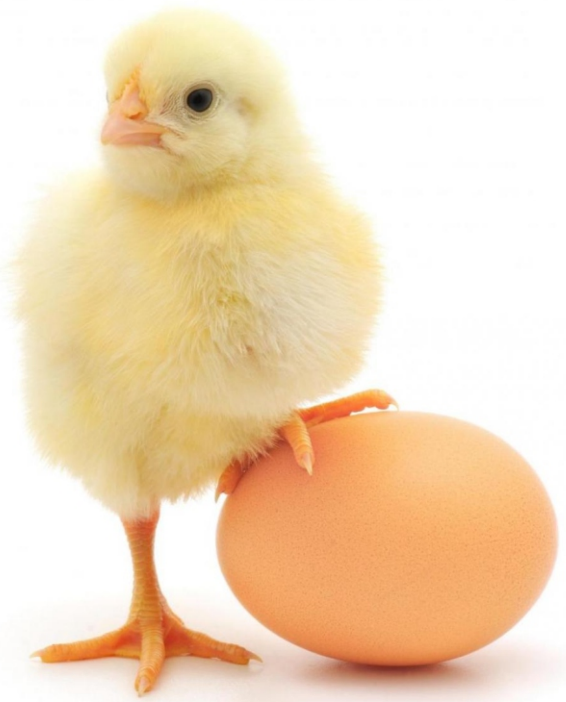
ISSN 1682-8356
ansinet.com/ijps



INTERNATIONAL JOURNAL OF POULTRY SCIENCE

OPEN ACCESS International Journal of Poultry Science

ISSN 1682-8356
DOI: 10.3923/ijps.2020.10.22

Research Article
Effect of Replacing Corn in Layer Quail Diets with Rice Husk Distillers' Dried Grain after Co-Culture Fermentation with *Saccharomyces cerevisiae* and *Candida tropicalis* on the Production Performance and Quality of Eggs

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Abstract
Background and Objective: The availability of ingredients as energy-rich feed sources, especially corn, is one of the obstacles in developing quail farms. The search for corn substitutes in feed formulations has received high attention from animal nutrition investigators. The present study aimed to explore the effect of rice husk distillers' dried grain (DDG) from a fermented co-culture with *Saccharomyces cerevisiae* and *Candida tropicalis* on the production performance and quality of quail eggs. **Materials and Methods:** The co-culture liquid fermentation of *S. cerevisiae* with *C. tropicalis* was carried out for 7 days using hydrolysed rice husk as feedstock at a temperature of 28-30°C and a relative humidity of 60-70% in the dark. A complete randomized block design was used to determine the effect of 6 proportions of rice husk DDG, namely, 0, 10, 20, 30, 40 and 50%, in quail feed formulations, each of which was repeated 5 times. After acclimatization and adaptation for 2 weeks, a total of 300 quails aged 35 days were divided into 6 groups and reared for 10 weeks in pens containing 10 birds each. Observations on mortality, body weight, feed consumption and egg production performance of the quails were carried out every week from the age of 49-104 days. Observations of the internal quality and nutritional composition of eggs was carried out at the age of 104 days. **Results:** The results of this study indicate that the proportion of rice husk DDG up to 50% in feed formulation has no significant ($p>0.05$) effect on the mortality, body weight, feed consumption, egg weight, albumen and yolk indexes, Haugh unit, protein content, total fat, carbohydrate content and quail egg total cholesterol but the amount of eggs, daily egg production, egg mass production and yolk egg colour from quails fed with 40-50% rice husk DDG was significantly ($p<0.05$) lower than those observed for quails fed with 0-30% rice husk DDG. The low energy content and high crude fibre in rice husk DDG are the main constraints for higher utilization of rice husk DDG or for replacing all corn in quail diets. **Conclusion:** This study concluded that rice husk DDG from a fermented co-culture with *S. cerevisiae* and *C. tropicalis* can be used to replace as much as 30% or 40-50% of corn in quail diets.

Key words: *Candida tropicalis*, distillers' dried grain, rice husks, *Saccharomyces cerevisiae*, quails

Citation: T. Sopandi and A. Wardah, 2020. Effect of replacing corn in layer quail diets with rice husk distillers' dried grain after co-culture fermentation with *Saccharomyces cerevisiae* and *Candida tropicalis* on the production performance and quality of eggs. Int. J. Poult. Sci., 19: 10-22.

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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 interest of Indonesian farmers in rearing quails for commercial marketing continues to increase, which is reflected in the increasing quail populations. The quail population in Indonesia over the past 5 years has continued to increase from 12,692 birds in 2014 to 14,877 birds in 2018[1]. However, the availability of energy-rich feed ingredients, especially corn, is one of the obstacles in developing quail farms in Indonesia.

Corn is the main choice in conventional poultry feed formulations and is used as a source of carbohydrates because of its high energy content, low fibre content, high palatability and pigment content and it contains several essential fatty acids[2]. The high, competitive demand for corn to meet the needs of the food and biofuel industries is increasing, causing its supply to be insufficient and the price to be expensive, thus reducing the efficiency of poultry production[3,4]. The scarcity and high price of corn cause quail farmers in Indonesia to often have difficulty formulating and making nutritionally sufficient rations. The increasing prices and limited supply of corn as a source of energy for animal feed have encouraged poultry nutrition experts to look for alternative energy sources to replace corn[5].

The exploration of inexpensive energy source ingredients is very important to reduce the cost of poultry feed[6], which can reach 70% of the total cost of feed[7]. On the other hand, the use of DDG for various purposes, including as an ingredient for livestock feed, is very important to maximize profits in the bioethanol industry[8]. Distillers' dried grain (DDG), as the main by-product of bioethanol production, is known to be good sources of protein, energy, vitamins, dissolved minerals and amino acids for poultry[9-11]. DDG from bioethanol production is generally used in poultry feed as a source of energy, protein, amino acids, vitamins and phosphorus and to reduce the use of corn, soybean meal and inorganic phosphorus[9-13]. However, due to the varied nutritional content of DDG[14-19], the proportion of its use differs in the formulation of feed that has been reported by previous investigators. Some investigators report that corn DDG can be added in poultry feed formulations up to 20% as long as the nutritional profile, especially amino acids, is sufficient[20-22]. Corn DDG was utilized in laying poultry feed at up to 15% of the total ration without negative side effects on the production and quality of eggs[23,24]. Moreover, other investigators report that the utilization of corn DDG as poultry feed is limited to only 5% because of the total low digestibility ratio and high levels of crude fibre[25]. Additionally, it was reported that corn DDG can be added in rations of approximately 9-15% without causing negative effects on the production performance of laying hens[17,23,26-29]. Substitution of 10% of corn with corn DDG in the feed formulation significantly improved the quality of eggs[30]. Utilization of 20% DDG corn in the ration was shown to reduce body weight during the maintenance of laying hens that were 30-42 weeks old[18]. Our previous results showed that the DDG of rice husks that underwent co-culture fermentation with *S. cerevisiae* and *C. tropicalis* can be used in the formulation of broiler chicken rations to replace 15% of the total rations. The aim of this study was to explore the effect of DDG from rice husks, a by-product of bio-ethanol production, after co-culture fermentation with *S. cerevisiae* and *C. tropicalis* on the production performance and quality of quail eggs.

MATERIALS AND METHODS

Preliminary treatment of rice husks: A total of 50 kg of rice husks was dried in the sun for 3 days, finely ground to 80 mesh and steamed at 130°C for 3 h. The rice husk powder was then soaked in a 500 L mixture of water and 2.5% sulfuric acid for 24 h. The acid-hydrolysed rice husks were washed twice with water to remove sulfuric acid and filtered and the residue was dried in an oven at 80°C to a constant weight. Dried, hydrolysed rice husks were immersed in 1% NaOH at a ratio of 1:10 for 24 h, washed 2 times with water to remove NaOH and filtered and the residue was dried in a cabinet dryer at a temperature of 80°C to a constant weight. Dried, hydrolysed rice husks were collected, homogenized and stored in a refrigerator at 10°C until use.

Microorganisms and culture conditions: Yeasts of *S. cerevisiae* and *C. tropicalis* were used in this study. Each yeast was maintained in potato dextrose agar media and were periodically rejuvenated every 3 months.

Fermentation: The fermentation process was carried out using liquid fermentation techniques such as those conducted by Sopandi and Wardah[31]. A total of 20 kg of hydrolysed rice husk flour was put into a 150 L plastic drum and 1.0 kg molasses, 1.0 kg urea, 60.0 g NaNO3, 100 g NH_4NO_3, 20.0 g KH_3PO_4, 14.0 g $MgSO_4{\bullet}7H_2O$ and sterile water until the volume reached 100 L were added to the mixture. The mixture was stirred and the pH was adjusted to 5.5 with the addition of NaOH. Then, the drum was tightly closed and left for 24 h. The mixture was inoculated with 2 L medium containing 10^6 spores mL^{-1} of *S. cerevisiae* and 10^6 spores mL^{-1} of *C. tropicalis*. The inoculates were incubated for 7 days at

28-30°C and a relative humidity of 60-70% in the dark. After fermentation, the medium was harvested and distilled at 70-75°C until thick and then dried in a cabinet dryer at 60°C until reaching a constant weight. Then, the fermented rice husks were ground in a mill to obtain DDG flour from the rice husk hydrolysate. The nutrient content of the DDG rice husk was chemically analysed to determine the dry weight, crude protein content, crude fat content, carbohydrate content and calcium and phosphorus levels according to protocols outlined by the Association of Official Analytical Chemists[32].

Feed formulation: The layer quail feed formulation was designed to replace part or all of the corn with rice husk DDG in their diets. All feed formulation ingredients were first proximately analysed[32] before being mixed in a dry state and formed into granules. A total of 6 feed formulations were evaluated in this study with different rice husk DDG proportions, namely, 0 (P_1), 10 (P_2), 20 (P_3), 30 (P_4), 40 (P_5) and 50% (P_6) rice husk DDG in the diets. Each feed formulation was analysed for proximate composition according to AOAC[32] recommendations, consisting of dry weight, crude protein, crude fat, carbohydrate, calcium and phosphorus determination.

Experimental design: The study was conducted using a completely randomized design with 6 treatments of rice husk DDG proportions (0, 10, 20, 30, 40 and 50%) in the quail diet, each of which was repeated 5 times. A total of 300 35-day-old female quails (*Coturnix coturnix japonica*) obtained from local breeders were randomly divided into 6 treatment groups. All quails were reared in groups in wire cages measuring 50×30×30 cm (l×w×h), each containing 10 birds for 10 weeks at 27-28°C with 18 h lighting. Each cage was provided with an automatic plastic feeder and drinker. Feeding and drinking was performed on an *ad libitum* basis.

Data collection: Data collection on the performance of quail day production (QDP), feed consumption (g bird^{-1}) and egg weight (g egg^{-1}) was carried out every week from 7 weeks (49-56 days) to 15 weeks (98-104 days). Data collection of the ratio of feed conversion, egg mass (egg production×egg weight), eggshell thickness and weight, albumen index, yolk index and egg yolk colour were carried out on day 104. Analysis of the egg protein, fat, carbohydrate and cholesterol content was carried out on day 104 using a composite of 3 eggs from each experimental unit. Yolk colour observations were carried out using a DSM yolk colour fan with a rating of 1 for pale yellow to 12 for dark orange[33]. Analysis of the quail cholesterol content was performed using KIT from DiaSys (Diagnostic System) with the Liebermann Burchard method[34].

Statistical analysis: All observations of the research variables underwent one-way variant analysis at a significance of 0.05 according to the complete randomized design using SPSS software version 20. Further tests were conducted using the Tukey honest difference test to determine the location of the differences between treatments if the treatment had a significant effect ($p<0.05$) on the observed variables.

RESULTS

The results of the proximate analysis in Table 1 showed that the metabolic energy content (2677.71 kcal g^{-1}) in rice husk DDG from the fermented co-culture by *S. cerevisiae* with *C. tropicalis* was lower than that of corn (3070.52 kcal g^{-1}). However, the crude protein content (9.23%), crude fat content (2.82%), carbohydrate content (46.24%), crude fibre content (12.34%) and phosphorus content (0.34%) in rice husk DDG were higher than those of corn 8.65, 2.58, 30.12, 2.42 and 0.28%, respectively. Replacing corn with rice husk DDG that underwent co-culture fermentation by *S. cerevisiae* with *C. tropicalis* in layer quail feed formulations (Table 2) shows that increasing the proportion of rice husk DDG reduced the metabolic energy content but increased the crude protein and crude fibre contents.

Mortality and body weight: The results in this study (Table 3) show that 100% replacement of corn with rice husk DDG or

Table 1: Chemical composition of ingredients for layer quail feed formulation

Ingredients	Metabolic energy* (kcal kg^{-1})	Crude protein (%)	Crude fat (%)	Carbohydrates (%)	Crude fibre (%)	Phosphorus (%)	Calcium (%)
Yellow corn	3070.52	8.65	2.58	30.12	2.42	0.280	0.020
Pollard	1356.19	15.81	4.33	12.50	7.82	1.290	0.130
Soybean meal	2240.78	20.45	0.98	21.22	6.34	0.650	0.270
Fish meal	2650.89	47.56	9.67	23.45	1.09	2.820	2.500
Rice husk DDG	2677.71	9.23	2.82	46.24	12.34	0.340	0.010
Meat bone meal	2848.58	42.67	10.53	25.66	4.56	5.130	10.300
Blood meal	5380.65	45.23	2.34	51.18	1.48	0.002	0.004

*Calculated

Table 2: Layer quail feed formulation

Ingredients	Feed formulations P_1	P_2	P_3	P_4	P_5	P_6
Yellow corn (%)	50.00	40.00	30.00	20.00	10.00	0.00
Soybean meal (%)	19.00	19.00	19.00	19.00	19.00	19.00
Rice husk DDG (%)	0.00	10.00	20.00	30.00	40.00	50.00
Meat bone meal (%)	10.00	10.00	10.00	10.00	10.00	10.00
Fish meal (%)	10.00	10.00	10.00	10.00	10.00	10.00
Pollard (%)	5.00	5.00	5.00	5.00	5.00	5.00
Blood meal (%)	6.00	6.00	6.00	6.00	6.00	6.00
Total	100.00	100.00	100.00	100.00	100.00	100.00
Nutrient composition						
Dry matter (%)	98.91	97.19	98.17	97.89	97.04	97.42
Crude protein (%)	20.74	20.80	20.85	20.91	20.97	21.03
Crude fat (%)	3.85	3.06	3.08	3.11	3.13	3.15
Crude fibre (%)	3.46	4.45	5.44	6.44	7.43	8.42
Calcium (%)	1.35	1.36	1.34	1.34	1.34	1.34
Phosphorus (%)	1.07	1.08	1.08	1.09	1.10	1.10
Metabolic energy* (kcal kg^{-1})	2901.60	2862.32	2823.04	2783.76	2744.48	2705.19

*Calculated, **All formulations contained a premix (0.4%) of Vitamin A: 3000 IU kg^{-1}, Vitamin D_3: 500 IU kg^{-1}, Vitamin E: 10 IU kg^{-1}, Vitamin K_3: 1 mg kg^{-1}, Vitamin B_1: 2 mg kg^{-1}, Vitamin B_2: 5 mg kg^{-1}, Vitamin B_6: 1 mg kg^{-1}, Vitamin B_{12}: 0.002 mg kg^{-1}, Vitamin C: 20 mg kg^{-1}, Ca-D-pantothenate: 0.48 mg kg^{-1}, Nicotinic acid: 15 mg kg^{-1} and Folic acid: 0.25 mg kg^{-1}

Table 3: The effect of replacing corn in the diet with rice husk DDG from a fermented co-culture with *S. cerevisiae* and *C. tropicalis* on mortality and live weights of the layer quails

Parameters	Feed formulation P_1	P_2	P_3	P_4	P_5	P_6
Mortality (%)	2.0	0.0	2.0	4.0	0.0	0.0
Initial live weight (g $bird^{-1}$) at 49 days	134.53±35.87	136.89±23.16	134.23±32.25	135.89±35.18	137.45±14.23	138.56±14.65
End live weight (g $bird^{-1}$) at 104 days	159.80±13.60	157.78±17.66	154.10±10.61	154.43±11.42	157.37±15.81	158.85±13.36
Average daily gain (g $bird^{-1}$ day^{-1})	0.45±0.33	0.39±0.32	0.31±0.15	0.35±0.19	0.39±0.28	0.41±0.28

use of 50% rice husk DDG fermented under co-culture conditions by *S. cerevisiae* with *C. tropicalis* in total quail diets had no significant effect (p>0.05) on mortality, body weight or average daily gain. Quail mortality found in this study was not caused by the effect of replacing corn with rice husk DDG because in the groups fed a proportion of 10, 40 or 50% rice husk DDG in feed formulations, there was no quail death.

Egg production performance: The results of this study indicate that the replacement of corn with rice husk DDG from a fermented co-culture with *S. cerevisiae* and *C. tropicalis* in layer quail diets had no significant effect (p>0.05) on the egg weight but had a significant effect (p<0.05) on the egg number, quail day production (QDP) and egg mass production. The quail egg weight found in this study ranged from 9.66-9.81 g egg^{-1}. The results of the study (Table 4) show that the QDP for each week observed in quails fed a proportion of 40 or 50% rice husk DDG was significantly (p<0.05) lower than that of the quails fed with 0, 10, 20 or 30% rice husk DDG. The number of eggs produced by quail fed a proportion of 40% rice husk DDG (5.49±1.89 eggs $bird^{-1}$ $week^{-1}$) and 50% rice husk DDG (2.29±1.99 eggs $bird^{-1}$ $week^{-1}$) was significantly (p<0.05) lower than that observed for quails fed a proportion of 0% (6.46±1.88 eggs $bird^{-1}$ $week^{-1}$), 10% (6.52±1.95 eggs $bird^{-1}$ $week^{-1}$), 20% (6.58±1.93 eggs $bird^{-1}$ $week^{-1}$) or 30% (6.52±1.95 eggs $bird^{-1}$ $week^{-1}$) rice husk DDG. Table 4 also shows that egg mass production in the groups fed a proportion of 40% (53.43±19.59 g $pens^{-1}$ day^{-1}) or 50% (52.02±0.65 g $pens^{-1}$ day^{-1}) rice husk DDG was significantly (p<0.05) higher than that observed for the groups fed 0% (63.19±20.81 g $pens^{-1}$ day^{-1}), 10% (63.82±20.36 g $pens^{-1}$ day^{-1}), 20% (64.25±20.27 g $pens^{-1}$ day^{-1}) or 30% (64.72±22.31 g $pens^{-1}$ day^{-1}) rice husk DDG. There was no significant difference (p>0.05) in the number and mass production of eggs between the feed formulations containing proportions of 0, 10, 20 and 30% rice husk DDG.

Feed consumption: The present study (Table 5) indicates that increasing the proportion of rice husk DDG in feed formulations tended to reduce feed consumption but without

Table 4: The effect of replacing corn in the diet with rice husk DDG from a fermented co-culture with *S. cerevisiae* and *C. tropicalis* on the production performance of the layer quails

	Feed formulation					
Parameters	P_1	P_2	P_3	P_4	P_5	P_6
Egg weight (g egg^{-1})	9.66±0.61	9.73±0.46	9.70±0.55	9.81±0.91	9.68±0.54	9.75±0.56
Number of eggs (eggs $bird^{-1}$ $week^{-1}$)	6.46±1.88^b	6.52±1.95^b	6.58±1.93^b	6.52±1.95^b	5.49±1.89^a	2.29±1.99^a
Quails day production (%) at age						
49-55 days	37.14±0.93^b	39.14±0.98^b	40.57±0.91^b	40.29±1.12^b	35.71±1.35^a	33.71±0.94^a
56-62 days	50.28±1.25^b	51.14±1.30^b	53.71±1.31^b	53.43±1.11^b	36.57±0.76^a	36.29±1.26^a
63-70 days	56.29±1.03^b	56.86±1.39^b	55.71±1.82^b	57.14±1.41^b	49.71±1.18^a	47.43±1.07^a
71-77 days	67.14±1.20^b	68.29±1.21^b	69.14±1.34^b	67.71±1.44^b	58.29±1.22^a	57.14±1.23^a
84-90 days	74.57±1.12^b	76.29±1.31^b	75.14±1.04^b	74.86±0.61^b	65.14±0.87^a	62.86±2.05^a
91-97 days	82.57±0.74^b	81.14±1.13^b	82.29±1.11^b	80.86±2.08^b	66.29±1.40^a	64.29±2.02^a
98-104 days	82.86±0.79^b	83.71±1.01^b	84.29±0.65^b	82.29±0.94^b	71.14±2.11^a	68.57±1.73^a
Egg mass production (g $pens^{-1}$ day^{-1})	63.19±20.81^b	63.82±20.36^b	64.25±20.27^b	64.72±22.31^b	53.43±19.59^b	52.02±0.65^b

Mean values and standard deviations with different superscript letters in the same row show significant differences (p<0.05)

Table 5: The effect of replacing corn in the diet with rice husk DDG from a fermented co-culture with *S. cerevisiae* and *C. tropicalis* on the consumption and feed conversion of layer quails

	Feed formulation					
Parameters	P_1	P_2	P_3	P_4	P_5	P_6
Feed consumption (g $bird^{-1}$ day^{-1})	18.70±1.15	18.63±0.99	18.69±1.13	18.53±1.14	18.51±1.04	18.47±0.96
Feed conversion (g g^{-1})	3.38±1.42^a	3.30±1.33^a	3.26±1.26^a	3.25±1.29^a	4.05±1.69^b	4.25±1.86^b

Mean values and standard deviations with different superscript letters in the same row show significant differences (p<0.05)

Table 6: The effect of replacing corn in the diet with rice husk DDG from a fermented co-culture with *S. cerevisiae* and *C. tropicalis* on quail egg quality

	Feed formulation					
Parameters	P_1	P_2	P_3	P_4	P_5	P_6
Eggshell weight (g egg^{-1})	1.99±0.11^b	2.09±0.18^b	2.02±0.19^b	1.95±0.18^b	1.90±0.17^a	1.81±0.09^a
Eggshell thickness (mm)	0.28±0.030^b	0.27±0.04^b	0.29±0.03^b	0.28±0.04^b	0.24±0.03^{ab}	0.22±0.02^a
Yolk index	41.31±0.71	41.19±1.69	40.95±1.36	41.80±1.83	40.11±0.33	40.26±0.70
Albumen index	9.80±0.43	9.57±0.86	9.64±0.97	9.96±0.90	9.50±1.03	10.11±1.18
Haugh unit	80.17±2.46	80.44±2.19	80.23±2.33	80.30±2.47	80.19±2.14	80.36±1.98
Yolk color	10.52±0.23^b	10.36±0.33^b	10.52±0.30^b	10.16±0.55^{ab}	9.64±0.17^a	9.60±0.32^a

Mean values and standard deviations with different superscript letters in the same row show significant differences (p<0.05)

Table 7: The effect of replacing corn in the diet with rice husk DDG on the nutrient composition of whole quail eggs

	Feed formulation					
Parameters	P_1	P_2	P_3	P_4	P_5	P_6
Protein (%)	12.01±1.15	13.17±1.64	11.85±1.76	13.73±2.33	11.99±1.81	12.41±1.27
Total fat (%)	9.16±1.18	8.73±1.59	8.44±0.74	7.54±1.82	7.85±1.63	8.18±1.28
Carbohydrates (%)	0.32±0.04	0.28±0.06	0.37±0.07	0.26±0.08	0.24±0.12	0.34±0.08
Total cholesterol (mg dL^{-1})	678.39±99.49	646.03±154.77	626.43±175.99	694.05±59.55	657.25±143.41	621.65±147.98

Mean values and standard deviations with no different superscript letters in the same row show no significant differences (p<0.05)

significance (p>0.05) up to the proportion of 50%. This study also shows that quail feed conversion with a proportion of rice husk DDG of 40-50% was significantly (p<0.05) higher than that of a proportion of 0-30%.

Egg quality: The present study (Table 6) shows that replacing corn with rice husk DDG from a fermented co-culture with *S. cerevisiae* and *C. tropicalis* up to a proportion of 40-50% in the diet significantly (p<0.05) reduced the weight and thickness of eggshells compared to those observed with no rice husk DDG supplementation. However, the results of this study (Table 6) show that replacing corn with rice husk DDG had no significant effect (p>0.05) on the albumen index, yolk index and Haugh unit. Table 6 also shows that the colour of the yolk from quails fed with a proportion of 40 or 50% rice husk DDG from a fermented co-culture with *S. cerevisiae* and *C. tropicalis* was significantly (P<.05) lighter than that observed in the yolks from quails fed a rice husk DDG proportion of 0-30%.

Chemical composition of whole egg quail: This study (Table 7) shows that replacing corn with rice husk DDG had no significant effect (p>0.05) on the protein, carbohydrate total fat and total cholesterol, contents of whole quail eggs.

DISCUSSION

The nutrient composition of rice husk DDG from a fermented co-culture with *S. cerevisiae* and *C. tropicalis* in this study (Table 1) was different from the results of our previous study[19]. The difference is thought to be due to differences in feedstock and fermentation conditions. Variability in DDG nutrient composition can be caused by a number of factors, including differences in processing technology between bioethanol plants and variability in the chemical composition of the feedstock[35-38].

The crude protein content in all quail feed formulations in this study was in accordance with the recommendations of NRC[14]. The metabolic energy content of the quail feed formulated in this study was in accordance with the recommendations of Shim and Vohra[39] and Prabakaran[40], who reported that the energy requirements of layer quails for tropical climates are 2550 kcal kg^{-1} and 2650 kcal kg^{-1}, respectively.

This study indicates that supplementing feed with rice husk DDG from a fermented co-culture with *S. cerevisiae* and *C. tropicalis* at a proportion of 50% or replacing all corn in the diet did not significantly influence quail mortality and quail weight. Live weights of quails in this study were within the range of quail weights reported by Randall and Bolla[41], which is 120-160 g $bird^{-1}$. However, the average live weight was relatively smaller than the live weight of quails that other investigators have previously reported. Bagh *et al.*[42] reported that the average weight of sexually mature (starting to lay eggs for the first time) quails aged 6 weeks in 3 strains of grey, brown and white quail were 173.79, 168.23 and 172.62 g $bird^{-1}$, respectively. The difference is thought to be due to genetic variation and maintenance management, including lighting, feed nutrition and different environmental conditions. Nasifar *et al.*[43] have reported that animal growth is influenced by their genetic makeup and the environment. Akbas *et al.*[44] reported that there was a high correlation between genetics and permanent environments and Japanese quail body weight. In addition, differences in quail weight can also be caused by differences in the age and weight of the mother eggs. Vali *et al.*[45] reported that there was a high positive correlation between weight and age in 2 Japanese quail strains.

The results in this study indicate that quails began to sexually mature at 49 days (Table 3). Thus, the quail body weight increased during the period from 49 days to 104 days but the increase was relatively low and there was no significant difference ($p>0.05$) between the diets. Growth rates vary considerably depending on the livestock type, sex, age, strain, rearing management, environmental temperature and quality and quantity of feed[46]. Female quails usually start laying eggs at the age of 52-60 days with a live weight of 115-125 g $bird^{-1}$. The weight increase continues to approximately 120-130 g $bird^{-1}$ and is partially stable at a weight of approximately 150-160 g $bird^{-1}$. Moreover, Arora and Samples[47] reported that quails gained weight in the laying period and stabilized after reaching weights of approximately 120-125 g $bird^{-1}$, sometimes stabilizing at approximately 140-150 g $bird^{-1}$. Farooq *et al.*[48] and Petek *et al.*[49] reported that quail weight was strongly influenced by the weight of the parent egg.

The weight range of the eggs in this study was smaller than the range of the weight of quail eggs reported by Randall and Bolla[41], which had an average of approximately 10 g egg^{-1} or 10% quail body weight. The difference is thought to be due to differences in quail age and quail live weight. Quail egg weight is influenced by quail age and increases with quail age[45,50-52].

This study indicates that egg production performance in quails fed a proportion of 40-50% rice husk DDG from a fermented co-culture with *S. cerevisiae* and *C. tropicalis* in the diet was significantly ($p<0.05$) lower than that of quails fed 0-30% rice husk DDG. Referring to the nutrient content of the feed formulation in Table 2, the low number of eggs, QPD and egg mass production in quails fed with a proportion of 40-50% rice husk DDG is thought to be due to the low energy and high crude fibre contents in rice husk DDG compared to those of corn. Some investigators have reported the effects of energy content and crude fibre content in feed on the performance of quail egg production. Fulfilment of the energy and protein requirements in feed is very important to obtain optimum quail production performance[53,54]. Some investigators recommended an energy content in layer quail feed of approximately 2700-3000 kcal kg^{-1} with crude protein 18-22%[14,54,55-57]. Filho *et al.*[58] reported that the metabolic energy requirements for maintaining European and Japanese quails decreased with an increase in temperature from 18-28°C. However, some investigators reported that decreasing the ratio of metabolic energy to crude protein in layer quails can disrupt egg production performance[59-61].

The QDP in this study was 84.29% lower than that reported by Bagh *et al.*[42], which was 87.67%. The difference in the highest egg production is thought to be due to differences in various factors that affect the performance of poultry production, including age and quail strains. Bagh *et al.*[42] reported that the mean QDP of 3 strains of *Coturnix coturnix japonica*, grey, brown and white, at 16 weeks were 66.31, 87.67 and 75.0%, respectively. Poultry performance is influenced by various factors, such as genetics, nutrition, health, rearing environment, management, age of poultry and egg weight at incubation[62,63].

The low feed efficiency in the quails fed a proportion of 40-50% rice husk DDG in this study is thought to be due to the effect of high levels of crude fibre. Araújo and Silva[64] reported that the use of feed containing high fibre can cause a decrease in feed efficiency in the layer period. In general, the flow rate of feed nutrients in the digestive tract and feed consumption is influenced by the type and formulation of the feed[65]. Poultry respond quickly to changes in feed fibre content through modification of intestinal length and organ weights to change feed rates in the digestive tract. Increasing the content of insoluble fibre in feed can reduce the length of the small intestine[66,67], reduce periventricular weight[68] and increase the weight and contents of the gizzard[69,70], which generally indicates the development of digestive tract functions[69,71]. Fibre will accumulate in the gizzard and inhibit the rate of feed flow in the proximal part of the gastrointestinal tract[68,69]. The presence of crude fibre particles can also reduce the flow of fine particles from the feed[72,73]. Sarıçiçek *et al*[74] reported that the high content of crude fibre in rations can reduce nutrient intake because the digestive tract of non-ruminant animals has limited ability to utilize crude fibre. Mateos *et al*[75] suggest using a moderate crude fibre content because a high crude fibre content in poultry feed can reduce energy consumption, increase digested transit rates, reduce nutrient digestibility and interfere with poultry performance. High levels of dietary fibre can increase the decay of intestinal epithelial cells, secretion of mucosa into the intestine and loss of endogenous amino acids[76]. Fibre content plays an important role in poultry feed and a minimum content is needed to maintain the physiological functions of the digestive tract[77]. High fibre consumption by monogastric animals can reduce nutrient utilization and low net energy value[78]. Roberts *et al.*[79] reported that an increased fibre content in feed can cause digestive disorders in feed nutrition and is generally associated with poor performance and decreased egg weight. Araújo and Silva[64] reported that the use of feed containing high fibre can cause a decrease in poultry weight gain at the end of the growth phase and egg production.

Referring to the nutritional composition of each feed formulation in Table 2, the lower weight and eggshell thickness of eggs from quails fed a proportion of rice husk DDG of 40-50% is thought to be due to the effect of a high crude fibre content in both feed formulations. The shell is formed in the eggshell gland (uterus) and consists of approximately 95% calcium carbonate in the form of calcite[80]. The calcium content in feed plays an important role in obtaining optimal egg production and quality[81]. Fibre can bind minerals in complex matrices and reduce mineral absorption in the digestive tract of poultry[82]. Eggshells are formed in the poultry uterus through the deposition of calcium carbonate in the membrane to make eggshells; therefore, poultry require calcium available in feed[83]. The strength and thickness of an eggshell is influenced by the level of calcium in the diet[84]. Calcium and phosphorus are needed in sufficient quantities because their excess or deficiency causes an inhibition of eggshell formation. Rath *et al.*[85] reported that higher fibre levels in poultry foods can reduce calcium absorption in the intestine. The quality of eggshells can be improved by supplementation of calcium in the feed[86]. The present study indicates that there was no significant difference ($p>0.05$) between the albumen index, yolk index and Haugh unit in replacing corn with rice husk DDG at proportions from 0-50%, presumably because there was no difference in the crude protein content in all feed formulations. Albumen is an important factor that influences the egg's internal quality[87] and albumen and Haugh units show egg quality[88,89]. The albumen height and Haugh unit are influenced by the viscosity and thickness of the albumen[90]. Protein and amino acid intake in feed significantly influences the egg Haugh unit[91]. Some investigators report that the albumen index, yolk index and Haugh units are affected by age[50-52] and the duration and temperature of storage[92].

This study (Table 6) shows that the colour of egg yolks from quails fed with a proportion of 40-50% rice husk DDG from a fermented co-culture with *S. cerevisiae* and *C. tropicalis* was lighter than that observed for egg yolks from quails fed 0-30% rice husk DDG. The decrease in yolk colour is thought to be due to the low carotenoid or xanthophyll content in rice husk DDG. Yolk colour is an important aspect of egg quality and is strongly influenced by xanthophyll content, such as lutein and zeaxanthin, in feed[93-95]. Poultry cannot synthesize egg yolk pigments; therefore, the colour of an egg yolk is highly dependent on the fat-dissolved pigments contained in the feed[96]. Ghazvinian *et al.*[56] reported that egg yolk colour was affected by carotenoid content in feed, which mostly came from xanthophylls and some carotene and cryptoxanthin. Corn is the main source of carotenoids, such as lutein, β-cryptoxanthin, zeaxanthin, α- and β-carotene and β-zeacarotene, in poultry feed[97,98]. Corn contains approximately 20 mg kg^{-1} of xanthophylls[95]. Xanthophylls can increase the yellow-red pigmentation in egg yolk layers[99,100]. Moreover, the distillers' dried grains with solubles (DDGS) nutrient content varies greatly between the different raw materials used, processing methods and the amount of solvents used[36,101]. Shin *et al.*[101] reported that the concentration of xanthophylls in corn DDGS varied from approximately 447-1,586 μg kg^{-1}. Bacchetti *et al*.[102] reported that the lutein content was approximately 231-496 μg kg^{-1} and the zeaxanthin content was approximately 1,762-2,183 μg kg^{-1} among 5 corn sources (*Zea mays* L.). Salim *et al*.[103] reported that the xanthophyll content in DDGS ranged approximately from 23.26-54.40 mg kg^{-1}. Roberson *et al*.[104] reported the large differences in xanthophyll content in 2 samples of corn DDGS, which were 29.75 and 3.48 mg kg^{-1}. Roberson *et al*.[104] also showed that the addition of 10% corn DDGS in a laying hen diet increased the colour of egg yolk. Lumpkins *et al*.[26] reported that supplementing feed with 15% corn DDGS did not affect the colour of yolk eggs.

This study indicates that replacing corn with rice husk DDG from a fermented co-culture with *S. cerevisiae* and *C. tropicalis* had no effect on the nutritional composition of the whole quail egg. The nutritional content (protein, carbohydrate and fat) of the whole quail eggs in this study was different from the results of previous studies that have been reported by several investigators. Rahmad and Wiradimadja[105] reported that the protein and fat contents of Japanese quail eggs were 13.1 and 11.1%, respectively, almost the same as those reported by Panda and Singh[106]. Dudusola[107] reported crude protein and crude fat contents in quail eggs of 11.98 and 11.91%, respectively. Tunsaringkarn *et al*.[108] reported carbohydrate, fat and protein contents in quail eggs of 4.01, 9.89 and 12.70%, respectively. Thomas *et al*[109] reported a protein content in quail eggs of 13.30%. The difference in protein and fat content in eggs is thought to be caused by the effects of genetic variation, feed and environmental maintenance. Clum *et al*.[110] suggested that variations in the cholesterol content and proximate composition (crude protein, crude fat, carbohydrate, ash and water contents) of eggs caused by differences in species or genetics, feed and the environment. The cholesterol content of whole quail eggs found in this study was approximately 621.65-694.05 mg dL^{-1}. The cholesterol content of the whole quail eggs in this study was different from the results of previous studies that have been reported by several investigators. Jalaludeen *et al*.[111] reported that the cholesterol content of quail eggs was 844 mg 100 g^{-1}. Fakai *et al*.[112] reported that the cholesterol content of *C. ypsilophora* quail eggs was 691.81 mg dL^{-1}. Wardah *et al*.[113] reported that the cholesterol content of quail eggs was 364 mg g^{-1}. The egg cholesterol content is mainly regulated endogenously (de novo synthesis) and is almost independent of feed treatment[114]. Changes in quail egg cholesterol content can also be caused by phytogenic administration in feed, such as supplementation of powdered *Phyllanthus buxifolius* leaves[113]. Egg quality is influenced by a number of internal factors, such as genetics, age and egg-laying period and external factors, such as nutrient levels, microclimate parameters and maintenance management[115].

In general, the maximum proportion of DDG in poultry feed found in this study was different from some previous study reports. Swiatkiwicz and Koreleski[23] reported that the proportion of corn DDGS in a layer hen diet was a maximum of 15%. Salim *et al*.[103] and Sun *et al*.[116] reported that the proportion of corn DDGS in a poultry diet was a maximum of 20%. Dinani *et al*.[117] reported that the proportion of corn DDGS in a poultry diet was a maximum of 7.5-10%. El-Hack *et al*.[118] reported that the proportion of corn DDGS in a layer hen diet was a maximum of 12%. Sopandi and Wardah[31] reported that rice husk DDG from a fermented co-culture with *S. cerevisiae* and *C. tropicalis* could be utilized in broiler diets at a proportion of 15%. The difference was thought to be due to differences in poultry species, DDG feedstock, fermenter microorganisms, composition of ingredients, including the nutrient content of dietary feed, rearing management and environmental conditions.

CONCLUSION

This study concludes that rice husk DDG fermented by a co-culture with *S. cerevisiae* and *C. tropicalis* can be utilized in quail diets up to a proportion of 30% without causing adverse effects on egg production and quality performance. The low metabolic energy content and high crude fibre in rice husk DDG are the main limiting factors to replacing all corn in the quail diet. Efforts to reduce crude fibre and increase the metabolic energy content and carotenoids in rice husks DDG still need to be done to improve the utilization of DDG as animal feed, especially to replace corn.

ACKNOWLEDGMENTS

This study was supported in part by a 2019 Competency Research Grant from Direktorat Riset dan Pengabdian Masyarakat and by Kementerian Riset, Teknologi dan Pendidikan Tinggi, Indonesia.

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