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Influence of feed supplementation with *Tropaeolum majus* L. on growth performance and urine isothiocyanate concentration of piglets

Einfluss von Kapuzinerkresse (*Tropaeolum majus* L.) als Futterzusatzstoff in der Ferkelaufzucht auf Wachstum und antimikrobiell wirksame Isothiocyanatkonzentration im Urin

Abstract

Tropaeolum majus L. is a herb with antimicrobial activity in humans, caused by the degradation product benzyl-isothiocyanate derived from enzymatic cleavage of glucotropaeolin. Piglets were fed diets with a graded supplementation of *Tropaeolum majus* for five weeks. *Tropaeolum majus* was supplemented at an upper dosage level of 1 g/kg with the feed, equaling 48.7 mg/kg glucotropaeolin, which resulted in a benzyl-isothiocyanate concentration in the urine of up to 2.4 mg/L, which is high enough to control a broad range of bacteria. On average 3.1 – 7.3% of the glucotropaeolin taken up by the animals was excreted as bioactive benzyl-isothiocyanate. Supplementation of the feed with *Tropaeolum majus* had no effect on growth performance of piglets.

Key words: *Tropaeolum majus*, Pigs, Glucotropaeolin, Supplementation

Zusammenfassung

Die Kapuzinerkresse (*Tropaeolum majus* L.) ist eine Heilpflanze, die eine stark antimikrobielle Wirkung zeigt und im Humanbereich gegen Blasenentzündungen bereits wirkungsvoll eingesetzt wird. Die antimikrobielle Wirkung wird dabei durch das Abbauprodukt des Inhaltsstoffes Glucotropaeolin, durch das Benzylisothiocyanat, erzielt.

In einem fünfwöchigen Versuch mit Ferkeln wurde getestet, inwieweit sich ein Futterzusatz von glucotropaeolin-haltigen Kressesamen auf die Futteraufnahme und die Gewichtszunahme von Ferkeln auswirkte. *Tropaeolum majus* wurde in drei unterschiedlichen Mengen zugesetzt und eine Kontrollgruppe ohne Futterzusatz wurde untersucht, wobei eine maximale Konzentration von 1 g/kg Futter zugesetzt wurde, was einer Menge von 48,7 mg Glucotropaeolin/kg entsprach. Aus dieser Glucotropaeolingabe resultierte eine Isothiocyanatkonzentration im Urin von maximal 2,4 mg/L, welche hoch genug ist, um ein breites Spektrum an Bakterien zu kontrollieren. Im Durchschnitt wurden 3,1 – 7,3% des verabreichten Glucotropaeolins in Form von bioaktivem Benzylisothiocyanat mit dem Urin wieder ausgeschieden. Kapuzinerkresse hatte als Futterzusatz keinen Einfluss auf das Wachstum und die Futteraufnahme der Ferkel.

Stichwörter: *Tropaeolum majus*, Ferkel, Glucotropaeolin, Futterzusatzstoffe, Heilpflanzen

Introduction

Alternative strategies to stabilize health and performance of livestock have gained interest since the ban of antibiot-

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ics as feed additives in the European Union in 2006. Weaning and the start of fattening are critical stages in pig development characterized by stress-related health problems and gastro-intestinal diseases like diarrhoea, enteritis or *Escherichia coli* enterotoxaemia (PLUSKE et al., 1997). Different medical herbs such as oregano, clove, thyme, peppermint and lemon grass have been tested with respect to their stabilizing or health promoting effects (GOLLNISCH, 2002), but results have been inconsistent. The mode of action of most plant components is not fully understood and procedures for the standardization of the plant material are not available (GOLLNISCH, 2002). *Tropaeolum majus* was chosen as a feed supplement in the present experiment because it is a plant whose efficacy, safety, quality, toxicology and clinical relevance has been described by a phyto-pharmaceutical working group (ANONYMOUS, 1985) and guidelines for cultivation and conditioning of the harvested plant material have been elaborated (BLOEM et al., 2007).

The antimicrobial mode of action of *Tropaeolum majus* (ANONYMOUS, 1985) is related to its benzyl-isothiocyanate (benzyl-ITC) content, which results from the enzymatic degradation of the aromatic glucosinolate (GSL) glucotropaeolin (GTL) (TRIPATHI and MISHRA, 2007). The anti-nutritional effects of the degradation products from GSLs have been reviewed by TRIPATHI and MISHRA (2007). Most investigations refer to rapeseed meal, which contains a broad range of GSLs with progoitrin as the major GSL. The degradation product *goitrin* is responsible for the morphological and physiological changes of the thyroid (TRIPATHI and MISHRA, 2007). The antinutritional effects need to be clearly distinguished from any therapeutic effect of low-dose amendments of the medical plant *Tropaeolum majus*, which contains GTL as a single GSL. For pigs no adverse effects have been reported when the total GSL content is below 0.78 µmol/g diet (TRIPATHI and MISHRA, 2007). In the current experiment the maximal total GSL content was 0.12 µmol/g diet.

The aim of the present study was to investigate if *Tropaeolum majus* has any influence on feed intake and growth performance of pigs. In addition the ITC concentration in urine was measured to check whether its concentration was high enough to show antimicrobial potential.

Materials and methods

Piglet experiment

A total of 80 piglets (40 females and 40 castrated males) of a commercial hybrid line (German Landrace/Large White x Piétrain) were used. Piglets were weaned at 21 days with a mean body weight (BW) of 8.3 ± 1.16 kg and were divided into four groups according to individual weight and sex with 20 piglets in 5 pens for each group. The experiment started at 25 days of age and lasted 35 days. The diets were based on cereals and soybean protein and were formulated according to standard recommendations (GfE, 1987) and offered in mashed form. Pigs had ad libitum access to feed and water.

Tropaeolum majus was administered to the feed in the form of seeds. Seed material containing 48.7 ± 2.3 mg/g GTL and feed were ground to a particle size of 1 mm (CONDUX cutting mill with 1 mm screen) to achieve a homogeneous mixture. The amount of supplementation was calculated on the basis of recommendations for humans (ANONYMOUS, 1985); 43.2 mg benzyl-ITC (130 mg GTL)/day for a 60 kg BW person. This value equals 2.17 mg GTL per kg BW. There were four experimental treatments; a control diet without feed supplementation, and 3 additional groups that received the recommended amount of benzyl-ITC and GTL or 25% less or more. Considering the GTL content of the seeds, BW of the piglets and feed intake, a corresponding rate of 0, 0.6, 0.8 and 1.0 g ground seed material was added per kg feed, corresponding to 0, 29.2, 39.0 and 48.7 mg GTL/kg diet (labeled as GTL-0, GTL-30, GTL-40 and GTL-50). BW of the pigs in the pen (n = 20 per group) and feed consumption per pen (n = 5 per group) were recorded weekly. The average daily gain was calculated by dividing the weekly weight gain by the number of days.

Additionally, one piglet per treatment was kept in a metabolic cage throughout the experiment to collect urine (Fig. 1). Two times during the experimental period (from 38 to 42 days of age and from 54 to 60 days of age) feed intake, urine excretion and benzyl-ITC release were balanced for these piglets. Generally, the urine was collected over 24 h and sampled in the morning. During the first 16 days (27 to 42 days of age) and during the last week of experimentation (54 to 60 days of age) urine was sampled daily; sampling in between was carried out twice a week to monitor changes in the ITC concentration.

Chemical analyses

The metabolizable energy of the feed was calculated using the equation given by the GfE (1987) based on digestible nutrients using feed nutrient and digestibility values from the literature (DLG, 1991).

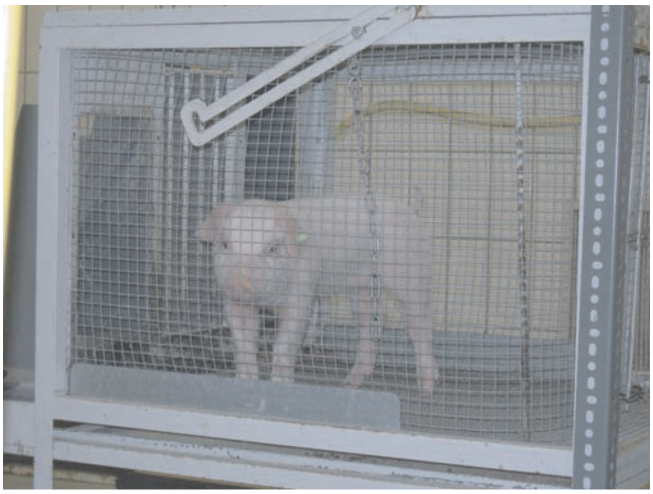

Fig. 1. Piglet kept in a metabolic cage.

The ITC content in the urine was determined according to Ye et al. (2002).

Statistical analyses
The experimental data were analyzed as a completely randomized design using the GLM procedures of SAS (2002-2003). Tukey's multiple range tests were applied to ascertain any significant differences between group means. Limits of significance for all critical ranges were set at $P < 0.05$.

Results and Discussion

Growth performance of piglets
Average daily gain over 35 days and for all piglets was 342 g/d. The mean values of the groups did not differ significantly (Tab. 1). Corresponding values for daily feed intake and the efficiency of the metabolizable energy were on average 566 g and 23.68 MJ/kg weight gain, respectively. Differences between the means of the groups proved to be statistically not significant for the shown parameters. Therefore it can be concluded that feed supplementation with *Tropaeolum majus* had no growth promoting effect on piglets.

Urine analysis
ITCs were found in the urine of all piglets that received *Tropaeolum majus* with the feed (Tab. 2), while in the control no ITC was determined. ITCs were not accumulated in the urine over time and neither time effects, nor an interaction between time and dosage was observed. The highest ITC concentration with a medium value of 1.8 mg ITC/L was determined in the urine of the piglet that received the medium dosage of GTL. The balance of GTL uptake and ITC and urine excretion revealed that the amount of urine excretion can vary significantly and that it affects the ITC concentration of the urine. At an age of 38 to 42 days no significant differences in the ITC excretion in relation to dosage were observed but at 54 to 60 days of age a dose-dependent excretion of ITC became obvious. At that time the pig with the highest GTL dosage excreted significantly more urine than the piglet with the medium dosage causing a higher dilution and thus a lower ITC concentration in the urine. Moreover differences in mastication can cause significant differences in GTL breakdown and excretion of breakdown products as shown by Shapiro et al. (2001) who found a 1.5-fold higher excretion of dithiocarbamates when the food was thoroughly chewed. Therefore individual feed uptake and drinking behavior had a strong influence on ITC concentration in the urine and it is necessary to record the absolute excretion of ITC when looking for dose effects.

The ITC concentration of the urine is important for antimicrobial potential. Concentrations of 0.5 – 50 µg/mL benzyl-ITC are reported to control a broad range of bacteria and 0.3 – 12 µg/mL act fungistatically (Anonymous, 1985). The results of the experiment revealed that the ITC concentration determined in the urine was sufficiently high to yield an antimicrobial effect in all treatments where the feed was supplemented with *Tropaeolum majus* (Tab. 2). A percentage of conversion from GSL to ITC of on average 5% is in the lower range of values reported by Shapiro et al. (1998) who determined a conversion of 10 ± 5% for humans. It might be assumed that the lower conversion was caused by a faster stomach passage in piglets compared to humans. The results indicated that feed supplementation with *Tropaeolum majus* can have a health effect in pigs.

Tab. 1. Growth performance of piglets fed *Tropaeolum majus* supplemented feed

	Treatment [a]					
	GTL-0	GTL-30	GTL-40	GTL-50	S.E.M.	*P*
Average daily gain (g/d)						
46th day of age	277	261	260	251	7.65	0.70
60th day of age	354	334	341	342	7.98	0.85
Feed intake (g/animal d)						
After 3 weeks (46th day of age)	412	407	408	404	14.68	1.00
After 5 weeks (60th day of age)	574	568	561	561	17.85	0.99
ME efficiency[b] (MJ/kg BW gain)						
After 3 weeks (46th day of age)	22.16	22.18	22.14	22.64	0.35	0.95
After 5 weeks (60th day of age)	23.75	24.00	23.36	23.63	0.35	0.93

[a)] GTL-0 control without feed supplementation with *T. majus*; GTL-30, GTL-40, GTL-50 different dosage of feed supplementation with *T.majus*, corresponding to round up 30, 40 and 50 mg GTL/kg diet.
[b)] ME efficiency = Metabolizable energy:gain ratio

Tab. 2. Isothiocyanate (ITC) concentration in the urine of piglets, which got glucotropaeolin (GTL) with the diet and balance of GTL uptake and daily ITC excretion in relation to the amount of urine.

Time dependent ITC excretion [mg/L]		n	Treatment[a] GTL-0	GTL-30	GTL-40	GTL-50	*P*
1st week		6	0.0	0.15	2.39	2.13	0.0012
	S.E.M.		(0.0)	(0.06)	(0.69)	(0.54)	
2nd week		7	0.0	0.67	1.36	1.18	≤ 0.001
	S.E.M.		(0.0)	(0.15)	(0.12)	(0.20)	
3rd week		4	0.0	0.78	2.44	1.28	0.0639
	S.E.M.		(0.0)	(0.16)	(0.96)	(0.21)	
4th week		2	0.0	0.57	1.89	1.34	0.1006
	S.E.M.		(0.0)	(0.03)	(0.53)	(0.22)	
5th week		7	0.0	0.69	1.42	1.02	≤ 0.001
	S.E.M.		(0.0)	(0.13)	(0.19)	(0.19)	
Medium ITC excretion over 35 days (26 to 60 days of age)							
[mg/L]		26	0.0	0.56	1.80	1.36	≤ 0.001
	S.E.M.		(0.0)	(0.08)	(0.24)	(0.16)	
Urine dependent ITC excretion: 38 to 42 days of age (corresponding to results for 2nd and 3rd week)							
GTL-uptake	[mg/d]	1	0.0	13.0	18.9	23.3	–[b]
Urine	[g/d]	5	405.8	331.6	223.0	212.8	0.1370
	S.E.M.		(74.9)	(45.5)	(51.1)	(50.3)	
ITC-excretion	[mg/d]	5	0.0	0.31	0.30	0.24	0.0034
	S.E.M.		(0.0)	(0.03)	(0.06)	(0.08)	
Relative ITC-excretion	[%]	5	0.0	7.3	4.8	3.1	≤ 0.001
	S.E.M.		(0.0)	(0.70)	(0.88)	(1.00)	
Urine dependent ITC excretion: 54 to 60 days of age (corresponding to results for 5th week)							
GTL-uptake	[mg/d]	1	0.0	24.73	31.10	39.17	–[b]
Urine	[g/d]	7	446.1	726.6	329.4	734.3	0.0098
	S.E.M.		(60.1)	(101.4)	(31.8)	(122.7)	
ITC-excretion	[mg/d]	7	0.0	0.44	0.46	0.73	0.0017
	S.E.M		(0.0)	(0.06)	(0.06)	(0.20)	
Relative ITC-excretion	[%]	7	0.0	5.4	4.4	5.6	0.0010
	S.E.M.		(0.0)	(0.68)	(0.61)	(1.50)	

[a] GTL-0 control without feed supplementation with *T. majus*; GTL-30, GTL-40, GTL-50 different dosage of feed supplementation with *T.majus*, corresponding to round up 30, 40 and 50 mg GTL/kg diet. [b] GTL uptake was only determined once during the time-period of balance.

Conclusions

Supplementing the diet of piglets for 35 days with *Tropaeolum majus* at a rate from 29 to 49 mg GTL/kg did not affect performance of the piglets but resulted in concentrations of benzyl-ITC in the urine with potential antimicrobial effect.

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Abbreviations

ITC, isothiocyanate, GSL, glucosinolate, GTL, glucotropaeolin, BW, body weight

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