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Ileal and Rectal Digestibility of Nutrients in Diets Based on Leucaena *(Leucaena leucocephala (Lam.) de Wit)* for Pigs. Influence of the Inclusion of Zeolite

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Abstract: A change over design was used to study in two experiments the effect of inclusion of a Cuban natural zeolite (5%) in a soybean/grain meal formulated to contain 20% leucaena (*Leucaena leucocephala (Lam.) de Wit)* meal. Responses criteria were apparent ileal and rectal digestibility and N balance indices in 6 Yorkshire×Landrace castrate male pigs each weighing on average 50 kg of initial live weight. In experiment 1, inclusion of 5% zeolite in the diet, determined a decrease in rectal digestibility of organic matter from 83.3-77.4% ($p<0.10$) and from 78.3-72.4% ($p<0.05$), respectively. In experiment 2, ileal digestibility of energy and N increased ($p<0.05$) respectively when 5% zeolite was added to the diet. Faecal concentration of total short chain fatty acids and ammonia were significantly ($p<0.05$) higher in pigs fed the diet containing zeolite (103 and 30 mmol/100 g DM) than when the animals were given the control feed (85 and 20 mmol/100 g DM) indicating a net capture of these metabolites by zeolite. In intact animals, a trend for retention of N to be higher in the diet with zeolite than in the other with no zeolite (48.4 and 47.0% of N intake) was noted. It is suggested that the inclusion of 5% of a Cuban natural zeolite in the diet is beneficial for digestive processes in the pig when this animal species is fed tree foliage as leucaena leaves. The incorporation of zeolite to this type of diet could influence pig digestive processes in a positive manner, probably in N economy. Other trials could be conducted to fix the optimal level of inclusion of zeolite in this type of diets.

Key words: Pigs, leucaena, zeolite, digestibility, ileum, faeces

INTRODUCTION

The use of zeolites in diets for pigs is a practice in full expansion (Poulsen and Oksberg, 1995; Uygongco *et al*., 1999; Yannakopoulos *et al*., 2000; Sardi *et al*., 2001; Prvulovic *et al*., 2007) among other causes, because it sequestering properties, which can in turn influence in a positive manner on pig health and environmental conditions (Nielsen *et al*., 1980). Zeolites are hydrated aluminosilicates which are characterized by a high internal surface area and high cation exchange capacity. In this connection, zeolites and among them, clinoptilolite, can remove ammonia from pig slurry by trapping and exchanging it in its crystalline structure (Beck, 1974; Milic *et al*., 2005).

There is not enough information concerning digestive processes taking place in pigs fed on diets formulated to contain zeolites (Leung, 2004). However, there are some previous reports claiming that zeolites exert a favorable action on the gastrointestinal tract of pigs therefore determining better digestibility of nutrients as measured at the rectum site (Collings *et al*., 1980; Cool and Willard, 1982; Shurson *et al*., 1984). This improvement effect on N metabolism has been observed too by Paolo *et al.* (1999) with pigs fed diets containing sepiolite, another aluminosilicate clay. In fact, these favorable effects of clinoptilolite and other clays on the pig gastrointestinal environment were also claimed by Pond *et al.* (1988). In this connection, the use of zeolite obtained from different Cuban deposits has been evaluated from the point of view of its balance of N and energy (Ly *et al*., 1996). Furthermore, it has been reported an improvement of ileal digestibility of N in pigs fed conventional diet, with no negative changes in either DM or organic matter at this site of the alimentary canal (Ly and Castro, 1997). On the other hand, it is known than in diets of leucena foliage, controversial results have been derived from the use of

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graded level of different types of feeds prepared using leucaena for feeding pigs, mainly due to the presence or not of mimosine, a toxic non protein amino acid present in leucaena tissues, or in any case, to the high cell wall content in leucaena foliage (Ly *et al*., 1998; Thompson, 2006). Previous observations (M. Castro, unpublished data) have suggested a possible positive effect of zeolite on digestive processes in pigs fed leucaena foliage diets.

The aim of the present communication is to report ileal and rectal digestibility parameter of pigs used in one investigation where the effect of including or not a Cuban natural zeolite was evaluated in a conventional diet containing leucaena foliage meal.

MATERIALS AND METHODS

General: A change over design (Gill, 1978) was used to study in two experiments the effect of inclusion of a Cuban natural zeolite (5%) in a soybean/grain meal formulated to contain 20% leucaena (*Leucaena leucocephala* (*Lam.*) *de Wit*) meal. The leucaena foliage was obtained after cutting terminal branches (30 cm length) from a periodically cut leucaena (cv, ipil-ipil) plantation. Cutting interval was of approximately every 3 months. The branches were chopped, spread over a plate and sun dried for 3-4 days. As a result of a natural shedding process, only dry leucaena leaves were collected, therefore discarding all type of stalks. Zeolite was obtained from a Cuban commercial deposit and it was essentially clinoptilolite in nature. Details concerning the evaluated diets are presented in Table 1.

Animals and housing: Responses criteria were either rectal or ileal digestibility indices and N balance, in 12 Yorkshire×Landrace castrate male pigs weighing on average 50 kg of initial live weight. In experiment 1, 6 intact animals were housed in adjustable metabolism cages of the Dummerstoff type and were on every treatment during 14 days, nine corresponding to adaptation to diets and five for quantitative collection of faeces and urine, by the common procedures of the Institute, fully described elsewhere (Ly *et al*., 1998). The animals were individually were fed twice at 9.00 h and 13.00 h. The level of daily feed intake was 0.1 kg DM, per kg $W^{0.75}$. Drinking water was provided *ad libitum.* In experiment 2, 6 animals were surgically prepared by large intestine ablation according to the method of Green *et al.* (1987) as applied by Domínguez *et al.* (2000). The pigs were housed in the same metabolism cages above mentioned, during seven days previous to surgery and thereafter until the end of the experiment. Recovery period was of approximately two weeks and it was considered to be ended when animals ate its daily ration in a complete form. Then the animals were fed the same type of diet in the same manner as described in experiment 1. Every period consisted of 12 days of adaptation to diet and two days where ileal digesta was continuously collected every three hours periods, weighed, carefully mixed and a 10% aliquot was frozen until analysis (Ly *et al*., 1998).

Table 1: Characteristics of the experimental diets (% in dry basis)

	Zeolite, %	
Ingrendients	-	5
Maize meal	59.40	56.41
Soybean meal	17.60	16.72
Leucaena foliage meal	20.00	19.00
Zeolita	-	5.00
$CaPO_4H.2H_2O$	1.00	0.95
$CaCO_3$	0.50	0.48
NaCl	0.50	0.48
Vitamins[1]	0.50	0.48
Trace elements[1]	0.50	0.48
Análisis		
Dry matter	88.06	89.23
Ash	5.21	9.95
Organic matter	94.79	90.05
Crude fibre	10.05	9.55
N	2.29	2.18
Gross energy, kjoule g^{-1} DM	18.13	17.22

[1]According to NRC (1998) recommendations

Analysis: After thawing and thoroughly homogenized all samples of frozen faeces, pH was determined by a glass electrode in a digital pHmeter, then faecal sub-samples were used by suspending the fresh faeces in distilled water (1:4 by weight), mixing and squeezing the resulting slurry through three cheese cloth layers to obtain a diluted faecal liquor. In this resulting faecal sub-sample, total Short Chain Fatty Acid (SCFA) and ammonia were estimated by steam distillation in a commercial distilling machine, after treatment with either Pennington reagent, consisting of a saturated $MgSO_4$ solution in concentrate H_2SO_4, or concentrated NaOH (40%) as previously described by Phimmasan *et al.* (2004). Analysis of DM, ash, crude fibre and N were conducted in feed and faeces as recommended by the AOAC (1995). The organic content in samples was considered as the difference of 100 minus ash percentage. In acidified ($pH<3$) representative samples of urine, the N content was determined by the Kjeldahl procedure described by AOAC (1995) whereas gross energy in feed and faeces was assayed in an adiabatic bomb calorimeter. All analyses were conducted in duplicate.

The analysis of variance technique was used for contrasting means of treatments, according to Steel *et al.* (1997) taking into consideration Gill (1978) suggestions for a change over design applied to physiology of digestion studies conducted in pigs.

RESULTS AND DISCUSSION

Experiment 1: Faecal characteristics of pigs are shown in Table 2. Values of pH were significantly ($p<0.05$) higher in faeces of pig fed zeolite and this is in agreement with other authors (Collings *et al*., 1980; Cool and Willard, 1982; Ly *et al*., 1996, 2004; Ly and Castro, 1997). Faecal output of fresh material and water had no influence of treatment. As compared to several experiments using cereals for feeding pigs (Martínez *et al*., 2004) data listed in Table 2 for faecal output of fresh material had values rather low. However, Díaz *et al.* (2007) and Ly *et al.* (2004) reported a similar faecal output (equivalent to about 790 g fresh material kg^{-1} DM intake) in pigs fed 20% leucaena foliage in diets based either on sugar cane molasses or maize. In the current investigation, it was observed a slight increase in faecal ouput of materials when 5% zeolite was introduced in the diet. This would be a logical consequence of excretion of companion products induced by, or linked to the undigested zeolite. On the other hand, Zimmerman (1995) found an increase in faeces volume when pigs were fed clinoptilolite based diets, in line with the trend observed in the current experiment. Faecal concentration of SCFA and ammonia were significantly higher ($p<0.05$) in the diet containing zeolite (103 and 30 mmol 100/g DM) as compared to that with no zeolite (85 and 20 mmol/100 g DM), therefore indicating a trapping effect of zeolite on these metabolites. Evidently, faecal ouput in both types of compounds increased in the diet containing leucaena and zeolite. There are a consensus on the ability of zeolites for retaining ammonia in its molecular architecture (Nielsen *et al*., 1980; Venglovsky *et al*., 1998; Milic *et al*., 2005) but reports related to the property of zeolites for trapping SCFA are absent in our knowledge. In this connection, Poulsen and Oksbjerg (1995) observed a reduction in 30% of fermentation in the large intestine when clinoptilolite was added to the diet of pigs. *In vitro* apparent binding of other anions such as phosphate, has been reported by Thilsing *et al*. (2006) and the adsorption capacity of zeolites to bind several materials has been observed by Venglovsky *et al.* (1998).

Table 3 shows apparent digestibility of diets up to faeces. It was found that when zeolite was included in the diet, rectal digestibility of organic matter decreased from 83.3-77.4% ($p<0.10$) and N, from 78.3-72.4% ($p<0.05$). There was not significant effect of treatment on rectal digestibility of crude fibre and energy. If presence of zeolite in the diet should not be taken into account, then digestibility indices would appear to be higher than those from the control diet. On the other hand, N retention tended to be higher in the diet with zeolite as compared to the control (48.4 and 47.0% of intake). In support of present results, Shurson *et al.* (1984) observed that increasing graded levels of clinoptilolite in the diet reduced linearly N digestibility in piglets, but at the same time, an improvement in biological value of protein was patent. Data from the present studies are in accordance with Fokas *et al.* (2004) who found that in pigs given 2% clinoptilolite in the diet, an improvement in N retention of pigs was very evident (Fokas *et al*., 2004). The same findings were obtained by Parisini *et al.* (1999) when using 2% sepiolite. However, Poulsen and Oksbjerg (1995) did not observe any improvement in N retention of pigs fed clinoptilolite, although this zeolite elevated N excretion in faeces and lowered N excretion in urine.

Table 2: Faecal characteristics in pigs fed leucaena foliage meal and zeolite[1] (experiment 1)

	Zeolite, %		
	-	5	SEM±
Animals	6	6	-
Faecal indices			
pH	6.20	6.40	0.09*
DM, %	25.3	27.0	1.1
SCFA, mmol/100 g DM	85.2	103.4	12.4*
NH_3, mmol/100 g DM	20.8	30.0	5.1*
Faecal output per kg DM intake			
Fresh material, g	735	859	99
Water, g	549	627	105
SCFA, mmol	158	239	35*
NH_3, mmol	38	70	15+

[1]Predominantly clinoptilolite, +$p<0.10$; *$p<0.05$

Table 3: Rectal digestibility and N retention in pigs fed leucaena foliage (20%) and a Cuban natural zeolite[1] (experiment 1)

	Zeolite, %		
	-	5	SEM±
Animals	6	6	-
Digestibility, %			
Dry matter	81.4	76.8	1.6+
Crude fibre	48.3	42.2	6.6
Organic matter	83.3	77.4	1.3+
Energy	83.5	75.0	3.0
N	78.3	72.4	1.6*
Balance of N, %			
Retention:intake	47.0	48.4	3.3
Retention:digestion	60.1	62.0	3.4

[1]Predominantly clinoptilolite, +$p<0.10$; *$p<0.05$

Table 4: Ileal characteristics in pigs fed leucaena foliage meal and zeolite[1] (experiment 2)

	Zeolite, %		
	-	5	SEM±
Animals	6	6	-
Ileal indices			
pH	6.82	7.03	0.10
DM, %	13.2	14.9	1.3
SCFA, mmol/100 g DM	33.2	45.0	5.2*
NH_3, mmol/100 g DM	7.6	11.6	2.3*
Faecal output per kg DM intake			
Fresh material, g	2 601	2 208	353
Water, g	2 258	1 879	254
SCFA, mmol	113	148	12*
NH_3, mmol	26	38	6+

[1]Predominantly clinoptilolite, +$p<0.10$; *$p<0.05$

Experiment 2: The characteristics of ileal digesta is presented in Table 4. Ileal flow of digesta appeared to be low when zeolite was added to the diet. However, this influence was not significant. These findings are in contrast with previous results found in pigs fed graded level of leucaena foliage meal introduced in diets based on maize meal (Ly *et al*., 2004). The explanation for this information is not apparent, but it could be attributed to a somewhat zeolite counteracting effect on the cell wall included in the diet by leucaena. In this connection, relatively undigested, but non mineral materials such as guar gum and cellulose, in fact increase gut fill (Owusu-Asiedu *et al*., 2006) and it could be thought that these types of non starch polysaccharides should determine an increased ileal flow in pigs fed these type of materials included in the diet, therefore provoking an opposite effect to that caused by zeolite. On the other hand, it was found that either SCFA or ammonia concentration in the ileal content of pigs fed zeolite increased in a significant manner ($p<0.05$) as compared to the other, control diet. As a consequence and increase in ileal flow of SCFA ($p<0.05$) and ammonia ($p<0.10$) was then observed. This same phenomenum was encountered in experiment 1, strongly suggesting that the binding effect of zeolite was operative in the small intestine of pigs.

Ileal digestibility of energy and N increased ($p<0.05$), respectively when 5% zeolite was added to the diet (Table 5). There was no treatment effect on DM, organic matter and crude fibre digestibility at the ileum site. Ileal digestibility of leucaena foliage meal has been observed to be rather low in pigs, as compared to that of diets where no fibre sources were present (Ly *et al*., 1997; Buy and Lindberg, 2000). However, Buy and Lindberg (2000) found a considerably low ileal digestibility of crude fibre and other nutrients when pigs were given 15% leucaena leaves in the diet. This result, as compared to that of the current investigation, could be understood from the point of view of differences to be encountered when old or new tree leaves are going to be fed to pigs. The ageing process affects negatively nutrient digestibility of tree foliages (Samkol *et al*., 2005). On the other hand, the use of zeolite as additive to diets containing leucaena meal indicated a beneficial effect on nutrient digestibility, particularly for N fraction. Perhaps this improvement in N utilization at the ileal site could be related to some extent to zeolite properties of trapping undesirable, antinutritional substances present in leucaena. In fact, Zimmerman (1995) provided information supporting the hypothesis that clinoptilolite can sequester N within the faeces. In this connection, Smith (1980) reported a positive action of zeolite added to diets in order to minimize zearalenone toxicosis in rats and pigs. According to the experimental evidence derived from the present investigation, the sequestering action of zeolites high in clinoptilolite could be operative before and after the ileocaecal valve of pigs.

Table 5: Ileal digestibility and N retention in pigs fed leucaena foliage (20%) and a Cuban natural zeolite[1] (experiment 2)

	Zeolite, %		
	-	5	SEM ±
Animals	6	6	-
Digestibility, %			
Dry matter	65.7	67.1	2.2
Crude fibre	14.0	16.3	5.7
Organic matter	72.4	74.9	1.8
Energy	72.5	76.2	1.5*
N	61.6	65.9	1.4*

[1]Predominantly clinoptilolite, *$p<0.05$

Table 6: Digestion in the large intestine of pigs fed leucaena foliage (20%) and a Cuban natural zeolite[1]

	Zeolite, %	
	-	5
Animals	6	6
Digestion in large intestine, %[2]		
Dry matter	19.3	12.6
Crude fibre	71.0	61.4
Organic matter	13.6	3.9
Energy	13.2	1.0
N	21.3	8.9

[1]Predominantly clinoptilolite, [2]Percent of total digestion in the digestive tract

General: Table 6 lists the estimation of digestion in the large intestine, when ileal digestibility values were substracted from those of rectal digestibility). It was very evident that if zeolite was incorporated to feed, then pigs modified to a great extent digestion in caecum and colon. This was probably due to changes in retention time of digesta in the large intestine, which is known can strongly vary according to different factors (Van Leeuwen and Jansman, 2007; Wilfart *et al*., 2007). Moreover, the data reported herein could show that the small intestine is not necessarily the site where a decrease in digestion occurs when diets plus zeolite are given to pigs. According to this, a reduction in the contribution of the large intestine to total digestibility of DM and crude fibre was evident, accounting for 34.7 and 13.5%, respectively. In fact, it was observed in the present investigation that organic matter and energy digestibility was practically abolished in animals given leucaena foliage plus zeolite. Similar findings were previously observed in pigs fed leucaena meal and sugar cane molasses (Ly *et al*., 1998). These results should be in fully agreement with Poulsen and Oksberg (1995) who claimed a sharp reduction in SCFA production in the large intestine of pigs fed clinoptilolite. In a similar status, N disappearance in the large intestine lowered from 21.3-8.9% of total digested N and this represented an overall decrease of 52.8%. As it is well known, organic matter disappearance beyond the ileo-caecal valve in pigs determines some recuperation of energy for the host in form of SCFA, but it is considerable annulled by ammonia absorption, which in turn must be eliminated by the animal through urea in the so energy expensive Krebs-Henseleit cycle.

CONCLUSION

It is suggested that the inclusion of 5% of a Cuban natural zeolite in the diet is beneficial for digestive processes in the pig when this animal species is fed tree foliage such as leucaena leaves. The incorporation of zeolite to this type of diet could influence pig digestive processes in a positive manner, probably in N economy. Other trials could be conducted to fix the optimal level of inclusion of zeolite in this type of diets.

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