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VOLATILE FATTY ACIDS PRODUCTION OF GROWING RABBITS FEED WITH DIFFERENT LEVELS OF BEET PULP

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ABSTRACT

The caecum has a major role in rabbits' development, is the site where microbial digestion of carbohydrates and proteins occurs and yet it is characterized by an abundant colonization of bacterial flora. Volatile fatty acids (VFA) are end products of fermentation in the caecum of rabbits, which provides as an important source of energy. This study aimed to evaluate the production of volatile fatty acids of growing rabbits fed different levels of inclusion of beet pulp (0, 9.41, 18.85, 32.94, 42.67 g/kg). Samples of the caecum content of 12 slaughtered rabbits randomly selected from each group were collected. The caecum content was secured in *falcon* tubes with the subsequent addition of 1ml of metaphosphoric acid solution 25% and frozen in freezer at -18ºC. The samples were transferred to centrifuge tubes and were homogenized for 10 minutes at 3000rpm in a refrigerated centrifuge, the supernatants were collected and filtered into millipore filters of 0.45 µm and the aliquots were then analyzed for acetic, propionic and butyric acids through the gas chromatograph CG Shimadzu 17A. 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. There was no statistical difference between the groups for acetic ($P>0.001$) and butyric acids ($P>0.001$). For propionic acid, a linear increase (10.51 to 17.63 µg/ml, $P<0.001$) was observed as the inclusion of beet pulp in the diet of growing rabbits increases. It is observed that the inclusion of beet pulp in the diet of growing rabbits exerts a beneficent effect on animal cecum, improving the development of the microbiota, resulting in a greater fermentation of substrates and greater production of volatile fatty acids in growing rabbits.

Key words: acids, acetic, propionic, butyric, fibre.

INTRODUCTION

The caecum has a major role in rabbits' development, is the site where microbial digestion of carbohydrates and proteins occurs and yet it is characterized by an abundant colonization of bacterial flora (Xiccato *et al.,* 2011). Volatile fatty acids (VFA) are end products of fermentation in the caecum of rabbits, which provides as an important source of energy (Marty e Vernay, 1984). Soluble fibers (hemicelluloses and pectins) promote the activity of the microbiota (Rodriguez-Romero *et al.,* 2011), and a balance between insoluble and soluble fibers can favor digestive health of rabbits (Gómez-Conde *et al.*, 2009; Trocino *et al.*, 2011).

This study aimed to evaluate the production of volatile fatty acids of growing rabbits fed 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

Composition of the diets are shown in table 1. Samples of the cecal content of 12 rabbits randomly selected from each group were collected from slaughtered animals at the end of the experimental period. After bloodletting, the caecums were removed and its content were secured in *falcon* tubes previously identified with the subsequent addition of 1ml of metaphosphoric acid solution 25% and frozen in freezer at -18ºC. The samples were transferred to centrifuge tubes and were homogenized for 10 minutes at 3000rpm in a refrigerated centrifuge, the supernatants were collected and filtered into millipore filters of 0.45 µm and transferred into previously identified *eppendorfs.* Aliquots were then analyzed for acetic, propionic and butyric acids through the gas chromatograph CG Shimadzu 17A. The experiment was conducted using a completely randomized design, animals were distributed in five dietary treatments (Very low beet pulp - VLBP, Low beet pulp - LBP, Medium beet pulp - MBP, High beet pulp - HBP, Very high beet pulp – VHBP). All procedures were approved by the Animal Use Ethics Committee of the Federal University of Minas Gerais, on protocol 123/2016.

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;vitaminK3:500.00IU;vitaminE:5000.00IU;zinc:14.00g.

Chemical Analyses

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). The 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 the solubilization of cellulose with sulfuric acid and analyzed according to Maertens (2002), AOAC (2000), procedure 973,187, and Van Soest *et al.* (1991), respectively, using the sequential process and filter bag system (Ankom Technology, New York). Hemicelluloses were calculated from the calculation NDFa-ADF e cellulose from ADF-lignins. The 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).

Statistical Analysis

The hypothesis model of normality and homegeinity of variance was 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. Results 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 the inclusion of beet pulp in the production of volatile fatty acids of growing rabbits

Table 2 shows the results of evaluating the production of volatile fatty acids in growing rabbits fed different levels of beet pulp. The relationship between fatty acids was maintained as presented in the literature, where there was higher production of acetic acid, butyric acid and propionic acid in this order. There was no statistical difference between the groups for acetic (P>0.001) and butyric acids (P>0.001). For propionic acid, a linear increase (10.51 to 17.63 µg/ml, P<0.001) was observed as the inclusion of beet pulp in the diet of growing rabbits increases.

It is observed that positive effects occur in the cecum of rabbits fed soluble fibers, due to the higher production of volatile fatty acid as demonstrated in the present study, this fact explains the greater development of the gastrointestinal tract of animals possibly due to the use of butyric acid as the main source of colonocytes energy (Vernay, 1987).

Table 2: Contribution of volatile fatty acids (acetic, propionic and butyric) of caecal content of growing rabbits fed different levels of beet pulp

	Treatments[†]					SEM	Probability	
	VLBP	**LBP**	**MBP**	**HBP**	**VHBP**		Linear	Quadratic
Acetic (µg/ml)	47,36	53,90	54,75	54,80	65,81	2,22	0,016	0,708
Propionic (µg/ml)	10,51	12,14	15,20	15,90	17,63	0,71	<0.001	0,530
Butyric (µg/ml)	14,36	24,20	25,38	21,04	23,47	1,16	0,044	0,023
Total VFA (µg/ml)	72,24	90,23	95,34	91,74	106,91	3,44	0,002	0,544
Acetic (%)	65,56	62,32	57,06	59,23	61,68	0,94	0,051	0,008
Propionic (%)	14,45	12,45	16,44	18,34	16,50	0,64	0,018	0,613
Butyric (%)	19,98	25,23	26,50	22,43	21,82	0,79	0,750	0,007

Very low beet pulp - VLBP, Low beet pulp - LBP, Medium beet pulp - MBP, High beet pulp - HBP, Very high beet pulp - VHBP; SEM: standard average error;

Figure 1: Production of volatile fatty acids of growing rabbits feed with different levels of beet pulp

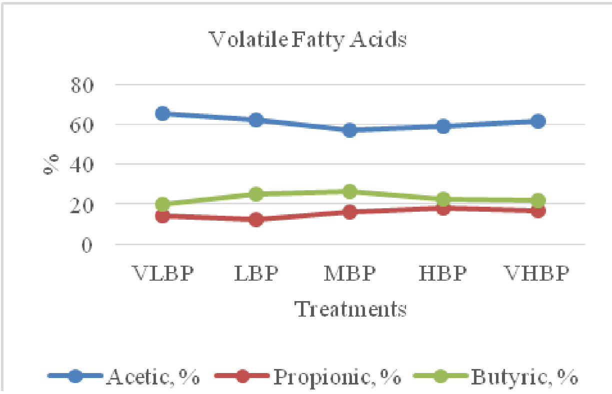

Figure 1 shows the values of the percentages of the production of volatile fatty acids for growing rabbits fed with different levels of beet pulp. In this study, higher full cecum weight was observed (5,81 a 7,72 %PA, P<0,001), also there was a reduction of caecum pH (5,81 a 5,70, P = 0,043). In addition an improvement in the digestibility of NDF (44,00 a 49,95%, P=0,007) e FDA (32,61 a 39,80%, P=0,009) was found, as the inclusion of beet pulp increases in the diet of rabbits, and it is possible to infer that higher levels of beet pulp positively influenced the development of cecum, due to the higher caecal content observed (4,28 a 6,13 %PA, P< 0,001) and amoniacal nitrogen (1.47 to 1.95, P=0.003), and provided greater fermentation and production of volatile fatty acids.

Evaluating high inclusions of beet pulp in the diet, authors observed higher proportions of acetate and lower butyrate rates, which may be associated with a higher availability of substrates fermented by cellulolytic bacteria and also to a lower activity of amylolytic microflora in the cecum of animals (Falcão-e-Cunha *et al.*, 2004; Xiccato *et al.*, 2011). However other studies have not demonstrated effects of soluble fiber levels on the behavior of cecal fermentation (Gómez-Conde *et al.*, 2009; Trocino *et al.*, 2011; Belenguer *et al.*, 2011).

CONCLUSIONS

It is observed that the inclusion of beet pulp in the diet of growing rabbits exerts a beneficent effect on animal caecum, improving the development of the microbiota, resulting in a greater fermentation of substrates and greater production of volatile fatty acids in growing rabbits.

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