Evaluation of two external markers for measurement of ileal and total tract digestibility of pigs fed human-type diets

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ABSTRACT: External markers for determination of nutrient digestibility have often been evaluated in conventional dry feeds but less often in conventional feeds such as human-type diets used in animal model studies. In the present study, 5 ileal-cannulated pigs were fed 5 types of soft bread-based diets supplemented with Cr₂O₃ and AIA as digestibility markers for 1 wk in a Latin square design. Ileal contents were collected twice for 5 h and a fecal grab sample was obtained once per week. Ileal and total tract digestibility of OM and nonstarch polysaccharides (NSP) based on the 2 markers were compared by linear regression. Across dietary treatments and site of collection, high correlation existed between digestibility values obtained with Cr₂O₃ and AIA, resulting in a R² > 0.77 (P < 0.001) and a linear relation close to unity. For ileal samples, the correlation was poor, particularly for NSP, which had R² = 0.09 (P = 0.14) whereas OM had an R² = 0.52 (P <0.001). On the other hand, fecal grab samples led to R² > 0.92 (P < 0.001) for both OM and NSP. However, AIA gave higher values than Cr₂O₃, particularly in samples with lower digestibility. The discrepancy is presumably caused by analytical difficulties due to a high fecal ash contents or interference with other components in the human-type diets.

Key words: acid insoluble ash, animal models, chromic oxide

INTRODUCTION

Indigestible markers are widely used to measure digestibility of nutrients and energy. Numerous studies have compared digestibility values obtained using markers such as Cr₂O₃, TiO₂, and AIA in pigs. Steady state conditions are prerequisite for obtaining reliable estimates (Agudelo et al., 2010). Furthermore, methodological considerations include risk of separation of marker and dietary components, intestinal content, or feces, particularly when cannulation techniques are used (Köhler et al., 1990; Mroz et al., 1996). Using liquid, nonhomogenous, or semimoist feed induces a greater risk of inhomogeneous distribution of marker within the diet. The objective of the present study was to compare digestibility of OM and nonstarch polysaccharides (NSP) between Cr₂O₃ and AIA using soft bread-based diets.

MATERIALS AND METHODS

The experimental diets consisted of 5 types of soft breads: (i) white wheat bread, (ii) dark milled rye bread, and (iii) whole kernel rye bread, which were commercial available soft breads provided by Schulstad Lantmannen A/S (Hammerholmen, Hvidovre, Denmark), and 2 experimental wheat flour-based breads supplemented with either (iv) concentrated β-glucan from oats (PromOat) and Vitacel WF600 (J. Rettenmaier & Söhne GmbH, Rosenberg, Germany) or (v) arabinoxylan (AX) from wheat produced at a local bakery (Konditor-Bageren, Ørum, Denmark). All breads except AX were supplemented with whey protein concentrate (Lacprodan 87; Arla Foods Ingredients Amba, Viby J, Denmark) to adjust CP content of the diet. All bread mixtures were supplemented with a mixture of vitamins and minerals, and markers were added at a concentration corresponding to 2 to 3 g Cr₂O₃/kg DM and 5 to 6 g Cr₂O₃/kg DM and 5 to 6 g
AIA/kg DM, respectively. Feed was portioned and stored at −20°C until consumption.

The animal experiment was conducted according to protocols approved by the Danish Animal Experiments Inspectorate, Copenhagen, Denmark. Five female crossbred (Duroc × Danish Landrace × Yorkshire pigs; 58 ± 2.8 kg) were fitted with a simple T-cannula 15 cm anterior to the ileocecal junction. After a 1-wk recovery, pigs were randomly assigned to experimental diets and fed 3 times daily at 0900, 1400, and 1900 h with 1 treatment per wk according to a Latin square design and a gradual change from 1 diet to the next over 2 d. Total daily feed intake was 1725 g DM/d and drinking water was provided ad libitum. Ileal digesta was collected continuously for 5 h after the morning meal on days 6 and 7, frozen immediately after collection, pooled, and freeze-dried prior to further analysis. Two drops of an aqueous solution of 0.02% sodium azide were added to the collection bags to prevent microbial activity. A fecal grab sample was taken on day 5 and freeze-dried. Chemical analyses were performed in duplicate. The DM, ash, and AIA were analyzed according to AOAC methods. Chromic oxide was analyzed as described by Schürch et al. (1950) and NSP according to Bach Knudsen (1997). In the current study, 2 markers were compared by linear regression across pigs, sampling time, and dietary treatments. Data were analyzed using PROC REG in SAS 9.2 (SAS Institute Inc., Cary, NC). Statistical significance was accepted at $P < 0.05$.

**RESULTS AND DISCUSSION**

Previously, similar total tract digestibility values of mixed foods were obtained in pigs and humans using either total collection or AIA as digestibility marker (Rowan et al., 1991). In the present study, pigs were used as an animal model for humans.

For both OM and NSP, better agreement existed between the estimates relative to AIA and Cr$_2$O$_3$ for the fecal spot samples than the ileal content, even though ileal content was collected twice for 5 h (Table 1; Figure 1). Hence, although $R^2$ between AIA and Cr$_2$O$_3$ was fairly high based on digestibility values across sample site, the $R^2$ was markedly reduced compared to fecal samples alone due to the very poor correlation for ileal samples. This finding contrasts with Yin et al. (2001) and others who have previously reported good agreement between AIA and Cr$_2$O$_3$ in estimations of ileal digestibility in traditional pig feed.

The OM and NSP digestibility across sampling site and ileal OM digestibility was not different from unity between markers (Table 1). However, when evaluating total tract digestibility alone, a strong correlation existed between the 2 marker estimates but a clear deviation from unity with an intercept significantly higher than zero and a slope below 1. A similar deviation has not previously been reported. A possible explanation for this apparent difference in the present diets could be the high total tract digestibility leading to high ash concentrations (14 to 44 g/kg DM) and possibly underestimation of AIA content or interference with other dietary components.

Moughan et al. (1991) found that naturally occurring AIA was a suitable marker for estimating total tract digestibility of DM, OM, and GE in young pigs provided that fecal grab samples were taken for at

| Table 1. Regression parameters (intercept, slope, and $R^2$) of digestibility coefficients (DC) for OM and nonstarch polysaccharides (NSP) calculated relative to AIA vs. DC calculated relative to Cr$_2$O$_3$ for both sample sites combined (ileum and total tract) and for ileal and feces samples separately, in which $DC_{(AIA)} = DC_{(CrO_3)} \times b + a$ |
|---|---|---|---|---|---|---|
| **Intercept (a)** | **Slope (b)** | **Model** |
| **Estimate** | **SE** | **$P$-value** | **Estimate** | **SE** | **$P$-value** | **$R^2$** | **$P$-value** |
| **DC$_{AIA}$** | **OM** | -0.10 | 0.077 | 0.20 | 1.13 | 0.089 | <0.001 | 0.77 | <0.001 |
| **NSP** | 0.04 | 0.042 | 0.40 | 1.00 | 0.074 | <0.001 | 0.80 | <0.001 |
| **DC$_{Ileum}$** | **OM** | 0.01 | 0.162 | 0.96 | 0.98 | 0.197 | <0.001 | 0.52 | <0.001 |
| **NSP** | 0.16 | 0.068 | 0.027 | 0.36 | 0.235 | 0.14 | 0.09 | 0.14 |
| **DC$_{Total}$** | **OM** | 0.29 | 0.041 | <0.001 | 0.71 | 0.045 | <0.001 | 0.92 | <0.001 |
| **NSP** | 0.28 | 0.023 | <0.001 | 0.72 | 0.030 | <0.001 | 0.96 | <0.001 |

Figure 1. Ileal and total tract digestibility of OM (left panel) and nonstarch polysaccharides (NSP; right panel) calculated relative to AIA vs. digestibility calculated relative to Cr$_2$O$_3$ (regression parameters in Table 1). Closed symbols and double cross = ileal digestibility; open symbols and single cross = total tract digestibility for the following breads: cross = white wheat; circle = milled rye; triangle = kernel rye; diamond = wheat flour plus arabinoylan; square = wheat flour plus β-glucan.
least 5 consecutive d. Then, the estimated digestibility was comparable to total collection and higher than with Cr$_2$O$_3$ as reference. In contrast, Mroz et al. (1996) found that total tract digestibility calculated relative to Cr$_2$O$_3$ gave results more similar to those obtained from intact pigs compared to total collection from steered ileocecal valve-cannulated pigs, presumably attributed to digesta leakage from the cannula.

In the present study, spot sampling of feces led to fairly similar result using AIA and Cr$_2$O$_3$, as also previously reported by Yin et al. (2001), whereas ileal collection resulted in scattering of results with poor correlation. For the estimated least square means of dietary treatments (data not shown), AIA led to higher SE than Cr$_2$O$_3$ in the ileum (0.017 vs. 0.012 for OM and 0.071 vs. 0.054 for NSP, respectively) but lower SE in feces (0.007 vs. 0.010 for OM and 0.037 vs. 0.052 for NSP). Based to the $R^2$ obtained from regression analysis and SE associated with digestibility coefficients of OM and NSP, we conclude that the 2 markers provided similar precision at the level of marker used for this type of diet.

**LITERATURE CITED**


