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Research Article
Nutritive Value, *in vitro* Fermentation Characteristics and Nutrient Digestibility of Agro-industrial Byproducts-based Complete Feed Block Enriched with Mixed Microbes

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Abstract

Objective: This study was carried out to evaluate the nutritive value, *in vitro* fermentation characteristics and nutrient digestibility of agro-industrial byproducts-based complete feed block enriched with mixed microbes. **Methodology:** The complete feed blocks were mainly composed of agricultural and food industry byproducts such as rice straw, palm oil frond, tofu waste, cassava waste, sago starch and molasses. Four treatments were A, complete feed block without microbe; B, complete feed block containing *Lactobacillus plantarum*, *Saccharomyces cerevisiae* and *Pseudomonas aeruginosa*; C, complete feed block containing *L. plantarum*, *S. cerevisiae* and *Acinetobacter baumannii*; D, complete feed block containing *L. plantarum*, *S. cerevisiae*, *P. aeruginosa* and *A. baumannii*. Lactic acid bacteria, yeast and cellulolytic bacteria were added to the feed block at 10^7-10^9 CFU g^{-1}. All complete feed blocks were formulated to be isonitrogenous. About 500 g of mixed ingredients were transferred into a hydraulic press to makes block of size 15×10×8 cm. **Results:** The feed blocks contained 2.4×10^6 CFU g^{-1} *L. plantarum*, 6.5×10^4 CFU g^{-1}, 2.7×10^6 CFU g^{-1} *S. cerevisiae*, 2.7×10^6 CFU g^{-1} *P. aeruginosa* and 1.4×10^5 CFU g^{-1} *A. baumannii*. Crude protein content was similar (11.6%) for 4 feed blocks. Addition of cellulolytic bacteria in feed block reduced ($p<0.01$) Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF) contents. Feed blocks containing mixed microbes (C and D) had higher ($p<0.01$) acetic acid, propionic acid, butyric acid, total Volatile Fatty Acids (VFA) concentrations as well as total gas production compared with feed block without microbe (A). Feed block containing combination of *L. plantarum*, *S. cerevisiae*, *P. aeruginosa* and *A. baumannii* had the highest Dry Matter (DM), Organic Matter (OM) and NDF digestibility. **Conclusion:** Combination of mixed microbes in the complete feed block decreased fiber fraction contents and improved *in vitro* fermentation activity and the nutrients digestibility.

Key words: Byproducts, feed block, fermentation, microbe, rumen

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

In line with the shortage of grasses as a result of the change of land for plantations, housing and industry, it would require many efforts to utilize agricultural and food processing industry byproducts as feed source for ruminants. The utilization of agricultural byproducts for increasing ruminants production has been received greater research attention within the past few decades because of the higher quantities of those byproducts.

Poor palatability and low bulk density apart from low nutritive value are restricting the utilization of the agricultural byproducts as animal feeds. During these scarcity periods, there is need for easily available feed that can meet nutritional requirements at low cost and is easy to transport. Nutritive value of poor quality and bulky roughages can be improved by densified complete feed block[1]. This technology offers a means to increase milk production, decrease in environmental pollutants, increase in income of farmers, decrease in labour requirement and time for feeding and reduction in transportation cost of straw[2].

Complete feed is comprised of forage, concentrate and other supplementary nutrients in desired proportion capable to fulfill nutrient requirements of animals. The feeding of complete feed stabilises rumen fermentation, minimises fermentation loss and ensures better ammonia utilisation[3].

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. Seo *et al.*[4] revealed that micro-organisms such as *Lactobacillus*, *Streptococcus* and *Enterococcus* are commonly used as probiotics for ruminants. Oyeleke and Okusanmi[5] reported that *Pseudomonas aeruginosa* was found in the rumen of cow, sheep and goat approximately 9% of the bacteria species and was able to hydrolyse cellulose. El-Sheikh[6] and Chang *et al.*[7] noted that *Acinetobacter* sp. was found in the rumen of Korean native cattle and it belonged to cellulolytic bacteria. Santoso *et al.*[8] concluded that the addition of *Lactobacillus plantarum*, *Saccharomyces cerevisiae*, *Acinetobacter baumannii* and *P. aeruginosa* in concentrate improved fermentation activity and the digestibility of nutrients *in vitro*. Therefore, the present study was carried out to evaluate the nutritive value, *in vitro* fermentation characteristic and nutrient digestibility of agro-industrial byproducts-based complete feed block treated with addition of mixed microbes.

MATERIALS AND METHODS

Preparation of complete feed block: Rice straw and palm oil frond were collected from paddy field and palm oil plantation areas, respectively in Prafi district, Manokwari regency, Indonesia (longitude 133°48′ E and latitude 00°53′ S) with a mean altitude of 128 m a.s.l. Tofu and cassava wastes were obtained from small-scale food industry located in Manokwari and Prafi districts. In order to prepare complete feed block, rice straw and palm oil frond were dried under the sun and then chopped with a chopper machine to 1-2 cm lengths. Tofu waste 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 Wiley mill. *Lactobacillus plantarum* was isolated from *Pennisetum purpureophoides* that has been used in the previous study by Santoso *et al.*[8,9] that *L. plantarum* was cultured using MRS broth at 30°C for 48 h[9], meanwhile *S. cerevisiae* was cultured using malt extract broth at 30°C for 48 h[10]. Cellulolytic bacteria i.e., *P. aeruginosa* and *A. baumannii* were isolated from rice straw and palm oil seed waste, respectively and were cultured using CMC. The ingredients of feed block were manually mixed by hand and then sprayed on top with a culture of lactic acid bacteria, yeast and cellulolytic bacteria at 10^7-10^9 CFU g^{-1} (Table 1).

The experiment was arranged in a completely randomized design with four treatments and three replications per treatment. Four treatments were A, complete feed block without microbe; B, complete feed block

Table 1: Ingredients composition of the complete feed blocks

	Feed blocks			
Ingredients (%)	A	B	C	D
Rice straw	15.0	15.0	15.0	15.0
Palm oil fronds	25.0	25.0	25.0	25.0
Cassava waste	13.0	11.0	11.0	11.0
Tofu waste	17.0	15.0	15.0	15.0
Molasses	20.0	20.0	20.0	20.0
Sago starch	7.0	7.0	7.0	7.0
Urea	1.5	1.5	1.5	1.5
Mineral mixture*	1.5	1.5	1.5	1.5
L. plantarum	-	1.0	1.0	1.0
S. cerevisiae	-	1.0	1.0	1.0
P. aeruginosa	-	2.0	-	1.0
A. baumannii	-	-	2.0	1.0

*Composition (per kg): Calcium: 270 g, Phosphorus: 189 g, Manganese: 12 g, Vitamin A: 300.000 IU, Vitamin D3: 50.000 IU, Vitamin E: 100 mg, Vitamin K: 100 mg, Trace elements (Zn, Mn, Fe, Cu, I, Co, Mo, Se): 20 g. A: Complete feed block without microbe, B: Complete feed block containing *L. plantarum*, *S. cerevisiae* and *P. aeruginosa*, C: Complete feed block containing *L. plantarum*, *S. cerevisiae* and *A. baumannii*, D: Complete feed block containing *L. plantarum*, *S. cerevisiae*, *P. aeruginosa* and *A. baumannii*

containing *Lactobacillus. plantarum, Saccharomyces cerevisiae* and *Pseudomonas aeruginosa*, C, complete feed block containing *L. plantarum, S. cerevisiae* and *Acinetobacter baumannii* and D, complete feed block containing *L. plantarum*, *S. cerevisiae*, *P. aeruginosa* and *A. baumannii*. All complete feed block were formulated to be isonitrogenous (12% CP, DM basis). About 500 g of mixed complete feed was transferred into a hydraulic press to makes block of size 15×10×8 cm. The physical characteristics of the feed blocks were evaluated in terms of keeping quality and texture.

Donor animals: Rumen fluid was obtained from two rumen fistulated Ongole crossbreed cattle preconditioned for 3 weeks with 6.8 kg DM of king grass (*Pennisetum purpureophoides*) to meet their maintenance requirement[11]. Rumen liquor was collected with a manual suction apparatus before the morning feeding and strained through four layers of cheesecloth into a pre-warmed thermos flask.

Analysis of *in vitro* rumen fermentation characteristics: *In vitro* gas production was determined in line with the method of Menke and Steingass[12] previously described by Santoso *et al.*[8,13]. Briefly, oven-dried samples of about 300±5 mg were weighed into 100 mL glass syringes (Model Fortune, Häberle Labortechnik, Lonsee-Ettlenschieβ, 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 (35 g $NaHCO_3$, 4 g $(NH_4)HCO_3$ L^{-1}), macromineral solution (5.7 g Na_2HPO_4, 6.2 g $KHPO_4$, 0.6 g $MgSO_4.7H_2O$ L^{-1}) and micromineral solution (13.2 g $CaCl_2.2H_2O$, 10 g $MnCl_2.4H_2O$, 1 g $CoCl_2.6H_2O$, 0.8 g $FeCl_2.6H_2O$ per 100 mL distilled water). The medium was than reduced by addition of reducing agent (2 mL of 1 mol L^{-1} NaOH, 285 mg $Na_2S.7H_2O$/47.5 mL distilled water). The 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 syringes were 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 1, 2, 4, 6, 8, 12, 24, 36 and 48 h of incubation. At the end of the incubation period, approximately 10 mL of syringe contents were sampled. The pH of medium was immediately recorded using a pH digital meter (Hanna, Hi 8520, Ronchi di Villafranca, Italy). Subsequently, 0.2 mL of sub-samples were pipetted into 1.5 mL micro centrifuge tube containing 1 mL of 25 g/100 mL (w/v) metaphosphoric acid and centrifuged at 9000×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^{-1} (w/v) NaCl.

Determination of *in vitro* nutrients digestibility: Determinations of Dry Matter (DM), Organic Matter (OM) and Neutral Detergent Fiber (NDF) digestibility were conducted using *in vitro* procedure[14] as previously demonstrated by Santoso *et al.*[8,13]. A total 25 mL of rumen liquor-buffer mixtures in a 1:4 (v/v) ratio were dispensed in 100 mL glass tubes that contained 250 mg dry sample. Triplicates of blank (with no feed sample) and standard (Pangola grass) samples were 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 $NaHPO_4.12H_2O$, 0.47 g NaCl, 0.57 g KCl, 0.04 $CaCl_2$, 0.12 g $MgSO_4.7H_2O$ per 1000 mL distilled water. After gassing CO_2 in the tube, corks were tightly placed over the tubes and 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. Therefore, the 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). The remaining residue was ashed at 550°C to determine *in vitro* OM digestibility (IVOMD).

Chemical analysis: Dried samples were used to determine DM, OM and Crude Protein (CP) according to procedure of AOAC[15]. The fiber content i.e., NDF and Acid Detergent Fiber (ADF) were analyzed using method of Van Soest *et al.*[16] with some modifications i.e., NDF was determined without the use of μ-amylase and sodium sulfite.

Statistical analysis: Data were subjected to one-way analysis of variance using the general linear model of SAS (SAS Institute Inc., Cary, NC). Duncan's multiple range test was used to separate treatment means with a significance level of 5%.

RESULTS AND DISCUSSION

Nutritional contents of complete feed blocks: The nutrient contents of the complete feed blocks are presented in Table 2. The results show that addition of mixed microbes in complete feed blocks significantly ($p<0.01$) reduced NDF and ADF contents. The feed D had the lowest NDF and ADF contents, whereas the control feed (A) had the highest. The lower NDF and ADF content might be partly caused by

Table 2: Nutrient contents of complete feed blocks

	Feed blocks					
Variables	A	B	C	D	SEM	p-value
Dry matter (%)	78.7	78.0	76.8	78.0	0.79	0.47
Organic matter (%)	91.5	91.4	91.3	90.1	0.33	0.06
Crude protein (%)	11.7	11.5	11.7	11.6	0.19	0.78
NDF (%)	42.8^{a}	41.3^{ab}	40.6^{ab}	39.1^{b}	0.50	0.01
ADF (%)	31.9^{a}	30.3^{b}	30.3^{b}	28.5^{c}	0.24	0.01
L. plantarum (CFU g^{-1})	-	1.00×10^{4}	7.20×10^{6}	1.00×10^{4}		
S. cerevisiae (CFU g^{-1})	-	2.80×10^{4}	2.70×10^{4}	1.40×10^{5}		
P. aeruginosa (CFU g^{-1})	-	5.00×10^{6}	-	4.20×10^{5}		
A. baumannii (CFU g^{-1})	-	-	2.60×10^{5}	2.30×10^{4}		

NDF: Neutral detergent fiber, ADF: Acid detergent fiber, A: Complete feed block without microbe, B: Complete feed block containing *L. plantarum*, *S. cerevisiae* and *P. aeruginosa*, C: Complete feed block containing *L. plantarum*, *S. cerevisiae* and *A. baumannii*, D: Complete feed block containing *L. plantarum*, *S. cerevisiae*, *P. aeruginosa* and *A. baumannii*. SEM: Standard error of mean, Mean values with different superscript letters within the same row are significantly different ($p<0.01$)

addition of cellulolytic bacteria into the feed block. Oyeleke and Okusanmi[5] reported that *Pseudomonas aeruginosa* was found in the rumen of cow, sheep and goat and was able to hydrolyse cellulose. El-Sheikh[6] and Chang *et al.*[7] noted that *Acinetobacter* sp. was found in the rumen of Korean native cattle and it was belonged to cellulolytic bacteria. Additionally, Van Soest[17] stated that microbial enzymes can reduce the levels of NDF.

The average DM content in all complete feed blocks was 77.9%, which were similar to complete feed based on palm oil fronds[18]. The OM content in all complete feeds blocks in the experiment varied from 90.1-91.5% which were similar to the previous study[19]. The average CP content was 11.6%, which is above the threshold value of 7%. Minson and Milford[20] revealed that the digestibility declines when the animals are fed herbage with a CP content below 7% because microbial activity in the rumen becomes depressed by the lack of nitrogen.

The population of microbes in the complete feed block were 10^{4}-10^{6} CFU g^{-1} of *L. plantarum*, 10^{4}-10^{5} CFU g^{-1} of *S. cerevisiae*, 10^{5}-10^{6} CFU g^{-1} of *P. aeruginosa* and 10^{4}-10^{5} CFU g^{-1} of *A. baumannii*. The population of *S. cerevisiae* in the feed feed block was lower than *S. cerevisiae* in the concentrate as previously reported by Santoso *et al.*[8]. Lower population of *S. cerevisiae* in the feed block could be due to limited available fermentable carbohydrate.

In the present study, the colour and texture of feed blocks were not changed and the mould was not grown during 3 months of storage. This finding was comparable with those of Samanta *et al.*[19] who concluded there was no visible change in colour, texture and no mould growth was noticed during 6 months of storage.

***In vitro* rumen fermentation characteristics:** The pH value, concentrations of NH_3-N and individual and total VFA are shown in Table 3. The pH value was similar ($p>0.05$) among the four feed blocks. By contrast, Santoso *et al.*[8] noted that addition of Lactic Acid Bacteria (LAB), yeast and cellulolytic bacteria in concentrate was changed pH value in the *in vitro* fermentation. Average pH value in the medium for all feed treatments ranged from 6.94 to 6.96 which were in the optimal pH range of 6.7 ± 0.5 required to maintain normal cellolysis[17] and were above 6.0, which required for microbial protein synthesis[21].

In the rumen protein and other nitrogenous compounds are broken down into ammonia and peptides. The ammonia is used by the microbes for their protein synthesis. For normal microbial activity 5-7 mg NH_3-N/100 mL rumen liquor is required[22] although later experiments show that 5-8 mg/100 mL rumen liquor could be sufficient for fiber digestion[23]. Addition of mixed microbes had no significant effect ($p>0.05$) on concentration of NH_3-N. However, in the present experiment, the ammonia N in all treatments of feed block was sufficient to ensure optimum microbial growth and fiber digestion.

The addition of LAB, yeast and cellulolytic bacteria in feed block D resulted in higher ($p<0.01$) production of acetic acid, propionic acid, butyric acid and total VFA than the feed block A (control). Generally, as cellulose and hemicellulose levels increase, relative to the amounts of soluble carbohydrate and starch levels, the acetate: propionate ratio also increases as a function of both increased acetate and decreased propionate[24]. Moreover, Dijkstra[25] concluded that fermentation of structural carbohydrates, compared to fermentation of starch, yield high amounts of acetic acid and low amounts of propionic acid.

Table 3: *In vitro* fermentation characteristics in the supernatant after 48 h of incubation

	Feed blocks					
Variables	A	B	C	D	SEM	p-value
pH	6.94	6.96	6.96	6.96	0.01	0.72
NH_3-N (mM)	33.50	34.60	33.00	33.60	0.92	0.69
Acetic acid (A) (mM)	58.70^{c}	64.20^{bc}	68.30^{ab}	73.10^{a}	1.23	0.01
Propionic acid (P) (mM)	21.60^{b}	23.90^{ab}	25.60^{a}	25.60^{a}	0.55	0.01
Butyric acid (mM)	9.80^{c}	9.80^{c}	12.30^{b}	14.70^{a}	0.31	0.01
A : P	2.70	2.70	2.70	2.80	0.09	0.23
Total VFA (mM)	90.30^{d}	97.90^{c}	106.10^{b}	113.30^{a}	1.26	0.01
Total gas 48 h (mL)	59.30^{c}	61.40^{b}	63.60^{b}	68.20^{a}	0.50	0.01

A: Complete feed block without microbe, B: Complete feed block containing *L. plantarum*, *S. cerevisiae* and *P. aeruginosa*, C: Complete feed block containing *L. plantarum*, *S. cerevisiae* and *A. baumannii*, D: Complete feed block containing *L. plantarum*, *S. cerevisiae*, *P. aeruginosa* and *A. baumannii*. VFA: Volatile fatty acids. SEM: Standard error of mean, Mean values with different superscript letters within the same row are significantly different (p<0.01)

Table 4: *In vitro* digestibility of dry matter, organic matter and neutral detergent fiber of the complete feed block

	Feed blocks					
Variables	A	B	C	D	SEM	p-value
IVDMD (%)	51.7^{c}	54.4^{b}	55.6^{b}	58.2^{a}	0.50	0.01
IVOMD (%)	64.6^{c}	66.8^{b}	67.5^{b}	70.3^{a}	0.41	0.01
IVNDFD (%)	31.4^{c}	35.5^{b}	35.3^{b}	38.3^{a}	0.29	0.01

A: Complete feed block without microbe, B: Complete feed block containing *L. plantarum*, *S. cerevisiae* and *P. aeruginosa*, C: Complete feed block containing *L. plantarum*, *S. cerevisiae* and *A. baumannii*, D: Complete feed block containing *L. plantarum*, *S. cerevisiae*, *P. aeruginosa* and *A. baumannii*. IVDMD: *In vitro* dry matter digestibility, IVOMD: *In vitro* organic matter digestibility, IVNDFD: *In vitro* neutral detergent fiber digestibility. Mean values with different superscript letters within the same row are significantly different (p<0.01)

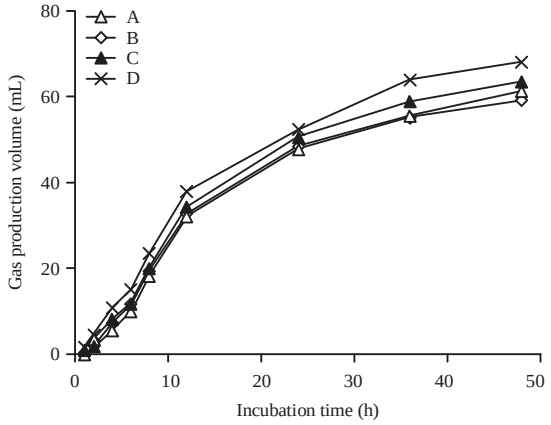

Fig. 1: Pattern of *in vitro* gas production affected by addition of mixed microbes in complete feed block at different incubation time

As a known LAB, *L. plantarum* is expected to increase lactic acid production in feed block B, C and D, which may stimulate the growth of lactate utilizing microorganisms, leading to the production of propionic acid. This finding is consistent with Soriano *et al.*[26] who reported that addition of *Lactobacillus mucosae* in substrate of brewers' grain increased the *in vitro* concentration of propionic acid.

Gas production can be used as an indicator of feed degradation in the rumen. Beuvink and Spoelstra[27] noted that there was a significant correlation between OM digestibility, VFA concentrations and gas production. Table 3 shows that the feed block enriched with LAB, yeast and cellulolytic bacteria (D) had the highest (p<0.01) total gas production during a 48 h compared to other feed block. These results concur with Tang *et al.*[28] who reported an increased rate of gas production and cumulative gas volume for cereal straws by addition of yeast culture. Yeast supplementation to low quality basal forages promoted the growth of fibrolytic bacteria through its ability to scavenge oxygen and production of metabolites such as peptides, amino acids and branched-chain organic acids in the rumen[29]. Besharati[30] concluded that addition of *S. cerevisiae* at 2.5 g kg^{-1} DM in biscuit waste improved *in vitro* gas production parameters. The ability of yeast to increase *in vitro* gas production has been also reported by various authors with different roughages[31,32]. Pattern on *in vitro* gas production affected by addition of mixed microbes in complete feed block at different incubation times are shown in Fig. 1.

Dry matter, organic matter and neutral detergent fiber digestibility: Combination of lactic acid bacteria, yeast and cellulolytic bacteria in complete feed block significantly (p<0.01) increased *in vitro* DM, OM and NDF digestibility (Table 4). Complete feed block with two species of cellulolytic bacteria such as *P. aeruginosa* and *A. baumannii* had the

highest DM, OM and NDF digestibility compared to other feed block. The higher digestibility of NDF could be due to addition of cellulolytic bacteria into the feed block. Oyeleke and Okusanmi[5] revealed that *P. aeruginosa* was found in the rumen of cow, sheep and cow approximately 9% of the bacteria species and was able to hydrolyse cellulose. Santoso *et al*.[8] reported that concentrate containing *L. plantarum*, *S. cerevisiae* and *A. baumannii* had the highest *in vitro* digestibility of dry matter, organic matter and neutral detergent fiber as compared to other concentrate. Similar result has been reported by Lila *et al*.[33] that the addition of *S. cerevisiae* increased *in vitro* dry matter degradability. Zain *et al*.[34] concluded that the addition of some microbes as probiotics in feed could stimulate the microbes of the rumen and improve the digestibility of feed in ruminant livestock. Moreover, Chaucheyras *et al*.[35] noted that *S. cerevisiae* showed an ability to provide growth factors, such as organic acids or vitamins, thereby stimulating ruminal populations of cellulolytic bacteria.

CONCLUSION

Results indicate that combination of LAB, yeast and cellulolytic bacteria reduced NDF and ADF contents in complete feed block, thereby increased *in vitro* digestibility of DM, OM and NDF as compared to control feed block. The complete feed block containing LAB, yeast and cellulolytic bacteria was effective in modifying ruminal fermentation patterns by increasing concentrations of acetic acid, propionic acid, butyric acid and total VFA.

SIGNIFICANCE STATEMENTS

- In this study nutritive value, *in vitro* fermentation characteristics and nutrient digestibility of complete feed block enriched by mixed microbes were evaluated
- Combination of LAB, yeast and cellulolytic bacteria in complete feed block had the lowest NDF and ADF contents and the highest concentrations of acetic acid, propionic acid, butyric acid, total VFA as well as digestibility of DM, OM and NDF
- The process of feed block making from low bulk density roughage resources may has advantage of reducing the storage space and transportation cost

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