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Application of Fermented Sword Beans (*Canavalia ensiformis* L.) on Rhizopus oligosporus Production Performance and Quality of Quail Eggs

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Abstract: Scarcity and high cost of raw materials for poultry feed are the main problems that can hamper poultry production. An alternative ingredient that can be used in feed is koro beans. The aim was to determine the effect of rations containing fermented jack beans, Rhizopus oligosporus, on the production performance and quality of quail eggs. The analysis used a completely randomized design consisting of 5 treatments; Each treatment was repeated 4 times and each replication consisted of 15 quail. Four types of rations contained fermented sword bean at 7.5, 15, 22.50 and 30% and the control ration did not contain fermented sword bean. The parameters observed included feed consumption, egg production, feed conversion, shell thickness, egg yolk color and egg yolk cholesterol. The level of fermented sword bean in the ration of laying quail did not have a significant effect ($p>0.05$) on feed consumption and eggshell thickness. However, egg weight, daily egg production, feed conversion, yolk color intensity and yolk cholesterol were significantly influenced by fermented treatment ($p<0.05$). Sword beans fermented by R. oligosporus can be used as a food ingredient in egg-laying quail feed formulations to increase the quantity and quality of quail eggs.

Keywords: *Canavalia ensiformis* L; Fermentation; Production performance; Quail

Introduction

One of the most important aspects of the management of "Five Animal Husbandry Businesses" is feed (Fatmona et al., 2024). The main problem is that the feed provided is commercial feed which is quite expensive (Fatmona et al. 2023). Scarcity and high prices of raw materials for poultry feed are the main problems that can hamper poultry production (Haque et al., 2020; Hafez & Attia, 2020). It is very important to implement strategies that can increase raw material production and minimize the problem of providing raw materials for poultry feed because poultry feed competes for raw materials with human needs and other livestock needs. In addition, the price of commercial feed continues to increase along with dependence on commercial feed from other countries. Therefore, it is necessary to carry out research regarding the potential of local raw materials which have not been widely utilized. The main factor that influences poultry productivity is feed. One alternative feed ingredient is sword bean. The direct use of koro beans is because they are anti-nutrients which can interfere with the metabolism and absorption of food in livestock. On the one hand, the body needs important elements apart from functioning as food or medicine, for example those containing omega 3 or what are called functional foods (Nur et al., 2024; Shaikh, 2022; Sajid Arshad et al., 2021).

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These anti-nutrients have an impact on livestock growth and production, therefore it is necessary to process sword bean beans through fermentation before being given to livestock (Indrati, 2022; Das et al., 2022). Fermentation is a method that uses microbes to convert substrates into desired products, such as improving nutritional quality or removing toxins and antinutrients (Xiang et al., 2019; Senanayake et al., 2023; Elhalis et al., 2023). This research uses the microbe used in fermentation, namely R. oligosporus, which is the same microbe used to ferment soybeans, because both substrates have the same physical structure. R. oligosporus produces digestive enzymes such as amylase, protease and lipase which can convert complex compounds into components so that they are more easily absorbed by the body (Masahid et al., 2021; Ababor et al., 2023).

Research on the ability of R. oligosporus to increase nutritional content and reduce antinutrients through the solid media fermentation process has been carried out by Sharma et al. (2020), Sánchez-García et al. (2022) and Shi et al. (2017). These authors indicated that the fermentation process used a dosage of 0.20% (w/w). R. oligosporus was fermented for 72 hours resulting in the greatest increase in nutritional content and reduced antinutrient content by more than 50%; cyanide and tannin content reduced by more than 75%. The current research examines the effects of using sword beans fermented by R. oligosporus to increase the production performance and quality of quail eggs.

Method

The main ingredients in this research were sword bean products fermented by R. oligosporus and 300 quail aged 1 month (not yet laying eggs), 20 per cage. Research procedure: Fermentation of the sword bean by R. oligosporus is carried out as follows: Sort the sword bean (C. ensiformis L.), sword bean (C. ensiformis L.), (2) Add 2000 mL of distilled water into 1000 g of sorted sword koro nuts, Soak the sword koro nuts 3 times for 24 hours, then steam, Change the soaking water 6 times for 24 hours, then wash and peel the sword koro nuts Boil sword beans for 30 minutes, then air dry until excess moisture is removed, Inoculate the beans with 0.2% R. oligosporus inoculum and mix well by stirring, Pack in a perforated plastic bag 2 cm thick to create an aerobic atmosphere, Incubate in a fermentation room at a temperature of 30 for 72 hours, After the incubation period is complete, dry and ready to be mixed with ingredients appropriate to the treatment that will be given to experimental animals. The treatment is as follows:

R0: Diet without fermented sword bean (control)
C R1: Diet containing 7.5% fermented swordtail beans
C R2: Diet containing 15.0% fermented sword bean
C R3: Food containing 22.5% fermented swordtail beans
C R4: Diet containing 30.0% fermented sword bean.

The diet is formulated to be isocaloric and iso protein aqueous and contains lysine, methionine, calcium, and phosphorus content. Consisting of four replications of each treatment, each replication used 15 quail for a total of 300 quail. The composition of the experimental feed and the nutrient and energy content that can be metabolized are presented in Table 1.

The variables measured in this research are production performance and egg quality:

Production Performance Analysis

Feed consumption (g quailG1 dayG1): Calculation of quail feed consumption is carried out every week; Quail feed consumption was calculated using the equation Quail daily production (QDP) (%): Daily production of quail is calculated based on the equation. Daily production of quail is expressed in percentage form using the following equation: Egg weight (g eggsG1): Egg weight is the average egg weight calculated as the total egg weight divided by the number of eggs produced. Egg weighing is done every day. Feed conversion ratio: The feed conversion ratio is calculated weekly using the following equation:

Egg Quality Analysis

Eggshell thickness (mm): The thickness of the eggshell is measured with a tricle micrometer after the inner eggshell has been removed; Egg Yolk Intensity: The color intensity of egg yolk is measured based on standard egg yolk color using the Roche Yolk Color Fan with a score range from 1-15; the scale ranges from light yellow to dark yellow; Egg yolk cholesterol: The cholesterol content of egg yolk was analyzed using the Liebermann Burchard method. The research materials were egg yolk, chloroform solution, anhydrous acetic acid solution, concentrated sulfuric acid solution, standard cholesterol solution and alcohol solution (3:1). Research equipment includes a centrifuge, water bath, UV-visible spectrophotometer, oven, and glassware.

The way cholesterol analysis works is by chloroform extracting cholesterol from egg yolk, which will react with acetic acid anhydride and concentrated sulfuric acid to make the product more colorful. The absorbance of the product is measuring a wavelength of 420 nm. Absorbance is proportional to cholesterol concentration. Experimental design and statistical analysis: The experiment used a completely randomized design. Several analyzes were carried out followed by Duncan's multiple range test to determine the effect of using fermented sword bean in the diet.

Table 1. The Variables Measured in this Research are Production Performance and Egg Quality

Food material	R0	R1	R2	R3	R4
Corn	46.45	45	44	42	41
B. soybeans	30	22.50	15	7.50	0
Q. Fish	3.30	4.20	5.30	6.40	7150
KKF	0	7.50	15	22.50	30
Bran	9.20	9.75	9.65	10.55	10.45
Grit	2.25	2.25	2.25	2.25	2.25
M. coconut	4.00	4	4	4	4
Lysine	0	0	0	0	0
CaCO3	2.35	2.35	2.35	2.35	2.35
Topmix	0.50	0.50	0.50	0.50	0.50
Q. Bones	1.80	1.80	1.80	1.80	1.80
Methionine	0.15	0.15	0.15	0.15	0.15
NaCl	0	0	0	0	0.00
	100	100	100	100	100
EM (kcal/kg)	2900	2907	2920	2923	2936
PK (%)	20.12	20.03	20.02	20.04	20.03
Arginine (%)	1.32	1.14	0.95	0.77	0.59
Glycine + Serine (%)	1.92	1.69	1.47	1.25	1.03
Histidine (%)	0.53	0.46	0.40	0.33	0.27
Isoleucine (%)	0.83	0.72	0.62	0.51	0.41
leucine (%)	1.70	1.48	1.28	1.07	0.86
Lysine (%)	1.11	0.95	0.79	0.64	0.48
Methionine (%)	0.45	0.43	0.40	0.38	0.36
Methionine+cystine (%)	0.81	0.76	0.72	0.67	0.63
Phenylalanine (%)	0.95	0.81	0.68	0.55	0.41
Phenylalanine+tyrocene (%)	1.76	1.52	1.29	1.06	0.83

Result and Discussion

Production performance: Average feed consumption for laying quail in this study ranged from 22.45 ± 0.26 to 22.59 ± 0.04 g dayG1. The presence of sword beans in the ration had no significant effect ($p>0.05$) on feed consumption of laying quail. These findings indicate that the concentration of fermented sword bean in the feed does not affect the amount of feed consumed by laying quail; even feed containing 22.5% fermented sword bean has good palatability. This observation is due to the results of fermenting animal feed with R. oligosporus improving the taste, aroma, texture and digestibility and fermentation also reduces antinutrients (Karangora et al., 2021).

Table 2. Treatment of Giving Sword Nuts in Feed with Different Formulations

	Treatments				
Variables	R_0	R_1	R_2	R_3	R_4
Feed consumption (g quailG^1 dayG^1)	22.45±0.26^a	22.55±0.75^a	22.52±0.05^a	22.59±0.04^a	22.56±0.05^a
Egg weight (g eggG^1)	10.79±0.70^b	11.13±0.28^{ab}	10.90±0.60^b	11.83±0.36^a	10.93±0.37^b
QDP (%)	69.86±0.75^b	68.12±0.80^b	68.92±1.76^b	72.59±1.87^a	61.78±2.29^c
Feed conversion ratio	3.03±0.04^{bc}	3.11±0.04^b	3.07±0.08^b	2.92±0.05^c	3.42±0.12^a
Egg yolk intensity	6.22±0.32^c	6.85±0.17^b	6.95±0.41^b	7.00±0.14^b	8.00±0.16^a
Eggshell thickness (mm)	0.19±0.04^a	0.20±0.07^a	0.21±0.01^a	0.23±0.02^a	0.16±0.01^a
Egg yolk cholesterol value (mg/100 g)	147.02±12.18^b	127.08±11.08^a	130.77±6.60^a	122.69±8.61^a	122.30±9.9^a

The results of feed consumption in this study are different from the results of research conducted by (Saraswati & Tana, 2015), where the feed consumption of laying quail ranges from 19.9-20.7 g quailG1 dayG1. However, the results of our study were not significantly different from the results of research Nuraini et al. (2020) which obtained feed consumption values ranging from 20.96-23.82 g for quailG1 dayG1 at 6-13 weeks of age. The ration given to each treatment in this study had the same energy content and protein content. The average body weight and age of quail are also relatively the same, so harvest capacity does not differ significantly (Abdul-Majeed & Abdul-Rahman, 2021; François et al., 2021). The average weight of quail eggs for birds aged 6-13 weeks is between 10.79-11.93 g eggsG1 (Aryee et al., 2020). The presence of fermented sword beans in the ration had a significant effect ($p<0.05$) on the weight of quail eggs. Feed containing fermented cowpeas

produced significantly different eggs compared to the control (without sword beans). Feed with a concentration of 22.5% fermented sword bean (R3) was significantly different from other treatments.

Overall, the average egg weight in this study was within the normal range according to (Iqbal et al., 2016) research (from 10-15 g). The weight of quail eggs in this study was higher compared to studies with different material treatments (Pratiwi et al., 2023). This finding was caused by research on sword bean products fermented with R. oligosporus, such as tempeh, which were proven to contain amino acids that were easily digested (Khairi et al., 2023). Rhizopus oligosporus, apart from producing metabolites that inhibit the growth of Aspergillus flavus, also produces proteases that can convert proteins into peptides. Fermentation with R. oligosporus is able to hydrolyze phytic acid in the substrate which causes the nutritional content in sword jack beans to decrease, thereby increasing the availability of calcium and phosphorus for egg shell formation (Emkani et al., 2022; Aldania & Suparno, 2024). (as well as vitamin B12 and other minerals) and contribute to egg weight gain.

The results are consistent with several previous studies which showed that fermentation with R. oligosporus produces the enzyme phytase which breaks down phytate and macromolecules in soybeans (a type of grain) into components that are more easily absorbed by the body (Teoh et al., 2024). Rhizopus sp. can break down the alkaloids or toxins contained in jatropha seeds, namely lectins, phorbolester esters, tannins, phytates, saponins and antitrypsin. The factors that influence egg weight are food composition with sufficient protein and amino acids that are easily absorbed (Macelline et al., 2021). Protein consumption from fermented sword bean products affects egg weight. The greater the protein consumption by laying quail, the greater the weight of the eggs produced (Herlina et al., 2016)

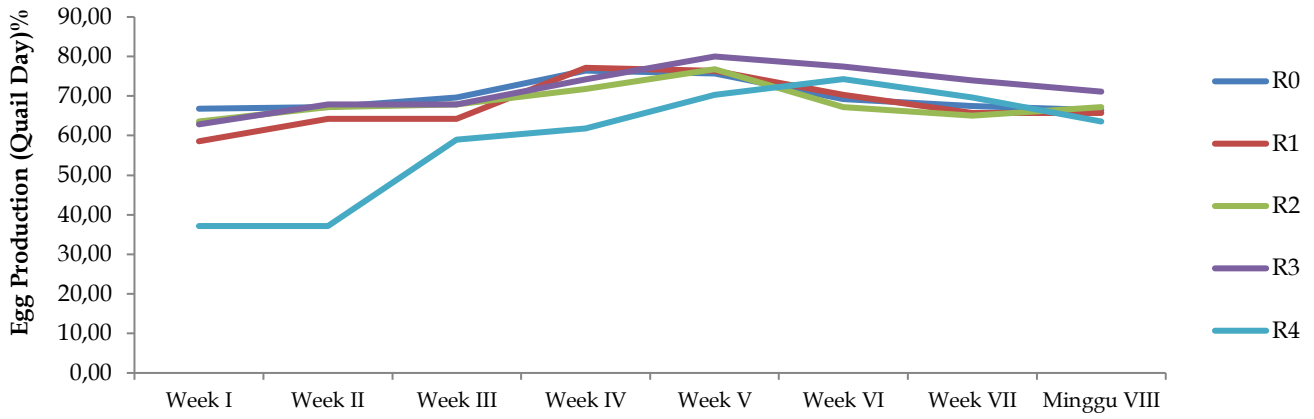

Figure 1. Effect of treatment on quail day egg production (QD%)

The average daily production of quail is between 69.86-72.59%. Egg production in this study shows similarities with research conducted by Rangkuti et al. (2022) which reported quail egg production of 71.43 ± 3.65%. However, egg production in this study was higher compared to research conducted by Yaman et al. (2020) where quail aged 6-10 weeks had an average production of 51.3%. This difference occurs due to differences in nutritional intake and length of research, so it can be concluded that giving fermented sword bean flour can increase quail production. Table 2 shows that the highest average production occurred in the 11th week in the treatment with a ration containing 22.50% fermented sword bean (R3). Quail can reach peak production of 96% if their maintenance and feeding are managed properly and correctly19. The high level of production in this study was caused by Choi et al. (2021) the influence of the fermented sword bean product which contains protein, amino acids and several minerals which are easily absorbed by quail because complex compounds are broken down into simple compounds.

Protein is very important for egg formation because 50% of the dry matter in eggs is protein. Providing amino acids ensures continued protein synthesis, which is essential during egg production15. Rhizopus oligosporus is a fungus that can produce protease or peptidase enzymes, an enzyme that catalyzes the hydrolysis of peptide bonds into short oligopeptides and free amino acids, which are more easily absorbed by the body compared to long proteins. Thus, the availability of nutrients needed by laying quail can be increased by the presence of this fungus. Egg production reached the maximum value in the 22.50% treatment. compared to

the control treatment without fermented sword bean. In addition, fermented cowpeas have the advantage of producing isoflavone compounds, which are very important in improving the health, production and quality of quail eggs.

Feed conversion means that livestock are able to consume food into desired output such as egg production within a certain period of time. The feed conversion rate shows the level of feed use efficiency. A small conversion indicates efficient use of feed and conversely, a large conversion value indicates inefficient use of feed. The results of the analysis of variance showed that the provision of sword bean in the ration had a significant effect ($p<0.05$) on the feed conversion ratio of laying quail. The treatment with 22.50% fermented jackfruit nuts was significantly different from the other treatments and gave the lowest feed conversion value of 2.29. These results prove that the use of sword beans at a concentration of 22.50% provides a high level of feed efficiency. Factors that influence feed conversion are those related to consumption levels such as feed quality, feeding methods and livestock health management. Egg quality: Egg yolk color is problematic, especially in quail eggs. Several studies have been conducted to improve the color of quail egg yolks. The intensity of egg yolk in food containing fermented sword bean ranged from 6.85 to 8.00, while the intensity of egg yolk in food without fermented sword bean was around 6.22 (Table 2).

The color of egg yolk in the diet containing 30% fermented sword bean was significantly different ($p<0.05$) from other treatments; The intensity of the yellow color of the eggs was 30% higher than other treatments. Egg yolk intensity scores increased with the addition of fermented sword bean to the diet. This shows that the pigment contained in the sword bean fermented by R. oligosporus functions to increase the egg yolk color score. The egg yolk color score obtained indicates good egg quality. When the egg yolk color reaches a score of between 7 and 8, then the egg is classified as good quality. A study by Yunitasari et al. (2023). found that feed added with katuk leaf powder containing 697.40 ppm beta-carotene increased the egg yolk color score to 8-9. The lower egg yolk color score in this study occurred due to higher egg production which caused the xanthophyll in the feed to be dispersed among more egg yolks, causing the egg yolk color score to decrease. Egg yolk color is influenced by several factors other than xanthophyll, namely strain, variety, cage position, poultry health management, stress and additional food ingredients.

The color of egg yolk is orange yellow due to the presence of carotenoids which contain large amounts of zeaxanthin, cryptoxanthin, and lutein (xanthophyll). Each bird has a different ability to convert the pigment carotene into egg yolk color. The higher the poultry consumption, the higher the level of carotenoid pigments in producing egg yolks with a deeper color. Table 2 shows that the average egg shell thickness was not significantly influenced by the feed treatment containing fermented cowpeas. This result is because the calcium content of the fish meal used in the feed formulation is sufficient for the formation of egg shells and the feed treated with the treated feed formulation contains the same calcium and phosphorus. The formation of egg shells requires calcium and phosphorus. These results are consistent with those of (Peterson et al., 2020), who reported a mean eggshell average of 0.23 mm for 12-week-old quail.

Adequate eggshell thickness can be achieved if there is a balance between calcium and phosphorus in the feed. The increase in calcium retention is due to increased efficiency in calcium utilization as a result of the fermentation process. Thus, the balance of calcium and phosphorus in the feed is sufficient to meet the needs of egg shell formation in quail. This balance does not produce a decreasing effect on calcium requirements. Phosphorus metabolism in the body will only run if calcium and phosphorus are available in balanced amounts. Cholesterol levels in egg yolks in this study were relatively low and the treatments showed average cholesterol levels that were significantly lower than controls. This finding is due to the presence of isoflavones in fermented jackfruit nuts which reduce egg yolk cholesterol and increase egg production. This observation is consistent with the results of Hertamawati et al. (2021) research, where the addition of 0.50% isoflavone extract to quail feed can reduce egg yolk cholesterol and increase egg production.

Isoflavones have a structure and biological function similar to phytoestrogens. The phytoestrogens contained in fermented jackfruit mimic the function of the hormone estrogen, which encourages reproductive growth in preparation for the formation of egg cells. Phytoestrogens are plant substrates that have estrogen-like activity. The isoflavone (phytoestrogen) content in nuts is believed to improve liver function and result in increased vitellogenin production. Vitellogenin stimulates the expansion of the ovarian follicular hierarchy, so that the number of follicles increases. Vitellogenin is synthesized in the liver, enters the bloodstream and is absorbed by the oocyte cytoplasm. The decrease in LDL levels is thought to be related to the number of developing follicular hierarchies.

If more follicles develop, yolk-forming substances such as cholesterol will be distributed throughout the follicles causing the average egg cholesterol level to decrease. The hormone estrogen causes a decrease in egg cholesterol levels. Developing follicles produce estrogen, which suppresses the activity of the HMG-

CoA enzyme and inhibits cholesterol biosynthesis. The use of fermented koro beans as feed for laying hens can increase egg production and quality. In the food of laying quail workers, 22.50% of the total feed ingredients can be added with fermented jack beans. Fermented jack beans should be added to the feed of laying quail over 1 month of age. One limitation of this study is that the effect of drying temperature on nutritional composition during the process of making fermented jack bean flour products has not been studied.

This research found that the use of a 22.50% concentration of sword beans fermented with R. oligosporus in laying quail feed could be useful for increasing the production and quality of laying quail eggs. This research will help researchers to uncover critical areas of investigation for the use of fermented sword bean in egg-laying quail feed formulations that cannot be explored by many researchers. Thus, a new theory regarding the use of sword beans as a food additive in the formulation of laying quail feed may be obtained.

Conclusion

The use of fermented swordtail beans in laying quail feed is safe up to a level of 22.5%. Feed containing 22.5% fermented sword bean can increase egg production and weight and reduce quail feed conversion value and quail egg cholesterol levels. Jack beans fermented by R. oligosporus can be used as a feed additive for laying quail with feed formulations to increase the quantity and quality of quail eggs.

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Author Contributions

S. R: Conceptualization, Data Curation, Formal Analysis, Investigation, Methodology, Writing – original draft. S. F.: Conceptualization, Data Curation, Formal Analysis, Investigation, Methodology, Project Administration, writing – original draft, Writing – review & editing. S. W: Conceptualization, Funding Acquisition, Investigation, Methodology, Project Administration.

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Conflicts of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work described in this paper.

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