can provide an idea of livestock's ability to utilize feed because the high and low digestibility of feed ingredients reflects the amount of feed that can be digested by livestock (Zhang et al., 2017).

The results of the statistical analysis of egg weight data did not have an effect (P ≥ 0.05), which means that egg weights in all treatments were not different when compared to the control group. The average egg weight in the control group was 11.10 g/egg, while the other three treatments were 10.74 in a row; 11.37; and 11.22 g/egg. Thus, the substitution of *A. diaperinus* flour to 15% in this study did not affect the size of the egg weight. This size is still within normal limits because according to (Silaban et al., 2019), quail egg weight ranges from 10 - 11.91 g / egg, or about 8% of the mother's body weight. Similarly, when compared to other studies, the figure is even higher than the average egg weight in commercial/standard feed treatments. For example, the average egg weight in the first week of the control group in the study of Ibrahim et al. (2018) was only 9.63 g /egg; research by Akramullah et al. (2023) was 9.79 g/item; and 10.93g/item was in Li et al. (2023). In the Simple Additive Weighting method, the weight of quail eggs is grouped into five criteria, namely Very Low, Low, Medium, High, and Very High. In this case, quail eggs weighing 10 – 11 g/egg belong to the very high-quality category (Abadi et al., 2018).

The quality of the eggshell has an important role in egg production. A quality shell must be strong enough to prevent damage during packing and transportation (Ketta & Tůamová, 2016). One of the indicators of the quality of the eggshell is its thickness. Table 4 shows the average thickness of quail eggshells in each treatment. Compared to the control group, the results of statistical analysis showed that the substitution of *A. diaperinus* flour did not affect the thickness of the shell (P ≥ 0.05). The average shell thickness in the control group was 0.18 mm, while the treatment group ranged from 0.15- 0.18 mm. These data show that feeding *A. diaperinus* flour in feed can still support the consistency of eggshell thickness at the beginning of laying. According to Abadi et al. (2018), the thickness of eggshells is classified into several categories, and thicknesses between 0.13 mm – 0.2 mm are included in the low category. Quality eggshells are influenced by many factors, including genotype, age, ovipositional time, and the balance of minerals and Ca - P elements in the feed. The thickness of the eggshell is also related to the duration of the formation, but it is more influenced by genotype (Ketta & Tůamová, 2016). The higher the concentration of calcium in the feed, the better the quality of the centipede and the thickness of the egg centipede (De Souza et al., 2016). Gül et al. (2022) found a decrease in shell thickness with age in laying quails. It is stated that at the age of 56-60 weeks, the eggshell is thicker (0.372 mm) than at the age of 20-24 weeks (0.354 mm). According to the study, eggshell quality is lower in old quails than in young quails, while egg weight, feed consumption, and follicle-stimulating hormones increase significantly as quails age. Increased levels of energy and protein in feed can increase the thickness and strength of egg shells (Lotfi et al., 2018).

Overall, the result of this study analysis showed that *A. diaperinus* larval flour can be used as a source of quail animal protein, a substitute for fish meal. For sustainable utilization, it is necessary to think about how to change people's perception of harmful *A. diaperinus* insects into a source of protein with high economic value, as well as how to provide larval flour on an industrial scale.

**Conclusion**

Feeding larvae of *A. diaperinus* up to the level of 15% in the feed does not affect feed consumption, egg weight, and shell thickness, but affects the weight gain of female quails during the *grower* phase. Thus, *A. diaperinus* larvae have the potential to become an alternative protein source for quails, if larval meal can be continuously available on an industrial scale as well as fish meal.

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