Animal Feed Science and Technology 165 (2011) 191–200

Contents lists available at ScienceDirect

Animal Feed Science and Technology

journal homepage: www.elsevier.com/locate/anifeedsci

In vitro fibre fermentation of feed ingredients with varying fermentable carbohydrate and protein levels and protein synthesis by colonic bacteria isolated from pigs

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ARTICLE INFO

Article history:
Received 4 November 2009
Received in revised form 4 October 2010
Accepted 6 October 2010

Key words:
Fibre
Fermentation
Bacterial protein synthesis
Pig

ABSTRACT

An *in vitro* experiment was carried out using the gas technique to study the fermentation characteristics of different feed ingredients differing in their fermentable carbohydrate and protein composition by colonic bacteria isolated from pigs. The effect on *in vitro* bacterial protein synthesis was also evaluated. The ingredients used were wheat bran (WB), wood cellulose (Solka-floc®, SF), peas, pea hulls (PH), pea inner fibre (PIF), sugar beet pulp (SBP), flax seed meal (FSM) and corn distillers dried grains with solubles (DDGS). The samples were pre-treated with pepsin and pancreatin and the hydrolyzed substrates were then incubated with pig faeces in a buffered mineral solution. The nitrogen source in the buffer solution (NH_4HCO_3) was replaced by an equimolar quantity of ^{15}N-labeled NH_4Cl, used for the determination of the rate of bacterial protein synthesis. Gas production, proportional to the amount of fermented carbohydrate, was recorded for 48 h and modelled. The fermented product was subjected to short-chain fatty acids (SCFA) analysis. The source of fibre affected the *in vitro* dry matter degradability (*IVDMD*), the fermentation kinetics and the gas production profile ($P<0.05$). The highest ($P<0.001$) *IVDMD* values were observed for peas (0.80) and FSM (0.70), whereas SF was essentially undegraded (0.06). The fractional rate of degradation appeared to be lower ($P<0.001$) for WB and DDGS (0.07 and 0.05 h, respectively) and highest for SBP (0.20 h). Peas started to ferment rapidly (lag time 1.3 h). Half gas production ($T/2$) was achieved sooner for PIF (8.4 h) and was the longest for DDGS (19.8 h). The total gas production was the highest for PH, followed by SF, PIF and peas (276, 266, 264 and 253 ml/g DM incubated, respectively) and the lowest for FSM and WB

Abbreviations: ACE, acetic acid; ADF, acid detergent fibre; AOAC, Association of Official Analytical Chemists; BUT, butyric acid; BCFA, branched-chain fatty acids; BNI, bacterial nitrogen incorporation; CHO, carbohydrates; CP, crude protein; DDGS, corn distillers dried grains with solubles; DF, dietary fibre; DM, dry matter; FSM, flax seed meal; GC, gas chromatography; iCP, indigestible protein; *IVDMD*, *in vitro* dry matter degradability; N, nitrogen; NDF, neutral detergent fibre; NSP, non-starch polysaccharides; PRO, propionic acid; PH, pea hulls; PIF, pea inner fibre; RS, resistant starch; SBP, sugar beet pulp; SF, Solka-floc®; SCFA, short-chain fatty acids; WB, wheat bran.

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0377-8401/$ – see front matter
doi:10.1016/j.anifeedsci.2010.10.002

(130 and 124 ml/g DM incubated, respectively). There was no difference (P>0.05) in SCFA production after the fermentation of SF, P, PH, PIF and SBP (ranging from 3.8 to 4.5 mmol/g DM incubated) while WB and FSM yielded lowest (P<0.05) SCFA. The bacterial nitrogen incorporation (BNI), both at *T*/2 and after 48 h of fermentation was the highest (P<0.001) for PIF (18.5 and 15.6 mg/g DM incubated, respectively) and the lowest for DDGS and WB. In conclusion, peas and pea fibres had higher rates of fermentability, produced more SCFA and had high bacterial protein synthesis capacity. They thus have the potential to be included in pig diets as a source of fermentable fibre to modulate the gut environment and reduce nitrogen excretion.

1. Introduction

Swine nutritionists are working on strategies to reduce nitrogenous gaseous emission from pig facilities. To deal with this socio-technical issue, different approaches have been investigated (Aarnink and Verstegen, 2007). One of them consists in increasing fermentable dietary fibre (DF) in the pig diets. The non-starch polysaccharides (NSP) and resistant starch (RS) that escape small intestinal digestion are fermented by the caecal and colonic microbiota (Cummings and Englyst, 1987). Increased intestinal fibre fermentation has been reported to reduce the ammonia emission from manure (Mroz et al., 2000; Nahm, 2003) by shifting nitrogen (N) excretion from urine to faeces (Kirchgessner et al., 1994; Zervas and Zijlstra, 2002; Bindelle et al., 2009). This shift in the N excretion pathway is due to an increase in ammonia uptake by bacteria in the large intestine (Mosenthin et al., 1992). In addition, the fermentation of DF in the pig intestines increases the production of short-chain fatty acids (SCFA) (Houdijk et al., 2002), since DF is the main substrate for microbial fermentation in gut. Thus, DF inclusion might be an effective approach to modulate the gut environment and, at the same time, to reduce the footprint of pork production on the environment (Verstegen and Williams, 2002). However, most of the functional properties of DF have been obtained with isolated fibres or with wheat bran and sugar beet pulp, while these characteristics are also affected by the source of fibre and the fibrous matrix (Pieper et al., 2008; Jha et al., 2010). Moreover, the inclusion of isolated fibre in pig diets on a regular basis to reduce ammonia emission or modulate the gut environment is not economically justified. Thus, the use of feed ingredients with desirable properties instead of isolated fibre in pig rations to achieve these functional benefits makes more sense. However, many fibrous ingredients have not been investigated in this perspective yet. Many feed ingredients with highly fermentable DF are also rich in indigestible protein (iCP), which impairs the positive effect of DF fermentation on ammonia emission (Le et al., 2005). Again, limited information is available on the interaction of DF and crude protein (CP) fermentation, especially when it resides in the matrix of feed ingredients.

Bindelle et al. (2009) recently concluded that *in vivo* N excretion shifts observed with graded concentrations of fermentable DF in pig diets were correlated with enhanced bacterial N uptake in the large intestine, as measured by an *in vitro* fermentation technique. They suggested that the *in vitro* technique can be used to predict the N excretion shift from urine to faeces by measuring the bacterial nitrogen incorporation (BNI). *In vitro* models also provide information on different fermentation variables for a large number of ingredients in relatively short time. They are also cost-effective, easy to develop and non-invasive (Coles et al., 2005). In this method, part of the soluble CHO is lost during the filtration process which is practically inevitable in such an *in vitro* system and is considered to be the limitation of the method (Bindelle et al., 2007a,b, 2009). However, a significant part of the S-NSP is not washed out during the filtration step. This fraction is probably embedded in the insoluble fibre matrix. Nevertheless the model can generate valuable information as has been confirmed by results obtained with the method being comparable to data obtained *in vivo* (Bindelle et al., 2009). The present study was carried out to evaluate the fermentation characteristics of feed ingredients with varying fermentable fibre and indigestible protein content. It also aimed at evaluating their possible influence on the intestinal environment and nitrogen excretion, as indicated by the BNI, using an *in vitro* technique. The investigation was based on the hypotheses that feed ingredients differing in their content and type of fermentable fibre and indigestible protein affect the fermentation kinetics and their end products profile as well as bacterial protein synthesis in the pig's gastrointestinal tract.

2. Materials and methods

The animal handling for the study was performed in accordance with the recommendations of the Canadian Council on Animal Care (CCAC, 1993) as specified in the Guide to the Care and Use of Experimental Animals and the Standard Animal Care Protocol (No. 970019) approved by the University of Saskatchewan Committee on Animal Care and Supply.

2.1. Ingredients

Eight feed ingredients with potentially high levels of fermentable fibre content were studied: wheat bran (WB), wood cellulose (Solka-floc®, SF), peas, pea hulls (PH), pea inner fibre (PIF), sugar beet pulp (SBP), flax seed meal (FSM) and corn distillers dried grains with solubles (DDGS). They were selected based on their diversity of fibre and protein content. Their