IN VITRO GAS PRODUCTION OF DIETS WITH INCLUSION OF SEAWEED (*LITHOTHAMNIUM* SP.) FLOUR FOR WHITE NEW ZEALAND RABBITS

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ABSTRACT

Thirty White New Zealand rabbits were fed a standard fattening diet with the following composition: crude protein 16.5%, ADF 17.5%, and digestible energy 2568 kcal/kg. This diet was fed *ad libitum* since weaning. The reference diet was used as a control for comparison of diets with increasing levels (0.25, 0.50, 0.75 and 1.0%) of seaweed (*Lithothamnium* sp.) flour. The seaweed flour replaced an equivalent amount of inert aluminium veterinary phyllosilicate in the reference diet. The aim of the present work was to investigate the relationship between increasing levels of seaweed flour, degradability of dry and organic matter, and fermentation gas production using the caecal content from rabbits as a source of inoculum. The completely randomized design was used with five treatments and six repetitions. The means were compared by Scott-Knott test at a probability level of 5%. Caecal inocula from rabbits fed with higher level of seaweed flour produced more gas. This result shows that seaweed flour could influence caecal microbiota, but this was not confirmed by degradability of dietary organic matter and dry matter.

Key-words: Seaweed, Additives, Feed, Fermentation, Gas production, Nutrition.

INTRODUCTION

Lithothamnium sp. is known as seaweeds, which are very abundant in certain cold and temperate seas. One possible reason for the activity of this material is the availability of mineral elements, particularly calcium carbonate and magnesium carbonate. These support physiological functions of the body in various ways, for example: constituents of skeletal structure, regulation of physical properties of colloidal systems (viscosity, diffusion, osmotic pressure), acid-base regulators, and components and activators of enzymes and other active biological systems.

In the rabbit, the caecum is the largest digestive compartment (40% of the whole digestive tract), and represents an organ for fermentation. It is colonized by an abundant bacterial flora. *In vivo* digestibility experiments are expensive, time-consuming and require large amounts of feed. Therefore, it is of great importance to develop new methods to estimate the nutritive value of feeds in an effortless and less costly way, as alternatives to *in vivo* trials. The cumulative gas production technique (GPT) was used to evaluate the nutritive value of ruminant feeds according to their fermentation kinetics (Pell and Schofield, 1993; Cone *et al.*, 1994; Theodorou *et al*., 1994). This technique has been applied successfully also in other animal species, for example in horses (Macheboeuf *et al.*, 1997), chickens (Kwakkel *et al*., 1997), and rabbits (Calabrò *et al*., 1999, Calabrò *et al*., 2000, Ferreira, 2003). The measurements of gas produced at certain intervals during incubation supply very detail information about the extent of microbial degradation and fermentation kinetics of a feedstuff. Therefore, they represent an option to the studies of feed characteristics in the animal nutrition (Getachew *et al*., 1998).

The aim of the present research was to show the relationship between increasing levels of seaweed flour, degradability of dietary dry and organic matter, and fermentation gas production using the caecal content from rabbits as a source of inoculum.

MATERIALS AND METHODS

Thirty New Zealand White rabbits (both sexes) of the experimental strain of the Veterinary School at Federal University, Minas Gerais, Brazil were used. Animals were fed a standard fattening diet, in accordance with recommendation of De Blas and Mateos (1998). See Tables 1, 2 and 3 for diets composition. Diets were always fed *ad libitum* since weaning. The reference diet was used for comparison between diets with increasing levels (0.25, 0.5, 0.75, 1.0%) of seaweed (*Lithothamnium* sp.) flour. The seaweed flour replaced an equivalent amount of inert aluminium veterinary phyllosilicate in the reference diet. All the diets were pelletized to have the shape of granules 12-15 mm long and 4-5 mm of diameter. Five inocula were prepared with caecal contents of each treatment group (reference diet, 0.25, 0.50, 0.75, 1.0% of *Lithothamnium* sp.). Inocula were prepared by mixing the caecal contents of six New Zealand White rabbits. The caeca were isolated by tying up the two extremities with a nylon string to prevent losses of digesta. The diets offered for rabbits were used as substrates in the *in vitro* fermentation.

Table 1: Ingredient composition of diets (%)

	1-(0.0%)	*2-(0.25%)*	*3-(0.50%)*	*4-(0.75%)*	*5-(1.0%)*
Alfalfa hay	35.09	35.09	35.09	35.09	35.09
Wheat bran	25.00	25.00	25.00	25.00	25.00
Soybean meal	10.53	10.53	10.53	10.53	10.53
Corn meal	6.69	6.69	6.69	6.69	6.69
Ground Ear Corn	15.00	15.00	15.00	15.00	15.00
Soybean oil	1.00	1.00	1.00	1.00	1.00
Dicalcium phosphate	0.57	0.57	0.57	0.57	0.57
Limestone	0.71	0.71	0.71	0.71	0.71
Salt	0.50	0.50	0.50	0.50	0.50
Al-phyllosilicate	2.00	1.75	1.50	1.25	1.00
Lithothamnium sp.	0.00	0.25	0.50	0.75	1.00
Sugarcane molasses	2.00	2.00	2.00	2.00	2.00
DL-methionine	0.11	0.11	0.11	0.11	0.11
L-lysine	0.30	0.30	0.30	0.30	0.30
Mineral and vitamin premix[1]	0.50	0.50	0.50	0.50	0.50

[1]Premix provided per kg diet: vitamin A, 12 000 IU; vitamin D_3, 1 000 IU; vitamin E acetate, 50 mg; vitamin K_3, 2 mg; biotin, 0.1 mg; Fe, 100 mg; Cu, 20 mg; Mn, 50 mg; Co, 2 mg; I, 1 mg; Zn, 100 mg; Se, 0.1 mg; Robenidine, 66 mg

Table 2: Chemical composition of reference diet

Nutrients	%
DM	88.87
CP	16.50
ADF	17.50
Ca	0.90
P	0.60
Lysine	0.74
Methionine + cysteine	0.60
DE (kcal/kg)	2568

Table 3: Mineral profile of *Lithothamnium* sp.

Calcium	34%	Sodium	300 ppm	Arsenic	<1 ppm
Magnesium	2.4%	Manganese	125 ppm	Lead	<1 ppm
Phosphorous	0.08%	Cobalt	6 ppm	Chromium	13 ppm
Potassium	0.10%	Copper	10 ppm	Cadmium	0.2 ppm
Sulphur	0.45%	Zinc	37 ppm	Mercury	<50 ppb
Iron	20 ppm	Selenium	1 ppm	Aluminium	<1 ppm
Boron	16.5 ppm	Molybdenum	<3 ppm	Iodine	160 ppm

Source: Caucareous material (2003)

Three flasks were used per a treatment and flasks containing only caecal liquid and medium (buffer)

like blanks, so the gas produced by caecal contents without substrate was discounted from the total gas production (two control flasks were used per each inoculum tested). Immediately after the slaughter, the caecal liquid was withdrawn manually. In the laboratory, the caecal liquid was filtered, passing by two layers of cotton cloth under CO_2 atmosphere, and kept in water bath at 39ºC. To each flask, 90 ml of medium (Theodorou *et al.*, 1994) and 1 g of substrate were added. Into each flask 10 ml of inoculum was injected using a graduated syringe with needle. As soon as the inoculum was injected, the needle was kept fixed at the top for a while to enable the escape of accidental gas injected. After that, the flasks were hand shaken and placed into a thermostat at 39ºC (time zero). The pressure of gases accumulated in the flasks was measured by means of a pressure transductor connected to a digital gauge which was connected to a computer in order to record the data. The read-outs were done initially every two hours and, after the 12th hour, every three hours until the 24th hour (2nd, 4th, 6th, 8th, 10th, 12th, 15th, 18th and 24th hour). At the end of fermentation, the flasks were removed from the thermostat and put into a refrigerator at 4ºC. The solid and liquid matter of each flask was separated by filtration using a vacuum pump. The dry matter degraded was measured after drying at 100ºC until the constant weight was obtained. The organic matter degraded was measured through the difference after ashing (6 hours/500ºC). The model of Maurício *et al*. (1999) was used to describe the gas production curve using gas production rate (µ), *"lag time"* (L) and potential of gas production (A).

The data of gas production were analyzed by ANOVA employing the SAS software (1990). The means were compared by Scott-Knott's test at the level of 5%, using the entirely random design.

RESULTS AND DISCUSSION

Table 4 and Figure 1 show the non-cumulative gas production. In the early hours (0-4th hr) of fermentation microorganisms are adapting to the new substrate and this can cause variation of results (Ferreira, 2003). This variation could affect gas production measurement (inoculum 4) and degradability of substrate organic matter in early hours of fermentation. In the later phase of fermentation the greatest gas production can be observed from the 10th to the 15th hour of fermentation ($P<0.05$) using the inoculum 5. This result shows that seaweed flour could influence caecal microbiota, but this was not confirmed by degradability of organic matter (OM) and dry matter (DM) ($P>0.05$; see Table 6).

Table 3: Means of non-cumulative gas production (ml) for different inocula. Time courses until the 24th hr of fermentation

Inoculum/time(h)	2 h	4 h	6 h	8 h	10 h	12 h	15 h	18 h	21 h	24 h
1 (reference diet)	12.26 ^{a}	10.74	9.87	8.08	7.19 ^{a}	6.52^{a}	7.68 ^{a}	7.08	5.71	5.34
2 (0.25%)	11.05 ^{a}	10.04	9.69	7.61	6.44 ^{a}	5.86 ^{a}	8.04 ^{a}	8.04	6.12	5.56
3 (0.50%)	11.02 ^{a}	11.14	10.03	7.80	6.83 ^{a}	6.40 ^{a}	7.99 ^{a}	8.64	7.02	6.68
4 (0.75%)	16.00 ^{b}	11.67	9.73	8.11	7.58 ^{a}	6.24 ^{a}	7.02 ^{a}	7.56	6.45	5.99
5 (1.0%)	11.73 ^{a}	10.53	9.64	8.03	9.06 ^{b}	8.59 ^{b}	10.21 ^{b}	8.31	6.14	5.20

Means with different small letters in the same column differ significantly ($P<0.05$)

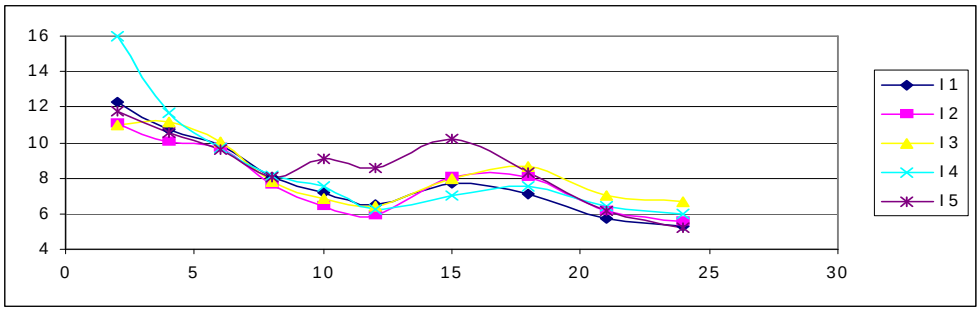

Figure 1: Non-cumulative gas production (ml) for different inocula until 24th hr of fermentation

Table 5 and Graph 2 show cumulative gas production for different inocula. Inoculum 5 had an increased gas production after the 15th hour, followed inoculum 4 after the 18th, and inocolum 3 after the 21st hour. These results showed that some influence of *Lithothanium* sp. on caecal microbiota exists.

Table 5: Means of cumulative gas production for different inocula until the 24th hr of fermentation

Inoculum/Time(h)	2 h	4 h	6 h	8 h	10 h	12 h	15 h	18 h	21 h	24 h
1 (reference diet)	12.26	23.01	32.88	40.96	48.14	54.66	62.34^{a}	69.42^{a}	75.13^{a}	80.46^{a}
2 (0.25%)	11.05	21.09	30.78	38.38	44.82	50.68	58.72^{a}	66.76^{a}	72.87^{a}	78.43^{a}
3 (0.50%)	11.02	22.17	32.20	40.00	46.83	53.23	61.22^{a}	69.86^{a}	76.87^{a}	83.55^{b}
4 (0,75%)	16.00	27.67	37.40	45.50	53.08	59.32	66.34^{a}	73.90^{b}	80.36^{b}	86.35^{b}
5 (1.0%)	11.736	22.27	31.91	39.94	49.00	57.59	67.80^{b}	76.11^{b}	82.25^{b}	87.46^{b}

Means with different small letters in the same column differ significantly (P<0.05)

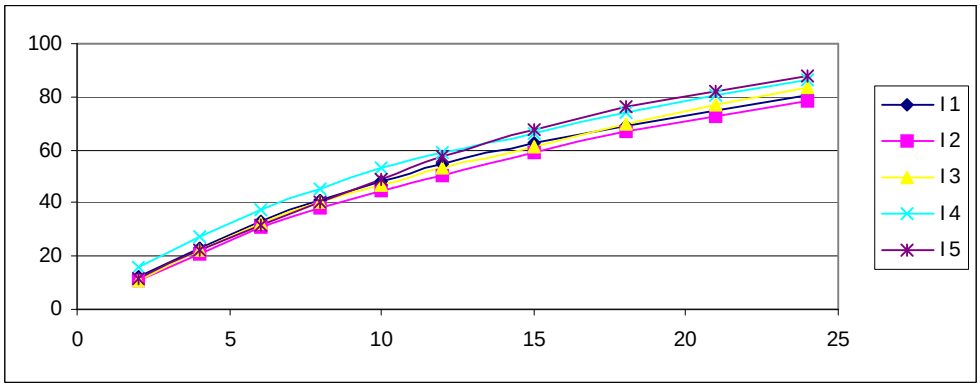

Figure 2: Cumulative gas production (ml) for different inocula until the 24th hr of fermentation

Table 6: Degradability (%) of dietary organic matter (DOM) and dry matter (DDM)

Inoculum / time	6h		12h		24h	
	DDM	DOM	DDM	DOM	DDM	DOM
1 (reference diet)	37.30 ^{Ba}	43.83 ^{Ca}	39.97^{B}	48.24 ^{B}	48.14 ^{A}	55.16 ^{A}
2 (0.25%)	37.84 ^{Ba}	45.03 ^{Ca}	43.53 ^{B}	51.03 ^{B}	50.53 ^{A}	56.57 ^{A}
3 (0.50%)	35.99 ^{Ca}	44.85 ^{Ca}	42.71^{B}	48.57 ^{B}	47.56 ^{A}	53.80 ^{A}
4 (0.75%)	35.65 ^{Ca}	43.16 ^{Ca}	40.22^{B}	47.49 ^{B}	47.95 ^{A}	56.33 ^{A}
5 (1.0%)	28.38 ^{Cb}	35.94 ^{Cb}	44.56^{B}	53.05 ^{B}	50.62^{A}	56.67 ^{A}

Means with different small letters in the same column or different capital letters in the same line differ significantly (P<0.05)

CONCLUSIONS

It is difficult to interpret our results exactly due to the non-existence of studies on the *Lithothamnium* sp. use. Caecal inocula from rabbits fed with higher level of seaweed flour produced more gas. However, further studies are required to investigate the effects of *Lithothanium* sp. on rabbit caecal microbiota.

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