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Addition of inulin, alfalfa and citrus pulp in diets for piglets: Influence on nutritional and faecal parameters, intestinal organs, and colonic fermentation and bacterial populations

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ABSTRACT

The effects of the inclusion of inulin, fresh alfalfa and fresh citrus pulp in diets for piglets on intake, nutritional and faecal parameters, digestive tract size, fermentation kinetics and colonic microbial populations was evaluated. Twenty-four cross-breed piglets (initial BW: 9.75 ± 1.63 kg) in a randomized complete block design were housed in metabolic cages and assigned to one of 4 treatments: 100% corn and soybean meal control diet (CON), 97% CON+3% inulin (INU), 95.5% CON+4.5% fresh alfalfa (ALF) and 95.5% CON+4.5% fresh citrus pulp (CIT). The experiment consisted of a 12 d adaptation period followed by 12 d for samples collection. The last 2 days of the experiment all animals were euthanized, the digestive tract of each animal was removed and an individual sample of colonic digesta was collected. Feed intake tended to be lower in the fibre supplemented groups ($P=0.098$), and was lower in ALF than in CIT ($P=0.026$). Groups receiving fibres tended to excrete more faeces ($P=0.088$), evacuated softer faeces ($P=0.041$) and presented a lower digestibility of CP ($P=0.003$). Retention of N was higher in the CON group than in fibre containing diets ($P=0.009$), and lower for ALF than for CIT ($P=0.034$). Colonic pH was lower in CIT and ALF treatments than in INU ($P=0.016$), *in vitro* gas volume was higher in the fibre-supplemented groups than in CON ($P=0.048$), but no differences between colonic bacterial counts were noticed among treatments. From the data obtained we conclude that the addition of fresh alfalfa and citrus pulp produce some beneficial effects in terms of promoting an increase in the hindgut fermentation, but negative aspects related to poorer nutrients absorption and N utilization can limit the usage of these fibrous sources.

1. Introduction

Dietary fibre is the sum of polysaccharides that escape enzymatic digestion of the host animal and include resistant starch, soluble and insoluble fibre and lignin (Metzler and Mosenthin, 2008). The inclusion of fermentable dietary fibre in the diet of pigs has different nutritional and physiological effects. Fibre alters the passage rate of digesta and may have undesirable implications diminishing feed consumption, daily gain and digestibility of

Abbreviations: ; A, asymptotic *in vitro* gas production; ADF, acid detergent fibre expressed inclusive of residual ash; ALF, control diet plus 4.5% fresh alfalfa; BW, body weight; CIT, control diet plus 4.5% fresh citrus pulp; CON, control diet; CP, crude protein; DM, dry matter; EE, ether extract; GIT, gastrointestinal tract; INU, control diet plus 3% inulin; NDF, neutral detergent fibre expressed inclusive of residual ash; OM, organic matter; OMD, organic matter disappearance; R_{max}, maximal rate of the *in vitro* gas production $T1/2$, half time of the asymptotic *in vitro* gas production; T_{max}, time of occurrence of R_{max}; WHC, water-holding capacity measured by filtration method

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nutrients (Owusu-Asiedu et al., 2006). However, the inclusion of moderate amounts of fermentable fibre in the diet may have positive effects in the consumer related to modification of the physicochemical properties of digesta and the enhancement in the production of organic acids in the gut, which are able to inhibit the growth of intestinal pathogens such as *Escherichia coli*, *Salmonella* spp. and *Clostridium* spp. in young pigs (Montagne et al., 2003; Lallès et al., 2007; Bindelle et al., 2008; Molist et al., 2014). Additionally, the inclusion of fermentable fibrous ingredients in the diet of pigs may produce a shift in N excretion from urine to faeces as a consequence of microbial protein synthesis in the hindgut and thus, a reduction in ammonia emissions (Monteiro et al., 2010).

Citrus pulp is rich in soluble fibre such as pectins (Bampidis and Robinson, 2006) that are rapidly fermented in the hindgut microbiota of monogastrics, and alfalfa is rich in insoluble fibre (cellulose) but also has fructans and up to 13% of pectins (Jung and Lamb, 2004). Both fibrous feedstuffs may be beneficial for young pigs in terms of intestinal health, microbiota profile and gut development (Molist et al., 2014). The functional activities in the gut metabolism of these supplements may have similarities with prebiotics, selectively fermented ingredients of the diet that allows changes in composition and activity of gastrointestinal microbiota conferring benefits to host wellbeing and health (Gibson et al., 2004).

Different authors (Loh et al., 2006; Lærke et al., 2007; Weber et al., 2008; Chen et al., 2013) refer to fibre sources as citrus pulp and alfalfa, including these fibrous sources in the diets in dehydrated or purified form (i.e. pectins). However, in some places of South America, industries that process fruits for juice sometimes do not have the necessary equipment to dehydrate or purify by-products, generating waste products, with the consequent environmental risks. At the same time, small farms near industries, usually use fresh by-products and pastures as supplemental feeds in swine production systems, without a clear reference about the benefits and limitations of their incorporation to the diets. Our hypothesis was that fresh citrus pulp and alfalfa included in moderate amounts in the diet of growing pigs could have a similar biological activity than inulin, a commercial and known prebiotic. The aims of this study were to evaluate the inclusion in the diet of piglets of two fibrous feedstuffs of different fibre characteristics (alfalfa and citrus pulp) and a prebiotic (inulin) on (1) intake and faecal characteristics; (2) digestibility of nutrients and N balance; and (3) GIT size, colonic fermentation and colonic microbial populations.

2. Materials and methods

All procedures were approved by the Bioethics Committee of Facultad de Veterinaria (UdelaR). The experiment was conducted at the Experimental Farm of Facultad de Veterinaria (San José Department, Uruguay; 34°S, 55°W).

Table 1
Ingredient and chemical composition of experimental diets.

	CON	INU	ALF	CIT
Ingredient (g/kg DM)				
Corn	521	505	497	497
Soybean meal	401	389	383	383
Olein	25	24	24	24
Dicalcium phosphate	15	15	14	14
Calcium carbonate	12	12	12	12
Salt	20	19	19	19
Vitamin–mineral mix[a]	3.0	2.9	2.9	2.9
L-Lysine	2.5	2.4	2.4	2.4
DL-Methionine	1.0	1.0	1.0	1.0
Inulin[b]	–	30	–	–
Alfalfa[c]	–	–	45	–
Citrus pulp[d]	–	–	–	45
Calculated digestible AA (g/kg DM)				
Lysine	13.6	13.2	13.2	13.0
Methionine	4.1	4.0	4.0	4.0
Threonine	7.9	7.6	7.7	7.6
Triptophan	2.5	2.4	2.5	2.4
Analysed nutrient composition (g/kg DM)				
Dry matter	891.7	893.0	775.0	741.7
Ash	84.1	71.4	76.2	71.7
Organic matter	915.9	928.6	923.8	928.3
Crude protein	222.3	192.3	191.1	183.5
Neutral detergent fibre	125.3	135.9	156.6	145.1
Acid detergent fibre	37.7	41.3	54.4	49.1
Ether extract	72.7	67.3	69.8	71.8
Metabolizable energy, (kcal/kg)[e]	3623.4	3636.8	3558.1	3624.9
WHC[f], g water/g dry feed	1.99	2.29	2.15	2.54

[a] Supplied per kg of feed: Vit. A, 9,000 IU; Vit. D3, 1,200 IU; Vit. E, 12 IU; Vit. B1, 1.2 mg; Vit. B2, 2.1 mg; calcium panthotenate, 9 g; Vit. B6, 1.8 mg; Vit. B12, 0.02 mg; nicotinic acid, 9 mg; Vit. K3, 0.7 mg; biotin, 0.6 mg; choline, 0.1 g; antioxidant, 72 mg; Mg, 0.15 g; Cu, 11 mg; Co, 0.45 mg; Zn, 0.1 g; Mn, 11 mg; Fe, 45 mg; Se, 0.1 mg; I, 0.6 mg.
[b] Frutafit HD, Saporiti (average chain length: ≥ 9 monomers).
[c] Alfalfa (g/kg DM): DM: 242.2; Ash: 93.5; CP: 214.9; NDF: 296.3; ADF: 150.3; EE: 36.1.
[d] Citrus pulp (g/kg DM): DM: 162.4; Ash: 37.6; CP: 66.4; NDF: 159.9; ADF: 105.8; EE: 32.2.
[e] Calculated according to Rostagno et al. (2005).
[f] Water-holding capacity by filtration according to Kyriazakis and Emmans (1995).

2.1. Animals and feeding

Twenty-four castrated crossbreed male piglets (Landrace × Large White) of 40 days of age (initial BW 9.75 ± 1.63 kg) from a commercial farm (Colonia Department, Uruguay) were individually allocated in metabolism crates (0.9 × 1.20 m^2). Animals were blocked by weight and assigned to one of four treatments (Table 1) with six pigs per treatment. The control diet (CON) was based in corn and soybean-meal, formulated following the nutritional recommendations proposed by FEDNA (2006). The piglets were provided with CON or diets in which CON was partly replaced with 3% inulin (INU), 4.5% fresh alfalfa (ALF) and 4.5% fresh citrus pulp (CIT). The inclusion of fibre sources was calculated on a dry matter basis. The level of inulin used was selected considering positive effects reported by others (Loh et al., 2006), and a pre-experimental period of 7 days was conducted to set the highest alfalfa and citrus level to be included in the diets without refusals by piglets. Therefore, the diets were not