JSAT
Journal of Science and Agricultural Technology
Research Article
https://doi.org/10.14456/jsat.2021.9
e-ISSN 2730-1532, ISSN 2730-1524

The use of different levels of mulberry leaf meal with the broken-riceberry-based diet for semi-free range layers

Phonthakon Huikhiaw[1], Thaweesin Saengdao[1], Sakda Phayom[1] and Nitima Chalermsan[1*]

[1]Department of Animal Science and Fishery, Faculty of Science and Agricultural Technology, Rajamangala University of Technology Lanna, Phitsanulok Campus, 52 Moo. 7 Tambon Ban Krang, Mueang Phitsanulok District, Phitsanulok, 65000 Thailand.

***Corresponding author**: nokgapood@gmail.com*

Received: March 20, 2021. Revised: August 18, 2021. Accepted: August 18, 2021

ABSTRACT

The present study investigated the effect of semi-free range laying hen fed dietary mulberry leaf meal mixed plus broken riceberry on egg performance, egg quality, and health. A randomized complete block experimental design was assigned, which block was a housing model (sunshine and shade). One hundred sixty laying hens (59 weeks of age) were divided into four groups in each house (20 birds of each group). The laying hens in each group were randomly assigned to 4 dietary treatments as follows: Diet 1, 2, 3, and 4, which were used broken riceberry as an energy source and mixed with 0, 2, 4, and 6 % sun-dried mulberry leaf meal, respectively. There were two consecutive 28 day periods to collect data based on egg production and egg quality. In the last period, the feces of each group were randomly collected to determine the number of microorganisms (total plate count, coliform, salmonella, and lactic acid bacteria). The results showed that there were no statistical differences among the four groups on egg production percentage, daily feed intakes, feed conversion ratio per 1 kilogram of egg weight, feed cost per 1 kilogram of egg weight, egg weight, albumen height, haugh unit, eggshell thickness, and the number of coliforms and lactic acid bacteria in feces ($P>0.05$). However, the egg yolk color scores were the highest, followed by diet 3, 2, and 1, respectively ($P<0.05$). The total plate count and salmonella in the feces of the laying hen fed with diet 4 were the lowest ($P<0.05$). In addition, feed cost per 1 kilogram of egg weight of the group fed with diet 4 was slightly lower than other groups.

Keywords: semi-free range layer, broken riceberry, mulberry leaf meal

INTRODUCTION

Nowadays, free-range laying hen (*Gallus gallus domesticus*) egg production is increasing within Thailand in response to consumer demand. Free-range chicken systems raise the chicken by letting them run freely or putting them together in a huge barn (floor pen egg); the chickens can move well and express any feelings. That is good for the animal's well-being or welfare, and then the eggs will be called “free-range eggs.” Besides, feeding laying hens using locally sourced ingredients is another option for laying hens to produce free-range eggs and reduce the cost of animal feed. (Intralamongkol, 2010).

Riceberry or Jasberry Rice (*Oryza sativa*) is the healthiest rice that most farmers cultivate without chemical pesticides. It is a registered rice variety from Thailand, a Jao Hom Nin (JHN) crossbred, a local non-glutinous purple rice, and Khoa Dawk Mali 105 (Hom Mali rice). The variety was created by the Rice Science Center, Kasetsart University, Thailand, after four years of research for nutritional properties, anthocyanin stability, physical and cooking properties. The outcome is a deep purple whole grain rice with softness and a palatable aftertaste. (Agriculture Editor, 2014). Riceberry has been a popular brown rice substitute due to its health-promoting properties. Inducing people to consume more whole grain rice varieties could help ameliorate food-related chronic diseases like diabetes, heart disease, high blood cholesterol, obesity, and cancers (Sripaya, 2015). Riceberry is rich in nutritional value, with water-soluble mainly anthocyanin and lipid-soluble antioxidants, such as carotenoid, gamma oryzanol, and vitamin E. The nutritional properties of riceberry are concentrated in its bran, with only a small fraction in its endosperm. It is true of all cereals, meaning that it is best to consume whole rather than polished grains (Sirichokworrakit et al., 2015). In the polishing process of riceberry brown rice will have 10-15 percent of the broken riceberry. It is reported that broken riceberry can be used as an energy source in laying quail diets (Chalermsan et al., 2017). However, Chalermsan et al. (2017) reported that although the broken riceberry is dark in color, most of the colorants in the rice are dissolved in water, making them unable to accumulate in the yolk.

It may make the yolk pale than the corn. If a natural colorant found in locally grown plants is used as an ingredient in feed, it can increase the color of the yolk of the egg. Therefore, if broken riceberry are used together with mulberry leaf meal in laying hens, another natural source of locally grown protein and pesticide is not used. At the right level, it should be a way to produce new feed ingredients. That is both a source of energy, protein, colorants and health supplements for laying hens. It is consistent with organic livestock production for future farmers.

Mulberry leaf (*Morus alba*) may be an alternative source of dietary protein for poultry production. It is a tree fodder that grows well in the tropics and subtropics. Mulberry leaves are used for raising silkworms in the sericulture industry. Mulberry leaf meal is rich in protein (15-35%), minerals (Ca 2.42-4.71%; P 0.23-0.97%) and metabolizable energy (1,130-2,240 kcal/kg) with absence of or negligible anti-nutritional factors. Mulberry leaf contains carotene, which can be converted with varying efficiency by animals to vitamin A and xanthophylls, which may have potential in egg yolk pigmentation (Sarita et al., 2006).

The present study investigated the effect of semi-free range laying hen dietary mulberry leaf meal mixed with broken riceberry on egg performance, egg quality, and chicken health.

MATERIALS AND METHODS

This study was conducted at a poultry farm and the laboratory of the agricultural safety center, Rajamangala University of Technology Lanna, Phitsanulok Campus, Phitsanulok, Thailand. One hundred sixty ISA-Brown laying hens (59 weeks of age) were randomly assigned to dietary treatments using a randomized complete block experimental design. The dietary treatments were: Diet 1 (control group), 2, 3, and 4 used different levels of broken riceberry as main energy source and mixed with 0, 2, 4, and 6 percent of mulberry leaf meal, respectively. The treatment consisted of two blocks with 20 laying hens each. Broken riceberry was collected from brown rice milling that was grown without pesticides. Mulberry leaves were collected from the local area of Tumbol Bankrang, Mueang district, Phitsanulok province, and Buongsampan district, Phetchaboon province, Thailand. The leaves were initially cut into small pieces and then sun-dried for about 2-3 days. The sun-dried mulberry leaves were milled into a powder. The diets were formulated following the recommendation of the National Research Council (NRC, 1994) to satisfy the nutrients requirement of the laying hens. Diets consisted of 0 (control), 2, 4, and 6 % sun-dried mulberry leaf meal and were fed for two consecutive eight-week periods with free access for clean water consumption. Feed compositions and calculated nutrient content of experimental diets are present in Table 1.

Proximate analyses were carried out on the sun-dried mulberry leaf sample to determine: dry matter (DM), crude protein (CP), ether extract (EE), ash, crude fiber calcium (Ca), and phosphorus (P), using standardized procedures of the Association of Official Analytical Chemists (AOAC, 2000). Gross energy was analyzed by an AC500 Bomb calorimeter. The chemical composition (% DM) of mulberry leaves used in the present experiment is presented in Table 2.

During the experimental period, eggs production was collected and weighed daily. Data on feed intake were collected weekly. Egg production was recorded daily, but quality characteristics of eggs were determined every four weeks (one period). In the last three days of each period, four eggs of each group were collected for quality determination. Egg weight, albumen height, and haugh unit were analyzed by automatic Egg Quality Tester (Egg Multi – Tester model EMT-5200, Robotmation Co., Ltd., Japan). Yolk color score was measured by Roche yolk color fan, Switzerland. Eggshell thickness was measured by a digital eggshell thickness tester (Mitutoyo Co., Ltd., Japan).

Last week of this experiment, feces of each group was collected from the hens' vent after experimental feeding in the morning to determine microorganism count (total plate count, coliform, salmonella, and lactic acid bacteria). The method of determining microorganism count was described by Downes and Ito (2001).

Data were analyzed by analysis of variance (ANOVA). The significant differences between the treatment means were calculated by Duncan's Multiple Range Test. All analyses were performed by SAS Program (SAS, 1990).

Table 1. Feed compositions and calculated nutrient content of experimental diets of semi-free-range layers.

Feed ingredients (kg)	Diet 1	Diet 2	Diet 3	Diet 4
Broken riceberry	59.28	58.09	56.98	55.82
Rice bran	10.00	10.00	10.00	10.00
Soybean meal	15.45	14.68	13.88	13.10
Fish meal	5.00	5.00	5.00	5.00
Mulberry leaf meal	0.00	2.00	4.00	6.00
Oyster shell	8.60	8.50	8.38	8.27
Methionine	0.30	0.30	0.30	0.30
Palm oil	0.62	0.68	0.71	0.76
Salt	0.50	0.50	0.50	0.50
Premix	0.25	0.25	0.25	0.25
Total (kg.)	100.00	100.00	100.00	100.00
Calculated nutrient contents				
Crude protein (%)	16.00	16.00	16.00	16.00
Metabolizable energy (Kcal/kg)	2,900	2,900	2,900	2,900
Calcium (%)	3.75	3.75	3.75	3.75
Available Phosphorus (%)	0.36	0.36	0.36	0.35
Crude fiber (%)	3.61	3.64	3.67	3.70
Lysine (%)	1.41	1.44	1.47	1.50
Methionine + cystine (%)	0.51	0.51	0.51	0.51
Tryptophan (%)	0.18	0.18	0.18	0.18
Threonine (%)	0.51	0.51	0.50	0.50
Price (baht/kg)	14.11	13.92	13.72	13.53

RESULTS AND DISCUSSION

The chemical composition (%DM) of the mulberry leaf meal used in the present experiment is presented in Table 2. It showed that mulberry leaf meal contains 22.30 % protein, 4,180 Kcal/kg, 11.29 % crude fiber, 2.43 % calcium, and 0.62 % phosphorus. Similarly, Sarita et al. (2006) reported that Mulberry leaf meal is rich in protein (15-35%), minerals (Ca: 2.42 - 4.71%; P: 0.23- 0.97%). Besides, the report of Al-kirshi et al. (2010) showed that crude protein, crude fiber, Calcium, and phosphorus of mulberry leaf meal at four weeks of age were 29.80, 11.11, 2.73, and 0.28 %, respectively.

Table 2. Chemical compositions and gross energy of sun-dried mulberry leaf meal (%DM)

Chemical compositions	Mulberry leaf meal
Dry matter (%)	88.92
Crude protein (%)	22.30
Ether extracted (%)	5.12
Ash (%)	11.00
Crude fiber (%)	11.29
Nitrogen free extracted (%)	50.29
Calcium (%)	2.43
Phosphorus (%)	0.62
Gross energy (Kcal/kg)	4,128

Productive Performances

The result regarding productive performance is shown in Table 3. The egg production percentage (hen day and hen house production), daily feed intake, feed conversion ratio, feed cost per egg weight, and survival percentage did not differ significantly (P>0.05) among the layer groups fed the broken riceberry as energy source diets supplemented with different levels of mulberry leaf meal. The result indicated that providing a mulberry leaf meal up to 6 % in the laying hen diet had no detrimental effect on egg production performance. These results agree with the previous report of Kamruzzaman et al. (2012, 2014), who indicated that feeding of mulberry leaf meals up to 9 percent did not affect egg production, daily feed intake, egg weight, and feed conversion ratio. These results showed a reduction in feed intake with increased dietary mulberry leaf meals in the diets for laying hens. Hamdan et al. (2013) reported that the high fiber content in leaves reduced digestion and absorption of chickens, resulting in lower feed intake. Besides, a decrease in feed intake for increased levels of the mulberry leaf may be due to bulkiness and unpalatable taste, which may affect the hen's appetite. It is likely that using broken riceberry as the primary energy source, with mulberry leaves in the laying hen diet at 6 %, had the best feed conversion rate to 1 kilogram of eggs, and the cost of feed per 1 kilogram of egg production was lower than all groups. It may be because birds fed higher levels of the mulberry leaf meal in the diet were less fed, but the egg productivity did not decrease much.

Table 3. The effects of dietary treatments on egg production performance of semi-free-range layers.

Item	Mulberry leaf meal level in diets (%)				P-value
	0	2	4	6	
Hen-day production (%)	85.80 ± 1.64	86.01±6.14	82.77±3.15	84.73± 3.66	0.883
Hen-house production (%)	85.80 ± 1.64	86.01±6.14	82.68± 3.28	84.73± 3.66	0.877
Dairy feed intake (g/day)	118.21± 3.65	112.34± 1.39	114.77± 0.84	109.61± 7.87	0.446
Feed conversion ratio	2.38± 0.15	2.31± 0.23	2.45± 0.22	2.26± 0.07	0.804
Feed cost/egg weight (Baht/kg)	33.66± 2.11	32.23± 3.27	33.67± 2.98	30.56± 0.96	0.693
Survival percentage (%)	100 ± 0.00	100 ± 0.00	97.50± 3.53	100 ± 0.00	0.500

Egg Quality

The effects of dietary treatments on egg quality of semi-free-range layers are shown in Table 4. The results indicated that egg weight, albumen height, haugh unit, and eggshell thickness did not differ significantly (P>0.05) among the four groups. Likewise, Kamruzzaman et al. (2014) reported that the inclusion of mulberry leaf meal up to 9 % in the diet of laying hens had no effect on egg size, and the results are consistent with the Tateno et al. (1999). They found no significant difference in egg size after the hens were exposed to the diet's 15 % mulberry leaf meal. In contrast, Al-kirshi et al. (2010) reported the effects of inclusion of 0, 10%, 15%, and 20% of mulberry leaf meal in the diet on productive performance and egg quality of laying hens; they found that shell thickness and albumen weight did not affect (P>0.05), but haugh units increased as the level of mulberry leaf meal increased. However, laying hens fed broken riceberry as the primary source of energy at 2-6 % mulberry leaf meal were higher in yolk color score than that the control group (0 % mulberry leaf meal), especially the hens fed a diet with 6 % mulberry leaf meal had the highest yolk color score. It agreed with the results of Seeang (1997) and Al-kirshi et al. (2010) for the effects of mulberry leaf meal on egg yolk color score when mulberry leaf meal was given as part of the diet to laying hens. They found that feeding mulberry leaf meal improved the yolk color when compared with control (0 % mulberry leaf meal). Because mulberry leaves contain carotene that can be converted with varying efficiency by animals to vitamin A as well as a xanthophyll, these may have potential effects in the pigmentation of egg yolk (Sarita et al. 2006). Moller et al. (2000) reported that increases in egg yolk color are due to a more incredible content of carotenoids in egg yolk. The increase in egg yolk coloration indicated the high bio-availability of the xanthophyll in the leaf meals (Udedibie and Opara, 1998).

Table 4. The effects of dietary treatments on egg quality of semi-free-range layers.

Item	Mulberry leaf meal level in diets (%)				P-value
	0	2	4	6	
Egg weight (g)	59.33± 0.44	57.73± 0.96	58.43± 3.53	58.95±0.15	0.867
Albumen height (mm.)	6.95 ± 0.78	6.41 ± 0.29	7.44 ± 0.54	7.31 ± 0.95	0.394
Haugh unit	82.26 ± 5.01	79.17± 2.11	85.04± 1.35	85.51± 5.83	0.421
Yolk color score	2.00 ± 0.41^{c}	3.11± 0.44^{bc}	4.25±0.29^{ab}	4.93±0.10^{a}	0.015
Eggshell thickness (mm.)	0.47 ± 0.01	0.49± 0.01	0.47± 0.01	0.48± 0.01	0.550

^{abc} Means with different superscripts within the same row are significantly different (P<0.05)

Microorganism Count in Feces

The effects of dietary treatments on microorganism count in feces of semi-free-range layers are in Table 5. The results showed that the total plate count and *salmonella spp*. in feces of the hens fed broken riceberry as the main source of energy, with 6 % mulberry leaf meal the lowest among four groups (P<0.05). The number of coliforms and lactic acid bacteria (beneficial microorganisms in the digestive tract) in the feces of 4 groups of birds were no different from each other (P>0.05). However, it is likely that the hens fed dietary increased levels (2-6 percent) of mulberry leaf meal, had a decrease in the number of coliform microorganisms in feces, and an increase in the number of lactic acid bacteria compared to the control group because the mulberry leaves are antibacterial substance. Antibacterial potential of the mulberry leaves extracted was screened against *Staphylococcus aureus, Pseudomonas aeruginosa, Streptococcus faecium, Escherichia coli, Neisseria gonorrheae and Proteus vulgaricus* etc. (Omidiran et al., 2012). In addition, Devi et al. (2013) reported that the leaf extracts of mulberry (Morus alba) were tested for antimicrobial activity against various bacterial strains and fungal strains compared to standard drugs. Results of the antimicrobial activity revealed that the extracts showed noticeable antimicrobial activity. Besides, in the study of Bharani et al. (2010), the extract of mulberry leaves has a significant effect on the humoral and cell-mediated immunity in experimental animals. Mulberry leaves contain biological active substances, including saponin, triterpenes (lupeol), sterols (β-Sitosterol), bioflavonoids (rutin, moracetin, quercetin-3-triglucoside and isoquercitrin), alkaloid and γ-aminobutyric acid (Srivastava, et al., 2006; Omidiran et al., 2012; Devi et al., 2013) as well as therapeutic activities. This particular has widely established and hidden therapeutic uses. The significant benefit of this medicinal plant is antidiabetic, immunomodulatory, antimicrobial, antioxidant and anticancer. (Devi et al., 2013)

Table 5. The effects of dietary treatments on microorganism counts in feces of semi-free-range layers. (log of cfu/g)

Item	Mulberry level in diets (%)				P-value
	0	**2**	**4**	**6**	
Total plate count	15.41±0.01^{a}	15.17±0.02^{ab}	14.84 ±0.05^{b}	14.35±0.24^{c}	0.008
Coliform	8.69 ±0.23	8.54±0.29	8.35±0.38	8.04+0.06	0.307
Salmonella	6.07±0.20^{a}	5.10±0.10^{b}	5.80±0.16^{a}	4.50±0.31^{c}	0.009
Lactic acid bacteria	11.75±0.39	12.22±0.05	12.32±0.16	12.17±0.05	0.164

^{abc} Means with different superscripts within the same row are significantly different (P<0.05)

CONCLUSIONS

Based on the present study results, it can be concluded that the supplement of mulberry leaf meal is possible by up to 6 % in broken riceberry based-diets for semi-free-range layers without adverse effects on production and egg quality, but tended to improve the rate of conversion of feed, lower feed costs per 1 kg of egg, and improve pigmentation of egg yolk compared to the control. In addition, the diet that contained 6% mulberry leaf meal could have inhibited the growth of coliform and salmonella but enhanced the increase of lactic acid bacteria that is beneficial to animal health. Thus, feeding diets mixed with mulberry leaf meal with broken riceberry should be an alternative feeding regime to use local feed resources for safe egg production.

REFERENCES

Agriculture Editor. 2014. Riceberry new varieties of fragrant rice changing the lives of Thai farmers. Panyachon Publisher, Bangkok.

Al-kirshi, R., Alimon, A.R., Zulkifli, I., Sazili, A., Zahari, M. W., and Ivan, M. 2010. Utilization of mulberry leaf meal (*Morus alba)* as protein supplement in diets for laying hens. Ital J Anim Sci. 9 (51): 265 – 267.

AOAC. 2000. Association of Office Analytical Chemist. 17thed. Association of Official Chemist, Inc. Washington, D.C.

Bharani, S.E., Asad, M., Dhamanigi, S.S., and Chandrakala, G.K.. 2010. Immuno modulatory activity of methanolic extract of *Morus alba Linn*. (Mulberry) leaves. Pak. J. Pharm. Sci. 23:63-68.

Chalermsan, N., Kajitphetjarat, S., Kajitphetjarat, K., and Jaree, T. 2017. The use of riceberry broken rice mixed with moringa leaf meal in laying quails diets. Agricultural Sci. J. 4(2) (Suppl): 128-129.

Devi, B., Sharma, N., Kumar, D., and Jeet, K. 2013. *Morus Alba* Linn: A phytopharmacological review. Int. J. Pharm. Sci, 5: 14-18.

Downes, F.P., and Ito., K.2001. Compendium of Methods for the Microbiological Examination of 4th ed. America Public Health Association, Washington D.C.

Hamdan, H., Yunianto, V.D., and Sukamto, B. 2013. The effectivity of fermented mulberry leaves with rumen liquor as broiler feed on final body weight, dry matter and crude fiber digestibility, and metabolic energy. Anim. Prod. 15(3): 173-179.

Intralamongkol, J. 2010. Organic free range laying hen production. Organic Livestock Center. Breeding Division, Department of Livestock Development, Bangkok.

Kamruzzaman M.D., Rahman, M.S., Asaduzzaman, M.D., and Zaminur Rahman M.D. 2012. Significant effect of Mulberry leaf (*Morus alba*) meal in the reduction of egg-yolk cholesterol. Bangladesh Res. Pub. J. 7(2): 153-160.

Kamruzzaman. M., Khatun, M.A., Islam, M.S., Rahman, M.Z., and Yeasmin, T. 2014. Effect of dietary mulberry leaf meal on egg quality of laying hens. J. Sci Technol. 12: 12 – 17.

Moller, A.P., Biard, C., Blount, J.D., Houston, D.C., Ninni, P., Saino, N., and Surai, P.F. 2000. Carotenoid dependent signals: indicators of foraging efficiency, Immunocompetence or detoxification ability?. Avian Poult. Biol. Rev. 11 (3):137- 159.

NRC. 1994. Nutrient Requirements of Poultry 9th Revised Ed. Academy of Sciences, Wachington DC. Omidiran, M.O., Baiyewu, R.A., Ademola, I.T., Fakorede, O.C., Toyinbo, E.O., Adewumi, O.J., and Adekunle, E.A. 2012. Phytochemical analysis, nutritional composition and antimicrobial activities of white mulberry (*Morus alba*). Pak J Nutr. 11(5): 456-460.

Sarita, S., Rashmi, K., Anju, T., and Prakash, R. 2006. Nutritional quality of leaves of some genotypes of mulberry (*Morus alba*). Int. J Food Sci Nutr. 57(5/6): 305-313.

SAS. 1990. SAS/STAT User's Guide (Vol. 2). SAS Institute Incorporated, Cary, NC.

Seeang, S. 1997. Study on using mulberry leaves as protein supplement in laying hen diet. M.Sc. Dissertation. Kasetsart University, Bangkok.

Sirichokworrakita, S., Phetkhuta, J., and Khommoon, A. 2015. Effect of partial substitution of wheat flour with Riceberry flour on quality of noodles. pp 1006-1012. In Procedia - Social and Behavioral Sciences. 7th World Conference on Educational Sciences, (WCES-2015), 5-7 February, 2015, Novotel Athens Convention Center, Athens, Greece.

Sripaya, C. 2015. Riceberry: Good rice is useful. Access (26 July 2010). Available: http://www.sininrice.com/ insight.html.

Srivastava, S., Kapoor, R., Thathola, A., and Srivastava, R. P. 2006. Nutritional quality of leaves of some genotypes of mulberry (*Morus alba*). Int. J Food Sci Nutr. 57(5-6): 305-313.

Tateno, H., Yatabe, T., and Iso, M. 1999. Studies on foliage of unused resources. Effects of mulberry leaves on egg production as a poultry food. Bull. Ibaraki Prefect. Poult. Exp. Stn. 33:15-20.

Udedibie, A.B.I., and Opara, C.C. 1998. Responses of growing broilers and laying hens to the dietary inclusion of leaf meal from *Alchornia cordifolia*. Anim. Feed Sci. Technol. 71:157-164.