Effect of Mao (*Antidesma thwaitesianum* Muell. Arg.) seed supplementation on *in vitro* rumen protozoal population and digestibility using gas production technique

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ABSTRACT: This experiment was to investigate the effect of mao seed (MS) levels on digestibility and rumen gas production by using *in vitro* gas production techniques. The experimental design was a completely randomized design (CRD) and the dietary treatments were MS supplementation at 0, 4, 8, 12, 16 and 20 mg with 0.5 g of roughage and concentrate ratio at 60:40. The results revealed that the intercept value (a), gas production from the insoluble fraction (b), gas production rate (c), potential extent of gas production (a+b) and cumulative gas production (72 h of incubation) were not significantly different among treatments (P>0.05). Supplementing with MS had not affected on *in vitro* dry matter degradability (IVDMD), true digestibility and NH_3-N concentrations (P>0.05). Moreover, supplementing MS linearly decreased in *in vitro* organic matter degradability (IVOMD). Populations of protozoa tended to decrease when increasing level of MS (P=0.06). Based on this study, it could be concluded that supplementation of MS at 8 mg could be efficiently utilized in rumen in term of reduced protozoal population and maintaining the digestibility and *in vitro* gas production.

Keywords: Mao seed, gas production technique, digestibility, protozoal population

Introduction

Manipulation of the rumen microbial ecosystem for reducing methane emissions with improved efficiency of the digested energy utilization by ruminants is some of the most important goals for animal nutritionists (Patra et al., 2006). Recently, the use of plant containing secondary compounds such as tannins and saponins with antimicrobial properties which could be exploited in animal nutrition to improve rumen ecology, manipulate rumen fermentation and improve feed efficiency (Anantasook et al., 2013a). Mao or called Mamao or Makmao is classified in the family *Stilaginaceae*, genus *Antidesma*. Thai *Antidesma* composes of 18 species (Hoffmann, 1999). However, *A. thwaitesianum* Muell Arg. is favorable to be consumed and sold in the local market in Thailand because of its good color and taste (Puangpronpitag et al., 2008). Mao grows well over a variety of soil types and is naturalised in Africa, Australia, tropical Asia

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and islands in the Pacific Ocean (Poungpronpitag et al., 2011). Recently, products such as Mao juice and Mao wine have become more popular in Thailand. This industry generates a large number of by-products, most notably Mao seed (MS) and Mao marce (MM). Supplementation of wastes products from the Mao into diet of animals could increase the efficiency of by-products utilization in the feeding systems. Suphanphuwong et al. (2012) reported supplementation Mao pomace (seed and marce) at 8 g/d could improve feed intake and digestibility of dry matter (DM) and neutral degergent fiber (NDF) in goats. Moreover, Gunun et al. (2014) reported that Mao pomace supplementation did not influence the digestibility and *in vitro* gas production. However, no investigations have been performed on supplementation of MS in concentrate diets of ruminants. Therefore, the objective of this experiment was to investigate the effect of MS levels on rumen kinetics gas production, protozoal population and digestibility of nutrients by using *in vitro* gas production technique.

Materials and methods

The experimental design was a completely randomized design (CRD) and the dietary treatments were MS supplementation at 0, 4, 8, 12, 16 and 20 mg with 0.5 g of roughage and concentrate ratio at 60:40. Rice straw was used as a roughage source. MS was collected from Mao juice and Mao wine at Department of Food Science and Technology, Faculty of Natural Resources, Rajamangala University of Technology -Isan, Sakon Nakhon Campus, Thailand; and sundried for 2 days. Samples of MS, roughage and concentrates were dried at 60 °C, then ground to pass a 1-mm sieve (Cyclotech Mill, Tecator, Sweden) and used for chemical analysis and in the *in vitro* gas test. The samples were analyzed for dry matter (DM), ash and crude protein (CP) using the procedures of AOAC (1995), neutral detergent fiber (NDF) and acid detergent fiber (ADF) according to Van Soest et al. (1991). Content of condensed tannins in MS was analyzed by using the modified vanillin-HCl method based on Burns (1971). Crude saponins were measured using methanol extraction following the method of Kwon et al. (2003) and modified by Poungchompu et al. (2009). The ingredients and chemical compositions of concentrate, rice straw and MS used in the *in vitro* experiment are shown in **Table 1**.

Two male, rumen-fistulated crossbred beef cattle with body weight of 400±30 kg were used as rumen fluid donors. Beef cattle rumen fluid was collected from animals fed with concentrate (16% CP) at 0.5% of BW in two equal portions, at 08:00 h and 16:00 h and rice straw was fed on *ad libitum* basis. The animals were kept in individual pens and clean fresh water and mineral blocks were offered as free choice. The animals received the diets for 14 d before the rumen fluid was collected. On day 15, 1000 ml rumen liquor was obtained from each animal before the morning feeding. Ruminal fluid from each animal was mixed with the artificial saliva solution of Menke and Steingass (1988) in a proportion 2:1 (ml/ml) at 39°C under continuous flushing with CO_2 and 40 ml of rumen inocula mixture were added into each bottle under CO_2 flushing. Bottles were sealed with rubber stoppers and aluminum caps and incubated at 39°C (72 h) for *in vitro* gas test.

During the incubation, data of gas production was measured immediately after incubation at 0, 1, 2, 4, 6, 8, 12, 18, 24, 48 and 72 h by using a glass syringe. Cumulative gas production data were fitted to the model of Ørskov and McDonald (1979) as follows:

$y = a + b\,[1\text{-}e^{(-ct)}]$

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 produced at time "t". Fermentation liquor was sampled at 2 and 4 h post inoculations and then filtered through four layers of cheesecloth. Samples were divided into 2 portions; 1 portion was used for NH_3–N analysis by Kjeltech Auto 1030 Analyzer (AOAC, 1995). A second portion was used for direct counts of protozoa using methods of Galyean (1989) by haemocytometer (Boeco, Singapore). The *in vitro* degradability and *in vitro* true digestibility were analyzed by standard method (Tilley and Terry, 1963; Van Soest et al., 1991; Blümmel et al., 1997). All data from the experiment were analyzed as a completely randomized design using the GLM procedure of SAS (1998).Trend of MS levels responded was performed by orthogonal polynomials.

Results and Discussion

The *in vitro* gas production technique is remarkable boost and data on rumen fermentation kinetics of numerous feeds are available (Infascelli et al., 1998). Gas kinetics, cumulative gas production, IVDMD, IVOMD, true digestibility and NH_3-N concentrations of each substrate treatments are presented in **Table 2.** It was found that gas production from intercept value (a) ranged from -3.4 to -4.8 and was not significantly different among treatments (P>0.05). Moreover, gas production rate (c) was also not altered However, gas production from the insoluble fraction (b) and potential extent of gas production (a+b) tended to linearly increased when supplementation of MS (P=0.09), and was highest at 12 mg MS (78.7 and 74.5, respectively). This could be due to supplementation of MS at 4-12 mg could reduce loss of nutrients. Cumulative gas production at 72 h was not significantly different among treatments (P>0.05).

The IVDMD, IVOMD, true digestibility and NH_3–N concentrations and protozoal population are given in **Table 3.** The results show that IVDMD and true digestibility were not affected by MS supplementation (P>0.05). In contrast, IVOMD was linearly reduced when increasing levels of MS supplementation (P<0.05). This result was probably due to the increased fiber contents, since the MS contained 58.7% and 48.6% of NDF and ADF, respectively. Similarly, Gunun et al. (2014) reported that IVOMD was decreased when increasing level of mao pomace. Ruminal NH_3-N concentrations were 20.7–21.7 mg% and were close to those previously reported by Kang and Wanapat (2013; 16.4-22.6 mg% *in vitro*) and was not significantly different among treatments (p>0.05). Moreover, the amount of protozoa decreased linearly at 4 h post-feeding with increased level of MS ($p<0.01$). These results are consistent with Anantasook et al. (2013b) reported that populations of protozoa decreased with supplementation with rain tree pod meal containing condensed tannins and saponins using real-time PCR assays in dairy steers. Similary, Pilajun and Wanapat (2012) reported that the number of protozoal population was decreased by mangosteen peel supplementation in swamp buffalo. Anti-protozoa activities of saponins, seem to be mediated by their capacity to form irreversible complexes with cholesterol in the protozoal cell membrane to cause destruction of cell membrane, cell lysis and death (Francis et al., 2002). In addition, saponins may reduce the activity of methanogen for a lower supplying of hydrogen, also reduce rumen methane emissions.

Table 1 Ingredients and chemical composition of concentrate, rice straw and mao seed (MS).

Item	Concentrate	RS[1]	MS[2]
Ingredient	----------------------% of DM	----------------------	
Cassava chip	61.7	-	-
Soybean meal	11.9	-	-
Whole cottonseed	11.2	-	-
Rice bran	8.3	-	-
Urea	2.4	-	-
Molasses	2.5	-	-
Salt	0.5	-	-
Sulfur	0.5	-	-
Mineral and vitamin mixture	1.0	-	-
Chemical composition			
Dry matter, g/kg	94.8	93.4	33.1
	----------------------% of DM	----------------------	
Organic matter	92.3	91.2	95.4
Crude protein	16.1	3.2	10.4
Neutral detergent fiber	27.0	70.9	58.7
Acid detergent fiber	17.9	57.1	48.6
Ash	7.7	8.8	4.6
Condensed tannins	-	-	9.9
Crude Saponins	-	-	9.6

[1] Rice straw; [2] Mao seed

Table 2 The effect of mao seed (MS) supplementation on gas kinetics and cumulative gas production (72 h)

MS (mg)	Gas kinetics				Gas[1]
	a	b	c	a+b	
0	-4.6	64.8	0.03	60.4	44.5
4	-4.0	70.1	0.02	66.0	43.3
8	-4.5	73.3	0.02	68.9	46.8
12	-4.2	78.7	0.02	74.5	46.1
16	-4.8	69.6	0.02	64.7	48.2
20	-3.4	62.8	0.03	59.4	46.0
SEM	0.36	4.50	0.002	1.60	2.02
Orthogonal polynomial[2]					
L	NS	0.09	NS	0.09	NS
Q	NS	NS	NS	NS	NS
C	NS	NS	NS	NS	NS

ns = non-significant

[1]Cumulative gas production at 72 h (ml/0.5 g DM substrate)

[2]Linear (L), quadratic(Q) and cubic(C) effects of different levels of MS supplementation

Table 3 The effect of Mao seed (MS) supplementation on *in vitro* degradability, true digestibility, NH_3-N and protozoal population

MS (mg)	Degradability (%)		True digestibility (%)	NH_3-N	Protozoa, 10^5		
	IVDMD	IVOMD		(Mg/dl)	2 h	4 h	Means
0	58.2	68.9	60.2	21.7	1.6	2.6	2.1
4	56.1	64.9	58.7	21.7	1.1	2.1	1.5
8	59.6	66.3	59.6	21.4	0.5	1.2	0.8
12	56.9	63.9	59.5	22.4	1.4	1.0	1.3
16	57.9	64.8	60.3	20.8	1.3	1.1	1.1
20	59.1	65.9	55.1	20.7	1.0	1.1	1.0
SEM	2.41	0.74	2.40	0.45	0.19	0.09	0.12
Orthogonal polynomial[2]							
L	NS	*	NS	NS	NS	**	0.06
Q	NS	NS	NS	NS	NS	NS	NS
C	NS	NS	NS	NS	NS	NS	NS

ns = non-significant, * $p<0.05$, ** $p<0.01$

[2]Linear (L), quadratic(Q) and cubic(C) effects of different levels of MS supplementation

Conclusion and Suggestions

Based on this experiment, it could be concluded that supplementation of MS at 8 mg resulted in decreased protozoal populationin and maintaining *in vitro* kinetics gas and digestibility. However, these findings should be applied further in *in vivo* experiment in order to enhance ruminant production.

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