The addition of feed additive in beef cattle ration on in vitro fermentation characteristics

W A Hartina¹, R Ridwan², D Diapari³, R Fidriyanto², A Jayanegara³

¹ Study Program of Nutrition and Feed Science, Graduate School of IPB University, Bogor, 16680, Email: wiwinaprihartina@gmail.com
² Research Center For Biotechnology, National Research and Innovation Agency (BRIN), Cibinong, 16911, Indonesia
³ Department of Nutrition and Feed Technology, Faculty of Animal Science-IPB University, Bogor, 16680, Indonesia

Corresponding author e-mail: rony_biotech@yahoo.com

Abstract. The effects of feed additives are increasing feed digestibility, balance of rumen microbial community, stimulating the immune response and livestock productivity. This study aimed to determine the effects of feed additives combination in the rumen fermentation. The method used in this study was a block randomized design with 9 treatments and 3 replications. The experiment using Theodorou In vitro method for 48 hours with parameters such as pH, kinetics gas and methane production, DMD, OMD, NH₃, and partial VFA. The treatment were P0; control (Basal Diet 70% Concentrate + 30% Forages), P1; P0 + Premix, P2; P1 + Probiotic, P3; P1 + Enzyme, P4; P1 + Plant Extract, P5; P1 + (Probiotics + Enzyme), P6; P1 + (Probiotics + Plant Extract), P7; P1 + (Probiotic + Enzyme + Plant Extract), and P8; P1 + (Enzyme + Plant Extract). The results showed kinetics gas, methane production, NH₃, and partial VFA were significantly affected (P <0.05), while the value of pH, DMD, and OMD was not significant. In conclusion, the addition of mix feed additives can affect the kinetics gas and methane production. However, it does not affect the level of pH, dry matter and organic matter digestibility.

Keywords: feed additive, probiotic, enzyme, plant extract, rumen fermentation

1. Introduction

The use of feed supplements and feed additives incorporates a useful impact on the health and productivity of placental, particularly inhibiting the expansion of morbific microorganism within the alimentary canal, modulating the response, increasing fiber degradation and fermentation, and increasing the expansion of placental productivity. The availability of a decent magnitude relation formulation and additives is often influenced by the ratio between concentrate and forage. The existence of excellent ration formulations and feed additives will have an effect on the fermentation characteristics within the first stomach. The aim of this study was to work out the impact of a mix of feed supplements and feed additives in beef rations on the characterization within the first stomach fermentation.

Ruminant production and feed at tropic unit intervals is restricted to forage grazing, crop residues, and agro-industrial by merchandise with very low concentrate allowances [1]. Nutritionist of ruminant have develope several ways of feed supplementation such as the us of antibiotic growth promotors to increase by limiting production the results of morbific infections in ruminant productivities [2]. However, its use is greatly reduced, due to its implications for health and the environment [3].

The gastrointestinal tract of ruminants has a wide array of species of microorganisms, some of which square measure is directly or indirectly critical to animal welfare [4]. The rumen, leading vat
fermentative, also a sophisticated system biological in which the fermentation, degradation, and transformation of feed ingredients into merchandise takes place by microorganisms [5]. This fermentation vat provides an associated anaerobic environment, a continuous temperature of thirty-eight to 41°C, and a pH scale of 5.5 to 6.9 for microbes [6].

Probiotics are non-pathogenic and non-toxic living microorganisms whose units are capable of providing beneficial results to the host animal at the appropriate size [7]. Probiotic microorganisms were determined to improve the first gastric condition, increase dry matter intake (DMI), feed potency, with body weight gain (WG) in ruminants [8]. It can also block the growth of moribund organisms, stimulate the system through bacteriocin secretion, and modulate the balance of microorganisms in the digestive tract [9]. Probiotics suitably improve the digestion of fiber building structural carbohydrates such as the many hemicellulose related saccharides out there as an energy supply to the animal host.

The feed sources available in the tropics have low feedability (poorly fermented) generally and unbalanced essential nutrients such as molecules, leading to low feed intake as a result of the slow rate of passage through the stomach and poor performance of the animal [10]. Thus, supplementation to increase the eating power of low-quality forage will increase the productivity of ruminants. Probiotics are reported as an alternative to antibiotics [11] to increase live body weight in ruminants by increasing nutrient utilization potential, increasing chemical element retention, and reducing excretion of essential nutrients. [12] reported fourteen different types of analysis that live yeast supplementation increased feed potency in cattle with respect to the third dimension.

Herbal plants turn out secondary metabolites, square measure is biologically active by providing protection against predatory attacks [13] and these metabolites are referred to as phytochemical feed additives, phytobiotics, or flavorings and biological science compounds [14] have been investigated as alternatives to antibiotics and growth promoters in nutrition of the ruminant. The biological activities of this phytochemical feed additive are as a support and process of stomach fermentation, modulation of microbiota, improving digestion and absorption of nutrients through the hyperbolic activity of processing enzymes, weakening aerophilic processes and growth of infective organisms and lastly, increasing energy utilization in the placenta.

2. Material and methods
The method used in this study was a block randomized design with 9 treatments and 3 replications of treatment using Theodore In vitro methods for 48 hours, with parameters such as pH, kinetic gas and methane production, DMD, OMD, NH3 and partial VFA. The rations used were 70% concentrate and 30% forage, with a combination of 1% DM (dry matter) and the addition of premix, probiotics (Lactobacillus plantarum 1010 CFU/ml), commercial enzymes (Superzyme from PT. Bright International) which contains xylanase, protease, cellulose, glucanase, invertase, amylase, mannanase, and pectinase, and plant extract Melastoma candidum per treatment. The treatments were P0; control (Basal Diet 70% Concentrate + 30% Forage), P1; P0 + Premix, P2; P1 + Probiotic, P3; P1 + Enzyme, P4; P1 + Plant Extract, P5; P1 + (Probiotics + Enzyme), P6; P1 + (Probiotics + plant extract), P7; P1 + (Probiotic + Enzyme + Plant Extract), P8; P1 + (Enzyme + Plant Extract).

2.1 In vitro fermentability test
This experiment used an in vitro modified method from Thedorou [15]. 0.5 gram substrate and each treatment were put into the incubation tube, then the incubation tube was added to 33 mL of buffer solution and 17 mL of rumen fluid (ratio 2: 1). Rumen fluid was obtained from fistulated cattle and was taken in the morning before feeding. After the adaptation to the experimental feed, the rumen fluid was taken filtered using 4 layers of gauze and mixed with a McDougall buffer solution. The samples were incubated with CO2 for 10 minutes, so that the atmosphere became anaerobic. The incubation tube was tightly closed and put into the incubator water bath (39°C) and incubated for 48 hours. The bottle was shaken in every 1 hour.

2.2 Kinetics gas and methane production
Gas measurements were carried out every 2, 4, 6, 8, 10, 12, 24, and 48 hours using a 50 ml gas syringe by injecting a needle into the rubber cap of the in vitro bottle. The volume calculation was recorded after the volume of gas in the syringe was constant via manual reading. After reading the
gas volume, the total volume of gas (ml) in the sample would be known. The total volume of gas
gained can be used to estimate the calculation of the rate of gas production and the maximum total
gas production. Estimation of this calculation used Orskov's equation [16]. Methane production used
an analysis with methane analyser at 12, 24, and 48 hours.

2.3 In vitro digestibility analysis
The measurement of the digestibility of the covering in vitro dry matter (IVDMD) and organic matter
digestibility (IVOMD). The procedure started with the process of fermentative digestion in
anaerobic such as implementation procedures for the testing of the rumen microbes and fermentability,
the incubation was only conducted for 48 hours, then proceeded with the process of digestion
enzymatically with the enzyme pepsin aerobic for 48 hours. The process of fermentative digestion was
centrifuged at 6000 rpm for 10 minutes, the supernatant discarded and the residue plus solution
pepsin-HCL and the mixture incubated for 48 hours. The mixture was then filtered with filter paper
Whatman with the help of a vacuum pump. The filtrate was then inserted into the cup of porcelain that
had been known its weight, subsequently was dried in an oven 130°C for 6 hours with the cup to the
determination of the value of the dry matter, and then the cup was put back into the furnace to 600°C
to determine the ash content and organic matter.

2.4 N-NH3 and VFA analysis
10µl of the sample was put into a test tube which was then added 1.5 ml of phenol solution and 1.5 ml
of NaOCl solution, then boiled for 15 minutes, then analyzed using a spectrophotometer. The results
from the spectrophotometer were then divided by 17 to determine the NH3 concentration [37].

Analysis VFA partial in a total of 2 ml sample of rumen fluid that has been centrifuged was then
added with 30 mg 5-sulfosalicylic acid dehydrate and centrifuged at 13000 rpm for 10 minutes 4°C.
The sample was then filtered using a nylon syringe (25mm, 0.45µm). As much as 1 ml of fluid was
inserted into the GC-MS vial. 1 µl of the liquid was injected into GCMS (GC-MS-QP2010 SE) using a
MEGA-WAX MS column (025-02530). An analysis was carried out with split mode, split ratio 5, purge
flow 3ml/min, and injector temperature 250°C. Column temperature was set at 100°C held for 9
minutes, then the temperature was increased to 200°C at a speed of 10°C/min and held for 10 minutes.

2.5 Data analysis
This study used a block randomized design with 9 treatments and 3 replications. The data obtained
were analyzed using analysis of variance (ANOVA). The differences among the treatments (P<0.05)
were then proceeded with Duncan by using the IBM SPSS Statistic 20 software.

3. Results and discussion

3.1 Fermentability characteristic
The pH value was measured after the incubation process in nine treatments ranged at 6.85-6.95 (Table
1) and statistically did show non-significant (P>0.05). The value shows the stability of the pH of the
rumen measured from the observations. It is indicated that the use of premix, probiotic, enzyme, and
plant extract not significantly effect the pH of the fermentation, so that the fermentation can be run
normally. The acidity degree on nine treatments have shown that the pH of the rumen was in normal
conditions to work optimally. The conditions of the normal pH of the rumen in order to work optimally
is 6.0-6.9 [17]. However, there are three treatments exceeding the ranges of 6.93, 6.94, 6.95. The pH
value is within the range of normal pH of the activity of the microorganisms of the rumen, namely 5.5-
7.0 [18].

Potential gas production (B) in addition of premix, probiotic, enzyme, and plant extract
significantly increased the incubation (P<0.05; Table 1). Gas production rate (c) and lag time (L)
showing no significant effects of premix, probiotic, enzyme, and plant extract addition as well as the
interaction were observed. Gas production showed asimilar trend with the dry matter digestibility, in
which the better result was the combination of premix, probiotic, enzyme, and plant extract. Gas
production was a direct result of substrate fermentation (CO2 and CH4) [19]. In general, the
length of the incubation process occurred, the more gases were produced. The rate of gas production
decreased as the length of incubation increased since the amount of available substrate for digestion was depleted. The increase of total gas production is usually followed by the increase of CH4 production (in mL) because CH4 is a component within the gas.

**Table 1. Kinetics of gas production and methane productions.**

| Treatment | Variable | B  | C  | M12 | M24 | M48 | pH  |
|-----------|----------|----|----|-----|-----|-----|-----|
| P0        | 89.79<sup>ab</sup> | 0.07<sup>ac</sup> | 0.09<sup>a</sup> | 1.87<sup>a</sup> | 3.85<sup>bc</sup> | 6.86<sup>a</sup> |
| P1        | 95.91<sup>c</sup> | 0.073<sup>bc</sup> | 0.00<sup>a</sup> | 2.93<sup>ab</sup> | 3.60<sup>a</sup> | 6.86<sup>a</sup> |
| P2        | 96.01<sup>c</sup> | 0.074<sup>c</sup> | 0.19<sup>a</sup> | 2.31<sup>bc</sup> | 3.13<sup>a</sup> | 6.94<sup>a</sup> |
| P3        | 88.81<sup>ab</sup> | 0.068<sup>abc</sup> | 0.75<sup>b</sup> | 3.20<sup>bc</sup> | 5.07<sup>c</sup> | 6.89<sup>a</sup> |
| P4        | 92.72<sup>bc</sup> | 0.065<sup>abc</sup> | 0.94<sup>b</sup> | 3.26<sup>bc</sup> | 5.23<sup>c</sup> | 6.95<sup>a</sup> |
| P5        | 89.08<sup>bc</sup> | 0.064<sup>abc</sup> | 0.80<sup>b</sup> | 3.23<sup>bc</sup> | 5.15<sup>c</sup> | 6.86<sup>a</sup> |
| P6        | 84.60<sup>c</sup> | 0.061<sup>abc</sup> | 0.68<sup>b</sup> | 3.50<sup>c</sup> | 4.83<sup>c</sup> | 6.87<sup>a</sup> |
| P7        | 86.33<sup>c</sup> | 0.06<sup>a</sup> | 0.76<sup>b</sup> | 2.63<sup>abc</sup> | 4.76<sup>c</sup> | 6.95<sup>a</sup> |
| P8        | 88.77<sup>ab</sup> | 0.063<sup>abc</sup> | 0.77<sup>b</sup> | 3.48<sup>c</sup> | 4.66<sup>bc</sup> | 6.90<sup>a</sup> |
| P-value   | 0.45 | 0.07 | 0.00 | 0.96 | 0.67 | 0.098 |

B= potential gas productions; c= gas production rate; M12= Methane 12 hours; M24= Methane 24 hours; M48= Methane 48 hours; P0: control (Basal Diet 70% Concentrate + 30% Forages), P1: P0 + Premix, P2; P1 + Probiotic, P3; P1 + Enzyme, P4; P1 + Plant Extract, P5; P1 + (Probiotics + Enzyme), P6; P1 + (Probiotics + plant extract), P7; P1 + (Probiotic + Enzyme + Plant Extract), P8; P1 + (Enzyme + Plant Extract).

<sup>a,b,c</sup> Different superscripts in the same row with various letters show significant differences (p <0.05).

The resulting analysis of variance shows that the use of P1 (premix without feed supplementation) and P2 (premix combined with probiotics) was decreased in methane gas production shows significant (P<0.05) effect compared with others. Methane is the end product of rumen fermentation and is considered to be the total energy loss consumed by ruminants, representing 6-10% of the total energy [20] which contributes to the greenhouse effect [21]. In ruminants, 20% of methane is produced by the decomposition and manure 80% of methane is produced during fiber fermentation, mainly cellulose [22]. This percentage may vary, depending on the composition of the ruminant feed [23], by reducing CO2 to maintain a low concentration of H+ in the environment can be done by the process of methanogenesis [24]. In the P1 and P2 treatments, there was a decrease in methane gas at 48 hours with the mechanism being more energy-efficient. Probiotics have a role in inhibiting the production of CH4, either directly or indirectly, in the methanogenesis process. The direct mechanism is by providing inhibition of metabolites such as bacteriocin produced by probiotics directly to methanogen cells, so that the process is disrupted. While the indirect effect on the stimulation of the preparation of metabolites such as lactate is to be used by lactate users to produce propionate, so that the need for H for methanogens is reduced. Feed additives that have been developed can be used to increase livestock productivity by directly affecting rumen productivity. [25] seen from trial data involving dairy cows and growing cows fed a high-concentration diet, and calculated that probiotics increased productivity by 7-8%. In vitro, probiotics can directly reduce CH4 reduction, where probiotics are a potential technology for CH4 reduction [26]. However, the results of in vitro on CH4 reduction is not consistent [27], and there are no reports in the literature about the production of CH4 in vivo after probiotic supplementation.

Given that the probiotic is a feed additive that needs to be fed every day, they seem only suitable for systems where feed supplements are given regularly or for lactating dairy cows. This combined with the limited evidence that probiotics directly affect CH4 emissions, suggests that they have limited utility to reduce CH4 in ruminants.

### 3.2 In vitro digestibility characteristic

Digestibility of dry material which is high in ruminants indicates the high substance of nutrients that are digested by the microbial rumen [28]. Dry matter digestibility describes the feed protein,
carbohydrate, fat, and mineral compounds that can be digested by livestock. Organic matter digestibility describes the digestibility of organic matter in food ingredients except minerals. The DMD value in this study ranged from 60.92%-67.09%, and the IVOMD was 61.81%-65.93%. In Vitro Dry Matter Digestibility (IVDMD) and In Vitro Organic Matter Digestibility (IVOMD) are shown in Table 2.

### Table 2. In vitro dry matter and organic matter digestibility.

| Treatment | IVDMD   | IVOMD   |
|-----------|---------|---------|
| P0        | 67.38b  | 68.98b  |
| P1        | 65.10ab | 65.64ab |
| P2        | 64.91ab | 65.99ab |
| P3        | 64.69ab | 65.41ab |
| P4        | 60.66a  | 62.11a  |
| P5        | 65.11ab | 66.02ab |
| P6        | 64.42ab | 65.74ab |
| P7        | 65.35ab | 66.23ab |
| P8        | 65.27ab | 66.13ab |

IVDMD = in vitro dry matter digestibility; IVOMD = in vitro organic matter digestibility; P0: control (Basal Diet 70% Concentrate + 30% Forages), P1; P0 + Premix, P2; P1 + Probiotic, P3; P1 + Enzyme, P4; P1 + Plant Extract, P5; P1 + (Probiotics + Enzyme), P6; P1 + (Probiotics + plant extract), P7; P1 + (Probiotic + Enzyme + Plant Extract), P8; P1 + (Enzyme + Plant Extract).

The value of digestibility of the dry material obtained range 60.65% - 67.38% and organic matter digestibility range from 62.11% - 68.98%. The value of the digestibility of the normal range is about 50% - 60% [29]. The productivity of ruminants is based on feed with high forage consumption and high concentrates aimed at increasing nutritional value, taking into account the ruminant production system. [30].

The addition of this combination with other feed supplements was not different from the other treatments, except with P4. During P4 treatment, there was a decrease in digestibility because there was an additional plant extract (Melastoma candidum) which contained flavonoids, which functioned as antibacterial. Therefore, the addition of plant extract can inhibit the mechanism of rumen bacterial activity in degrading substrates.

The main enzymes used for this purpose are xylanase [31] and cellulose [32], which were purified from bacterial and fungal fermentation cultures [33]. Several enzymes have been developed as additives in silage, straw, and agricultural by-products, then evaluated as a fibrolytic which becomes an additive in animal feed [34]. [34] proved that supplementation of fibrolytic enzymes can increase in vitro dry matter digestibility and dry matter digestibility in vitro and organic matter digestibility in vitro silage agricultural waste corn, rice straw, and wheat straw. In supporting fiber digestion, exogenous enzymes are needed that can increase fibrolytic activity in rumen microbial activity to the cell wall matrix. [35].

#### 3.3 VFA partial and NH3 concentration

The results of beef cattle ration based on a combination of premix, probiotic, enzyme, and extract plant had an effect (p <0.05) on propionic acid, butyric acid, valeric acid, iso valeric acid, n valeric acid, acetic acid, and iso butyric acid. The effect of combination of premix, probiotic, enzyme, and extract plant level on partial VFA is shown in Table 3.

The three majors of volatile fatty acids (VFA) produced are acetic acid, propionic acid, and butyric acid. The ratio of various VFA produced depends on types of feed digested in the rumen [30]. Acetate is an end product from of the fermentation of fiber in the rumen. Propionic acid, acetic acid, and butyric acid have a function in rumen fermentation. In glucogenesis, propionic acid is the substrate. Acetic acid is an important substrate for lipogenesis, and acetic acid with butyric acid is mainly used in the citric acid cycle for energy sources by host animals [31].


2nd International Conference on Animal Production for Food Sustainability 2021
IOP Conf. Series: Earth and Environmental Science 888 (2021) 012074
doi:10.1088/1755-1315/888/1/012074

Table 3. VFA partial and NH3 analysis.

| Parameters          | Treatment |
|---------------------|-----------|
|                     | P0        | P1        | P2        | P3        | P4        | P5        | P6        | P7        | P8        |
| Acetic acid (C2)    | 65.44d    | 62.92c    | 63.02c    | 62.48bc   | 62.81c    | 61.12ab   | 61.16cd   | 60.57d    | 60.46d    |
| Propionic acid (C3) | 22.28a    | 23.49abcd | 22.96abc  | 22.65ab   | 22.16a    | 23.98bcd  | 24.26cd   | 24.68ad   | 24.81d    |
| Butyric acid (C4)   | 0.70a     | 1.02b     | 1.07b     | 1.24cd    | 1.17bcd   | 1.29d     | 1.16bcd   | 1.07b     | 1.10bc    |
| Butyric acid (C4)   | 9.32a     | 9.57ab    | 9.86bc    | 10.28ed   | 10.33ed   | 10.29d    | 10.01bc   | 10.54d    | 10.53d    |
| Butyric acid (C5)   | 1.22a     | 1.71b     | 1.76bc    | 2.04d     | 2.07d     | 2.00d     | 2.02d     | 1.84bcd   | 1.62b     |
| Valeric acid (i-C5) | 1.00a     | 1.28b     | 1.33b     | 1.31b     | 1.47c     | 1.33b     | 1.37bc    | 1.29b     | 1.47d     |
| A/P                 | 2.94f     | 2.68cdef  | 2.74cdef  | 2.76cdef  | 2.84cdef  | 2.55abcd  | 2.52abcd  | 2.46b     | 2.44a     |
| N-NH3               | 3.19ab    | 3.11ab    | 3.12ab    | 2.98a     | 3.47b     | 2.94a     | 3.77b     | 3.21ab    | 3.51ab    |

P0; control (Basal Diet 70% Concentrate + 30% Forages), P1; P0 + Premix, P2; P1 + Probiotic, P3; P1 + Enzyme, P4; P1 + Plant Extract, P5; P1 + (Probiotics + Enzyme), P6; P1 + (Probiotics + plant extract), P7; P1 + (Probiotic + Enzyme + Plant Extract), P8; P1 + (Enzyme + Plant Extract).

Different superscripts in the same row with various letters show significant differences (p <0.05).

The proportion of rumen VFA depends on feed composition, forage ratio, protein content, feed processing, and feeding time [38]. [39] demonstrated in a simple in vitro study that the observed change in the acetate : propionate ratio after administration of the concentrate was due mainly to food (75%) and to a lesser extent pH (25%). The separation of these two factors involved in the control of rumen fermentation is important for a full understanding of rumen function, for developing more accurate mathematical models, and for designing strategies to prevent and control rumen acidosis.

Rumen NH3 product depends on dietary protein, the rate of degradation, and time after eating. Feed protein that enters the rumen will be fermented by proteolytic microbes (bacteria and protozoa). Bacteria and protozoa produce proteolytic enzymes such as proteases, peptides and deaminases that digest proteins into amino acids, peptides, and ammonia [40].

4. Conclusion

In conclusion, the addition of mix feed additive premix, probiotic, enzyme and plant extract can affect the kinetics gas, methane production, and VFA partial. However, it does not affect the level of pH, dry matter, organic matter, and ammonia (NH3).

References
[1] Adegoke TA, Abioye AA. 2016. Assessment of Existing and Potential Feed Resources for Improving Livestock Productivity in Niger. Int J Agric Res, 11
[2] Reti KL, Thomas MC, Yanke LJ, Selinger LB, Inglis GD. 2013. Effect of Antimicrobial Growth Promoter Administration on The Intestinal Microbiota of Beef Cattle. Gut Pathog, 5(8)
[3] Gaggia F, Mattarelli P, Biavati B. 2010. Probiotics and Prebiotics in Animal Feeding For Safe Food Production. Int J Food Microbiol;141:S15e28.
[4] Stover MG, Watson RR, Collier R. 2016. Pre- and Probiotic Supplementation In Ruminant Livestock Production. p. 25e36.
[5] McSweeney C, Mackie R. 2012. Microorganisms and ruminant digestion: state of knowledge, trends and future prospects. Background Study Paper. Roma.
[6] Dehorigy BA. 2003. Rumen Microbiology. Nottingham: Nottingham University Press.

[7] Ezema C. 2013. Probiotics In Animal Production: a review. J Vet Med and Anim Health;5(11):308e16.

[8] Elghandour MMY, Salem AZM, Castaneda JSM, Camacho LM, Kholif AE, Chagoya JCV. 2015. Direct-Fed Microbes: A Tool for Improving The Utilization of Low-Quality Roughages In Ruminants. J Integr Agric;14:526e33.

[9] Khan RU, Shabana N, Kuldeep D, Karthik K, Ruchi T, Mutassim MA. 2016. Direct-Fed Microbial: Beneficial Applications, Modes of Action And Prospects As A Safe Tool for Enhancing Ruminant Production And Safeguarding Health. Int J Pharm;12:220e31.

[10] Wanapat M. 1990. Nutritional Aspects of Ruminant Production in Southeast Asia With Special Reference To Thailand. 549

[11] Callaway TR, Anderson RC, Edrington TS, Genovese KJ, Bischoff KM. 2004. What Are We Doing About Escherichia coli O157: H7in cattle? J Anim Sci, 82:93e9.

[12] De Ondarza MB, Sniffen CJ, Dussert L, Chevaux E, Sullivan J, PAS Walker N. 2010. Case Study: Multiple-Study Analysis of the Effect of Live Yeast on Milk Yield, Milk Component Content and Yield, and Feed Efficiency. Prof Anim Sci;26:661e6.

[13] Iason G. 2005. The Role of Plant Secondary Metabolites in Mammalian Herbivory: Ecological Perspectives. Proc Nutr Soc, 64:123e31.

[14] Kumar M, Kumar V, Roy D, Kushwaha R, Vaiswani S. 2014. Application of Herbal Feed Additives In Animal Nutrition-A Review. Int J Lives Res, 4:e18.

[15] Theodorou, MK, Brooks AE. 1994. Evaluation of A New Laboratory Procedure for Estimating the Fermentation Kinetics of Tropical Feeds. Annual Reports. AFRC Inst. Hurley. Meidenhead, UK.

[16] Orskov ER, McDonald P. 1979. The Estimation of Protein Degradability In the Rumen from Incubation Measurements Weighted According to Rate of Passage. J of Agri Sci. 92:499-503.

[17] D.N. Kamra. 2005. Current science 89, 124-135

[18] Castillo-González, A., Burrola-Barraza, M., Domínguez-Viveros, J., & Chávez-Martínez, A. 2014. Rumen Microorganisms and Fermentation. Archivos de Medicina Veterinaria, 46(3), 349–361.

[19] Getachew, G., M. Blummel, H.P.S. Makkar and K. Becker, 1998. In Vitro Gas Measuring Techniques For Assessment of Nutritional Quality of Foods: A Review. Anim. Feed Sci. Technol., 72: 261-281.

[20] Mohammed N, N Ajisaka, ZA Lila, K Haru, K Mikuni, K Haru, S Kanda, H Itabashi. 2004. Effect of Japanese Horseradish Oil on Methane Production and Ruminal Fermentation in Vitro and In Steers. J Anim Sci 82, 1839-1846.

[21] Garnsworthy PC, J Craigo, JH Hernandez-Medrano, N Saunders. 2012. On-Farm Methane Measurements During Milking Correlate with Total Methane Production by Individual Dairy Cows. J Dairy Sci 95, 3166-3180.

[22] Vergé XPC, JA Dyer, RL Desjardins, D Worth. 2007. Greenhouse Gas Emissions From the Canadian Dairy Industry In 2001. Agricultural Systems 94, 683-693.

[23] Rotz CA, F Montes, DS Chianese. 2010. The Carbon Footprint of Dairy Production Systems Through Partial Life Cycle Assessment. J Dairy Sci 93, 1266-1282.

[24] Bodas R, N Prieto, R García-González, S Andrés, FJ Giráldez, S López. 2012. Manipulation of Rumen Fermentation and Methane Production by Plant Secondary Metabolites. Anim Feed Sci Tech 176, 78-93.

[25] Wallace R.J.; Newbold, C.J. 1993. Rumen Fermentation and Its Manipulation: The Development of Yeast Cultures As Feed Additives. In: Alltech’s Annual Symposium, 9, USA. Proceedings... 1993. P.173-192.

[26] Frumholz, P.P.; Newbold, C.J.; Wallace, R.J. 1989. Influence of Aspergillus Oryzae Fermentation Extract on The Fermentation Of A Basal Ration In The Rumen Simulation Technique (Rusitec). Journal of Agriculture Science, V.113, P.169.

[27] Martin, S.A.; Nisbet, D.J.; Dean, R.G. 1989. Influence of A Commercial Yeast Supplement On The In Vitro Ruminal Fermentation. Nutrition and Reproduction International, V.40, P.395.
[28] N.I Said. 2014. Kecearnaa NDF dan ADF Ransum komplit dengan kadar Protein Berbeda pada Ternak Kambing Marica, Bachelor thesis, Universitas Hasanudin.

[29] T. Sutardi. 1988. Ketahanan Protein Bahan Pakan Terhadap Degradasi oleh Mikroba Rumen dan Manfaatnya Bagi Peningkatan Produktivitas Ternak. Seminar Penelitian dan Hasil Penelitian Penunjang Pengembangan Peternakan Tradisional. Lembaga Penelitian Peternakan, Bogor.

[30] Giraldo LA, ML Tejido, MJ Ranilla, MD Carro. 2007. Effects of Exogenous Cellulose Supplementation on Microbial Growth and Ruminal Fermentation of a High-Forage Diet in Rusitec Fermenters. J Anim Sci 85.

[31] Goncalves TA, ARL Damásio, F Segato, TM Alvarez, J Bragatto, LB Brenelli, APS Citadini, MT Murakami, R Ruller, AF Paes Leme, RA Prade, FM Squina. 2012. Functional characterization and synergic action of fungal xylanase and arabinofuranosidase for production of xylooligosaccharides. Bioresource Technol 119, 293-299.

[32] Morgavi DP, KA Beauchemin, VL Nserekro, LM Rode, TA McAllister, AD Iwaasa, Y Wang, WZ Yang. 2001. Resistance of Feed Enzymes to Proteolytic Inactivation by Rumen Microorganisms and Gastrointestinal Proteases. J Anim Sci 79, 1621-1630.

[33] Beauchemin KA, D Colombatto, DP Morgavi, WZ Yang. 2003. Use of Exogenous Fibrolytic Enzymes to Improve Feed Utilization by Ruminants. J Anim Sci 81, 37-47.

[34] Tang SX, GO Tayo, ZL Tan, ZH Sun, LX Shen, CS Zhou, WJ Xiao, GP Ren, XF Han, SB Shen. 2008. Effects of Yeast Culture and Fibrolytic Enzyme Supplementation on In Vitro Fermentation Characteristics of Low-Quality Cereal Straws. J Anim Sci 86, 1164-1172.

[35] Nserekro VL, DP Morgavi, LM Rode, KA Beauchemin, TA McAllister. 2000. Effects of Fungal Enzyme Preparations on Hydrolysis and Subsequent Degradation Of Alfalfa Hay Fiber by Mixed Rumen Microorganisms In Vitro. Anim Feed Sci Technol 88, 153-170

[36] Conway, E.J. 1962. Microdiffusion Analysis and Volumetric Error. 5th Edition. Crosby Lockwood, London.

[37] N.K.P. Souza, E. Detmann, S.C. Valadares Filho, V.A.C. Costa, D.S. Pina, D.I. Gomes, A.C. Queiroz, H.C. Mantovani. 2013. Accuracy of the Estimates of Ammonia Concentration in Rumen Fluid Using Different Analytical Methods. Arq. Bras. Med. Vet. Zootec., v.65, n.6, p.1752-1758.

[38] Czerkawski, J.W. 1986. An Introduction to Rumen Studies. Programon Press. England.

[39] Russell, J.B. and D.B. Wilson. 1996. Why Are Ruminal Cellulolytic Bacteria Unable to Digest Cellulose at Low pH?. J. Dairy Sci. 79: 1503-1509.

[40] Chuzaimi, S. dan J.V. Bruchem. 1990. Fisiolegi Nutrisi Ruminansia. Universitas Brawijaya. Malang.

Acknowledgements

The author is would grateful thank to the Research Grand DIPA IPH- Research Center for Biotechnology National Research and Innovation Agency (BRIN) – and to the Ministry of Research and Technology, Republic of Indonesia, for funding this research through the National Research Priority (PRN-LPDP), 2020, contract number No.44/E1/PRN/2020.