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Effect of Heat-treatment on Ruminal Protein Degradability of Wolffia Meal (*Wolffia globosa* L. Wimm)

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ABSTRACT

The objective of this research was to reduce ruminal protein degradability of Wolffia meal (*Wolffia globosa* L. Wimm.) by roasting at 150°C for 2 to 8 min. Two Thai-indigenous x Brahman crossbred cattle were used to determine the *in situ* ruminal degradability of heat-treated Wolffia meal. The ruminal degradation characteristics of Wolffia meal were determined using nylon bags incubated in rumen in reverse order for 72, 48, 24, 12, 8, 4 and 2 h. The results found the decreased rapidly soluble Crude Protein (CP) fraction ($p<0.05$) and the increased potentially degraded CP fraction ($p<0.05$) of Wolffia meal roasted at 150°C for 6 to 8 min. However, protein degradability of Wolffia meal was not reduced by heat treatment. The results indicated that roasting at 150°C for 6 to 8 min could not protect Wolffia meal's protein degradation by rumen microorganism.

Key words: Duckweed, protein degradation, roasting, ruminant

INTRODUCTION

Wolffia meal (*Wolffia* spp.) 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 rom 13-38 tons/ha/year, which is a rate exceeding single-crop soybean production six to ten fold (Skillicorn *et al*., 1993). Furthermore, the protein content (29.9-45 g/100 g) of Wolffia meal grown in enriched water containing mineral media or effluents from agricultural waste lagoons, is greatly increased over that from natural waters low in nutrient (Skillicorn *et al*., 1993; Huque *et al*., 1996). Its protein content (Skillicorn *et al*., 1993) and amino acid profile (Chantiratikul *et al*., 2010a) were comparable to those of soybean meal. Thus, Wolffia meal can be used as a source of protein in animal diets.

Utilization of duckweed species, namely *Lemna gibba, Lemna perpusilla, Spirodela punctata* and *Lemna minor* as a protein source, has been studied in ruminant animals such as cattle (Chewewattanagool, 2002) and sheep (Damry *et al*., 2001). However, there is little scientific work to study on ruminal protein degradability of duckweed. Leng *et al*. (1995) suggested that duckweed would be a source of ruminally degradable protein and/or minerals to support rumen microbial growth. Huque *et al*. (1996) also revealed that duckweed proteins were highly degraded in the rumen of cattle. On the other hands, *Spirodela punctata* has been found to be a valuable source of undegradable protein for ruminants (Damry and Nolan, 2002; Damry *et al*., 2001). Protein of Wolffia meal has been firstly reported to be extensively degraded in the rumen of bull by Huque *et al*. (1996). This result indicated that only small amounts of amino acids would have been available from Wolffia meal for absorption in the small intestine. Therefore, reduction of Wolffia meal's protein degradable in the rumen is needed to be studied. For many years different physical and chemical methods have been researched to reduce rumen protein degradability. Heat treatments seem to be more effective and practical than chemical ones protecting protein from ruminal degradation (Mustafa *et al*., 2000). Furthermore, heat treatments have been reported to significantly reduce ruminal protein degradability in many feedstuffs (Ljokjit *et al*., 2003; McNiven *et al*., 2002). Thus, the objective of this research was to decrease protein degradability of Wolffia meal (*Wolffia globosa* L. Wimm., accession number GQ221774) by roasting at 150°C for 2 to 8 min.

MATERIALS AND METHODS

This study was conducted during October 2009 to September 2010. Two Thai-indigenous x Brahman crossbred cattle with an average body weight of 400±30 kg were used to determine the *in situ* ruminal degradability of Wolffia meal. The animals were fitted with a rumen fistula. The experimental animals were dewormed by Ivomectin (1 mL/30 kg BW) and injected with AD_3E vitamin-complex (1 mL/50 kg BW) before the beginning of the study. The cattle were housed in individual pen and fed twice daily (830 and 1630 h) in equal aliquots at 1.5% DM of body weight. The basal diet was formulated to meet nutrient requirements of beef cattle (NRC, 1996) and prepared in total mixed ration with rice straw as a roughage source (Table 1). Drinking water was freely available. The experiment consisted of 14 day adjustment period and 14 day determination of *in situ* ruminal degradability.

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 sieve. Ground Wolffia meal was roasted in a roaster at 150°C for 2, 4, 6 and 8 min. A portion of each sample was ground though a 1 mm screen sieve and used for analysis of Dry Matter (DM), Organic Matter (OM), Crude Protein (CP) using the procedure of AOAC (1990). The Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF) and Acid Detergent Insoluble Nitrogen (ADIN) were determined according to Van Soest *et al*. (1991).

Approximately 4 g of untreated and heat-treated (at 150°C for 2, 4, 6 and 8 min) Wolffia meal was weighed and put into a nylon bag (9×15 cm) with an average pore size 45 μm (International Feed Resources Unit, Aberdeen, UK). The samples were prepared in four replicates. All nylon bags were soaked into the water for 5 min to exclude the air and then introduced into the rumen of each cannulated cattle in reverse order for 72, 48, 24, 12, 8, 4 and 2 h. After incubation, the bags, including the 0-h bags were removed and immediately rinsed under tap water until the water was cleared. The samples were dried at 60°C until a constant weight was achieved. The same incubated samples within cattle were pooled and ground through a 1 mm screen prior to analysis of DM, OM and CP according to AOAC (1990).

Dry matter degradability was calculated as the difference between the original sample and the weight of the residue. Nutrient degradability was calculated as the difference between the amount nutrient (g) of the original and the residual samples (Maiga *et al*., 1996). Dry matter, OM and CP ruminal degradability characteristic were calculated according to the monoexponential equation

Table 1: Ingredients and chemical composition of the basal diet

Items	(%)
Ingredients	
Rice straw	30.00
Cassava meal	38.80
Soybean meal	20.50
Wolffia meal	2.50
Molasses	5.00
Urea	0.50
Dicalcium phosphate	1.50
Sulfur	0.20
Salt	0.50
Premix[1]	0.50
Total	100.00
Chemical composition	
Dry matter	91.70
Crude protein	12.73
Ash	10.96
Metabolizable energy[2] (MJ kg^{-1})	12.11

[1]The premix provided per kilogram of diet: 10,000 IU vitamin A; 2,000 IU vitamin D3; 20 IU vitamin E; 0.01 g Cu; 0.08 g Mn; 0.04 g Zn; 0.05 g Fe; 0.0008 g I; 0.0003 g Co; 0.0003 g Se; 0.005 g Ethoxiquin; and 0.05 g SiO_2, [2]ME = Metabolisable energy

proposed by Orskov and McDonald (1979). The equation used was p= a+b ($1-e^{-ct}$) where, P is the degradation at t time (%), a is the intercept at zero time of the degradation curve (%), b is the fraction of DM, OM and CP which will be degraded potentially (%), c is the degradation rate constant of fraction b (/h) and t is time of incubation (h).

The effective degradability (ED) of DM, OM and CP were estimated using the equation of Orskov and McDonald (1979) ED = a+b {c/(c+k)} where, a, b and c are as defined previously. k is the fractional outflow rate, assumed to be 0.08 h^{-1}.

Statistical analyses: Chemical compositions of the test samples were analyzed in Completely Randomize Design using the analysis of variance (ANOVA) procedure of SAS (SAS, 1996). Ruminal degradation data was analyzed in Randomize Completely Block Design using the ANOVA procedure of the SAS program. The effect of roasting time on ruminal degradation characteristics was tested in Completely Randomize Design by the ANOVA of the SAS program. Means were separated by Duncan's New Multiple Range Test (Steel and Torries, 1980).

RESULTS AND DISCUSSION

Wolffia meal cultivation has been recently commercialized in northeastern Thailand. The cultivated Wolffia meal was successfully tested as protein replacement for soybean meal in diets of poultry (Chantiratikul *et al.*, 2010a-c). On the other hand, there is insufficient information of using Wolffia meal as a protein source in the diet of ruminants. The first report that was studied on the potentiality of Wolffia meal as a feed for cattle found that protein of Wolffia meal was mostly degraded in the rumen after incubation for 72 h (Huque *et al.*, 1996). The current study tried to reduce protein degradation of Wolffia meal by roasting at 150°C for 2 to 8 min. The results indicated that heat treatment did not alter DM, OM, Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF) contents of Wolffia meal. However, Acid Detergent Insoluble Nitrogen (ADIN) of Wolffia meal increased significantly ($p<0.05$) from 7.58 to 21.08% of N when Wolffia

Table 2: Chemical composition (% of DM) of heat-treated Wolffia meal

Chemical Composition	Heat-treated Wolffia meal (min) 0	2	4	6	8	SEM
Dry matter	93.42	93.78	94.29	94.77	93.64	0.24
Crude protein	35.55	34.55	34.64	33.22	34.15	0.38
Organic matter	83.72	83.48	83.64	83.33	83.09	0.11
NDF	28.23	28.41	28.45	28.23	28.49	0.06
ADF	23.07	23.43	24.58	23.65	23.35	0.26
ADIN (% of N)	7.58[c]	7.80[c]	14.15[b]	19.19[a]	21.08[a]	2.80

[abc]Means in the same row with different superscripts are significantly different ($p<0.05$), NDF = Neutral detergent fiber, ADF = Acid detergent fiber, ADIN = Acid detergent insoluble nitrogen

Table 3: Dry matter degradability characteristics and effective degradability of heat-treated Wolffia meal

Item[1]	Heat-treated Wolffia meal (min) 0	2	4	6	8	SEM
a (%)	35.19	33.18	31.53	30.59	29.07	0.76
b (%)	59.02	61.23	63.21	64.49	64.65	0.75
c (/h)	0.0662	0.0675	0.0687	0.0687	0.0687	0.0033
ED (%)	61.42	60.60	59.87	59.92	58.63	0.59

[1]a = Rapidly soluble fraction, b = Potentially degradable fraction, c = Fractional degradation rate of fraction b, ED = Effective degradability at an outflow rate (fraction/h) of 0.08 h^{-1}

Table 4: Organic matter degradability characteristics and effective degradability of heat-treated Wolffia meal

Item[1]	Heat-treated Wolffia meal (min) 0	2	4	6	8	SEM
a (%)	29.52[a]	26.86[ab]	23.28[bc]	22.25[bc]	19.25[c]	1.11
b (%)	69.59[c]	72.11[bc]	75.37[ab]	76.19[ab]	79.72[a]	1.14
c (/h)	0.0575	0.055	0.0575	0.0588	0.0575	0.003
ED (%)	52.28	55.60	54.67	53.87	51.87	0.74

[abc]Means in the same row with different superscripts are significantly different ($p<0.05$), [1]a = Rapidly soluble fraction, b = Potentially degradable fraction, c = Fractional degradation rate of fraction b, ED = Effective degradability at an outflow rate (fraction/h) of 0.08 h^{-1}

Table 5: Crude protein degradability characteristics and effective degradability of heat-treated Wolffia meal

Item[1]	Heat-treated Wolffia meal (min) 0	2	4	6	8	SEM
a (%)	37.81[a]	34.70[a]	32.85[ab]	28.57[b]	27.89[b]	0.97
b (%)	62.75[b]	65.45[b]	67.20[ab]	71.46[a]	72.02[a]	0.95
c (/h)	0.0739	0.075	0.0754	0.0763	0.0768	0.0032
ED (%)	65.24	65.96	64.89	62.96	62.76	0.81
RUP (%)	34.76	34.04	35.11	37.04	37.24	0.81

[ab]Means in the same row with different superscripts are significantly different ($p<0.05$), [1]a = Rapidly soluble fraction, b = Potentially degradable fraction, c = Fractional degradation rate of fraction b, ED = Effective degradability at an outflow rate (fraction/h) of 0.08 h^{-1}, RUP = Ruminal undegradable protein

meal was roasted at 150°C for 2 to 8 min (Table 2). Similarly McNiven *et al.* (2002) found the increased ADIN (3 to 9.5% of N) in soybean heated at 195°C for 3 to 6 min. The increased ADIN in some feedstuffs also found with increased heat intensity (McNiven *et al.*, 2002; Pereira *et al.*, 1988). The present and previous results consistently demonstrated that the Maillard reaction depends on treatment temperature and treatment time. Additionally, moisture and reducing agents may be limiting for Maillard reaction (Martins *et al.*, 2001; Bach, 1997). The increase in ADIN when heating feedstuffs is attributed to the formation of Maillard products, which decreases rumen protein degradation (Ljokjit *et al.*, 2003).

The protein of untreated Wolffia meal in the present study was highly degraded (98%) after incubated in the rumen for 72 h. Huque *et al.* (1996) also found Wolffia meal's protein was mostly degraded in the rumen of bull. On the other hand, the results in sheep demonstrated that about half of the protein in duckweed (*Spirodela punctata*) was degraded in the rumen (Damry and Nolan, 2002). The different rumen degradable protein of untreated Wolffia meal and *Spirodela punctata* may be due to the incubation period in the rumen, drying conditions of duckweed or ruminal conditions between animal species (Damry *et al.*, 2001).

The rapidly soluble DM fraction, the potentially degraded DM fraction, breakdown rates (c) of the DM fraction and effective DM degradation of Wolffia meal were not influenced ($p>0.05$) by heat treatment (Table 3). Heat treatment significantly decreased ($p<0.05$) the rapidly soluble OM and CP fractions, but markedly increased ($p<0.05$) the potentially degraded OM and CP fractions. However, fractional degradation rate of OM and CP fractions, effective OM and CP degradations and ruminal undegradable protein (RUP) of Wolffia meal were not changed ($p>0.05$) by heat treatment (Table 4, 5). The present results reflect that roasting at 150°C for 2 to 8 min did not alter DM degradability, but partly affected CP degradability characteristics of Wolffia meal. Other studies similarly observed the decreased soluble fraction and the increased potentially degraded CP fraction of soybeans (Nowak *et al.*, 2005; Chouinard *et al.*, 1997) and whole cottonseeds (Arieli *et al.*, 1989) by heat treatment as a result of rapidly degradable protein fraction denatured and became slowly degradable fractions (Van Soest, 1987). In the current study, ADIN and potentially degraded CP fraction increased 11.61-13.50 and 8.71-9.27%, respectively. However, RUP slightly increased (2.28-2.48%) when Wolffia meal was roasted at 150°C for 6 to 8 min. The results indicated that roasting at 150°C for 6 to 8 min could not strongly protect protein of Wolffia meal from microbial degradation. Haugen *et al.* (2006) implied that ADIN might not be a good predictor of the indigestible dietary protein in some forages. Additionally, ADIN can be digested in some extent (Arieli *et al.*, 1989). Van Soest (1987) suggested that an optimum heat intensity and time depend on many factors: moisture content, carbohydrate content and composition, protein content, the presence of sulphite and therefore, optimum parameters of heat treatment and time vary from one dietary protein to another. Therefore, further research is needed to investigate the optimum heat intensity and duration to reduce ruminal protein degradation of Wolffia meal.

CONCLUSION

Roasting at 150°C for 6 to 8 min decreased ($p<0.05$) the rapidly soluble CP fraction, but increased ($p<0.05$) the potentially degraded CP fraction of Wolffia meal when compared with those of untreated Wolffia meal. However, heat treatment did not decrease ruminal degradation of Wolffia meal's protein.

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