Bioavailability of glucosinolates in feed; uptake and metabolism of intact glucosinolates by pigs

Frandsen H.B.[1], Jensen S.K.[2], Kristensen, N.B.[2], Maribo, H.[3], Sørensen J.C.[1] and Sørensen H.[1]
1) Department of Basic Science and Environment, Biochemistry and Natural Product Chemistry, Faculty of Life Sciences, University of Copenhagen, Thorvaldsensvej 40, DK 1871- Frederiksberg C, Denmark
2) Faculty of Agricultural Sciences, Aarhus University, Blichers Allé 20, P.O.box, DK-8830 Tjele, Denmark
3) Danish Agriculture & Food Council, Pig Research Centre, Axeltorv 3, 1609 Copenhagen V, Denmark

Abstract
Glucosinolates are known as bioactive compounds with nutritional effects defined by their structure and dietary concentration. These effects vary from being beneficial to becoming health risks for monogastric animals and humans. It is often mentioned, but without experimental documentation, that intact glucosinolates only have limited or no biological effects whereas it is claimed that the bioactivities only are a result of glucosinolate transformation products, especially the products of myrosinase catalyzed glucosinolate transformations.
The present study was performed to evaluate the bioavailability of intact glucosinolates, when fed to pigs with a standard feed, which do not contain other crucifer constituents than the added glucosinolates. In one diet, a pig was fed with a diet containing benzylglucosinolate (10 mmol) and phenethylglucosinolate (3 mmol), and in another diet, a pig was fed with a diet containing sinalbin (10 mmol) and glucosinolates from rapeseed (5 mmol). Blood samples were taken from the portal, hepatic and arterial vein continuously before and until 6 hours after feeding. The next day the pigs were sacrificed and the digesta from the GI tract were collected.
The results of this study revealed conversion of glucosinolates into nitriles in the stomach, absorption of intact glucosinolates to the blood, glucuronation of sinalbin in the liver and excretion of intact glucosinolates as well as sinalbinglucuronide to the urine.

Introduction
Glucosinolates are alkyl aldoxime-O-sulphate esters with a β-D-thioglucopyranosyl group at the aldoxime carbon in Z-configuration to the ester group. These compounds are present in all plants of the order Capparales (Bellostas *et al*., 2007) co-occurring with myrosinase isoenzymes (EC.3.2.1.147). Upon cell membrane disruption, mixing of glucosinolates and myrosinase in the presence of water results in glucosinolate transformation into a variety of compounds depending on the structure of the parent glucosinolate and reaction conditions (Bellostas *et al*., 2007). However, non-enzymatic transformations of glucosinolates can also occur, and lead to formations of nitriles in environments with reducing conditions and the presence of e.g. Fe^{2+}, ascorbic acid and thiol groups (Bellostas *et al*., 2009). These reactions could thus take place in the stomach, where the pH is approximately 3 and reducing agents like e.g. thiols and ferro ions from the feed would be present.
Individual glucosinolates and glucosinolate derived products call for special attention owing to their bioactivities and different biological effects which are closely defined by their specific chemical structures, their bioavailability and concentrations in the target tissues, cells or organisms (Andersen *et al*., 2010 and refs cited therein). It is thus well known, that goitrin (5R)-5-vinyloxazolidine-2-thione), and the structural isomeric 5-phenyloxaxolidine-2-thiones exhibit quite different effects on the xenobiotica metabolism as revealed from biological values determined by in vivo balance trials (Andersen *et al*., 2010). Toxicological studies performed by other research groups with determination of LD_{50} values demonstrate as well, that the biological effects largely depend on the structure of the glucosinolates and derived products, especially those produced as oxazolidine-2-thiones, dithiocarbamates, thioureas and nitriles, whereas only limited information are available concerning effects caused by products of indol-3-ylmethylglucosinolates (Bellostas *et al.*, 2007; Andersen *et al.*, 2010 and refs cited therein).
Intact glucosinolates present in diets without the presence of active myrosinase are as well the source of bioactive compounds. An excellent review on bioavailability of glucosinolates with special focus on isothiocyanate derived products has been published by Holst and Williamson (2004). The formation of isothiocyanates follow myrosinase catalysed hydrolysis of glucosinolates (Bellostas *et al*., 2009) but non-enzymatic transformations of glucosinolates can also occur (Bellostas *et al.*, 2008). Results from several in vivo studies confirm a decrease in ingested glucosinolate concentrations as these passes from the stomach to the small intestine (Freig *et al*., 1988; Michaelsen *et al*., 1994; Elfoul *et al*., 2001).

This loss could be due to transformation in the stomach (vide supra) or due to absorption of glucosinolates to the blood from the small intestine. In vitro investigations indicates thus that the glucosinolate transport across the intestine wall is facilitated or passive (Michaelsen *et al*., 1994). Moreover, recent work has been able to confirm the presence of intact glucosinolates in plasma (Song *et al*., 2005; Bheemreddy and Jeffery, 2007; Cwik *et al*., 2010), however in much lower concentrations than found in consumed diets.
The purpose of the present work was a continuation of previously performed studies of the transformation of intact glucosinolates in the digestive tract and to follow the absorption and possible biotransformation of these compounds.

Materials and methods
Two 70 kg pigs (LYxD) with permanent catheters were fed a standard diet; in the diet for pig A benzylglucosinolate (10 mmol) and phenethylglucosinolate (3 mmol) were added, and in the diet for pig B, sinalbin (10 mmol) and glucosinolates from rapeseed (5 mmol) were added. Blood samples were taken from the portal, hepatic and arterial vein continuously before and until 6 hours after feeding, and a urine sample was collected during the day and during sacrification. The next day the pigs were sacrificed and the digesta from the GI tract were collected. The material and methods for analytical work performed on the biological samples has been described elsewhere; preparation of crude extracts, (Sørensen *et al*., 1999), crude extracts analyses by MECC (Bellostas *et al*., 2006) and analysis of desulfoglucosinolates by MECC (Bjergegaard *et al*., 1995).

Results and discussion
In order to follow possible glucosinolate transformation it was chosen to feed one pig with benzylglucosinolate and phenethylglucosinolate, which both contain an aromatic ring, and thus a chromophore which makes the glucosinolates as well as possible produced nitrilles easy to detect with the applied methods. The other pig was fed rapeseed glucosinolates and also sinalbin, which also have the advantage of an aromatic ring.
Crude extracts of digesta from stomach, duodenum, jejunum, ileum, caecum and colon were analyzed by micellar electrokinetic capillary chromatography (MECC). Phenethyl- and benzyl glucosinolates as well as their corresponding nitriles were detected in digesta from stomach and duodenum. In the portal vein sinalbin was detected, and in the hepatic vein it was possible to follow a transformation product which had a similar UV spectrum to that of sinalbin. This product was identified by LC-MS and NMR to be sinalbin glucuronide, corresponding to O-glucuronation of phenols in phase II xenobiotic reactions.
In the urine samples it was possible to detect progoitrin (2.0 µmol/g DM), epiprogoitrin (0.03µmol/g DM), napoleiferin (0.1 µmol/g DM), gluconapin (1.17 µmol/g DM), sinalbin (3.36 µmol/g DM) and sinalbin glucuronide (8.26 µmol/g DM) by MECC, whereas isothiocyanates and other products expected from myrosinase catalysed glucosinolate hydrolyses were not present in agreement with the lack of myrosinases (Bellostas *et al*., 2009).
Methanol-water extracts of plasma and urine samples were purified by column chromatography and on-column desulfated by sulfatase and following desulfoglucosinolates were then analysed by MECC .The plasma from pig A did not contain detectable levels of glucosinolates, but the plasma from the portal vein of pig B did. Over time, starting with 90 min from the feeding time, sinalbin and sinalbin glucuronide could be detected (Figure 1).

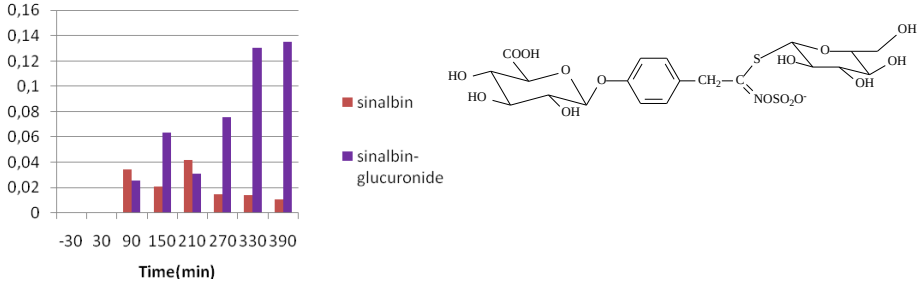

Figure 1: Relative content of sinalbin and sinalbin glucuronide in portal vein at different time from feeding time determined by MECC of desulfated samples from anion exchange. Structure of sinalbin glucuronide is shown in the right part of the figure.

Sinalbin glucuronide was thus detected in plasma from the hepatic vein together with minor concentrations of intact sinalbin sampled 1.5 hour after ingestion, and samples taken after 5.5 and 6.5 hours respectively showed increase of 10 and 13 times the concentration of sinalbin.
Similar crude extracts of urine samples were analysed with MECC, and these samples were desulfated on an anionic exchanger. Urine from pig B collected few hours after feeding had a relatively

high content of progoitrin (2.0 µmol/g DM freeze dried urine), epiprogoitrin (0.03µmol/g DM), napoleiferin (0.1 µmol/g DM), gluconapin (1.17 µmol/g DM), sinalbin (3.36 µmol/g DM) and sinalbin glucuronide (8.26 µmol/g DM) detected by MECC. The results have shown that minor amounts of intact glucosinolates are absorbed via the gut epithelium to the blood which, however, only accumulated the compounds to low concentrations and for a short time. Then the compounds are metabolized and/or excreted with the urine. The results now obtained are in good agreement with other studies (Cwik *et al*., 2010; Song *et al*., 2005). Detection of compounds in plasma and urine should be seen as a balance between absorption rate and excretion, dilution in blood, and metabolism.

Glucosinolates and glucosinolate transformed products have different biological effects depending on the specific chemical structures (Andersen *et al*., 2010). It is well known that goitrin, the myrosinase catalyzed product from progoitrin, has a negative effect on thyroidea, and thus the growth of animals (Bille *et al*., 1983), while epigoitrin from enzyme catalyzed epi-progoitrin does not affect thyroidea, although the biological value is reduced (Andersen *et al*., 2010). The fact that these two compounds, which are structurally identical except for being stereoismers, have different biological effects shows how specific the mechanisms are.

Toxicological studies of LD50 values produced by other groups (refs. cited in Andersen *et al*., 2010) demonstrate that the biological effect very much depend on the structure of the glucosinolate or derived product, especially the enzyme catalyzed transformation products oxazolidine-2-thiones, dithiocarbamates and thioureas, but also nitriles cause an effect on the biological value (Andersen *et al*., 2010). For most glucosinolates a higher concentration of intact glucosinolates are tolerated before reduction in the biological value is seen; compared to experiments where myrosinases were present (Andersen *et al*., 2010). A reduction in the biological value would be associated with absorption of appreciable amounts of bioactive xenobiotics, and it is thus interesting to gain more insight into the possible non-enzymatic transformation of the intact glucosinolates into nitriles e.g. in the stomach, and possible absorption of intact glucosinolates or derived products.

Conclusion

This study revealed conversion of glucosinolates into nitriles in the stomach, absorption of intact glucosinolates to the blood, glucuronation of sinalbin in the liver and excretion of intact glucosinolates as well as sinalbin glucuronide to the urine. Further studies will be focused on investigations of the percentage of non enzymatic transformation in the stomach as well as the bioavailability of glucosinolates, and glucosinolate derived compounds and their biological effects.

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