Efficacy Extract and Hydrolysate Protein from Brown Cricket (*Gryllus assimilis*) as an Antibiotic for Improving Performance of Quails

Anna Lidiyawati[1], Suyadi Suyadi[2], Eko Widodo[2], and Muhammad Halim Natsir[2]

[1]Doctoral Program of Animal Science, Faculty of Animal Science, Brawijaya University,
[2]Faculty of Animal Science, Brawijaya University, Malang 65145, Indonesia

Abstract. Antimicrobial resistance (AMR) is a growing global issue, necessitating the restriction of antibiotic use and the identification of safer alternatives. Insect extracts and hydrolysates are known to contain many bioactive peptides with antibacterial, antiparasitic, and antiprotozoal properties. This study aimed to determine the influence of extracts and hydrolysate proteins from brown crickets (*Gryllus assimilis*) on the antibiotic production performance of quails. A total of 400 female quails aged 6 weeks were reared, fed, watered, and vaccinated according to the recommendations in colony cages for 60 days. The quails were separated into four treatment groups with five replicates each, each containing 20 quails. T0 = basal feed, T1 = basal feed with antibiotic 50 mg/kg, T2 = basal feed with insect extract 10 ml/kg, and T3 = basal feed with insect hydrolysate protein 10 ml/kg. The extracts and hydrolysate proteins were administered via feed. The variables measured were productivity (feed consumption, egg weight, and feed conversion). The results of this study indicated a significant effect on egg weight ($p<0.05$), although there was no significant effect on feed consumption and feed conversion ratio. In conclusion, insect hydrolysate protein can be utilized as a feed additive and as an alternative to antibiotics.
Keywords: antibiotic, brown cricket, extract, hydrolysate protein, quails.

1 Introduction

Quail farming is a specialized domain within the poultry industry that focuses on breeding and rearing quails for their eggs and meat. Quail products, particularly their eggs and meat, are highly valued for their substantial nutritional content and are rich in proteins, vitamins, and minerals. Within the context of quail farming, antibiotics play a crucial role in disease prevention and growth promotion. However, the use of antibiotics presents significant risks, including the presence of antibiotic residues in products and the growing threat of antibiotic resistance. This has led to the investigation of alternatives, such as probiotics, prebiotics, phytogenics, and antimicrobial peptides, which are considered potential substitutes because of their capacity to enhance gut health and immunity without leaving residues [1]. More stringent regulations on antibiotic use are also encouraging a shift towards more sustainable practices, aiming to reduce dependence on antibiotics while maintaining animal health and production efficiency. This challenge requires immediate attention to ensure the sustainability of quail farming.

The sustainability of quail farming is inherently dependent on support from the feed sector, particularly the utilization of alternative feeds that offer potential benefits. In this regard, insect-derived proteins are a promising option. Insect proteins from species such as black soldier flies, house flies, mealworms, and crickets provide a source of essential amino acids, antimicrobial peptides, and other bioactive compounds that are advantageous for poultry [2]. A primary advantage of incorporating insect protein into poultry feed is its nutritional profile, which is comparable to that of conventional protein sources, such as fish and soybean meals. Insects can efficiently convert organic waste into nutrient-rich biomass, thereby positively affecting the environment and offering a sustainable alternative to traditional feed ingredients. Furthermore, insect meal is abundant in essential amino acids, such as lysine and methionine, which are vital for optimal poultry growth and health. Crickets, akin to other Orthoptera insects, are recognized for their potential application as poultry feed, providing an alternative protein source to sustainably support the increasing demand for poultry products [3].

Antimicrobial peptides (AMPs) derived from insects are increasingly recognized as viable alternatives to traditional antibiotics owing to their distinctive properties and notable antimicrobial efficacy. Insects synthesize a diverse array of AMPs, which are essential components of innate immune defense. These small, positively charged peptides demonstrate broad-spectrum activity against bacteria, viruses, and fungi, rendering them promising candidates for addressing the escalating challenge of antibiotic resistance [4]. Protein hydrolysis, which involves the breakdown of proteins into peptides or amino acids, can significantly affect peptide bioactivity. Hydrolysis alters peptide structures, potentially enhancing or diminishing their antimicrobial activity. Modifying hydrolysis conditions and employing strategies such as d-amino acid substitution or peptide cyclization can enhance peptide stability and bioactivity, thereby increasing their efficacy for therapeutic applications [5]. Most previous studies have focused on the use of single protein extracts or hydrolysates on performance or on other insect species, leaving a clear research gap regarding the functional advantages of hydrolysates over crude extracts in laying hens [6]. This study aimed to explore the potential of cricket protein extracts and hydrolysates as sustainable alternatives to antibiotics in quail farming, focusing on their effects on growth performance, egg quality, and health.

2 Materials and methods

2.1 Ethical Approval

This experiment was conducted according to the guidelines of the Animal Care and Research Ethics Committee at the Institut Biosains Universitas Brawijaya, Indonesia, under ethical number (No 004-KEP-UB-2024).

2.2 Study Period and Location

This study was conducted from October to November 2024 at the Andira Farm House, Wajak, South Malang, Indonesia. The insect sample was a brown adult cricket (*Gryllus assimilis*) produced by cricket farmers in East Java, Indonesia. The age of the brown crickets was 28 d.

2.3 Management of Experimental Birds and Design

The house was cleaned, disinfected, and dried for two weeks before arrival. Before the arrival of the drinkers and feeders, they were cleaned and disinfected. A total 400 of 60-day-old quail were obtained from the Andira farm. The initial weights of the quails were measured before they were randomly assigned to one of five treatments (T0, T1, T2, and T3) with five replicates of 20 quails each in a completely randomized design. The birds were provided with feed and water ad libitum for 8 weeks.

2.4 Preparation of Test Ingredient

The average age of the brown crickets was 28 days. Crickets were killed by CO_2 treatment oven-dried at 50°C for two days, and then ground as described above [7].

2.5 Extract Cricket

Cricket powder was extracted using the maceration method, with 200 g of powder macerated in 2000 mL of 96% ethanol at a 10:1 ratio. Maceration was chosen for its effectiveness in extracting a broad range of bioactive compounds, particularly those that are heat-sensitive [8]. The mixture was stirred to ensure full immersion of the powder in the solvent, and the process was repeated daily for five days until the solvent became nearly clear. The extract was filtered using Whatman No. 1 filter paper, and the filtrate was concentrated using a rotary evaporator at 40ºC. The cricket extract slurry sample was freeze-dried at -73ºC.

2.6 Cricket Protein Hydrolysates (CPH)

CPH were prepared following the protein hydrolysis procedure described by [9], with modifications. Brown cricket hydrolyzed flour was produced by mixing 90 g of dead brown crickets with 180 mL of distilled water (1:2) and then mashing the mixture using a blender. The mashed sample was added with CH_3COOH and 1 M NaOH were added to the mashed sample until the pH of the solution reached 7. Papain enzm was added according to the recommended use of (15 g/kg sample) so that the enzyme could work optimally. PAYA is a meat tenderizer that contains papain. Enzymatic hydrolysis of insects using papain offers a sustainable processing method that may result in protein hydrolysates with a higher amino acid content. The cricket solution was incubated with 50ºC pH 7 for 6 h at pH 7. The enzyme was then inactivated at a temperature of 90ºC for 20 min. After that, a 4000rpm centrifuge was carried out for 30 minutes to produce a supernatant/Hydrolysate of Cricket protein. The cricket protein hydrolyzate slurry sample was dried by freeze drying at -73ºC.

2.7 Experimental Treatments

Isonitrogenous and isocaloric diets were formulated for all birds. T0 (basal feed), T1 (basal feed + antibiotic /zinc-bacitracin 50 mg/kg), T2 (basal feed + cricket extract 10 ml/kg), and T3 (basal feed + cricket hydrolysate protein 10 ml /kg). The extract and hydrolysate proteins were administered through the feed.

2.8 Data Collection

Laying quails were individually weighed at the 8th week of dietary treatment to monitor live weight changes throughout the experiment. Feed intake was recorded weekly on a per cage basis. During this period, the laid eggs per cage were counted and individually weighed daily, and the average egg weight was calculated. Egg production was computed as the number of laid eggs/numbers of laying quails × 100. Defected eggs (i.e., broken, without solid shells, or with unusual shapes) were counted daily, and the percentage of total laid eggs/cage was calculated. The feed conversion ratio was calculated as kg of feed consumed/kg of eggs produced.

2.9 Data Analysis

The collected data were subjected to Analysis of Variance (ANOVA) according to [10]. Significant differences between the treatment means were determined using Duncan's multiple range test.

3 Results and discussion

The inclusion of hydrolysate protein in the diet can affect quail performance, as demonstrated in Table 1. This impact is evident in the alterations in key parameters, such as egg weight, which indicate enhancements in the nutritional value of the feed.

Table 1. Gross Composition for Experimental Layer Diet (g/100 kg)

Ingredient	**Layer**
Maize	540
Soybean	280
Fishmeal	84
Pollard	72
Vegetable oil	12
Limestone	3.48
MCP	1.9
Salt	2.46
Choline cholride	0.1
Premix	4
Lysin	0.1
Methionine	0.1
CP (%)	20.06
ME (Kcal/kg)	2822.90

Table. 1. presents the performance metrics of quails administered feed supplemented with the cricket protein extract and hydrolysate. The analysis revealed no significant differences among the treatments with respect to feed intake or HDP parameters. However, significant differences were observed in egg weight and FCR ($p<0.05$).

Table 2. Effect of extract and hydrolysate protein from brown crickets on quail performance.

Variabel	**Treatment**			
	T0	**T1**	**T2**	**T3**
Feed intake (g/ek/hr)	22.38 ± 2.33	21.98 ± 1.35	20.52 ± 0.92	21.07 ± 1.17
Egg weight (g/ek/hr)	9.30 ± 0.50^{a}	10.69 ± 0.70^{b}	10.34 ± 0.43^{b}	10.26 ± 0.71^{b}
HDP (%)	72.30 ± 0.06	72.30 ± 0.06	72.30 ± 0.04	73.84 ± 0.08
Egg Mass	69.57 ± 7.47	78.32 ± 8.94	75.83 ± 3.21	71.69 ± 15.89
FCR	3.3 ± 0.55	2.85 ± 0.277	2.72 ± 0.16	2.97 ± 0.64

^{a,b,ab} Means along the same row with different superscripts are significantly different ($P <0.05$).

Feed consumption serves as an indicator of livestock preference for the provided feed, particularly when introducing new feed containing specific ingredients. The results showed that the administration of the extracts and hydrolysate protein from crickets did not significantly influence the performance parameters of Japanese quails. The results of this study are in agreement with those of [11], in which earthworm hydrolysate supplementation in laying duck feed did not affect feed consumption. However, this differs from the study by [12], which stated that the use of hydrolyzed cottonseed protein (HCP) in quail feed showed significant results in terms of consumption parameters, with the addition of 7 g of HCP increasing feed intake by 21.4 g/bird/day. The results indicated that there were no significant differences between the treatments because the energy and protein content of the feed were equal across the treatments, resulting in similar feed consumption. Protein content and energy intake affect the amount of feed consumed. In addition, many other factors influence feed intake, including genetic factors such as body weight, strain, age, sex, and energy content in the feed. The chitin content in the cricket extract was within tolerable limits for livestock and did not affect consumption. This is in line with [13], who stated that administering cricket chitin at a dose of 0.5 g/kg does not affect feed intake but significantly improves growth performance, carcass quality, and broiler organ characteristics compared to chitosan.

The antibiotic administered at T1, bacitracin, aims to improve production efficiency by suppressing pathogenic microbes in the digestive tract, thus reducing nutrient competition between microbes and the host (quails). It reduces intestinal inflammation, making nutrient (protein, fat, and mineral) absorption more efficient. It also enhances the balance of gut microflora, boosts the production of short-chain fatty acids, and supports the health of the intestinal epithelium [2]. In treatments T2 and T3, the egg weights were almost identical. This indicates that providing extract and protein hydrolysates in quail feed has the same effect on increasing the egg weight. This differs from the findings of [14], who stated that BSF supplementation in quail feed did not have a significant effect on quail egg weight. Cricket protein hydrolysate is rich in essential amino acids that are important for growth and development. These amino acids are crucial for supporting egg production and improving egg quality by affecting factors such as yolk diameter, yolk weight, and albumen content, which are directly related to egg weight and internal quality [5]. Cricket proteins are also known to contain several vitamins and minerals that are important for metabolic processes and support the reproductive health of quails. These nutrients contribute to the overall egg quality by influencing factors such as shell weight and thickness, which are important for egg quality. This is because bioactive peptides derived from protein hydrolysates can influence vitellogenesis by affecting hormonal regulation, increasing nutritional support, modulating the immune response, and potentially directly affecting oocyte development. This effect is influenced by the interaction between estrogen and other hormones involved in yolk protein synthesis in the ovaries. The antioxidant and antimicrobial activities of peptides can support a healthy reproductive system, thereby indirectly enhancing vitellogenesis [15].

The addition of protein hydrolysate to treatment T3 showed significant results compared with the other treatments. This indicates that the amino acid content in cricket protein hydrolysate can be optimally utilized by quails, thereby improving their egg quality. Cricket protein hydrolysate contains short-chain amino acids that result from hydrolysis. These amino acids are easily absorbed and used as raw materials for egg formation, particularly in the egg white. This is in line with a study by [12], who stated that the addition of 5 g/kg of Hydrolyzed Cottonseed Protein to the feed can increase albumin protein. At T0, egg white weight differed from that at T3, whereas at T1, the egg white weight was almost the same.

4 Conclusion

Research has indicated that administering protein extracts and hydrolysates to Japanese quails does not negatively impact their performance or egg quality. Interestingly, the addition of 10 ml of cricket protein hydrolysate per bird to their diet yielded results similar to those obtained using antibiotics.

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