Journal of Animal and Feed Sciences, 4, 1995, 153–160

Studies on enzymatic fractionation, chemical composition and biological effects of dietary fibre in rape seed (*Brassica napus* L.)

3. Degradation of dietary fibre components of rape seed by microbial enzymes and its influence on nutrient utilization by rats and chickens

P. Ochodzki[1], Maria Rakowska[1], Barbara Rek-Cieply[1], Charlotte Bjergegaard[2] and H. Sørensen[2]

[1] *Department of Biological Evaluation of Plant Products, Institute of Plant Breeding and Acclimatization, Radzików, 05-870 Błonie, Poland*
[2] *Chemistry Department, Royal Veterinary and Agricultural University, 40-Thorvaldsensvej, DK 1871-Frederiksberg C, Denmark*

(Received 9 November 1994; accepted 28 April 1995)

ABSTRACT

Microbial enzyme preparations, especially Viscozyme 120L (Novo Nordisk A/S, Denmark) added *in vitro* to milled, defatted rape seed, following enzymatic pepsin and pancreatin digestion induced evident changes in the level of insoluble (IDF) and soluble (SDF) dietary fibres (DF). An appreciable part of IDF in oilseed rape was thus solubilised reducing its content from 28.9 to 13.0%, while SDF increased from 1.6 to 3.7%. Viscozyme 120L treatment also reduced the level of DF-associated ash and protein (N x 6.25).

Feeding experiments with rats and chickens given Viscozyme 120L – treated full fat rape seed meal were performed in order to evaluate the effect on utilization of protein (rat trials) and growth performance (trials with chickens). The digestibility of protein was unchanged when the diet included 40% enzyme-treated full fat rapeseed meal, whereas there was a tendency for the biological value of the protein to increase when compared with the results obtained using a control diet containing untreated rapeseed meal. Experiments with chickens fed 15% enzyme-treated full fat rapeseed meal showed an increase of 10-day-body mass gain from 208 g (without enzyme addition) to 240 g (with Viscozyme 120L preincubation).

KEY WORDS: biological value, chicken, dietary fibre, double low oilseed rape, exoenzymatic degradation, rat

INTRODUCTION

DF in rape seed low in glucosinolates are the main components limiting the use of rape seed meal in feed mixtures for poultry and young pigs (Bell, 1993; Slominski and Campbell, 1990; Bjergegaard et al., 1991a; Danielsen et al., 1994). The DF components have a broad capability of binding different nutrients, including proteins (Ochodzki et al., 1995) and amino acids, as well as minerals as calcium, magnesium, zinc, and iron (Bjergegaard et al., 1991b; Bjergegaard, 1993; Casterline and Ku, 1993; Weber et al., 1993). This may result in a reduction of the availability of nutrients in feed mixtures containing rapeseed meal.

Bell and Keith (1989) used β-glucanase and pentosanase from *Aspergillus niger* in pigs fed Canola and low mucilage rapeseed meals. Slight improvement of daily body mass gain and feed gain ratio was obtained on a Canola diet while no improvement was noted on "low mucilage" rapeseed meal.

Boisen and Fernandez (1991) showed that in vitro digestion of dry matter of rapeseed meal was of the same range compared to dry matter ileal digestion of cannulated pigs. Additional treatment with Viscozyme caused further digestion of the dry matter of RSM, which was of the same range as faecal digestibility in pigs.

Slominski and Campbell (1990) showed 37% better digestion of nonstarch polysaccharides in laying hens after treatment with Novo's commercial enzyme SP 249. Alloui et al. (1994) used several exoenzymes (among others Energex Novo) in diets for chickens. In spite of the fact that 5-hour preincubation of the RSM with Energex resulted in a 10% decrease in acid detergent fibre (ADF) and 11% in neutral detergent fibre (NDF), there was no effect on growth and feed utilisation in chickens.

The objective of this study was to investigate the breakdown of fibre compounds of unprocessed defatted rapeseed meal by exoenzymes with different properties, and the resulting liberation of fibre associated compounds – proteins and minerals. The trials on N-balance in rats and growth test on chicken were performed testing the extent of the biological effects of enzyme-treated unprocessed rape seed.

MATERIAL AND METHODS

Double low oilseed rape (spring type) of the variety J0024-1-2-5 was obtained from the Polish Plant Breeding Station Małyszyn, milled and defatted by extraction with n-hexane in a Soxlet apparatus. After defatting, the rape seed samples contained 42.3% protein (N x 6.25) of air-dried material (8% moisture).

Three different microbial enzyme preparations: Viscozyme 120L, Celluclast 1.5L, and Novozyme 188 (Novo Industri A/S Copenhagen, Denmark) were used in two types of digestion systems in vitro:

System 1:

1 g of defatted rapeseed meal was incubated in 50 ml of 0.1 M phosphate buffer with pepsin (100 mg, 2 h, pH = 2, 40°C), pancreatin (100 mg, 2 h, pH = 6.8, 40°C) and one of the microbial enzyme preparation (0.1 ml Viscozyme 120L, 0.5 ml Celluclast 1.5L or 0.1 ml Novozyme 188 3 h, pH = 5.0, 40°C. A rape seed sample treated as above, except for the microbial enzyme addition, served as a reference sample.

System 2:

The second type of *in vitro* digestion was performed as described above, except for the order of enzyme treatment, using the microbial enzyme preparation before pepsin and pancreatin. A rape seed sample treated as above, except for the microbial enzyme addition, served as a reference sample.

Termamyl (thermostable α-amylase) was excluded in both system I and II, as the starch content in rape seed is very low (1-2%). Distinction between IDF and SDF in the rape seed samples was performed by filtration and ethanol precipitation as described by Asp et al. (1983). Ash and protein, determined gravimetrically and by the Kjeldahl method (N x 6.25), respectively, were subtracted from the residue weight in order to calculate the corrected IDF and SDF content in the starting material.

Noncellulosic polysaccharide (NCP) content and composition of insoluble fractions after enzymic treatment were determined. Hydrolysis in 2N TFA 1 h, 121°C (Albersheim, 1967) was carried out, aldononitrile acetate derivatives were prepared according to Mc Ginnis (1982) with mesoerythritol as the internal standard. A gas chromatograph Varian 3700 with 30m, 0.53mm ID DB-Wax megabore capillary column was used for GLC analyses. All data were integrated on a HP 3395 integrator.

The biological experiments were performed with the enzyme-treated full fat rapeseed meal on rats as well as chickens. Six-week-old male Wistar rats weighing from 65 to 70 g were kept in N balance cages for a 9-day period. True protein digestibility (TD), biological value (BV), and organic matter digestibility (DOM) were measured according to Eggum (1973). The experimental diets contained 40% rape seed meal (cv. Bolko) as the only source of protein (9.7% in air dried material) as well as N-free wheat starch, vitamin and mineral mixture according to the requirements for rats (NRC, 1973). The fat content of the diets was about 18%, giving an excess caloric value. In experimental diet 1, preincubation of the full fat rapeseed meal with Viscozyme 120L (25 ml of Viscozyme 120L + 75 ml H_2O, added to 900 g of milled seeds) was performed for 3.5 h at 35°C, prior to preparing the diets. Experimental diet 2 contained the same amount of Viscozyme 120L as used in diet 1, but no preincubation was

performed. The control diet included untreated full fat rapeseed meal in the same amount as in the experimental diets.

Analysis of IDF and SDF according to the enzymatic gravimetrical principle (Asp et al., 1983) normally includes treatment of plant material with Termamyl (starch degradation), pepsin (protein degradation), and pancreatin (combination of activities). In the present study, Termamyl treatment was excluded since rape seed has a very low starch content. On the other hand, sample treatment was supplemented by incubation with microbial enzyme preparations having a wide range of cell wall degrading activities: Viscozyme 120L, arabinase, cellulase, β-glucanase, hemicellulase, xylanase and pectinase activity; Celluclast 1.5L, cellulase activity; and Novozyme 188, cellobiase activity. The level of IDF and SDF in defatted rapeseed meal was determined for samples with and without this additional enzyme treatment, using one of three different microbial enzyme preparations either before or after pepsin and pancreatin treatments.

The feeding trial with chicken was performed using eight broilers per group, selected according to body mass on the 4th day after hatching. The birds were placed in heated plastic cages (30^{0}C) – one bird in each cage. The diets were composed of 85% Starter type meal (composed of, %: wheat 40, maize 20, soyabean meal 20, wheat bran 10 and protein-vitamin-mineral concentrate 10; protein content 23%, metabolic energy 10.64MJ and 15% full fat rapeseed meal (cv. Bolko) (control diet) or 15% Viscozyme 120L-treated full fat rapeseed meal as described above (rat trials, experimental diet 1). The feed intake of chickens and body mass gain after 10 days of experiment were measured. The statistical evaluation of data was performed by using QuattroPro 3.0, Borland International Inc., USA.

RESULTS AND DISCUSSION

The use of microbial enzymes in general reduced the IDF level, whereas the level of SDF was increased (Table 1). The explanation of these changes lays in the enzymatic cleavage of DF components (mainly hemicelluloses and cellulose) into smaller molecules, changing their solubility properties toward more soluble compounds. Part of IDF will thus be found in SDF. Initial release of protein and other compounds in the DF fraction by pepsin and pancreatin treatments was shown to be essential for the effect of microbial enzymes, as revealed from the results in Table 1. So, reductions in DF-associated compounds, especially the ash content, was much lower using Viscozyme 120L, Celluclast 1.5L, or Novozyme 188 as the first step compared to the use of the microbial enzymes as the final treatment. All together, Viscozyme 120L treatment had the most marked effect on IDF level, reducing IDF from 28.9 to 13% when used as a final step, and from

TABLE 1

Content of dietary fibre and associated compounds in defatted rape seed cv. J0023-1-2-5 after treatment with enzymes in two systems, %

Enzyme treatment	System 1*			System 2**		
	Ash	Protein N x 6.25	Fibre	Ash	Protein N x 6.25	Fibre
	IDF fraction					
Reference (pepsin-pancreatin)	5.3	9.7	28.9	3.3	8.7	27.5
+ Viscozyme	0.6	6.3	13.0	1.6	6.4	17.4
+ Celluclast	3.4	9.0	19.7	2.4	9.4	25.5
+ Novozyme	3.1	7.0	25.9	2.5	7.4	27.7
	SDF fraction					
Reference (pepsin-pancreatin)	11.2	4.3	1.6	4.6	3.6	1.2
+ Viscozyme	4.7	2.7	3.7	5.1	1.8	3.1
+ Celluclast	5.7	10.0	3.3	5.7	5.5	2.6
+ Novozyme	7.1	3.0	2.9	4.1	1.5	2.7

Enzyme treatment conditions:

* Pepsin 2 hours, pH = 2.0, pancreatin 2 hours, pH = 6.8, microbial enzymes 3 hours, pH = 5.0

** Microbial enzymes 3 hours, pH = 5.0, pepsin 2 hours, pH = 2.0, pancreatin 2 hours, pH = 6.8

Temperature for all treatments was 40°C

TABLE 2

Content of carbohydrates of insoluble dietary fibre residues after exogenic enzyme hydrolysis, % of dry rapeseed meal

Enzyme treatment	Rhamnose	Fucose	Arabinose	Xylose	Mannose	Glucose	Galactose	Total
Reference (pepsin-pancreatin)	0.42	0.21	4.59	1.96	2.98	0.64	1.45	12.25
+ Viscozyme	0.10	0.02	0.54	0.50	2.14	0.48	0.50	4.26
+ Celluclast	0.30	0.10	3.25	1.25	2.74	0.81	1.01	9.30
+ Novozyme	0.32	0.14	3.57	1.71	2.57	0.64	1.21	10.0

27.5 to 17.4% when used prior to pepsin and pancreatin treatments. This is not surprising because of the much wider range of enzymatic activity of Viscozyme 120L compared with that of Celluclast 1.5L and Novozyme 188. Analysis of monosaccharide composition of noncellulosic polysaccharides (Table 2) shows mannose as the main enzyme-resistant saccharide and Viscozyme as the most active polysaccharide-degrading enzyme. After Viscozyme digestion, mannose compounds constituted 50% of the remaining polysaccharides. The content of arabinose decreased from 45.9 mg in reference sample residues to 5.4 mg in residues after Viscozyme treatment, while decrease of the mannose content from 29.8 to 21.4 mg, respectively, was observed.

TABLE 3

Influence of Viscozyme pretreatment of rapeseed meal on protein and organic matter utilization in rats, %

Diet	TD	BV	NPU	OMD
Control	86.3±1.0	96.4	83.2	89.3±1.0
Experimental diet 1 (+ Viscozyme, + preincubation)	86.6±1.6	100	86.6	90.4±0.7
Experimental diet 2 (+ Viscozyme, – preincubation)	85.6±0.9	100	85.6	90.1±0.6

The data discussed above resulted in the choice of Viscozyme 120L for enzyme treatment of full fat rapeseed meal used in the animal trials. The N balance experiments with rats showed no improvement of TD whereas BV was increased by Viscozyme 120L treatment of rape seed (Table 3). The effect was independent of whether preincubation of the rape seed meal with Viscozyme 120L for 3.5 h was performed prior to mixing or Viscozyme 120L was added directly to the feed mixture without preincubation. The lack of effect on TD was surprising in the light of the marked reduction found for proteins associated to the IDF and SDF fractions after Viscozyme 120L treatment (Table 1). A possible explanation may be that the enzyme treatment of rape seed for the animal trials was performed on the full fat rapeseed meal without incubation with other enzymes, the opposite of what was done in the *in vitro* systems described above.

It would be interesting to know how much DF-associated protein left in the rape seed meal after the preincubation is available for the animal. It should be said that the DF fraction must have been affected to a certain degree as revealed from the positive effect of Viscozyme 120L treatment on BV. Another possibility is that the slightly positive effect on BV, and thereby on NPU, is an effect of the amino acids released by hydrolysis of Viscozyme 120L in the stomach and intestine of the rats. Further investigations are, however, required in order to understand the actual effect on rape seed DF components leading to this result.

TABLE 4

Influence of Viscozyme preincubation of rape seed on biological parameters in chicken fed a diet containing 15% rape seed (composition of diet, see text)

Diet	10 days body weight gain, g	Feed utilisation index g/g weight gain
Control	214±15	2.03±0.18
Experimetal diet	240±26	1.93±0.1

The results from the feeding trials with chickens are shown in Table 4. It was found that the 10-day-body mass gain increased by 15% (from 213 to 240 g) when chickens were fed the experimental diet containing 15% enzyme-treated full fat rapeseed meal. Feed utilization was only slightly improved (ca. 5%), decreasing from 2.03 to 1.94 g/g. In experiments with dehulled rape seed from aqueous enzymatic rape seed processing based on similar cell wall degrading enzymes, a lack of improvement of TD values in piglets was observed (Danielsen et al., 1994).

CONCLUSION

Microbial enzymes with cell wall degrading activities, e.g. Viscozyme 120L, proved to affect the solubility properties of rape seed DF, reducing the IDF level while increasing the level of SDF. The concomitant reduction in DFassociated compounds, including proteins had, however, only a minor effect on protein utilization using enzyme-treated rapeseed meal in N-balance trials with rats, whereas the growth rate of chickens proved to be increased compared with chickens fed a control diet. The differences of the digestive tract of rats and chickens may explain some of the differences observed. Further studies will be necessary in order to identify the components in DF responsible for the negative relationship between DF intake and utilization of nutrients. Investigation of the positive effect of Viscozyme 120L may also contribute to elucidating this observation.

ACKNOWLEDGMENTS

This study was supported by the Polish Scientific Research Committee (KBN) (Project No PB 1129/5/91).

We would like to thank Ms. Czeslawa Guzik, Anna Jankowska and Elżbieta Durawa for skillful technical assistance.

REFERENCES

Albersheim P., Nevins D.J., English P.D., Karr A., 1967. A method for the analysis of sugars in plant cell-wall polysaccharides by gas-liquid chromatography. Carbohyd. Res. 5, 340-345

Alloui O., Chibowska M., Smulikowska S., 1994. Effects of enzyme supplementation on the digestion of low glucosinolate rape seed meal in vitro, and its utilization by broiler chicks. J. Anim. Feed Sci. 3, 119-128

Asp N.G., Johansson C.G., Hallmer H., Siljeström M., 1983. Rapid enzymatic assay of insoluble and soluble dietary fiber. J. Agric. Food Chem. 31, 476-482

Bjergegaard C., Eggum B.O., Jensen S.K., Sørensen H., 1991a. Dietary fibres in oilseed rape: Physiological and antinutritional effects in rats of isolated IDF and SDF added to a standard diet (1). J. Anim. Physiol. Anim. Nutr. 66, 69-79

Bjergegaard C., Jensen S.K., Sørensen H., 1991b. Dietary fibres in rapeseed: Properties and effects on the digestibility of rapeseed meal. GCIRC-Congress, Saskatoon, Canada II, pp. 448-453

Bjergegaard C., 1993. Dietary fibres in rapeseed and peas – analytical methods and characterisation, Ph.D. Thesis Chemistry Department, Royal Veterinary and Agricultural University, Copenhagen, Denmark, pp. 206

Boisen S., Fernandez J.A., 1991. In vitro digestibility of energy and amino acids in pig feeds. Proceedings V International Symposium on Digestive Physiology in Pigs, Wageningen (Doorwerth), Netherlands, pp. 231-236

Casterline J.L., Ku Y., 1993. Binding of zinc to apple fiber, wheat bran, and fiber components. J. Food Sci. 58, 365-368

Danielsen V., Eggum B.O., Jensen S.K., Sørensen H., 1994. Dehulled protein-rich rapeseed meal as a protein source for early weaned piglets. Anim. Feed Sci. Technol. 46, 239-250

Eggum B.O., 1973. A study of certain factors influencing protein utilization in rats and pigs. 406. Beretn. Statens Husdyrbrugsforsøg, Copenhagen, Denmark, pp. 10-30

McGinnis G.D., 1982. Preparation of aldononitrile acetates using N-methyl imidazole as catalyst and solvent. Carbohyd. Res. 108, 284-292

NRC National Research Council, Committee on Animal Nutrition, Nutrient requirements of rats 1973, National Academy of Science. Washington. D.C.

Slominski B.A., Campbell L.D., 1990. Non-starch polysaccharides of canola meal: Quantification, digestibility in poultry and potential benefit of dietary enzyme supplementation. J. Sci. Food Agric. 53, 175-184

Weber C.W., Kohlhepp E.A., Idouraine A., Ochoa L.J., 1993. Binding capacity of 18 fiber sources of calcium. J. Agric. Food. Chem., 41, 1931-1935

STRESZCZENIE

Badania nad enzymatycznym frakcjonowaniem, składem chemicznym i działaniem biologicznym włókna pokarmowego rzepaku podwójnie ulepszonego. 3. Degradacja składników włókna pokarmowego pod wpływem enzymów grzybowych i ich wpływ na wykorzystanie składników żywieniowych przez szczury i kurczęta

Preparaty enzymów grzybowych, a zwłaszcza Viscozyme 120L (Novo Nordisk A/S, Dania) dodane do odtłuszczonego rzepaku trawionego wcześniej pepsyną i pankreatyną, wywołują wyraźne zmiany w zawartości i składzie frakcji nierozpuszczalnej (IDF) i rozpuszczalnej (SDF) włókna pokarmowego. Ilość IDF po zastosowaniu dodatkowo Viskozymu zmniejszała się z 28.9 do 13.0%, a SDF wzrastała z 1.6 do 3.7%. Zmniejszała się ilość popiołu i białka związanego z IDF. Zastosowanie enzymów grzybowych przed pepsyną i pankreatyną zmniejszało skuteczność ich działania. W doświadczeniach bilansowych na szczurach otrzymujących diety z 40% dodatkiem zmielonych nasion inkubowanych z Viskozymem lub bez dodatku nie stwierdzono zmian w strawności białka (TD), natomiast wystąpiła pewna tendencja do wzrostu wartości biologicznej białka w porównaniu z rzepakiem nie inkubowanym. W doświadczeniu wzrostowym kurczęta karmione dietą zawierającą 15% zmielonych nasion w grupie żywionej rzepakiem inkubowanym z Viscozymem (240 g/10dni/kurczę) przyrastały lepiej w porównaniu z kurczętami otrzymującymi rzepak nie inkubowany (208 g/10 dni/kurczę).