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LIVESTOCK SCIENCE

Livestock Science 108 (2007) 280-283

www.elsevier.com/locate/livsci

# Effect of nucleotides and carob pulp on gut health and performance of weanling piglets $\stackrel{\leftrightarrow}{\sim}$

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### Abstract

Nucleotides and carob pulp were tested as possible alternatives to antimicrobial growth promoters for newly weaned pigs. Four dietary treatments were used: basal diet, nucleotides, carob pulp and nucleotides + carob pulp. Performance was measured in a group of 72 pigs. Another group of 60 pigs was used for histological measurements of the jejunal mucosa and microbiological measurements by RFLP in ileum and caecum at different post-weaning intervals. Treatment did not affect performance. On the other hand, the morphology parameters of the jejunum were significantly (P < 0.001) affected by day after-weaning. No effects (P > 0.05) of treatment were observed on these parameters. The similarity (intra and inter-group) of the intestinal microbiota decreased with day after-weaning (P < 0.001) and was also affected by dietary treatment (P < 0.05). The nucleotide diets had the highest similarities in the ileum whereas the carob pulp diets had the lowest in the caecum. It is concluded that both nucleotides and carob pulp are able to modulate the changes in microbiota composition after weaning and that whereas nucleotides act in the ileum, carob pulp acts in the caecum.  $\mathbb{C}$  2007 Elsevier B.V. All rights reserved.

Keywords: Nucleotides; Carob pulp; Weaning piglets; Jejunal mucosa; Intestinal microbiota; Alternatives to antibiotics

### 1. Introduction

At weaning, pigs are exposed to several stressful conditions which alter their physiology and impair their normal growth. This situation has traditionally been dealt with antimicrobial growth promoters but their ban has encouraged the research for alternative products. Amongst these, carob pulp has been suggested to prevent diarrhoea (Lizardo et al., 2002) and nucleotides may support high demand tissues like the intestinal mucosa and the immune system (Martínez-Puig et al., 2005). The aim of this work was to evaluate the effects of nucleotides and carob pulp in diets for weanling piglets on performance and on several gut health parameters.

# 2. Materials and methods

The experimental procedures with animals described in this study were approved by IRTA's Ethical Committee on Animal Experimentation.

#### 2.1. Experimental

A total of 132 piglets  $(7.2\pm0.1 \text{ kg}; 26\pm2 \text{ days-old})$  that had not been offered creep feed during lactation were used. They were assigned to one of four treatments:

<sup>☆</sup> This paper is part of the special issue entitled "Digestive Physiology in Pigs" guest edited by José Adalberto Fernández, Mette Skou Hedemann, Bent Borg Jensen, Henry Jørgensen, Knud Erik Bach Knudsen and Helle Nygaard Lærke.

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 $<sup>1871\</sup>text{-}1413/\$$  - see front matter @ 2007 Elsevier B.V. All rights reserved. doi:10.1016/j.livsci.2007.01.080

Table 1

Effect of nucleotides, carob pulp or the combination of both on the morphology and the number of intraepithelial lymphocytes in the jejunal mucosa, and the relative weight of the empty small intestine

	Days	Basal diet	Nucleotides	Carob pulp	Nucleotides and carob	P values <sup>a</sup>		
						D	Т	$D \times T$
Villus height (μm)	0	689 <sup>ab</sup>	591 <sup>ab</sup>	699 <sup>a</sup>	513 <sup>b</sup>	0.001 (31.5)	0.536 (28.2)	0.880 (63.0)
	1	522	466	423	508	× /		
	2	440	435	445	421			
	6	345	338	332	319			
	14	447	392	483	433			
Villus width (μm)	0	128 <sup>a</sup>	101 <sup>b</sup>	122 <sup>ab</sup>	120 <sup>ab</sup>	0.001 (3.9)	0.098 (3.5)	0.539 (7.8)
	1	114	114	109	121			
	2	119	114	125	113			
	6	120	126	135	136			
	14	144 <sup>ab</sup>	125 <sup>b</sup>	137 <sup>ab</sup>	150 <sup>a</sup>			
Crypt depth (µm)	0	216	215	224	178	0.001 (11.3)	0.808 (10.1)	0.596 (22.7)
	1	176	166	202	196			
	2	160	186	191	203			
	6	265	317	278	279			
	14	340	305	317	293			
IEL (per 100 enterocytes)	0	4.0	5.4	3.7	3.0	0.001 (0.72)	0.210 (0.65)	0.481 (1.45)
	1	4.2	6.1	4.2	5.6			
	2	4.5	6.7	5.3	6.8			
	6	6.4	4.6	6.7	6.9			
	14	7.5 <sup>b</sup>	$8.8^{\mathrm{b}}$	7.8 <sup>b</sup>	13.3 <sup>a</sup>			
Small intestine relative weight (%BW)	0	1.9 <sup>b</sup>	2.6 <sup>a</sup>	2.6 <sup>a</sup>	$2.0^{b}$	0.001 (0.10)	0.061 (0.09)	0.037 (0.21)
	1	$2.0^{ab}$	1.6 <sup>b</sup>	1.9 <sup>ab</sup>	2.3 <sup>a</sup>			
	2	1.5 <sup>b</sup>	1.8 <sup>ab</sup>	1.7 <sup>ab</sup>	2.1 <sup>a</sup>			
	6	2.4 <sup>b</sup>	3.1 <sup>a</sup>	3.3 <sup>ab</sup>	3.1 <sup>a</sup>			
	14	3.9	3.5	3.7	3.6			

<sup>ab</sup>: means in the same row with different letters differ significantly (P < 0.05).

<sup>a</sup> Responses were obtained from ANOVA including the main effects: day post-weaning (D), dietary treatment (T) and their interaction  $(D \times T)$ . Values in brackets are pooled Standard Errors.

basal diet (BD), diet containing nucleotides (N), diet containing carob pulp (C) and diet containing both nucleotides and carob pulp (NC). Performance was measured at days 0, 14 and 28 post-weaning using 72 pigs that were kept in a 24-pen room (3 pigs/pen). The remaining 60 piglets, kept in a 12-pen room (5 pigs/pen), were used for histological and microbiological measurements. One animal per pen was killed at days 0, 1, 2, 6 and 14 post-weaning for sampling. The empty small intestine was weighed and villus height and width, crypt depth and intraepithelial lymphocytes (IEL) in jejunal mucosa (3 m from pylorus) were measured as described by Torrallardona et al (2003). Microbiota in ileal and caecal digesta was evaluated by restriction fragment length polymorphism (RFLP). Intra-group similarity was calculated by comparing the RFLP profiles from animals in the same treatment group. Inter-group similarity relative to the profiles at weaning (day 0) was calculated by comparing the RFLP profiles from animals in each treatment group with those of all the pigs on day 0. Finally, biodiversity was also estimated as the number of sequences compatible with those deposited in

the SSU Unal.gb file from the Ribosomal Database Project (Maidak et al., 1997).

# 2.2. Diets

A pre-starter basal diet (formulated to contain 12.5 g Lys and 10 MJ NE<sub>f</sub>/kg) was fed between days 0-14 and contained (g/kg): barley: 300, wheat: 297, peas: 50, whey: 80, wheat bran: 25, soy concentrate: 40, maize starch: 40, potato protein: 50, maize gluten meal: 22, sunflower meal: 25, vegetable oil: 31, molasses: 10 and amino acids, vitamins and minerals premix: 30. A starter diet (formulated to contain 11.4 g Lys and 10 MJ  $NE_f$ / kg) with basically the same ingredient composition as the pre-starter diet was used between days 15-28. Feed was presented in pelleted form and was offered ad libitum. Maize starch in diet BD was partially replaced with 30 g/ kg of carob pulp (BioNulpro®, Alimcarat S.A., Spain) in diets C and NC. A nucleotide rich yeast extract containing 25% of free nucleotides (Nucleoforce®, Bioibérica S.A., Spain) was added on top of diets N and NC at 1500 mg/kg.

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