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Research Article
The Effect of Diets Containing Jack Bean (*Canavalia ensiformis* L.) Fermented by *Rhizopus oligosporus* on the Production Performance and the Egg Quality of Quail

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Abstract

Background and Objectives: The scarcity and high prices of raw materials for poultry feed are major problems that can hinder the production of poultry. One alternative ingredient that can be used in feed is jack beans. This study was conducted to determine the effect of diets containing jack beans fermented by *Rhizopus oligosporus* on the production performance and the egg quality of quail. **Materials and Methods:** The main materials used in this research were jack bean products after fermentation by *R. oligosporus* and 1-month-old quail(not yet egg-laying). The study was carried out using a completely randomized design consisting of 5 diet treatments; each treatment was replicated 4 times and each replication contained 15 quail. Four kinds of diet contained fermented jack beans at 7.5, 15.0, 22.5 and 30.0% and the control diet did not contain fermented jack beans. The observed parameters included feed consumption, egg production, feed conversion, eggshell thickness, egg yolk color and egg yolk cholesterol. **Results:** The level of fermented jack beans in the diets of layer quail had no significant effect ($p>0.05$) on feed consumption and eggshell thickness. However, egg weight, daily egg production, feed conversion, egg yolk color intensity and egg yolk cholesterol were significantly affected by the jack bean treatment ($p<0.05$). **Conclusion:** The use of fermented jack beans in the feed formulations of layer quail is safe up to 22.5%. Feeds containing 22.5% fermented jack beans can increase egg weight and egg production and reduce the feed conversion ratio of quail and the cholesterol levels of quail eggs. Jack beans fermented by *R. oligosporus* can be used as a food ingredient in the feed formulations of layer quail to increase the quantity and quality of quail eggs.

Key words: Feed conversion, feed formulation, jack beans, quail, *Rhizopus oligosporus*

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The scarcity and high prices of raw materials for poultry feed are major problems that can hinder the production of poultry. It is essential to implement strategies that increase the production of raw materials and minimize the problem of providing raw materials for poultry feed because poultry feed competes for raw materials with human needs and the needs of other livestock. In addition, the price of commercial feeds continues to increase along with our dependency on commercial feeds from other countries. Therefore, we need to identify alternatives by investigating the potential of local raw feed materials that have not been widely used.

One alternative feed ingredient is jack beans. The direct use of jack beans as an animal feed has limitations because antinutrients can disrupt the metabolism and absorption of food in livestock. These antinutrients impact the growth and production of livestock and it is therefore necessary to process the jack beans via fermentation before feeding them to livestock.

Fermentation is a method that uses microbes to convert a substrate into a desired product, such as improving the quality of nutrients or removing toxins and antinutrients. The microbe used in the fermentation process in this study is *R. oligosporus*, the same microbe used to ferment soybeans, because the two substrates have the same physical structure. *R. oligosporus* produces digestive enzymes such as amylases, proteases and lipases that are able to break down complex compounds into components that are simpler and more easily absorbed by the body[1].

Research on the ability of *R. oligosporus* to increase nutrient content and reduce antinutrients by a solid media fermentation process was carried out by Utami *et al.*[2]. These authors demonstrated that a fermentation process using a 0.2% (w/w) dose of *R. oligosporus* during a 72 h fermentation produced the greatest increase in nutrient content and reduced the antinutrient content by more than 50%; the cyanide and tannin contents were reduced by more than 75%. The current study examined the effects of using jack beans fermented by *R. oligosporus* on the production performance and egg quality of quail.

MATERIALS AND METHODS

The main materials used in this study were jack beans products after fermentation by *R. oligosporus* and 300 1-month-old quail (not yet egg-laying) housed with 20 birds per cage.

Research procedure: The jack bean fermentation by *R. oligosporus* was conducted as follows: (1) Sort the jack beans *(C. ensiformis* L.), (2) Add 2000 mL of distilled water to the 1000 g sorted jack beans, (3) Soak the beans3 times for 24 h, then steam, (4) Replace the soaking water 6 times in 24 h, then wash and peel the beans (5) Bring the jack beans to a boil for 30 min, then air dry them until excess moisture is removed, (6) Inoculate the beans with 0.2% *R. oligosporus* inoculum and mix thoroughly by stirring, (7) Package in a perforated, 2 cm thick plastic bag to create an aerobic atmosphere, (8) Incubate in a fermentation chamber at 30°C for 72 h, (9) After the incubation period is complete, the dried product is drilled and ready to be mixed with other food ingredients in accordance with the treatment to be given to the experimental animals. The treatments were as follows:

- R0: Diets without fermented jack beans (control)
- R1: Diets containing 7.5% fermented jack beans
- R2: Diets containing 15.0% fermented jack beans
- R3: Diets containing 22.5% fermented jack beans
- R4: Diets containing 30.0% fermented jack beans

The diets were formulated to be isocaloric and iso protein aceous and to have similar lysine, methionine, calcium and phosphorus contents. Each treatment consisted of four replications and each replication consisted of 15 quail, for a total of 300 quail. The composition of the experimental diets and the nutrient and metabolizable energy contents are presented in Table 1. The variables quantified in this experiment were production performance and egg quality.

Production performance analysis

- **Feed consumption (g quail^{-1} day^{-1}):** Quail feed consumption calculations were carried out weekly. Quail feed consumption was calculated using the following equation from Maknun *et al.*[3]:

$$\text{Feed consumption} = \frac{\text{Initial feed weight (g)} - \text{residual feed weight (g)}}{\text{No. of days}}$$

- **Quail daily production (QDP) (%):** Quail daily production was calculated based on a equation from Zahra *et al.*[4]. Quail daily production is expressed as a percentage using the following equation:

$$\text{QDP (\%)} = \frac{\text{Total daily egg production}}{\text{Total number of quail}} \times 100\%$$

Table 1: Formulation and metabolizable energy of experimental feeds

Feed Ingredients (%)	R_0	R_1	R_2	R_3	R_4
Corn	46.00	45.00	44.00	42.00	41.00
Soybeans flour	30.00	22.50	15.00	7.50	0.00
Fish meal	3.00	4.20	5.30	6.30	7.30
Rice bran	10.00	9.80	9.70	10.70	10.70
Fermented jack bean flour	0.00	7.50	15.00	22.50	30.00
Coconut oil	4.00	4.00	4.00	4.00	4.00
$CaCO_3$	2.35	2.35	2.35	2.35	2.35
Premix flour	0.50	0.50	0.50	0.50	0.50
Bone meal	1.80	1.80	1.80	1.80	1.80
Methionine	0.10	0.10	0.10	0.10	0.10
Grit	2.25	2.25	2.25	2.25	2.25
	100.00	100.00	100.00	100.00	100.00
Metabolizable energy (kcal kg^{-1})	2895.00	2908.00	2920.00	2923.00	2935.00
Crude protein (%)	20.00	20.00	20.00	20.00	20.00
Arginine (%)	1.32	1.17	1.01	0.86	0.70
Glycine+serine (%)	1.91	1.86	1.80	1.75	1.68
Histidin (%)	0.53	0.51	0.48	0.46	0.43
Isoleucin (%)	0.83	0.74	0.66	0.57	0.48
Leucine (%)	1.69	1.63	1.56	1.49	1.42
Lysine (%)	1.12	1.07	1.01	0.96	0.90
Methionine (%)	0.44	0.47	0.50	0.53	0.55

- Egg weight (g egg^{-1}): Egg weight is the average egg weight calculated as the total egg weight divided by the number of eggs produced. The eggs were weighed each day

- **Feed conversion ratio:** The feed conversion ratio was calculated weekly using the following equation[5]:

$$\text{Feed conversion ratio} = \frac{\text{Total feed intake(g)}}{\text{Total egg production(g)}}$$

Egg quality analysis:

- **Eggshell thickness (mm):** Eggshell thickness was measured by a tricle micrometer after the inner eggshell was removed
- **Egg Yolk Intensity:** The intensity of yolk color was measured based on the standard color of the yolk using a Roche Yolk Color Fan with a range of scores from 1-15; the scale ranges from light yellow to dark yellow
- **Egg yolk cholesterol:** The cholesterol content of the egg yolk was analyzed using the Liebermann Burchard method[6]. The materials included egg yolk, a chloroform solution, an anhydrous acetic acid solution, a concentrated sulfuric acid, a standard cholesterol solution and an alcohol/ether solution (3:1). The tools used included a centrifuge, water bath, UV-visible spectrophotometer, oven and glassware. The working principle of cholesterol analysis is that chloroform extracts cholesterol from the yolk, which will react with the acetic acid anhydride and concentrated sulfuric acid to form a colored product. The absorbance of the product was measured at a wavelength of 420 nm. The absorbance is proportional to the cholesterol concentration

Experimental design and statistical analysis: The experiment used a completely randomized design. Multiple analyses were carried out followed by a Duncan's multiple range test to determine the effect of using fermented jack beans in the diet.

RESULTS AND DISCUSSION

Production performance: The average feed consumption of layer quail in this research ranged from 22.45±0.26 to 22.59±0.04 g $quail^{-1}$ day^{-1}. The presence of the fermented jack beans in the diet had no significant effect ($p>0.05$) on the feed consumption of layer quail. This finding demonstrates that the concentration of fermented jack beans in the feed does not influence the amount of feed consumed by layer quail; even diets containing 22.5% fermented jack beans had good palatability. This observation is because the results of animal feed fermentation with *R. oligosporus* improves its taste, aroma, texture and digestibility and fermentation also reduces antinutrients[7].

The feed consumption results of this study differed from the results of research conducted by Saraswati and Tana[8], in which the feed consumption of layer quail ranged from 19.9-20.7 g $quail^{-1}$ day^{-1}. However, our results were not significantly different from those of Triyanto[9],

who obtained feed consumption values ranging from 20.96-23.82 g quail^{-1} day^{-1} at age 6-13 weeks. The diets in each treatment of the current study had the same energy and protein content. The average body weight and age of the quail were also relatively similar, so the crop capacity is not significantly different.

The average weight of the quail eggs for birds from 6-13 weeks of age were between 10.79-11.93 g egg^{-1}. The presence of fermented jack beans in the diets had a significant effect ($p<0.05$) on the weight of quail eggs. Feed containing fermented jack beans produced significantly different egg weights compared with the control (without fermented jack beans). Feeds with a concentration of 22.5% fermented jack beans (R3) were substantially different from the other treatments. Overall, the average egg weight in this study was still within the normal range as given by Pangestuti[10] (from 10-15 g). The egg weights of quail in this study were higher than those in a study by Lutfi *et al.*[11], who produced egg weights from 11.09-11.33 g. This finding is because research on jack bean products fermented with *R. oligosporus*, such as tempeh, have been shown to contain easily digestible amino acids. *Rhizopus oligosporus*, in addition to producing metabolites that inhibit the growth of *Aspergillus flavus* competitors, also produces proteases that can break down proteins into peptides. Fermentation with *R. oligosporus* is able to hydrolyze the phytic acid in the substrate that causes the nutrient content in jack beans to decrease, thereby increasing the availability of calcium and phosphorus for eggshell formation (as well as vitamin B12 and other minerals) and contributing to the weight gain of the egg[12].

These results are consistent with several previous studies demonstrating that fermentation with *R. oligosporus* produces phytase enzymes that break down phytate and the macromolecules in soybeans (a type of grain) in to components that are more easily absorbed by the body[13]. *Rhizopus* sp. can break down alkaloids or poisons contained in jatropha seeds, namely, lectin, phorbolester/diterpene esters, tannins, phytate, saponin and antitrypsin[14]. Several factors influence egg weight, one of which is the composition of the diet such as the adequacy of proteins and amino acids that are easily absorbed[15]. The consumption of protein from fermented jack bean products affect egg weight. The greater the protein consumption by layer quail, the greater the weight of the eggs produced[16].

The average daily production of quail was between 69.86-72.59%. Egg production in this study was similar to that in a study carried out by Sudrajat *et al.*[17], who reported an egg production by quail of71.43±3.65%. However, production in this study was higher than a study by Eishu *et al.*[18], in which quail aged 6-10 weeks had an average production of 51.3%. These differences occurred because of differences in the nutrient intake and the duration of the study, so it can be concluded that feeding powdered fermented jack bean products can increase quail production.

Table 2 shows that the highest average production occurred at week 11 in the treatment with diets containing 22.5% fermented jack beans (R_3). Quail can reach a peak production of 96% if their maintenance and feeding is managedcorrectly[19]. The high level of production in this study was due to the effect of fermented jack bean products containing protein, amino acids and some minerals that were easily absorbed by the quail because complex compounds were broken down into simple compounds.

Protein is crucial for egg formation because 50% of the dry matter in eggs is protein. Providing amino acids guarantees the continuity of protein synthesis, which is very important during egg production[15]. *Rhizopus oligosporus* is a fungus that can produce protease enzyme or peptidase, 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 with long chain proteins[20]. Thus, the availability of nutrients required by laying quail can be enhanced by the action of this fungus. Egg production reached its maximum value in the 22.5% treatment compared with the control treatment without fermented jack beans. In addition, fermented jack beans have

Table 2: The average production performance and egg quality of quail

	Treatments				
Variables	R_0	R_1	R_2	R_3	R_4
Feed consumption (g quail^{-1} day^{-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 egg^{-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}	122.3.0±9.9^{a}

Numbers followed by a different superscript in the same line indicate significant differences

the advantage of producing isoflavone compounds, which are very important in improving the health, production and egg quality of quail.

Feed conversion is the ability of livestock to convert food consumption into desired outputs such as egg production within a given time period[21]. The feed conversion rate indicates the level of efficiency of food use. Small conversion values indicate efficient feed use and conversely, large conversion values indicate inefficient feed use. The results of analysis of variance showed that the presence of fermented jack beans in the diets had a significant effect ($p<0.05$) on the feed conversion ratio of layer quail.

The treatment with 22.5% fermented jack beans was significantly different from the other treatments and provided the lowest feed conversion value of 2.29. This result proves that the use of fermented jack beans at a concentration of 22.5% provides a high level of feed efficiency. Feed conversion rates are influenced by various factors related to consumption levels such as food quality, feeding procedures and livestock health[22].

Egg quality: The color of the yolk is problematic, particularly in quail eggs. Some studies have been conducted to improve the color of quail egg yolks. The egg yolk intensity in diets containing fermented jack beans ranged from 6.85-8.00, whereas the egg yolk intensity in the diet without fermented jack beans was approximately 6.22 (Table 2).

The egg yolk color in diets with30% fermented jack beans was significantly different ($p<0.05$) from the other treatments; the intensity of the yolk color at the 30% level was higher than the other treatments. The egg yolk intensity score increased with the addition of fermented jack beans to the diet. This finding indicates that the pigment contained in the jack beans fermented by *R. oligosporus* plays a role in increasing the yolk color score. The yolk color scores obtained in this study indicate good quality eggs. When the color of the yolk reaches a score between 7 and 8, the egg is classified as good quality[23]. A study by Wiradimadja *et al.*[24] found that feeds with added katuk leaf powder containing 697.40 ppm beta-carotene increased the yolk color score to 8-9. The lower color score of the yolks in this study occurred because higher egg production caused the xanthophyll in the feed to be dispersed among more egg yolks, causing the yolk color score to decrease. In addition to xanthophyll, the color of the yolk is influenced by strain, variety, caging, health, stress and additional food ingredients[23].

The color of the egg yolk is orange-yellow due to the presence of carotenoids that contain great quantities of zeaxanthin, cryptoxanthin and lutein (a xanthophyll). Each poultry breed has a different ability to convert carotene pigments to egg yolk color[25]. Poultry that consume higher levels of carotenoid pigments produce egg yolks with more intense color[26].

Table 2 shows that the average eggshell thickness was not significantly affected by the feed treatments containing fermented jack beans. This result is because the calcium content of the fish powder used in the feed formulation is sufficient for eggshell formation and the treated feed formulations contained the same calcium and phosphorus content. Calcium and phosphorus are two minerals essential for eggshell formation. These results are consistent with those of Chantsavang *et al.*[27], who reported an average eggshell thickness 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 increased calcium retention is caused by an 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 eggshell formation in the quail. This balance does not produce the decreased effect on calcium requirements. Phosphorus metabolism in the body will only proceed if calcium and phosphorus are appropriately balanced[28].

The cholesterol levels in the egg yolks in this study were relatively low and the treatments exhibited average cholesterol levels that were significantly lower that the control. This finding was due to the presence of isoflavones in the fermented jack beans that reduced yolk cholesterol and increased egg production. This observation is consistent with the results of a study by Hertamawati[29], in which the addition of 0.5% isoflavone extract to quail feed reduced yolk cholesterol and increased egg production.

Isoflavones have a biological structure and function similar to phytoestrogens[30]. The phytoestrogens contained in fermented jack beans mimic the function of the hormone estrogen, which promotes the growth of the reproductive organs in preparation for egg formation. Phytoestrogens are plant-based substrates that have estrogen-like activities[31].

The isoflavone (phytoestrogen) content in fermented jack beans is believed to improve liver function and result in increased vitellogenin production. Vitellogenin stimulates an expansion of the hierarchy of ovarian follicles, such that the number of follicles increases. Vitellogenin is synthesized in the liver, enters the bloodstream and is absorbed by the cytoplasm of the oocyte[32]. Decreased LDL levels are thought to be related to the large number of follicular hierarchies that develop. If more follicles develop, yolk-forming substances such as cholesterol will be distributed to all follicles causing

the average egg cholesterol level to be reduced[33]. A decrease in the cholesterol levels of eggs is also caused by the hormone estrogen. Developing follicles produce estrogen, which suppresses the activity of HMG-CoA enzymes and inhibits cholesterol biosynthesis.

The use of fermented jack beans in the diets of laying quail can improve egg production and egg quality. Fermented jack beans can be added to the diet of laying quail at a level of22.5% of the total feed ingredients. Fermented jack beans should be added to the diets of laying quail over the age of 1 month. One limitation of this study is that the effect of the drying temperature on the nutrient composition during the process of producing fermented jack bean flour products has not yet been studied.

CONCLUSION

The use of fermented jack beans in laying quail feed formulations is safe up to the level of 22.5%. Feeds containing 22.5% fermented jack beans can increase egg weight and egg production and reduce the feed conversion values of quail and the cholesterol levels of quail eggs. Jack beans fermented by *R. oligosporus* can be used as food additives in layer quail feed formulations to increase the quantity and quality of quail eggs.

SIGNIFICANCE STATEMENT

This study discover the use of a 22.5% concentration of jack beans fermented by *R. oligosporus* in the feed of laying quail that can be beneficial to improve the production performance and egg quality of laying quail. This study will help the researcher to uncover the critical areas of investigation for the use of fermented jack beans in the feed formulations of laying quail that many researchers were not able to explore. Thus a new theory on the use of fermented jack beans as a food additive in layer quail feed formulations may be arrived at.

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