The 17th Asian-Australasian Association of Animal Production Societies
Animal Science Congress

Proceedings

22-25 AUGUST 2016
CONGRESS VENUE: FUKUOKA JAPAN

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1 Hasanuddin University, Indonesia; 2 Department of Livestock Services, Indonesia

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Clinic for Obstetrics Gynecology Andrology and Artificial Insemination of Domestic Animals, Faculty of Veterinary Medicine, Mahasarakham University of Technology, Thailand

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1 Animal Husbandry Faculty of Brawijaya University, Indonesia; 2 Animal Husbandry Faculty of Kanjuruhan University, Indonesia; 3 Beef Cattle Research Station, Pasuruan Indonesia

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Oral Session 11: Animal Nutrition (Ruminants) (1)

Tuesday, 23 August 14:30-16:30 Room N302

Chair: Indah Prihartini  Faculty of Agriculture and Animal Science, University of Muhammadiyah Malang

O-11-1  Effect of Kaempferia galanga L. on in vitro nutrients digestibility, ruminal fermentation and methane production  
Ashi Kurniawati, Widodo, Wayan T Artama, Lies Mira Yusniati

1 Faculty of Animal Science Universitas Gadjah Mada, 2 Faculty of Veterinary Universitas Gadjah Mada
O-11-1
Effect of Kaempferia galanga L. on in vitro nutrients digestibility, ruminal fermentation and methane production

With Kurniawan1,*, Witiw1, Wayan Thankana1, Iis Mira Yusti1
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2Faculty of Veterinary, Universitas Gadjah Mada, Indonesia

The study was conducted to evaluate the effect of Kaempferia galanga L. on nutrient digestibility, ruminal fermentation and methane production. Dry powder of Kaempferia galanga L. was added to substrate (steam hemic, rice bran, wheat, and 1:20 EM based), at different levels of 0 (control), 5, 10, 15, and 20% in the feed, and was incubated with 5 cm3 of inoculum. Methane production was determined by gas production method. The results showed that the addition of Kaempferia galanga L. powder in the feed increased the methane production.

O-11-2
EVALUATION ON INNOCULUM OF CYTOPHAGA Sp AS PROBIOTIC IN Ruminant by IN VITRO GAS PRODUCTION APPROACH

Indah Purnawati
Faculty of Agriculture and Animal Husbandry, University of Muhamaruddin Nalang

Cytophaga Sp has a high ability to degrade lignin and shows potential as probiotic for swine. The aim of this research was to evaluate the supplement of inoculum Cytophaga Sp as probiotics using in vitro gas production. The research method used a randomized complete block design, with 180 replications divided into 4 treatments, ie, with and without supplement of inoculum Cytophaga Sp (S), and with inoculum Cytophaga Sp (L). Gas production was observed at 0, 4, 8, 12, 16, 24, 30, 48, 72, and 96 hours. Production of VFA and N2O were measured using the supernatant while the digestibility and microbial processes were measured using the inoculum Cytophaga Sp production. The results showed that the supplement of inoculum Cytophaga Sp fluctuated, but the use of inoculum Cytophaga Sp increased gas production and reduced the gas production rate.

O-11-3
The Effect of Rejected Soybean Supplementation with Different Treatments on Ongongse Crossbred Cattle Feed

Ramie, S., Urso, R., Sarat, R.
1Faculty of Agriculture Science, Sadaat Mada University,
2Faculty of Agriculture Science, Sadaat Mada University

The study was carried out to determine the effect of rejected soybean in the undegraded protein supplemented with different treatments on cattle feed. The rejected soybean seed was prepared as protein treated with rice bran (RRSB) and divided into 6 groups of treatment: 1) control; 2) RSB; 3) RSB + 20% rice bran; 4) RSB + 40% rice bran; 5) RSB + 60% rice bran; 6) RSB + 100% rice bran. The results showed that the rice bran supplementations in the RSB treatments increased the protein digestion and improved the carcass characteristics.

O-11-4
Effects of nitrate, extracted chitosan or shrimp shell meal on degradability and in Vitro gas production

Thanh Nguyen1, Thanh2, Metha Wanapat2
1Department of Animal Science, Veterinary Medicine, College of Agriculture and Forestry, An Giang University, Vietnam
2Nutritional and Nutritional Research and Development Center (NUTREC), Department of Animal Science, College of Agriculture, Chonburi University, Thailand

This study was undertaken to determine the effects of nitrate, extract chitosan or shrimp shell meal on degradability and in vitro gas production. The experimental design was a 2x2 factorial arrangement in a completely randomized design with two factors as two E.C ratios (60:40 and 40:60) and the nitrogen source in several levels and levels of additives (DON: 20; 40; 60; 80 mg/kg of DM). The results showed that the addition of nitrate, chitosan, or shrimp shell meal increased the degradability and reduced the in vitro gas production.

KEYWORDS: Kaempferia galanga L., inoculum, Cytophaga Sp, probiotics, gas production, ruminal fermentation, soybean supplementation, nitrogen, rice bran, chitosan, shrimp shell, gas production.
Effect of Kaempferia galanga L. on in vitro nutrients digestibility, ruminal fermentation and methane production

Asih Kurniawati1, Widodo1, Wayan T Artama2, Lies Mira Yusiati1

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Objective
Antibiotics such as monensin have been used to manipulate rumen fermentation and successfully increase feed efficiency and animal production. The effect of monensin on rumen fermentation was briefly explained by Russell and Strobel (1989). The inclusion of antibiotics in animal rations as has been limited due to the occurrence of multi-drug resistant bacteria which may be a risk to human health (Gustafson and Bowen, 1997). In recent years, plant bioactive compounds as natural feed additives have been studied as antibiotic alternatives for improving ruminal fermentation and nutrient utilization. The antimicrobial effects of some phytochemical compounds such as essential oils have been previously demonstrated (Janssen et al., 1987).

Several plants containing phytochemical reduced methane production and increased VFA production (Bodas et al., 2008). Plant essential oil from various sources has been intensively studied during the last decades by ruminant scientists aiming to develop rumen modifiers for manipulating rumen fermentation. Blended plant essential oil, cinnamaldehyde, eugenol, and capsicum in feedlot cattle diet have the similar effect with monensin (Geraci et al., 2012) improve growth performance and health, by optimizing rumen fermentation and increase immune system status (Compiani et al., 2013). Among the essential oil, thyme and cinnamon oil and their main active components (thymol and cinnamaldehyde, respectively) have potential antimicrobial activity against ruminal microorganisms (Calsamiglia et al., 2007; Benchaar and Greathead, 2011) and have potency as monensin alternate (Khorrami et al., 2015). The effects of essential oil in characteristics of rumen fermentation depend on dose. Varies response were showed among different natural extracts and pure essential oil as well as the concentration of essential oil which included in the fermentation (Macheboeuf et al., 2008).

*Kaempferia galanga* L. is a plant which widely used as herb in cooking in Indonesia, where it is called *kencur*, and especially in Javanese and Balinese cuisine. It has different essential oil component from other herbs previously mentioned. The major chemical constituents of volatile oil obtained by water distillation of dried rhizome of *Kaempferia galanga* L. were identified as ethyl-p-methoxyxinnamate (31.77%), methyl cinnamate (23.23%), carvone (11.13%), eucalyptol (9.59%) and pentadecane (6.41%), respectively (Tewtrakul et al., 2005). Whereas Kumar (2014) reported the major constituents of the *Kaempferia galanga* L oil were ethyl cinnamate (29.48%), ethyl-p-methoxyxinnamate (18.42%), γ - cadinene (9.81%), 1. 8-cineole (6.54%), δ -carene (6.19%), borneol (5.21%), ethyl-m-methoxyxinnamate (2.15%), camphene (1.58%), linoleoyl chloride (1.35%) and α-pinene (1.32%). Differences plant propagation resulting the different concentration of essential oil component. The main component of *Kaempferia galanga* L. ethyl p-methoxyxinnamate in quantity were 82.01% and 71.77% respectively for essential oil component of *Kaempferia galanga* L. rhizomes with conventional and in vitro propagation (Sahoo et al., 2014). Essential oil from *Kaempferia galanga* L. had antimicrobial activity toward Gram positive and Gram negative bacteria (Tewtrakul et al., 2005). Moreover, to our knowledge, no research has done on *Kaempferia galanga* L. related to rumen fermentation. Therefore this research was conducted to studied the effect of *Kaempferia galanga* L. in ruminal feed fermentation and methane production.

Methodology

*Feed, treatments and in vitro fermentation*

The effect of *Kaempferia galanga* L. on nutrients digestibility, ruminal fermentation, and methane production were studied in this research using batch culture of in vitro gas production technique. Feed sample for in vitro fermentation consist of Pennisetum purpureum, which cut before flowering stage, rice bran and wheat pollard, obtained from feed shop, with ratio 60:20:29 based on dry matter. *Kaempferia galanga* L. meal was prepared by drying fresh rhizomes in dryer incubator at 55°C and grounded to pass through a 1 mm pore size sieves. Additions of *Kaempferia galanga* L. were based on the final concentration of essential oil in fermentation media i.e. 0, 25, 50, 75, and 100 mg/L.

Inoculum for the in vitro gas production was obtained from two ruminal canulated Ongole grade cattle fed a diet...
consisting of *Pennisetum purpureum* and beef cattle concentrate 60:40 DM basis TDN 88.57% and CP 9.34%. Rumen fluid was collected before morning feeding, and squeezed through polyester cloth into a vacuum flask thermos, and immediately sent to the laboratory.

Serum bottles, 125 ml, were used for in vitro incubations. Bottles were set into three triplicate bottles, one set for dry matter digestibility (DMD) and organic matter digestibility (OMD) determination, gas and methane production, one set for crude protein digestibility (CPD), and one set for rumen fermentation parameter. Sufficient anaerobic media was prepared the day before the incubation according to Theodorou *et al.* (1994). Sixty three milliliters of media was added into serum bottles which previously filled with 700 mg of substrate and *Kaempferia galanga* L. powder according to the treatments and continue flushed by oxygen-free carbon dioxide. Bottles were sealed immediately with butyl rubber stopper plus aluminum crimp cap and pre-warmed overnight at 39°C. In the next morning, rumen fluid were collected, and 7 ml was added into each bottle using 10 ml plastic syringe. Bottles then incubated for 24 h at 39°C. Bottle head space gas pressure were zeroing before incubation by inserting 0.6 mm needle attached to a pressure transducer.

At the end of incubation gas were collected using calibrated syringe and 5 ml of gas were transferred into 5 ml plain vacuum tube (Becton Dickinson Vacutainer System) for methane analysis. DMD, OMD and CPD were determined by filtered the bottle content, and residual feed were collected for residual nutrients analysis, including DM, OM and CP. Procedure for nutrient analysis according to AOAC (2005). Sample for protozoa calculation were prepared by pipetting 1 ml of bottle content and be added to 0.8ml of formaldehyde saline (1ml of 37% formaldehyde + 9 ml 0.9% NaCl). One microliter sample then transferred to haemocytometer for direct calculation under microscope according to method explained by Diaz *et al.* (1993). For ammonia measurement 1 ml of bottle content were preserve with 1 ml NaCl 20% and be frozen until later analysis of ammonia base on phenol hypochlorite reaction as explained by Chaney and Marbach (1962). Media, as much as 1 ml for VFA analysis were added into tube containing 1 ml of 20% metha-phosphoric acid and stored in freezer for further analysis using gas chromatography. Prior to sampling for ammonia, VFA, microbial protein and protozoa, pH media were measured. Rumen microbial protein was determined by Lowry method (Alexander and Griffiths, 1993). Microbial cell were separated from residual feed by centrifugation 1.5 ml of bottle content at 500g. Cell were precipitated from supernatant by spin down at 15,000 g. Pellets were re-suspension in physiology solution and re centrifuge. Re-suspension was repeated for twice. The last suspension was subjected for protein determination.

**Calculation and statistical analysis**

Parameters studied were nutrients digestibility including dry matter digestibility (DMD), organic matter digestibility (OMD), crude protein digestibility (CPD) (expressed in %), carboxymethyle cellulose (CMCase) (expressed in U), total gas production (ml), total VFA, acetate, propionate and butyrate concentration as mmol/100mL, rumen microbial protein, ammonia concentration as mg/100L, methane production as ml/g DM digested, and protozoa number. Data were subjected to one-way analysis of variance with level of *Kaempferia galanga* L. as the treatment factor. Comparisons between means were analysis using t-test of Duncan Multiple Range test.

**Result**

Dry mater, organic matter and crude protein digestibility were not affected by addition of *Kaempferia galanga* L. at all treatments level (Table 1). CMCase, represent fibrolitic enzymes also was not affected by the treatments even there was a tendency in treatments groups have lower activity except treatment group of *Kaempferia galanga* L. addition equal to 75 mg/L of essential oil. Fermentations with addition of *Kaempferia galanga* L. have not differences on volume of gas production per dry matter digested compared to control (P<0.01) (Table 1.) Pattern of DMD fluctuation similar with the pattern of gas production per DM degraded (Figure 1.). Effect of essential oils on nutrient digestibility were varies. Talebzadeh *et al.* (2012) reported addition of *Zataria multiflora* essential oils at level 150 to 600 mg/L have no effect on IVDMD but linearly decreased IVOMD. Main component of *Zataria multiflora* essential oil were carvacrol, p-cymene and thymol. Other result from screening plant experiment in ‘rumen up’ project showed varies value of IVDMD by addition of different plant on in vitro rumen fermentation, some plant showed have no different effect on IVDMD compared to control, some plants increase and some plants decreased (Bodas *et al.*, 2008). Plant secondary metabolites activities were influenced by their chemical nature and concentration. Activities of essential oils also depend on their component and dose. Thymol have no effect on NDF and ADF digestibility at level 5, and 50 mg/L but significantly reduced at level 500 mg/L, while eugenol have no effect on NDF and ADF at dose 5, 50 and 500mg/L. *Zataria*
multiflora essential oil reduced gas production at level 300 mg/L and up, reduced VFA and ammonia at level 600 mg/L, and reduction of rumen biomass start at level 150 mg/L (Talebzadeh et al., 2012). Unlike of Zataria multiflora, Kaempferia galanga L. did not affect DMD digestibility and other nutrients as well as gas production. Gas production commonly used to predict DMD, in this research DMD pattern parallel to gas production pattern. Main component of Kaempferia galanga L. essential oil were ethyl-p-methoxycinnamate, and methylcinnamate, carvone, eucalyptol and pentadecane, respectively (Tewtrakul et al., 2005; Kumar, 2014) which differ from previous study. The differences of essential oil component are responsible for their differences effect on enzyme activity, gas production, pH, VFA, ammonia, microbial protein and protozoa number. CMCase activity was not differing among treatments. Unaltered of CMCase activity by essential oil treatments suggested that essential oils may not affect fibrolitic microbes but may depressed activity of amilolytic and proteolitic bacteria by suppressed the colonization and digestion of readily degrades substrates (Wallace et al., 2002).

pH, total and individual VFA as well as ratio of acetate to propionate, microbial protein and ammonia concentration as shown in Table 2., they were not affected by increasing level of Kaempferia galanga L. in the diet. Overall, methane produced in fermentation with Kaempferia galanga L. were lower compared to control. Significant decreasing of methane occurred at level of Kaempferia galanga L. addition equal to final essential oil concentration 50 and 100mg/L media (P<0.01). Protozoa number in treated fermentation have higher protozoa number (P<0.05). Significant higher number appeared at level 50 mg/L and up.

Essential oil did not affect proteolysis, the first step of protein degradation in rumen, but decreased rate of ammonia production, deamination step (Wallace et al., 2002). In contrast, Kaempferia galanga L. did not alter ammonia might due to it concentration and structure of main essential oil component. Major constituents of Kaempferia galanga L. essential oil, is esters and terpenoid compounds (Kumar, 2014), ethyl-p-methoxycinnamate, and methylcinnamate, derivatives of cinamic acid (Baser and Buchbauer, 2010). Cinnamaldehyde other derivative of cinamic acid which given in in vitro fermentation at doses 1 to 5 mmol/L equal to 132.16 to 660.88 mg/L reduced ammonia significantly (Macheboeuf et al., 2008), in agreement with Busquet et al. (2006), high level of cinnamaldehyde, 300mg/L and 3000mg/L reduced ammonia concentration while at low level 3 and 30 mg/L did not effect on ammonia. Structure changes of essential oil component will alter its activity. Biohydrogenation transform of Ethyl p-methoxycinnamate a major constituent of the Kaempferia galanga L. rhizome essential oil, to ethyl p-hydroxycinnamate increased the potential inhibition, bactericidal, and fungicidial toward bacteria and fungi (Omar, 2014).

Total VFA, acetate, propionate, butyrate, ratio acetate to butyrate, and rumen microbial protein did not affected by Kaempferia galanga L. Cinnamaldehyde reduced VFA and its component at level 3000 mg/L and up (Busquet et al., 2006) 300 mg/L and up except acetate did not affected up to level 660 mg/L (Macheboeuf et al., 2008). Dose up to 100 mg/L of Kaempferia galanga L. essential oil might not enough to change the VFA production and profile, but methane production and protozoa number had been modify. Methane productions per digested dry matter decrease around 14.98% to 32.29% due to the addition of Kaempferia galanga L. Several essential oil component reduced methane at different level, carvacrol at 225mg/L, cinnamaldehyde at 265 mg/L, and thymol at 300mg/L (Macheboeuf et al., 2008).

Addition of Kaempferia galanga L. increase the number of protozoa level 50 and 75mg/L of essential oil. At level 75 mg/L protozoa number was the highest, then a little bit decreased at level 1000 mg/L. Essential oil at low level might become stimulator for some species of protozoa, and at high level the effect on protozoa population is not drastic (Patra and Xaxena, 2009).

Conclusions
Kaempferia galanga L. in the diet equal to essential oil level 25 to 100mg/L does not effect on DMD, OMD, CPD, gas production, pH, total VFA, acetate, propionate, butyrate, rumen microbial protein, and ammonia concentration, whereas methane production were lower in fermentation with addition of Kaempferia galanga L. in the diet, and protozoa number increase at dose 50 up to 100mg/L.

Acknowledgments
Authors gratefully acknowledge Directorate General of Higher Education (DGHE), Ministry of Research Technology and Higher Education for awarding Doctoral scholarship. This research is part of doctoral research. Authors also acknowledge Nutritional Laboratory, Faculty of Animal Science, Universitas Gadjah Mada for the supports and
provide the facilities for this research

**KEYWORD**: Kaempferia galanga L., Essential oil, Ruminal fermentation, Methane production, nutrients digestibility

**Figure 1.** Patterns of dry matter digestibility and gas production of in vitro rumen fermentation added *Kaempferia galanga* L. (gas Production (---), dry matter digestibility (—-))

**Table 1.** Effect of *Kaempferia galanga* L. on nutrients digestibility and carboxymethyl cellulase activity

<table>
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<th>Parameters</th>
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<tr>
<td></td>
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<tr>
<td>Dry matter digestibility (%)</td>
<td>49.72±6.637</td>
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<tr>
<td>Organic matter digestibility (%)</td>
<td>49.12±5.543</td>
</tr>
<tr>
<td>Crude protein digestibility (%)</td>
<td>56.02±3.909</td>
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<tr>
<td>DM digestibility (%)</td>
<td>174.36±23.487</td>
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<tr>
<td>CMC ase</td>
<td>2.30±0.130</td>
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**Table 2.** Effect of *Kaempferia galanga* L. on parameters of rumen fermentation

<table>
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<tr>
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<tr>
<td>pH</td>
<td>6.7±0.049</td>
</tr>
<tr>
<td>Total VFA (mmol/100mL)</td>
<td>15.11±2.477</td>
</tr>
<tr>
<td>Acetate</td>
<td>10.77±1.536</td>
</tr>
<tr>
<td>Butyrate</td>
<td>1.34±0.095</td>
</tr>
<tr>
<td>Propionate</td>
<td>3.00±0.846</td>
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<tr>
<td>Acetate/propionate</td>
<td>3.66±0.522</td>
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<tr>
<td>Rumen microbe (mg/L)</td>
<td>319.36±9.400</td>
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<tr>
<td>Ammonia (mg/10mL)</td>
<td>25.44±0.076</td>
</tr>
<tr>
<td>Methane production/DM digested (ml/g)*</td>
<td>8.61±0.897*</td>
</tr>
<tr>
<td>Protozoa (cel 10⁴)*</td>
<td>8.52±0.080*</td>
</tr>
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CERTIFICATE OF PRESENTATION

This is to certify that

Asih Kurniawati
Widodo
Wayan T Artama
Lies Mira Yusiati

made an oral presentation on the following paper at

the 17th Asia-Australasian Association of
Animal Production Society Animal Science Congress
held in Fukuoka, Japan from 22 to 25 August 2016

Effect of Kaempferia galanga L. on in vitro nutrients
digestibility, ruminal fermentation and methane production
(O-11-1)

Mitsuhiro Furuse, Ph.D.
President of the 17th AAAP Animal Science Congress