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International Journal of Animal Science, Husbandry and Livestock Production ISSN: 2141-5191 Vol. 5 (3)pp. 264-271 August 2019 . Available online at www.advancedscholarsjournals.org © Advanced Scholars Journals

Full length Research paper

Performance and egg yolk quality of laying hens fed rice bran of road paddy milling fermented by effective microorganisms (EM4)

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Accepted 22 July, 2019

The objective of this study was to evaluate feeding with road paddy milling rice bran (FRPMRB) fermented by effective microorganisms (EM4) on laying performance and egg yolk quality. A total of 150 laying hens were allocated to 5 groups of 6 replications, raised in individual cages from 25 to 32 weeks of age. Five dietary treatments were assigned, T0 (standard diet); T1 to T4 were standard diets with 5, 10, 15 and 20% FRPMRB. Egg production was higher (p<0.05) in 10 and 15% FRPMRB than 5 and 20% levels although similar to the control diet. However, feed intake, feed utilization and egg weight did not differ among the treated birds. Egg yolk cholesterol levels were not affected by the treatments (p>0.05). Diets fermented by EM4 produced a similar cost of production to the control diet. The inclusion of 15% FRPMRB offered the highest income over feed cost of US$0.437 per kg of eggs compared with other diets.

Key words: Egg production, egg yolk cholesterol, fermented rice bran, laying performance, road paddy milling,

INTRODUCTION

Rice bran is one of the raw materials for poultry rations. As an agricultural by –product, it is usually purchased by farmers in the form of fine rice bran from the common rice mills. However, this rice bran is too expensive even unaffordable for small growers. Alternatively, small farmers fed rice bran which is grounded by the road paddy millers. This trend becomes farmer's choice because this milling service proactively comes to sell this sort of rice bran at a relatively cheap price. Since mixing with husks and also it is difficult to be separated, very high fiber and course rice bran is available in quality and thus fermentation to improve the nutrient value for poultry feeding is a desirable approach. It has been reported that the enhancement of the nutritional value of some agro industrial by-products is by fermentation (Iyayi and Aderolu 2004; Sugiharto and Ranjitkar, 2019). Fermentation of rice bran has shown to increase protein content, decrease crude fiber (Sivamaruthi et al., 2018) increased mineral contents particularly calcium, and free of toxicity (Ryan et al., 2011), increased phosphor (Metzler and Mosenthin, 2008), inhibited the intestinal inflammatory syndromes (Islam et al., 2017) and improved immunity (Sadeghi et al., 2015). In general, feeding fermented product may improve animal performance and health (Walugembe et al., 2015). A feeding trial to molting hens, Naqvi et al. (2000) reported a direct use of 2% effective microorganisms (EM4) in a commercial layer diet increased egg production by 2.3 eggs per day. In broiler chickens, the supplementation of CECT 4043 *Lactobacillus casei* into a basal diet improved feed efficiency and stimulated growth rate (Fajardo et al., 2012). Furthermore, the administration of EM4 in drinking water (Haq et al., 2000) and in a commercial ration (Ali et al., 2014) reduced feed intake, improved feed efficiency and increased intestinal weight in broilers. Fermented fibrous diets are also a strategy to reduce serum and carcass cholesterols of broilers (Kalavathy et al., 2006) as well as the cholesterol of egg yolk content (Haq et al., 2000; Hassan et al., 2013; Kim et al., 2017). However, to best of our knowledge, a study implementing EM4 for fermenting rice bran which is

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grounded by road paddy milling and feeding to layer chickens is not available. Therefore, this study was conducted to evaluate whether this efforts could be effective to improve laying performance and reduce egg yolk cholesterol of layer chickens.

MATERIALS AND METHODS

Ethical approval

The present study was approved by the Animal Ethics Committee of Faculty of Animal Science, Mataram University.

Preparation of EM4 -fermented rice bran

Rice bran was procured from the road paddy miller (Figure 1).

Figure 1. Road paddy miller

Figure 2. Fermentation process

As pre-experimental study through preparation of fermented feedstuffs, two hundred and fifty ml of red sugar was added into 2500 ml water. Effective microorganisms (EM4) solution which was procured from a local supplier was diluted with red sugar solution in three levels (20, 25 and 30 ml) and then stirred until homogenous. The resultant solution was mixed in 5 kg road paddy milling rice bran (RPMRB). The mixture was then put into a plastic bag and was firmly tied (anaerobic fermentation) for 2, 4 and 8 days of incubation. This method adopted Tellew et al (2013) with a slight modification (Figure 2).

We controlled the pH and room temperature between 35 and 40^{o}C. Of these treatments, eight days of incubation with the mixed solution of red sugar and 25 ml EM4 produced the highest crude protein and lowest crude fiber levels. The proximate composition of unfermented common rice bran (UCRB) and fermented road paddy milling rice bran (FRPMRB) were determined in duplicate for dry matter (DM), crude protein, crude ash and crude fiber (AOAC 1990) and then nutritionally compared. The results are presented in Table 1. Furthermore, these values were used to formulate the experimental diets as presented in Table 2.

Birds, diets and experimental design

The study was conducted at the farm of the Faculty of Animal Science Mataram University in a completely randomized design.

A total of twenty wk-150 layer chickens, obtained from a commercial breeding farm were assigned randomly into 5 treatments and six replicates of consisting 5 birds (30 birds per treatment). They were then placed in individual bamboo cages (width × length × height = 25 cm x 35 cm x 35 cm) under uncontrolled climate conditions in a poultry house of teaching farm. A diet containing 0, 5, 10, 15 and 20% of the fermented road paddy milling rice bran (FRPMRB) was fed to commercial laying hens for 8 weeks (25 to 32 week of age).

A commercial type basal diet was formulated to meet the standard diet for laying hens as feed mill recommendation (T0) as the control group which was the mixture of protein concentrate, yellow corn and common rice bran (3:5:2) without the inclusion of FRPMRB. Whilst T1 to T4 were the basal diet with a combination between the common rice bran and FRPMRB (Table 2).

Feed and water were provided *ad libitum* throughout the experimental period. The birds were not treated to any health prevention or other medications. Light was provided for 12 h/day from 06:00 pm to 06: am throughout the experimental period.

Laying performance parameters

Table 1. Chemical composition of unfermented and fermented rice bran (DM-basis)

Composition (%)	**UFRB**	**FRPMRB**
Dry matter	94.94	89.85
Crude protein	6.83	8.02
Crude fiber	33.43	28.74
Ether extract	3.04	5.86
Ash	9.65	15.27

UFRB- Unfermented rice bran ; FRPMRB- Fermented road paddy miller rice bran by EM4

Table 2. Ingredients (%) and calculated nutrient content of experimental diets

	Dietary treatments				
	T0	**T1**	**T2**	**T3**	**T4**
Ingredient (%)					
Commercial protein concentrate (CPC)	30	30	30	30	30
Yellow corn (YC)	50	50	50	50	50
Common rice bran (CRB)	20	15	10	5	0
Fermented road paddy milling rice bran (FRPMRB)	0	5	10	15	20
Total	100	100	100	100	100
Calculated nutrient content					
Metabolizable energy (ME) (kcal/kg)	2752	2686	2673	2660	2647
Crude protein (CP) %	17.63	17.46	17.19	16.93	16.66
Crude fiber (CF) %	5.50	6.50	7.48	8.46	9.43
Calcium (Ca) %	3.03	3.03	3.03	3.03	3.02
Available phosphor (AP) %	0.71	0.66	0.61	0.56	0.51

Feed intake (FI) was calculated by subtracting feed residue weights from feeders of the total feed. Egg production (EP-%) was recorded daily and was calculated on weekly basis of cumulative production. Egg weight (EW-g) was recorded on individual egg basis. Egg mass production (EM-g/bird/d) was calculated by multiplying average egg weight and egg production percentage. Feed conversion ratio (FCR) was calculated by dividing the total of feed consumed with the total egg mass. The experimental feeding lasted for 8 weeks from 24 to 32 weeks of age.

Chemical analysis

Proximate analysis using method of AOAC (1990) for both common and fermented rice bran and egg yolk protein and fat. Egg yolk cholesterol as one of important quality indicator for healthy poultry products was analyzed using the Liebermen-Burchard method and was tested by a cholesterol kit (ILab 300).

Statistical analysis

The data collected were subjected to analysis of variance (ANOVA) following the General Linear Model (GLM) procedure of SAS (SAS Institute, 1985). The differences between the means of groups were identified by the test of Tukey's test at 5% significant level.

Economic analysis

The present study also considered the economic efficiency of the experimental diets which was based upon the differences in both selling revenue and feeding cost (income over feed cost- IOFC). This was calculated according to the price of the experimental diets and egg production during the year of the study 2018.

RESULTS AND DISCUSSION

Laying performance

This study showed that the fermented rice bran levels in layer diets ($p<0.05$) affected laying performance (Table 3). Although the feed intake, feed conversion ratio and egg weight of layer chickens were not influenced ($p>0.05$) by the diets, there were increases ($p<0.05$) in hen-day egg production and egg mass by the inclusion of fermented road paddy milling rice bran (FRPMRB). Birds receiving 10 (T2) and 15% (T3) FRPMRB increased egg production by 3.7% (from 62.7 to 66.4%) and 2.9% (from 62.7 to 65.6%) but reduced by 6.5% (from 62.7 to 56.2%) and 9.3% (62.7 to 53.4%) by feeding 5 and 20% FRPRB respectively compared to the control groups. Thus, T2 treated group showed higher increase in egg production

Table 3. Laying performance of commercial chickens fed FRPMRB

Parameter	Fermented road paddy milling rice bran					
	0 (T0)	5% (T1)	10% (T2)	15% (T3)	20% (T4)	SEM
Feed intake (g/bird/d)	120	120	124	125	124	1.5
FCR (g/g)	3.266	3.615	3.247	3.198	3.981	0.07
Egg production (%)	62.7[a]	56.2[ab]	66.4[a]	65.6[a]	53.4[b]	1.55
Egg mass (g/hen/d))	36.74[ab]	33.19[b]	38.19[a]	39.08[a]	31.01[b]	0.95
Egg weight (g)	58.11	58.88	57.24	59.15	58.1	0.43

SEM – standard error of mean; [a-b] means with different superscript within column dietary treatments differed significantly at $p<0.05$

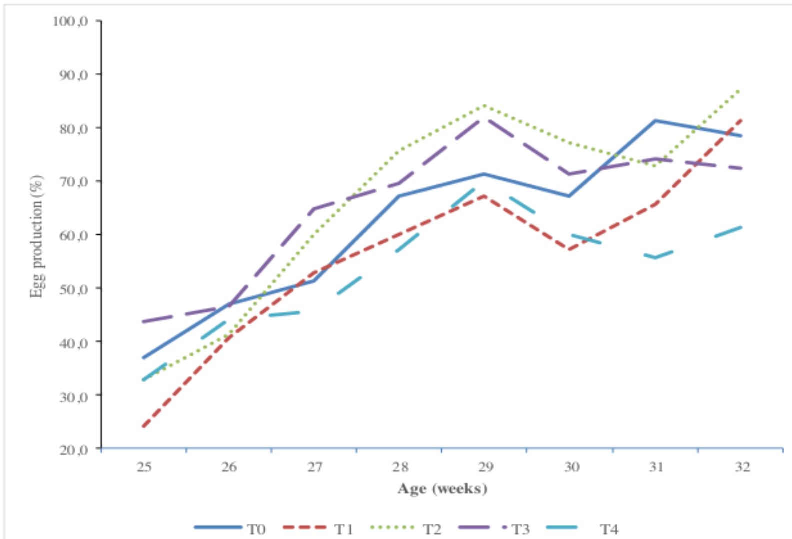

Figure 3. Weekly egg production of laying hens subjected to different levels of FRPMRB from 25 to 32 weeks of age

and this trend was also nearly steady on higher levels and achieved a sharp at 29 weeks compared to other groups (Figure 3) although a slight reduction at 31 weeks was observed. At the end of experiment, T2 continues to increase at higher levels than others. In the same manner, egg mass increased by 1.45 g (36.74 to 38.19 g) and 2.34 g (from 36.74 to 39.08 g) but reduced by 3.55 g (from 36.74 to 33.19 g) and 5.73 g (from 36.74 to 31.01 g) compared to the control treatment. Thus, the highest egg production of treated birds was fed by 10% FRPMRB, implying the enough nutrients are available to be utilized and absorbed because of better nutrient digestibility of the fibrous diet. Several authors (Chiang et al., 2010; Fasuyi and Olumuyiwa, 2012; Mateos et al., 2012; Freitas et al., 2014) observed that a reduction in nutrient digestibility with high fiber diet might be correlated with the limited number of the cellulolytic bacteria growth. The growth of *Lactobacillus* in the crop and ceca was dependent on the levels and source of fiber. Mateos et al. (2012) Jiménez-Moreno et al. (2009) reported the higher fermentation capability of sugar beet pulp than oat hull by the resident microflora. In addition, the high level of fiber in the diet impaired the absorption of the nutrient because of increasing the thickness of the unstirred water layers of the mucosa (Jiménez-Moreno et al., 2009). In this study feeding with high inclusion rate of FRPMRB at 20% level might limit the growth of beneficial microbes particularly *Lactobacillus spp* (Dinza, 2017) and *Lactobacillus spp* were the major bacterial population in the gastrointestinal tract found especially ileum and caecum when feeding with fermented fibrous diets (Zulkifli et al., 2009; Baniyah et al., 2018; Rezaeia et al., 2018). A study of Yan et al. (2017) indicated the noticeable role of cecal microbiota in the feed efficiency of chickens and recommended the uses of *Lactobacillus* to improve the feed efficiency of host. Although this study

Table 4. Egg yolk quality fed fermented rice bran road paddy miller

Parameter	Fermented road paddy milling rice bran					SEM
	0 (T0)	**5% (T1)**	**10% (T2)**	**15% (T3)**	**20% (T4)**	
Yolk protein (%)	12.6^{ab}	11.8^{b}	11.2^{b}	12.2^{ab}	13.4^{a}	0.238
Yolk fat (%)	7.7	8.3	8.5	6.5	8.1	0.245
Cholesterol (mg/dl)	57.3^{b}	70.3^{b}	61.3^{b}	67.0^{b}	90.3^{a}	6.64

SEM – standard error of mean; $^{a\text{-}b}$ means with different superscript within column dietary treatments differed significantly at p<0.05

did not account for the number of the beneficial microbes in the gut, it might be possible that the poor laying rate at 20% FRPMRB is because of less digestible fiber diets. As supported by Chiang et al. (2010) who reported higher *Lactobacilli* counts in colon and ceca digesta from broilers fed the fermented diet than birds fed the control and non-fermented feed. It has been reported that moderate amounts of fiber diets improves the development of organs, enzyme production, and nutrient digestibility in poultry (Mateos et al., 2012; Walugembe et al., 2015; Dinza, 2017).

It is generally accepted that increase in feed intake may be attributed with an increase in rate passage of the high-fiber diets which is associated with high insoluble fiber (Panaite et al., 2016; Rezaeia et al., 2018). However, the present study observed that fed high-fiber diets did not affect feed intake. The possible reason for this result is that fermented feed provide enough nutrient content (Alam et al., 2018) because enriched amino acids and increased antioxidant activities (Rashad et al., 2011), changes in palatability (Ryan et al., 2011) and reduced anti nutritional factors (Mateos et al., 2012) at proportional levels. Although no differences in feed utilization were observed between treatments laying hens receiving the fermented diets at 15% level exhibited the lowest FCR values and were considered the most efficient at converting feed into egg mass. Another possible reason to explain the improvement in fermented fed birds at moderate levels, as mentioned, could be the growth of *Lactobacillus spp* affecting villus height (Sarikhan et al., 2010) which is responsible for nutrient absorption (Teirlynck et al., 2009; Rezaeia et al. 2018). The review by Jha and Berrocoso (2015) and Sugiharto and Ranjitkar (2019) clearly pointed out that dietary fiber and fermentation in non-ruminant feeding offered favorable values on performance, gastro intestinal tract microecology, gut morphology, immune system and welfare

Egg yolk quality

In terms of egg yolk quality, a significant ($p<0.05$) difference was observed among the yolk protein and yolk cholesterol whilst yolk fat was not affect by the diets (Table 4). T4 group (20% FRPMRB) showed the highest yolk protein, cholesterol and relatively higher yolk fat than other FRPMRB groups. However, the lowest cholesterol level was the control group. Whilst T2 and T3 were in the moderate levels of all parameters of egg qualities. The obtained results of the present study are unexpected and not in agreement with the findings of Hassan et al. (2013), Kim et al. (2017) and Alam et al. (2018), who reported that egg yolk cholesterol reduced ($p<0.05$) when diets containing fermented or high fibrous compound diet were fed. It is difficult to clarify why the higher egg cholesterol value was observed in the fermented diet groups. This difference might be due to the low moisture of the fermented substrate, affecting the beneficial bacteria to grow at maximum levels. Kim et al. (2017) in Hy-Line Brown laying hens demonstrated a decrease in cholesterols from 128.2 to 95.8 (mg/dl) with *Lactobacillus* fermented rice bran. They inoculated with 5 L of prepared *Lactobacillus* for 4 kg of dried rice bran, whilst in the present study applied 2.5 L for 5 kg of rice bran. Consequently, fermented rice bran remained to contain a relatively high dry matter (Table 1). Previous studies reported lowering of plasma concentration of LDL (Low Density Lipoprotein) and triglyceride levels was due to a high crude fiber diet and a reduction in egg cholesterol levels was as a result of reduced bile excretion (Bogusławska-Tryk et al., 2016). Lowering of cholesterol levels was also due to the ability of insoluble fiber binding to the bile lipids in the gut (Kritchevski, 1987). A reduced level of cholesterol has also been observed in the fat, muscle and carcass of broilers fed *Lactobacillus culture* (Kalavathy et al., 2006). This suggests that feeding high crude fiber which stimulate *Lactobacillus* population inhibit cholesterol metabolism is dependent on the number of existing bacteria and the moisture of the substrate culture. Therefore, to elucidate this results, further research on the fermentation process is desirable particularly in terms of moisture for a diet containing high fiber of agricultural by-products which is low in quality.

Income over feed cost

Table 5 presents a simple economic calculation of FRPMRB feeding. There were no significant economic changes noticed in terms of the cost of per kg feed with fermented rice bran. However, the cost of kg total feed and IOFC were significantly higher ($p<0.05$) for T0, T2 and T3 compared to T1 and T4. Of all tested diets, the

Table 5. Cost of production and IOFC of commercial layer chickens fed different fermented rice bran of road paddy miller

Parameter	Fermented road paddy milling rice bran					
	0% (T0)	5% (T2)	10% (T3)	15% (T4)	20% T4	SEM
Feed conversion ratio (g/g)	3.266	3.615	3.247	3.198	3.981	0.07
Cost of kg feed (US$)	0.443	0.443	0.444	0.444	0.444	0.00
Cost of total feed (US$)	1.446[a]	1.602[b]	1.440[a]	1.420[a]	1.769[c]	60.4
Selling price of kg egg (US$)	1.857	1.857	1.857	1.857	1.857	0.00
IOFC (US$)	0.411[a]	0.255[b]	0.417[a]	0.437[a]	0.088[c]	0.11
Cost of kg feed (IDR)	6,200	6,205	6,210	6,215	6,220	0.00
Cost of total feed (IDR)	20,249[b]	22,431[ab]	20,163[b]	19,875[c]	24,761[a]	150
Selling price of kg egg (IDR)	26,000	26,000	26,000	26,000	26,000	0.00
IOFC (IDR)	5,751[a]	3,569[b]	5,836[a]	6,124[a]	1,238[c]	200.14

IOFC: Income over feed cost US$ = 14000 IDR; SEM – standard error of mean; [a-c] means with different superscript within column dietary treatments differed significantly at $p<0.05$

income over feed cost of US$ 0.416 and 0.437 per kg of eggs were found in moderate levels of FRPMRB (10 and 15%), but the most economic benefit was at 15% FRPMRB level. It is suggested that moderate levels of FRPMRB are potential to be included in formulating layer diets. Results of this study are in consonance with Abubakar et al. (2007) who fed laying hens with supplementing yeast into rice bran diets. The higher net incomes were obtained with 30, 35% rice bran and 30% rice bran with yeast compared to the control and 35% rice bran supplemented with yeast.

CONCLUSIONS

Performance of laying hens increased by feeding a diet containing fermented road paddy milling rice bran at medium levels. Egg yolk cholesterol was not affected by the dietary treatments. The most economic values were obvious for feeding the fermented diets at 15% inclusion.

CONFLICT OF INTEREST

The authors declared there was no any conflict of interest regarding the publication of this paper

AUTHOR'S CONTRIBUTION

Hari Permadi designed the work, analyzed, wrote and prepared the manuscript. Syamsuhaedi assisted and supervised the work and Budi Indarsih finalized the manuscript.

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