

# In vitro assessment of digestibility LRRD Caribu - paper only

*by Agus Susanto*

---

**Submission date:** 01-Apr-2023 12:04PM (UTC+0700)

**Submission ID:** 2052713380

**File name:** n\_vitro\_assessment\_of\_digestibility\_LRRD\_Caribu\_-\_paper\_only.pdf (159.27K)

**Word count:** 4583

**Character count:** 23319

## ***In vitro* assessment of digestibility and rumen fermentation of ammoniated rice straw based beef diet, supplemented with extract of garlic and *Sapindus rarak***

C H Prayitno, Suwarno and A Susanto

Faculty of Animal Science, Jenderal Soedirman University. PO Box 53123 Purwokerto, Indonesia

[caribu\\_prayitno@yahoo.co.id](mailto:caribu_prayitno@yahoo.co.id)

25

### **Abstract**

The study was conducted to investigate the effect of garlic extract (GE) and *Sapindus rarak* extract (SRE) on rumen fermentation and rumen microbial structure in vitro experiment. Randomized completely block design was used in this study. There were 6 treatments and 5 blocks included. The materials of this study consisted of rumen fluid of beef cattle. The diet of beef cattle was composed of 40% ammoniated rice straw and 60% concentrates. The experimental treatments were, CARS : Concentrate:ammoniated rice straw (60:40), G250 : CARS + 250 ppm GE, G250-SR : CARS+ 250 ppm GE + 0.18 % SRE, G500-SR : CARS + 500 ppm GE + 0.18% SRE, G750-SR : CARS + 750 ppm GE + 0.18% SRE, and G1000-SR : CARS + 1000 ppm GE + 0.18% SRE.

Supplementation of GE and SRE did not affect the pH and DM and OM digestibility and it decreased crude fiber digestibility as much as 13.0% - 16.6%. Supplementation of 250 ppm GE or 250 ppm GE and 0.18% of SRE in beef cattle decreased acetate, protozoa population and increased propionate. Supplementation of 250 ppm GE and 0.18% of SRE in the ammoniated rice straw based beef diet resulted in the increase of rumen fermentation efficiency as indicated by the low production of acetate and methane, and high production of propionate.

**Key words:** herbal, methane, microbial rumen

### **Introduction**

Garlic (*Allium sativum*) contains complex mixture of bioactive compounds, including allicin ( $C_6H_{10}S_2O$ ), diallyl sulphide ( $C_6H_{10}S$ ), diallyl disulfide ( $C_6H_{10}S_2$ ) and allyl mercaptan ( $C_3H_6S$ ). These compounds are able to manipulate rumen fermentation such as decreasing the proportion of acetate and increasing the proportion of propionate and butyrate, inhibiting methanogenesis and decreasing the  $CH_4$ :VFA ratio (Busquet et al 2005). Kongmun et al (2010) reported that garlic powder was more effective than coconut oil in reducing protozoa population. Active ingredients in garlic are able to limit the growth of protozoa. Busquet et al (2005) stated that Archaea has unique membrane layer that stabilizes cell membrane function. The synthesis of this membrane is catalyzed by hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase. Garlic is strong inhibitor of HMG-CoA reductase (Gebhardt and Beck 1996), with an effect on isoprenoid synthesis causing Archaea membrane become unstable and the cell die. Kim et al (2012) proved that garlic extract decreased methane production by more than 25%. According to Amagase (2006), bioactive compounds of garlic extract were: alliin, allicin, diallyl disulfide (DADS) and S-Allyl-L-cysteine. Kongmun et al (2010) showed that the molar proportion of

propionate was influenced by garlic supplementation. Supplementation of garlic powder as much as 16 mg/kg DM of feed resulted in the lowest total VFA and highest proportion of propionate and significantly lower CH<sub>4</sub> production.

Guo et al (2008) stated that crude saponins have been shown to inhibit protozoa causing the reduction of methanogens presumably by lowering methanogenic activity of protozoal-associated methanogens. Holtshausen et al (2009) proved that the negative effect of saponin supplementation on IVNDFD increased with the increase of dose rate, which suggests that lower levels of supplementation may attenuate the negative effects on fibre digestion. Cardozo et al (2005) reported that the supplementation of 30 mg/L garlic extract in the diet of beef cattle decreases acetate and increases propionate. Most fibrolitic rumen bacteria, which are generally acetate and butyrate producers, are sensitive to low rumen pH. In contrast, the amylolytic bacteria are acid-tolerant and are responsible for most of the production of propionate in the rumen. Saponins from fruits of *Sapindus rarak* have been reported by Wina et al (2005) and Suharti et al (2010) as defaunating agents. Addition of *S. rarak* saponins through *in vitro* decreased significantly protozoa counts. The symbiotic of protozoa with archea in the rumen is well established (Finlay et al 1994). However, there is only 37% of the archea that live symbiotically with protozoa, the rest live freely in the rumen ecosystem.

The objective of this study was to evaluate the effects of supplementation of GE and SRE in beef diet on rumen fermentation in order to improve fermentation efficiency.

## Materials and methods

This research was conducted experimentally using randomized block design, with 6 treatments and 5 blocks. The treatments were:

- CARS: beef cattle feed (concentrate: ammoniated rice straw, 60:40 with CP: 10.9%, TDN: 63.03%, CF : 23.3%, NDF: 32.04%);
- G250 : CARS + 250 ppm GE;
- G250-SR : CARS + 250 ppm GE + 0.18 % SRE;
- G500-SR : CARS + 500 ppm GE + 0.18% SRE;
- G750-SR : CARS + 750 ppm GE + 0.18% SRE;
- G1000-SR : CARS + 1000 ppm GE + 0.18% SRE.

## Location and duration

The experiment was conducted in the laboratory of Feedstuff of Animal Science Faculty, University of Jenderal Soedirman, Purwokerto, Central Java province, Indonesia, from March to July 2013.

## Preparation of ammoniated rice straw

Fresh, air-dry rice straw was sampled, weighed and put in an oven (60<sup>o</sup> C, 24 hours) for dry matter content determination. A certain weight of the air-dry rice straw was chopped 5-7 cm of length, and then it was put into a plastic bag. Urea was weighed (3% of rice straw, dry matter basis), and mixed, diluted thoroughly in water (33% of rice straw, dry matter basis). The urea solution was poured thoroughly on to the chopped rice straw, the bag was sealed and kept in a shaded room for 30 days, until it was ready to use for in-vitro digestibility study.

## In vitro fermentation

The rumen fluid for this experiment was collected from a non-fistulated Onggole-Cross cow fed a diet consisted of ammoniated rice straw and concentrate mixture (40:60 %). The rumen fluid

12 was filtered through double layer cheese cloth for *in vitro* studies. 12 The substrate for *in vitro* rumen fermentation was a mixture of concentrate feed and dried milled ammoniated rice straw. The concentrate mix consisted of rice brand, coconut cake meal, cassava waste, wheat pollard, mineral premix, and NaCl (CP: 12.9%, TDN: 64.03%, CF: 23.3%, NDF: 32.04%).

*In vitro* fermentation was conducted according to the method of Tilley and Terry (1963). Into each 100 mL fermentation tube, 500 mg substrate, 40 mL McDougall buffer and 8 mL rumen fluid were added. The McDougall buffer contained NaHCO<sub>3</sub> (58.8 g), Na<sub>2</sub>HPO<sub>4</sub>·7H<sub>2</sub>O (42 g), KCl (3.42 g), NaCl (2.82 g), MgSO<sub>4</sub>·7H<sub>2</sub>O (0.72 g), CaCl<sub>2</sub> (0.24 g) and 30 H<sub>2</sub>O per 6 L. The mixture was stirred and flushed with O<sub>2</sub>-free carbon dioxide and the tubes were then sealed with a rubber cork with the gas release valve. All the fermentation tubes were incubated in a shaker water bath at 39 °C for 24 h.

#### Preparation of garlic extract (GE) and *Sapindus rarak* extract (SRE) (modified methods of Suharti et al 2010)

Garlics were obtained from a local market in Purwokerto, Central Java, Indonesia. At the first phase, gar

*Sapindus rarak* fruits were obtained from Surakarta, Central Java province. The first stage of the preparation of SRE, was initiated by separating seeds from the fruit. Then the seed-free fruits were put in an oven at a temperature of 60° C for 4 days, and in warm condition, the *Sapindus rarak* fruits were immediately milled. The SR powder was macerated in methanol (1:4 w / v) overnight. Further the methanol was evaporated in an evaporator-rotary so that the residue was separated from the extractor. The extraction was repeated once more to produce a crude extract. The residue was then dried on a freeze drier and stored at -4°C. This method would result in SRE yield of 42.5% with 81.9% saponin content.

#### Protozoa and bacteria counts

After 24 h incubation, 1 mL of aliquot of each treatment was taken for protozoa and bacteria counts. One mL of aliquot was mixed with 1 mL of methyl green formaldehyde (35% of formaldehyde, distilled water, methylgreen and NaCl) (Ogimoto and Imai 1981). The stained sample with methyl green formaldehyde was kept in cool temperature (17 °C) and the protozoa population was directly counted in a counting chamber (0.1 mm) with a microscope (40 x magnitudes) and then was exposed to a 30x20 cm screen. Protozoa population, Colony Forming Unit (CFU), was calculated using a formula:

$$P = \frac{1 \times 1000 \times C \times DF}{0.1 \times 0.065 \times 16 \times 5}$$

P: Protozoa population

C: protozoa number, colony.

DF: dilution factor (=2)

To count the number of bacteria population, the dilution method was used at 24-hour incubation. As much as 0.05 mL of aliquot was added to the 4.95 ml diluted medium. A serial dilution 10<sup>-6</sup>, 10<sup>-7</sup> and 10<sup>-8</sup> was made using of Brain Heart Infusion (BHI) medium (Gerard-Champod et al 2009). The count unit of bacteria in term of colony forming unit, was calculated using a formula:

$$B = \frac{\text{colony number}}{0.05 \times 10^x \times 0.1}$$

B: Bacteria population

X: dilution factor.

Measurement of total gas was based on the method of Menke and Steingass (1988) and the phases of measurements were as follows: (1) Piston was smeared with vaseline before inserting it into the tube, (2) A total of 230 mg of feed treatment was put into Menke tube basin, (3) Preparation of medium that was made of 400 mL of distilled water; 0.1 mL micromineral solution; 200 mL of buffer solution; 200 mL macromineral solution; rezazurin 0.1 mL and 40 mL of reducing solution, (4) one section of rumen fluid was mixed with 2 parts of medium, stirred at 39°C with magnetic stirrer, (5) A total of 30 mL solution hose was inserted into Menke tube and locked with a plastic clip, (6) Incubated for 24 h and the scale was read every 6 h.

### Methane (CH<sub>4</sub>) production

The CH<sub>4</sub> production was calculated from stoichiometry of the main VFA formed during fermentation, i.e: acetate (C<sub>2</sub>), propionate (C<sub>3</sub>), and butyrate (C<sub>4</sub>) as follows:  

$$CH_4 = 0.45 C_2 - 0.275C_3 + 0.40 C_4$$
 (Moss et al 2000).

C<sub>2</sub> = concentration of acetate, C<sub>3</sub>= concentration of propionate, C<sub>4</sub>= concentration of butyrate.

### Statistical analysis

The data was analyzed by one-way ANOVA (Steel and Torrie 1995).

## Results and discussion

The results (table 1) showed that GE and SRE supplementation on feed resulted in similar digestibility of dry matter and organic matter as those of control feed. The means ranges of dry and organic matter digestibility were 60.4%-67.5% and 61.6%-67.5%, respectively. This result showed that the increasing garlic extract supplementation did not result in the decrease of microbe's activity of digesting feed. The results of this study was similar to the findings of Manasri et al (2012), who found that there were no significant differences in IVDMI, IVDMD, IVOMD, IVCFD, and pH of beef cattle feed supplemented with garlic pellet. Anassori et al (2012) report, their in-vitro study using sheep rumen fluid found out that dietary garlic oil decreased OMD. Komun et al (2010) found an increase of IV (True) D when 16 mg of garlic powder was included in an in-vitro study using rumen fluid of swamp buffaloes.

**Table 1.** Effect of supplementation of GE and SRE on digestibility, VFA, total gas and rumen microbes

Items	Treatments						SEM	Prob
	CARS	G250	G250-SR	G500-SR	G750-SR	G1000-SR		
pH	6.80 <sup>a</sup>	6.70 <sup>b</sup>	6.50 <sup>b</sup>	6.60 <sup>b</sup>	6.50 <sup>b</sup>	6.60 <sup>b</sup>	.10	.000
IVDMD (%)	65.6 <sup>a</sup>	64.6 <sup>a</sup>	64.9 <sup>a</sup>	62.9 <sup>b</sup>	61.40 <sup>b</sup>	60.42 <sup>c</sup>	2.29	.000
IVOMD (%)	65.8 <sup>a</sup>	63.4 <sup>a</sup>	64.4 <sup>a</sup>	63.5 <sup>a</sup>	61.8 <sup>b</sup>	61.6 <sup>b</sup>	2.14	.015
IVCFD (%)	77.8 <sup>a</sup>	74.9 <sup>b</sup>	70.08 <sup>c</sup>	71.8 <sup>c</sup>	70.8 <sup>c</sup>	72.8 <sup>bc</sup>	2.30	.000
TVFA (%)	160 <sup>a</sup>	150 <sup>c</sup>	146 <sup>c</sup>	153.2 <sup>b</sup>	140 <sup>d</sup>	160 <sup>a</sup>	5.02	.000
Gas production (ml)	10.02 <sup>a</sup>	6.82 <sup>c</sup>	2.13 <sup>d</sup>	11.09 <sup>a</sup>	7.11 <sup>c</sup>	9.10 <sup>b</sup>	0.55	.000
Protozoa (10 <sup>6</sup> cell/ml)	31.5 <sup>a</sup>	15.00 <sup>c</sup>	10.42 <sup>d</sup>	11.4 <sup>d</sup>	16.00 <sup>bc</sup>	19.00 <sup>b</sup>	0.64	.000
Bacteria (log 10 /ml)	9.46 <sup>a</sup>	9.69 <sup>b</sup>	9.57 <sup>b</sup>	9.75 <sup>c</sup>	9.94 <sup>c</sup>	9.38 <sup>a</sup>	0.17	.000

IVDMD= In vitro dry mater digestibility, IVOMD = In vitro organic mater digestibility, IVCFD= In vitro crude fiber digestibility, TVFA= total volatile fatty acids.

abc means in the same row without common letter are different at P<0.05

In general, the results of this study showed that the supplementation of GE, either alone, or in

combination with SRE, decreased crude fibre digestibility as much as 7.4 % (Table 1). The decrease in crude fibre digestibility was supposedly associated with the reduced fibre-degrading bacteria population; this is shown with the reduction of acetate concentration up to the supplementation level of 750 ppm. The reduction of acetate will limit the synthesis of methane due to the limited hydrogen ion. Anassori et al (2012), reported that the addition of garlic essential oil in feed immersed in sheep rumen fluid, and Manasri et al (2012) stated that inclusion of garlic pellet in feed incubated in beef cattle rumen fluid decreased IVNDFD and IVADFD, which was in line with the results of this study.

An in-vitro study by Kongmun et al (2010) reports that inclusion of garlic powder in feed immersed in the rumen fluid of swamp buffaloes decreased total volatile fatty acids (TVFA), relative to the control. However, Kim et al (2012) describes that TVFA production and rumen pH were not affected by garlic extract (GE) inclusion in feed that was incubated in Holstein cow rumen fluid. The results of this study showed that the increase of GE concentration up to 750 ppm, decreased the VFA concentration.

The result showed that the increase of GE supplementation was followed by the decrease of acetate concentration, except at 1000 ppm GE; it increased the concentration of VFA. These results showed that GE supplementation was able to change rumen fermentation from acetogenic to glucogenic, as it was showed by the increase in propionate concentration as much as 9.23%.

Generally, the supplementations decreased acetate:propionate ratio (Table 2). The decrease in acetate:propionate ratio was easily understandable (made sense) since the acetate concentrations decreased, in contrast, propionate concentration increased by the supplementation treatment. The decrease in acetate:propionate ratio by the supplementation was expected in this study, because this in-vitro study was targeted to obtain a proper, appropriate feed for beef cattle, and propionate is a simple precursor for the bio-chemical formation of meat-amino acids and protein. This result was similar with the finding of Kongmun et al (2010) that the inclusion of garlic powder in feed incubated in swamp buffalo rumen fluid, decreased acetate:propionate ratio, and that of Kim et al (2012) that the inclusion of GE (20mg/ml) decreased acetate:propionate ratio from 1.79 (control feed) to 1.32 (control feed + GE).

The results showed that the treatment significantly lowered total gas respectively for G250, G250-SR, G500-SR, G750-SR, G1000-SR, amounted to 29.03%, 38.1%, 80.76%, 0.60%, 35.5%, 17.5% (Table 1). These results indicated that the increased concentrations of the GE were not in line with the decrease in total gas. The highest decrease in the concentration of total gas due to the supplementation of 250 ppm GE + 0.18% SRE in feed was 80.76%. Increased concentrations of extract of GE above 250 ppm only lost a total gas of 28.5%. The decrease in total gas in this study illustrated that the tested treatment was able to improve rumen fermentation, because 30% of the total gas as a result of rumen fermentation, is methane. Anassori et al (2012) found a similar results; the addition of garlic oil in sheep feed, decreased rumen gas production. On the contrary, Kim et al (2012) found out that the inclusion of garlic whole plant methanol extract in feed immersed in rumen fluid of fistulated Holstein cow, increased gas production. The decrease in gas production due to the increasing levels of garlic was also found by Kongmun et al (2010).

The results showed that the supplementation treatment effectively decreased protozoa population (Table 1). The decrease of protozoa population relative to the control were 52.38%, 66.92%, 63.81%, 49.21%, and 39.68% relatively for G250, G250-SR, G500-SR, G750-SR, and G1000-SR. The decrease in protozoa population in this study indicated that the supplementation treatments were able to represent defaunating agents. This shows that GE and SRE contain compound that have the ability to reduce protozoa population. Some of the natural compounds that have characteristics of defaunating agents are saponins and tannins. Cheeke (1999) describes the mechanism of saponin in inhibiting the growth of protozoa and methanogens.

Newbold et al (1995) explain that between 15-37% of methanogens lives symbiotically with protozoa, therefore, the decrease in protozoa population will be followed by the decrease in methanogens population. Hart et al (2006) explains that allicin of garlic has the characteristic as an anti-methanogenic, which means that there is about 63% of the methanogens that is targeted by the allicin. Supplementation of 250 ppm GE and 0.18% SRE was the most effective in decreasing protozoa population relative to other treatments, which meant that the combination of 250 ppm of GE (allisin source) and 0.18% SRE (saponin source) was the most effective for the inhibition of protozoa growth. Kongmun et al (2010) also found similar results that protozoa counts decreased from 14.3 ( $\times 10^5$ ) CFU in control feed to 6.4 ( $\times 10^5$ ) CFU in control feed supplemented with 16 mg garlic powder.

The results showed that the treatment affect the concentrations of the tested rumen bacteria (Table 1). The limitations of this study was because the study did not observe the species of the bacteria, it only observed the total strains of bacteria. In previous study in dairy cows, the results showed that the combination of garlic extract and *Sapindus rarak* affected bacteria populations (Prayitno et al 2013). The dominant bacteria in the rice straw-based feed were fibre-degrading bacteria. It is reported by Kongmun et al (2010), inclusion of a mixture of garlic powder 4 mg, and coconut oil at 8 mg, increased *Ruminococcus albus*, a cellulolytic rumen bacteria, from 18.3 ( $\times 10^5$ ) CFU for control feed, to 33.8 ( $\times 10^5$ ) CFU for control feed plus the mixture of GP and CO. Kim et al (2012) also found similar results when GE (20 mg/ml) was included in feed; the treatment decreased methanogenic bacteria and increased fibrolytic bacteria. Manasri et al (2012) found rather different results than those of Kim et al (2012), when garlic pellet in feed, immersed in beef cattle rumen fluid, decreased methanogenic and total bacteria counts.

**Table 2.** Effect of supplementation of GE and SRE on volatile fatty acids

Items	Treatments						SEM	Prob.
	CARS	G250	G250-SR	G500-SR	G750-SR	G1000-SR		
Proportion of VFA (mM)								
Acetate	48.9 <sup>b</sup>	39.1 <sup>d</sup>	40.3 <sup>d</sup>	44.4 <sup>c</sup>	42.07 <sup>dc</sup>	47.6 <sup>b</sup>	3.21	.000
Propionate	27.4 <sup>c</sup>	29.6 <sup>b</sup>	29.9 <sup>b</sup>	29.2 <sup>b</sup>	29.7 <sup>c</sup>	31.09 <sup>a</sup>	1.69	.000
Butyrate	9.89 <sup>b</sup>	9.89 <sup>b</sup>	9.96 <sup>b</sup>	10.7 <sup>b</sup>	9.83 <sup>b</sup>	12.3 <sup>a</sup>	2.98	.000
Acetate:propionate (A:P)	1.79 <sup>a</sup>	1.32 <sup>d</sup>	1.35 <sup>d</sup>	1.52 <sup>c</sup>	1.52 <sup>c</sup>	1.53 <sup>c</sup>	0.16	.000
Methane (mM)	18.4 <sup>a</sup>	13.4 <sup>c</sup>	13.9 <sup>c</sup>	16.2 <sup>b</sup>	15.2 <sup>b</sup>	17.8 <sup>a</sup>	3.02	.000

*abcd* Means in the same row without common letter are different at  $P < 0.05$

The results of this study showed that GE supplementation as well as GE + SRE supplementation decreases

## Conclusions

- Based on this experiment, it could be conclude that supplementation of 250 ppm garlic extract, 0.18% SRE in the ammoniated rice straw based beef diet resulted in the increase of rumen fermentation efficiency as indicated by the low production of acetate and methane, and high production of propionate.

## References

Anassori E, Dalir-Naghadeh B, Pirmohammadi R, Taghizadeh A, Asri-Rezaei S, Farahmand-Azar S, Besharati M and Tahmoози M 2012 In vitro assesment of digestibility of forage based sheep diet, supplemented with raw garlic, garlic oil and monensin. Veterinary Research Forum. Volume 3, 5-11.

- Amagase H 2006** Clarifying the real bioactive constituents of Garlic. *Journal Nutrition*. 136:716S-725S. <http://jn.nutrition.org/content/136/3/716S.short>.
- Busquet M, Calsamiglia S, Ferret A and Kamel C 2005** Effects of cinnamaldehyde, garlic oil on ruminal microbial fermentation in a dual flow continuous culture. *Journal of Dairy Science*. Volume 88, 2508-2516. [http://www.journalofdairyscience.org/article/S0022-0302\(05\)72928-3/pdf](http://www.journalofdairyscience.org/article/S0022-0302(05)72928-3/pdf).
- Cardozo P W, Calsamiglia S, Ferret A and Kamel C 2005** Screening for the effects of natural plant extracts at different pH on in vitro rumen microbial fermentation of high-concentrate diet for beef cattle. *Journal of Animal Science*. Volume 83, 2572-2579. <http://www.journalofanimalscience.org/content/83/11/2572.full.pdf+html?sid=55a7ca12-fb76-4998-8c64-0556a35704d>
- Cheeke P R 1999** Actual and potential application of *Yucca schidigera* and *Quillaja saponaria* saponins in human and animal nutrition. *Proceeding of the American Society of Animal Science*. 1-10.
- Finlay B J, Esteban G, Clarke K J, Williams A G, Embley T M and Hirt R P 1994** Some rumen ciliates have endosymbiotic methanogens. *Federation of European Microbiological Societies (FEMS) Microbiology Letters*. Volume 117, 157-162. <http://femsle.oxfordjournals.org/content/117/2/157.abstract>.
- Gebhardt R and Beck H 1996** Differential inhibitory effect of garlic-derived organosulfur compounds on cholesterol biosynthesis in primary rat hepatocytes cultures. *Lipids*. Volume 31, 1269-1276. <http://link.springer.com/article/10.1007/BF02587912#page-1>.
- Gerard-Champod M M, Blanquet-Diot S, Mazuranok L and Alric M 2009** Enumeration of some cultivable bacterial groups and characterization of some abiotic variables in the jejunoileal content of Prim'Holstein veal calves. *Journal of Animal Science*. Volume 87, 1664-1675. <http://www.journalofanimalscience.org/content/87/5/1664.full.pdf+html?sid=befcf43-3d2d-4ca2-b61b-57d7277ee085>.
- Guo Y Q, Liu J X, Lu Y, Zhu W Y, Denman S E and McSweeney C S 2008** Effect of tea saponin on methanogenesis, microbial community structure and expression of *mcrA* gene, in culture of rumen micro-organisms. *Letter Applied Microbiology*. Volume 47, 421-426. <http://onlinelibrary.wiley.com/doi/10.1111/j.1472-765X.2008.02459.x/full>.
- Hart K J, Girwood S E, Taylor S, Yanez-Ruiz D R and Newbold C J 2006** Effect of allicin on fermentation and microbial populations in the rumen simulating fermentor Rusitec. *Reproduction Nutrition Development*. Volume 46 (supplement 1), 97-115.
- Holtshausen L, Chaves A V, Beacheamin K A, McGinn S M, McAllister T A, Odongo N E, Cheeke P R and Benchaar C 2009** Feeding saponin-containing *Yucca schidigera* and *Quillaja saponaria* to decrease enteric methane production in dairy cows. *Journal of Dairy Science*. Volume 92, 2809-2821. [http://www.journalofdairyscience.org/article/S0022-0302\(09\)70598-3/pdf](http://www.journalofdairyscience.org/article/S0022-0302(09)70598-3/pdf).
- Hook S E, Andre-Denis G W and McBride B W 2010** Methanogens: methane producers of rumen and mitigation strategies. *Archaea*. 2010/945785. <http://downloads.hindawi.com/journals/arch/2010/945785.pdf>.
- Inthapanya S, Preston T R and Leng R A 2011** Mitigating methane production from ruminants: effect of calcium nitrate as modifier of the fermentation in an in vitro incubation using cassava root as the energy source and leaves of cassava or *Mimosa pigra* as source of protein. *Livestock Research for Rural Development*. Volume 23 (2) <http://www.lrrd.org/lrrd23/2/sang23021.htm>.
- Kim, E T, Kim H, Min K S and Lee S S 2012** Effect of plant extracts on microbial population, methane emission and ruminal fermentation characteristics in In Vitro. *Asian-Australasian Journal of Animal Science*. Volume 25, 806-811. <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4093095/>,
- Kongmun P, Wanapat M, Pakdee P and Nawanukraw C 2010** Effect of coconut oil and garlic powder on *in vitro* fermentation using gas production technique. *Livestock Science*. Volume 127, 38-44. <http://www.thaiscience.info/Article%20for%20ThaiScience/Article/2/Ts-2%20effect%20of%20coconut%20oil%20and%20garlic%20powder%20on%20in%20vitro%20fermentation%20using%20gas>
- Manasri N, Wanapat M and Mavanukraw C 2012** Improving rumen fermentation and feed digestibility in cattle by mangosteen peel and garlic pellet supplementation. *Livestock Science*. Volume 148, 291-295. <http://www.thaiscience.info/Article%20for%20ThaiScience/Article/2/Ts-2%20improving%20rumen%20fermentation%20and%20feed%20digestibility%20in%20cattle%20by%20mangosteen%20peel>
- Menke K H and Steingass H 1988** Estimation of the energetic feed value obtained from chemical analysis and gas production using rumen fluid. *Animal Research and Development*. Volume 28, 7-55.
- Moss A P, Jouany J P and Newbold J 2000** Methane production by ruminants: Its contribution to global warming. *Annual Zootechnology*. Volume 49, 231-253. <https://hal.archives-ouvertes.fr/hal-00889894/document>.
- Newbold, C J, Lassalas B and Jouany J P 1995** The importance of methanogens associated with ciliate protozoa in ruminal methane production in vitro. *Letters in Applied Microbiology*. Volume 4, 230-234. <http://onlinelibrary.wiley.com/doi/10.1111/j.1472-765X.1995.tb01048.x/abstract>.



**Ogimoto K and Imai S 1981** Atlas of Rumen Microbiology. Japan Scientific Societies Press, Tokyo.  
<http://www.cabdirect.org/abstracts/19871495708.html;jsessionid=C2E0C575B4CC540988728C55C761B784>.

**Prayitno C H, Suwarno and Subagyo Y 2013** Supplementation of *Sapindus rarak* and garlic extract in feed containing adequate Cr, Se, and Zn on rumen Fermentation. Journal of Animal Science and Technology (Media Peternakan). Volume 36, 52-57. <http://jesl.journal.ipb.ac.id/index.php/mediapeternakan/article/download/6846/5257>.

**Steel R G D and Torrie J H 1995** Principles and Procedure of Statistic. A Biometrical Approach. 2nd Ed. McGraw Hill International Book Co. London.

**Suharti S, Kurniawati A, Astuti D A and Wina E 2010** Microbial population and fermentaion characteristic in response to *Sapindus rarak* mineral block supplementation. Jurnal Animal Science and Technology (Media Peternakan). Volume 33, 150-154. <http://jesl.journal.ipb.ac.id/index.php/mediapeternakan/article/viewFile/3123/2082>.

**Tilley J M A and Terry R A 1963** A two stage technique for the *in vitro* digestion of forage. Journal of British Grassland Society. Volume 18,104-111. <http://onlinelibrary.wiley.com/doi/10.1111/j.1365-2494.1963.tb00335.x/abstract>.

**Wina E, Muetzel S, Makkar H P S and Becker K 2005** Saponins containing methanol extract of *Sapindus rarak* affect microbial fermentation, microbial activity and microbial community structure *in vitro*. Animal Feed Science and Technology. Volume 121, 159-174. <http://www.animalfeedscience.com/article/S0377-8401%2805%2900069-6/abstract?cc=y>.

Received 28 March 2015; Accepted 5 April 2015; Published 1 May 2015

[Go to top](#)

# In vitro assessment of digestibility LRRD Caribu - paper only

---

## ORIGINALITY REPORT

---

14%

SIMILARITY INDEX

9%

INTERNET SOURCES

11%

PUBLICATIONS

2%

STUDENT PAPERS

---

## PRIMARY SOURCES

---

- |   |   |    |
|---|---|----|
| 1 | <a href="http://www.iiste.org">www.iiste.org</a><br>Internet Source   | 1% |
| 2 | Afshar Mirzaei-Ag, Syed Alireza Sy, Hasan Fathi, Sohrab Rasouli, Mohammad Sadaghian, Mohamad Tarahomi. "Garlic in Ruminants Feeding", Asian Journal of Biological Sciences, 2012<br>Publication                     | 1% |
| 3 | P. Kongmun, M. Wanapat, P. Pakdee, C. Navanukraw, Z. Yu. "Manipulation of rumen fermentation and ecology of swamp buffalo by coconut oil and garlic powder supplementation", Livestock Science, 2011<br>Publication | 1% |
| 4 | <a href="http://www.nexusacademicpublishers.com">www.nexusacademicpublishers.com</a><br>Internet Source   | 1% |
| 5 | M. Wanapat, V. Chanthakhoun, K. Phesatcha, S. Kang. "Influence of mangosteen peel powder as a source of plant secondary compounds on rumen microorganisms, volatile fatty acids, methane and microbial              | 1% |

protein synthesis in swamp buffaloes",  
Livestock Science, 2014

Publication

---

6	Foiklang, Suban, Metha Wanapat, and Thitima Norrapoke. "In vitro rumen fermentation and digestibility of buffaloes as influenced by grape pomace powder and urea treated rice straw supplementation : In vitro Study of Grape Pomace Powder", Animal Science Journal, 2015. Publication	1 %
7	curis.ku.dk Internet Source	1 %
8	id.123dok.com Internet Source	1 %
9	Submitted to Universitas Brawijaya Student Paper	1 %
10	aadgc2018.tvu.edu.vn Internet Source	<1 %
11	vet.unne.edu.ar Internet Source	<1 %
12	W D Astuti, Y Widyastuti, R Fidriyanto, R Ridwan, Rohmatussolihat, N F Sari, Firsoni, I Sugoro. "In vitro gas production and digestibility of oil palm frond silage mixed with different levels of elephant grass", IOP	<1 %

# Conference Series: Earth and Environmental Science, 2020

Publication

13

[www.tesisenred.net](http://www.tesisenred.net)

Internet Source

<1 %

14

Castillejos, L.. "In vitro evaluation of effects of ten essential oils at three doses on ruminal fermentation of high concentrate feedlot-type diets", *Animal Feed Science and Technology*, 20080814

Publication

<1 %

15

Dona R. Jayawickrama, Piyatilak B. Weerasinghe, Dinesh D. Jayasena, Deshani C. Mudannayake. "Effects of supplementation of urea-molasses multinutrient block (UMMB) on the performance of dairy cows fed good quality forage based diets with rice straw as a night feeding", *Korean Journal of Agricultural Science*, 2013

Publication

<1 %

16

[scialert.net](http://scialert.net)

Internet Source

<1 %

17

Submitted to Higher Education Commission Pakistan

Student Paper

<1 %

18

Novie Setianto. "Systems thinking approach to develop smallholder beef farming in rural

<1 %

Java, Indonesia", 'University of Queensland Library', 2015

Internet Source

---

19

Sineenart Polyorach, Metha Wanapat, Anusorn Cherdthong, Sungchhang Kang. "Rumen microorganisms, methane production, and microbial protein synthesis affected by mangosteen peel powder supplement in lactating dairy cows", Tropical Animal Health and Production, 2016

Publication

---

<1 %

20

The Rumen Microbial Ecosystem, 1997.

Publication

---

<1 %

21

[jurnal.um-tapsel.ac.id](http://jurnal.um-tapsel.ac.id)

Internet Source

---

<1 %

22

[www.atlantis-press.com](http://www.atlantis-press.com)

Internet Source

---

<1 %

23

Amlan Kumar Patra, Devki Nandan Kamra, Neeta Agarwal. "Effects of extracts of spices on rumen methanogenesis, enzyme activities and fermentation of feeds in vitro", Journal of the Science of Food and Agriculture, 2009

Publication

---

<1 %

24

C.R. Soliva. "Effects of mixtures of lauric and myristic acid on rumen methanogens and methanogenesis in vitro", Letters in Applied Microbiology, 7/2003

Publication

<1 %

---

25 Hu, W.L.. "Effect of tea saponin on rumen fermentation in vitro", *Animal Feed Science and Technology*, 20050528 <1 %  
Publication

---

26 E. T. Kim, C. -H. Kim, K. -S. Min, S. S. Lee. "Effects of Plant Extracts on Microbial Population, Methane Emission and Ruminant Fermentation Characteristics in In vitro", *Asian-Australasian Journal of Animal Sciences*, 2012 <1 %  
Publication

---

27 [vuir.vu.edu.au](http://vuir.vu.edu.au) <1 %  
Internet Source

---

28 [www.ajas.info](http://www.ajas.info) <1 %  
Internet Source

---

29 Ehsan Anassori, Bahram Dalir-Naghadeh, Rasoul Pirmohammadi, Akbar Taghizadeh et al. "Garlic: A potential alternative for monensin as a rumen modifier", *Livestock Science*, 2011 <1 %  
Publication

---

30 P. Hutton, C. L. White, Z. Durmic, P. E. Vercoe. "Eremophila glabra is an Australian plant that reduces lactic acid accumulation in an in vitro glucose challenge designed to simulate lactic acidosis in ruminants", *animal*, 2009 <1 %  
Publication

---

31	archive.org Internet Source	<1 %
32	classes.uleth.ca Internet Source	<1 %
33	researcherslinks.com Internet Source	<1 %
34	www.lrrd.cipav.org.co Internet Source	<1 %
35	www.tdx.cat Internet Source	<1 %
36	M. Wanapat, S. Kang, P. Khejornsart, S. Wanapat. "Effects of Plant Herb Combination Supplementation on Rumen Fermentation and Nutrient Digestibility in Beef Cattle", Asian-Australasian Journal of Animal Sciences, 2013 Publication	<1 %
37	"Article", Reproduction Nutrition Development, 2004 Publication	<1 %
38	Zhao GY. "The suitability of a faecal suspension of sheep as inocula for the estimation of utilizable crude protein of feeds by in vitro incubation", Archives of Animal Nutrition, 4/1/2004 Publication	<1 %

---

Exclude quotes      On

Exclude matches      Off

Exclude bibliography      On