ILEAL FLOW OF N IN PIGS FED ON TORULA YEAST BASED DIETS

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SUMMARY

A 4x4 Latin square arrangement in a 2 x 2 factorial experiment was designed to study ileal flow of N in pigs fed on sugar cane high-test molasses based diets where the evaluated factors were torula yeast (Candida spp) prepared in two forms (dried, conventional, and liquid, cream), and two levels of feed intake (0.08 and 0.10 kg DM/kg$^{0.75}$ per day). The animals were four 40 kg castrate male pigs surgically prepared with a re-entrant cannula fitted in the terminal ileum.

There was no significant (P>0.05) interaction for any measurement concerning the evaluated factors. The nature of the examined types of torula yeast was more important in ileal digestibility of DM, organic matter and N (P<0.01) if compared to the effect of feed intake on these same indices (P<0.05). Accordingly ileal flow of materials was influenced in the same manner by the type of assayed yeast and feed intake. Ileal N digestibility of torula yeast either dry of liquid was 84.95 and 74.53%, respectively, whereas these values as determined by in vitro (pepsin/pancreatin) procedures were 92.52 and 88.64% for dry and liquid torula yeast, respectively.

A suggestion could be made that ileal nutrient digestibility of torula yeast in pigs, N compounds included, may be manipulated by varying the type of yeast and the level of feed intake. It should be of interest to search for other factors which should influence digestive processes in this animal species in order to increase efficiency of utilization of torula yeast.

***Key words**: pigs, ileum, digestibility, nitrogen, ammonia, torula yeast, sugar cane high-test molasses*

***Short title**: Ileal flow of N in pigs*

FLUJO ILEAL DE N EN CERDOS ALIMENTADOS CON DIETAS DE LEVADURA TORULA

RESUMEN

Se diseñó un cuadrado latino 4x4 en arreglo factorial 2x2 para estudiar el flujo ileal de N en cerdos alimentados con dietas basadas en miel rica de caña de azúcar, donde los factores fueron levadura torula (Candida spp) preparada en dos formas (seca, convencional, y líquida, en forma de crema), y dos niveles de consumo (0.08 y 0.10 kg MS/kg$^{0.75}$ por día). Los animales fueron cuatro cerdos machos castrados de 40 kg preparados quirúrgicamente con cánulas reentrantes colocadas al final del íleon.

No hubo interacción significativa (P>0.05) en ninguna de las medidas concernientes a los factores evaluados. La naturaleza de la levadura torula fue más importante en la digestibilidad ileal de MS, materia orgánica y N (P<0.01) que el nivel de consumo en estos índices (0.05<P<0.10). La digestibilidad ileal del N de la levadura seca y líquida fue 84.95 y 74.53% respectivamente, y estos valores determinados por procedimientos in vitro (pepsina/pancreatina) fueron 92.52 y 88.64%, respectivamente, para la levadura seca y líquida.

Pudiera sugerirse que la digestibilidad ileal de nutrientes de la levadura torula en cerdos, incluyendo la de los compuestos nitrogenados, puede ser manipulada haciendo variar el tipo de levadura y el nivel de consumo. Sería de interés buscar otros factores que pudieran influir en los procesos digestivos de esta especie animal con el fin de incrementar la eficiencia de utilización de la levadura torula.

***Palabras claves:** cerdos, íleon, digestibilidad, nitrógeno, amoníaco, levadura torula, miel rica de caña de azúcar*

***Título corto**: Flujo ileal de N en cerdos*

INTRODUCTION

A considerable research effort has been conducted concerning the use of torula yeast for feeding pigs in Cuba, particularly in those aspects relative to performance traits and digestibility of diets where torula yeast was either the only or the major source of protein (Figueroa and Ly 1990; Pérez 1997; Lezcano 2005).

In the particular case of the evaluation of digestive processes of pigs fed on torula yeast, although N digestibility of this

material is generally found to be high either at the ileal or at the rectal site (Boucourt 1982; Ly 1984; Maylin et al 1987; Díaz et al 1989; Figueroa et al 1990), it has been encountered that the level of feed intake may influence rectal digestibility of nutrients in conventional diets (Oude et al 1986). This can be applied to rectal N digestibility in particular in diets where torula yeast is the only source of protein (Piloto and Ly 2001). Since economical constrains usually prevent the use of torula yeast even as the major source of dietary protein, it is important to determine which factors could contribute to a better use of torula yeast in diets for feeding pigs, therefore reducing the cost of production of this animal species (Williams 1995).

This experiment was conducted to further evaluate N utilization by pigs given torula yeast and the influence of the methods of it production together with the effect of the level of feed intake on ileal digestibility of N.

MATERIALS AND METHODS

A 2x2 factorial arrangement following a 4x4 Latin square was designed to study ileal flow of N in pigs fed on sugar cane high-test molasses based diets were the evaluated factors were torula yeast prepared in two forms (dried, conventional, and liquid, cream) and two levels of feed intake (0.08 and 0.10 kg DM/kg$^{0.75}$ per day). Both types of torula yeast were of Cuban origin and were sent directed from the respective factories to the Institute. These torula yeasts (Candida spp) were grown on final cane molasses as substrate and contained in dry basis, 7.55% ash and 6.98% total N in the case of dry torula yeast (93.5% DM), and 9.75% ash and 6.28% total N in liquid torula yeast (15.5% DM). High-test sugar cane molasses was recently prepared and were sent from the sugar mill to the Institute too. As it is known, high-test sugar cane molasses does contain all carbohydrates from sugar cane, partially hydrolyzed to prevent crystal formation during storage after concentration by heat (Figueroa and Ly 1990). Details concerning the experimental diets are listed in table 1.
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Table 1. Characteristics of the diets (per cent in dry basis)

	Torula yeast	
	Dry	**Liquid**
Ingredients		
High-test sugar cane molasses	65.0	63.0
Dry torula yeast	33.5	-
Liquid torula yeast	-	35.5
NaCl	0.5	0.5
Premix[1]	1.0	1.0
Analysis		
Dry matter	83.98	31.48
Ash	6.81	7.61
Organic matter	93.19	92.39
N	2.56	2.56

[1] Vitamins and trace elements required according to NRC (1998)

The animals were four 40 kg Yorkshire castrate male pigs surgically prepared with a re-entrant chronic cannula fitted in the terminal ileum. The surgical procedure was conducted according to standard procedures (Markowitz et al 1964; Swindle 1998). Recovery period lasted approximately two weeks, when pigs apparently returned to its full feed intake capacity. Every experimental period consisted of seven days, the first six days used for adaptation of the animals to feeding level and diet, and the last day for collection of ileal digesta. The animals were weighed at the beginning of the experiment and thereafter every seven days in order to adjust feed intake according to the experimental schedule.

The pigs were housed in metabolism crates provided of a portable trough and a drinker. The dry torula yeast was stored in standard conditions, where liquid torula yeast was frozen until 24 hours prior to be given to the animals. This liquid torula yeast was subjected to an autolysis treatment at the factory and then added 0.2% formaldehyde (33% purity) prior to be frozen. The diet was prepared daily in the moment to be offered to the animals according to the feeding schedule, 0.08 or 0.10 kg DM/kg$^{0-.75}$ in one daily ration, given at 9:00 am.

Description of ileal digesta collection has been described elsewhere (Díaz et al 1989). Briefly, sample collection was made through plastic and flexible tubes attached to the barrel of the cannula, in order to quantitatively collect the digesta, which was deposited in a plastic reservoir placed in iced water, to prevent undesired fermentative activity of digesta. Ileal digesta were stored frozen at -5°C until analysis, then thawed, thoroughly mixed and a representative sample from every 24 hr was taken from pooled material for conduction of analyses.

DM and N concentration in ileal digesta was determined following standards procedures (AOAC 1990), starting from fresh materials, whereas ammonia determination in ileal samples was made according to the microdiffusion technique of Conway (1957) in supernatant aliquots of digesta after centrifuging the samples at 5 000 rpm for 15 minutes, then discarding the precipitate.

Ileal digestibility was determined by the usual methodology for direct determination of digestibility (Crampton and Harris 1969), whereas ileal output of materials was calculated considering daily flow adjusted to one kg of DM intake. In vitro digestibility of torula yeast was conducted by the two-step incubation method of Dierick et al (1985), using casein as protein standard. In this particular case, four samples from both torula yeast batches were obtained in every one of the four experimental periods of the investigation.

Data were subjected to analysis of variance according to Steel and Torrie (1980). In cases that significant differences ($P<0.05$) were encountered among means per treatment, these means were separated according to the multiple range and multiple F test of Duncan (Steel and Torrie 1980). The software package of Harvey (1990) was used for manipulation of data.

RESULTS

The pigs showed a healthy appearance and not feed refusal occurred during the experiment. There was no digesta leakage through the fistulas, and care was made to avoid any discomfort of the pigs throughout the described evaluation, which lasted 28 days.

There was no significant ($P>0.05$) interaction for any measurement concerning the evaluated factors. There was a significant ($P<0.01$) effect on ileal DM and N digestibility, when comparisons were made between dry and liquid torula yeast as the only source of protein (table 2).

Table 2. Ileal digestibility of diets. Effect of type of yeast

	Torula yeast		
	Dry	**Liquid**	**SE ±**
Ileal concentration			
DM, %	11.15	9.93	2.02
N, % in dry basis	4.25	4.51	1.01
NH_3, mmol/100 g DM	17.5	22.7	5.3
Ileal output, g/kg DM intake			
Fresh material	1 659	2 477	303*
Water	1 474	2 231	323*
Dry material	185	246	55
N	5.54	7.76	0.77*
Ammonia[1]	32.4	55.8	10.0*
Digestibility, %			
DM	81.5	75.4	1.7**
N	78.5	69.7	3.3**

[1] In mmol/kg DM intake
* P<0.05; ** P<0.01

The effect of the level of feed intake on ileal digestibility characteristics is shown in table 3. Increasing feed intake determined a significant (P<0.05) decrease in ileal DM digestibility. This same effect was only a non significant trend (P<0.10) for ileal N digestibility.

Ileal flow of DM and N showed a somewhat increase (P<0.05) in magnitude as determined by an increase in feed intake level, which in turn was accompanied by a slight, not significant (P>0.05) depression of ileal DM concentration (table 3). In contrast, a higher, significant (P<0.01) flow of ileal fresh material and water, was evident when feed intake level was the highest assayed. This phenomenum was very marked too in the case of ileal flow of ammonia (P<0.01).

Table 3. Ileal digestibility of diets. Effect of level of feed intake

	Feed intake, kg DM/kg$^{0.75}$		
	0.08	**0.10**	**SE ±**
Ileal concentration			
DM, %	12.6	8.48	2.66
N, % in dry basis	4.60	4.35	0.97
NH_3, mg/100 g DM	16.6	23.6	4.1
Ileal flow, g/kg DM intake			
Fresh material	1 325	3 113	285**
Water	1 158	2 849	308**
Dry material	167	264	50*
N	6.04	7.22	0.85*
Ammonia[1]	27.7	62.3	13.8**
Digestibility, %			
DM	83.3	73.6	2.8*
N	76.4	71.8	3.0$^+$

[1] In mmol/kg DM intake
$^+$ P<0.10; * P<0.05; ** P<0.01

It was found that in vitro digestibility of N was significantly (P<0.001) affected with respect to the nature of the evaluated types of torula yeast, otherwise found not to be low in dry torula yeast (table 4). Even in liquid torula yeast, in vitro N digestibility was relatively high. In the case of in vitro digestibility of casein, which was used as a standard material, the obtained values were practically complete. On the other hand, it is noteworthy to indicate that in vitro N digestibility as determined here, was considerably higher than the same indices for DM and organic matter.

Table 4. In vitro (pepsin/pancreatin) digestibility of torula yeast

	Digestibility, %		
	DM	**OM**	**N**
Torula yeast[1]			
Dry	80.87^a	81.48^a	92.52^a
Liquid	70.61^b	69.74^b	88.64^a
Casein	98.72^c	98.53^c	99.12^b
SE ±	1.73***	1.62***	2.82***

[1] Four representative samples from every yeast batch
*** P<0.001
^{abc} Means without letter in common in the same column differ significantly (P<0.05) among them

DISCUSSION

Types of yeast and ileal N digestibility

It has been found that different sources of protein may determine different ileal digestibility of DM and N in pigs (see for example, Heinz et al 1989; Nyachoti et al 1997), but there are very scarce knowledge related to digestive processes as influenced by different types of yeast, and even less when different methods of processing the same source of yeast is concerning. In this, as compared to dry torula yeast, limited information is really available concerning digestion of liquid torula yeast. With regard to Cuban dry torula yeast, Díaz et al (1990) observed that ileal digestibility of N was 75.4% in the dry yeast they used, assuming that N utilization from final molasses was negligible. On the other hand, Ly (1992) encountered that ileal digestibility of N of torula yeast accounted for 60.5-68.6% when given to growing pigs fed semipurified diets. Other results published by Apolonio et al (2003) in Brazil have indicated a higher ileal digestibility values for N of brewer yeast (Saccharomyces sp) as compared to sugar cane yeast (genus not specified), both in dry state. In these two types of yeast, ileal digestibility of N was 81.2 and 70.4% respectively according to Apolonio et al (2003). Other studies such as that of Heinz et al (1989) on presumably Candida and Saccharomyces type of yeast were in agreement with the herein reported values. In this connection, Heinz et al (1989) reported a value of 78.9% for ileal digestibility of yeast, determined by difference.

Maylin et al (1987) previously observed that formaldehyde preserved liquid torula yeast determined an evident decrease in in vitro N digestibility. This negative effect on rectal digestibility of N was observed by Ly et al (1990), when formaldehyde was added to a diet of syrup off and dry torula yeast offered to fattening pigs. This same effect could be present in the herein described experiment.

Several results provided from the Shinfield research team indicate that ileal flow of materials may be influenced by the type of diet given to pigs (Braude et al 1976; Low et al 1978; Low 1979). In this connection, the current investigation indicated that the nature of the protein source and the level of feed intake, as it was so in this investigation (see table 3), may influence ileal flow of materials in growing pigs.

Ammonia status in the distal ileum indicated that liquid torula based diets and increased feed intake level could determine either a high concentration or an increased flow of ileal ammonia. In fact, there was a clear influence of treatment on ammonia concentration in the ileum of the herein examined individuals, otherwise in agreement with that of other studies (Bolduan et al 1986). On the other hand, this same difference in detriment of the higher feed intake level examined in this experiment was also evident. A more pronounced presence of ileal ammonia, perhaps as consequence of bacterial activity in this site of gastrointestinal tract, could be linked to a certain decrease in ileal N digestibility of pigs, as it was encountered in this evaluation.

Feed intake and Ileal N digestibility

The absence of a marked effect of feed intake level on ileal digestibility of N was previously noted by others (Sauer et al 1982; Haydon et al 1984; Albin et al (2001). In this connection, very few changes in ileal flow of N have been observed in pigs after feeding a variety of diets (Low 1979). According to the general trend, otherwise confirmed here, that the level of feed intake has few if any influence on ileal digestibility of nutrients, particularly N compounds, it could be considered that differences found in nutrient digestibility at the rectal site, such as it have happened with torula yeast based diets (Piloto and Ly 2005), could be attributable to different responses of digestion in the large intestine of animals. This hypothesis merits to be confirmed in the case of torula, and perhaps other types of yeast given to pigs.

This assumption should arise from the fact that ileal flow of materials positively increases, and ileal DM concentration and apparent digestibility decreases, when feed intake increases to values which could be considered as describing an ad libitum status, as it happened in this experiment.

Ileal in vivo and in vitro digestibility interdependences

It has generally found that in vitro (pepsin/pancreatin) digestibility of N is relatively high, and the herein reported data are not an exception, in accordance with other previous Cuban estimations made with this type of material (Maylin et al 1985; Bouriche 1987). As illustration, Bouriche (1987) found a value for in vitro (pepsin/pancreatin) N digestibility of Cuban dry torula yeast, as high as 89.4%. On the other hand, It could be assumed that the assayed types of torula yeasts are representative of the Cuban microbial products, from the point of view of its chemical composition (López et al 2002; Macías et al 2002; Piloto and Macías 2005).

Ileal N digestibility of torula yeast was calculated assuming that ileal digestibility of N from molasses, 0.33% in dry basis in the current investigation, was negligible (Maylin et al 1987). For calculation, the two obtained values from dry and liquid products were used, after pooling results estimated from different feed intake. In these circumstances, average digestibility values were 84.95 and 74.53%. These data were not very near to the observed means when digestibility of N was determined by in vitro (pepsin/pancreatin) procedures in samples from the same torula yeast batches.

As it is well known, differences between observed in vitro and in vivo digestibility of N could be attributed to endogenous N present in ileal digesta of pigs as it has been claimed by several authors (Nyachoti et al 1997; Hess y Seve 1999; Stein et al 1999; Moter and Stein 2004). In this connection, Boisen and Fernández (1995) suggested that endogenous losses of protein and amino acids may vary considerably, in dependence on the chemical composition of the diet. The effect of endogenous N losses on ileal N digestibility was not examined in the current study.

In conclusions, a suggestion could be made that nutrient digestibility of torula yeast in pigs, N compounds included, may be manipulated by varying the type of yeast and the level of feed intake. It should be of interest to search for other factors which should be influencing digestive processes in this animal species in order to increase efficiency of utilization of torula yeast.

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