In vivo determination of effects on xenobiotica metabolism from dietary concentrations of xenobiotica including glucosinolates

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Abstract

Food and feed based on various types of plant materials and other food ingredients contain different types and concentrations of xenobiotica in addition to ubiquitous molecules. Xenobiotica comprise both essential nutrients as vitamins, some lipids, some amino acids, and antinutrients. Plants of the order Capparales comprising Brassicaceae and thus Brassica, oilseed rape and cabbage, contain structural different types and amounts of phenolics, glucosinolates, myrosinases (EC.3.2.1.147) and glucosinolate derived compounds. The effects from glucosinolates and glucosinolate derived products are especially important in relation to evaluation of the bioactivities of constituents present in diets including *Brassica*, rapeseed and cabbage. These compounds are often considered as antinutrients with potential toxic effects. However, some of the glucosinolates and/or products thereof can at some more or less unknown dietary concentrations be beneficial for the health. We have thus a need for experimental documentation of the dietary xenobiotica concentrations corresponding to borderlines where appreciable antinutritional effects are the result.

Balance trials with determinations of true protein digestibility (TD) and corresponding biological values (BV) was found to be of great value in experimental *in vivo* determination of xenobiotica effects of individual intact glucosinolates and their transformation products as basis for determination of acceptable dietary concentrations of the individual compounds. An acceptable dietary concentrations of glucosinolates was estimated to be 1 µmol/g feed dry matter (DM), but with very different levels of acceptable concentrations for the individual components.

Introduction

Xenobiotica can be beneficial or a health risk, antinutritional or toxic depending on chemical structure and concentration (Andersen *et al.*, 2010). The metabolism in all living organisms including animals and humans comprises complex groups of biomolecules, membranes and many enzyme systems which can be the target for xenobiotica binding and effects, especially in the liver, lungs and intestine (Vang *et al.*, 1995; Bonnesen *et al.*, 1999; Vang *et al.*, 2001). In food and feed from plant based materials many compounds are xenobiotic to the body, both essential nutrients like vitamins, lipids and amino acids which abut on antinutrient compounds (Bjerg *et al.*, 1984; Bjerg *et al.*, 1988; Eggum *et al.*, 1995; Bille *et al.*, 1983a; Bjerg *et al.*, 1989; Jensen *et al.*, 1991).

In plants of the order *Capparales*, and thereby the genera *Brassica* as oilseed rape, broccoli, cabbage, several xenobiotica are accumulated, such as phenolics, glucosinolates, myrosinase isoenzymes (EC3.2.1.147) and glucosinolate derived products (Bellostas *et al.*, 2007a; Bellostas *et al.*, 2007b; Andersen *et al.*, 2010). Glucosinolates are alkyl aldoxime-*O*-sulphate esters with a β-D-thioglucopyranosyl at the aldoxime carbon in Z-configuration to the ester group, which can be transferred into a variety of compounds depending on the structure of the glucosinolates, treatment conditions and possible action of myrosinase isoenzymes (Bellostas *et al*., 2008a; Bellostas *et al*., 2009), or in non-enzyme catalysed reactions (Bellostas *et al*., 2008b). In the last decades focus has been on glucosinolates and their potential biological effects. Glucosinolates or glucosinolate degradation products are most often considered as antinutrients and even toxic (Andersson *et al.*, 2008; Andersen *et al.,* 2010), while especially the broccoli glucosinolates are believed to be beneficial, and anticarcinogenic (Jeffery and Araya 2001; Vang *et al*., 2001). The chemical structure of the individual glucosinolates is, however, important for their specific bioactivity, which may be both positive and negative.

Rapeseed cakes are a widely used protein source in animal nutrition, but especially the monogastric animals are sensitive to too high concentration of glucosinolates or derived products present in the feed (Bille *et al.,* 1983a, Bjerg *et al.*, 1989; Andersen *et al.,* 2010). Feeding monogastric animals with rapeseed products produced from single low rapeseed (low content of erucic acid in the oil but high

content of glucosinolates; 50-150 µmol/g seed) or even from some of the double low rapeseed (less than 18 µmol/g but more than 8-10 µmol/g of glucosinolates in the seeds) give negative effects on the animals as a reduction in feed intake, enlargement of thyroidea, reduction in weight gain, and effect on liver (Bille *et al.*,1983a; Bjerg *et al.*, 1989; Schöne *et al*., 1997; Andersson *et al.*, 2008). The present work is a continuation of our previously performed investigations (*vide supra*) of structure-function of glucosinolates/glucosinolate derived products, with search for an *in vivo* method to reveal the concentration levels which gives effects on the xenobiotica metabolism – beneficial or risks for man and animals.

Balance trials as *in vivo* tools for determination of dietary effects on xenobiotica metabolism

One way to evaluate the biological effect of xenobiotica is the B. Eggum techniques based on balance trials (Bille *et al.*, 1983a, and refs. cited therein; Bille *et al.*, 1983b), by which the dose response also can be tested. These trials can be performed with use of optimized standard diet, where proteins are easily hydrolyzed and have the optimal amino acid composition and bioavailability. When measuring the intake and output the true protein digestibility (TD) and the biological value (BV) can be calculated.

True protein digestibility: $$TD\ \% = \frac{N_{conmsumed} - (N_{faeces} - N_{metabolic})}{N_{consumed}} x100$$

Biological value: $$BV\ \% = \frac{N_{comsumed} - (N_{faeces} - N_{metabolic}) - (N_{urine} - N_{endogene})}{N_{consumed}} x100$$

Net protein utilization: $$NPU\ \% = \frac{\mathrm{TD \times BV}}{100}$$

Thereby, both TD and BV are close to the theoretical value of 100 % which give a net protein utilization (NPU) = TD x BV = 100 %. This type of trials are efficient for *in vivo* determination of protein quality where a part of the standard protein is exchanged with the protein under evaluation, as imbalance in the amino acids will give reduction in BV. The balance trials have also been shown to be efficient to reveal xenobiotica in dietary concentrations which give effects on the xenobiotica metabolism either by disturbing and reducing BV or by xenobiotica effects on TD (Andersen *et al*., 2010).

Recently such a study was performed by Andersen *et al.*, (2010), where both individual glucosinolates were tested for effects on TD and BV, as well as the doses response and effects of individual glucosinolates + active myrosinase on the diets were investigated. These studies have given the basis for conclusions about the acceptable dietary concentrations of glucosinolates, which was found to be 1 µmol/g feed DM, but with very different levels of acceptable concentrations for the individual components.

Biological effects of glucosinolates

Rapeseed or *Brassica* glucosinolates is typically composed by a mixture of 10 – 15 glucosinolates, but most often quantitatively dominated by some few glucosinolates depending on the variety (Bellostas *et al.*, 2007b). The quantitative dominating glucosinolate of rapeseed is progoitrin, which is hydrolysed to goitrin ((5S)-5-vinyloxazolidine-2-thione) by myrosinase (Bellostas *et al.*, 2007a; Bellostas *et al.*, 2007b). Goitrin is known to affect thyroidea by inhibiting the enzymatical production of T3 and T4 hormones, which strongly affects growth (Bille *et al*.1983a, Bille *et al.*, 1983b; Tripati and Mishra, 2007). Another glucosinolate which is present in considerable concentrations is 4-hydroxyglucobrassicin, which has the thiocyanate ion as a degradation product (Bellostas *et al.*, 2007a). Thiocyanate ions also affect the thyroidea by inhibiting the uptake of iodide (Schöne *et al*., 1997).

Consumption of *Brassica* vegetables and broccoli glucosinolates has been associated with anticarcinogenic effects and other health related bioactivities (Jeffery and Araya, 2001). When investigating bioactivities focus has mainly been related to the presence of the aliphatic glucosinolate glucoraphanin and the indol-3-ylmethyl glucosinolates (Vang *et al*., 1995; Vang *et al*., 2001; Bonnesen *et al.*, 1999; Jeffery and Araya, 2001; Nho and Jeffery, 2001). Several authors have also investigated sulphoraphane and indol-3-carbinol, the enzymatic degradation products of glucoraphanin and glucobrassicin, for their capability to induce phase II detoxification enzymes in the xenobiotica metabolism (Jeffery and Araya, 2001; Vang *et al*., 2001; Nho and Jeffery, 2001). Induction of phase II enzymes, which was confirmed by these authors as an effect of sulphoraphane, has been related to cancer prevention.

Conclusion

To avoid unwanted or toxic concentrations of the xenobiotica in diets, it is recommended to use the mentioned balance trials as *in* vivo test procedure. The balance trials have been found to be of great value in experimental *in vivo* determination of xenobiotica effects of individual intact glucosinolates and their transformation products as basis for determination of acceptable dietary concentrations of the individual compounds.

The biological effects depend on the structure of the actual xenobiotica, and for glucosinolates this can be related to intact glucosinolates, non-enzymatic or enzyme catalyzed reaction products.

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