DIGESTIBILITY OF NON-TRANSGENIC AND TRANSGENIC OILSEED RAPE IN RABBITS

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Abstract - Thirty, seven-week-old hybrid rabbits were used to determine the nutritive value of two oilseed rapes: a non-transgenic double low cultivar (control) and its transgenic derivative (NMS8-RF3) obtained by gene-transfer technology. Digestible energy (DE) and the digestibility coefficients (DC) were estimated by the substitution method. The inclusion level of the oilseed rapes, at the expense of all basal ingredients, amounted to 30%. The oilseed rapes differed in fat content (44.3% *vs.* 41.42% on DM basis) and consequently in gross energy content: 29.00 and 28.29 MJ/kg DM for the non-transgenic and NMS8-RF3 seeds, respectively.
The protein, fat, NDF, ADF and energy digestibilities of both experimental oilseed rape diets were significantly ($P<0.001$) higher compared to the basal diet. However, differences in DC between the oilseed rape diets were small and not significant ($P>0.05$). Consequently, the DC of both oilseed rape seeds were quite similar. Protein and fat digestibilities were high: 78.7 and 92.9 % *vs.* 76.3 and 90.8% for control and NMS8-RF3 seeds, respectively. The energy digestibility of the non-transgenic oilseed rape tended ($P = 0.08$) to be higher than the transgenic entry. The DE content of both oilseed rape seeds was 23.61 for the control and 22.26 MJ/kg DM for the NMS8-RF3. This difference could be explained mainly by the different fat content. The DC of NDF and especially ADF of both oilseed rapes were surprisingly high (65 - 70%), indicating possible interactions between the basal diet and the oilseed rapes.
Especially during the first 3 days of the adaptation period, rabbits fed oilseed rape diets showed a severe decreased feed intake (45% of basal diet) and consequently lower gains. After one week the intake reached a normal level taking into account the increased dietary DE content. No significant differences in intake pattern between both oilseed rapes were observed.

INTRODUCTION

The world-wide production of *Brassica* oilseed crops is expanding faster than any other annual oilseed crop. Rapeseed has become the third most important world source of edible oil after soybean and palm (SHAHIDI, 1990). In 1991, the production was over 27 million tonnes of seeds (F.A.O., 1992). Rapeseed meal is used as a high quality protein source for livestock production. Rapeseed oil is used in a variety of edible and industrial products.
Because of the antinutrients in rapeseeds (PUSZTAI, 1989), genetic selection in the last decades has been emphasized to achieve cultivars low in erucic acid and glucosino lates. More recently there have been increased efforts to select triple low varieties, with a reduced tannin and fibre content.
Recent selection in view of seed yield increase is pursued by making use of hybridization. Oilseed rape is a crop capable of both self-pollination and cross-pollination. A pollination control system is therefore required to produce 100% F_1 hybrid seeds. Gene transfer technology has recently been used to develop a new hybridization system in oilseed rape (*Brassica napus* L. OLEIFERA) based on a new type of male sterility and fertility restoration (MARIANI *et al.*, 1990; MARIANI *et al.*, 1991; MARIANI *et al.*, 1992). A Nuclear Male Sterility (NMS) gene construct (containing the T-DNA derived from pTHW107) and a Restorer of Fertility (RF) construct (containing the T-DNA derived from pTHW118) have been introduced in an elite oilseed rape variety. This double low variety, contains little erucic acid and also a low content of glucosinolates (< 15 mM/g) (GEVES, 1993).
Oilseed rape from the original cultivar and its transgenic derivative (NMS8-RF3) were offered to growing rabbits in order to study their digestibility.

MATERIALS AND METHODS

Experimental animals and housing

Seven-week-old rabbits belonging to the final cross between the dam and sire line, of the Institute's own selected strain, were housed in a separate experimental rabbit house. In total 30 rabbits of both sexes were assigned randomly to the digestibility cages. They were individually caged in wire mesh cages (40 cm x 30 cm x 29 cm high). Cages were equipped with a nipple drinker and a 10 cm wide, outside placed feeder. During the experiment water was always available *ad libitum*. Artificial ventilation and heating was used to create optimal environmental conditions (17 ± 2°C). In the windowless experimental house, 9 hours of light was provided per 24 hours period.

Treatments and feeding

Untreated oilseed rape from the non-transgenic cultivar (as control) and the transgenic entry (NMS8-RF3) were included in a standard basal diet (Table 1). The inclusion level, at the expense of all basal ingredients amounted to 30%. The basal diet and the two experimental diets were pelleted (Æ 3.2 mm). In order to increase pellet quality of the experimental diets, all diets were pelleted three times. Temperature measured immediately after pelletization was always lower than 75°C.

The digestibility trial was performed according to the European reference method (PEREZ *et al.*, 1995). A preliminary adaptation period of 10 days was carried out before the 4 days balance trial. From the initial 10 replicates, 2 were discarded on each diet because of sanitary problems or having a relatively high or low feed intake.

Rabbits were always fed *ad libitum*. Faecal output was collected daily, pooled individually in plastic boxes and stored at -18°C until further analysis.

Table 1 : Ingredient composition of the basal diet

Ingredients	%	Ingredients	%
Alfalfa meal	28.3	Molasses (cane)	4.0
Wheat	13.0	Salt	0.1
Wheat middlings	30.0	Min.vit. mix	2.5
Sunflower meal (29%)	5.0	Methionine	0.05
Soybean meal (44%)	5.0	Coccidiostat	0.1
Flax chaff	12.0		

Analytical procedures

Faecal outputs were dried at 80°C for 24h, homogenized, equilibrated and ground before analysis. Samples of feeds and faeces were analyzed for dry matter (105°C until constant weight), ash (550°c for 5 h), nitrogen (KJELDAHL) and fat (Soxhlet petroleum ether extraction with a HCL-pretreatment) following AOAC (1990) proscriptions. NDF, ADF and ADL were determined subsequently according to the procedure of VAN SOEST *et al.* (1991). An amylolytic treatment with a thermostable amylase was used prior to NDF extraction. Gross energy was measured with an adiabatic bomb calorimeter (IKA, C 400). All results were calculated on a dry weight basis. Apparent whole-tract digestibility coefficients (DC) and the digestible energy (DE) content of diets were calculated from the respective dry matter intake and output, as well as their corresponding nutrient content. DC of oilseed rapes were estimated by the substitution method using the difference principle.

Statistical analysis

Digestibility coefficients and DE content of diets and oilseed rapes were analyzed statistically by a GLM procedure, using the SAS®/STAT version 6 (1990).

RESULTS AND DISCUSSION

Chemical composition of both oilseed rape entries and the different diets is given in Table 2. Seeds from the NMS8-RF3 entry had a comparable protein content (26.2%) but contained less fat (-2.9% on DM basis) and more ADL (+1.7%) than the non-transgenic oilseed rape. Consequently, the GE content differed 0.5 MJ between both seeds. Due to the high fat content of oilseed rape, experimental diets were very fat-rich (± 15%) and their pellet quality was weak. In order to prevent deblending and excessive meal production, diets had to be pelleted several times until a satisfactory quality was obtained.

Some zootechnical results are presented in Table 3. Although the number of rabbits in a digestibility trial is limited and the experimental period too short to judge the effect on these parameters, some indications concerning palatability or toxicity can be drawn. Due to a significant lower feed intake, daily weight gain was significantly ($P<0.001$) reduced in rabbits fed one of both oilseed rape diets. Especially in the first days of the adaptation period, rabbits did not like both experimental diets. On average the intake was only about 45% compared to the basal diet. After one week the intake reached a normal level, taking into account the high DE content of the oilseed rape diets (Table 4). Numerous studies have clearly demonstrated that rabbits try to adjust their voluntary feed intake in response to changes in dietary energy concentration (LEBAS *et al.*, 1975). During the balance trial, although feed intake was significantly lower, daily DE intake on both oilseed rapes was higher (0.18 and 0.25 MJ, respectively) which explains why some compensatory growth occurred during the balance period. Between both oilseed rape diets, differences in intake and gain were small and did not reach the significance level.

Table 2 : Chemical composition (% of DM) of the oilseed rapes and experimental diets

	Oilseeds rapes		Diets		
	Control	MS8-RF3	Basal	30% control	30% MS8-RF 3
DM	94.0	94.3	89.4	91.9	91.9
OM	94.7	95.3	91.4	92.5	92.7
Ash	5.3	4.7	8.6	7.5	7.3
Crude protein	26.3	26.2	17.4	19.9	19.9
Crude fat	44.3	41.4	2.9	15.6	14.8
NDF	40.1	40.2	40.4	39.8	39.9
ADF	25.5	25.6	23.0	23.5	23.5
ADL	14.0	15.7	8.3	9.9	10.6
GE (MJ/kg)	29.00	28.29	18.36	21.40	21.23

Table 3 : Zootechnical results (in g/rabbit/day)

	Basal diet	Control diet	MS8-RF3 diet	SEM	P
Start weight (g)	1583	1563	1552	23.7	>0.1
Final weight (g)	2259^{a}	2097^{b}	2103^{b}	30.4	0.03
Feed intake (g/d)					
* Adaptation period (0-3d)	145.3^{A}	60.6^{B}	68.2^{B}	5.6	<0.001
(3-7d)	170.0^{A}	102.2^{B}	98.2^{B}	3.0	<0.001
(7-10d)	182.8^{A}	131.8^{B}	124.2^{B}	3.4	<0.001
* Balance trial (10-14d)	188.0^{A}	148.1^{B}	157.4^{B}	4.1	0.003
* Total period (0-14d)	172.6^{A}	112.8^{B}	114.3^{B}	3.2	<0.001
Weight gain (g/d)					
* Adaptation period (10-14d)	49.8^{A}	34.2^{B}	33.0^{B}	1.4	<0.001
* Balance trial (10-14d)	44.7	48.0	55.3	2.7	>0.1
* Total period (0-14d)	48.3^{a}	38.1^{b}	$39.4b^{b}$	1.4	0.02
Feed efficiency					
0-14 days	3.60^{A}	2.97^{B}	2.92^{B}	0.07	0.001

Treatment means followed by a different letter are significant from each other :a,b: P<0.05; A,B: P<0.01

Dietary digestibilities and DC of oilseed rapes are presented in Table 4 and 5. With the exception of ash, all digestibilities of the oilseed rape diets were significantly higher (P<0.001) compared to the basal diet. However, DC differences between both oilseed rape diets were small except for gross energy. The energy digestibility of the diet containing the original cultivar was 1.3 points higher (P<0.05) compared to the transgenic diet. The DC of both oilseed rapes were quite similar as concerns their protein, fat and fibre digestibilities (Table 5). The digestibility of protein was comparable with oilseed rape meal (77%) (MAERTENS *et al.*, 1988). The digestibility of the quantitative most important fraction, the lipids, was very high: 92.9 and 90.8%, respectively and in the same range of soybean oil (MAERTENS *et al.*, 1986).

The energy digestibility of the non-transgenic seeds tended (P=0.08) to be higher than the transgenic entry. An explanation could be searched in the protein and fat digestibility which was somewhat lower probably due to the increased ADL content of the transgenic line. The difference in digestible energy content amounted to 1.3 MJ/kg which could mainly be explained by the higher fat content (+2.9%) of the original non-transgenic variety.

The DC of NDF and ADF of both oilseed rape diets and consequently of the seeds was very high. The DC of ADF was about 65% while previously in oilseed rape meals an average value of the DC of crude fibre of only 10% was determined (MAERTENS *et al.*, 1988). An explanation for the unrealistic high fiber digestibilities found here, has to be searched in the methodology used. The DC of oilseed rapes are calculated with the assumption that no interactions between basal diet and the test feedstuffs occur (additivity principle). However, interactions can be expected when high-fat containing feedstuffs are included (SANTOMA *et al.*, 1987; FEKETE *et al.*, 1990). As a result improved DC of the nutrients were found, e.g. due to changes of the retention time in the digestive tract (GIDENNE *et al.*, 1987; GIDENNE & PEREZ, 1994) although the work of FERNANDEZ *et al.* (1994) does not support this hypothesis.

Table 4 : Apparent digestibility coefficients of the diets (%)

	Basal diet	Control diet	MS8-RF3 diet	SEM	P value
DM	57.2^{A}	63.4^{B}	62.4^{B}	0.3	<0.001
OM	57.8^{A}	64.6^{B}	63.7^{B}	0.4	<0.001
Ash	51.3^{A}	47.8^{B}	46.7^{B}	0.7	<0.001
Crude protein	72.1^{A}	75.2^{B}	74.2^{B}	0.5	<0.001
Crude fat	70.8^{A}	88.5^{B}	86.2^{B}	1.2	<0.001
NDF	25.2^{A}	39.8^{B}	38.6^{B}	0.8	<0.001
ADF	16.8^{A}	33.9^{B}	32.7^{B}	0.8	<0.001
Gross energy	56.1^{A}	66.8^{Ba}	65.5^{Bb}	0.4	<0.001
Dig. energy (MJ/kg DM)	10.30^{B}	14.30^{Ba}	13.90^{Bb}	0.1	<0.001

Treatment means followed by a different letter are significant from each other: A,B: P<0.01

Table 5 : Digestibility coefficients of the different oilseed rapes for rabbits

	Non-transgenic control	MS8-RF3	SEM	P value
Digestibility (%)				
Dry matter	77.7	74.6	1.3	>0.1
Organic matter	80.3	77.2	1.4	>0.1
Ash	31.0	23.0	3.4	>0.1
Crude protein	78.7	76.3	1.1	>0.1
Crude fat	92.9	90.8	1.4	>0.1
NDF	72.5	68.7	3.1	>0.1
ADF	68.7	65.0	2.5	>0.1
Gross energy	81.4	78.7	1.0	0.08
Digestible energy (MJ/kg DM)	23.61^{A}	22.26^{B}	0.3	0.007

CONCLUSION

The inclusion of 30% oilseed rape in a basal diet for fattening rabbits had some negative effects on the feed intake of rabbits during the first days of administration. However, afterwards rabbits' feed intake on both oilseed rape diets was sufficient, taking into account the increased dietary DE content. The seeds from the transgenic entry showed at least similar zootechnical performances as the original non-transgenic cultivar. Mainly due to a lower fat content, the transgenic seeds had a lower DE content. The very high determined ADF digestibilities of both oilseed rapes indicate that when high fat containing feedstuffs are introduced in a basal diet, interactions between the raw material in test and the basal diet takes place.

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Digeribilità dei semi di colza e di una corrispondente cultivar transgenica nei conigli - Trenta conigli ibridi di 7 settimane d'età sono stati utilizzati per determinare il valore nutritivo di due semi di colza: una cultivar (doppio OO) non trangenica (controllo) e la sua corrispondente transgenica (NMS8-RF3) ottenuta con la bio-tecnologia del trasferimento dei geni. L'energia digeribile (ED) ed i coefficienti di digeribilità (CD) sono stati determinati con il metodo della sostituzione. Il livello di inclusione dei semi di colza, in sostituzione di tutti gli ingredienti di base, è stato del 30%. I semi di colza differivano nel contenuto di grasso (44,3% e 41,42% sulla s.s.) e di conseguenza nel contenuto in energia grezza: 29,00 e 28,29 MJ/kg s.s. per la linea non transgenica e per quella transgenica, rispettivamente.
La digeribilità di proteine, lipidi, NDF, ADF ed energia di entrambe le diete contenenti di colza è stata statisticamente superiore (P<0,001) in confronto alla dieta di base. In ogni caso, le differenze dei CD tra le due diete contenenti la colza non sono risultate statisticamente significative (P>0,05); di conseguenza, i CD delle due semi sono, in pratica, risultati uguali. La digeribilità delle proteine e dei grassi è stata abbastanza elevata: 78,7 e 92,9% *versus* 76,3 e 90,8 per semi di controllo e per i semi NMS8-RF3, rispettivamente. La digeribilità dell' energia di colza non transgenica he mostrato la tendenza ad essere più elevata (P=0,08) di quella della linea transgenica. Il contenuto in ED dei semi di colza è risultato 23,61 (controllo) e 22,26 MJ/kg DM (NMS8-RF3); tale differenza potrebbe essere giustificata dal diverso contenuto in grassi. I CD del'NDF e specialmente del'ADF delle due semi di colza sono stati sorprendentemente elevati (65 - 70%), il che indica una probabile interazione tra la dieta base e le diete con i semi di colza.
Specialmente durante i primi 3 giorni del periodo di adattamento, i conigli alimentati con le diete a base di colza hanno mostrato una forte diminuzione nell'assunzione dell'alimento (45% della dieta base) e, di conseguenza minori incrementi di peso. Dopo una settimana, il consumo di alimento ha raggiunto livelli normali, tenendo presente anche l'aumentato contenuto di ED della dieta. Non si sono riscontrate differenze statisticamente significative tra i due diversi tipi di colza per quanto riguarda il consumo di alimento.
