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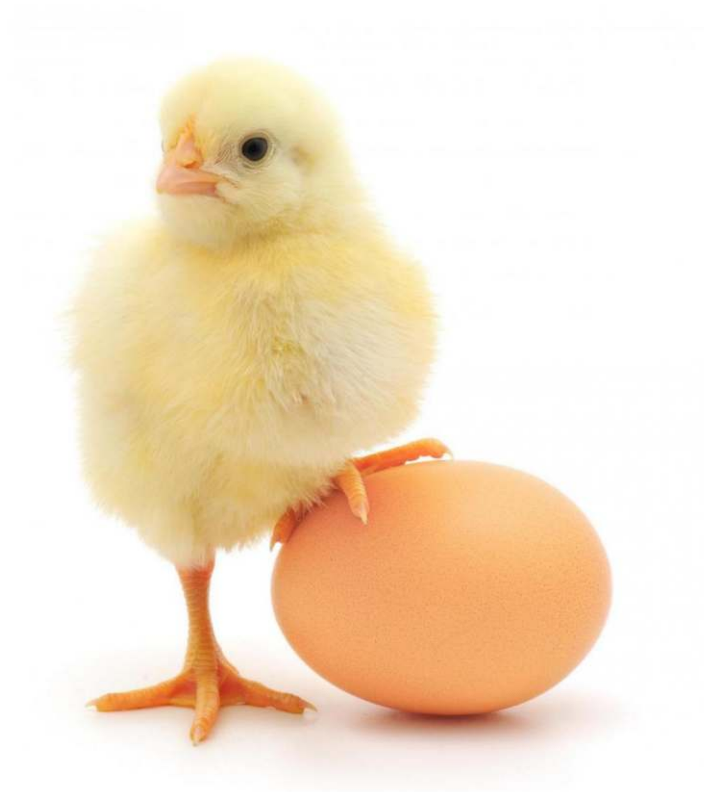

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Effect of Replacement of Protein from Soybean Meal with Protein from Wolffia Meal [*Wolffia globosa* (L). Wimm.] on Performance and Egg Production in Laying Hens

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Abstract: Wolffia meal [*Wolffia globosa* (L). Wimm.] was examined as a protein replacement for Soybean Meal (SBM) in the diets of laying hens. A total of 180 Rohman laying hens of 71 weeks old of age were randomly allocated into 5 groups; each group contained 4 replicates with 9 hens per replicate according to Completely Randomized Design. The dietary treatments were T1 = control diet; T2, T3, T4 and T5 = 25, 50, 75 and 100% of the CP from SBM was replaced by the CP of Wolffia meal, respectively. The results found the decreased feed intake and ME intake ($p<0.05$) when CP from SBM was replaced by CP from Wolffia meal. Total replacement of CP from SBM with CP from Wolffia meal decreased ($p<0.05$) egg production of laying hens. Egg yolk pigmentation increased ($p<0.05$) with increasing replacement of CP from SBM with CP from Wolffia meal. The findings reflected that Wolffia meal can be used as protein replacement for CP from SBM at 75% in the diet of laying hens.

Key words: Duckweed, egg production, yolk pigmentation, layers

INTRODUCTION

The cost of animal feed generally accounts for 60% or more of the total production cost of raising poultry (Teguia and Fon Fru, 2007). It has been predicted that the price of protein sources, especially SBM, will rise further owing to increased demand from rapidly expanding economies like China and other South-East Asian counties (Robinson and Sigh, 2001). Therefore, the investigation of locally produced alternative protein sources is seriously and urgently needed for feed cost reduction. Wolffia meal (*Wolffia* spp.) is a small circular floating weed about 0.5-1.5 mm in length that lives in tropical and subtropical lakes and marshes. It is monocotyledons and classified as a higher plant, or macrophytes (Landolt, 1986). Wolffia meal, one of the duckweed species, belongs to the botanical family *Lemnaceae*. The family consists of five genera *Landoltia*, *Lemna*, *Spirodela*, *Wolffia* and *Wolffiella* (Les *et al*., 2002). Wolffia meal has been used as a vegetable in the Indochinese peninsular for many generations (Bhanthumnavin and Mcgarry, 1971). Interestingly, the actual dry matter yield from commercial-scale cultivation of *Lemna*, *Spirodela* and *Wolffia* species in Bangladesh ranges from 13-38 tons/ha/year, which is a rate exceeding single-crop soybean production six to ten fold (Skillicorn *et al*., 1993). Furthermore, Wolffia meal grown in enriched water containing mineral media or effluents from agricultural waste lagoons, the protein content (29.9-45 g/100 g) is greatly increased over that from natural waters low in nutrient (Rusoff *et al*., 1980; Skillicorn *et al*., 1993; Huque *et al*., 1996). Its protein content (Skillicorn *et al*., 1993) and amino acid profile (Rusoff *et al*., 1980) were comparable to those of SBM. Thus, Wolffia meal can be possibly used as a source of protein in animal diets. Several scientific papers reported that duckweed species (*Lemna gibba* and *Lemna minor*) were successfully used as a protein source in poultry diets (Jhori and Sharma, 1979; Islam *et al*., 1997; Sokantat, 1990; Men *et al*., 2001; Men *et al*., 2002). On the other hand, the first report on Wolffia meal

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(*Wolffia arrhiza*) revealed that the inclusion of 15% Wolffia meal in the diets of layers produced no significant differences in performance and egg production, but increased ($p<0.05$) yolk pigmentation (Haustein *et al*., 1990). Presently, there is insufficient information using Wolffia meal in poultry diets. Therefore, the purpose of this study was to investigate the effects of replacing Crude Protein (CP) from SBM with CP from cultivated Wolffia meal [*Wolffia globosa* (L)] Wimm., accession number GQ221774) on the performance and egg production in laying hens.

MATERIALS AND METHODS

The experiment was conducted in an evaporative system housing with an internal temperature of 24°C. Internal lights were on continuously. A total of 180 Rohman laying hens, 71 weeks old, were randomly allocated into 5 groups; each group contained 4 replicates with 9 hens per replicate and placed in wire cages, three hens per cage. Feeders between the different cages were separated by plastic sheets to avoid cross contamination of dietary treatments. Water from nipple drinkers was freely available in the cages.

Fresh Wolffia meal [*Wolffia globosa* (L). Wimm.] was purchased from a local producer, who cultivated Wolffia meal as human food and dried under sunlight for 1-2 days. Dried Wolffia meal was ground through a 2 mm. screen and stored in air tight bags. Prior to preparation of the experimental diets, the Wolffia meal was determined for chemical composition (AOAC, 1999) and amino acid using an Amino Acid Analyzer (Table 2).

The dietary treatments were T1 = control diet; T2, T3, T4 and T5 = 25, 50, 75 and 100% of the CP from SBM was replaced by the CP of Wolffia meal, respectively. All diets were formulated to meet the nutrient requirement of laying hens according to NRC (1994) as presented in Table 1. The used Metabolizable Energy (ME) value of Wolffia meal for dietary treatment formulation was 1,302 kcal/kg (Islam *et al*., 1997). The hens received the diets *ad libitum* throughout the 8 experimental weeks.

Feed consumption and egg production were recorded daily. The dietary treatments were randomly collected at the end of each week for determination of chemical composition (AOAC, 1999). The feed conversion rate was calculated as kilograms of feed consumed per kilogram of eggs. Hens were weighed at the beginning and the end of the experimental period for estimation of body weight changes. Eight eggs from each experimental group were sampled weekly (two eggs per replicate) and determined for egg weight and yolk color using a yolk color fan (Roche, Switzerland). Haugh units and eggshell thickness were measured using albumen height gauge (TSS-QCD instrument, England) and micrometer (395-541-30 BMD-25DM, Mitutoya, Japan), respectively.

Statistical analysis: The data of feed intake, feed conversion rate, egg production and egg quality were analyzed by the analysis of variance technique appropriate for Completely Randomized Design (SAS, 1996). The model used was:

$$Y_{ij} = \mu + T_i + \varepsilon_{ij}$$

Where: Y_{ij} = observation, μ = population mean, T_i = diet effect (I = 1-5) and ε_{ij} = residual error. The differences among the means of each parameter were compared by Duncan's New Multiple Range Test (Steel and Torries, 1980). A probability level of $p<0.05$ was considered to be statistically significant.

RESULTS

The analyzed chemical compositions of all dietary treatments (Table 1) were identical and met nutrient requirements recommended by NRC (1994). The CP content in cultivated Wolffia meal was 29.61 g/100 gDM. Amino acid profile indicated that Wolffia meal contained lysine and methionine 5.00 and 1.45 g/100 gCP, respectively (Table 2).

Daily feed intake and ME intake of laying hens reduced ($p<0.05$) when CP from SBM was replaced by CP from Wolffia meal. However, replacement of CP from SBM with CP from Wolffia meal did not significantly affect ($p>0.05$) CP intake and feed conversion rate/kg of the eggs of laying hens. Body weight changes of hens fed diets containing 0, 25, 50, 75 and 100% of CP replacement from Wolffia meal were -0.09, -0.04, -0.03, -0.03 and -0.19 kg, respectively (Table 3).

Complete replacement of CP from SBM with CP from Wolffia meal dramatically decreased ($p<0.05$) egg production of laying hens. Egg weight, haugh units and eggshell thickness were not altered ($p>0.05$) by inclusion of wolffia meal in the diets. Interestingly, egg yolk color increased ($p<0.05$) with increasing replacement of CP from SBM with CP from Wolffia meal (Table 4).

DISCUSSION

The CP content of cultivated Wolffia meal in this study was similar to the values previously reported to range from 29.9-31.25 g/100 gDM (Chowdhury *et al*., 2000; Huque *et al*., 1996). The CP value of Wolffia meal could be varied depending mostly on nitrogen concentration of the water upon which it grows (Skillicorn *et al*., 1993, Leng, 1999). Rusoff *et al*. (1980) found that CP content of Wolffia meal collected from anaerobic dairy waste lagoon was 36.5 g/100 g. On the other hand, Wolffia meal grew in natural water sources in northeastern Thailand contained 20.4 gCP/100 g (Chareontesprasit and Jiwyam, 2001). Although, the variation of CP level in Wolffia meal was found, aforementioned information indicated that Wolffia meal, regardless of sources, was high in CP content.

Table 1: Feed ingredient (kg/100 kg) and chemical composition (g/100 g DM) of dietary treatments

	Dietary treatments[1]				
Ingredients	T1	T2	T3	T4	T5
Corn	53.00	53.00	53.00	53.00	53.00
Rice bran	14.20	11.46	8.70	5.80	2.73
Full fat soybean	9.30	10.20	11.13	12.10	13.13
Soybean meal (44%CP)	8.00	6.00	4.00	2.00	0.00
Wolffia meal	0.00	3.04	6.07	9.10	12.14
Fish meal	4.00	4.00	4.00	4.00	4.00
DL-Methionine	0.10	0.10	0.10	0.10	0.10
Dicalcium phosphate	1.00	1.00	1.00	1.00	1.00
Oyster shell meal	7.90	7.90	7.90	7.90	7.90
Soybean oil	2.00	2.80	3.60	4.50	5.50
Salt	0.25	0.25	0.25	0.25	0.25
Premix[2]	0.25	0.25	0.25	0.25	0.25
Chemical composition					
Dry matter	96.05	96.46	96.80	96.41	97.47
Crude protein	15.98	16.03	15.91	16.84	16.75
Ether extract	6.01	6.00	6.03	7.26	7.43
Crude fiber	2.45	2.46	2.53	2.74	2.78
Ash	11.84	10.84	13.28	12.75	12.80
ME[3] (kcal/kg)	2,913.34	2,908.11	2,908.15	2,901.64	2,903.50

[1]T1 = control; T2, T3, T4 and T5 = 25, 50, 75 and 100 % CP from SBM were replaced by CP from Wolffia meal, respectively.
[2]Vitamin-mineral premix provide (per kg diet): 10,000 IU vitamin A, 2,000 IU vitamin D_3, 11 mg vitamin E, 1.5 mg vitamin K_3, 1.5 mg thiamin, 4 mg riboflavin, 10 mg pantothenic acid, 0.4 folic acid, 4 mg pyridoxine, 22 mg niacin, 0.4 mg colabamin, 0.1 mg biotin, 60 mg Fe, 70 mg Mn, 50 mg Zn, 8 mg Cu, 0.5 mg Co, 0.7 mg I, 0.1 mg Se. [3]Calculated value

Table 2: Chemical composition and amino acid profile of Wolffia meal

Chemical composition (g/100g DM)	Wolffia meal	Soybean meal[1]
Dry matter	4.74	88.20
Crude protein	29.61	44.00
Ether extract	4.00	0.80
Crude fiber	8.76	7.00
Ash	16.01	-
Amino acid (g/100 g CP)		
Lysine	5.00	6.11
Methionine	1.45	1.41
Cystine	1.15	1.50
Methionine + Cysteine	2.60	-
Threonine	3.65	3.91
Valine	5.47	4.70
Isoleucine	3.75	4.45
Leucine	7.16	7.70
Phenylalanine	4.56	4.91
Histidine	1.89	2.66
Arginine	5.57	7.14

[1]NRC (1994)

The information of amino acid composition of Wolffia meal presently is very scarce. Rusoff *et al.* (1980) found that Wolffia meal contained 3.37 g lysine/100 gCP and 0.87 g methionine/100 gCP. The current results of lysine and methionine contents in Wolffia meal were higher than those in a previous report (Rusoff *et al.*, 1980). However, lysine and methionine in *Lemna minor*, one of the duckweed species, ranged from 5.00-5.93 and 1.38-1.400 g/100 gCP, respectively (Dewanji, 1993; Hanczakowski *et al.*, 1995) which were similar to those of Wolffia meal in this study. Hanczakowski *et al.* (1995) suggested that dried duckweed can supply farm animals with sufficient amounts of all required amino acids except methionine. A shortage of isoleucine in case of layers may also occur. Consistently, Rusoff *et al.* (1980) recommended that duckweed was a good source of lysine but deficient in methionine when compared to those of the FAO reference pattern (FAO, 1973). Therefore, methionine level should be carefully checked when Wolffia meal is used as protein source in the diets of laying hens.

Wolffia meal was lower in CP, lysine, isoleucine, histidine and arginine contents, but comparable in the remaining amino acids contents as compared to those of SBM (Table 2). Skillicorn *et al.* (1993) reported that duckweed protein had higher concentrations of essential amino acids, lysine and methionine, than most plant proteins. However, Leng (1999) mentioned that duckweeds are good sources of essential amino acids, but are not enriched in any particular amino acid in comparison with the usual protein sources used in animal production. Amino acid composition in duckweeds generally relied on level and source of nitrogen in nutrient media, pH of the water and other factors (Goopy and Murray, 2003), including ribose bisphosphate carboxylase enzyme (Leng, 1999).

Numerous studies have reported the utilization of duckweed in diets of broilers (Sokantat, 1990; Haustein *et al.*, 1992; Islam *et al.*, 1997) laying hens (Haustein *et al.*, 1990; Nolan *et al.*, 1997), ducks (Men *et al.*, 2001; Men *et al.*, 2002; Khandaker *et al.*, 2007) and Japanese quails (Sokantat, 1990). Most of those works evaluated

Table 3: Feed intake, CP intake, ME intake and feed conversion rate of laying hens fed diets containing different levels of protein replacement from Wolffia meal

	Dietary treatments[1]					
Parameters	T1	T2	T3	T4	T5	SEM
Feed intake (g/d)	97.61[a]	95.76[a]	88.03[ab]	88.21[ab]	80.38[b]	2.02
CP intake (g/d)	15.60	15.35	14.01	14.86	13.46	0.30
ME intake (kcal/d)	284.35[a]	278.99[a]	256.45[ab]	257.00[ab]	234.18[b]	5.88
Feed conversion rate/kg eggs	2.17	2.10	1.96	1.96	2.01	0.03
Body weight changes (kg)	-0.09	-0.04	-0.03	-0.03	-0.19	-

[a-b]Means on the same row with different superscripts are significantly different (p<0.05).

[1]T1 = control; T2, T3, T4 and T5 = 25, 50, 75 and 100 % CP from SBM were replaced by CP from Wolffia meal, respectively

Table 4: Egg production and egg quality of laying hens fed diets containing different levels of protein replacement from Wolffia meal

	Dietary treatments[1]					
Parameters	T1	T2	T3	T4	T5	SEM
Egg production (%)	71.83[a]	72.87[a]	70.98[a]	74.07[a]	61.76[b]	2.24
Egg weight (g)	64.47	63.97	65.12	66.59	66.12	0.50
Haugh units (%)	77.00	78.00	75.94	79.88	79.38	0.79
Eggshell thickness (mm)	0.335	0.345	0.340	0.328	0.323	0.003
Egg yolk colour	5.78[e]	7.24[d]	8.63[c]	9.72[b]	10.40[a]	0.39

[a-e]Means on the same row with different superscripts are significantly different (p<0.05).

[1]T1 = control; T2, T3, T4 and T5 = 25, 50, 75 and 100 % CP from SBM were replaced by CP from Wolffia meal, respectively

duckweed species of *Lemna minor*, *Lemna gibba*, *Lemna perpusilla* and *Spirodela punctata* as protein sources in poultry diets. On the other hand, using sewage-grown Wolffia meal, the smallest duckweed, in the diets of laying hens was reported only by Haustein *et al.* (1990). Wolffia meal is usually used as a food source in northern and northeastern Thailand. It is mainly collected from natural ponds. Recently, small commercial Wolffia meal cultivation farms have been established and their productions have never been studied as a protein source in poultry diets. This was the first report using cultivated Wolffia meal as a protein replacement for SBM in diets of laying hens. Haustein *et al.* (1990) found that feed intake, egg production and egg weight of laying hens were maintained by inclusion of 15% dietary Wolffia meal. However, the present results found the reductions of feed intake, ME intake and egg production (p<0.05) when CP from SBM was totally replaced by CP from Wolffia meal or using 12% dietary Wolffia meal. Although, CP intake of hens was not significantly different among dietary treatments, CP intake of hens fed a diet containing complete replacement of CP from SBM with CP from Wolffia meal was lower than that of NRC (1994) recommendation (14-15 gCP). Daily ME intake of hens in this study was also lower than the recommended level (292-305 kcal) for laying hens with 60-70% of egg production (NRC, 1994). Additionally, the body weight of hens fed diets containing complete replacement of CP from SBM with CP from Wolffia meal markedly decreased (-0.19 kg) as compared to that (-0.03 to -0.09 kg) of hens fed the rest diets at the end of the experiment. Therefore, decreased egg production occurred when CP from SBM was totally replaced by CP from Wolffia meal. The present result of egg quality is in agreement with Haustein *et al.* (1990) who revealed that the optimal level of *Lemna gibba* in the diets for laying hens was 15%, but even at 40% egg quality was not affected.

The increment of yolk pigmentation with increasing CP replacement from Wolffia meal in this study is consistent with the findings in the following studies: *Lemna gibba* (Haustein *et al.*, 1990) and *Spirodela punctata* (Nolan *et al.*, 1997) in laying hens and *Lemna perpusilla* in Jinding layer ducks (Khandaker *et al.*, 2007). Duckweed has high concentrations of pigments, particularly beta carotene (120-627.2 mg/kg) and xanthophyll (261-1000 mg/kg) (Haustein *et al.*, 1990; Dewanji, 1993; Skillicorn *et al.*, 1993; Hanczakowski *et al.*, 1995). The high concentrations are probably due to the anatomical structure of this plant and the high contribution of leaves to its total biomass (Hanczakowski *et al.*, 1995). The current result indicated that Wolffia meal was a good source of natural pigment for laying hens.

Conclusion: Complete replacement of CP from SBM with CP from Wolffia meal decreased (p<0.05) feed intake, ME intake and egg production. Egg yolk pigmentation increased (p<0.05) with increasing CP replacement from Wolffia meal. The results obviously indicated that 75% of CP from SBM can be replaced by CP from Wolffia meal in the diet of laying hens.

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