FROM SEED TO CURE: ASPECTS OF CULTIVATION, PREPARATION AND ADMINISTRATION OF *TROPAEOLUM MAJUS* L.

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Abstract

Nasturtium (*Tropaeolum majus* L.) is a herb with a proven antimicrobial activity due to benzyl-isothiocyanate, the degradation product of glucotropaeolin. A non-destructive harvest of leaves in combination with a gentle drying procedure at 40 °C delivered the highest concentration of extractable glucotropaeolin. In an experiment with piglets, direct and graded supplementation of *T. majus* with feed was performed over a period of five weeks. *T. majus* was supplemented at an upper dosage of 1 g kg^{-1} with the feed, equaling 48.7 mg kg^{-1} glucotropaeolin, which resulted in a benzyl-isothiocyanate concentration in the urine of up to 16 µmol l^{-1}. This concentration ought to be high enough to control a broad range of bacteria. Up to 7.3% of the glucotropaeolin taken up by the animals was excreted as bioactive benzyl-isothiocyanate via the urine. No effect was observed on the intestinal microbiota and supplementation with *T. majus* had also no effect on growth performance of healthy piglets.

Alternative strategies to stabilize health and performance of livestock animals gained in importance since the ban of antibiotics as feed additives in animal nutrition in the European Union in 2006. Phytopharmaceuticals with a proven efficiency in humans may offer a special prospect in animal nutrition, too. Different medical herbs such as oregano, clove, thyme, peppermint, fennel, caraway, lemon grass and many others have been tested with respect to their stabilizing or health promoting effects but results proved to be inconsistent (Gollnisch 2002). A possible reason for incoherent findings can be a lack of quality control, particularly the missing quantification of active ingredients in the supplemented herbs.

Tropaeolum majus is an herbal plant that meets 'Commission E' standards (Blumenthal *et al.* 1998) and the phytopharmaceutical activity is well documented (Anon, 1985). Guidelines for cultivation and conditioning of the harvested plant material have been elaborated, too (Bloem *et al.* 2007). The antimicrobial activity of *T. majus* is caused by benzyl-isothiocyanate (benzyl-ITC) the degradation product of glucotropaeolin (GTL). GTL is an aromatic glucosinolate (GSL), which is produced in high concentrations in leaves (Lykkesfeldt and Lindberg-Møller 1993). In *T. majus* GTL

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is the single GSL. When the plant tissue is disrupted GTL is immediately hydrolyzed by the endogenous enzyme myrosinase, which is separated from the GTL in the intact tissue, and a range of breakdown products is released including the biologically active benzyl-ITC, which causes antimicrobial, antifungal and virostatic effect.

The GTL-content is a highly variable parameter because of the fast decomposition after injury of the plant tissue. Therefore the harvest procedure and the post-harvest treatment are of special relevance when growing GSL-containing crops for phytopharmaceutical use. Besides harvest conditions and post-harvest treatment, other relevant parameters influence the GSL-content in crops such as sulfur fertilization, light intensity, temperature, growth stage of the crop, plant part, interactions with pathogens and genetic variability (Rosa *et al.* 1997; Schnug 1997).

From results, which were obtained from 2001 to 2003 at two different sites in Germany (Hessisch Oldendorf and Braunschweig) the influence of different factors on variation of the GTL-content of *T. majus* was determined (Fig. 1). The harvesting technique and the drying procedure are the factors that caused the highest variation in the GTL-content because of GTL degradation during harvest and processing of the plant material. So, high drying temperatures can cause a complete loss of GTL. In addition, it is important to harvest leaf material prior to stems as leaves have a 3 to 6-fold higher GTL-content than stems (Bloem *et al.* 2007). Sulfur fertilization with 100 kg ha^{-1} S enhanced the GTL-content in leaves and seeds significantly by factor 1.4 – 2.1, and 1.6 – 1.8, respectively (Bloem *et al.* 2007). Site-specific characteristics such as soil type and local climatic conditions in different years also influence the GTL-content (Fig. 1). Precipitation, temperature and sunshine hours had no significant effect on the GTL-content during three years of experimentation in Germany. *T. majus* is a plant, which originates from the tropical climate of South America and the impact of climatic conditions on the GTL-content was shown in a comparative cultivation of *T. majus* in Egypt and Germany. The GTL-content proved to be eight times higher under semi-arid conditions in Egypt compared to the temperate conditions in Germany (Bloem *et al.* 2002).

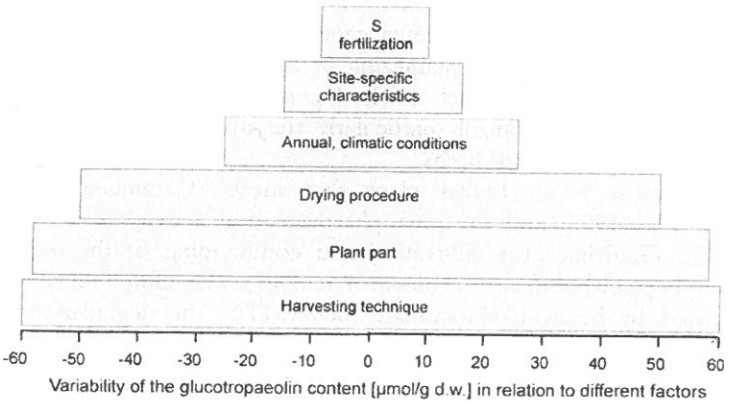

Fig. 1. Influence of different factors of variation with significant influence on the GTL-content of *T. majus*. (data basis: Results from two sites, Braunschweig and Hessisch Oldendorf in the years 2001-2003).

For maintaining a high content of GTL a harvesting technique, which preserves the intact cell structure such as gentle drying at 40 °C proved to deliver the highest concentration of GTL when mainly leaf material was harvested by hand with a sickle and S was fertilized in adequate amounts. Up to now no experimentation has been carried out with piglets to study the potential of *T. majus* to promote health and growth performance. In a feeding experiment with weaned piglets, the influence of feed supplementation with *T. majus* on animal performance and excretion of the bio-active benzyl-ITC was investigated in relation to the GTL dose (Bloem *et al.* 2008). The therapeutic effect of low-dose amendments of the medical plant *T. majus* needs to be clearly distinguished from the antinutritional effects caused by high GSL-contents in feedstuff like extracted rapeseed meal. Dose-effect relationships with GSLs have been determined for all groups of livestock animals with view to growth performance, quality and sensory features of meat products when GSL-containing feedstuff was administered (Tripathi and Mishra, 2007). For pigs no adverse effects were reported up to a total GSL-content of 0.78 μmol g^{-1} diet (Tripathi and Mishra 2007). In the present experiment the maximum total GSL-content accounted for 0.12 μmol g^{-1} diet and thus was distinctly below the critical threshold.

In an experiment with piglets, direct and graded supplementation of *T. majus* with the feed was performed over a period of five weeks (Bloem *et al.* 2008). Four experimental groups each consisting of 20 piglets were studied: One control group without feed supplementation, one group that received 25% less than the recommended dosage (75% GTL), one which received the recommended dosage (100% GTL) and one that received 25% more (125% GTL). Additionally, one piglet per treatment was kept in a metabolic cage throughout the experiment to collect urine. Considering the GTL-content of the seeds, body weight of the piglets and feed intake a corresponding amount of 0, 0.6, 0.8 and 1.0 g ground seed material was added to 1 kg feed, equaling to at maximum 48.7 mg kg^{-1} TL. This resulted in a maximum benzyl-ITC concentration in the urine of 16 μmol/L. In the control group no ITCs were determined in the urine while in the other three groups a mean value of 3.8 (75% GTL), 12.7 (100% GTL), 9.3 μmol l^{-1} (125% GTL) was determined. The highest concentration was found in the group that received the medium GTL-content; this result reflects the differences in drinking behavior of the piglets. Two times during the experimental period a balance between GTL intake and ITC excretion was determined. Up to 7.3% of the GTL taken up by the animals was excreted as bioactive benzyl-ITC via the urine (Fig. 2). The balance also revealed that the highest amount of ITC was excreted by the piglet with the highest dose of GTL. The ITC concentration of the urine is an important criterion for the antimicrobial potential of the feed supplementation. Concentrations of 0.5 – 50 μg ml^{-1} benzyl-ITC are reported to control a broad range of bacteria and 0.3 – 12 μg ml^{-1} acted fungistatic (Anon, 1985). The results of the experiment revealed that the ITC concentration in the urine was sufficiently high to yield an antimicrobial effect in all treatments where the feed was supplemented with *T. majus* (Bloem *et al.* 2008). No significant effect of the feed supplementation was observed on the intestinal microbiota (Pieper *et al.* 2007) but the *Shannon* index of biodiversity increased slightly with the dose of *T. majus* supplementation, which is an indication for a more stable microbial community. Feed supplementation with *T. majus* had no effect on growth performance of healthy piglets and the feed intake was not affected. It was observed that the piglets with the highest

rate of feed supplementation with *T. majus* watered more, very likely because of the hot taste of the feed caused by benzyl-ITC.

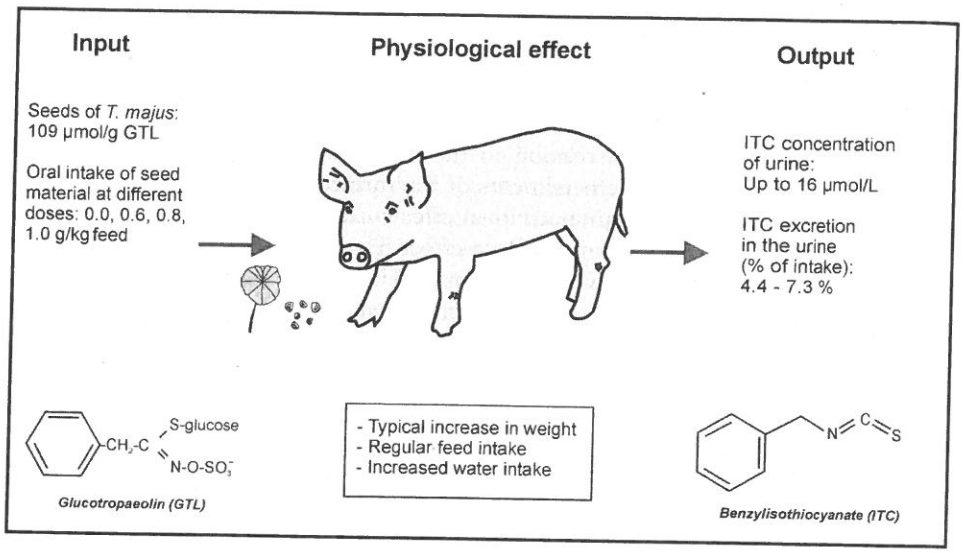

Fig. 2. Influence of *Tropaeolum majus* as feed additive on the performance of piglets as well as isothiocyanate excretion with the urine.

The results of the presented feeding experiment provide clear evidence that feed supplementation with *T. majus* has no adverse effects on growth performance and feed intake of piglets. Feed supplementation with *T. majus* yielded benzyl-ITC concentrations in the urine that ought to be sufficiently high to unfold antimicrobial activity. Adaptation of application rates from human studies proved to be adequate for adjusting the dose of bio-active GTL in animal nutrition. The proof whether *T. majus* has a health promoting effect in animal nutrition needs verification. Thus further experiments are required to test the therapeutic effect of *T. majus*. Of special interest is the prophylactic and curative effect of *T. majus* in feed supplementation of sows which suffer from the Mastitis-Metritis-Agalactia-syndrome as the disease shows similarities with urethral infections in humans, where *T. majus* proved to have significant health effects.

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