OPEN ACCESS
Journal of Animal Health and Production

Research Article

Effect of Tannin-rich *Mimosa pigra* Leaf Meal in Molasses-based Blocks on Feed Intake, Rumen Parameters, and Blood Metabolites of Dairy Heifers

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Abstract | The study was aimed to evaluate the influence of condensed tannin in tannin-rich *Mimosa pigra* leaf meal (TMLM) as a nitrogen binder in molasses-based blocks (MBB) on feed intake, rumen parameters, and blood metabolites of dairy heifers. Four crossbred Holstein heifers with 328.12 ± 1.96 kg average body weight were assigned in a 4x4 Latin square design to dietary treatments with four different levels of TMLM in MBB at 0, 75, 150, and 300 g/kg dry matter, respectively. Heifers were fed green chopped guinea grass (*Panicum maximum* TD58) for *ad libitum* intake as the basal feed. The feeding trial lasted for 80-d. The results indicated that grass intake, total feed intake, neutral detergent fiber intake, and acid detergent fiber intake significantly increased in response to the advanced degree of TMLM inclusion MBB (P<0.05). However, none of the TMLM inclusions had a significant impact on rumen parameters, blood biochemical profiles, and complete blood counts (P>0.05), except the number of eosinophils (P<0.05). In conclusion, TMLM was beneficial for dairy heifers at 150 g/kg dry matter in MBB to improve feed intake and allow the development of dairy feeding strategies.

Keywords | Tannin-rich, *Mimosa pigra* Leaf Meal, Molasses-based Blocks, Heifer

Received | March 15, 2021; **Accepted** | May 09, 2021; **Published** | October 01, 2021
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Citation | Wittayakun S, Innaree W, Chainetr W, Thongtip N (2021).Effect of tannin-rich *Mimosa pigra* leaf meal in molasses-based blocks on feed intake, rumen parameters, and blood metabolites of dairy heifers. J. Anim. Health Prod. 9(4): 463-470.
DOI | http://dx.doi.org/10.17582/journal.jahp/2021/9.4.463.470
ISSN | 2308-2801

INTRODUCTION

The ideal goals of dairy feeding programs have been focused on how to increase the usages of available local feed resources to provide fermentable rumen carbohydrates, nitrogen, and minerals, particularly concerning smallholder dairy farms in the tropics, especially during the dry season. Supplementation of molasses-based blocks is a widely simple feeding technique that primarily aims to supply a small amount of nitrogen from urea together with non-structural carbohydrate in the form of molasses for rumen microbes to use as substrates for microbial protein synthesis (George Kunju, 1988; Katulski et al., 2017). Although supplementation of molasses-based blocks has shown several advantages, the rapid conversion of urea to ammonia in the rumen may cause potential problems such as poor feed intake and urea toxicity if excess urea is being absorbed rapidly post-feeding (Mirza et al., 2002). Giant sensitive tree (*Mimosa pigra* L.), a leguminous shrub, and a serious weed in the tropical regions have been referred to as a potential feed ingredient due to its availability, yield, and nutrient contents (Wittayakun et al., 2017). The leaf yield of giant sensitive tree accounts for 22.52 % of plant weight, which is high in crude protein (18.87 to 21.80%) and condensed tannins (3.11 to 9.89 %) (Hong et al., 2008). Many research results reported the benefits of condensed tannins as a potential substance to reduce nitrogen degradation in the rumen by forming strong complexes ruminants resulting in a balance of degradation rate and optimize microbial protein synthesis (Dey et al., 2006; Dey and De, 2014). We hypothesized that condensed tannins of tannin-rich *Mimosa pigra* leaf meal (TMLM) would reduce nitrogen degradation by forming strong complexes with urea nitrogen in molasses-based blocks (MBB), and have consequence positive effects in replacement dairy heifers particularly when feeding was based on guinea grass. Therefore, the objective of this study was to evaluate the effects of TMLM inclusion as a condensed tannin source in MBB and if this would affect feed intake, nutrient intake, rumen parameters, blood biochemical profiles, metabolites, or complete blood counts of dairy heifers.

MATERIALS AND METHODS

Experimental design and management

Four crossbred Holstein Friesian (93.75%) heifers, averaged 328.12±1.96 kg body weight, were assigned in a 4x4 Latin square design. The experimental periods consisted of four consecutive periods which overall trial lasted for 80 days. Each period consisted of 14-days as an adapt period and 6-days of sampling and measurements. Treatments were molasses-based block containing either tannin-rich *Mimosa pigra* leaf meal (TMLM) at 0, 75, 150, or 300 g/kg dry matter (Table 1). The *Mimosa pigra* L. leaves were collected in Lampang province (N18°17'32.35", E 99°29'33.97"), northern Thailand. The molasses-based block (MBB) was prepared according to George Kunju (1988), then packed into molds, and allowed 10 to 14 days to become harden. Purple guinea grass or green panic grass (*Panicum maximum* TD58) was grown at Lampang campus, and harvested at approximately 60 days of regrowth age by tractor-combine double chop harvester; then fed twice at 08.00 and 16.00 h daily. Diets were expected to maintain the expected average daily gain at 0.60 kg/day of heifers based on the recommendation of NRC (2001). Heifers were individually kept in a corral of 1.5 x 4.0 m^2, each with free access to drinking water. Animal care and procedures were performed according to the guidelines of the Animal for Thai Scientific Purposes Acts, B.E. 2558 or A.D. 2015 (TGG, 2015).

Sampling and measurement

Feed offered and refused were weighed and recorded daily throughout the trial. All heifers were weighed twice (day 1 and day 20) of each period. During the last 3-days of the data collection period, feed samples were collected, dried at 60°C for 72 h; ground, and composited to analyze for dry matter (DM), crude protein (CP), ether extract (EE), and ash (AOAC, 1990). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were measured by the method of Van Soest et al. (1991). Organic matter (OM) was calculated as follows: OM = 100 – Ash %. Condensed tannin was examined by the method of Broadhurst and Jones (1978). On the last day of each period at 4 h post-feeding, rumen fluid was sampling using an esophageal tube, filtered through four layers of sheet cloth, and measured pH with a portable pH meter (pHtestr 30®, EUTECH Instruments, Singapore). The population of rumen microorganisms; including bacteria, protozoa, and fungi, was measured by total direct count, followed the method described by Galyean (1989). Blood samples were taken from a coccygeal vein at 4 h after feeding and subsequent analysis for biochemical profiles using Auto Biochemistry Analyzer (Model TC 220, Tecom Science Corporation, China); for electrolytes using Electrolyte Analyzer (Model URIT-910C Plus, URIT Medical Electronic Co, Ltd., China); for complete blood using Automated Hematology Analyzer (Model URIT-3000 Plus, URIT Medical Electronic Co, Ltd., China).

Statistical analysis

Data were analyzed using the general linear procedure for Analysis of Variance (ANOVA), and significance was set at P-value less than 0.05. Treatment means were compared by the least significant difference (LSD) (SPSS 2006).

RESULTS

Characteristics of experimental diets

Nutrient composition and physical characteristics of experimental diets are present in Table 2. The mean concentration of CP was relatively low in the 0 g/kg TMLM but high in the 300 g/kg TMLM while the amount of EE was in contrast due to the high-fat content of the RB ingredient. All dietary treatments were low in fiber contents, as indicated in terms of NDF, and ADF. In contrast, ash contents were high in all treatments in response to some feed ingredients that high in inorganic contents, such as dicalcium phosphate, sulfur, premix, and lime.

Feed intake and nutrient intake

Feed intake, nutrient intake, and average body weight of heifers are present in Table 3. Increasing TMLM in MBB had no significant effects on daily MBB intake ($P>0.05$). However, daily grass intake and total DM intake were significantly influenced by increasing of TMLM as well as intake of neutral detergent fiber (NDF), and acid detergent fiber (ADF) ($P<0.05$). Heifers fed grass with a higher proportion of TMLM tended to consume more metabolizable energy (ME) than those other groups. The condensed tannin intake was numerically greater for heifers fed the higher inclusions of TMLM compared with those fed the lower levels of TMLM ($P<0.05$).

Table 1: Ingredients of experimental diets, %DM

Items	**TMLM, g/kg DM**			
	0	75	150	300
TMLM	0	7.5	15	30
Urea	10	10	10	10
Molasses	30	30	30	30
RB	30	22.5	15	0
Ground corn	5	5	5	5
Salt	5	5	5	5
Dicalcium phosphate	4	4	4	4
Sulfur	0.5	0.5	0.5	0.5
Premixed	0.5	0.5	0.5	0.5
Lime	15	15	15	15
Total	100	100	100	100

TMLM: tannin-rich *Mimosa pigra* leaf meal; RB: rice bran

Table 2: Nutrient contents and physical characteristics of diets, %DM

Nutrient contents	**TMLM, g/kg DM**				**TMLM**	**RB**	**Grass**
	0	75	150	300			
DM	88.03	84.85	86.20	88.24	78.12	91.74	44.37
OM	71.55	72.44	72.53	71.27	92.97	92.12	81.85
CP	37.44	46.17	45.96	48.56	21.88	13.15	7.86
EE	2.87	1.19	1.22	0.92	4.66	14.50	1.56
NDF	8.39	5.10	6.13	11.75	56.25	19.58	62.31
ADF	4.17	4.61	8.37	12.51	43.82	8.24	47.72
Ash	28.44	27.55	27.46	28.72	7.03	7.88	19.47
ME, Mcal/kgDM	2.69	2.68	2.62	2.55	2.03	2.62	1.97
Condensed tannin		5.55	10.94	21.37	6.60		
Physical characteristics							
Weight, kg	2.70	2.90	2.57	2.61			
Density, g/cm^3	2.95	3.17	2.91	2.86			

TMLM: tannin-rich *Mimosa pigra* leaf meal; RB: rice bran; DM: dry matter; OM: organic matter; CP: crude protein; EE: ether extract; NDF: neutral detergent fiber; ADF: acid detergent fiber; GE: gross energy; ME: metabolizable energy by calculation according to the equation of Donker (1989) as ME (Mcal/kgDM) = 2.76 – 0.00165 (gADF/kgDM)

Table 3: Feed intake and nutrient intake of heifers offered tannin-rich *Mimosa pigra* leaf meal (TMLM).

Items	**TMLM, g/kg DM**				SEM	***P*-value**
	0	75	150	300		
Dry matter intake						
- Molasses-based blocks						
kg/h/d	0.29	0.33	0.25	0.26	0.08	0.570
% BW	0.08	0.10	0.07	0.07	0.03	0.498
$g/kgBW^{0.75}$	3.79	4.32	3.25	3.20	1.14	0.517
- Grass						
kgDM/h/d	7.59^{a}	7.17^{a}	8.11^{ab}	8.59^{b}	0.55	0.045
% BW	2.32^{ab}	2.23^{a}	2.52^{bc}	2.66^{c}	0.15	0.031
$g/kgBW^{0.75}$	98.30^{ab}	94.61^{a}	106.56^{bc}	112.65^{c}	6.55	0.029
- Total intake						

kgDM/h/d	7.88	7.51	8.36	8.85	0.54	0.054
% BW	2.41^{a}	2.34^{a}	2.60^{ab}	2.74^{b}	0.16	0.045
g/kgBW$^{0.75}$	102.09^{a}	98.94^{a}	109.82^{ab}	115.85^{b}	6.70	0.042
Nutrient intake, kg/h/d						
kgDM/h/d	7.88	7.51	8.36	8.85	0.54	0.054
OM	6.42	6.11	6.82	7.22	0.44	0.053
CP	0.70	0.72	0.75	0.80	0.05	0.178
EE	0.13	0.12	0.13	0.14	0.00	0.059
NDF	4.75^{a}	4.48^{a}	5.07^{ab}	5.38^{b}	0.34	0.043
ADF	3.63^{a}	3.43^{a}	3.89^{ab}	4.13^{b}	0.26	0.041
ME, Mcal/d	15.74	15.03	16.63	17.59	1.07	0.062
Condensed tannin, g/d		18.67^{a}	27.37^{ab}	55.65^{b}	16.96	0.019
Condensed tannin, g/kgDM		2.50^{a}	3.33^{a}	6.00^{b}	1.45	0.007
Average BW, kg	329.12	325.37	329.87	328.12	7.07	0.818

TMLM: tannin-rich *Mimosa pigra* leaf meal; BW: body weight; OM: organic matter; CP: crude protein; EE: ether extract; NDF: neutral detergent fiber; ADF: acid detergent fiber, ME: metabolizable energy by calculation according to the equation of Donker (1989) as ME (Mcal/kgDM) = 2.76 – 0.00165 (gADF/kgDM)

^{ab} Superscripted means within a row that are not same differ at ($P<0.05$)

Table 4: Rumen parameters of heifers offered tannin-rich *Mimosa pigra* leaf meal (TMLM).

Items	**TMLM, g/kg DM**				**SEM**	***P*-value**
	0	75	150	300		
Rumen pH	7.42	7.50	7.80	7.79	0.27	0.220
Rumen microbial population count, cell/ml						
Bacteria, x 10^{11}	3.93	4.97	6.64	3.65	1.64	0.138
Protozoa, x 10^{6}	3.13	2.40	2.49	2.42	0.44	0.163
Fungal zoospore, x10^{5}	1.67	1.45	1.17	1.15	0.24	0.119

TMLM: tannin-rich *Mimosa pigra* leaf meal

Table 5: Blood biochemical profiles of heifers offered tannin-rich *Mimosa pigra* leaf meal (TMLM).

Items	**TMLM, g/kg DM**				**SEM**	***P*-value**
	0	75	150	300		
Glucose, mmol/L	4.06	4.37	4.28	4.06	0.15	0.136
Urea, mmol/L	3.93	4.26	4.13	4.26	0.53	0.786
Total Protein, g/L	77.00	75.75	74.00	75.50	3.25	0.653
Triglyceride, mmol/L	0.72	0.75	0.75	0.76	0.05	0.791
Cholesterol, mmol/L	3.32	3.37	3.47	3.19	0.10	0.054
Creatinine, µmol/L	86.19	86.85	93.26	81.54	19.57	0.864
SGOT, µkat/L	1.42	1.46	1.21	2.64	1.14	0.361
SGPT, µkat/L	0.29	0.36	0.36	0.35	0.05	0.330
Calcium, mmol/L	2.15	1.93	2.13	2.04	0.17	0.362
Potassium, mmol/L	4.50	4.57	4.72	4.77	0.43	0.798
Sodium, mmol/L	139.50	140.00	139.25	140.00	0.85	0.551
Chloride, mmol/L	101.25	103.50	101.75	104.00	2.04	0.265

TMLM: tannin-rich *Mimosa pigra* leaf meal; SGOT = serum glutamic-oxaloacetic transaminase; SGPT = serum glutamic pyruvate transaminase

Table 6: Complete blood counts of heifers offered tannin-rich *Mimosa pigra* leaf meal (TMLM).

Items	TMLM, g/kg DM				SEM	*P*-value
	0	75	150	300		
Hb, g/L	79.25	80.25	78.66	78.75	8.65	0.938
Hct, %	23.20	23.12	23.46	22.45	2.55	0.924
RBC, 10^{12}/L	4.31	4.64	4.41	4.35	0.53	0.820
MCV, fL	50.52	50.17	52.60	51.12	1.14	0.273
MCH, fmol	1.12	1.09	1.10	1.17	0.07	0.503
MCHC, mmol/L	22.54	21.92	21.03	23.00	1.10	0.614
RDW, %	19.05	19.45	18.46	18.30	1.26	0.555
WBC, 10^9/L	12.85	14.40	11.26	12.00	2.73	0.495
PMN, %	44.25	33.25	33.00	33.25	9.17	0.427
Lymphocyte, %	44.25	57.25	49.66	56.50	8.25	0.200
Monocyte, %	2.00	1.50	1.66	1.50	0.75	0.744
Eosinophil, %	9.50^{a}	8.00^{a}	5.33^{ab}	2.00^{b}	2.54	0.031

TMLM: tannin-rich *Mimosa pigra* leaf meal; Hb = hemoglobin; Hct = hematocrit; RBC = red blood cell count; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; RDW = red blood cell distribution; WBC = white blood cell; PMN = polymorphonuclear neutrophil

^{ab} Superscripted means within a row that are not same differ at (P<0.05).

Rumen parameters, biochemical blood profiles, and complete blood counts

Rumen parameters of heifers offered various levels of TMLM in MBB are presented in Table 4. The average rumen pH and microbial populations were not significantly affected by the inclusion of TMLM (P>0.05). Similarly, the inclusion of TMLM did not influence blood biochemical profiles (P>0.05; Table 5).

Complete blood counts of dairy heifers fed TMLM are presented in Table 6. In this trial, increasing levels of TMLM did not have a significant effect on complete blood counts as expressed in term of hemoglobin, hematocrit, red blood cell count, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, red blood cell distribution, white blood cell, polymorphonuclear neutrophil, lymphocyte, and monocyte (P>0.05). However, increasing levels of TMLM had significantly affected the number of eosinophils when the inclusion of TMLM in MBB was greater than 150 g/kg DM (P<0.05).

DISCUSSION

TMLM may influence feed intake, rumen parameters, and blood metabolites of dairy heifers for daily feeding regime. The findings from this study suggest that the inclusion of TMLM in MBB can increase the feed intake of dairy heifers. The results agree with many previous trials, but those were based on low-quality roughages (Plaizier et al., 1999; Mirza et al., 2004; Cherdthong et al., 2014a; Cherdthong et al., 2014b). In this trial, only green panic grass (*Panicum maximum* TD58) was offered as the sole roughage while urea was used as the main nitrogen source, approximately 10% DM of feed ingredients in MBB. After feeding, urea may undergo hydrolysis rapidly in the rumen into ammonia via microbial degradation, especially within the first hour after feed consumption. Then, ammonia may be actively absorbed by rumen bacteria to utilize as a nitrogen source via microbial amino acid biosynthesis and a carbon skeleton from ruminal carbohydrates. The greater efficiency of these processes depends on the synchrony of ammonia released together with available ruminal carbohydrates, leading to improved feed intake and productive performance (Golombeski et al., 2006; Taylor-Edwards et al., 2009; Giallongo et al., 2015). The application of TMLM may induce the forming of indigestible complexes between urea and condensed tannin by digestive enzymes of bacteria, which have impacts on reducing nitrogen degradation to parallel ruminal carbohydrate digestion resulting in improvement of non-protein nitrogen utilization in the rumen (Dey et al., 2006; Dey and De 2014; Cidrini et al., 2019; Zhang et al., 2019). Besides, condensed tannin alters urinary nitrogen excretion to the feces routes that are minimized nitrogen excretion from a dairy farm and beneficial to the environment (Zhang et al., 2019). According to many works, tannin-rich leaves may interfere with palatability and reduce feed intake if the inclusion of tannin-rich leaves containing condensed tannin greater than 50 g/kg DM due to their astringent taste (Mueller-Harvey 2006). In this study, the level of condensed tannin was low and ranged from 2.50- 6.00 g/kg of dry matter intake resulting in no interference on feed intake. Our results contrast with the results of Dschaak et al. (2011) who demonstrated that condensed tannin in dairy cows fed high forage diets decreased feed, and nutrient intake. Besides, the lack of effects of condensed tannin on dry matter intake has been reported (Pathak et al., 2013; Piñeiro-Vázquez et al., 2018). The inclusion of TMLM did not increase crude protein intake as expected and slightly below those recommended by NRC (2001) because of low MBB consumptions in all groups. These imply low palatability of MBB due to the astringent taste of TMLM and high inorganic ingredients such as lime. The increasing consumption of NDF and ADF of the green chopped guinea grass was consistent with many previous works (Mirza et al., 2004; Golombeski et al., 2006; Taylor-Edwards et al., 2009; Giallongo et al., 2015).

Rumen pH can be used as an indicator for monitoring the healthy rumen function of ruminants. In the present study, rumen pH reflected adequate dietary fiber consumption for chewing and salivation mechanisms of heifers. Besides, some heavy alkali ingredients such as lime may cause rumen pH of all treatments above seven. The inclusion of TMLM in MBB did not influence rumen microbial populations. However, the bacterial population tended to increase continuously when the inclusion rate of TMLM was added and up to 150 g/kg DM, but tended to decline sharply after the inclusion rate of TMLM was more than 150 g/kg DM. This could be the sign of optimal synchrony of nitrogen utilization by rumen cellulolytic bacteria due to its slow release (Russell et al., 2009). The lack of significant difference for the protozoa population may indicate the low availability of dietary starch (Santra and Pathak, 2001). This result agrees with Piñeiro-Vázquez et al. (2018), who observed no change in the population of rumen protozoa in heifers fed condensed tannin from *Leucaena leucocephala*. Normally, the protozoa population changes dramatically with high starch or fat diets (Zhang et al., 2015; Panyakaew et al., 2020). The lack of difference in the population of fungal zoospores despite higher consumption of grass may have been due to condensed tannin in diets. Saminathan et al. (2019) reported that population, community structure, and diversity of fungi were decreased by the addition of condensed tannins.

Blood biochemical profiles can be used to monitor the metabolism and health status of ruminant animals. Plasma glucose is synthesized mainly in the liver using propionic acid as a precursor by the gluconeogenesis pathway, especially in response to high grain feeding. Because of low MBB consumption in all groups, no difference in blood glucose was detected in this trial. Besides, variation in glucose levels may be an indicator to detect animal stress (Wheelock et al., 2010). The inclusion of TMLM in this study resulted in no changes in blood urea nitrogen; this may be contributed to lower consumption of MBB than expected due to palatability interference of some feed ingredient. Therefore, ammonia nitrogen to be absorbed across the rumen wall into the portal vein to process the urea cycle is the limit. Feeding with tanniniferous supplements is generally a decrease in blood urea nitrogen (Mkhize et al., 2018; Zhang et al., 2019) in response to the formation of nitrogen complexes condensed tannin in the rumen and enhancing protected protein supply into the small intestine (Min and Hart 2003). Although high in inorganic contents of MBB, no sign of impaired kidney function was detected in response to total protein and creatinine levels. Inclusions of TMLM in MBB did not affect liver function due to SGOT and SGPT levels and blood electrolytes, including calcium, potassium, sodium, and chloride. A complete blood count (CBC) is an optional tool to evaluate overall animal health status and detect a wide range of disorders in response to TMLM feeding. According to the results, high levels of TMLM inclusion caused a lower number of eosinophils in CBC. This finding contradicts Pathak et al. (2013), who reported higher mean eosinophil counts with condensed tannin of leaf meal mixture in sheep. Reduction in eosinophils may impair the efficiency of body immune systems and associate the risk of infections.

CONCLUSION

The TMLM inclusion in MBB in dairy heifers significantly increased grass intake, total feed intake, NDF, and ADF intake without any adverse effects on rumen parameters, blood biochemical profiles, and complete blood counts (except eosinophils). The results of the current study suggest that TMLM inclusion in MBB should limit not more than 150g/kg dry matter for feeding dairy heifers as a condensed tannin source with green chopped guinea grass as the basal diet.

CONFLICT OF INTEREST

The authors wish to declare that they had no conflict of interest.

AUTHORS CONTRIBUTION

All authors contributed equally.

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