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Effect of *Mucuna pruriens* leaves in dairy cow feed on gas production, digestibility and rumen fermentation by using *in vitro* gas production technique

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Abstract The results showed that the *Mucuna pruriens* leaves at 10% in feed affect dry matter and organic matter digestibility which higher than with the control group ($p<0.05$). However, *M. pruriens* leaves in all level were not different among dietary treatments on the gas production from the immediately soluble fraction, the gas production from the insoluble fraction, and the gas production rate constant for the insoluble fractionand the potential extent of gas productio. Therefore, it can use *M. pruriens* leaves at 10% in dairy cow feed.

Keywords: *Mucuna pruriens*, Gas production, Digestibility, Rumen fermentation

Introduction

Dairy farming is an important occupation in Thailand because people tend to increase milk consumption and milk is widely as a nutritious drink for people of all ages. Especially, among children (Zhang *et al.*, 2021). In addition, the Thai government has the policy to supported farmers to enhance dairy production. However, farmer has still faced many issues in the dairy production sector such as management, disease, breed and feed. Especially, feed costs have an increased. Most of the farmers in Thailand depend on natural pastures but the roughages are poor quality and insufficient of roughage for animal so require protein supplementation (Muinga and Saha, 2003) but the ingredient as protein source in concentrate was *expensive* such as soybean meal, Palm kernel meal, fish meal and cottonseed meal (Abdeltawab and Khattab, 2018). Agricultural residues include rice straw, wheat straw and corn stover, which are used as animal feed. In addition, native plant was used to feed animals, it consists of Leucaena, Mata Raton, Mulberry and Mucuna.

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Mucuna (*Mucuna pruriens*) is widespread in tropical and sub-tropical regions of the world and with a great potential for animal feeding. This is the high protein content which can used as replacements for soybean meal in animal feeding. The chemical and nutritional composition of Mucuna contain 25-35% crude protein, 31.2-39.5 % of starch and 21.3 % neutral detergent fiber (Martínez-Pérez *et al.,* 2008). Several research studies have been carried out on the development about using *M. pruriens* leaves in animal feed to increase the use of alternative protein sources and reduces feed cost. According to Muinga and Saha (2003) who found that cow fed Mucuna forage tended to increased production performance and live bodyweight and Mucuna hay has been fed both to dairy cattle to increase milk production and meat production (Mbuthia and Gachuiri, 2003). However, the studies and research about *Mucuna pruriens* leaves in dairy cow feed is limited and unclear. Therefore, the objective of was to investigate the effect of *M. pruriens* leaves meal as a protein sourse in dairy cow feed on gas production, digestibility and rumen fermentation by using *in vitro* gas production technique.

Materials and methods

Study area

The study is carried out in Ubon Ratchathani University, Ubon Ratchathani Province which locates in the north-eastern region of Thailand during June 2020- June 2021.

Experimental design and dietary treatments

The experiments was performed in a Completely Randomized Design (CRD). The basal diet was 40:60 ratio of Ruzi grass (*Brachiaria ruziziensis*) and concentrate. The ingredients composition of the basal diet is shown in Table 1. There were four dietary treatments consisting basal diets without *Mucuna pruriens* (the control group; T1) and T2, T3, T4 were basal diets using *M. pruriens* at 10, 20 and 30% respectively. Dietary treatments were incubated at 70 ℃ for three day and grounded into a powder form and passed through a 0.5 mm sieve.

Rumen and substrate inoculums

Rumen fluid was collected from three healthy dairy cows, which were placed on a routine basis for at least 1 week and fed by the same basal diet for 5

days before sampling by collection through a suction pump. The rumen fluid was collected and filtered through two layers of cheesecloth. *In vitro* fermentation was used in this study according to the technique described by Makkar *et al*. (1995). The rumen fluid (660 ml) was added to warm (39 ℃) and reduced medium consisting of 1,095 ml distilled water, 730 ml rumen buffer solution (417 mM $NaHCO_3$ and 51 mM NH_4HCO_3), 365 ml macro mineral solution (46 mM KH_2PO_4, 40 mM Na_2HPO_4, 38 mM NaCl and 2 mM $MgSO_4 \cdot 7H_2O$), 0.23 ml micromineral solution (505 mM $MnCl_2 \cdot 4H_2O$, 898 mM $CaCl_2 \cdot 2H_2O$, 42 mM $CoCl_2 \cdot 6H_2O$ and 341 mM $FeCl_2 \cdot 6H_2O$), 1 ml of 4mM resazurin and 60 ml freshly prepared reduction solution (145 mM $Na_2S \cdot 9H_2O$ and 3.7 ml 1 M-NaOH). The mixture was kept stirring and continuously filled with CO_2 to ensure anaerobic condition at 39 ℃ on a hot plate. Approximately 30 ml of the rumen-fluid medium was transferred into serum bottles incubated with dietary treatments (200 mg) at 39 ℃ for 72 h.

Table 1. Ingredient composition of the basal diet

Item	% of fresh weigh
Ingredients	
Maize	25.0
Cassava chip	22.0
Rice bran	10.0
Soybean meal	26.4
Palm kernel meal	15.0
Mineral premix	0.5
Salt	0.5
sulfur	0.1
Dicalcium	0.5

Data collection and analysis

Dietary treatments were analyzed for dry matter (DM), Ash, crude protein (CP) and ether extract (EE) using the procedure of AOAC (1997), while neutral detergent fiber (NDF) and acid detergent fiber (ADF) were also determined as described by Van Soest *et al*. (1991).

Gas production during the incubation, the kinetics of gas production were recorded at 2, 4, 6, 8, 12, 18, 24, 36, 48 and 72 h. Cumulative gas production data were fitted to the model of ∅rskov and McDonald (1979) as follows:

$$Y = a + b\ (1 - e^{(ct)})$$

Where a = the gas production from the immediately soluble fraction, b = the gas production from the insoluble fraction, c = the gas production rate constant for the insoluble fraction (b), t = incubation time, (a+b) = the potential extent of gas production, y = gas production at time't'. At 24- and 48-h post inoculation, a set of samples were determined *in vitro* true digestibility according to Van Soest and Robertson (1985).

Statistical analysis

All data were analyzed as a Completely Randomize Design (CRD) using the SAS (1998). The significant differences between treatments were compared by Duncan's New Multiple Range Test (Steel and Torrie, 1980). Difference among treatment with $P<0.05$ was accepted as statistical differences.

Results

The chemical compositions of basal diet and *Mucuna pruriens* leaves is shown in Table 2. The results showed the crude protein content in basal diet and *M. pruriens* leaves were 19.2% and 24.3% and 4.5% and 2.4% EE of dry matter basis.

Table 2. The chemical composition of basal diet and *Mucuna pruriens* leaves

Item	Basal diet	*Mucuna pruriens* leaves
DM (%)	96.5	17.4
Chemical composition		
OM	91.8	94.6
Ash	8.2	5.40
CP	19.1	24.3
EE	4.5	2.40
NDF	32.2	66.2
ADF	27.5	44.8

DM = Dry Matter, OM = Organic Matter, CP = Crude Protein, EE = Ether Extract
NDF = Neutral Detergent Fiber, ADF = Acid Detergent Fiber

Kinetic of gas production

The estimated parameters of gas production are shown in Table 2 and cumulative gas production profiles are shown in Figure 1. The results showed

that the gas production from the insoluble fraction (b) the potential extent of gas production (d) and effective gas production potential (EP) were significant different among treatments (P<0.05), of which the *Mucuna pruriens* leaves at 30% that was lower than with the control group and *M. pruriens* leaves at 10%. However, the gas production from the immediately soluble fraction (a), the gas production from the insoluble fraction (b), the gas production rate constant for the insoluble fraction (c) were not significant different among treatments (P>0.05). However, the gas production at different incubation times (GP 2, 4, 6, 8, 12, 18, 24, 36, 48 and 72 h) (Figure 1).

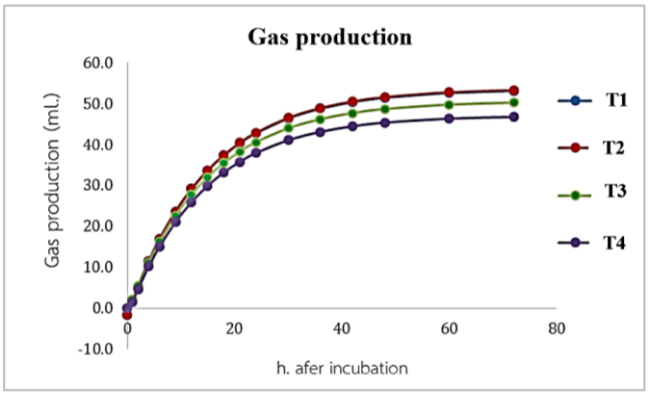

Figure 1. Effect of *Mucuna pruriens* leaves on gas production at different times of incubation**:** T1 = Basal diet (Control group), T2 = Basal diet used *Mucuna pruriens* leaves at 10%, T3 = Basal diet used *Mucuna pruriens* leaves at 20%, T4 = Basal diet used *Mucuna pruriens* leaves at 30%

Table 3. Effects of *Mucuna pruriens* leaves on gas production in dietary treatments

Item	Dietary treatments				SEM	P-value
	T1	T2	T3	T4		
Gas production characteristics						
a	-1.718	-1.755	-1.320	-1.775	0.13	0.61
b	55.2^{a}	55.4^{a}	51.9^{ab}	48.8^{b}	1.01	0.04
c	0.0682	0.0681	0.0686	0.0699	0.001	0.88
d	53.5^{a}	53.6^{a}	50.5^{b}	47.0^{c}	0.96	0.03
Effective gas production potential (EP)						
	30.1^{a}	30.1^{a}	28.7^{ab}	26.6^{b}	0.49	0.02

The significant at P < 0.05

a = the gas production from the immediately soluble fraction, b = the gas production from the insoluble fraction, c = the gas production rate constant for the insoluble fraction, d = the potential extent of gas production,

EP = a + [bc/(k + c)] where k = 0.05 (Ørskov and McDonald, 1979)

Table 4. Effects of *Mucuna pruriens* leaves on *in vitro* digestibility

Items	Dietary treatments				SEM	P-value
	T1	T2	T3	T4		
In vitro dry matter digestibility, IVDMD (%)						
24 h	45.5[a]	46.0[a]	40.4[b]	40.0[b]	1.07	<0.01
48 h	52.5[a]	56.1[a]	46.9[b]	47.0[b]	1.51	<0.01
In vitro organic matter digestibility, IVOMD						
24 h	47.7[b]	55.3[a]	47.3[b]	41.5[c]	1.87	<0.01
48 h	63.5[b]	65.5[a]	59.4[c]	58.8[d]	1.05	<0.01
Metabolizable energy, ME (Mcal/kgDM)						
24 h	2.07[a]	2.08[a]	2.01[ab]	1.92[b]	0.02	0.03
48 h	2.38[a]	2.39[a]	2.29[ab]	2.18[b]	0.03	0.02
Total digestible energy, TDN						
24 h	56.7[a]	56.7[a]	55.3[ab]	53.3[b]	0.52	0.04
48 h	63.3[a]	63.7[a]	61.5[ab]	59.1[b]	0.66	0.02

Significant at $P < 0.05$, Significantly at $P < 0.01$
T1 = Basal diet (Control group), T2 = Basal diet used *Mucuna pruriens* leaves at 10%,
T3 = Basal diet used *Mucuna pruriens* leaves at 20%, T4 = Basal diet used *Mucuna pruriens* leaves at 30%

In vitro organic matter digestibility (IVOMD) in basal diet used *M. pruriens* leaves at 10% (T2) was higher than the other groups ($P<0.01$). Moreover, *in vitro* dry matter digestibility was not affected between the control group and basal diet used *M. pruriens* leaves at 10% but basal diet used *M. pruriens* leaves at 20% and 30% were lower than with the control group ($P<0.01$). Metabolizable energy (ME) and total digestible energy (TDN) incubated at 24 h and 48 h were significant between dietary treatments ($P<0.05$) by the basal diet used *Mucuna pruriens* leaves at 30% was lower the other group. However, basal diet used *M. pruriens* leaves at 10, 20% was no different with control group.

Discussion

The crude protein content in *M. pruriens* leaves is and 24.3% and 4.5% EE of dry matter basis. This results similarly reported by Ujowundu (2010) who reported the *M. pruriens* leaves contain 25%crude protein of dry matter basis. Basal diet was also formulated to contain 22.1% CP, 4.5% EE, 97.8% OM, 32.2 % NDF and 27.5% ADF of dry. The results of the gas production

from the immediately soluble fraction (a) were -1.718, -1.755, -1.320 and -1.775 respectively that result was minus because the treatment diets are resting state. This result agrees with Yeanpet *et al.* (2021). The basal diet used *M. pruriens* leaves at 30% affected on the gas production from the insoluble fraction (b) the potential extent of gas production (d) and effective gas production potential (EP) lower than with the control group and basal diet used *M. pruriens* leaves at 10% that *M. pruriens* leaves contain 12.5% crude fiber of dry matter basis (Ezeokonkwo and Okafor, 2015), whereas soybean meal used as a source of protein in basal diet has higher fiber content (≈ 5%of DM) (Stein *et al.,* 2008). The high fiber content in the dietary treatments effect on digestion and kinetic of gas production. However, there are only a few studies about *M. pruriens* leaves on digestibility in cow. While *M. pruriens* leaves at 10% no different with control group while the gas production from the immediately soluble fraction (a), the gas production from the insoluble fraction (b), the gas production rate constant for the insoluble fraction (c) were not significant difference among treatment.

The *in vitro* fermentation profiles were significantly different of *Mucuna pruriens* leaves for *in vitro* organic matter digestibility (IVOMD) at 24 and 48 h. *Mucuna pruriens* leaves at 10% higher than with another group but *M. pruriens* leaves at 30% lower than with other group this result suggests that *M. pruriens* leaves consist polyphenols, trypsin inhibitors, phytate, saponins, lectins, and tannin able to bind to proteins, thus lowering their digestibility. (Lampariello *et al.*, 2011). *Mucuna pruriens* at 30% had negative effect on metabolizable energy (ME) and total digestible energy (TDN) at 24 h and 48 h because in *vitro* dry digestibility in *M. pruriens* leaves at 30% lower than another group. While basal diet used *Mucuna pruriens* at 10, 20% was no different with control group (Muinga and Saha, 2003)

This study shown the results that the *M. pruriens* leaves at 10% enhance *in vitro* dry matter and organic matter digestibility and which has not a negative impact of kinetic of gas production of cow can use *M. pruriens* leaves at 10% in dairy cow feed. However, the issue regarding *M. pruriens* leaves supplement in feed on digestibility has not yet been completely settled. Further studies are warranted to understand these relationships

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