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## Effect of Wolffia Meal [*Wolffia globosa* (L). Wimm.] As a Dietary Protein Replacement on Performance and Carcass Characteristics in Broilers

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**Abstract:** Crude Protein (CP) from Wolffia meal [*Wolffia globosa* (L). Wimm.] was used to replace CP from Soybean Meal (SBM) in the diets of broilers to determine its effect on the broiler's productive performance and carcass characteristics. Using Completely Randomize Design, 384 ten-day-old broilers were divided into 4 treatments; each treatment consisted of 3 replicates of 16 birds each. The treatments were T<sub>1</sub>: control diet, T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub>: 25, 50 and 75% of CP from SBM was replaced by CP from Wolffia meal, respectively. The results indicated that feed intake, average daily gain, feed conversion ratio, protein efficiency ratio and carcass characteristics of control group were better ( $p < 0.05$ ) than other groups. Skin pigmentation of broilers increased with increasing CP replacement from Wolffia meal. It was concluded that less than 25% of CP from SBM could be replaced by CP from Wolffia meal in broiler diets.

**Key words:** Duckweed, protein source, performance, pigmentation, poultry

### INTRODUCTION

Wolffia meal (*Wolffia* spp.) is a tiny water plant about 0.5-1.5 mm in length that grows very well on lakes, marshes and sewage ponds. It is monocotyledons and is classified as higher plant (Landolt, 1986). Wolffia meal, one of duckweed species, belongs to the botanical family Lemnaceae, consisting of five genera *Landoltia*, *Lemna*, *Spirodela*, *Wolffia* and *Wolffiella* with over forty identified species (Les *et al.*, 2002). Natural Wolffia meal has been used as a vegetable by the Burmese, Laotians and the people of northern and northeastern Thailand for many generations (Bhanthumnavin and McGarry, 1971). Generally, Crude Protein (CP) content of Wolffia meal relies mainly on nitrogen concentration of the water (Skillicorn *et al.*, 1993, Leng, 1999). Therefore, CP content (29.9-45 g/100 g) of Wolffia meal grown in enriched water containing mineral media or effluents from agricultural waste lagoons (Rusoff *et al.*, 1980; Skillicorn *et al.*, 1993) was higher than that (20.4 g/100 g) of Wolffia meal from natural water low in nutrient (Chareontesprasit and Jiwyam, 2001). Its protein content (Skillicorn *et al.*, 1993) and amino acid profile (Rusoff *et*

*al.*, 1980) were comparable to those of soybean meal. Thus, Wolffia meal can be possibly used as protein source in animal diets.

Many studies previously demonstrated that duckweed species namely *Lemna gibba*, *Lemna perpusilla*, *Spirodela punctata* and *Lemna minor* were successfully used as a protein source in poultry diets (Jhori and Sharma, 1979; Haustein *et al.*, 1990, Sokantat, 1990; Haustein *et al.*, 1992; Islam *et al.*, 1997; Nolan *et al.*, 1997; Men *et al.*, 2001; Men *et al.*, 2002). On the other hand, utilization of Wolffia meal in poultry diet was merely reported by Haustein *et al.* (1990). They found that feed intake, egg production and egg weight of laying hens were maintained by inclusion of 15% dietary Wolffia meal. Presently, there is no available information of using Wolffia meal in the diets of broiler chicks. Therefore, this study was designed to examine the effects of replacing CP from Soybean Meal (SBM) with CP from cultivated Wolffia meal [*Wolffia globosa* (L) Wimm., accession number GQ221774] on the productive performance and carcass characteristics in broilers.

## MATERIALS AND METHODS

The experiment was conducted in accordance with the principles and guidelines approved by the Masahasakham University Animal Care and Use committee. Three hundred eighty four male broilers (Arbor Acres) were obtained at 1 d of age. Broilers were vaccinated for Newcastle disease and infectious bronchitis at the Hatchery. After feeding control diet until 10 d of age, broilers were randomly assigned to 4 groups, with 6 replicates of 16 broilers each. Broilers were kept in evaporative cooling system housing. Internal light was provided for 24 h/d. The room temperature was 32°C at the first week and was gradually decreased to 25°C from d 30 until the end of the trial.

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 2 mm. screen and stored in air tight bags for preparation of the experimental diets. The chemical composition of used Wolffia meal was previously reported by Chantiratikul *et al.* (2010).

The dietary treatments were T1 = control diet; T2, T3 and T4 = 25, 50 and 75% of the CP from SBM was replaced by the CP of Wolffia meal, respectively. All diets were isonitrogenous and isocaloric and formulated to meet the nutrient requirement of broiler according to NRC (1994) as presented in Table 1 and 2. The used Metabolizable Energy (ME) value of Wolffia meal for dietary treatment formulation was 4.39 MJ/kg (Poonpan *et al.*, 2009). The broilers received diets and water for *ad libitum* consumption throughout the experimental period.

The dietary treatments were randomly collected at the end of each week for determination of chemical composition (AOAC, 1999). Feed consumption was weekly recorded. Body weight was determined for d 10 to 21, d 21 to 42 and d 0 to 42 intervals. Feed Conversion Ratio (FCR) was calculated as kilograms of feed consumed per kilogram of gain. Protein Efficiency Ratio (PER) was calculated as grams of weight gain per gram of protein intake. The mortality of broilers was recorded during the entire experiment.

On d 42, 18 chickens from each treatment were weighed live, stunned and slaughtered by neck cutting and

Table 1: Feed ingredient and chemical composition of starter diets

Ingredients (%)	Dietary treatments <sup>1</sup>			
	T1	T2	T3	T4
Corn	49.00	43.80	38.55	33.00
Rice bran	4.00	4.00	4.00	4.30
Full fat soybean	15.70	16.85	18.10	19.30
Soybean meal (44%CP)	17.00	12.75	8.50	4.25
Wolffia meal (29.6%CP)	0.00	6.35	12.65	18.95
Fish meal	7.50	7.50	7.50	7.50
Soybean oil	4.20	6.15	8.10	10.10
Dicalcium phosphate	0.75	0.80	0.90	1.00
Oyster shell	1.25	1.20	1.10	1.00
DL-methionine	0.10	0.10	0.10	0.10
Sodium chloride	0.25	0.25	0.25	0.25
Premix <sup>2</sup>	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00
<b>Nutrient composition (%)</b>				
Crude protein	22.96	23.26	23.74	22.41
Ash	7.82	7.87	8.49	9.49
Metabolizable energy <sup>3</sup> (MJ/kg)	13.40	13.40	13.39	13.40
Ether extract	8.15	10.54	12.22	15.28
Crude fiber	3.58	4.09	4.08	4.49
Calcium	1.02	1.03	1.02	1.02
Total phosphorus	0.70	0.80	0.90	1.00
Available phosphorus	0.45	0.45	0.45	0.46
Lysine	1.34	1.35	1.35	1.35
Methionine	0.53	0.53	0.53	0.53
Methionine + Cystine	0.88	0.87	0.86	0.85

<sup>1</sup>T1 = control; T2, T3 and T4 = 25, 50 and 75% CP from SBM were replaced by CP from Wolffia meal, respectively.

<sup>2</sup>Vitamin-mineral premix provide (per kg diet): 10,000 IU vitamin A, 2,000 IU vitamin D<sub>3</sub>, 11 mg vitamin E, 1.5 mg vitamin K<sub>3</sub>, 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. <sup>3</sup>Calculated value

Table 2: Feed ingredient and chemical composition of grower diets

Ingredients (%)	Dietary treatments <sup>1</sup>			
	T1	T2	T3	T4
Corn	56.95	53.27	49.10	45.00
Rice bran	5.40	5.00	5.10	5.30
Full fat soy	14.00	15.00	16.00	16.80
Soybean meal (44%CP)	13.00	9.75	6.50	3.25
Wolffia meal (29.6%CP)	0.00	4.85	9.65	14.50
Fish meal	5.50	5.50	5.50	5.50
Soybean oil	2.85	4.35	5.85	7.35
Dicalcium phosphate	0.45	0.48	0.55	0.60
Oyster shell	1.35	1.30	1.25	1.20
Salt	0.25	0.25	0.25	0.25
Premix <sup>2</sup>	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00
<b>Nutrient composition (%)</b>				
Crude protein	20.46	20.39	19.07	19.40
Ash	6.11	6.81	7.45	8.52
Metabolizable energy <sup>3</sup> (MJ/kg)	13.39	13.40	13.40	13.39
Ether extract	8.44	10.10	11.83	13.03
Crude fiber	3.03	3.63	3.65	3.77
Calcium	0.91	0.90	0.91	0.91
Total phosphorus	0.61	0.68	0.76	0.84
Available phosphorus	0.35	0.34	0.35	0.35
Lysine	1.12	1.13	1.13	1.13
Methionine	0.38	0.38	0.38	0.38
Methionine + Cystine (%)	0.69	0.69	0.68	0.67

<sup>1</sup>T1 = control; T2, T3 and T4 = 25, 50 and 75% CP from SBM were replaced by CP from Wolffia meal, respectively.

<sup>2</sup>Vitamin-mineral premix provide (per kg diet): 10,000 IU vitamin A, 2,000 IU vitamin D<sub>3</sub>, 11 mg vitamin E, 1.5 mg vitamin K<sub>3</sub>, 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. <sup>3</sup>Calculated value

exsanguinated. Carcasses were plucked, eviscerated and weighed. Afterwards, carcasses were processed by removing wings, skinless breast muscle and legs. The heart, liver and abdominal fat were removed from the viscera. The gizzard was cut open and rinsed of its content. All separated organs and components were weighed and calculated for determination of carcass characteristics. Breast skin and shank colours were measured using RYC fan (F. Hofmann-La Roche Ltd, Basel, Switzerland), showing the range of colours from 1 (very light yellow) to 15 (very dark yellow).

**Statistical analysis:** The data of productive performance and carcass characteristics were analyzed by analysis of variance technique appropriate for Completely Randomized Design (SAS, 1996). The model used was:

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

Where:

$Y_{ij}$  = Observation

$\mu$  = Population mean,

$T_i$  = Diet effect (I = 1 to 4)

$\epsilon_{ij}$  = Residual error

The differences among 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

Feed intake and average daily gain of broilers significantly decreased ( $p < 0.05$ ) when CP from SBM was replaced by CP from Wolffia meal. Therefore, FCR and PER of broilers fed control diet were better than

( $p < 0.05$ ) those of broilers fed diets containing CP replacement from Wolffia meal. The result showed that mortality rate of broilers was not affected by inclusion of dietary CP from Wolffia meal (Table 3).

Carcass characteristics and skin colour of experimental broilers are summarized in Table 4. The body live weight and percentage of carcass decreased ( $p < 0.05$ ) when CP from SBM was replaced by CP from Wolffia meal. Percentages of leg, wing, breast, heart, gizzard and abdominal fat were not different among dietary treatments. The liver as percent of BW of broilers fed diets containing CP replacement from Wolffia meal was higher ( $p < 0.05$ ) than that of broilers fed control diet.

Breast and shank skin colours increased dramatically ( $p < 0.05$ ) with increasing dietary CP replacement from Wolffia meal.

## DISCUSSION

Wolffia meal is generally high in CP content (Huque *et al.*, 1996; Chowdhury *et al.*, 2000). However, naturally grown Wolffia meal has been rarely studied as protein replacement and supplement in animal diets. The main reason is probably due to the difficulty of its mass collection for the experiment. Recently, the small commercial Wolffia meal cultivation farms have been established in northeastern Thailand. The cultivated Wolffia meal was successfully used as protein replacement for SBM in diets of laying hens (Chantiratikul *et al.*, 2010).

The previous report found that broilers fed at level above 15% of dietary *Lemna gibba* had decreased feed consumption and productive performance (Haustein *et al.*, 1992; 1994). Furthermore, Sokantat (1990) suggested that CP from duckweed (*Lemna* spp.) should

Table 3: Effect of replacing protein from soybean meal with protein from Wolffia meal on productive performance in broilers

Item	Dietary treatments <sup>1</sup>				SEM
	T1	T2	T3	T4	
<b>Feed intake (g/bird/day)</b>					
10 to 21 d	62.04 <sup>bc</sup>	64.16 <sup>a</sup>	63.00 <sup>ab</sup>	60.64 <sup>c</sup>	0.36
21 to 42 d	178.40 <sup>a</sup>	166.56 <sup>b</sup>	153.17 <sup>c</sup>	145.63 <sup>d</sup>	2.83
10 to 42 d	120.22 <sup>a</sup>	115.36 <sup>b</sup>	108.08 <sup>c</sup>	103.14 <sup>d</sup>	1.48
<b>Average daily gain (g/bird/day)</b>					
10 to 21 d	48.50 <sup>a</sup>	41.32 <sup>b</sup>	38.76 <sup>c</sup>	36.59 <sup>d</sup>	0.98
21 to 42 d	82.69 <sup>a</sup>	76.02 <sup>b</sup>	67.06 <sup>c</sup>	61.23 <sup>d</sup>	1.84
10 to 42 d	65.59 <sup>a</sup>	58.67 <sup>b</sup>	52.91 <sup>c</sup>	48.91 <sup>d</sup>	1.36
<b>Feed conversion ratio (feed/gain)</b>					
10 to 21 d	1.28 <sup>c</sup>	1.56 <sup>b</sup>	1.63 <sup>ab</sup>	1.66 <sup>a</sup>	0.03
21 to 42 d	2.16 <sup>c</sup>	2.19 <sup>c</sup>	2.29 <sup>b</sup>	2.39 <sup>a</sup>	0.02
10 to 42 d	1.72 <sup>d</sup>	1.87 <sup>c</sup>	1.96 <sup>b</sup>	2.02 <sup>a</sup>	0.03
<b>Protein efficiency ratio</b>					
10 to 21 d	3.41 <sup>a</sup>	2.77 <sup>b</sup>	2.59 <sup>c</sup>	2.70 <sup>bc</sup>	0.07
21 to 42 d	2.27 <sup>a</sup>	2.24 <sup>ab</sup>	2.30 <sup>a</sup>	2.17 <sup>b</sup>	0.02
10 to 42 d	2.59 <sup>a</sup>	2.40 <sup>b</sup>	2.40 <sup>b</sup>	2.34 <sup>b</sup>	0.02
<b>Mortality rate (%)</b>					
10 to 42 d	0.00	1.04	1.04	1.04	0.43

<sup>1</sup>T1 = control; T2, T3 and T4 = 25, 50 and 75% CP from SBM were replaced by CP from Wolffia meal, respectively.

<sup>abcd</sup>Means in the same row with different superscripts are significantly different ( $p < 0.05$ )

Table 4: Effect of replacing protein from soybean meal with protein from Wolffia meal on carcass characteristics and skin colour in broilers

Item	Dietary treatments <sup>1</sup>				SEM
	T1	T2	T3	T4	
Body live weight (g)	2494.45 <sup>a</sup>	2270.00 <sup>b</sup>	2172.22 <sup>bc</sup>	2020.00 <sup>c</sup>	43.40
BW after plucking (g)	2328.89 <sup>a</sup>	2097.22 <sup>b</sup>	1993.33 <sup>bc</sup>	1870.56 <sup>c</sup>	41.62
BW after plucking (% BW)	93.35 <sup>a</sup>	92.40 <sup>ab</sup>	91.80 <sup>b</sup>	92.60 <sup>ab</sup>	0.22
Carcass dressing (% BW)	80.31 <sup>a</sup>	77.90 <sup>b</sup>	77.40 <sup>b</sup>	77.77 <sup>b</sup>	0.41
Legs (% carcass weight)	26.33	27.58	26.93	27.08	0.27
Wings (% carcass weight)	9.80	10.04	10.12	10.27	0.09
Breast (% carcass weight)	20.51	20.24	20.49	20.63	0.26
Liver (% BW)	1.76 <sup>b</sup>	1.96 <sup>a</sup>	1.93 <sup>a</sup>	2.01 <sup>a</sup>	0.03
Heart (% BW)	0.61	0.61	0.64	0.68	0.01
Gizzard (% BW)	1.32	1.49	1.45	1.42	0.04
Abdominal fat (% BW)	2.60	2.21	2.25	2.35	0.08
Breast skin colour	1.28 <sup>c</sup>	2.39 <sup>b</sup>	2.61 <sup>b</sup>	4.00 <sup>a</sup>	0.23
Shank skin colour	2.56 <sup>d</sup>	3.50 <sup>c</sup>	4.39 <sup>b</sup>	5.28 <sup>a</sup>	0.25

<sup>1</sup>T1 = control; T2, T3 and T4 = 25, 50 and 75% CP from SBM were replaced by CP from Wolffia meal, respectively.

<sup>abcd</sup>Means in the same row with different superscripts are significantly different ( $p < 0.05$ )

not replace CP from SBM at the level higher than 10% in starting broiler and 20% in finishing broiler diets. The above findings are in agreement with the present result using Wolffia meal as protein replacement in the diet of broilers. Replacement of CP from SBM with CP from Wolffia meal obviously depressed feed intake, performance and carcass percentage of broilers. Those results were directly reflected by the worsened FCR and PER of broilers fed diets containing CP replacement from Wolffia meal.

Wolffia meal was lower in lysine, isoleucine and arginine contents as compared to those of SMB (Chantiratikul *et al.*, 2010). Hanczakowski *et al.* (1995) and Rusoff *et al.* (1980) recommended that dried duckweed was deficient in methionine. Therefore, amino acid balance should be seriously considered when Wolffia meal was used as protein replacement in the diet. The present results could possibly explain by amino acid imbalance in the diets. Dietary amino acid imbalance leads to increased amino acid catabolism that mainly takes place in the liver (McDonald *et al.*, 1996), resulting in reductions in feed intake and growth performance (Peng *et al.*, 1972; Davis and Austic, 1982). Additionally, metabolic effects of the amino acid imbalance may cause the accumulation of lipids in the liver and the enlargement of liver (Yasukawa and Yoshida, 1980). Consequently, proportion of the liver per body weight of broilers increased ( $p < 0.05$ ) with increasing CP replacement from Wolffia meal in the diets (Table 4).

Duckweed contains 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; Haustein *et al.*, 1994; Hanczakowski *et al.*, 1995). Therefore, skin pigmentation of broilers in the current study increased with increasing inclusion of dietary Wolffia meal. This result is consistent with the previous study in broilers

using *Lemna gibba* (Haustein *et al.*, 1994). Other duckweed species [*Spirodela punctata*, *Lemna perpusilla* and *Wolffia globosa* (L. Wimm.)] have been also found to increase pigmentation in yolk of laying hens (Haustein *et al.*, 1990; Nolan *et al.*, 1997; Chantiratikul *et al.*, 2010).

**Conclusion:** Replacement of CP from SBM with CP from Wolffia meal negatively affected ( $p < 0.05$ ) performance and carcass characteristics in broilers. However, the skin pigmentation of broilers increased with increasing inclusion of dietary Wolffia meal. The results indicated that less than 25% of CP from SBM could be replaced by protein from Wolffia meal in broiler diet.

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