

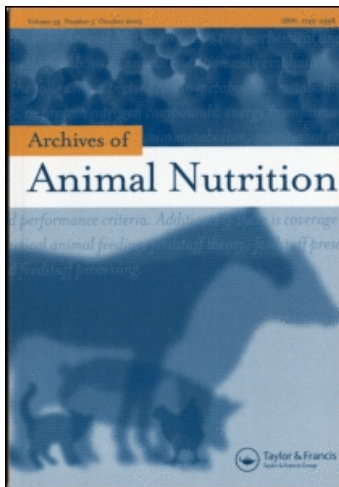
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Hartwig Böhme <sup>a</sup>; Eike Rudloff <sup>b</sup>; Friedrich Schöne <sup>c</sup>; Wolfgang Schumann <sup>d</sup>; Liane Hüther <sup>a</sup>; Gerhard Flachowsky <sup>a</sup>

<sup>a</sup> Institute of Animal Nutrition, Federal Agricultural Research Centre (FAL), Braunschweig <sup>b</sup> Federal Centre for Breeding Research on Cultivated Plants, Groß Lüsewitz <sup>c</sup> Agricultural Institution of Thuringia, Jena <sup>d</sup> Research Centre for Agriculture and Fishery of Mecklenburg-Vorpommern, Gülzow, Germany

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## Nutritional assessment of genetically modified rapeseed synthesizing high amounts of mid-chain fatty acids including production responses of growing-finishing pigs

HARTWIG BÖHME<sup>1</sup>, EIKE RUDLOFF<sup>2</sup>, FRIEDRICH SCHÖNE<sup>3</sup>,  
WOLFGANG SCHUMANN<sup>4</sup>, LIANE HÜTHER<sup>1</sup>, &  
GERHARD FLACHOWSKY<sup>1</sup>

<sup>1</sup>Institute of Animal Nutrition, Federal Agricultural Research Centre (FAL), Braunschweig, <sup>2</sup>Federal Centre for Breeding Research on Cultivated Plants, Groß Lüsewitz, <sup>3</sup>Agricultural Institution of Thuringia, Jena, and <sup>4</sup>Research Centre for Agriculture and Fishery of Mecklenburg-Vorpommern, Gülzow, Germany

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### Abstract

The nutritive value of genetically modified myristic acid-rich rapeseed, in which a acyl-thioesterase gene inserted, was studied. Crude nutrients, amino acid and fatty acid profiles as well as mineral and glucosinolate contents were determined and compared with those of the non-transgenic parental cultivar. The concentration of crude nutrients, minerals and amino acids were found to be within the range of natural variance. The myristic and palmitic acid content increased from 0.1–11.4% and from 3.6–20%, respectively, at the expense of oleic acid, which decreased from 68.6–42.6% of total fatty acids. The glucosinolate contents increased from 12.4 µmol/g in the parental plant to 19 µmol/g DM in the GM-plant. Full-fat rapeseed of both cultivars was incorporated in pig diets at a level of 15%, and the digestibility and the production efficiency were tested under *ad libitum* feeding conditions with ten pigs each over the growing finishing period from 32–105 kg BW. The experimental diets did not show significant differences in digestibility and energetic feeding value. However, feed intake and weight gain decreased presumably due to the increasing glucosinolate intake associated with the feeding of transgenic rapeseed. The dietary fatty acids profile influenced the fatty acid profile of body fat. Myristic acid accumulated in back fat and intramuscular fat while the oleic acid content decreased. The increased glucosinolate intake affected the weight of thyroid glands and their iodine concentration.

**Keywords:** *GM-rapeseed, glucosinolates, fatty acid profile, digestibility, pigs*

### 1. Introduction

In the last decade numerous studies on feeds from the so-called first generation of genetically modified plants have been reported, as recently reviewed by Flachowsky et al. (2005, 2007). When such transgenic plants (with input traits) are used in animal feeding their constituents and their availability of nutrients, do not differ from their conventional counterparts.

In contrast, animal-experimental studies on the nutritional assessment of genetically modified feedstuffs with modifications of their chemical composition, i.e. an increase of desirable substances or a decrease of anti-nutrients, are as yet infrequently reported. The approach to assess these plants as feedstuffs of the 'second generation' is still under discussion (Flachowsky & Böhme 2005) because the concept of substantial equivalence is not applicable (Organisation for Economic Co-operation and Development [OECD] 1993), and there are indications that special attention has to be paid on unexpected side effects, i.e. an increase of undesirable substances affecting animal and human health negatively (Cellini et al. 2004). This paper deals with composition and animal investigations, to compare the concentration of nutrients, anti-nutrients and the production efficiency of the transgenic rapeseed variety differing in its fatty acid profile when compared with seeds of the non transgenic parental line. This construct was developed for industrial purposes (mineral oil replacement), the co-products such as press cake and extracted meal are intended to be used as feedstuffs. Unfortunately the amounts of available rapeseed were limited for technical treatment like extraction. Therefore, full-fat rapeseed was used for this preliminary study.

## 2. Material and methods

### 2.1. Rapeseed

The investigations were conducted with full-fat rapeseed of the isogenic line 'Drakkar' and the transgenic cultivar 'Line TM5', into which the acyl-thioesterase gene of *Cuphea lanceolata* was inserted. This is a wild plant originating from Central America. The gene constructed can interfere with the fatty acid metabolism and enable the synthesis of myristic acid in rapeseed on a consequent of a modified fatty acid profile.

The non-transgenic and the transgenic line were grown on the experimental field of the Federal Centre for Breeding Research on Cultivated Plants in Groß-Lüsewitz (Germany) during the 2003 growing season. As both crops (isogenic and transgenic) were grown on an experimental scale, sowing and harvesting were performed with plot machines. The seeds were dried in the open air and passed through a roll crusher to destroy their germination capacity before analyses and animal feeding.

### 2.2. Analyses

Three representative samples of the isogenic and transgenic cultivars were analysed for dry matter, organic matter, crude protein, ether extract and fibre fractions as well as for starch and sugar according to the VDLUFA-methods (Naumann & Bassler 1993). The corresponding procedures recommended by the VDLUFA were applied for the determination of amino acids, fatty acids and minerals. The analysis of glucosinolates followed the official EU-method, which is a HPLC-procedure laid down in the commission regulation (EWG 1990). The iodine content in the organs of pigs was measured applying the Intra-Coupled-Plasma-technique in combination with a mass spectrometer (ICP- MS). Details including sample preparation were described by Leiterer et al. (1997).

### 2.3. Feeding trial

A feeding trial with a total of 20 crossbred hybrid pigs was designed. The dietary treatments were the two rapeseed sources incorporated in the diet at a level of 15%, which had proved to be the maximum rate under practical feeding conditions (Schöne et al. 1997). The diets for

the growing period (32–69 kg BW) and the finishing period (69–105 kg BW), which were formulated to fulfil the DLG-recommendations (DLG 1991), are listed in Table I. The pigs were kept under single feeding conditions; the feeding regime was *ad libitum*.

The digestibility of the experimental diets was tested by balance trials, which were designed in accordance with the guideline of the Committee for Requirement Standards of the Society of Nutrition Physiology (GfE 2005). Each diet was tested on five male castrated pigs. The collection period of faeces lasted 10 days, which was preceded by an adaptation phase of 14 days. The feed intake was kept constant during the collection period. The energy supply amounted to 2.3 times maintenance requirements. The supply of micro nutrients met the recommendation of the German Society of Nutrition Physiology (GfE 1987). The animals were fed twice daily. Excreta were totally collected and stored frozen at  $-18^{\circ}\text{C}$  until analysis. To study the effects of the fatty acid supply on fat accretion the slaughtered pigs were subjected to carcass grading (ALZ 2004). The following parameters were recorded: carcass weight, carcass length, back fat thickness, leaf fat weight, eye muscle area, fat thickness above the eye muscle and lean meat content. Additionally, the weights of liver, kidney, heart and thyroid gland were recorded. Samples were taken from these organs to study the iodine status of the pigs as affected by the glucosinolate intake.

#### 2.4. Statistics

The statistics were carried out using the SAS-system for Personal Computers (1988). To test differences between both variants, the *t*-test was chosen. Means with different superscript capitals within treatments differ significantly at a confidence level of 99%, means with different lowercase superscripts indicate significant differences at a confidence level of 95%.

### 3. Results and discussion

#### 3.1. Chemical composition

The content of selected constituents of the transgenic rapeseed and the non-modified comparator are shown in Table II.

The analyses of proximates indicate differences between both cultivars, but as they are small apart from protein, they were considered biologically not relevant. The fat content which contributes essentially to the nutritive value was analysed to be 3.9% lower for the transgenic cultivar compared to the non-transgenic one, the protein content was 4.6% higher. Compositional differences in the mineral content due to the genetic modification were also analysed. Considering the compositional variance worldwide as presented in the OECD

Table I. Composition of the experimental diets.

Diet	Grower	Finisher
Wheat [%]	35.0	37.0
Barley [%]	40.0	40.0
Potato protein concentrate [%]	7.0	5.0
Rapeseed (iso- or transgenic) [%]	15.0	15.0
Premix* [%]	3.0	3.0

\*Supplements per kg diet: 12 000 IU vitamin A; 1 200 IU vitamin D<sub>3</sub>; 36 mg vitamin E; 1.1 mg vitamin B<sub>1</sub>; 3 mg vitamin B<sub>2</sub>; 3 mg vitamin B<sub>6</sub>; 15 mg Cu; 1.5 mg I; 120 mg Fe; 60 mg Mn; 120 mg Zn; 0.6 mg Co; 0.5 mg Se.

Consensus Document (2001), the values all fed with these associated with rape varieties generally. To study any effects on protein quality the amino acid profile was determined. The results showed that no marked differences of the amino acids measured were found between the Drakkar and the TM5 seed. The fatty acid profiles of the transgenic rapeseed showed the expected increase of myristic and palmitic acid at the expense of oleic acid (Table II).

In the genetically modified cultivar the total glucosinolate (GSL) content was increased by more than 50% (Table III). However, the GSL-pattern was not affected. The predominant compound was analysed as progoitrin, which forms 60% of total glucosinolates. This substance is known to be particularly toxic because it is hydrolyzed in the digestive tract to goitrin. Consequently, the anti-nutritional effect of TM5 rapeseed is assumed to be higher than that of Drakkar rapeseed (Lange et al. 1995).

Table II. Chemical constituents of the isogenic (cultivar Drakkar) and transgenic rapeseed (line TM5) ( $n=3$ ).

	Isogenic rapeseed	Transgenic rapeseed
Dry matter [%]	93.7	93.5
Crude ash [g/100 g DM]	4.2	4.7
Crude protein [g/100 g DM]	22.8	27.4
Ether extract [g/100 g DM]	47.9	44.0
Crude fibre [g/100 g DM]	10.6	9.6
ADF [g/100 g DM]	16.3	14.8
NDF [g/100 g DM]	19.7	18.6
Starch [g/100 g DM]	2.9	2.8
Sugar [g/100 g DM]	4.4	4.5
<i>Minerals</i> [g/kg DM]		
Calcium	4.39	4.19
Phosphorus	7.36	8.41
Potassium	6.27	8.29
Sodium	1.63	1.84
Manganese	3.22	3.92
<i>Amino acids</i> [g/16 gN]		
Lysine	5.61	5.74
Methionine	1.86	1.97
Cystine	2.26	2.75
Threonine	4.31	3.99
<i>Fatty acids</i> [g/100 g total fatty acids]		
Lauric (C12:0)	1.5	1.7
Myristic (C14:0)	0.1	11.4
Palmitic (C16:0)	3.6	20.2
Stearic (C18:0)	0.5	0.8
Oleic (C18:1)	68.6	42.6

Table III. Contents of glucosinolates (GSL) [ $\mu\text{mol/g}$ ] of isogenic and transgenic rapeseed ( $n=3$ ).

	Alkenyl GSL	Indolyl GSL	Total GSL
Isogenic rapeseed	9.6	3.6	13.2
Transgenic rapeseed	16.3	4.1	20.4

### 3.2. Feeding and digestion experiments

Although high amounts of rapeseed were incorporated in the diets, adverse effects on health and behaviour of the pigs were not observed. The nutrient content, the apparent digestibility and the energetic feeding value of the experimental diets are listed in Tables IV and V.

The chemical composition and digestibility did not show important differences. Because of the higher protein content of transgenic rapeseed (Table II) the diets with transgenic rapeseed contained more protein (Table IV). The low digestibility of ether extract of both rapeseed sources (Table V) cannot be explained presently and needs further studies. However, an improved ether extract digestibility was observed between the grower and finisher diets, which can be attributed to the improved capacity of fat digestion with age (Wiseman 1984). Correspondingly, the digestibility of the organic matter improved, overall the aim of an isoenergetic and isonitrogenic formulation of the diets was achieved. The energy concentration in the dry matter of the grower diet amounted to 14.0 MJ ME/kg when Drakkar rapeseed was incorporated and 14.1 MJ ME/kg when the TM5 rapeseed was used. The corresponding ME-contents for the finisher diets were found to be 14.8 and 14.6 MJ per kg DM, respectively. It can be concluded that the genetic modification did not affect the digestibility of nutrients and the energetic feeding value.

The results of growth performance, obtained from the growing-finisher trial support this thesis (Table VI).

Health problems were not observed and no pigs had to be withdrawn from the trial so that all animals were subjected to statistical analysis. The results demonstrate a tendency towards

Table IV. Chemical composition of the experimental diets (g/kg DM;  $n=3$ ).

	Grower diet		Finisher diet	
	Isogenic rapeseed	Transgenic rapeseed	Isogenic rapeseed	Transgenic rapeseed
Organic matter	936	938	944	943
Crude protein	206	222	188	197
Ether extract	84	78	90	81
Crude fibre	60	59	55	55
N-free extract	586	579	611	610
Starch	453	451	459	463
Sugar	25	24	24	25

Table V. Digestibility of crude nutrients [%] and energy content [MJ ME/kg DM] of the experimental diets ( $n=5$ ).

	Grower diet		Finisher diet	
	Isogenic rapeseed	Transgenic rapeseed	Isogenic rapeseed	Transgenic rapeseed
Organic matter	79.7 ± 1.5	80.3 ± 0.7	82.3 ± 1.6	82.2 ± 0.7
Crude protein	82.5 ± 2.8	83.2 ± 1.5	85.1 ± 2.4	84.3 ± 0.3
Ether extract	37.6 ± 10.6	37.4 ± 4.4	50.8 ± 5.0	46.6 ± 4.9
Crude fibre	42.2 ± 1.6	44.5 ± 3.5	35.6 ± 7.0	40.4 ± 2.8
N-free extract	88.6 ± 0.8	88.6 ± 0.5	90.3 ± 0.8	90.0 ± 0.3
Energy content	14.00	14.78	14.13	14.61

lower feed intake because the transgenic rapeseed with the higher glucosinolate content (see Table III) was fed. The average BWG corresponds to the feed intake, so that feed and energy efficiency were not affected by the rapeseed source. This was in accordance with the investigations conducted with hamsters, which were fed myristic-rich and conventional rapeseed oil at a level of 15% in the diet. After the feeding period of six weeks significant differences in body weight gain and feed conversion were not detected (Eder & Brandsch 2002).

The results of slaughter performance correspond to the different production level in both groups (Table VII).

From the various parameters including back fat thickness, leaf fat weight and meat:fat-area of the *M. longissimus dorsi* muscle measured between rib 13 and 14, it can be concluded that the pigs fed the transgenic rapeseed had leaner carcasses. The parameter meat percentage was estimated to be significantly higher ( $p < 0.05$ ), obviously due to the lower feed intake.

The transfer of dietary fatty acids into body fat considering the total intake during the growing- finishing period is presented in Table VIII.

The fatty acid intake (FAI) was estimated from the feed intake (FI), the crude fat content (EE) and the FA profile of the diets according to the equation deduced by Kratz (2003):

$$\text{FAI [g]} = \text{FI [kg]} \cdot 0.9 \text{ EE [g/kg]} \cdot \text{FA [\%]}$$

This equation is based on the assumption that crude fat consists totally of triglycerides and that the average portion of glycerine in triglycerides amounts to 10%.

Table VI. Growth performance of pigs (33–105 kg BW) fed isogenic or transgenic rapeseed ( $n = 10$  per group).

	Isogenic rapeseed	Transgenic rapeseed
Feed intake [kg/d]	2.47 ± 0.23	2.35 ± 0.22
Energy intake [MJ ME/d]	32.4 ± 2.9	30.6 ± 2.9
BWG [g/d]	832 ± 75	795 ± 59
FCR [kg/kg]	2.97 ± 0.21	2.95 ± 0.18
Energy conversion ratio [MJ ME/kg BWG]	39.0 ± 2.8	38.6 ± 2.3

Table VII. Slaughter performance and weight of organs ( $n = 10$  per group).

	Isogenic rapeseed	Transgenic rapeseed
Back fat thickness [cm]	1.67 ± 0.20	1.61 ± 0.52
Dressing percentage [%]	81.1 ± 1.9	80.2 ± 0.8
Leaf fat [kg]	1.13 ± 0.05	1.04 ± 0.05
Meat area <i>longissimus dorsi</i> muscle [cm <sup>2</sup> ]	52.33 ± 19.08	56.28 ± 8.47
Fat area <i>longissimus dorsi</i> muscle [cm <sup>2</sup> ]	19.08 ± 1.25	18.19 ± 2.08
Meat percentage [%]	58.3 <sup>b</sup> ± 2.5	61.1 <sup>a</sup> ± 2.1
Thyroid gland weight [g]	9.6 ± 1.8	11.0 ± 1.8
Thyroid gland weight [mg/kg BW]	90 <sup>b</sup> ± 17	107 <sup>a</sup> ± 15
Liver weight [g]	1478 ± 123	1500 ± 109
Liver weight [g/kg BW]	13.8 <sup>b</sup> ± 0.6	14.6 <sup>a</sup> ± 1.2
Kidney weight [g]	343 ± 44	321 ± 30
Kidney weight [g/kg BW]	3.2 ± 0.2	3.1 ± 0.3

<sup>a,b</sup>Mean values in the same line not followed by the same superscript are significantly different ( $p < 0.05$ ).

As expected, the different intake of medium-chain fatty acids influenced the fatty acid profile of body fat. With an increased intake the myristic and palmitic acid accumulated in back fat and intramuscular fat and the oleic content decreased with lower intake ( $p < 0.01$ ).

The glucosinolate content was 1.9 mmol/kg in the isogenic diet and 2.9 mmol/kg in the transgenic one. The different glucosinolate intake affected the weight of thyroid gland and liver significantly ( $p < 0.05$ ), when related to body weight, whereas the kidney weights remained unaffected (Table VII).

The higher glucosinolate intake associated with feeding the transgenic rapeseed influenced the iodine concentration in the thyroid glands, although the iodine intake was almost the same in all diets, amounting to 1.5 mg/kg concentrate (Table IX). These results are in accordance with previous investigations published by Schöne et al. (1997) demonstrating that the amount of iodine stored in the thyroid gland depends only to a small extent on the dietary supply, but

Table VIII. Influence of total fatty acid intake on the fatty acid profile of back fat and intramuscular fat of pigs ( $n = 10$ ).

	Isogenic rapeseed	Transgenic rapeseed
<i>Total intake of fatty acids [kg]</i>		
Lauric (C12:0)	0.20 <sup>b</sup> ± 0.02	0.18 <sup>a</sup> ± 0.02
Myristic (C14:0)	0.01 <sup>A</sup> ± 0.00	1.27 <sup>B</sup> ± 0.12
Palmitic (C16:0)	1.06 <sup>A</sup> ± 0.10	2.62 <sup>B</sup> ± 0.24
Stearic (C18:0)	0.11 <sup>a</sup> ± 0.01	0.12 <sup>b</sup> ± 0.01
Oleic (C18:1)	10.15 <sup>B</sup> ± 0.91	5.19 <sup>A</sup> ± 0.49
<i>Back fat [% of total fatty acids]</i>		
Lauric (C12:0)	2.12 ± 0.07	2.11 ± 0.06
Myristic (C14:0)	1.47 <sup>A</sup> ± 0.16	1.94 <sup>B</sup> ± 0.13
Palmitic (C16:0)	19.69 <sup>A</sup> ± 0.99	21.04 <sup>B</sup> ± 1.17
Stearic (C18:0)	10.39 ± 0.86	10.95 ± 0.89
Oleic (C18:1)	50.47 <sup>B</sup> ± 1.45	46.72 <sup>A</sup> ± 1.18
<i>Intramuscular fat [% of total fatty acids]</i>		
Lauric (C12:0)	1.73 <sup>A</sup> ± 0.06	1.86 <sup>B</sup> ± 0.10
Myristic (C14:0)	1.48 <sup>A</sup> ± 0.17	1.77 <sup>B</sup> ± 0.16
Palmitic (C16:0)	21.01 ± 1.04	21.79 ± 0.91
Stearic (C18:0)	10.98 ± 0.99	11.34 ± 1.15
Oleic (C18:1)	50.53 <sup>B</sup> ± 1.09	47.90 <sup>A</sup> ± 1.93

Mean values in the same line not followed by the same superscript are significantly different (<sup>a,b</sup> $p < 0.05$ ; <sup>A,B</sup> $p < 0.01$ ).

Table IX. Iodine status of growing finishing pigs fed rapeseed differing in the glucosinolate content ( $n = 10$ ).

Rapeseed	Isogenic rapeseed	Transgenic rapeseed
Total glucosinolate intake [mmol]	422 <sup>A</sup> ± 39	606 <sup>B</sup> ± 57
<i>Iodine concentration</i>		
Thyroid gland [µg/g]	795 <sup>b</sup> ± 77	561 <sup>a</sup> ± 63
Liver [µg/kg]	31 ± 4	29 ± 3
Kidney [µg/kg]	25 ± 2	27 ± 3

Mean values in the same line not followed by the same superscript are significantly different (<sup>a,b</sup> $p < 0.05$ ; <sup>A,B</sup> $p < 0.01$ ).

primarily on the glucosinolate content of the diet. The decrease of feed intake, the increased liver and thyroid gland weights (see Table VII) are substantial indicators for increased metabolic burdens.

#### 4. Conclusion

The results of the chemical analyses demonstrate that the introduction of the acyl-thioesterase gene did not greatly affect the content of crude nutrients. This was not only demonstrated by main nutrients, but also for amino acid profiles and cell wall constituents. The content of minerals remained also unaffected. The modified fatty acid profile resulted in an increased content of medium-chain fatty acids at the expense of oleic acid. Normally such full-fat rapeseed is not used in animal feeding, but the limited amounts did not allow technical treatments like extraction. The increased glucosinolate content of GM-rapeseed cannot be sufficiently explained and needs further studies. From batch to batch differences have been observed for glucosinolates.

There are indications that the introduction of the acyl-thioesterase gene did not affect the digestibility of nutrients and the nutritive value as the balance experiments have shown. These results are in agreement with earlier investigations of genetically modified inulin- synthesizing potatoes (Böhme et al. 2005).

Comparing the diets with their high rapeseed inclusion level, the transgenic rapeseed lowered feed intake and weight gain and had a significant anti-thyroid effect due to the higher glucosinolate content.

These investigations support the thesis that genetic engineering of plants with the aim to change their composition might be associated with an increase of undesirable substances. Therefore, compositional investigations are not sufficient to assess the nutritional value or the bio-safety of genetically modified feedstuffs of the second generation. The results support the necessity of comprehensive feeding studies with the target species with specific experimental designs (Flachowsky & Böhme 2005).

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