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The Effect of Probiotics on High Fiber Diet in Rumen Fermentation Characteristics

N F Sari*, R Ridwan, Rohmatussolihat, R Fidriyanto, W D Astuti and Y Widyastuti

Research Center for Biotechnology, Indonesian Institute of Sciences, Jl Raya Bogor km 46, Cibinong 16911, Indonesia

*e-mail: nurulfitrisari@gmail.com

Abstract. The productivity of cattle must be achieved through feed efficiency which has strong correlation with the rumen work. The efforts to increase productivity of cattle can use probiotics as feed additive in animal feeding. Probiotics are increasingly used in commercial animal production operations to profitably alter the gastrointestinal flora, thus improving animal health and productivity. This study aimed to investigate the effects of different type of probiotics on high fiber diet in live weight change, rumen fermentation, the total rumen of bacteria, and the total of lactic acid bacteria. Three fistulated cattle received a daily ration 2% from body weight (DM) of *Pennisetum purpureum* (70% of DM), concentrates (30% of DM), and mineral mix (1% of DM). The experiment was arranged as a crossover design with six treatments and three sampling periods as replications. The treatments were (R0) control diet, (R1) control diet + 10 mL of *Lactobacillus plantarum* TSD-10 per day, (R2) control diet + 10 mL of *L. plantarum* MX-16 per day, (R3) control diet + 10 mL of *L. brevis* SPCE-39 per day, (R4) control diet + 10 mL of mix *Lactobacillus plantarum* TSD-10, *L. plantarum* MX-16, and *L. brevis* SPCE-39 per day, (R5) control diet + 10 mL of commercial probiotics per day. Neither pH nor the total of lactic acid bacteria (LAB) were significantly affected by the treatment. Among others treatment, R3 showed a tendency body weight gain. The total of rumen bacteria was affected significantly for R2, R3, R4, and R5 compared with R0. The acetate to propionate ratio were affected for R3, R4, and R5, but were not affected significantly for R2 and R3 compared with R0. The butyrate ratio and rumen ammonia concentration were not affected by the treatment. In conclusion, probiotics change the population of the rumen bacteria. In addition, based on metabolism characteristics, particularly on propionate production and C2/C3 ratio, R3 was shown the best probiotics candidate on high fiber diet. Nevertheless, the difference pattern in volatile fatty acid indicates that different mechanisms may be involved.

Keywords: probiotics, cattle, lactic acid bacteria, rumen fermentation

1. Introduction

The growth number of the human population has increased the need to improve the productivity of the cattle. For this reason, most efforts in the field of animal production, have been directed to raise the productivity of ruminant using biotechnology approach to manipulate the ruminal ecosystem [1]. The use of probiotics can manipulate the process of rumen fermentation to maximize the production efficiency while decreasing energy loss [2]. This appears to be strongly driven by the ban of most of

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the antibiotic feed additives within the European Union, because of speculated risk for generating antibiotic resistance in pathogenic microbiota [3], chemical residues in animal products, release of antibiotics into the environment [4;5] and also the advent of present-day organic agriculture.

Probiotics, in general as reported by different authors, have the ability to enhance intestinal health by stimulating the development of a healthy microbial ecosystem [6;7], increase digestive capacity and their bio-availability [8], prevent enteric pathogens from colonizing the intestine [9], restore the gut microflora [7], lower pH, and improve mucosal immunity and nutrient absorption [10;6]. As a result, they should increase productivity and the general health of ruminants.

The ruminants highly rely on a symbiosis between the host and the rumen microbes, with the microorganisms supplying protein, vitamins and short-chain organic acids for the animal host [11]. Most of the energy in the ruminants are derived from microbial origins, such as the absorption of energy, the formation of glucose in the liver and the digestion of protein in the abomasum. In ruminants that function normally, in fact, as much as 90% of the protein that reaches the small intestine and up to 50% of the host's energy needs is provided by reticulorumen microbial cells [12]. Microbial communities are involved in the digestion and fermentation of plant polymers, which are very important in adult herbivores [6].

The rumen is a complicated ecosystem where nutrients consumed by the microorganisms for example bacteria, protozoa, and fungi are digested anaerobically. The main end products of fermentation are volatile fatty acids (VFA) and microbial biomass, which are used by ruminants. The interaction between microorganisms and host animals produces a symbiotic relationship that allows ruminants to digest fiber-rich and low-protein feeds. In the rumen, the environment supports microorganisms to provide the enzymes needed to digest nutrients. Ruminants have the ability to convert low-quality fibrous ingredients into products such as meat, milk, and fiber, which are useful for humans. The ability of rumen microorganisms to produce enzymes needed for the fermentation process allows ruminant animals to obtain energy contained in forages efficiently [13]. However, the rumen fermentation process is not entirely efficient because it produces several end products such as methane gas [14]. A symbiotic relationships exist in the rumen which provides the environment necessary for the formation of microorganisms and substrates needed for their maintenance. In turn, microorganisms provide nutrients to the ruminant host to produce energy [15]. The objective of this study was to investigate the effects of probiotics on a high fiber diet in rumen fermentation characteristics.

2. Material and Methods

2.1. Animal Care

Feeding trials were carried out using three fistulated Ongole cattle. The use of the cattle in this experiment was approved No. 9879/WK/HK/XI/2015 by the Ethics Clearance Committee of Indonesian Institute of Sciences.

2.2. Preparation of Probiotic Candidates

Lactobacillus plantarum TSD-10, *L. plantarum* MX-16, *L. brevis* SPCE-19 were chosen as probiotic candidates. The probiotic candidates from previous study were grown on the specific probiotic medium. The MRS is used as specific probiotic medium for the production of lactic acid bacteria [6]. The quality of candidate probiotics is done by calculating the number of colonies using TPC method.

2.3. Diet and Feeding

This experiment was used a high fiber feed formulation with a proportion of *Pennisetum purpureum* (70% of DM), concentrate (30% of DM), and mineral mix (1% of DM). *Pennisetum purpureum* were provided by the plant collection of the Research Center for Biotechnology at Indonesian Institute of Sciences. Concentrate was made from the following formulation: 30% of rice bran, 13% of pollard bran, 2% of soybean meal, 15% of coconut milk, 10% of palm kernel mill, 7% of coffee pulp, 20% of cassava flour waste, 2% of molasses, and 1% of mineral mix.

The experiment was arranged as a crossover design with six treatments and three sampling periods as replications. The treatments were :
R0: control diet
R1: control diet + 10 mL of *Lactobacillus plantarum* TSD-10 per day
R2: control diet + 10 mL of *L. plantarum* MX-16 per day
R3: control diet + 10 mL of *L. brevis* SPCE-39 per day
R4: control diet + 10 mL of mix among *Lactobacillus plantarum* TSD-10, *L. plantarum* MX-16, and *L. brevis* SPCE-39 per day
R5: control diet + 10 mL of commercial probiotics per day.

Each treatment was administered for 17 days, and rumen samples were collected on days 7, 12, and 17 as replication sampling periods. All cattle were given amounts of feed equal to 2% dry matter of their body weight (360 kg). The Nutrient and chemical composition of diets are shown in Table 1.

2.4. Chemical and pH analysis

Samples of P.purpureum and fermented concentrate were analyzed for dry matter (DM) and ash according to AOAC [17]. Crude protein (CP), crude fiber (CF), ether extract (EE) were determined followed the manufacturing procedure of FOSS. The NH_3-N concentration was determined by a Conway micro-diffusion method. Volatile Fatty Acids (VFA) were determined in centrifuged samples (1mL) by Gas Chromatography (GC) as described by Goering and Van Soest [18]. pH measurement of mix buffer rumen fluids was measured using pH meter (Jenway Model 3505, UK). Prior to the measurements, pH meter was calibrated by using pH 7 buffer solution and it was prepared carefully as the manufacturers instructions. All chemical composition analyses were conducted in triplicate.

2.5. Microbiology analysis

Total lactic acid bacteria and rumen bacteria were counted as colony forming units (CFU) per mL. The lactic acid bacteria were grown on MRS in agar medium and counted by using total plate count [19], while the rumen bacteria on 98-5 agar medium were grown anaerobically by following the method of roll tube [20]. Incubation was done at 38^0C for 3-4 days.

2.6. Statistical analysis

The experiment design was a block randomized. The statistical analysis of data was performed, using SPSS 16 for Windows. The difference among groups was determined by one-way ANOVA analysis. Significance was defined at $P<0.05$. Microbial diversity data were analyzed descriptively.

3. Results and discussions

3.1. Probiotics Production

Probiotics have been defined by the FAO/WHO as live microorganisms which in the appropriate amounts will give the health benefit on the host. In addition, it has specificity to the host, able to survive in the preparation process and the storage period can live and play in the target organ, must be consistently controlled for the observed response and can eliminate pathogenic microbes and optimize the immune system response of host [21;6]. Some of lactic acid bacteria (LAB) strains species belong to genera *Lactobacillus*, which are considered as a beneficial effect to the host. Therefore, we used three strains of *Lactobacillus plantarum* collected from biotechnology culture collection, Indonesian Institute of Sciences as candidate probiotics.

Table 1. The total of LAB Population during in vivo

Treatment	Strain	LAB Population (CFU/ml)
R0	Control	
R1	*L. plantarum* TSD-10	3.4×10^8
R2	*L. plantarum* MX-16	2.8×10^8
R3	*L. brevis* SPCE-39	7.8×10^7
R4	Mix R1-R3	5.9×10^8
R5	Commercial probiotics	1.0×10^6

The total population of probiotics candidate was shown in Table 1 which range from 1,0 x 10^6 – 5.9 x 10^8 CFU/ml. The volume of probiotics used in all treatments in PO fistulated cattle was 10 ml/head/day. This probiotic assumption is an early study related to survival or probiotic survival in rumen in vivo conditions.

3.2. Chemical and pH analysis

Chemical analysis of feed material is very important to evaluate the nutrient content of feed. proximate analysis is one of parameter to determine the quality of feed material. The nutrition composition of feed material that used in this study is shown in Table 2.

Table 2. Chemical composition (g/kg DM)

Item[a]	(%) DM	(%) ASH	(%) OM	(%) CF	(%) EE	(%) CP
Pennisetum purpureum	87.94	17.49	82.51	32.28	2.02	10.17
Concentrate	92.19	12.17	87.83	20.46	3.56	13.93
TMR (30% C, 70 *Pennisetum purpureum*)		15.89	84.11	28.74	2.48	12.10

DM= dry matter; OM = organic matter; CF= crude fiber; EE= ether extract ; CP = crude protein; TMR = total mix ratio aData are the means of three replicate analysis.

The pH rumen indicates rumen conditions that are appropriate or inappropriate for the growth of rumen microbes. This study showed that the use of different types of probiotics did not affect the pH of rumen (Table 3). The pH of the rumen is still within the normal range of 6.24 -6.58. The same pH values as control showed that the use of types of probiotics does not interfere rumen fermentation activity. According to Dehority [22], the rumen's normal pH is about 5.5-7.0. The pH value represents the amount of acid produced by microbial activity in the rumen [23;24]. The lowest pH value is generally achieved about 2-6 hours after meals, in accordance with the maximum acid production. Changes in pH are affected by time after meal, lactic acid supply, feed properties, and feeding frequency of animal [22;25].

3.3. The Total Rumen Bacteria and LAB

Provision of probiotics in fistulated cattle as a model of experimental cattle tends to show a positive effect when given on a high fiber formulation. This can be seen from the C_2/C_3 ratio R3, R4, and R5 has the lowest value compared to other treatment which can lead to increase the productivity.

The rumen microbiological profile of the probiotics treatment showed that R2, R3, R4, and R5 had significantly different from R0 and R1 (Table 3). However, the probiotics did not affect the total of LAB among all treatment. This can be explained; although the total LAB counts did not change, it does not mean that the existing population did not change. The total LAB counts might be same, but the LAB population might be different.

Table 3. The comparison among body weight, pH total rumen bacteria, and total lactic acid bacteria during in vivo in each treatment

Treatment	Body Weight	pH	The Total of Rumen Bacteria (Log 10 CFU/ml)	The Total of LAB (Log 10 CFU/ml)
R0	363.33	6.51	8.29a	7.23
R1	362.00	6.24	7.39a	6.33
R2	360.67	6.32	6.84b	6.48
R3	365.78	6.43	7.36b	6.84
R4	360.11	6.58	7.27b	6.84
R5	362.83	6.38	7.36b	6.89

LAB = lactic acid bacteria; R0; Control, R1; *L. plantarum* TSD-10, R2; *L. plantarum* MX-16, R3; *L. brevis* SPCE-39, R4; Mix R1-R3, R5; Commercial Probiotics

3.4. NH_3 and VFA Production

Ammonia is an important component for the synthesis of amino acids and microbial cell proteins. Protein from feed will be degraded by proteolytic bacteria with the help of protease enzyme into peptide then hydrolyzed to amino acid, and amino acid will go through deamination process to become ammonia. According to McDonald et al. [26] states that ammonia is produced in conjunction with several small peptides and free amino acids, then it is utilized by rumen organisms for microbial protein synthesis. In addition, some of the amino acids will be further digested into organic acids, ammonia (NH_3), and carbon dioxide (CO_2). The results of the average concentration of NH_3 are presented in Table 4. This study showed the effect of probiotics on high fiber feed formulation on N-NH_3 production gave no significant difference for all treatment of probiotic and control. This showed that rumen microbial activity tends to be stable in utilizing source N on high fiber formulations.

The effect of probiotics on high fiber feed formulation leads to the formation of high production of acetic acid. The acetate to propionate ratio was affected by R3, R4, and R5, but was not affected significantly for R2 and R3 compared with R0. The butyrate ratio and rumen ammonia concentration were not affected by the treatment. The ratio of C2/C3 shows a pattern of microbial fermentation in the rumen that has the potential to form acetic acid or propionate. The lowest C2/C3 ratio is produced by R3, R4, and R5 as shown in Table 4. The low of C2/C3 ratio is due to the high amount of fermentable organic matter enabling the formation of higher propionic acid than acetic acid. However, it is recommended to study the molecular approach to identify the rumen population. As reported by Moran [27] that the fermentative digestion of carbohydrates especially starch will produce lactic acid, this lactic acid will be converted to propionic acid by microbial lactate utilization, such as *Propionibacteria* sp*, Veillonella alkalescens,* and *Peptostreptococcus elsdeini*. The low of C2/C3 ratio will stimulate fattening and tend to body fat formation. In the treatment of R0, R1, and R2 showed higher C2/C3 ratio values than other treatments; this indicates that the microbial fermentation pattern leads to the formation of acetic acid because the carbohydrates contained in the feed are still dominated by fibrous carbohydrates and microbes that grow well are cellulolytic and hemicellulolytic microbes.

Table 4. Production of Volatile Fatty Acid (% mM) and NH_3 (mM) During Each Treatment

Variable	Treatment					
	R0	R1	R2	R3	R4	R5
C_2	63.72^{a}	62.85^{a}	66.99^{a}	56.40^{b}	56.71^{b}	57.46^{b}
C_3	21.15^{b}	20.61^{b}	18.95^{b}	25.27^{a}	23.70^{a}	25.21^{a}
C_4	10.20	11.05	9.89	10.90	11.95	10.12
Iso-C_4	2.90^{c}	3.19^{c}	2.50^{c}	4.27^{a}	4.34^{a}	3.77^{ab}
C_5	0.71^{ab}	$0.90a^{b}$	0.61^{b}	0.98^{ab}	1.00^{ab}	1.15^{a}
Iso-C_5	1.32^{b}	1.40^{b}	1.07^{b}	2.19^{a}	2.30^{a}	2.30^{a}
C_2/C_3	3.01^{a}	3.05^{a}	3.54^{a}	2.23^{b}	2.39^{b}	2.28^{b}
NH_3	10.77	9.68	10.32	10.86	10.01	9.03

R0; Control, R1; *L. plantarum* TSD-10, R2; *L. plantarum* MX-16, R3; *L. brevis* SPCE-39, R4; Mix R1-R3, R5; Commercial Probiotics ; C_2 = acetate ; C_3 = propionate ; C_4 = butyrate ; Iso-C_4 = Iso butyrate ; C_5 = valeric acid ; Iso-C_5 = Iso-valeric acid ; NH_3 = ammonia ; $^{a-c}$Different superscripts within the same column are significantly different at $P<0.05$

4. Conclusion

Based on this study, it could be concluded that probiotics in high fiber diet can change the population of the rumen bacteria. In addition, based on metabolism characteristics, particularly on propionate production and C2/C3 ratio, R3 was shown the best probiotics candidate on high fiber diet. Nevertheless, the difference pattern in volatile fatty acid indicates that different mechanisms may be involved.

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