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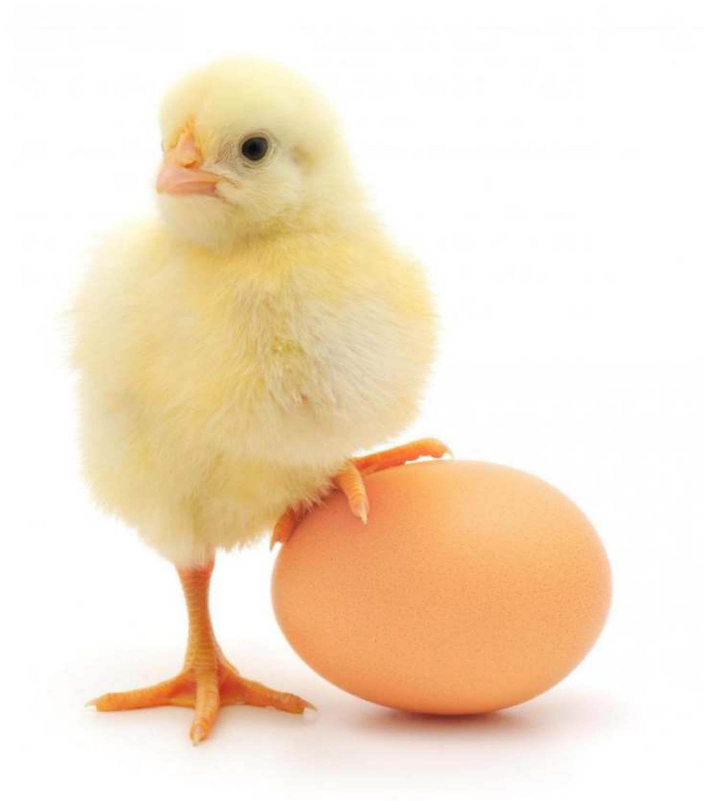

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Research Article
Production Performance and Carcass Percentage of Broilers Fed Distillers Dried Grain From Rice Husks With Co-culture Fermentation of *Saccharomyces cerevisiae* with *Candida tropicalis*

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Abstract
Background and Objective: Distillers dried grains of bioethanol production can be used as an alternative source of energy and protein for poultry feed. Sources of raw material for energy are very important to reduce the cost of poultry feed. The present study aimed to find suitable proportion of distillers dried grains from a rice husk with co-culture fermentation of *Saccharomyces cerevicea* with *Candida tropicalis* as feed ingredients for broiler chickens to replace corn. **Materials and Methods:** One hundred day-old chicks (DOC) were used in the present study. The experiment used a completely randomized design (CRD) with 6 treatments and different replications. Six formulations of treatment feed were made for the starter and finisher periods, each consisting of 0, 5, 10, 15, 20 and 25% rice husk DDG in broiler chicken rations. In the present study, the composition of DDG nutrients and formulated feed were analyzed according to AOAC recommendations. **Results:** Corn substitution with rice husk DDG up to 15% does not negatively affect the growth and weight of harvest, feed consumption and feed conversion, carcass, liver organ and gastrointestinal tract percentage and nitrogen retention of broiler chicken. However, replacement of corn with 20-25% of rice husk DDG can decreases growth and harvest weight, feed conversion and nitrogen retention of broiler chicken. **Conclusion:** The rice husk DDG can be used as a formulation material and replaces 15% of corn in broiler chicken rations.

Key words: Distillers dried grain, rice husk, *Saccharomyces cerevicea*, *Candida tropicalis*, broiler chicken, ration

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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

Lignocellulose biomass is an ideal raw material for the production of bioethanol as a liquid fuel that can be ordered[1]. Conversion of lignocellulose biomass including agricultural waste to bioethanol is an important choice for exploiting alternative energy sources and reducing air pollution[2,3].

The development of the bioethanol industry can produce by-products that are quantitatively potential as raw material for other industries including the animal feed industry. Utilization of distillers dried grains for various purposes including as raw material for livestock is very important to maximize the benefits of the bioethanol industry[4]. Distillers dried grains as the main by-product of bioethanol production are known to be sources of protein, energy, water soluble vitamins and minerals and good amino acids for poultry[5-7]. Distillers dried grains of bioethanol production can be used as an alternative source of energy and protein for poultry feed[8]. The previous study indicated that co-culture *Saccharomyces cerevicea* with *Candida tropicalis* can produce bioethanol from rice husk[9,10]. However, research on the potential use of distillers dried grains from rice husk fermentation by co-culture *S. cerevicea* with *C. tropicalis* as animal feed, especially poultry has never been carried out. Sources of raw material for energy are very important to reduce the cost of poultry feed[11]. Feed energy costs has reached 70% of the total cost of feed[12]. Distillers dried grain is generally used in poultry feed which functions as dietary energy, digestible amino acids and bioavailable phosphorus and can reduce the use of corn, soybean meal and inorganic phosphorus[13]. Increasing prices and non-availability of corn as a source of feed energy encourage poultry nutritionists to look for alternative energy sources for corn[14]. The present study aimed to find the suitable proportion of distillers dried grain from rice husk with co-culture fermentation of *S. cerevisiae* with *C. tropicalis* as feed ingredients for broiler chickens to replace corn.

MATERIALS AND METHODS

The present study was conducted at the Laboratory of Animal Physiology, Department of Biology, Faculty of Mathematical and Natural Science, University of PGRI Adi Buana Surabaya, Indonesia, from April-June 2018.

Preliminary treatment of rice husks: Local farm-sourced rice husks from Sidoarjo, Indonesia were air dried for 2 days and then ground to approximately 2-mm-diameter particles using a grinder mill. The milled rice husks were steamed at 130°C for 3 h, cooled at room temperature, mixed with 0.25% H_2SO_4 and autoclaved for 15 min at 121°C. The rice husk hydrolysate was cooled and stored at 1-5°C in the dark until it was used.

Fermentation: The rice husks hydrolyzate is dissolved in water, filtered in cotton cloth and the filtrate is dried. A total of 5 kg of rice husks hydrolyzate was added to a 100 L plastic drum added 1.0 molasses, 1.0 kg fishmeal, 60.0 g NaNO3, 100 g NH_4NO_3, 20.0 g KH_3PO_4 and 14.0 g MgSO4• $7H_2O$ and sterile water until the volume reached 100 L. The mixture was then stirred and the pH of the medium was adjusted by adding NaOH until the pH reached 5.5, tightly closed and left for 24 h. The mixture of medium was inoculated with 2 L of starter culture containing 10^6 mL^{-1} of *S. cerevisiae* and 10^6 mL^{-1} of *C. tropicalis spores*. The inoculated media was incubated for 7 days at 28-30°C and 60-70% relative humidity in the dark. After fermentation, medium was harvested and distilled at 70-75°C until thick and dried at 60°C, ground in miller and then sieved to obtain DDG meal. The fermentation process with new media is carried out repeatedly until distillers dried grain reaches approximately 75 kg. Distillers dried grain (DDG) rice husks was analyzed for proximate composition in accordance with Association of Official Analytical Chemists (AOAC)[15] recommendations consists of dry matter, crude protein, crude fat, carbohydrates, calcium and phosphorus.

Feed formulation: Broiler rations were formulated to replace part of the corn with rice husk DDG according to the range of broiler nutritional requirement as recommended by National Research Council (NRC)[16]. All feed ingredients in dry conditions were mixed and made in the form of granules for starter broiler (days 1-20) and pellets for the finisher period (days 21-42). A total of 6 feed formulations for starter and finisher broiler were made in this study with different proportion of rice husk DDG 0 (A), 5 (B), 10 (C), 15 (D), 20 (E) and 25% (F). Each feed formulation was analyzed for proximate composition in accordance with AOAC[15] recommendations consists of dry matter, crude protein, crude fat, carbohydrates, calcium and phosphorus.

Experimental design: This study was conducted in a completely randomized design (CRD) with 6 treatments of feed formulations (0, 5, 10, 15, 20 and 25% DDG in diets) and different replications. One hundred day old chicks (DOC) were randomly divided into 6 groups. The first four groups (A, B, C and D) each consisted of 17 heads broilers and 2 second groups (E and F) each consisted of 16heads. Each group was given formulated feeds (0, 5, 10, 15, 20 and 25% DDG) for both the starter and finisher period feed. All chickens were kept in individual cages bamboos (30×30×30 cm) for 42 days at 27-28°C. The birds were vaccinated at 4 days (eye drops), 14 and 21 days (intramuscular) against Newcastle disease. Each cage was equipped with a plastic feeder and a plastic drinker. The experimental diets and water were offered for *ad libitum* consumption.

Data collection: Collected data were feed intake (kg head^{-1}), live weight (kg head^{-1}), feed conversion, carcass percentage (% live weight head^{-1}), liver organ (% live weight head^{-1}), gut tract (% live weight head^{-1}) and nitrogen retention (%) of broilers. Feed intake and live weight of broiler were recorded weekly to assess feed conversion. Carcass, liver organ, gastrointestinal tract percentage were recorded at 41 days and nitrogen retention (%) was recorded at 40 days of rearing.

Data analysis: All observational data were analyzed using one-way analysis of variance for complete randomized design followed by Tukey's test to determine differences among treatment means at 5% level of significance (p<0.05).

RESULTS

Nutritional composition of rice husk DDG: Nutritional composition of DDG from rice husks with co-culture fermentation of *S. cerevisiae* with *C. tropicalis* is shown in Fig. 1. The rice husk DDG shows high carbohydrate (57.51%) but low in crude protein (9.43%) content. DDG rice husk also shows high fat, calcium and phosphorus content.

Formulation and nutrient ration: Ingredient and nutritional composition of the experimental diets fed to broilers from 1-21 days of age is shown in Table 1. Gradual replacement of corn with rice husk DDG in starter period of ration formulations decreased crude protein levels but increased carbohydrates, crude fat and energy.

Ingredient and nutritional composition of the experimental diets fed to broilers from 22-42 days of age is shown in Table 2. Like the starter period ration, the gradual

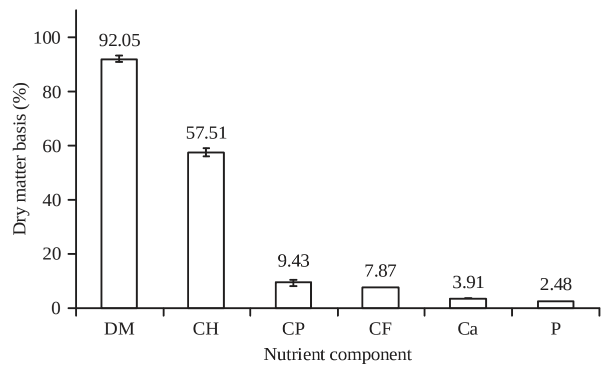

Fig. 1: Nutrient composition of rice husk DDG

DM: Dry matter, CH: Carbohydrate, CP: Crude protein, CF: Crude fat, Ca: Calcium, P: phosphorus

Table 1: Ingredient and nutritional composition of the experimental diets fed to broilers from 1-21 days of age (starter period)

	Formulation					
Ingredients	A	B	C	D	E	F
Rice bran (%)	1.00	1.00	1.00	1.00	1.00	1.00
Yellow corn (%)	50.00	45.00	40.00	35.00	30.00	25.00
Soybean meal (%)	10.00	10.00	10.00	10.00	10.00	10.00
Pearl millet (%)	1.00	1.00	1.00	1.00	1.00	1.00
Distiller dried grain (%)	0.00	5.00	10.00	15.00	20.00	25.00
Local fish meal (%)	25.00	25.00	25.00	25.00	25.00	25.00
Meat bone meal	11.50	11.50	11.50	11.50	11.50	11.50
Topmix (%)*	1.40	1.40	1.40	1.40	1.40	1.40
Bone meal (%)	0.10	0.10	0.10	0.10	0.10	0.10
Nutritional composition						
Crude protein (%)	22.24	21.96	20.92	19.68	19.64	19.27
Crude fat (%)	3.19	3.69	3.75	3.97	4.05	4.15
Carbohydrate (%)	46.27	49.08	49.25	49.33	49.72	51.66
Calsium (%)	2.18	2.24	2.29	2.34	2.4	2.45
Phosphorus (%)	1.66	1.69	1.73	1.76	1.79	1.82
Energy (kcal kg^{-1})**	3027.50	3173.70	3144.30	3117.70	3138.90	3210.70

*Topmix composition per kg as follows; Vitamin A: 1,000,000 IU, Vitamin B12: 400 mg, Vitamin D: 100,000IU, DL-methionine: 22,700 mg, Vitamin E: 700 mg, Antioxidant: 12,500 mg, Vitamin K3: 100 mg, Mg: 5.000 mg, Vitamin B1: 100 mg, Fe: 1,000 mg, Vitamin B2: 600mg, Cu: 200 mg, Vitamin B6: 50 mg, Mn: 1,500 mg, Niacin1: 000 mg, Zn: 1,000 mg, Panthothenic acid: 50 mg, Iodine: 10 mg and Choline: 1.000 mg, **Calculated

Table 2: Ingredient and nutritional composition of the experimental diets fed to broilers from 2-42 days of age (finisher period)

	Formulation					
Ingredients	A	B	C	D	E	F
Rice bran (%)	2.50	2.50	2.50	2.50	2.50	2.50
Yellow corn (%)	60.00	55.00	50.00	45.00	40.00	35.00
Soybean meal (%)	11.00	11.00	11.00	11.00	11.00	11.00
Pearl millet (%)	3.00	3.00	3.00	3.00	3.00	3.00
Distiller dried grain (%)	0.00	5.00	10.00	15.00	20.00	25.00
Local fish meal (%)	20.00	20.00	20.00	20.00	20.00	20.00
Meat bone meal	2.00	2.00	2.00	2.00	2.00	2.00
Topmix (%)*	1.40	1.40	1.40	1.40	1.40	1.40
Bone meal (%)	0.10	0.10	0.10	0.10	0.10	0.10
Nutritional composition						
Crude protein (%)	19.56	19.72	18.58	18.49	18.76	18.15
Crude fat (%)	2.98	4.17	4.39	4.53	4.51	4.69
Carbohydrate (%)	49.31	52.08	55.97	57.68	58.78	61.06
Calsium (%)	2.20	2.78	2.91	3.29	3.53	3.71
Phosphorus (%)	1.25	1.48	1.74	1.82	1.95	2.14
Energy (kcal kg^{-1})**	3023.00	3247.30	3377.10	3454.50	3507.50	3590.50

*Topmix composition per kg as follows; Vitamin A: 1,000,000 IU, Vitamin B12: 400 mg, Vitamin D: 100,000IU, DL-methionine: 22,700 mg, Vitamin E: 700 mg, Antioxidant: 12,500 mg, Vitamin K3: 100 mg, Mg: 5.000 mg, Vitamin B1: 100 mg, Fe: 1,000 mg, Vitamin B2: 600 mg, Cu: 200 mg, Vitamin B6: 50 mg, Mn:1,500 mg, Niacin: 1,000 mg, Zn: 1,000 mg, Panthothenic acid: 50 mg, Iodine: 10 mg and Choline: 1.000 mg, **Calculated

Table 3: Live weight of broiler chickens fed different proportion of rice husk DDG

Proportion of		Live weight (kg $head^{-1}$) of the week					
rice husk DDG (%)	Live weight DOC (kg $head^{-1}$)	1	2	3	4	5	6
0	0.03^{a}	0.13^{a}	0.32^{a}	0.55^{a}	0.96^{a}	1.48^{a}	1.76^{a}
5	0.03^{a}	0.12^{a}	0.33^{a}	0.52^{a}	0.95^{a}	1.49^{a}	1.78^{a}
10	0.04^{a}	0.12^{a}	0.34^{a}	0.51^{a}	0.93^{a}	1.46^{a}	1.74^{a}
15	0.03^{a}	0.11^{a}	0.31^{a}	0.50^{a}	0.92^{a}	1.51^{a}	1.73^{a}
20	0.04^{a}	0.11^{a}	0.29^{a}	0.49^{a}	0.88^{ab}	1.49^{a}	1.71^{a}
25	0.03^{a}	0.09^{a}	0.29^{b}	0.46^{b}	0.85^{b}	1.39^{b}	1.62^{b}

Table 4: Feed intake of broiler chickens fed different proportion of rice husk DDG

Proportion of	Accumulation feed intake (kg $head^{-1}$) at week					
rice husk DDG (%)	1	2	3	4	5	6
0	0.133	0.394	0.743	1.469	2.708	3.467
5	0.126	0.422	0.723	1.501	2.771	3.524
10	0.125	0.432	0.719	1.460	2.759	3.428
15	0.120	0.400	0.760	1.500	2.929	3.495
20	0.122	0.389	0.794	1.505	2.950	3.642
25	0.119	0.320	0.759	1.496	2.794	3.532

Data presented as the means of 16-17 replication (N = 16-17 bird each)

replacement of corn with rice husk DDG in the finisher period of ration formulation decreased crude protein content but increased carbohydrate, crude fat and energy.

Live weight: Present study showed that the use of rice husk DDG in the experimental ration significantly ($p<0.05$) decreased the live weight of broiler chickens. At ages 3-6 weeks (Table 3), the live weight of broiler chickens fed 25% of rice husk DDG was significantly ($p<0.05$) lower than those fed 0, 5, 10, 15 and 20% of rice husk DDG. There were no significant ($p>0.05$) differences in live weight of broilers fed 0, 5, 10, 15 and 20% of rice husk DDG but 25% was significantly ($p<0.05$) lower than those fed the other proportions (0, 5, 10, 15 and 20%) of rice husk DDG.

Feed intake: Table 4 shows feed intake of broiler chicken fed different proportion of rice husk DDG ration. Feed intake of broiler chicken was not significantly ($p>0.05$) affected by the different proportion of rice husk DDG.

Feed conversion: Table 5 shows that feed conversion ratio was significantly ($p<0.05$) affected by the different proportion

Table 5: Feed conversion of broiler chickens fed different proportion of rice husk DDG

Proportion of rice husk DDG (%)	Feed convertin at week 1	2	3	4	5	6
0	1.02^{a}	1.23^{a}	1.35^{a}	1.53^{a}	1.83^{a}	1.97^{a}
5	1.05^{a}	1.28^{a}	1.39^{a}	1.58^{a}	1.86^{a}	1.98^{a}
10	1.04^{a}	1.27^{a}	1.41^{a}	1.57^{a}	1.89^{a}	1.97^{a}
15	1.09^{a}	1.29^{a}	1.52^{a}	1.63^{a}	1.94^{a}	2.02^{a}
20	1.11^{a}	1.34^{ab}	1.62^{ab}	1.71^{b}	1.98^{ab}	2.13^{b}
25	1.13^{a}	1.39^{b}	1.65^{b}	1.76^{b}	2.01^{b}	2.18^{b}

Data presented as the means of 16-17 replication (N = 16-17 bird each). $^{a-b}$values in the same column with different superscripted letters are significantly different (p<0.05)

Table 6: Nitrogen retention of broiler chickens fed different proportion of rice husk DDG in ration

Proportion of rice husk DDG (%)	Nitrogen consumption (kg)	Nitrogen in faeces (kg)	Nitrogen retention
0	0.085^{a}	0.048^{a}	56.47^{a}
5	0.087^{a}	0.049^{a}	56.32^{a}
10	0.082^{a}	0.046^{a}	56.10^{a}
15	0.087^{a}	0.048^{a}	55.17^{a}
20	0.089^{a}	0.049^{a}	55.06^{a}
25	0.081^{b}	0.044^{b}	54.32^{b}

Data presented as the means of 6 replication (N = 16-17birds each). $^{a-b}$Values in the same column with different superscripted letters are significantly different (p<0.05)

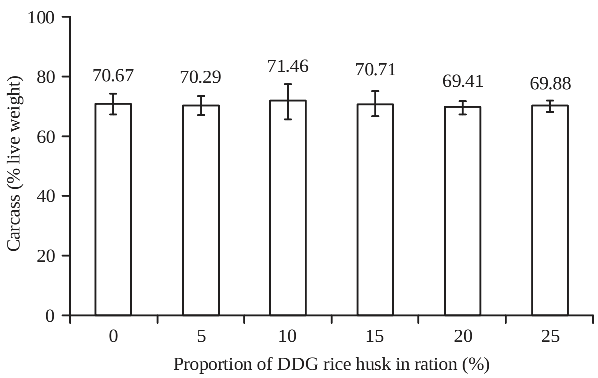

Fig. 2: Carcass percentage of broiler chicken fed rice husk DDG in ration

Data presented as the means of 16-17 replication (N = 16-17 bird each)

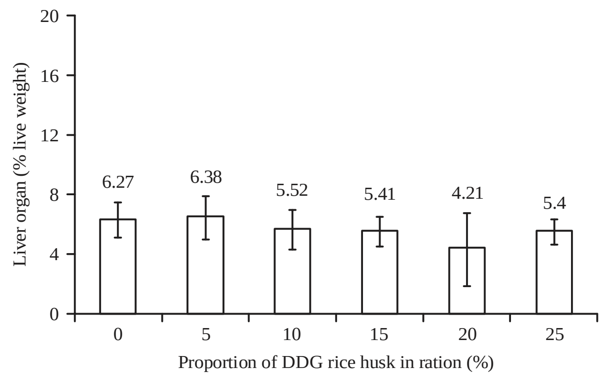

Fig. 3: Liver organ percentage of broiler chicken fed rice husk DDG

Data presented as the means of 16-17 replication (N = 16-17 bird each)

of rice husk DDG in the ration. There were no significant (p>0.05) differences in feed conversion ratio of broilers fed 0, 5, 10 and 15% of rice husk DDG but 25% was significantly (p<0.05) higher than those fed 0, 5, 10 and 15% of rice husk DDG. Feed conversion ratio of broiler fed 20% of rice husk DDG at week 2, 3 and 6 was not significantly (p>0.05) different from those fed 0, 5, 10, 15, 20 and 25%. However, at weeks 4 and 6, feed conversion ratio of broiler fed 20 and25% of rice husk DDG was significantly (p<0.05) higher than those fed 0, 5, 10 and 15% of rice husk DDG.

Carcass percentage: Figure 2 shows carcass percentage of broiler chicken fed different proportion of rice husk DDG in ration. The carcass percentage of broiler chicken was not significantly (p>0.05) affected by the proportion of rice husk DDG.

Liver organ: Figure 3 shows liver organ percentage of broiler chicken fed different proportion of rice husk DDG in ration. The liver organ percentage of broiler chicken was not significantly (p>0.05) affected by the different proportion of rice husk DDG in ration.

Gastrointestinal tract: Figure 4 shows gastrointestinal tract of broiler chicken fed different proportion of rice husk DDG in ration. The gastrointestinal tract of broiler chicken was not significantly (p>0.05) affected by the different proportion of rice husk DDG in ration.

Nitrogen retention: Table 6 shows that nitrogen retention was significantly (p<0.05) affected by the different proportion of rice husk DDG in the ration. There were no significant (p>0.05) differences in nitrogen retention of broilers fed 0, 5,

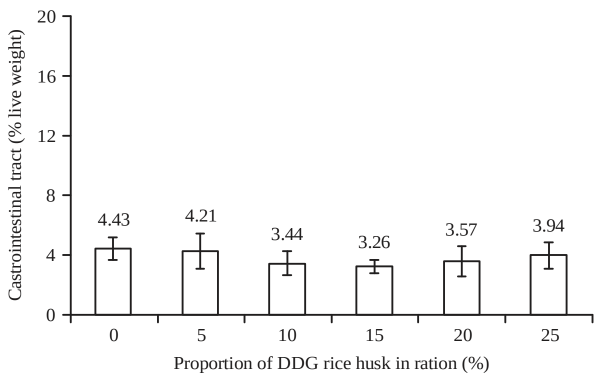

Fig. 4: Gastrointestinal tract percentage of broiler chicken fed rice husk DDG in ration
Data presented as the means of 16-17 replication (N = 16-17 bird each)

10 and 15% of rice husk DDG but 25% was significantly ($p<0.05$) lower than those fed 0, 5, 10, 15 and 20% of rice husk DDG.

DISCUSSION

The present study indicates that the crude protein content of DDG from rice husks with co-culture fermentation of *S. cerevisiae* with *C. tropicalis* is low but the content of energy, calcium and phosphorus are high. The calculated rice husk DDG energy content is 3385.50 kcal kg^{-1}. Some researchers reported variations in the composition of DDG nutrients. Shurson *et al.*[17] reported that corn DDG contained 29.2% crude protein, 3065 kcal kg^{-1} metabolic energy, 0.04% calcium and 0.83% phosphorus. Ning *et al.*[11] reported that distiller dried grain with soluble (DDGS) corn contented 27.81% crude protein and 4.94 Mcal kg^{-1} metabolic energy. Meanwhile, wheat bran DDGS contained 18.82% crude protein and 4.09 Mcal kg^{-1} metabolic energy. Distiller dried grain of corn contained 89.48-94% dry matter[16,18,19], 23.0-53.39% crude protein[1-21] and 2146-3554 kcal kg^{-1} metabolic energy[16,19,22,23]. Belyea *et al.*[24] and Shurson *et al.*[25] reported that the composition of DDG nutrients varied greatly and influenced by fermentation processes, raw materials and microorganisms. The present study indicated that the nutritional composition of all experimental feed formulations was in accordance with the range of nutritional composition recommended by NRC[16]. However, the replacement of corn by rice husk DDG decreased the crude protein content but increased the metabolic energy content of the experimental ration. The high proportion of rice husk DDG to replace corn produced low levels of crude protein and high metabolic energy in rations. In this study, it was assumed that the crude protein content in rice husk DDG was lower than the yellow corn. Lalujan *et al.*[26] reported that the crude protein content of the local yellow corn was 10-11%. Sudiastra and Suasta[27] reported that the crude protein of corn yellow was 14.35%. In this study, it was assumed that the metabolic energy content in rice husk DDG was higher than the yellow corn. Sudiastra and Suasta[27] also reported that the metabolic energy of yellow corn was 3294 kcal kg^{-1}.

The present study indicated that replacing corn with 15% rice husk DDG did not reduce the growth performance and harvest weight of broiler chickens. Decreased growth of broiler chickens appeared on corn replacement by 20-25%. This decrease occurs due to a decrease in crude protein levels in the ration. The proportion of DDG in broiler chicken rations that has been reported by several researchers varies. Cortes-Cuevas *et al.*[28] reported that the use of 6 or 12% corn DDG did not significantly effect the production performance of broiler chicken. The use of corn DDG in broiler and laying rations can reach 15%[7,29]. The corn DDG can be used in poultry feed up to 20% as long as nutritional profiles, especially amino acids, are sufficient in the ration[30-32].

This study indicated that corn replacement with 5-25% rice husk DDG does not significantly influence on feed intake of broiler chicken. However, replacing corn with 25% rice husk DDG significantly increased feed conversion. The results of this study are different from Thacker and Widyaratne[33] who reported that no significant differences were observed in feed intake and feed conversion ratio of broiler chickens fed 20.0% wheat DDGS. However, Wang *et al.*[5] reported that feeding 25.5% DDGS increased feed intake and decreased feed conversion ratio.

The present study indicated that the replacement of corn with 5-25% rice husk DDG did not affect negatively on the carcass, liver organ and gastrointestinal tract percentage of broiler chicken. These results are in agreement with Wang *et al.*[5] who reported that birds fed diets with 15% DDGS did not differ significantly in dressing percentage. Also Choi *et al.*[34] reported that, there was no negative effect of DDGS supplementation up to 15% on meat qualities.

The present study indicated that replacing corn with 25% rice husk DDG decreased nitrogen retention. This decrease was due to the lower crude protein content in the 25% rice husk DDG formulation than the other proportions (0, 5, 10, 15 and 20%). Low nitrogen consumption causes low nitrogen retention in broiler chickens. In previous studies, Leytem *et al.*[35] and Applegate *et al.*[21] has also reported linear decreases in nitrogen retention with increasing wheat DDGS levels in broiler diets.

In general, this study agrees with several previous studies which reported that the 15% of DDG in the diet did not have a negative effect on growth performance, carcass percentage and nitrogen retention of broiler chicken. Previous studies[5,36,37] agreed with the use of 15% DDG in the diet of broiler chicken.

CONCLUSION

Feeding 15% of rice husk DDG in rations does not have a negative effect on production performance and the carcass percentage of broiler. The distiller dried grain from rice husk with co-culture fermentation of *S. cerevisiae* with *C. tropicalis* can replace 15% of corn in broiler chicken rations.

SIGNIFICANCE STATEMENT

This study discovers that distiller dried grain from rice husk with co-culture fermentation of *S. cerevisiae* with *C. tropicalis* can utilize as feedstock for the preparation of broilers diet. This study will help the researchers to uncover the critical area of using distiller dried grain in broiler diet as feed stock.

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