

Screening of Probiotic Candidates from Rumen Bacteria Isolates for Improving Rumen Fermentation and Feed Digestibility

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Abstract – Gas production is the result of microbial activity in the rumen. Amount of gas production is an indication of digestible organic material. This experiment was aimed to determine the effect of rumen bacteria isolates on kinetics and fermentation indicators of a cattle diet (70:30 forage: concentrate ratio) to be candidate of probiotic. The *in vitro* gas production technique was applied. Samples were incubated at 39^o C for analysis gas fermentation products at 2, 4, 6, 8, 10, 12, 24, 48 and 72 h of incubation period. Aproximately 0.75 g of the diet was mixed with 75 ml of buffered rumen liquor and with one of forty seven rumen bacteria isolates. The rumen content was sampled through the fistula from three different cattles before morning feeding. Asymptotic gas production and kinetics were estimated by the Orskov's model. A randomized block design with three replicates was used. The results showed that some rumen bacteria isolates addition increased gas production, i.e. isolates number 23, 88, 93, 99 and 149. These isolates are promising to be selected as probiotic candidates for improving fermentation and feed digestibility in the rumen.

1. INTRODUCTION

A key role in ruminant nutrition is rumen fermentation processes by rumen micro-organisms. Gas production is the result of microbial activity in the rumen. Amount of gas production is an indication of digestible organic material. This experiment was aimed to determine the effect of rumen bacteria isolates on kinetics and fermentation indicators of a cattle diet (70:30 forage: concentrate ratio) to be candidates of probiotic.

2. METHODS

2.1 Chemicals

The chemicals of this research are rumen fluid, diet (70:30 forage : concentrate ratio), rumen bacteria isolates, culture media 98-5 (glucose, cellobiose, soluble starch 25%, yeast extract, maltose, trypticase peptone, mineral solution I, mineral solution II, resazurin 0.1 solution, rumen fluid, gliserol, cystein-ascorbic acid, Na₂CO₃ 8% and destiled water) buffer (NaHCO₃, Na₂HPO₄, KCl, NaCl, MgSO₄, CaCl₂), CO₂, destiled water.

2.2 Procedures

Rumen bacteria isolates were selected from rumen of cattle with high concentrate diet (30:70 forage : concetrare ratio). Cultivasi of bacteria use 98-5 medium, culturing rumen bacteria in anaerobic media. 100 ml 98-5 medium was contained glucose 0.05 g, cellobiose 0.05 g, soluble starch (25%) 0.05 g, yeast extract 0.05 g, maltose 0.03 g, trypticase peptone 0.2 g, mineral solution I 7.5 ml, mineral solution II 7.5 ml, resazurin (0.1 solution) 0.1 ml, rumen fluid 40 ml, gliserol 0,03 ml cystein-ascorbic acid 0.5 ml, Na₂CO₃ (8%) and distilled water 50 ml. Forty seven rumen bacteria isolates were selected from fifty five isolates. Rumen bacteria isolates were incubated for three days.

In vitro incubation of the diet was performed using metode by Theodore (1994). Diet sample was prepared by forage 70% (king grass (*Pennisetum purpureum*)) and concentrate 30% (cassava waste, soybean meal, CaCO₃, premix and NaCl) (table 1). King grass was chopped and dried until moisture content reached 12%. Then king grass was ground and sieved into 1 mm particle.

Aproximately 0,75 g of the diet was mixed with 75 ml of buffer and rumen liquor and with one of forty seven rumen bacteria isolates in 100 ml serum bottle. The rumen fluid (including solid material) was sampled through the fistula from three different cattles before morning feeding and filtered by passing through a nylon net. Samples were incubated in water bath at 39^o C for analysis gas fermentation product at 2, 4, 6, 8, 10, 12, 24, 48 and 72 h of incubation period. Asymtotic gas production and kinetics were estimated by the Orskov's model (gas = b. (1-exp^{-c.t})).

Treatments were arranged on randomized block design consisted of forty seven treatments (rumen bacteria isolates). Each treatment was conducted in three replications. Variables measured were *in vitro* total gas production and rate gas production. All data were analyzed by using SPSS software version 16.0.

Table 1 Composition of diet used in the experiment

Ingredients	Composition(%)
King grass	70
Cassava waste	15
Soybean meal	13.80
CaCO ₃	0.20
Premix	0.50
NaCl	0.50

3. RESULTS AND DISCUSSION

Rumen bacteria isolates addition increased gas production (Table 2 and Figure 1). It indicated that rumen bacteria isolates presence contributed to support ruminal microbes activity in producing of gas. Getachew *et al* [2] said the gas measuring technique has been widely used for evaluation of nutritive value of feed. Das and Qin in [1] said *in vitro* gas production is the indicator of digestibility and nutritive value of feed stuffs. The results showed that some rumen bacteria isolates addition increased gas production, i.e isolates number 23, 88, 93, 99 and 149 had the highest total gas production and rate gas production volume among treatments. These isolates are promising to be selected as probiotic candidates for improving fermentation and feed digestibility in the rumen.

Table 2 *In Vitro* gas production kinetics of various rumen bacteria isolates

No	Isolates	b	c	No	Isolates	b	c	No	Isolates	b	c
1	Blanko	68.18	0.007	18	50	183.42	0.048	35	94	188.56	0.046
2	Control	179.39	0.044	19	53	184.18	0.049	36	96	184.77	0.049
3	11	184.26	0.053	20	54	181.37	0.055	37	97	181.86	0.051
4	12	177.63	0.048	21	59	184.10	0.047	38	99	186.01	0.051
5	15	185.14	0.05	22	60	182.90	0.048	39	148	183.79	0.045
6	16.A	182.97	0.048	23	71	183.61	0.047	40	149	185.44	0.049
7	16.B	184.12	0.051	24	72	183.58	0.049	41	150	176.15	0.052
8	17	181.68	0.052	25	77	180.06	0.053	42	153	183.87	0.051
9	23	186.76	0.05	26	78	183.04	0.044	43	158	184.47	0.051
10	25	180.87	0.045	27	80	180.67	0.054	44	162	184.29	0.05
11	26	183.18	0.048	28	81	183.57	0.049	45	164	183.97	0.045
12	31	182.42	0.053	29	82	181.44	0.052	46	165	181.17	0.051
13	32	179.39	0.055	30	85	182.39	0.046	47	170	184.57	0.05
14	35	185.12	0.048	31	87	181.84	0.048	48	174	182.92	0.047
15	42	185.53	0.048	32	88	186.31	0.049	49	176	183.00	0.051
16	48	182.84	0.046	33	92	181.55	0.053				
17	49	184.01	0.05	34	93	185.73	0.05				

b (total gas production), c (rate of gas production)

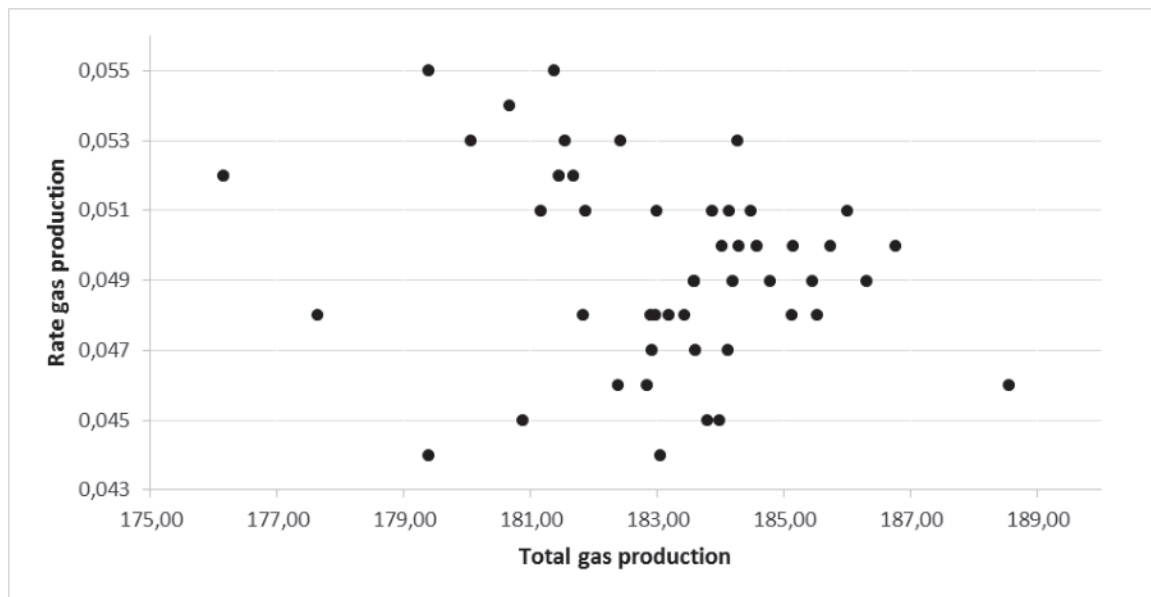


Figure 1 *In vitro* gas production kinetics of various rumen bacteria isolates

The result of fermentation of carbohydrates to acetate, propionat and butyrate, protein fermentation and fat fermentation are basically of gas production [2]. Digestibility is measure of gas production, decreasing of gas production that is mean decreasing of digestibility. Tanin was reduce gas production by obstructing degradation of protein and crude fiber [3].

4. CONCLUSIONS

It was concluded that *in vitro* total gas production and rate gas production of rumen bacteria isolates were increased. Rumen bacteria isolates had potential of improving rumen fermentation characteristics and probiotic candidates.

5. REFERENCES

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