

Influence of *Tropaeolum majus* supplements on growth and antimicrobial capacity of glucotropaeolin in piglets

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Abstract

Phytopharmaceuticals with a proven efficiency in humans may also offer a special prospect in animal nutrition. Nasturtium (*Tropaeolum majus* L.) is a herb with a proven antimicrobial activity, which is caused by benzyl-isothiocyanate the degradation product of glucotropaeolin. In an experiment with piglets, direct and graded supplementation of *T. majus* with the feed was performed over a period of five weeks. *T. majus* was supplemented at an upper dosage 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 16 µmol/L, which 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. Supplementation with *T. majus* had no effect on growth performance of piglets.

Keywords: Feed supplementation, Tropaeolum majus L., pigs, glucotropaeolin

Zusammenfassung

Kapuzinerkresse (*Tropaeolum majus* L.) als Futterzusatzstoff in der Ferkelaufzucht – Einfluss auf Wachstum und antimikrobielles Potenzial des Glucotropaeolins

Heilpflanzen, deren Wirksamkeit bereits am Menschen untersucht und bestätigt wurde, stellen vielversprechende Kandidaten als Futterzusatzstoffe in der Tierernährung hinsichtlich ihres Potenzials, die Gesundheit der Tiere zu fördern und zu stabilisieren, dar. Dabei können Dosierungsempfehlungen für den Menschen zu Grunde gelegt und auf das Tier übertragen werden. Kapuzinerkresse (*Tropaeolum majus* L.) ist eine anerkannte Heilpflanze mit einem hohen antimikrobiellen Potenzial. Dieses ist auf das Abbauprodukt Benzylisothiocyanat zurückzuführen, welches beim enzymatischen Abbau des in hohen Konzentrationen vorhandenen Inhaltsstoffs Glucotropaeolin gebildet wird. In einem Experiment mit Ferkeln wurden über 35 Tage Kressesamen dem Futter zugesetzt, um das Potenzial von Kapuzinerkresse als Futterzusatzstoff zu untersuchen. Die höchste Dosierung entsprach dabei einem Futterzusatz von 1g Kressesamen/kg Futter, äquivalent zu 48,7 mg Glucotropaeolin/kg Futter, was mit einer maximalen Konzentration von 16 µmol Isothiocyanat pro Liter im Urin verbunden war. Diese Konzentration ist vermutlich hoch genug, um eine breite Palette von Bakterien zu kontrollieren. Von der verabreichten Menge an Glucotropaeolin wurde maximal 7.3 % in Form von Isothiocyanat im Urin wiedergefunden. Der Zusatz von Kapuzinerkresse zum Futter hatte keinen Einfluss auf die Futteraufnahme und die Gewichtszunahme der Ferkel.

Schlüsselwörter: Futterzusatzstoffe, Tropaeolum majus L., Schweine, Glucotropaeolin

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Introduction

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. In growing piglets, weaning and the start of fattening are critical stages in the development of grower-finishers, which are characterized very often by stress-related health problems and gastro-intestinal diseases like diarrhea, enteritis or *Escherichia coli* enterotoxaemia (Lalles et al., 2004; Pluske et al., 1997). 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 (Gollnisch et al., 2001; Schöne et al., 2004; Wald et al., 2001), but results proved to be inconsistent (Gollnisch, 2002). Additionally, the exact mode of action of these plant components is not fully understood and likewise procedures for the standardization of cultivation and processing of the plant material are not available (Gollnisch, 2002). *Tropaeolum majus* was chosen as an experimental crop in this study because the physiological mechanisms involved in the phytopharmaceutical effectiveness of *T. majus* are well-known (Anon, 1985) and guidelines for cultivation and conditioning of the harvested plant material have been elaborated (Bloem et al., 2007).

In 1978, the German government established the 'Commission E', which investigated and affirmed the safety and effectiveness of 363 herbal plants; these monographs are today the basis for the registration of new and old phytopharmaceuticals. Nasturtium (*T. majus*) is a herbal plant that meets 'Commission E' standards. *T. majus* has a very broad range of action. Fresh leaves of *T. majus* were traditionally used for the treatment of infected wounds and the gall bladder, as diuretic, aphrodisiac, and as medicine against chronic diseases such as obstructive pulmonary disease, cystitis, pyelitis and infections of kidneys and bladder (Madaus, 1976; Müller, 1979; Weiss, 1980; Winter, 1955). More recently it was shown that *T. majus* has also an anticarcinogenic potential (Fahey et al., 1997; Pintão et al., 1995).

The antimicrobial effects of *T. majus* are related to its glucotropaeolin (GTL) content and its breakdown product, benzyl-isothiocyanate (benzyl-ITC). GTL is an aromatic glucosinolate (GSL), which is produced in high concentrations by *T. majus*. The benzyl-ITC is released upon hydrolysis of GTL by the endogenous enzyme myrosinase. Plants that contain GSLs also hold myrosinase, and both seem to be located in different compartments in the intact living tissue (Lüthy and Matile, 1984). When the plant tissue is disrupted GSLs are hydrolyzed by myrosinase activity, and a range of breakdown products is released including the biologically active ITCs. It is predominantly the ITC, which yields an antimicrobial, antifungal and virostatic effect. For the

release of benzyl-ITC the pH value of the medium during the degradation of GSLs is important; only under neutral pH conditions benzyl-ITC is formed. Additionally, the maximum efficiency of benzyl-ITC against bacteria was found in the range of pH of 6 to 8 (Halbeisen, 1954). Under acid conditions nitriles and amines may be formed, both with yet unknown in vivo effects at low concentrations.

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 glucosinolate contents in food stuff like rapeseed meal. Antinutritional effects of GSLs are well-known and reviewed by Tripathi and Mishra (2007). Most studies were conducted with rapeseed meal which contained high concentrations of GSLs before the introduction of double low oilseed rape varieties in the 1980s. Rapeseed meal generally contains several different GSLs with progoitrin, gluconapin and glucobrassicinapin as the dominant GSLs, which consequently deliver different degradation products. The degradation of GSLs yields thiocyanates, isothiocyanates, cyclic sulfur compounds and nitriles, which are goitrogenic. So-called cabbage goiter or struma inhibits the iodine uptake of the thyroid gland (Berdanier, 2002). In this respect, one of the most potent GSLs is progoitrin, which was identified as mainly responsible for the toxic effects of rapeseed meal on pigs (Griffith et al., 1998). *T. majus* only contains a single GSL the GTL and no adverse effects were reported in the few experiments which were conducted with *T. majus* as feedstuff (Tovar et al., 2005; Winter and Willeke, 1955).

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 $\mu\text{mol/g}$ diet (Tripathi and Mishra, 2007). In the here presented experiment the maximum total GSL content accounted for 0.12 $\mu\text{mol/g}$ diet and thus is distinctly below the critical threshold.

Up to now no experimentation has been carried out in piglets to study the capacity 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 dose rate of GTL during the first five weeks after weaning.

Materials and methods

A total of 80 piglets (40 females and 40 castrated males) of a commercial hybrid line ("BH2P", German Landrace/Large White x Piétrain) were used (Table 1). The piglets were weaned after 21 days with a mean live weight of 8.33 ± 1.16 kg. The pigs were divided equally into four

groups according to their individual weight and sex with 20 piglets in 5 pens per group. The experiment started 4 days after weaning and was carried out for 35 days.

Table 1:
Experimental design

	Treatment			
	1	2	3	4
Feed supplementation with nasturtium [mg GTL/kg]	control without GTL	29 mg GTL/kg	39 mg GTL/kg	49 mg GTL/kg
No. of boxes	5	5	5	5
No. of piglets	20	20	20	20
No. of females	10	10	10	10
No. of castrated males	10	10	10	10
No. of piglets in metabolic cages	1	1	1	1

The basic diet consisted of cereals and soybean protein, supplemented with minerals, vitamins, amino acids and phytase to meet the recommendations of the Society of Nutrition Physiology (GfE, 1987). The specific composition is shown in Table 2. Feed in meshed form and water were offered *ad libitum*.

Table 2:
Composition of the diet

Ingredient	Quota (g/kg)
Barley	339.35
Wheat	300.00
Maize, extruded	100.00
Soybean meal	150.00
Soybean protein concentrate	50.00
Soy oil	30.00
Min./Trace E./Vit. ¹	19.00
Amino acids ¹	11.50
Phytase	0.15

¹Ingredients per kg complete diet: Ca 4.5 g, P 1.4 g, Na 0.9 g, Mg 0.08 g, Fe 75 mg, Cu 15 mg, Mn 40 mg, Zn 50 mg, I 1 mg, Se 0.2 mg, Co 0.4 mg, vitamin A 10,000 IU, vitamin D3 1,000 IU, vitamin E 50 mg, vitamin B1 1 mg, vitamin B2 3.1 mg, vitamin B6 2.5 mg, vitamin B12 20 µg, vitamin K3 2 mg, vitamin C 50 mg, nicotinic acid 12.5 mg, folic acid 0.5 mg, choline chloride 125 mg, biotin 50 µg, lysin-HCl 6 g, DL-methionine 3 g, L-threonine 1 g, L-tryptophane 0.5 g

Feed supplementation with *T. majus*

Seed material from *T. majus* was ground to the same particle size as the feed (1 mm), thoroughly homogenized and the GTL content was determined prior to supplementation in 10 samples according to the method described by Bloem et al. (2007). The mean GTL content of the ground dry seed material was 48.7 ± 2.3 mg/g.

The dosage of the feed supplementation was calculated on basis of the recommendations of Blumenthal et al. (1998). A daily dosage of 43.2 mg benzyl mustard oil (130 mg GTL) is recommended for a person with a body weight of 60 kg (Blumenthal et al., 1998) equivalent to 2.17 mg GTL per kg body weight. Four experimental groups were studied: one control group without feed supplementation, one group that received 25 % less than the recommended dosage, one which received the recommended dosage and one that received 25 % more. 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.

Body weight 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 through the days.

Additionally one piglet per treatment was kept in a metabolic cage throughout the experiment to collect urine. The piglets were weaned after 21 days and the experiment started with the feed supplementation at an age of 25 days. From an age of 27 days on enough urine was excreted by the piglets to take samples for analyses. Two times during the experimental period, from 38 (equivalent to 14 days after start of the experiment) 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; in between sampling was carried out twice a week to monitor changes in the ITC concentration.

Chemical analyses

Dry matter, crude ash, crude protein (N x 6.25), ether extract (HCl digestion), crude fiber, sugar (according to Luff-Schoorl) and starch were analyzed according to standard methods (Naumann and Bassler, 1993). The metabolizable energy content of the feed was calculated by the equation given by GfE (1987) using the nutrient values of the feed and the digestibility values from feed tables (DLG, 1991).

Prior to analysis the urine samples were filtered and the pH value was recorded. The total ITC content was determined according to Ye et al. (2002) and measured by HPLC with UV detection at 365 nm. ITCs were measured isocratically with an eluent consisting of 85 % methanol/15 % water using a Supelcosil LC-18 HPLC column (150 x 2.1 mm, 5 µm, Sigma-Aldrich, Nr. 57934) and a flow of 3 mL/min. For the quantification a calibration curve was established, which was prepared from propyl-ITC.

Statistical analyses

The experimental data were analyzed as a completely randomized design using the GLM procedures of SAS (9.1 for WINDOWS, 2002-2003). Tukey's multiple range test was applied to ascertain any significant differences between group means. Limits of significance for all critical ranges were set at $P < 0.05$.

Results

Growth performance of piglets

No significant differences in the composition of the feed were found in relation to the supplementation with *T. majus* (Table 3). Values of on average 200 g protein and 15.9 MJ metabolizable energy (ME) per kg DM were determined.

Table 3:

Nutritional composition of feedstuff (g/kg DM) in relation to piglet test groups

	Treatment			
	1 control without GTL	2 29 mg GTL/kg	3 39 mg GTL/kg	4 49 mg GTL/kg
Dry matter	895	894	896	897
Crude ash	47	46	46	47
Crude protein	200	196	197	199
Ether extract	58	57	58	57
Crude fiber	42	43	40	43
Sugar	51	52	50	51
Starch	489	487	493	492
ME (MJ/kg DM)	15.9	15.8	15.9	15.8

Table 4:

Growth performance parameter of piglets fed with *T. majus* supplemented feed

	Treatment				LSD _{5%}
	1 control without GTL	2 29 mg GTL/kg	3 39 mg GTL/kg	4 49 mg GTL/kg	
Body weight (kg)					
Body weight (25 th day of age)	8.4	8.4	8.3	8.3	1.0
Body weight (60 th day of age)	20.8	20.0	20.2	20.3	2.6
Average daily gain (g/d)					
ADG after 1 week	193	194	163	164	58.4
ADG after 2 weeks	245	232	228	221	56.5
ADG after 3 weeks	277	261	260	251	56.9
ADG after 4 weeks	325	292	300	302	57.0
ADG after 5 weeks	354	334	341	342	59.4
Feed intake (g/animal d)					
After 1 week	275	268	261	268	67.2
After 2 weeks	311	302	296	295	95.0
After 3 weeks	412	407	408	404	118.8
After 4 weeks	468	443	440	448	119.1
After 5 weeks	574	568	561	561	144.5
ME efficiency (MJ/kg gain)					
ME:G ¹⁾ after 1 week	20.62	19.48	22.99	23.51	4.73
ME:G after 2 weeks	21.25	20.92	20.93	21.38	2.64
ME:G after 3 weeks	22.16	22.18	22.14	22.64	2.82
ME:G after 4 weeks	22.36	22.93	22.16	22.74	1.98
ME:G after 5 weeks	23.75	24.00	23.36	23.63	2.79

¹⁾ ME:G = Metabolizable energy:gain ratio

During the experiment 3 piglets died without any symptoms of disease or any visible relationship to the treatment. Average daily gain (ADG) over 35 days and for all piglets was 342 g/d. The mean values of the groups did not differ significantly (Table 4). Corresponding values for daily feed intake (FI) and the efficiency of the ME 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 (Table 4). Therefore it can be concluded that feed supplementation with *T. majus* had no effect on growth performance of piglets.

Urine analyses

ITCs were found in the urine of all piglets that received *T. majus* feed supplementation (Table 5) while in the control no ITC was determined. ITCs were not accumulated in the urine over time and no interactions between time and dosage were observed. The highest ITC concentration was measured in the urine of the piglet that received a medium dosage of GTL but differences in the ITC concentration in relation to treatment proved to be not significant (Table 5). Feed supplementation with *T. majus* had no significant influence on the pH in the urine and the pH was between 5.3 and 6.9 where a conversion into ITCs can take place and a high antimicrobial potential can be unfolded because the degradation of GTL into benzyl-ITC is a pH dependent process and highest in the neutral pH range.

The balance of GTL uptake and ITC and urine excretion revealed that the amount of urine excretion varied significantly between individual piglets. This resulted in varying ITC concentrations in the urine. At an age of 38 to 42 days no significant differences in the ITC excretion in relation to dosage and amount of urine were observed, but at 54 to 60 days of age a dose and volume dependent excretion of ITC became obvious (Table 6). 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. The cumulative excretion of ITCs with urine is shown in Figure 1, each for a 5 days interval at the start and end of the experiment. The results for the corresponding GTL/ITC balance are shown in Table 6. The results revealed that the total daily amount of ITC excretion increased with duration of the experiment (Figure 1) because the piglets took up a higher amount of food but this had no effect on the concentration of ITCs in the urine (Table 5). Individual feed uptake and drinking behavior had a stronger influence on ITC excretion and dominated over the impact of GTL dosage in the initial phase (Figure 1; Table 6). At the end of the experiment feed intake was higher and a distinct effect of GTL dosage on ITC excretion was determined.

At the start of the experiment about 3 to 7 % of the

GTL was excreted as ITC with urine, while at the end of the study all groups of animals showed a more uniform value of 4 to 5 % (Table 6).

Table 5:

Benzyl-isothiocyanate concentration and pH value in the urine of weaning piglets in relation to glucotropaeolin (GTL) supplementation

Supple- menta- tion	n	LSD _{5%}	Treatment			
			1 control without GTL	2 29 mg GTL/kg	3 39 mg GTL/kg	4 49 mg GTL/kg
			Isothiocyanate concentration [µmol/L urine]			
1 st week ^a	6	8.8	0.0	1.0	16.0	14.3
2 nd week	7	2.9	0.0	4.5	9.1	7.9
3 rd week	4	11.9	0.0	5.2	16.4	8.6
4 th week	2	10.7	0.0	3.8	12.7	9.0
5 th week	7	3.1	0.0	4.6	9.5	6.9
			pH value of the urine			
1 st week ^a	6	ns	6.0	6.6	6.5	5.9
2 nd week	7	ns	5.7	5.6	6.6	5.6
3 rd week	4	ns	5.6	5.3	5.6	5.8
4 th week	2	ns	5.5	6.9	6.3	5.8
5 th week	7	ns	5.5	6.6	5.5	5.4

^a in the first week only 6 measurements could be conducted because at the first day there was not enough urine for analysis

Table 6:

Balance of glucotropaeolin (GTL) uptake and isothiocyanate (ITC) excretion 14 to 18 days and 30 to 36 days after feed supplementation of piglets with nasturtium

Dosage of GTL supplementation	GTL uptake	ITC excretion	Urine excretion	Percentage of GTL which was excreted as ITC
				%
		µmol/d	g/d	
38 to 42 days of age				
Control	(0.0 mg/kg)	0.0	406	0.0
Low	(0.29 mg/kg)	29.0	332	7.3
Optimum	(0.39 mg/kg)	42.1	223	4.8
High	(0.49 mg/kg)	52.0	213	3.1
LSD_{5%}		a	1.12	2.52
54 to 60 days of age				
Control	(0.0 g/kg)	0.0	446	0.0
Low	(0.6 g/kg)	55.3	727	5.4
Optimum	(0.8 g/kg)	69.5	329	4.4
High	(1.0 g/kg)	87.5	734	5.5
LSD_{5%}		a	2.25	2.76

^a Anova was not carried out for GTL uptake as this parameter was not directly determined but calculated via the intake of feed on a weekly basis

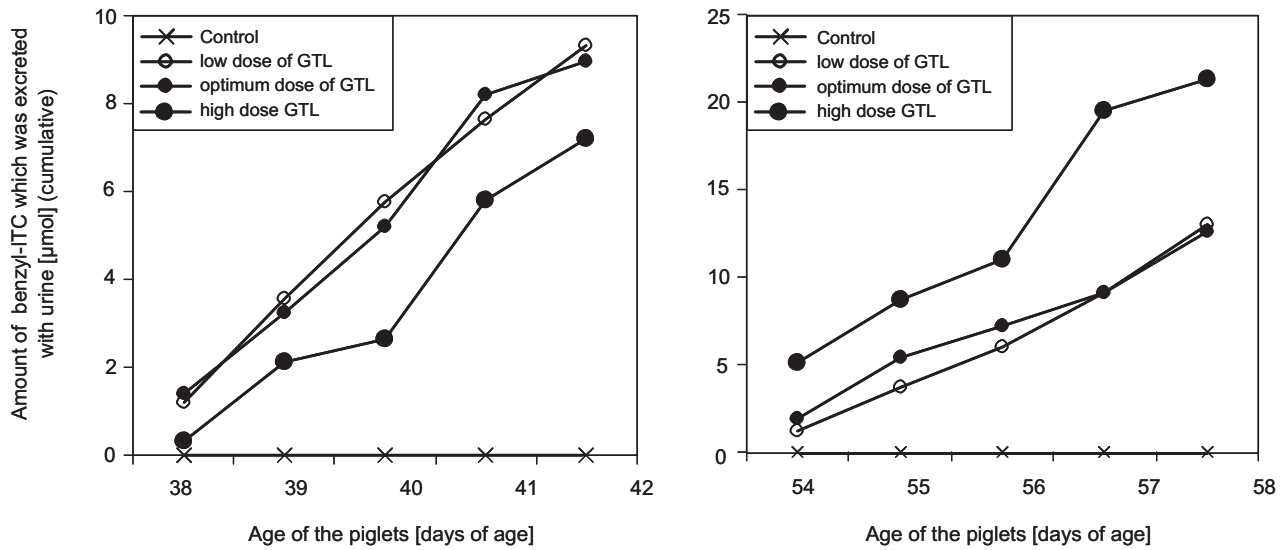


Figure 1:

Amount of benzyl-ITC, which was excreted with the urine of piglets during 5 days 14 and 30 days after start of the experiment in relation to GTL supplementation

Discussion

Feed supplementation with medical plants is a yet uncommon practice because scientific evidence for its effectiveness is regularly missing (Kijlstra and Eijck, 2006). Reasons for discrepancies include animal species, dosage and duration of the treatment, variability in the content of bioactive substances, and degradation of bioactive substances by inadequate processing techniques. For the medical plant *T. majus* both cultivation and processing guidelines have been elaborated (Bloem et al., 2007) and the medical efficacy of *T. majus* is well documented (Anon, 1985; Blumenthal et al., 1998).

The free bioactive form of benzyl-ITC can be found in lung, kidneys and in lower concentration in the spleen, while in liver and brain benzyl-ITC is bonded (Anon, 1985). After oral intake of benzyl-ITC, it is quickly resorbed in the stomach. *T. majus* preparations were usually administered in capsulated form so that degradation of GTL will take place in the small intestine and benzyl-ITC end up in kidneys and urine. In contrast, Shapiro et al. (2001) showed that differences in mastication can cause significant differences in GTL breakdown and excretion of breakdown products. The same authors found a 1.5-fold higher excretion of dithiocarbamates when the food was thoroughly chewed. These investigation reveal that for instance the bioavailability of GSLs and related compounds depends on several factors (Holst and Williamson, 2004) and this might explain why no dose-related differences were found at the start of the experiment (Table 6) when the piglets took up a lower amount of feed. 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 an important criteria for the antimicrobial potential of the supplementation. Concentrations of 0.5 to 50 μg/mL benzyl-ITC are reported to control a broad range of bacteria and 0.3 to 12 μg/mL acted fungistatic (Anon, 1985). The results of the experiment revealed that the ITC concentration in the urine was in all treatments where the feed was supplemented with *T. majus* sufficiently high to yield an antimicrobial effect (Table 5). On average 5 % of GTL was converted to ITC, which is in the lower range of values reported by Shapiro et al. (1998), who determined a conversion rate of 10 ± 5 % for humans. Most likely the lower conversion in piglets is caused by the faster uptake combined with a less intensive chewing of the feed and the generally quick gastrointestinal passage of the feed.

The results obtained in this first experiment with piglets are promising as it was shown that feed supplementation with *T. majus* had no adverse effect on growth performance and, equally important proved to unfold an antimicrobial capacity that should be sufficiently high to combat infectious diseases. Faecal samples of the piglets of the here presented experiment were also investigated for the composition of the intestinal microbial community but no differences were observed in relation to feed supplementation with *T. majus* (Pieper et al., 2007). Their data also yielded no clear proof whether *T. majus* has a health promoting effect in animal nutrition. Therefore further experiments are required and experimentation with sows

suffering from the Mastitis-Metritis-Agalactia-syndrome are supposed to deliver explicit results for the following reasons: First, benzyl-ITC has a proven effect on urethral infections in humans; second, *T. majus* can be administered as capsules so that a higher rate of benzyl-ITC will be effective and third, vegetative plant material can be used, which supposedly is more efficient than seeds. The reason is simply that the leaf material contains other secondary compounds with bioactive components such as glutathione and chlorophyll (Haneklaus and Schnug, 2004). In the presented experiment the use of leaf material was not possible as an uniform intake could only be warranted by mixing feed and seeds with same particle size. The use of fine-textured leaf material would have favored selective intake and segregation of feed and supplement in the feeder. Another alternative, the production of composed pellets has been evaluated before start of the experiment with the results that GTL was completely degraded during the manufacturing process, which included the amendment of water and heat.

Conclusions

The presented feeding experiment showed that supplementation of piglets with *T. majus* had 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 dosage of bio-active GTL in animal nutrition.

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