In vitro Nutrient Digestibility and Fermentation Characteristics of King Grass Combined with Concentrate-Containing Mixed Microbes

by Widayati Trisiwi W

Submission date: 12-Apr-2020 05:08PM (UTC+0800)
Submission ID: 1295525724
File name: Bu_Siwi_artile_2016_convert_pdf_to_word.pdf (224.15K)
Word count: 4044
Character count: 20837

Pakistan Journal of Nutrition 15 (8): 784-788, 2016
ISSN 1680-5194

In vitro Nutrient Digestibility and Fermentation Characteristics of King Grass Combined with Concentrate-Containing Mixed Microbes

B. Santoso[1], M.N. Lekitoo[1], B. Tj. Hariadi[1], T.W. Widayati[2] and H. Abubakar[3]
[1]Department of Animal Nutrition, [2]Department of Animal Production, [3]Department of Biology,
University of Papua, Manokwari, West Papua, 98314, Indonesia

Abstract: The main feed for ruminants is forage, which is composed of polysaccharides. Feed digestion in the rumen is mediated by microbes, thus the type and makeup of rumen microbes are an important factor that affects nutrient digestibility. This study aimed to evaluate the *in vitro* nutrient digestibility and fermentation characteristics of king grass combined with a concentrate that contained mixed microbes. *Lactobacillus plantarum, Saccharomyces cerevisiae* and two strains of cellulolytic bacteria (i.e., *Acinetobacter baumannii* and *Pseudomonas aeruginosa)* were added to the concentrate. Cellulolytic bacteria were isolated from waste from either rice straw or palm oil seeds. The concentrate was mainly composed of agricultural and food industry wastes, such as cassava waste, tofu waste and rice bran. The following four concentrates were made: A, concentrate without microbe; B, concentrate containing *L. plantarum* and S. *cerevisiae;* C, concentrate containing *L. plantarum*, S. *cerevisiae* and *P. aeruginosa* and D, concentrate containing *L. plantarum*, S. *cerevisiae* and *A. baumannii.* Bacteria and yeast were added to the concentrate at 10^6-10^7 *cfu*/*g*. *In vitro* nutrient digestibility assays were conducted using 250 mg substrate composed of king grass and concentrate (70: 30, OM). Our findings revealed that concentrate contained 7.2 x 10^6 *cfu*/*g L. plantarum,* 3 x 10^8 *cfu*/*g* S. *cerevisiae* and 8.6 x 10^7 *cfu*/*g* A. *baumannii* and *P. aeruginosa.* The OM digestibility was greater ($p<0.01$) for the grass substrate with concentrate containing *L. plantarum*, S. *cerevisiae* and cellulolytic bacteria than for the concentrate that only contained *L. plantarum* and S. *cerevisiae.* Moreover, NDF digestibility was greater ($p<0.01$) for the grass substrate combined with concentrate that contained mixed microbes compared with the concentrate without microbes. The addition of cellulolytic bacteria increased NH_3-N and acetic acid concentrations ($p<0.05$). We concluded that the addition of mixed microbes to the concentrate improved fermentation activity and the digestibility of nutrients *in vitro*.

Key words: By-products, concentrate, digestibility, rumen, cellulolytic

INTRODUCTION

There is growing interest in studying the potential uses of natural products as feed additives instead of chemical compounds, such as ionophores or antibiotics, as manipulators of rumen fermentation to improve fermentation activity. The usage of growth-promoting antibiotics in animal feed is banned in Europe because of potential risks that include the spread of antibiotic resistance genes or contamination of milk or meat with antibiotic residues (Hong *et al*., 2005).

The main feed for ruminants is forage, which is composed of polysaccharides. Feed digestion in the rumen is carried out by microbes, thus the type and population of microbes are important factors that affect the digestibility of nutrients. Recently, probiotics have been frequently evaluated for use as replacements for antibiotics. Probiotics are live microbial feed supplements that may beneficially affect the host animal upon ingestion by improving the balance of intestinal microbes (Fuller, 1989). Seo *et al.* (2010) reported that micro-organisms, such as *Lactobacillus, Streptococcus* and *Enterococcus*, are commonly used as probiotics for ruminants. Furthermore, *Saccharomyces cerevisiae* and *Aspergillus oryzae* are two primary fungal direct-fed micro-organisms (DFMs) that have been used as diet supplements in ruminants. Seo *et al.* (2010) also found that propionibacteria ferment lactic acid to yield propionic acid. Because propionic acid is the major precursor for gluconeogenesis, increasing propionic acid production in the rumen increases hepatic glucose production. Additionally, increased amounts of propionic acid may reduce the amount of hydrogen that is available for CH_4 production in the rumen. Newbold (1995) and Lila *et al.* (2004) found that the addition of S. *cerevisiae* to the ruminant microflora could improve animal production by promoting bacterial viability, reducing the amount of lactate and slightly reducing levels of CH_4 and hydrogen. In another study, Santoso *et al.* (2014) concluded that the addition of *L. plantarum, L. acidophilus* and S. *cerevisiae* in feed concentrate increased the amount of propionic acid, promoted *in vitro* nutrient digestibility and reduced *in vitro* CH_4 production. In previous *in vivo* studies, most of researchers directly fed probiotics to animals. However,

Corresponding Author: B. Santoso, Department of Animal Nutrition, University of Papua, Manokwari, West Papua, 98314, Indonesia

this method was less efficient when applied to a large number of animals. Therefore, this present study aimed to evaluate the *in vitro* nutrient digestibility and fermentation characteristics of king grass combined with a concentrate that contained a mixture of microbes.

MATERIALS AND METHODS

Concentrate preparation and treatments: Rice bran, tofu waste and cassava waste were obtained from small-scale food industry located in the Manokwari and Prafi Districts, Manokwari Regency, Indonesia. Tofu and cassava waste were dried in an oven at 60°C for at least 48 h and were ground to pass through a 1 mm sieve in a Wiley mill. *Lactobaci/lus plantarum* was isolated from *Pennisetum purpureophoides* that had been used in a previous study by Santoso *et al.* (2012). *L. plantarum* were cultured using MRS broth at 30°C for 48 h (Santoso *et al.*, 2013a), while S. *cerevisiae* was cultured using malt extract broth at 30°C for 48 h (Newbold, 1995). Cellulolytic bacteria were isolated from rice straw or palm oil seed waste and were cultured using CMC. Solid concentrate materials were manually mixed by hand and then sprayed on top with a culture of LAB, yeast and cellulolytic bacteria at 10^6-10^7 cfu/g. The four following concentrates were generated: (A) concentrate without microbes; (B) concentrate containing *L. plantarum* and S. *cerevisiae;* (C) concentrate containing *L. plantarum*, S. *cerevisiae* and *P. aeruginosa* and (D) concentrate containing *L. plantarum*, S. *cerevisiae* and *A. baumannii* (Table 1). A total of 5 treatments were used in this study as follows: (G) grass, (G+A) grass+concentrate A, (G+B) grass+concentrate B, (G+C) grass+concentrate C and (G+D) grass+concentrate D.

Donor animals: A total of two ruminally fistulated Ongole crossbreed cattle were used as rumen liquor donors. Animals were fed 6.8 kg DM king grass to meet their maintenance requirements and were adapted for 3 weeks prior to rumen liquor collection. Feed was offered twice daily at 08:00 and 16:00 h. Rumen liquor was collected before the morning feeding and strained through four layers of cheesecloth into a pre-warmed thermos flask.

***In vitro* gas production and CH_4 measurements:** *In vitro* gas production was determined according to the method of Menke and Steingass (1988), as previously described by Hariadi and Santoso (2010) and Santoso *eta/.* (2013b). Briefly, oven-dried samples of ~300±5 mg were weighed into 100 ml glass syringes (Model Fortune, Haberle Labortechnik, Germany) with pistons that were lubricated with Vaseline. Additionally, three parallel syringes that contained mixtures of rumen liquor-buffer without substrate served as blanks. The buffer solution contained carbonate buffer, macromineral solution and micromineral solution. Syringes were pre-warmed at 39°C overnight, before the addition of 30±1.0 ml of rumen liquor-buffer mixtures into each syringe. Each syringe was incubated in a water bath at 39°C for 48 h and were gently shaken every 8 h. The volume of gas that was released from each syringe was recorded before incubation (0 h) and at 1, 2, 4, 6, 8, 12, 24, 36 and 48 h of incubation.

To facilitate CH_4 measurements, glass syringes were fitted with an extra outlet containing a gas-tight septum for gas sampling, as described by Hariadi and Santoso (2010) and Santoso *et al.* (2013b). At 24 and 48 h of incubation, 100 ml gas was sampled from the headspace of an airtight syringe. Methane was determined based on the injection of 100 µl gas into a gas chromatograph (GC model 263-50, Hitachi Ltd., Ibaraki, Japan).

At the end of the incubation period, ~10 ml of syringe contents were sampled. The pH of the medium was immediately recorded using a pH digital meter (Hanna, Hi 8520, Ronchi di Villafranca, Italy). Subsequently, 0.2 ml sub-samples were pipetted into 1.5 ml micro centrifuge tubes containing 1 ml of 25 g/100 ml (w/v) metaphosphoric acid and centrifuged at 9000 x g for 10 min for volatile fatty acids (VFA) determination. For NH_3-N analysis, an additional 2 ml of sub-samples were added to 2 ml of 20 g/l (w/v) NaCl.

***In vitro* nutrient digestibility:** Measurements of DM, organic matter (OM) and neutral detergent fiber (NDF) digestibility were conducted using the *in vitro* procedure of Tilley and Terry (1963). A total of 25 ml rumen liquor-buffer mixtures in a 1:4 (v/v) ratio were dispensed in 100 ml glass tubes that contained 250 mg dry sample, which consisted of grass and concentrate (70:30, DM). Triplicate blank (with no feed sample) and standard (Pangola grass) samples were also included in each run. Rumen liquor was collected in the morning prior to feeding and strained through four layers of cheesecloth into a pre-warmed thermos flask. The buffer solution contained 9.8 g $NaHCO_3$, 9.3 g $NaHPO_4$•12Hp, 0.47 g NaCl, 0.57 g KCl, 0.04 $CaCl_2$ and 0.12 g $MgSO_4$•$7H_2O$ per 1000 ml distilled water. After gassing CO_2 into the tube, corks were tightly placed over the tubes, which were incubated in a water bath at 39°C for 48 h. After 48 h of microbial incubation, samples were incubated at 39°C for 48 h with acid-pepsin. Thereafter, contents were filtered through pre-weighed Gooch crucibles and dried at 105°C for 24 h. The percent loss in weight was determined and presented as *in vitro* DM digestibility (IVDMD) and *in vitro* NDF digestibility (IVNDFD) values. The remaining residue was ashed at 550°C to determine the *in vitro* OM digestibility (IVOMD).

Chemical analysis: Dried samples were used to determine DM, OM and CP according to procedure of the AOAC (2005). The fiber content (i.e., NDF and acid detergent fiber (ADF)) were analyzed using the method of Van Soest *et al.* (1991) with some modifications including the determination of NDF without the use of ~-amylase and sodium sulfite.

Statistical analysis: Data were analyzed following a completely randomized design with the GLM procedure of the SAS software package (SAS Institute Inc., Cary, NC). Duncan's multiple range test was used to identify significant differences between means.

RESULTS AND DISCUSSION

Chemical composition of feeds: The chemical compositions of the king grass and concentrate that we used in this study are presented in Table 1. The grass had 11.4% of CP, which is above the threshold value of 7%. Minson and Milford (1966) reported that digestibility declines when animals are fed herbage with a CP content below 7% because microbial activity in the rumen becomes depressed by the lack of nitrogen. Dry matter content of the concentrates showed a trend to be reduced by the addition of microbes to the culture because the added microbes were in a liquid form. Organic matter contents in all of the concentrates used in this experiment were similar, which varied from 93.8 to 94.7%. The addition of 1% urea in concentrates increased the CP content to 15%. The NDF and ADF contents in the concentrates were similar, as they varied from 59.4 to 61.9% and 16.0 to 17.8%, respectively. The population of LAB, yeast and cellulolytic bacteria in the concentrate ranged from 10^6 to 10^8 cfu/g, which was lower than concentration of probiotic of 5×10^8 cfu/g, which was used by Lila *et al.* (2004).

Fermentation characteristics: The pH value as well as the concentrations of NH_3-N and VFA are presented in Table 2. The pH values in the substrates that consisted of grass and concentrate containing *L. plantarum,* S. *cerevisiae* and *P. aeruginosa* (G+C) and concentrate containing *L. plantarum,* S. *cerevisiae* and *A. baumannii* (G+D) were lower ($p<0.01$) than the control substrate. The lower pH value could be a consequence of the higher total VFA concentration, which was a result of cellulolytic bacteria activity that suppressed the pH value. However, the pH values in all treatments ranged from 6.82 to 6.91, which were in the optimal pH range of 6.7±0.5 required to maintain normal cellulolysis (Van Soest, 1994) and were above 6.0, which is the threshold for microbial protein synthesis (Russell *et al.,* 1992). In a previous *in vitro* study, Lila *et al.* (2004) noted that the addition of probiotic that contained 5×10^9 S. *cerevisiae* cells/g had no significant effect on the pH value. By contrast, Mwenya *et al.* (2004) reported that supplementation of 4 g/d of S. *cerevisiae* significantly increased sheep rumen pH values. The ammonia concentration represents a balance between degradation of feed protein and the uptake of ammonia for the synthesis of microbial proteins. During fermentation in the rumen, feed protein can be degraded by microbes to NH_3N. Concentrations of NH_3N in grass combined with concentrate without microbe (G+A) were similar to those of a concentrate that contained *L. plantarum* and S. *cerevisiae* (G+B). Our present findings are supported by Lila *et al.* (2004) and Mwenya *et al.* (2004) who found that the concentration of ammonia N did not change after the addition of probiotic, i.e., S. *cerevisiae* or LAB. However, a higher NH_3-N concentration was observed in substrate grass combined with concentrate containing cellulolytic bacteria (G+C and G+B) compared with grass alone (G) or a combination of grass and concentrate without microbes (G+A). The concentration of NH_3-N in this present study ranged from 25.9 to 29.6 mg/100 ml, which was above the threshold value for maximum fiber digestion that was recommended by Abdulrazak *et al.* (2000).

The proportions of acetic acid (C2) and total VFA concentration were higher ($p<0.05$) in concentrate in which cellulolytic bacteria had been added (G+C and G+D). This finding is in accord with those of Lila *et al.* (2004) who found that the addition of S. *cerevisiae* increased the proportion of propionic acid and total VFA. Additionally, Mwenya *et al.* (2004) reported that proportion propionic acid and the concentration of total VFA in the rumen were similar between sheep fed either LAB or S. *saccharomyces* and control sheep. The increased proportion of propionic acid could be a consequence of increased lactic acid production by LAB. Furthermore, lactic acid can be converted by lactic acid-utilizing bacteria such as *Megasphaeraelsdenii,* to propionic acid. Increasing the proportion of propionic acid (C3), however, reduced the C2/C3 ratio in treatment groups G+B, G+C and G+D. Increasing the total VFA in concentrate by adding probiotic is supported by IVDMD data for treatments G+B, G+C and G+D.

Total gas production during a 48 h incubation was higher ($p<0.05$) in substrate grass combined with concentrate (G+B, G+C, G+D and G+E) compared with grass alone (G). Gas production can be used as an indicator of feed degradation in the rumen. Beuvink and Spoelstra (1992) concluded that there was a significant correlation between organic matter digestibility, VFA concentrations and gas production. However, our measurements of total gas production in this study are supported by IVDMD values, as presented in Table 3.

Nutrient digestibility: Table 3 shows the *in vitro* DM, OM and NDF digestibility of grass and concentrate-containing mixed microbes. IVDMD in the substrate that consisted of grass and concentrate was higher ($p<0.01$) compared with that of control feed. The addition of LAB and yeast in concentrate (G+B) increased the IVDMD value by 11% when compared with concentrate without mixed microbe (G+A). A combination of LAB, yeast and cellulolytic bacteria in concentrates (G+B, G+C and G+D) enhanced ($p<0.01$) IVNDFD by an average of 21.45% compared with concentrate without mixed microbes. This finding was comparable with those of Lila *et al.* (2004) who found that

Table 1: Ingredients of the concentrate and chemical composition of the grass and concentrates (%)

		Concentrates			
	G	A	B	C	D
Ingredients					
Cassava waste		42	38	36	36
Tofu waste		25	25	25	25
Rice bran		30	30	30	30
Salt		2	2	2	2
Urea					
L. plantarum			2	2	2
S. cerevisiae			2	2	2
P. aeruginosa				2	
A. baumannii					2
Chemical composition					
Dry matter	84.3	89.9	86.1	83.3	83.3
Organic matter	91.8	94.3	93.8	94.7	94.1
Crude protein	11.4	15.0	13.5	14.0	13.9
NDF	79.3	61.7	61.4	61.9	59.4
ADF	38.9	16.2	16.0	17.8	16.5
Hemicellulose	39.1	45.5	45.4	44.1	42.9
L. plantarum (cfu/g)			$7.2x10^6$	$7.4 x 10^6$	$7.0 x 10^6$
S. cerevisiae (cfu/g)			$3.0x10^8$	$3.1 x 10^8$	$3.0x10^8$
P. aeruginosa (cfu/g)				$8.6x10^7$	
A. baumannii (cfu/g)					$1.9x10^8$

G: Grass, A: Concentrate without microbe, B: Concentrate containing *L. plantarum* and *S. cerevisiae*, C: Concentrate containing *L. plantarum*, *S. cerevisiae* and *P. aeruginosa*, D: concentrate containing *L. plantarum*, *S. cerevisiae* and *A. baumannii*

Table 2: *In vitro* fermentation characteristics in the supernatant after 48 h of incubation

	Treatments						
	G	G+A	G+B	G+C	G+D	SEM	p-value
pH	6.91$_a$	6.87'	6.86'$_b$	6.83$_b$	6.82$_b$	0.01	0.01
NH_3-N (mg/100 ml)	25.9$_b$	26.3$_b$	28.5'$_b$	29.6'	29.5'	0.82	0.02
C2 (mol/100 mol)	72.7$_b$	75.3'$_b$	80.8'$_b$	86.0'	86.5'	3.37	0.05
C3 (mol/100 mol)	29.3	32.0	30.7	32.6	36.4	1.83	0.14
C4 (mol/100 mol)	8.3	9.7	10.7	11.6	9.4	1.15	0.39
C2/C3	2.5	2.4	2.6	2.4	2.6	0.17	0.74
Total VFA (mM)	110.2'$_b$	117.1$_{bc}$	122.2'$_b$	134.0'	128.6'$_b$	4.55	0.03
Total Gas 48 h (ml)	38.3$_b$	42.0'	42.3'	42.7'	42.7'	1.00	0.05

G: Grass, A: Concentrate without microbe, B: Concentrate containing *L. plantarum* and *S. cerevisiae*, C: Concentrate containing *L. plantarum*, *S. cerevisiae* and *P. aeruginosa*, D: concentrate containing *L. plantarum*, *S. cerevisiae* and *A. baumannii*
Mean values with different superscript letters within the same row are significantly different ($p<0.05$)

Table 3: *In vitro* digestibility(%) of dry matter, organic matter and neutral detergent fiber of feed

	Treatments						
	G	G+A	G+B	G+C	G+D	SEM	p-value
IVDMD	39.2$_b$	44.1'	44.5'	45.4'	46.1'	0.82	<0.01
IVOMD	49.4'		59.7'	57.7'$_b$	58.2'$_b$	1.17	<0.01
IVNDFD	25.5'		43.8'	43.4'	45.1'	1.47	<0.01

G: Grass, A: Concentrate without microbe, B: Concentrate containing *L. plantarum* and *S. cerevisiae* C: Concentrate containing *L. plantarum*, *S. cerevisiae* and *P. aeruginosa*, D: concentrate containing *L. plantarum*, *S. cerevisiae* and *A. baumannii*
Mean values with different superscript letters within the same row are significantly different ($p<0.01$)

the addition of S. *cerevisiae* increased *in vitro* dry matter degradability. In another study, Krisnan *et al.* (2009) concluded that the addition of probiotic collected from buffalo rumen in catalytic supplement increased the NDF digestibility in sheep. Chaucheyras *et al.* (1995) noted that S. *cerevisiae* showed an ability to provide grovvth factors, such as organic acids or vitamins, thereby stimulating ruminal populations of cellulolytic bacteria.

Conclusion: In this present study, the concentrate containing LAB, yeast and cellulolytic bacteria was effective in modifying ruminal fermentation patterns by increasing NH_3-N and total VFA concentrations along with the proportion of acetic acid. The addition of LAB, yeast and cellulolytic bacteria to the concentrate increased NDF digestibility *in vitro* when compared with the concentrate without added microbes.

ACKNOWLEDGMENTS

This study was funded by the Directorate General of Higher Education of the Indonesian Ministry of National Education via the "Hibah Kompentensi" scheme (Contract No. 45.i/UN42.2.1/PL/III/2014).

REFERENCES

AOAC (Association of Official Analytical Chemists), 2005. Official Methods of Analysis. 17th Ed. Washington: AOAC International.

Abdulrazak, S.A., T. Fujihara, J.K. Ondiek and E.R. Orskov, 2000. Nutritive evaluation of some Acacia tree leaves from Kenya. Anim. Feed Sci. Technol., 85: 89-98.

Beuvink, J.M.W. and S.F. Spoelstra, 1992. Interactions between substrate, fermentation and end-products, buffering system and gas production upon fermentation of different carbohydrates by mixed rumen microorganism *in vitro*. Appl. Microbial. Biotehnol., 37: 505-509.

Chaucheyras, F., G. Fonty, G. Bertin, J.M. Salmon and P. Gouet, 1995. Effect of *Saccharomyces cerevisiae* (Levucell SC), a microbial additive for ruminants, on lactate metabolism *in vitro*. Can. J. Microbial., 42: 927-933.

Fuller, R., 1989. Probiotics in man and animals. J. Appl. Bacterial., 66: 365-378.

Hariadi, Tj.B. and B. Santoso, 2010. Evaluation of tropical plants containing tannin on *in vitro* methanogenesis and fermentation parameters using rumen fluid. J. Sci. Food Agric., 90: 456-461.

Hong, H.A., L.H. Due and S.M. Cutting, 2005. The use of bacterial spore formers as probiotics. FEMS Microbial. Rev., 29: 813-835.

Krisnan, R., B. Haryanto dan K.G. Wiryawan, 2009. The effect of combined probiotics with catalyst supplementation on digestion and rumen characteristic in Priangansheep. Indonesian J. Anim. Vet. Sci., 14: 262-269.

Lila, Z.A., N. Mohammed, T. Yasui, Y. Kurokawa, S. Kanda and H. Itabashi, 2004. Effects of a twin strain of *Saccharomyces cerevisiae* live cells on mixed ruminal microorganism fermentation *in vitro*. J. Anim. Sci. 82: 1847-1854.

Menke, K.H. and H. Steingass. 1988. Estimation of the energetic feed value obtained from chemical analysis and *in vitro* gas production using rumen fluid. Anim. Res. Develop., 28: 7-55.

Minson, D.J. and R. Milford, 1966. The energy values and nutritive value indices of *Digitaria decumbens, Shorghum a/mum* and *Phaseo/us atropurpureus*. Aust. J. Agric. Res., 17: 411-423.

Mwenya, B., B. Santoso, C. Sar, Y. Garno, T. Kobayashi, I. Arai and J. Takahashi, 2004. Effects of including a 1-4 galactooligosaccharides, lactic acid bacteria or yeast culture on methanogenesis, energy and nitrogen metabolism in sheep. Anim. Feed Sci. Technol., 115: 313-326.

Newbold, C.J., 1995. Probiotics for ruminants. Ann. Zootech., 45: 329-335.

Russell, J.B., J.D. O'Connor, D.G. Fox, P.J. Van Soest and C.J. Sniffen, 1992. A net carbohydrate and protein system for evaluating cattle diets. I. Ruminal fermentation. J. Anim. Sci., 70: 3551-3561.

Santoso, B., A. Maunatin, B. Tj. Hariadi and H. Abubakar, 2013a. Isolation and identification of lactid acid bacteria originated from king grass *(Pennisetum purpureophoides)* as candidate of probiotic for livestock. JITV, 18: 131-137.

Santoso, B., E.W. Saragihand B.Tj. Hariadi, 2013b. Effect of water extract of plants containing tannin on *in vitro* methanogenesis and fermentation characteristics of the grass. J. Indonesian Trop. Anim. Agric., 38: 47-54.

Santoso, B., B.Tj. Hariadi, Alimuddin and D.Y. Seseray, 2012. Fermentation characteristic of rice crop residue-based silage treated by epiphytic and commercial LAB. J. Anim. Sci. Technol., 35: 60-66.

Santoso, B. B.Tj. Hariadi and H. Abubakar, 2014. The effect concentrate containing probiotic on fermentation characteristics, methanogenesis and *in vitro* nutrient digestibility. J. Indonesian Trop. Anim. Agric., 39: 210-216.

Seo, J.K., S.W. Kim, M.H. Kim, S.D. Upadhaya, D.K. Kam and J. K. Ha, 2010. Direct-fed microbials for ruminant animals. Asian-Aust. J. Anim. Sci., 23: 1657-1667.

Tilley, J.M. and R.A. Terry, 1963. A two stage technique for the *in vitro* digestion of forage crops. J. Br. Grassl. Soc., 18: 104-111.

Van Soest, P.J., 1994. Nutritional Ecology of the Ruminant. (2nd ed.). Comstock Publishing Associates a Division of Cornell University Press, Ithaca, NY, USA and London, UK, pp: 476.

Van Soest, P.J., J.B. Robertson and B.A. Lewis, 1991. Methods for dietary fiber, neutral detergent fiber and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci., 74: 3583-3597.

In vitro Nutrient Digestibility and Fermentation Characteristics of King Grass Combined with Concentrate-Containing Mixed Microbes

ORIGINALITY REPORT

50%	44%	31%	22%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	scialert.net Internet Source	12%
2	media.neliti.com Internet Source	6%
3	fapet.ipb.ac.id Internet Source	2%
4	conservancy.umn.edu Internet Source	2%
5	hdl.handle.net Internet Source	2%
6	www.koreascience.or.kr Internet Source	1%
7	e-sciencecentral.org Internet Source	1%
8	eprints.undip.ac.id Internet Source	1%

9	cdn.aphca.org Internet Source	1%
10	www.e-sciencecentral.org Internet Source	1%
11	www.performanceprobiotics.com.au Internet Source	1%
12	Adriani ., R. Asra, S. Novianti, Fatati .. "The Effect of Coleus amboinicus L. Supplementation on In vitro Digestibility", Pakistan Journal of Nutrition, 2019 Publication	1%
13	Submitted to CVC Nigeria Consortium Student Paper	1%
14	Saminathan, Mookiah, Chin Chin Sieo, Norhani Abdullah, Clemente Michael Vui Ling Wong, and Yin Wan Ho. "Effects of condensed tannin fractions of different molecular weights from a Leucaena leucocephala hybrid on in vitro methane production and rumen fermentation", Journal of the Science of Food and Agriculture, 2014. Publication	1%
15	Santoso, B.. "Ruminal fermentation and nitrogen metabolism in sheep fed a silage-based diet supplemented with Yucca schidigera or Y. schidigera and nisin", Animal Feed Science and	1%

Technology, 20060901
Publication

16	Submitted to Harper Adams University College Student Paper	1%
17	Mardalena, S. Syarif, S. Erina. "Molecular Characteristics and Identification of Lactic Acid Bacteria of Pineapple Waste as Probiotics Candidates for Ruminants", Pakistan Journal of Nutrition, 2016 Publication	1%
18	ajas.info Internet Source	1%
19	repository.up.ac.za Internet Source	1%
20	Smerjai Bureenok. "Additive effects of fermented juice of epiphytic lactic acid bacteria on the fermentative quality of guineagrass (Panicum maximum Jacq.) silage", Grassland Science, 9/2005 Publication	1%
21	iskv.in Internet Source	1%
22	Pirondini, Mario, Luca Malagutti, Stefania Colombini, Paola Amodeo, and Gianni Matteo Crovetto. "Methane yield from dry and lactating cows diets in the Po Plain (Italy) using an in vitro	1%

gas production technique", Italian Journal of Animal Science, 2012.
Publication

23	www.scribd.com Internet Source	1%
24	John E. Moore, Gerald O. Mott. "Structural Inhibitors of Quality in Tropical Grasses", Crop Science Society of America, 2015 Publication	1%
25	www.slu.se Internet Source	<1%
26	ijps.aizeonpublishers.net Internet Source	<1%
27	Submitted to Asian Institute of Technology Student Paper	<1%
28	Wang, H.. "Morphological fractions, chemical compositions and in vitro gas production of rice straw from wild and brittle culm1 variety harvested at different growth stages", Animal Feed Science and Technology, 20060804 Publication	<1%
29	G. Getachew, P.H. Robinson, E.J. DePeters, S.J. Taylor, D.D. Gisi, G.E. Higginbotham, T.J. Riordan. "Methane production from commercial dairy rations estimated using an in vitro gas technique", Animal Feed Science and	<1%

Technology, 2005
Publication

30	Wang, Zuo, Zhixiong He, Karen A. Beauchemin, Shaoxun Tang, Chuanshe Zhou, Xuefeng Han, Min Wang, Jinhe Kang, Nicholas E. Odongo, and Zhiliang Tan. "Comparison of two live Bacillus species as feed additives for improving in vitro fermentation of cereal straws : Live Bacillus feed addition", Animal Science Journal, 2015. Publication	<1%
31	www.pjbs.org Internet Source	<1%
32	www.omicsonline.org Internet Source	<1%
33	Ja Kyeom Seo, Seon-Woo Kim, Myung Hoo Kim, Santi D. Upadhaya, Dong Keun Kam, Jong K. Ha. "Direct-fed Microbials for Ruminant Animals", Asian-Australasian Journal of Animal Sciences, 2010 Publication	<1%
34	Wulansih D. Astuti, Komang G. Wiryawa, Elizabeth Wina, Yantyati Widyastuti, Sri Suharti, Roni Ridwan. "Effects of Selected Lactobacillus plantarum as Probiotic on In vitro Ruminal Fermentation and Microbial Population", Pakistan Journal of Nutrition, 2018	<1%

Publication

35	213.55.99.198 Internet Source	<1%
36	K.D. Deng, Y. Xiao, T. Ma, Y. Tu, Q.Y. Diao, Y.H. Chen, J.J. Jiang. "Ruminal fermentation, nutrient metabolism, and methane emissions of sheep in response to dietary supplementation with Bacillus licheniformis", Animal Feed Science and Technology, 2018 Publication	<1%
37	C. Promkot, M. Wanapat, C. Wachirapakorn, C. Navanukraw. "Influence of Sulfur on Fresh Cassava Foliage and Cassava Hay Incubated in Rumen Fluid of Beef Cattle", Asian-Australasian Journal of Animal Sciences, 2007 Publication	<1%
38	hrcak.srce.hr Internet Source	<1%
39	world-food.net Internet Source	<1%
40	Mwenya, B.. "Effects of including @b1-4 galacto-oligosaccharides, lactic acid bacteria or yeast culture on methanogenesis as well as energy and nitrogen metabolism in sheep", Animal Feed Science and Technology, 20040802 Publication	<1%

41	Ghasemi, E, M Khorvash, and A Nikkhah. "Short Communication: Effect of forage sources and *Saccharomyces cerevisiae* (Sc 47) on ruminal fermentation parameters", South African Journal of Animal Science, 2012. Publication	<1%
42	B. Pen, K. Takaura, S. Yamaguchi, R. Asa, J. Takahashi. "Effects of Yucca schidigera and Quillaja saponaria with or without β 1–4 galacto-oligosaccharides on ruminal fermentation, methane production and nitrogen utilization in sheep", Animal Feed Science and Technology, 2007 Publication	<1%
43	scholar.lib.vt.edu Internet Source	<1%
44	rnd.edpsciences.org Internet Source	<1%
45	www.scopemed.org Internet Source	<1%
46	dc.library.okstate.edu Internet Source	<1%
47	Rune Rødbotten, Kjell Ivar Hildrum, Jan Berg. "Effects of Concentrate Level on Carcass Composition, Tenderness and Growth Rate of Angus × NRF and Limousin × NRF Bulls", Acta	<1%

Agriculturae Scandinavica, Section A - Animal Science, 2002
Publication

---

48 G. Licitra, T.M. Hernandez, P.J. Van Soest. "Standardization of procedures for nitrogen fractionation of ruminant feeds", Animal Feed Science and Technology, 1996 <1%
Publication

---

49 ifzz.pl <1%
Internet Source

---

50 idosi.org <1%
Internet Source

---

51 journal.ugm.ac.id <1%
Internet Source

---

52 Mardalena ., E. Musnandar. "In vitro Evaluation of Oil Palm Fronds Fermented with Produren: A Durian Probiotic", Pakistan Journal of Nutrition, 2019 <1%
Publication

---

53 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 <1%
Publication

---

54 Submitted to Universität Hohenheim

	Student Paper	<1%
55	www.tandfonline.com Internet Source	<1%
56	Y. Castillo-Castillo, O. Ruiz-Barrera, M.E. Burrola-Barraza, Y. Marrero-Rodriguez et al. "Isolation and characterization of yeasts from fermented apple bagasse as additives for ruminant feeding", Brazilian Journal of Microbiology, 2016 Publication	<1%
57	Budi SANTOSO. "Effect of Yucca schidigera with or without nisin on ruminal fermentation and microbial protein synthesis in sheep fed silage- and hay-based diets", Animal Science Journal, 12/2004 Publication	<1%
58	Submitted to University of Stellenbosch, South Africa Student Paper	<1%
59	Submitted to Széchenyi Istvan University Győr Student Paper	<1%
60	Mustafa Oskay, Dilek Sarı. "Antimicrobial Screening of Some Turkish Medicinal Plants", Pharmaceutical Biology, 2008 Publication	<1%

61	Shawle Retta Kassa. "Role of probiotics in rumen fermentation and animal performance: A review", International Journal of Livestock Production, 2016 Publication	<1%
62	"Proceedings of the 10th International Symposium on the Nutrition of Herbivores", Advances in Animal Biosciences, 2018 Publication	<1%
63	X.S. Guo, L.M. Ding, R.J. Long, B. Qi, Z.H. Shang, Y.P. Wang, X.Y. Cheng. "Changes of chemical composition to high altitude results in Kobresia littledalei growing in alpine meadows with high feeding values for herbivores", Animal Feed Science and Technology, 2012 Publication	<1%

Exclude quotes	Off	Exclude matches	Off
Exclude bibliography	Off		

In vitro Nutrient Digestibility and Fermentation Characteristics of King Grass Combined with Concentrate-Containing Mixed Microbes

GRADEMARK REPORT

FINAL GRADE

/0

GENERAL COMMENTS

Instructor

PAGE 1

PAGE 2

PAGE 3

PAGE 4

PAGE 5