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GAS PRODUCTION AND *IN VITRO* DIGESTIBILITY OF DIFFERENT LEVELS OF INCLUSION OF BEET PULP IN DIETS OF GROWING RABBITS

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ABSTRACT

In vitro digestibility methods are important because they aim to simulate the digestion performed by the animals and are able to evaluate the digestibility of the nutritional principles of feed more quickly. This study aimed to evaluate the gas production and *in vitro* digestibility of growing rabbits fed with different levels of inclusion of beet pulp (0, 9.41, 18.85, 32.94, 42.67 g/kg). The technique of gas production was used, using the bags of Ankon. Samples of 0.5g were weighed in bags of Ankon F57 and used to measure the gas produced. The caecum content was diluted in a ratio of 1:1 (w/v) with a buffering mineral solution. The pressure generated was measured using a digital pressure transducer. For *in vitro* digestibility, the multienzymatic technique was used, with ANKON filter bags (F57). An amount of 0.5g of samples from the five dietary treatments, were weighed in filter bags. In the first step, the samples were incubated at 40º C for 1.5 h in a fresh solution of pepsin, in the second step, the samples were incubated at 40º C for 3.5 h in a fresh pancreatin solution. As the last step, the samples were incubated 40ºC for 24h in a fresh caecal content solution. The results were statistically analyzed through unidirectional ANOVA, with beet pulp levels as the main factor. For all data, linear and quadratic effects were studied for the inclusion levels of beet pulp using a polynomial contrast. No statistically significant differences were observed for the variables of B, C, A, TMTF, MFR and LAG (P>0.001). However, a significant linear behavior was observed as a reduction in degradability (51.3 to 39.7%, P<0.001) and in *vitro* digestibility (47.01 to 40.27%, P<0.001). same linear reduction of coefficients is observed as increases the inclusion of beet pulp in the diet of these animals for *in vivo* and *in vitro* methods.

Key words: degradability, digestibility, multienzymatic, dry matter, fibre.

INTRODUCTION

To determine the nutritional value of new feeds to be used in cuniculture, studies are needed for adequate diets to be developed. There are varied ways to evaluate the digestibility of feed and consequently their contribution. *In vivo* studies are more expensive, it is necessary a place to keep animals, large amount of feed, considerable number of animals, and in addition, time and specialized workforce making it difficult to carry out these studies (Pascual *et al.*, 2000). *In vitro* digestibility methods are important because they aim to simulate the digestion performed by the animals and are able to evaluate the digestibility of the nutritional principles of feed more quickly, economically and that still has a high correlation with the determinations made in *vivo.*

This study aimed to evaluate the gas production and *in vitro* digestibility of growing rabbits fed with different levels of inclusion of beet pulp (0, 9.41, 18.85, 32.94, 42.67 g/kg).

MATERIALS AND METHODS

Animals and experimental design

The substrates used in this evaluation were the same five diets of treatments (Very low beet pulp - VLBP, Low beet pulp - LBP, Medium beet pulp - MBP, High beet pulp - HBP, Very high beet pulp – VHBP) with beet pulp inclusions (0, 33, 66, 115, 149 g/kg). The technique of gas production was described by Theodorou *et al*. (1994) and adapted by Maurício *et al*. (1999), using the bags of Ankon (Ankom Technology, Macedon, NY, USA). Samples of 0.5g were weighed in bags of Ankon F57, sealed and placed in vials of 50ml previously washed with distilled water, dried in greenhouse and preheated at 39 ºC. The caecum content was diluted in a ratio of 1:1 (w/v) with a buffering mineral solution (Theodorou *et al*. 1994). The pressure generated by the accumulation of gas at the top of the vials was measured using a digital pressure transducer. Gas production was measured by readings after 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22 and 24 h of incubation. For *in vitro* digestibility, the multienzymatic technique was used (Pascual *et al*., 2000), with ANKON filter bags (F57) proposed by Abad *et al*. (2013). An amount of 0.5g of samples from the five dietary treatments, previously grounded to 1 mm, were weighed in filter bags. In the first step, the samples were incubated at 40º C for 1.5 h in a fresh solution of pepsin, in the second step, the samples were incubated at 40º C for 3.5 h in a fresh pancreatin solution, as the last step, after the second incubation an adaptation to the technique was made (Ferreira *et al*., 2017), where the cecal content diluted at a rate of 1:1 (w/v) with a mineral buffer solution (Theodorou *et al*., 1994) as an inoculum and incubated for 24h at 40ºC.

Chemical Analyses

Table 1: Composition of diets with different levels of beet pulp

	VLBP	LBP	MBP	HBP	VHBP
Ingredients (g/kg):					
Alfalfa hay	167,4	205,3	242,6	193,5	159,4
Corn grain 7.92%	232,5	169,4	106,2	64	0
Soybean meal 45%	104,7	101,3	98,1	114,2	117,2
Wheat Bran	177,1	186,6	196,2	161,2	187,3
Soybean hull	254,1	180,1	106,2	82,3	55,7
Beet Pulp	0	94,1	188,5	329,4	426,7
Molasses	20	20	20	20	20
Soybean Oil	25,1	26,5	27,9	21,5	20,6
Di-calcium phosphate	0	0	0	2,2	1,7
Limestone	6,8	4,4	2	0	0,03
L-Lysine	0,9	0,8	0,7	0,2	0

Salt:0.50;DL-Methionine:0.14;Premixvitamin:0.50: Folicacid:200.00mg;Pantothenicacid:3000.00mg:BHT:2500.00mg;cobalt:200.00mg;copper:40.00g;choline:120.00g;iron:20.00g;iodine:140.00mg;manganese:4000.00mg;niacin:7500.00mg;selenium:20.00mg;vitaminA:2000000.00IU;vitaminB1:400.00mg;vitaminB12:2000mcg;vitaminB2:1000.00mg;vitaminB6:mg;vitaminD3:200000.00IU;vi

The chemical composition of experimental diets was determined by the AOAC (2000) method for dry matter (MS; 934.01), ash (967.05), crude protein (PB; 986.06), ether extract (EE; 920.39) and calcium (Ca; 927.02). Neutral detergent fiber was analyzed using thermostable amylase included residual ash (NDFa), acid detergent fiber was expressed with residual ash (ADF) and lignin was determined by solubilization of cellulose with sulfuric acid and analyzed according to Maertens (2002), AOAC (2000), procedure 973,187, using the sequential process and filter bag system (Ankom Technology, New York). Hemicellulose were calculated from the NDFa-ADF and cellulose calculated from ADF-lignins. PH was measured using a digital pH-metr (model HI 221, Hanna Instruments, Woonsocket, RI, USA). Phosphorus (P) was measured through spectrometry (model E-225D, CELM, Barueri, SP, Brazil), shown in table 1.

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Statistical Analysis

The experiment was conducted using a completely randomized design. The hypothesis model of normality and homogeneity of variance were examined using the Shapiro-Wilk and Levene test, respectively. The results were statistically analyzed through unidirectional ANOVA, with beet pulp levels as the main factor. For all data, linear and quadratic effects were studied for the inclusion levels of beet pulp using a polynomial contrast. Answers were considered statistically different when $P<0.001$, and the results were presented by mean ± standard error (SEM). All statistical analyses were made using Software R (R Core Team 2019).

RESULTS AND DISCUSSION

Effect of inclusions of beet pulp in the cecotrophy of growing rabbits

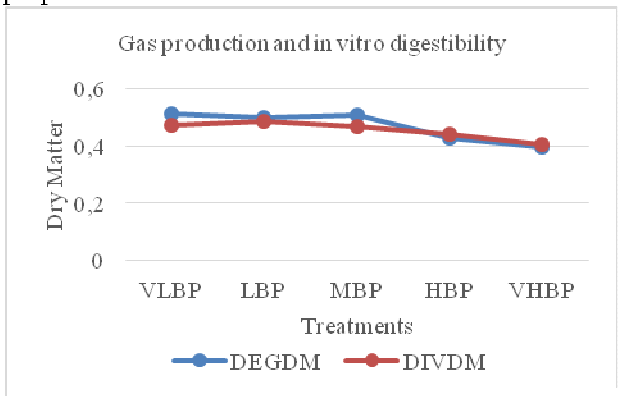
Table 2 shows the results obtained by in vitro analysis of gas production and digestibility. No statistically significant differences were observed for the variables of B, C, A, TMTF, MFR and LAG (P>0.001). However, a significant linear behavior was observed for the degradability and in *vitro* digestibility of dry matter (P<0.001) where, as the level of beet pulp in the diet increases, there is a reduction in degradability (51.3 to 39.7%, P<0.001) and in *vitro* digestibility(47.01 to 40.27%, P<0.001).

Table 2: Gas production and *in vitro* digestibility of growing rabbits feed with different levels of beet pulp

	Dietary treatments					SEM	Probability	
	VLBP	**LBP**	**MBP**	**HBP**	**VHBP**		Linear	Quadratic
B ml/g DM	85,43	101,81	96,03	97,95	127,8	4,83	0,021	0,423
C	3,94	3,85	4,75	4,19	5,09	0,17	0,026	0,806
A	0,091	0,088	0,099	0,093	0,076	0,005	0,536	0,220
TMTF, H.	26,08	27,39	23,20	26,05	29,18	1,40	0,685	0,350
MFR, ml/h	2,18	2,22	2,81	2,45	2,98	0,12	0,020	0,885
LAG, h	3,95	4,32	5,59	4,85	8,79	0,612	0,014	0,283
DEGDM (%)	0,513	0,498	0,509	0,429	0,397	0,013	<0,001	0,043
DIVDM (%)	0,470	0,483	0,465	0,441	0,403	0,006	<0,001	0,001

Very low beet pulp - VLBP, Low beet pulp - LBP, Medium beet pulp - MBP, High beet pulp - HBP, Very high beet pulp - VHBP; A - Decay in the specific rate of gas production; B - Maximum amount of gas produced; C - Specific rate of gas production; TMFR;h - Time for maximum fermentation rate; MFR - Maximum fermentation rate; LAG - Latency in fermentation at the beginning of incubation; DEGDM – dry matter degradability; DIVDM - Dry matter in vitro digestibility; SEM: standard average error;

Figure 1: Degradability and in vitro digestibility of growing rabbits feed with different levels of beet pulp



DEGDM – dry matter degradability; DIVDM - Dry matter in vitro digestibility

De Blas *et al.*, (2015), corroborated the present study when using the enzyme complex technique, already mentioned, to evaluate co-products for rabbit feeding, they observed good reliability of the data. It is demonstrated that the technique can be used in the evaluation of food since the values are in accordance with the recommended for experimentation with rabbits. It was observed that the digestibility coefficient of dry matter obtained from the adapted multienzymatic method and degradability by gas production presented lower values when compared to the dry matter (63,82 a 60,80%, P<0,05) obtained by the total collection technique. This can be explained due to the fact that the cecal content used for *in vitro* analysis and gas production were of animals that were not adapted to diets with high levels of soluble fiber or high inclusion of beet pulp as in total collection, possibly this caused dry matter digestibility values to be lower.

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Unlike the present study, Kara (2016), observed evaluating diets with inclusion of beet pulp and tomato residue, that there was an increase in gas production, possibly due to a higher fermentation intensity by the microbiota, and this effect is related to a higher soluble fiber content, insoluble pectins in water and lower levels of lignin and condensed tannins. Several authors are using the production of gases to predict digestibility of diets for rabbits and have found good results with the use of this technique, being shown to be the predominant *in vitro* technique today for the evaluation of diets for rabbits (Rodríguez-Romero *et al*., 2011).

CONCLUSIONS

It is observed that the caecum content used in both techniques has great importance in determining the in vitro degradability and digestibility of diets for growing rabbits, but the same linear reduction of coefficients is observed as increases the inclusion of beet pulp in the diet of these animals.

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