Replacement of soybean meal by kased-kok meal (*Neptunia javanica* Miq.) in the diet on gas production and ruminal degradability by using *in vitro* gas technique

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บทคัดย่อ:วัตถุประสงค์ของการศึกษาในครั้งนี้เพื่อศึกษาอิทธิพลของการทดแทนกากถั่วเหลืองด้วยกระเฉดโคกบดในสูตรอาหารต่อผลผลิตแก๊สและการย่อยสลายในกระเพาะรูเมนโดยใช้เทคนิค *in vitro*แก๊ส ใช้แผนการทดลองแบบสุ่มสมบูรณ์และอาหารทดลองคือการทดแทนกากถั่วเหลืองด้วยกระเฉดโคกบดที่ระดับ 30, 60 และ 100% ในสูตรอาหาร, ตามลำดับ ทำการทดลองโดยใช้อาหารในอัตราส่วนอาหารหยาบ:อาหารข้นที่ 40:60 ผลการทดลองพบว่า จลศาสตร์ผลผลิตแก๊สและผลผลิตแก๊สรวมไม่มีความแตกต่างกันทางสถิติ (P>0.05) ระหว่างทรีทเมนต์ การย่อยสลายของวัตถุแห้ง, อินทรียวัตถุและการย่อยได้ที่แท้จริงในทรีทเมนต์ที่ทดแทนกากถั่วเหลืองด้วยกระเฉดโคกบดที่ระดับ 30 และ 60% มีค่าไม่แตกต่างกับทรีทเมนต์ที่ใช้กากถั่วเหลือง 100% แต่อย่างไรก็ตามพบว่าเมื่อทดแทนกากถั่วเหลืองด้วยกระเฉดโคกบดที่ระดับ 100% จะส่งผลให้การย่อยสลายและการย่อยได้ที่แท้จริงลดลง ชีวมวลจุลินทรีย์และความเข้มข้นของแอมโมเนีย-ไนโตรเจนมีค่าสูงสุดเมื่อทำการทดแทนกากถั่วเหลืองด้วยกระเฉดโคกบดที่ระดับ 30% ดังนั้นจากการศึกษาในครั้งนี้พบว่าการทดแทนกากถั่วเหลืองด้วยกระเฉดโคกบดที่ระดับ 30% สามารถปรับปรุงผลผลิตแก๊ส, จลศาสตร์ผลผลิตแก๊ส, การย่อยสลายในกระเพาะรูเมน, ชีวมวลจุลินทรีย์ และความเข้มข้นของแอมโมเนีย-ไนโตรเจน

ABSTRACT: The aim of this study was to investigate the effect of replacement of soybean meal (SBB) by kased-kok (*Neptunia javanica* Miq.) meal, (KSKM) in the diet on gas production and ruminal degradability by using *in vitro* gas technique. The experimental design was a completely randomized design (CRD) and the dietary treatments were replacement of SBM by KSKM at 30, 60 and 100%, respectively with 0.5 g of roughage and concentrate ratio at 40:60.The gas production was recorded at 0, 1, 2, 4, 6, 8, 12, 18, 24, 48 and 72 h of incubation and was used for calculation of gas kinetics. The results revealed that gas kinetics and cumulative gas production were not significantly different among treatments (P>0.05).The *in vitro* degradability of DM, OM and true digestibility in treatment that replacement SBM with KSKM at 30 and 60% were not different with SMB group. However, increased level of replacement to 100%, the *in vitro* degradability and true digestibility were reduced. Microbial biomass was highest with KSKM replacement at 30% (19.6 mg). Moreover, NH_3-N concentrations were quadratically increased when increasing levels of KSKM in the diet and were highest with KSKM replacement at 30% (25.6 mg/ dL).Based on this study, it could be concluded that replacement of SBM by KSKM at 30% in the diets could improve *in vitro* gas production,gas kinetics, ruminal degradability, microbial mass and NH_3-N concentration.

Keywords: soybean meal, *Neptunia javanica* Miq., *in vitro*gas production, digestibility

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INTRODUCTION

Feeding is very important for livestock production,especially the energy and protein source. Soybean meal (SBM) is currently the most commonly used as protein source in animal feeds. (Yue and Zhou, 2008). Most of SBM used in feed formulation are imported, resulting in increasing production cost (Wanapat et al., 2013a). Moreover, the price of SBM has trend todramatically increase.

The high cost of protein sources, their restricted availability and theunpredictability of their markets, increase the need for alternativesources of protein in animal feed. Most published research on the use ofplant protein and/or carbohydrate, as a substitute of SBM, in animal feedshas focused on the inclusion of cottonseed meal (Margaridaet al., 2002), sunflower seed meal (Irshaid et al., 2003; Titi, 2003), *Leucaena leucocephala* and *Pithecellobium dulce*(Paengkoumand Paengkoum, 2009), *Vicia faba* L.*var. minuta*(Azazaet al., 2009) anddried tomato pomace(Yuangklanget al., 2010).

Neptunia is in Mimosaceae family (subfamily of leguminosae) are widely cultivated and extensively grown in Myanmar, Indochina region, Jawa, East Timor and Thailand. In Thailand found twospecies, *Neptunia javanica* Miq. and *Neptunia oleracea* Lour. (water mimosa). People consume *Neptunia oleracea* Lour. but not for *Neptunia javanica* Miq.(kased-kok)because it has higher fiber. It contain 23-25% crude protein, which could be a potential protein source for animal. However, little information is available on the use of *Neptunia javanica* Miq. mealin the animal feeds. Therefore, the aim of this work was to evaluate the effects of replacement of SBM by KAKM in feeds on gas production and ruminal degradability by using *in vitro* gas technique

MATERIALS AND METHODS

Experimental design and dietary treatments

The experimental design was a completely randomized design (CRD) and the dietary treatments were soybean meal replacement by dry *Neptunia javanica* Miq.(kased-kok)meal (KAKM) in the diet at 30, 60 and 100% with 0.5 g of roughage and concentrate ratio at 40:60. Rice straw was used as a roughage source. Concentrates were formulated to contain 14% and 76% of crude protein (CP) and total digestible nutrient (TDN), respectively. KAKM were dried at 60°C, then ground to pass a 1-mm sieve (Cyclotech Mill, Tecator, Sweden) and used for chemical analysis and use 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). The ingredients and chemical compositions of concentrate, rice straw and KAKM used in the *in vitro* experiment are shown in **Table 1**.

Animals and preparation of rumen inoculums

Two male, rumen-fistulated beef cattle with body weight of 500±30kg were used as rumen fluid donors. Beef cattle rumen fluid was collected from animals fed with rice straw ad libitum. 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. The rumen fluid was

filtered through four layers of cheesecloth into pre-warmed thermo flasks and then transported to the laboratory.

In vitro fermentation of substrates

Samples of 0.5 g of roughage and respective concentrate (**Table 1**) at ratio 40:60 were weighed into 50 ml serum bottles. For each treatment, three replications were prepared. 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.

Sample and analysis

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 werefitted to the model of Ørskov 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 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 centrifuged at 16,000 x*g* for 15 min, and the supernatant was stored at -20 °C before NH_3-N analysis using the micro-Kjeldahl methods (AOAC, 1995). The *in vitro* degradability was determined after termination of incubation, when the contents were filtered through pre-weighed Gooch crucibles and residual dry matter was estimated. The percent loss in weight was determined and presented as *in vitro* dry matter degradability (IVDMD). The dried feed sample and residue left above was ashed at 550 °C for determination of *in vitro* organic matter degradability (IVOMD)(Tilley and Terry, 1963). At 48 h postinoculationa set of treatment was determined *in vitro* true digestibility according toVan Soest et al. (1991).The true digestibility was used to calculate microbial mass according to the method of Blümmel et al. (1997).

Statistical analysis

All data from the experiment were analyzed as acompletely randomized design using the GLM procedure of SAS (1998). Differences between treatment means were determined by Duncan's New MultipleRange Test (Steel and Torrie, 1980), and differences among means with $P<0.05$ were accepted as representing statistically significant differences. Trend of KSKM levels responded was performed by orthogonal polynomials.

RESULTS AND DISCUSSIONS

The chemical compositions of concentrate, rice straw and KSKM are shown in **Table 1**. Concentrate and rice straw contained 14% and 3.2% CP, respectively. The KSKM contained 23.0% CP, 62.0% NDF and 41.0% ADF. Gas kinetics and cumulative gas production for each of the substrate treatments are presented in **Table 2**. It was found that gas production from soluble fractions

(a) ranged from -6.1 to -6.6 and was not significantly different among treatments (P>0.05). Moreover, gas production from the insoluble fraction (b), gas production rate constant for the insoluble fraction b (c), potential extent of gas production (a+b) and cumulative gas production were maintained when SBM was replaced with KSKM. In addition, replacing SBM with KSKM at 30 and 60% did not show a difference in *in vitro* degradability of DM, OM and true digestibility when compared with SBM treatment. This indicated that utilization of KSKM in feeds may provide essential nutrients for microbial growth and their activity. However, when the instead level of replacement was 100% the *invitro* degradability and true digestibility were reduced. This result was probably due to the increased fiber contents, since the KSKM contained 62.0% and 41.0% of NDF and ADF, respectively. Similarly, Cherdthong and Wanapat (2013) found that the *in vitro* digestibility was negatively correlated with NDF and ADF contents in the diet of swamp buffalo. Higher *in vitro* true digestibility reflects higher microbial biomass (Ørskov, 1994;Blu¨mmel et al., 1997; Infascelli et al., 2005; Zicarelli et al., 2011). A similar trend was also found in this experiment with KSKM replacement at 30% (19.6 mg of microbial biomass). The microbial produced in the rumen by microorganisms is the major source of protein for the ruminants and the prediction of efficiency of microbial production is very important in ruminant nutrition (Karabulutetal., 2007; Kongmun et al., 2010). Replacement of SBM by KSKM at 30% may provide essential nutrients for microbes, thus resulting in an improved efficiency of microbial protein synthesis. Ruminal NH_3-N concentrations in this study were 19.6 to 25.6 mg/dL and closer to the optimal ruminal NH_3-N range (15 to 30 mg/dL, Perdok and Leng, 1990; Wanapat et al., 2008; Gunun et al., 2013; Anantasook et al., 2013).

CONCLUSION

Based on this study it can be concluded that replacement of SBM by KSKM has positive effect on gas production and ruminal degradability. Replacement of SBM by KSKM at 30% in feed resulted in improved *in vitro* true digestibility, microbial mass and NH_3-N concentration. However, these findings should be investigated further in *in vivo* study.

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Table 1. Ingredients and chemical composition of concentrate, rice straw and dry *Neptuniajavanica*meal used in the experiment.

Item	SBM[1]	*Nep*.30	*Nep*.60	*Nep*.100	RS	*Nep.*
Ingredient, kg DM						
Cassava chip	72.0	68.0	61.0	55.0		
Rice bran	8.0	12.0	16.0	16.0		
Palm kernel meal	6.0	6.2	10.0	16.5		
Neptuniajavanica	0.0	4.4	9.1	13.2		
Soybean meal	14.0	10.0	4.4	0.0		
Urea	2.5	2.5	2.5	2.5		
Molasses	3.0	3.0	3.0	3.0		
Salt	0.5	0.5	0.5	0.5		
Sulfur	0.5	0.5	0.5	0.5		
Mineral premix	1.0	1.0	1.0	1.0		
Chemical composition						
Dry matter, %	87.7	87.9	88.1	89.0	93.4	28.2
	----------g/kg of dry matter----------					
Organic matter	92.9	92.5	92.8	92.8	95.4	91.2
Crude protein	13.9	13.6	13.7	13.7	3.2	23.0
Neutral detergent fiber	19.0	23.4	23.9	24.7	70.9	62.0
Acid detergent fiber	11.1	12.3	12.8	13.9	57.1	41.0
Total digestible nutrients[2]	76.2	76.1	76.0	76.0	45.0	-

[1]SBM = soybean meal, KSKM30, 60, 100 = soybean meal replacement by *Neptuniajavanica* meal30 %, 60% and 100%, RS = rice straw. *Nep.=Neptuniajavanica*,

[2]Calculated value.

Table 2. Effect of *Neptuniajavanica*as protein sourcesin the diet on gas kinetics, gas production, degradability and NH_3-N concentration from *in vitro* incubation with rumen fluid

Incubation time (h)	Treatment[1]				SEM	Contrast[2]		
	SBM	KSKM30	KSKM60	KSKM100		L	Q	C
Fermentation kinetic values[3]								
a	-6.6	-6.1	-6.1	-6.2	0.35	ns	ns	ns
b	73.9	66.6	68.3	62.7	1.77	ns	ns	ns
c	0.04	0.03	0.03	0.04	0.01	ns	ns	ns
a+b	67.3	60.5	62.2	56.5	1.46	ns	ns	ns
Gas (72 h) ml/0.5 g DM substrate	59.4	56.3	53.8	49.9	1.47	ns	ns	ns
In vitro degradability, %								
IVDMD	60.0^{ab}	65.3^{a}	59.1^{ab}	56.9^{b}	0.59	ns	*	ns
IVOMD	66.6^{ab}	72.3^{a}	66.8^{ab}	63.8^{b}	0.54	*	*	ns
True digestibility, %	60.0^{ab}	65.3^{a}	59.1^{ab}	56.9^{b}	0.05	ns	**	*
Microbial mass, mg	17.4^{b}	19.6^{a}	16.2^{c}	17.2^{b}	0.05	**	**	**
NH_3-N, (mg/ dL)	19.6^{a}	25.6^{c}	22.9^{b}	21.4^{ab}	0.19	ns	**	ns

[1]SBM = soybean meal, KAKM.30, 60, 100 = soybean meal replacement by *Neptuniajavanica* meal 30 %, 60% and 100%, *P<0.05, **P<0.01

[2]Linear (L), quadratic (Q), and cubic (C) effects of supplemented treatments.

[3]a=gas production from the immediately soluble fraction, b=gas production from the insoluble fraction, c=gas production rate constant for the insoluble fraction (b), (a+b)=potential extent of gas production.