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Quantification of soluble fibre in feedstuffs for rabbits and evaluation of the interference between the determinations of soluble fibre and intestinal mucin

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ABSTRACT

This work compared the quantification of soluble fibre in feeds using different chemical and *in vitro* approaches, and studied the potential interference between soluble fibre and mucin determinations. Six ingredients: sugar beet pulp (SBP), SBP pectins, insoluble SBP, wheat straw, sunflower hulls and lignocellulose, and seven rabbit diets, differing in soluble fibre content, were evaluated. In experiment 1, ingredients and diets were analyzed for total dietary fibre (TDF), insoluble dietary fibre (IDF), soluble dietary fibre (SDF), aNDFom (corrected for protein, aNDFom-cp) and 2-step pepsin/pancreatin *in vitro* DM indigestibility (corrected for ash and protein, *iv*DMi2). Soluble fibre was estimated by difference using three procedures: TDF–IDF (SDF_{IDF}), TDF–*iv*DMi2 (SDF_{ivDMi2}), and TDF–aNDFom-cp ($SDF_{aNDFom\text{-}cp}$). Soluble fibre determined directly (SDF) or by difference as SDF_{ivDMi2} were not different (109 g/kg DM, on average). However, when it was calculated as $SDF_{aNDFom\text{-}cp}$ the value was 40% higher (153 g/kg DM, $P < 0.05$), whereas SDF_{IDF} (124 g/kg DM) did not differ from any of the other methods. The correlation between the four methods was high ($r \geq 0.96$; $P \leq 0.001$; $n = 13$), but it decreased or even disappeared when SBP pectins and SBP were excluded and a lower and more narrow range of variation of soluble fibre was used. In experiment 2, the *iv*DMi2 using crucibles (reference method) were compared to those made using individual or collective ankom bags in order to simplify the determination of SDF_{ivDMi2}. The *iv*DMi2 was not different when using crucibles or individual or collective ankom bags. In experiment 3, the potential interference between soluble fibre and intestinal mucin determinations was studied using rabbit intestinal raw mucus, digesta and SBP pectins, lignocelluloses and a rabbit diet. An interference was observed between the determinations of soluble fibre and crude mucin, as contents of TDF and apparent crude mucin were high in SBP pectins (994 and 709 g/kg DM) and rabbit intestinal raw mucus (571 and 739 g/kg DM). After a pectinase treatment, the coefficient of apparent mucin recovery of SBP pectins was close to zero, whereas that of rabbit mucus was not modified. An

Abbreviations: aNDFom-cp, α-amylase neutral detergent fibre corrected for ash and protein; CP, crude protein; IDF, insoluble dietary fibre; *iv*DMi2, 2-step pepsin/pancreatin *in vitro* DM indigestibility corrected for ash and protein; *iv*DMi3, 3-step pepsin/pancreatin/viscozyme *in vitro* DM indigestibility corrected for ash and protein; SBP, sugar beet pulp; SDF, soluble dietary fibre determined directly; $SDF_{aNDFom\text{-}cp}$, soluble dietary fibre estimated as TDF–aNDFom-cp; SDF_{IDF}, soluble dietary fibre estimated as TDF–IDF; SDF_{ivDMi2}, soluble dietary fibre estimated as TDF–*iv*DMi2; SFF_{ivDMi3}, *in vitro* soluble and fermentable fibre estimated as TDF–ivDMi3; TDF, total dietary fibre.

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estimation of the crude mucin carbohydrates retained in digesta TDF is proposed to correct TDF and soluble fibre digestibility. In conclusion, the values of soluble fibre depend on the methodology used. The contamination of crude mucin with soluble fibre is avoided using pectinase.

1. Introduction

The influence of insoluble fibre, usually quantified as NDF (Mertens, 2003) on rate of passage and caecal fermentation has been well established in the rabbit (Gidenne, 1994; García et al., 2002). Soluble fibre affects caecal fermentation (Falcao-e-Cunha et al., 2004; Gómez-Conde et al., 2009; Rodríguez-Romero et al., 2011) and gut barrier function (Gómez-Conde et al., 2007) leading to lower mortality rate in rabbits (Trocino et al., 2013). However, there is no agreement in the method to quantify soluble fibre and the potential interference of soluble fibre with other substances when determining its digestibility (Graham et al., 1986). Soluble dietary fibre can be quantified directly (SDF, Prosky et al., 1985) or by difference between total dietary fibre (TDF) and NDF (Van Soest et al., 1991). When these methods are used, inaccuracies are unavoidable because of problems such as partial degradation of carbohydrates, incomplete extraction and precipitation of soluble fibre with the addition of ethanol or interference with other fractions of the feed (Hall et al., 1997; Prosky, 1999; McCleary et al., 2010; Martínez-Vallespín et al., 2011). Moreover, these methodologies might not evaluate correctly the true proportion of insoluble and soluble fibre in the digestive tract of the rabbit, because they prioritize the elimination of starch (utilization of hot buffers to gelatinize, hydrolyse and depolymerise the starch) and therefore the temperatures and pH values used are not within physiological ranges (Marlett et al., 1989; Monro, 1993). The two step pepsin/pancreatin i*n vitro* dry matter indigestibility (corrected for ash and protein, *iv*DMi2) can be used to measure the insoluble fibre content of ingredients under more physiological conditions. For example, the validated *in vitro* digestion proposed by Carabaño et al. (2008) to simulate small intestine digestion uses temperatures, pH and time similar than those existing in the rabbit gut. When the *in vitro* method is used the filtration step of the digested fractions is done using crucibles. However, it has been shown that the use of the ankom technology would simplify this technique as has been shown previously for NDF and ADF and other *in vitro* methodologies (Komarek et al., 1994; Fay et al., 2005).

The quantification of TDF and soluble fibre in ileal digesta or faeces might be affected by the contamination with endogenous substances (Wilfart et al., 2007), like mucin, an endogenous glycoprotein mostly constituted by carbohydrates (>700 g/kg, Mantle and Thakore, 1988) that covers the mucosa and resistant to digestion. Mucin is precipitated in ethanol, as occurs with soluble fibre, and it may result in a lower apparent ileal and faecal TDF and soluble fibre digestibility compared to the real one or even in negative digestibility values (Graham et al., 1986; Gidenne, 1992). Likewise, determination of crude mucin content in the digesta, by ethanol precipitation (Lien et al., 1997; Leterme et al., 1998; Libao-Mercado and de Lange, 2007; Piel et al., 2004) may be overestimated due to the lack of specificity as the residue may be contaminated with proteins and soluble fibre (Mañas and Saura-Calixto, 1993; Leterme et al., 1996).

The aim of this work was to confirm whether the quantification of soluble fibre using methods with different chemical and *in vitro* approaches renders similar results when using different fibrous ingredients and diets for rabbits. A second objective was to study the potential interference between soluble fibre and intestinal mucin determinations.

2. Materials and methods

2.1. Experiment 1

Seven rabbit diets and six ingredients were selected based on their soluble, insoluble and total dietary fibre and used to compare the accuracy of quantifying soluble and insoluble fibre content using different methodologies. The diets were based on sources of fibre currently used in rabbit feeds, whereas the ingredients were chosen to increase the range of variation of TDF. The ingredients used were wheat straw (Pagran, PITE S.A., Tordesillas, Spain), sunflower hulls (SOS Cuétara, Andújar, Spain), lignocellulose (Arbocel RC fine, Rettenmaier Ibérica S.L., Barcelona, Spain), sugar beet pulp (SBP, Fipec, Nordic Sugar, Copenhagen, Denmark), SBP pectins (Betapec RU 301, Herbstreith & Fox, Neuenbürg, Germany) and insoluble SBP. The latter ingredient was obtained by boiling SBP in a solution (13.5 L water with 0.4 kg sodium alkyl sulphate and 100 g EDTA per kg SBP) with a pH value of 7 (adjusted with NaOH) for 1 h. Afterwards, the mixture was filtered through nylon tissue (46 μm pore), washed with water overnight at room temperature to remove only the soluble constituents, dried at 70 °C and ground. Additionally, seven rabbit diets were used in the present study. Four of the diets contained 330 g aNDFom/kg DM and 161 g crude protein (CP)/kg DM. The control diet contained 360 g wheat starch, 154 g casein, with 180 g wheat straw and 180 g sunflower hulls per kg. A second diet was obtained by substituting 60 g of starch of the control diet by SBP pectins. Two more diets were obtained by substituting part of the fibrous sources (0.4) of the control diet by either SBP or by the insoluble SBP fibre, respectively. Another three diets contained 341 g aNDFom and 199 g CP/kg DM (Gómez-Conde et al., 2007) and were obtained by substituting part of the alfalfa hay by oat hulls and soybean protein concentrate or a mixture of SBP and apple pulp. Ingredients and diets were analyzed for TDF and insoluble dietary fibre (IDF), SDF, aNDFom corrected for CP (aNDFom-cp), *iv*DMi2 and 3-step pepsin/pancreatin/viscozyme *in vitro* DM indigestibility (*iv*DMi3 corrected for ash and CP).