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INFLUENCE OF DIETARY FIBER UPON *In Vitro* MICROBIAL CECAL FERMENTATION IN MEXICAN HAIRLESS AND MEXICAN CUINO PIGS

[INFLUENCIA DE LA FIBRA DIETÉTICA SOBRE LA FERMENTACIÓN CECAL *In Vitro* IN CERDOS PELONES Y CUINOS MEXICANOS]

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RESUMEN

El objetivo fue evaluar y comparar la fermentación cecal *in vitro* (por la técnica de producción de gas), en cerdos Pelón Mexicano y Cuino Mexicano, mediante la adición de los sustratos purificados de celulosa o almidón (0, 100, 200, 300 y 400 mg/g MS). Se sacrificaron 12 cerdos (PV=104±0.5 kg), seis de cada genotipo; de los cuales se colecto contenido cecal y se hizo un pool por cada dos cerdos en cada genotipo; posteriormente, por cada sustrato (celulosa o almidón) en su distinta concentración, se incubaron tres frascos por cada pool con inóculo y se hicieron tres series de incubación. El diseño experimental contemplo los efectos de genotipo, sustrato y concentración de sustrato adicionado, sobre las variables de fermentación *in vitro*. Los promedios se compararon con el método de Tukey. La producción de gas *in vitro* fue superior (P<0.05) (mL/g MS) para el cerdo Pelón Mexicano (206.8) vs Cuino (180.2). El ritmo fraccional de degradación (b, 0.094 y c, -0.0127) y tiempo de retardo (1.79) de los cerdos Pelon Mexicano fueron superiores que en los cuinos (b, 0.074; c -0.102) y tiempo de retardo (1.26) respectivamente; en la adición de carbohidratos como sustratos, el almidón fue superior (P<0.05) a la celulosa en la producción de gas (238.3 vs 148.7 mL/g MS); respecto a las fracciones b, c y Lag time, hubo diferencias (P<0.05) entre sustratos. La adición creciente de celulosa o almidón tuvo un efecto lineal (P<0.05) en la producción de gas, de su nivel cero mg (133.4 ± 2 ml gas / g MS) a 400 mg de almidón (349 mL gas/g MS) o celulosa (176 mL gas / g MS). El cerdo Pelón Mexicano tiene mayor fermentación microbiana cecal (P<0.05) que el Cuino, siendo mayor la producción de gas a las 9 y 12 h , así mismo hubo mayor producción de gas (fermentación cecal) al adicionar el almidón (P < 0.05) como sustrato.

Palabras claves: Cerdo Pelón Mexicano; Cuino Mexicano; Digestibilidad; Producción de Gas; In vitro; Celulosa; Almidón.

ABSTRACT

The objective of the present study was to evaluate and compare the *in vitro* cecal fermentation (by the gas production technique), in Mexican hairless pig (MHP) and Mexican cuino pig (MCP), adding cellulose or starch as substrates (0, 100, 200, 300 and 400 mg/g DM). 12 pigs were slaughtered (BW= 104±0.5 kg), six of each genotype were collected from the cecal contents and there was a pool for every two pigs in each genotype, and thereafter, for each substrate (cellulose or starch) in its different concentration, three flasks were incubated with inoculum for each pool and made three series of incubation. The experimental design use the effect of genotype, substrate and concentration of the substrate added on the variables of *in vitro* fermentation. The averages of the data were compared by Tukey's method. In vitro gas production was higher (P<0.05) (mL/g DM) for MHP (206.8) vs MCP (180.2). The degradation fractional rate rhythm (b, 0.094 and c, -0.0127) and lag time (1.79) of the MHP was higher than MCP (b, 0.074; c -0.102) and lag time (1.26); with the addition of carbohydrates as substrates, *in vitro* gas production of potato starch was higher (P<0.05) than cellulose (238.3 vs 148.7 mL/g DM); the fractions b, c and lag time, there were differences (P<0.05) between substrates. The increasing addition of cellulose or starch had a lineal effect (P<0.05) with the gas production, level cero mg

(133.46) to 400 mg of substrate (263.16 mL/g DM). MHP had a higher cecal fermentation than MCP, being higher the gas production at 9 and 12 h, there was higher gas production (cecal fermentation) when added starch as substrate.

Key words: Cellulose; Digestibility; Gas production; In vitro; Mexican cuino pig; Mexican hairless pig; Starch.

INTRODUCTION

In recent decades Mexican hairless pig (MHP) has been displaced by the introduction of improved breeds, causing genetic erosion and a diminution in the crossbreed population (Sierra-Vásquez, 2000). Currently, around 30 % of the genetic resources of the domestic animals in the world were classified as in high risk of extinction; MHP was also considered in danger. Pig is an important source of quality protein, the most utilized products are: ham, bacon, chops, sausages and sub-products. Mexico has some autochthonous rustic genotypes, which are known as Mexican hairless pig and Mexican cuino pig (MCP) both are native Mexican pig breeds (Lemus et al., 2003), the advantages they offer is a great adaptability to the environment (Sierra-Vásquez, 2000), which provides high rusticity, they are a source of broad biological diversity, so they are an option to cross-breeding selected genotypes (Lemus Flores et al., 2001), being an ideal genotype to integrate into sustainable production systems.

Glucose is the main source of energy from the absorption of carbohydrates in pigs, however there are some other nutrients such as volatile fatty acids (VFA) from intestinal fermentation which also provide energy that may reach up to 25% of the whole-animal energy requirements (Yen et al., 1991). There are several potential sources available for the bacteria in the large intestine and cecum of pigs, the fermentation will be according to the source, for instance digestible or highly fermentable carbohydrates might influence on the beneficial intestinal microbiota, particularly in relation to cecal fermentation (Williams et al., 2001; Morales et al., 2002); moreover, depending on the sort of fiber in the diet of the pigs, the fermentation will have different characteristics (Bindelle et al., 2007).

Cecal fermentation can be estimated by means of the technique of *in vitro* gas production (Theodorou *et al.*, 1994), using this method several factors can be studied and a number of treatments can be tested with a higher number of repetitions. The objective of the present study was to evaluate *in vitro* cecal fermentation in two pig genotypes (Mexican hairless and Mexican cuino) and to compare it by means of the addition of two sources of carbohydrates (cellulose and starch), increasing amounts as energy source for microbial fermentation.

MATERIALS AND METHODS

Animals and diets

Twelve pigs of two genotypes (Mexican hairless and Mexican cuino) were used, six per genotype, were fed *ad libitum* with standard diets for growing (75 d) and finishing (64 d) stages (Table 1); once the pigs reached mean live weight at slaughter (LW=104±0.5 Kg) they were slaughtered in a FIT (Federal Inspection Type) slaughterhouse in Cuautitlan, Mexico. Later on, the digestive organs were extracted from the abdominal cavity and cecal content was collected, samples were taken around 250 g from each pig, these samples were refrigerated (4ºC) until they reached the laboratory for their processing. The pigs always had good health conditions and the study was approved by the Committee of Ethics of the Faculty of Veterinary Medicine and Zootechnics of the Autonomous University of the State of Mexico.

Table 1. Ingredients (%) and chemical composition (g/100g DM) of the diets given to Mexican hairless pigs and Mexican cuino pigs in growth and finishing stages.

Ingredient (%)	**Growth**	**Finishing**
Corn grain	69.0	77.7
Canola meal	6.0	8.0
SoyBean meal	13.9	6.8
Bran	7.0	5.0
Vitamins and minerals	2.8	2.5
$CaCO_3$	1.0	0.5
NaCl	0.3	0.2
Chemical composition (g/100 g DM)		
OM	94.8	95.5
CP	17.3	15.6
Fat	4.8	3.8
NDF	14.7	15.9
ADF	4.7	4.9
Lignin	1.6	1.4

Substrates

Two purified carbohydrates were used, chosen according to their contents of total and insoluble fiber, potato starch (Fluka B-5650) (2.2g of CP, 1.9g of ashes, 783g of starch per kg of DM) and cellulose as fiber source (Sigma C- 6663) (881g of NDF, 791g of ADF, 82g of Lignin, 1g of CP and 1.9g ashes per kg of DM), which represents 0 and 1000 g/kg DM of the total of dietary and insoluble fibers for starch and cellulose, respectively.

In vitro gas production

The gas production was determined through the method proposed by Theodorou et al. (1994). Three 125-milliliter amber glass flasks per substrate (starch and cellulose) and genotype (Mexican hairless pigs and Mexican cuino pigs) were used for each substrate sample; 0.800g of DM of oat straw were introduced in the flasks, and cellulose and starch were added in different quantities (0, 100, 200, 300 and 400 mg/g of DM), then 90mL of buffer solution gassed with CO_2, and were kept in refrigeration (4°C, 12h); the following day 700mL of buffer solution and approximately 300g of cecal content were mixed, the cecal contents came from a pool of two pigs per genotype, obtaining three repetitions per genotype; it was filtered through four layer of gauze and later through glass wool, the temperature of the solution was kept at 39ºC, it was gassed with CO_2, then 10mL of the mixture were added to each flask, and the flasks were introduced in a heated bath (39ºC) and the registration of reads from gas production started at 3, 6, 9, 12, 18, 24 and 36 h using a pressure transducer (DELTA OHM :Manometer, 8804); in order to perform the corrections in cecal fermentation, three flasks without substrate were utilized as blanks and three flasks only with oat straw as standards. Gas production was determined using the average amount of gas produced by the hour. For the additive sum of gas production the equation proposed by France et al. (1993) was used (1993), $GP = a\ \{1- e\ –b^{(t-T)} – c^{(\sqrt{t}-\sqrt{T})}\}$, where GP: accumulated gas production (mL gas/g DM); a, asymptote of the curve (total gas production, mL); b (h^{-1}) and c ($h^{-1/2}$), constants of gas production and delay time before the beginning of fermentation (T).

Chemical composition

Substrates and diets were analyzed for DM (60 ºC, 48h), ashes, CP (N-Kjeldahl x 6.25), ethereal extract (Soxhlet) (AOAC, 1990), as well as NDF, ADF and lignin (Van Soest *et al.* 1991).

Statistical analysis

Pigs were distributed in an experimental design completely at random with a factorial arrangement, 2 x 2 (genotype x substrate), using 3 repetitions (cecal pool of two pigs per genotype). The means of each variable with significant effect ($P<0.05$) were compared by means of Tukey's test (Steel *et al.*, 1997). The effects of genotype, substrate and inclusion level were analyzed, as well as gas production by hour. A polynomial analysis was also carried out to evaluate the effect of the growing levels of substrates in gas production (SAS, 1999).

RESULTS

In Table 1 the chemical composition of diets is presented, showing that the content of protein and fat is superior in the growth diet than finalization, maintaining a similar content of NDF. As it is indicated in Figure 1, the cecal inoculum of MHP incubated with starch had a higher gas production than MCP with the same substrate; however, the same did not occur when it was incubated with cellulose. Nevertheless, when the hourly gas production is analyzed (Figure 2) the fermentation pattern is similar in the first hours (3 and 6 h; $P>0.05$); later on, it is observed that the higher fermentation ($P<0.001$) is produced at 9 and 12 h, and in this case we noticed that the gas production when starch is applied in MHP is superior to MCP; the same effect is observed for cellulose, with a diminution of gas production after 18 h of incubation. In Table 2 are show the gas production the effect of pig genotype and the source of substrate; the gas production, as well as the fractions b, c and T were higher ($P<0.05$) in Mexican hairless pigs; as to substrate, starch produced enhance more gas (238 mL) than cellulose (148.6 mL/g of DM); there were also differences ($P<0.05$) in fractions b and T, but not for c ($P>0.1$).

The addition of increasing amounts of cellulose or starch produced an effect per genotype, substrate and linear ($P<0.001$) in the production of gas (Table 3); conversely, for cellulose no linear effect was observed ($P>0.1$) for fractions b, c, and T; there was an effect due to genotype and substrate for variables b and c; in relation to starch addition, fraction b had a linear effect ($P<0.05$) as the amount increased, however fractions c and T were not affected ($P>0.1$) by the inclusion of increasing levels, but fraction c and t was not affected ($P> 0.1$) by the inclusion of increasing levels.

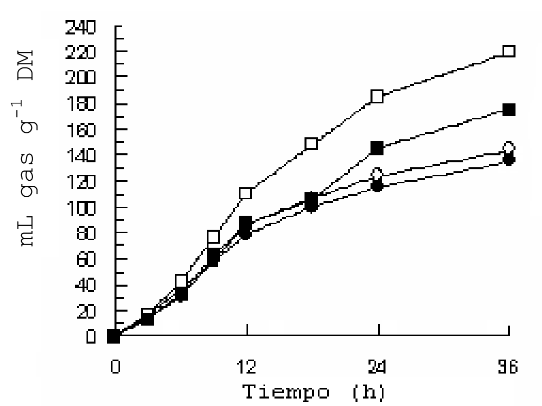

Figure 1. Evolution of the volume of gas produced (mL gas g^{-1} DM) by cecal fermentation of cellulose in Mexican hairless (○) and Mexican cuino (●) pigs or starch in Mexican hairless (□) and Mexican cuino (■) pigs.

In Table 4 show the hourly gas production (ml gas/h), MHP produces higher gas production (P<0.01) than MCP, except at 24 h of incubation (P>0.1), when substrates are compared there were not differences (P>0.1) from the first hours of incubation (3 and 6 h), yet later one sees that starch produces more gas than cellulose (P<0.001), as for the effect due to the level of addition of cellulose or starch it showed a significant effect (P<0.001) for all the hours of incubation, save the 18th hour.

DISCUSSION

Most of the starch that reaches into the large intestine is digested by α-amylase, yet a fraction of the dietary starch resists its degradation and flows into the posterior digestive tube. Indigestible carbohydrates (ICH) are the main substrate for the bacteria present in the large intestine to produce VFA which will work as energy source. The largest proportion of fermentable carbohydrates increases the process of digestion in the large intestine (Bach *et al.*, 1991) as it is seen in the addition of cellulose and starch in increasing amounts (Table 3). Diets with soluble carbohydrates have a higher capacity of retention of water and are more fermentable compared to diets with insoluble fiber (cellulose) (McBurney et al., 1985). The results of these authors coincide with the results of the present study, as starch produced a greater amount of gas than cellulose (Tables 3 and 4), they also coincide with the results by Jezieny *et al.* (2007) observing a higher production of gas (24h) with starch in respect to cellulose. According to the results of the present study it seems as though amylolytic bacteria have a higher activity in respect to cellulolytic ones, and these have higher activity between 9 and 12 h of incubation. Nevertheless, Bindelle *et al.* (2007), comparing the patterns of fermentation of cellulose and starch in commercial pigs, did not find differences (P>0.1) between substrates.

According to that observed in the present study it is probable that the microorganisms present in the cecum of MHP have a higher enzymatic activity than MCP, even than other commercial pigs, as it had higher fermentation of both starch and cellulose at 9 and 12 h (Figure 2). It has been verified that cecal digestion of Iberian pigs has higher enzymatic activity (Morales *et al.*, 2002) than commercial genotypes, which could reflect a selection of different species of bacteria by differences in the amount and type of substrates fermented and/or changes in the residence time of digestion; this may be similar in MHP, which presented a higher production of gas than MCP (Table 2 and 3). Moore *et al.* (1987) detected changes in fecal flora of pigs caused by diets high in fiber, marking differences in the patterns of fermentation on the quantity of substrate that reached cecum, which may be influenced by the sort of flora presented in this place. In the present study, the diets of the pigs had a 15 ± 1% of NDF, a value relatively high in fiber, however as both breeds of pigs intake the same level of fiber, it is clearly demonstrated that there is an effect on fermentation due to the genotype and the sort of substratum that reaches the cecum.

ICH have a greater fixation of bacterial N, this produces a faster fermentation (with a lower value of T), as it is appreciated when one compares the fermentation of cellulose versus starch Table 2 and Figure 2); these results are agree with those obtained by Bindelle *et al.* (2007) who used different substrates to study bacterial fermentation in pigs.

Table 2. *In vitro* cecal gas production of Mexican hairless pigs and Mexican cuino pigs with addition of cellulose and starch at different inclusion levels.

Variable	**Genotype**			**Carbohydrate**		
Fraction	Mexican hairless	Mexican cuino	SEM	Cellulose	Starch	SEM
a	206.78^x	180.18^y	3.70	148.67^x	238.29^y	3.70
b	0.094^x	0.074^y	0.001	0.094^x	0.075^y	0.001
c	-0.127^x	-0.120^x	0.016	-0.111^x	-0.183^x	0.016
T	1.79^x	1.26^y	0.06	1.39^x	1.66^y	0.06

^{x,y} Different letters in the same column differ P≤0.05.
a, Gas production (mL gas/g DM); b, degradation rate (h^{-1}); c, degradation rate (h½); T, delay time (h); SEM, Standard error mean .

Table 3**.** In vitro cecal gas production with addition of different levels of starch in Mexican hairless and Mexican cuino pigs

Substrate Fraction	Mexican hairless pig Levels (mg/g DM)						Mexican cuino pig Levels (mg/g DM)						Significance		
	0.0	100.0	200.0	300.0	400.0	SEM	0.0	100.0	200.0	300.0	400.0	SEM	G	S	L
Starch															
a	147.3	197.9	232.2	316.3	412.7	4.83	122.7	168.1	250.9	247.5	287.2	3.88	***	***	***
b	0.1495	0.0950	0.0834	0.0579	0.0588	0.09	0.0779	0.0686	0.0560	0.0539	0.0527	0.05	**	**	NS
c	-0.1881	-0.1239	-0.1190	-0.0804	-0.0967	0.18	-0.3004	-0.0787	-0.0619	-0.0977	-0.0346	0.15	NS	NS	NS
T	1.6448	1.7330	1.8879	1.7578	2.3753	0.25	0.9785	1.2279	1.1939	3.1955	0.6390	0.47	*	*	NS
Cellulose															
a	133.1	151.3	139.0	151.2	186.8	2.15	130.8	145.6	140.2	142.7	165.9	1.70	***	***	***
b	0.1117	0.0942	0.1060	0.1015	0.0897	0.04	0.0751	0.0916	0.0760	0.0930	0.0970	0.05	**	**	*
c	-0.1584	-0.1247	-0.1490	-0.1321	-0.1042	0.21	-0.0887	-0.0930	-0.0461	-0.1143	-0.1089	0.17	NS	NS	NS
T	1.9503	1.6989	1.9622	1.6365	1.2653	0.25	1.3074	1.0670	0.4256	1.3568	1.2492	0.29	*	*	NS

[d,e,f,g] Means with different letter in the same column are different *P≤0.05, **P<0.01, ***P≤0.0.001; [NS,] Non significant P>0.1.
a, gas production (mL/g DM); b, degradation rate (h^{-1}) c, degradation rate (h½); T, Lag time (h). SEM, Standard error mean
G, Genotype; S, Substrate; l, linear effect.

Table 4. *In vitro* gas production (ml gas/hour) adding cellulose and starch at cecal level in two pig genotypes.

	Genotype			Substrate			Significance		
Time (hours)	Mexican hairless	Mexican cuino	EEM	Cellulose	Starch	EEM	Genotype (G)	Substrate (S)	G*S
3	3.41[a]	3.12[b]	0.23	3.29	3.24	0.23	**	NS	***
6	4.00[b]	4.45[a]	0.35	4.08	4.38	0.35	**	T	***
9	6.84[a]	5.89[b]	0.36	5.54[b]	7.19[a]	0.36	***	***	**
12	6.69[a]	5.01[b]	0.55	5.02[b]	6.67[a]	0.55	***	***	*
18	3.35[a]	2.15[b]	0.44	2.21[b]	3.28[a]	0.44	***	***	***
24	3.11	3.17	0.53	1.78[b]	4.50[a]	0.53	NS	***	*
36	1.49[a]	1.36[b]	0.55	1.04[b]	1.80[a]	0.55	*	***	T

[ab] Means with different letter in the same row are different *P<0.05, **P<0.01, ***P<0.0.001; [T], P <0.1; [NS,] Non Significant P>0.1.
SEM, Standard error mean.

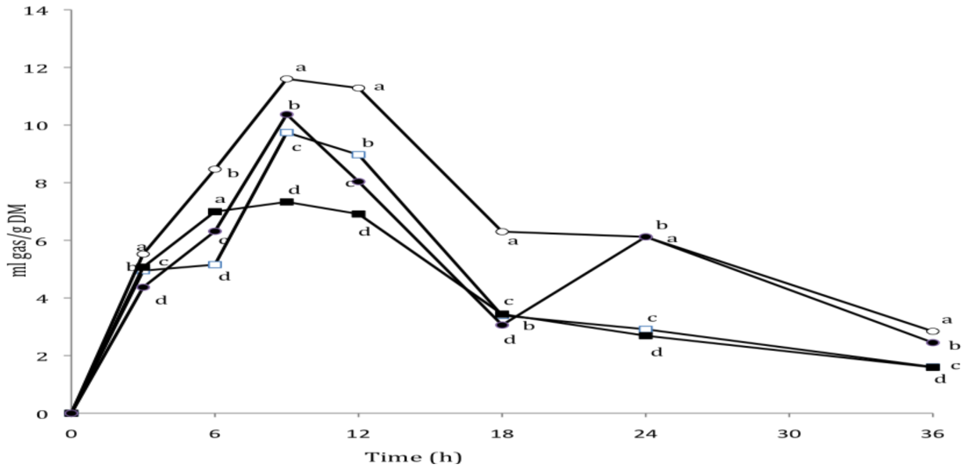

Figure 2. *In vitro* gas production (mL gas g^{-1} DM) at different incubation times due to cecal fermentation of starch in Mexican hairless (○) and Mexican cuino (●) pigs or cellulose in Mexican hairless (□) and Mexican cuino (■) pigs

CONCLUSIONS

The results of the present study make it evident that Mexican hairless pigs have a higher capability of cecal fermentation using starch and cellulose than cuino pigs, which makes us suppose that are more efficient to ferment soluble carbohydrates in the intestine, but also at cecal level, in diets with higher contents of fiber. These results reinforce the idea that more studies have to be carried out with the different genotypes in order to research on the digestion of carbohydrates as well as on the utilization of products from cecal fermentation as an energy source in the digestive gut of pigs.

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