POTENTIAL FOR IMPROVED UTILIZATION OF CANOLA MEAL BY MONOGASTRIC ANIMALS

A Thesis

Submitted to the Faculty

of

Graduate Studies

The University of Manitoba

by

Joseph Simbaya

In Partial Fulfilment of the

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of

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JOSEPH SIMBAYA

A Thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

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DEDICATION

To my mother Melise Ngao Nambela, who will never understand why it had to take so long.

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FOREWORD

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MANUSCRIPT I. Simbaya, J., Slominski, B.A., Rakow, G., Campbell, L.D., Downey, R.K. and Bell, J.M. 1995. Quality characteristics of yellow-seeded *Brassica* seed meals: Protein, carbohydrates and dietary fibre components. J. Agric. Food Chem. 43: 2062-2066.

MANUSCRIPT II. Simbaya, J., Slominski, B.A., Rakow, G. and Campbell, L.D. 1995. Nutritive value of yellow-seeded canola. Part I. Digestible protein, dietary fibre and the effect of moist heat treatment on meal quality. J. Sci. Food Agric. (to be submitted).

MANUSCRIPT III. Simbaya, J., Slominski, B.A., Campbell, L.D., Rakow, G. and Guenter, W. 1995. Nutritive value of yellow-seeded canola. Part II. Chemical composition and feeding quality of meals derived from newly selected varieties. J. Sci. Food Agric. Sci. (to be submitted).

MANUSCRIPT IV. Simbaya, J., Slominski, B.A., Guenter, W., Morgan, A. and Campbell, L.D. 1995. The effects of protease and carbohydrase supplementation on the nutritive value of canola meal for poultry: *In vitro* and *in vivo* studies. Anim. Feed Sci. Technol. (accepted).

ABSTRACT

The purpose of this research was to explore the potential for improved utilization of canola meal by monogastric animals. In study one, 26 yellow-seeded and 7 brown-seeded *Brassica* genotypes were evaluated for differences in chemical composition and dietary fibre profiles. On average, in comparison to brown-seeded, yellow-seeded genotypes contained more sucrose (8.7% vs 7.5%) and protein (44.5% vs 42.7%) and less dietary fibre (28% vs 33%). Dietary fibre was negatively correlated (r=-0.71) with protein content and its reduction in yellow-seeded samples was attributed to lower contents of lignin and polyphenols, cell wall protein and minerals associated with the fibre fraction. In a second study, selected *Brassica* genotypes were analyzed for digestible protein, soluble fibre, content of soluble phenolics and extract viscosity. Despite only minor differences in soluble fibre, soluble phenolics and extract viscosity, *B. rapa* and *B. napus* species had relatively high digestible protein content in comparison to *B. juncea* and *B. carinata* species. The measurements of digestible protein and dietary fibre as well as soluble phenolics and extract viscosity were poorly correlated. Based on chemical composition and digestible protein content, four *Brassica* cultivars were selected for use in a third study and the seeds were processed under optimal moist heat treatment conditions (108 +1°C for 20 min). The samples included yellow-seeded *B. rapa* (cv. Parkland), *B. napus* (cv. Y1016) and *B. juncea* (J4316) and brown-seeded *B. napus* (cv. Excel). With the exception of *B. rapa*, all samples had higher than commercial meals protein content with the yellow-seeded *B. napus* canola showing the highest true metabolizable energy value. The overall performance of broiler chickens fed the *Brassica* seed meals was similar to that of the commercial meal from yellow-seeded canola (control) except for *B. juncea* which had a relatively high content of undesirable aliphatic glucosinolates. Of the diets with comparable growth performance, birds fed the yellow-seeded *B. napus* canola showed the highest feed efficiency value. In a forth study an attempt was made to improve the utilization of canola meal by supplementation of broiler chicken diets with exogenous enzymes. A positive and synergistic effect was noted when a combination of protease, carbohydrase and phytase enzymes were supplemented to canola meal-based diets deficient in available phosphorus.

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LIST OF ABBREVIATIONS

AOAC	Association of Official Analytical Chemists.
ANOVA	Analysis of Variance.
AMEn	Nitrogen corrected apparent metabolizable energy.
CM	Canola meal.
CP	Crude protein.
DM	Dry matter.
IU	International unit.
LMWC	Low molecular weight carbohydrates.
MWCO	Molecular weight cut off.
NDF	Neutral detergent fibre.
NRC	National Research Council.
NSP	Non-starch polysaccharides.
SAS	Statistical analysis system.
SBM	Soybean meal.
SCFA	Short chain fatty acids.
SCN	Isothiocyanate ion.
SCWL	Single comb white leghorn.
SD	Standard deviation.
SDS	Sodium dedocyl sulphate.
SEM	Standard error of the mean.
TDF	Total dietary fibre.
TMA	Trimethylamine.
TMEn	Nitrogen corrected true metabolizable energy.

1. INTRODUCTION

Seeds of *Brassica* species, canola/rapeseed and mustard rape, have become important oilseeds in many temperate and high altitude climate zones where other oil crops can not thrive and grow (Bell, 1984). *Brassica* crops currently account for 13.2% of the world's edible oil output, which makes them the third most important source of edible oil after soybean and palm (Shahidi, 1990a). In comparison to soybeans, *Brassica* seeds are considerably smaller and contain a higher oil content of more than 40% (dry matter basis), with a profile of fatty acids well suited to modern human consumption (Ackman, 1990). The superiority of canola varieties to the original rapeseed cultivars is well known. Rapeseed cultivars had disadvantages in that they yielded oil which contained 25 to 45% erucic acid and the oil-extracted meal had 110 to 150 μmoles of aliphatic glucosinolates per gram (Bell, 1993a). As a result of genetic selections, current cultivars yield oil with less than 2% erucic acid and less than 30 μmoles of glucosinolates per gram of the meal.

Most of the oil derived from *Brassica* seeds is used in human food products such as margarine, salad and cooking oils (Downey, 1983). The meal which remains after oil extraction contains 38-44% (dry matter basis) of high quality protein with an amino acid profile comparable to that of soybean meal (Fenwick, 1982; Downey and Röbbelen, 1989). While in some Asian countries the high glucosinolate meal is still being used as an organic fertilizer, in Europe and Canada the improved quality meal is exclusively used as a protein supplement in livestock and poultry diets (Downey, 1983; Bell, 1984).

Despite the high protein content, the use of canola meal (CM) as a high quality protein supplement for poultry and other monogastric animals is still limited by high content of dietary fibre (30%)in the meal (Bell and Shires, 1982). Dietary fibre tends to dilute the nutrient content and has also been associated with reduced energy (Sarwar *et al.*, 1981) and protein digestibilities in CM-based diets (Bell, 1993a). The major fibre components in CM include cellulose (4-6%), non-cellulosic polysaccharides (13-16%), lignin and polyphenols (5-8%), and protein and minerals associated with the fibre fraction (Slominski and Campbell, 1990). The nutritive quality of CM may be improved by reducing fibre content through genetic selections, innovations in processing plants or by dietary means. Currently, plant breeding programs are directed towards selection for yellow-seeded varieties of low fibre content (Stringam *et al.*, 1974). Efforts in commercial processing are aimed at reducing fibre content by dehulling of the seed prior to oil extraction and animal nutritionists are examining the possibility of increasing nutrient digestibility through the use of cell wall degrading enzymes (Slominski and Campbell, 1990; Slominski *et al.*, 1993).

Recent reports on chemical composition of yellow-seeded canola indicated only minimal reduction in fibre content which, as opposed to brown-seeded type, was found to contain more of non-starch polysaccharides (NSP) at the expense of lignin and associated polyphenols (Slominski and Campbell, 1991a; Slominski *et al.*, 1994a). Despite the increased nutrient content in dehulled CM, the processing industry is yet to establish the appropriate conditions to improve percolation of hexane through the hull-free and thus very fine meal (Bell, 1993b). While the application of supplementary cell

wall degrading enzymes tend to increase NSP digestibility in laying hens (Slominski and Campbell, 1990), their effect on the performance of growing chickens still remains inconclusive (Slominski *et al.*, 1993).

Lack of visible improvements in energy and protein utilization from yellow-seeded canola, dehulled CM and enzyme supplemented conventional CM suggests that further research is needed to characterize the factors that influence meal quality. The major objective of this study was to provide detailed knowledge on chemical composition of canola with emphasis on the potential relationship between total and digestible protein and soluble and insoluble dietary fibre contents. The effect of various conditions of moist heat treatment on *in vitro* protein digestibility was studied in order to establish optimal parameters required for processing of canola seed. Such parameters were further employed for processing of the seed used in subsequent *in vivo* evaluation of selected samples of canola. Since a wide range of yellow- and brown-seeded strains/varieties were included in the study, it is believed that this research will provide valuable information for future canola selection programs. Knowledge of digestible protein and available amino acid contents and the profile of dietary fibre will assist in identifying the industrial enzyme preparations suitable for canola meal treatment. Therefore, the second objective of the study was to explore the potential for improved utilization of canola meal by supplementation of canola meal containing poultry diets with exogenous protease and carbohydrase enzymes.

2. LITERATURE REVIEW

2.1. CANOLA MEAL: ITS IMPORTANCE AND LIMITATIONS

2.1.1. Historical Perspectives

The real beginning of rapeseed cultivation is not certain as the domestication of the crop occurred at different times in different parts of the world. Domestication of rapeseed occurred whenever the economical value of *Brassica* weed seeds in cereal grain crops was appreciated by local populations (Boulter, 1983; Åppleqvist, 1972). Early Asian Sanskrit writings indicate rapeseed to have been an important oil crop as early as 2000-1500 B.C. Ancient civilizations used rapeseed oil for illumination, cooking, medicinal, paint and soap making (Åppleqvist and Olhson, 1972). Rapeseed cultivation was introduced to Japan from China directly or via the Korean Peninsula in 35 B.C. (Bell, 1982).

In Europe, rapeseed cultivation dates back to the 13th century, when it was mainly confined to the Netherlands where it was grown for land reclamations from the sea (Åppleqvist and Olhson, 1972). The crop was introduced to England in the 16th century for the same purpose probably by Dutch ship builders. In England rapeseed cultivation increased in 1752 due to a Parliamentary bill which emphasized making oil out of locally grown seeds equal to foreign oils (Bunting, 1986). Rapeseed cultivation spread to other European countries in the 18th century, especially those where olive oil and poppy seed trees were not grown. It was from this period that rapeseed oil became

the most important lamp oil in Europe until the end of the 19th century when cultivation declined due to the discovery of mineral oils. However, this was also the time when rapeseed production was spreading eastwards into Switzerland, Poland and Western Russia and northwards into the Scandinavian countries of Denmark and Sweden, where production continued well up to the end of the 19th century. In Western Europe, production started again during the first world war due to blockades on industrial oil imports and the increased demand for edible oils during the hostilities. This initiative did not last long as there was a decrease in rapeseed production after the war and most of the oil demand was being met by cheap imports from the colonies in Africa and Asia.

Production increased again in the early 1940s when imports could not reach Europe with the start of the second world war. After the world war II, Sweden was the first nation to establish a guaranteed price system on locally grown rapeseed oil to boost domestic production and avoid future shortages as was experienced during the war. Other nations adopted the guaranteed price scheme, though in the early 1960s, the European rapeseed industry could not compete with cheap Canadian imports. In 1960 the formation of the EEC and its support policy of promoting local agricultural crops saved the oil industry from collapsing. Since then, the European rapeseed industry has been on the increase up to the present.

In Canada, rapeseed cultivation started in 1936 when a Polish farmer at Shellbrook, Saskatchewan received seeds of *Brassica rapa* from a contact in Poland (Bell, 1982). These seeds became the resource material for yield and establishment trials by the Canadian Department of Agriculture. In 1942, seeds of *Brassica napus* from

Argentina were introduced through the USA to Saskatchewan farmers who grew the crop on contract (White, 1979). For a long time, the two types of rapeseed were grown and came to be known as *Polish* and *Argentine* rape, respectively (Bell, 1982). The original interest in rapeseed production was centred on its high oil content and the unique property of sticking to steam and marine engines (Bell, 1982).

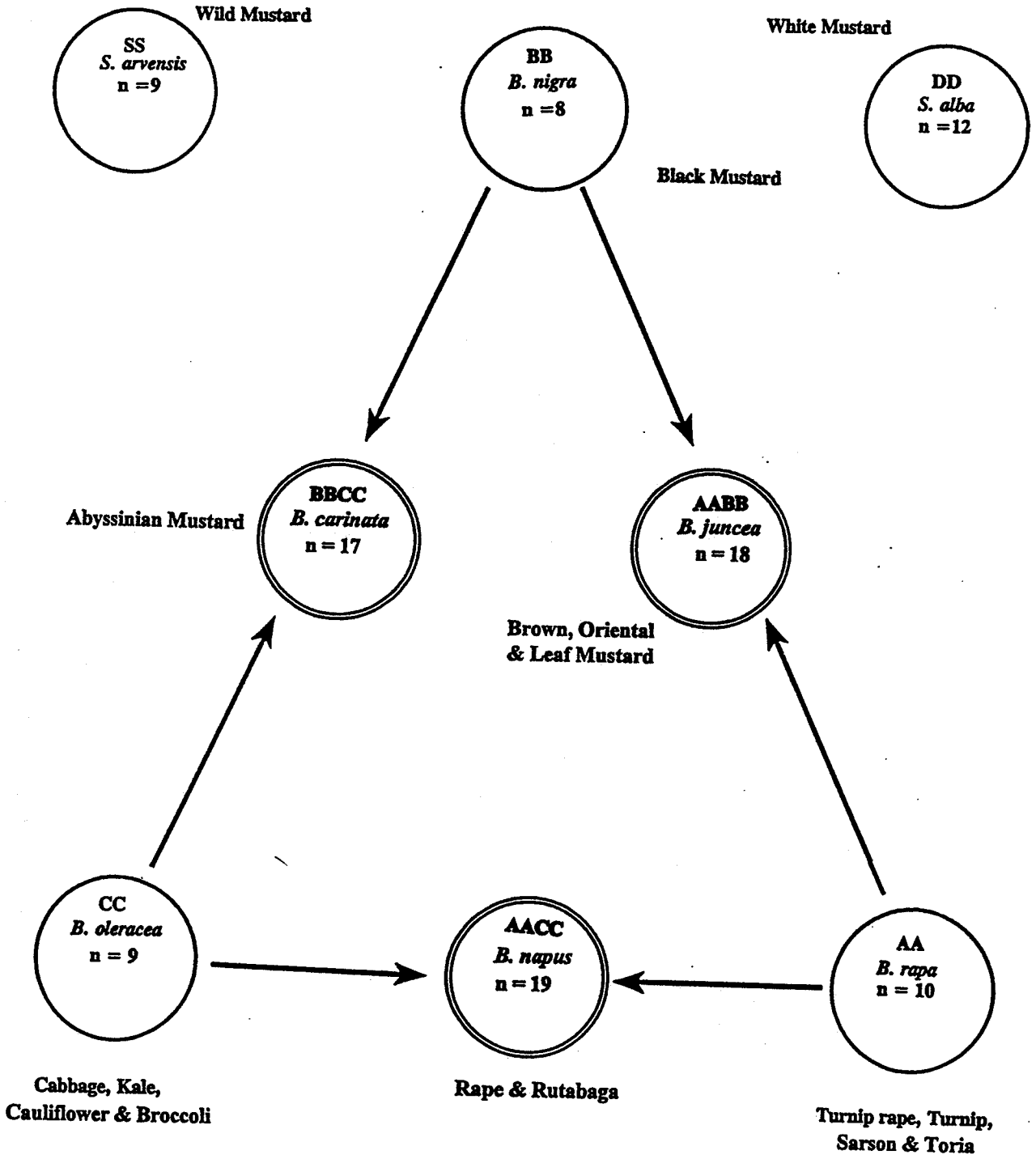
The increase in demand for industrial and human consumption created by war blockades led to increased local supply of rapeseed oil. But after the war, there was a decrease in the demand of industrial oil and there were concerns regarding the use of rapeseed oil for human consumption due to high erucic acid content which was considered a health hazard by the Food and Drug Directorate of the Department of National Health. While most of the oil was being exported to European markets, the limitations on erucic acid provided a motivation for the search of rapeseed strains with lower erucic acid content in their oil. Many cultivars were developed through plant breeding, and in 1970 a decision was made to change from growing high erucic acid varieties to low erucic acid varieties.

In parallel with expanded use of rapeseed oil on the domestic market, there was an urgent need to increase the use of rapeseed meal for animal feeding to make the crop more competitive (Bell and Wetter, 1979). But feeding of rapeseed meal to livestock and poultry resulted in thyroid disorders and was also associated with reduced feed intake and poor animal performance. This lack of response from rapeseed feeding set a stage for future research on meal improvement techniques. But progress on meal utilization was limited for many years by lack of analytical and appropriate processing techniques (Bell

and Wetter, 1979). With the analytical procedure developed by the Prairie Regional Laboratory, it was learnt that glucosinolates, which yield toxic and goitrogenic products upon hydrolysis, were the main factor limiting rapeseed meal utilization in swine and poultry. The break through came in the late 1960s when a variety "Bronowski" from Poland was found to contain only one seventh of glucosinolates normally present in rapeseed cultivars. This led to the development of low erucic acid and low glucosinolate varieties. By 1980, most of the rapeseed varieties grown in Canada were low erucic acid and low glucosinolate (double zero) cultivars. These were patented in 1981 under the name "canola" to differentiate them from the original rapeseed varieties.

2.1.2. Adaptation and Distribution

All current canola oil and meal producing varieties belong to the genus *Brassica* in the family of *Cruciferae*. Other members of the family and their cytogenetic relationships are presented in Fig. 1. The relationships have been verified by both chemotaxonomy and artificial hybridization of amphidiploids (Dass and Nybom, 1967). *Brassica* species appear to have evolved from a now extinct common ancestor in the Himalayan region (Hedge, 1976), though nearly all species seem to have secondary centres of origin and appear to have developed at different times wherever parental species coexisted. Due to natural selection and/or perhaps crossbreeding over several hundreds of years, the *Brassica* species have subdivided into different subspecies, forms and varieties or cultivars (Downey, 1983). Turnip rape (*Brassica rapa =campestris)* is the most variable and widely distributed of all the *Brassica* species (Thompson and


Wild Mustard
SS
S. arvensis
n =9
BB
B. nigra
n =8
White Mustard
DD
S. alba
n =12
Black Mustard
Abyssinian Mustard
BBCC
B. carinata
n = 17
AABB
B. juncea
n = 18
Brown, Oriental
& Leaf Mustard
CC
B. oleracea
n = 9
AACC
B. napus
n = 19
AA
B. rapa
n = 10
Cabbage, Kale,
Cauliflower & Broccoli
Rape & Rutabaga
Turnip rape, Turnip,
Sarson & Toria

Hughes, 1986). Its secondary centres of diversity range from the Atlantic Islands in the West to the shores of China and Korea in the East and from Norway in Northern Europe to Northern India in the South. The origin of *Brassica napus* is somehow obscure and it appears that the species have evolved at a much latter date; most probably in the Mediterranean region where the parental species of *B. rapa* and *B. oleracae* occurred together (Thompson and Hughes, 1986). The origins of *B. juncea* has also not been established with certainty, though most studies point to the Middle East as its secondary centre of diversity where *B. rapa* and *B. nigra* existed in proximity.

Because of their ability to survive and grow under relatively cold conditions, canola/rapeseed crops are restricted to the temperate regions of Canada, Northern Europe and the high altitude subtropical regions of China and the Indian subcontinent. As newly adapted varieties get released each year, there is a potential for Australia, the United States and perhaps South America to join the major rapeseed/canola producing regions of the world (Downey and Röbbelen, 1989). In Canadian and European temperate regions, *B. napus* and *B. rapa* are the predominant species grown whereas in China and the Indian subcontinent *B. rapa* (brown and yellow Sarson) and *B. juncea* (Toria) are the main species (Bunting, 1986). The Asian and European forms of *B. rapa* must have separated early in their development as the two forms differ significantly in their morphology and chemical compositions. Both European and Canadian *B. napus* and *B. rapa* varieties yield oil and meal of similar characteristics with each having spring and winter grown forms. In both cases, winter cultivars out yield spring ones by approximately 20%. There are also variations in seed yield between *B. rapa* and *B.*

napus varieties with the latter out yielding the former by approximately 20% and its seeds having more oil and protein.

Brassica carinata or Ethiopian mustard is yet to be incorporated into canola quality varieties and its production is still confined to its region of origin in North Eastern Africa in the Abyssinian highlands (Bunting, 1986). It should be pointed out, however, that its superior agronomic characteristics and resistance to pests and diseases has attracted attention in many current canola breeding programs. *Brassica juncea* forms are mostly grown in China and the Indian subcontinent though the recent increase in rapeseed production in Asia is mostly attributed to expanded use of *B. napus* varieties. In Canada and the United Kingdom, there has been an ongoing small scale cultivation of *B. juncea* varieties for commercial production of condiment mustard (Downey and Röbbelen, 1989). In recent years, *B. juncea* varieties have been undergoing trials for canola oilseed production in Canada, Australia and the United States (Shahidi, 1990b).

2.2. COMMERCIAL CRUSHING OF CANOLA SEED

In Canada, the seed that is destined for processing is either obtained directly from the farm or indirectly from primary grain elevators via the Canadian Grain Commission. Seeds of *B. napus* and *B. rapa*, the most common canola species grown, and since the two species yield oil and meal of similar quality characteristics (Beach, 1983; Downey, 1983), the seed is usually mixed on delivery and is processed as such.

2.2.1. Seed Cleaning

The first step in canola processing is cleaning of the seed from any docking materials such as sticks, leaves, stones, chaff and cereal grains (Beach, 1983; Unger, 1990). Metallic fragments in the seed are removed by passing the seed though a series of magnetic steel bars (Carr, 1989). The seed is then passed through a series of screens to remove undersized and oversized seeds with air aspiration being employed through out to remove dust particles (Brogan, 1986).

2.2.2. Seed Flaking

The cleaned seed, which usually contains less than 2.5% dockage, is flaked by passing through a series of roller mills adjusted to a narrow clearance of 0.2-0.3 mm to physically rapture the seed coat and some of the oil containing cells (Pickard, 1993). In Canada, where the canola seed is usually stored under freezing conditions, the seed must first be pre-conditioned to flaking by heating at 30-40°C indirectly or by direct hot air applications (Unger, 1990). Pre-conditioning of the seed is a pre-requisite for proper flake formation, screw pressing, cake formation, oil extraction and subsequent solvent removal from the oil extracted meal.

2.2.3. Cooking

The flaked seed is passed on into a series of closed cylindrical kettles stacked one on top of the other with each having a sweep type handle device for continuous mixing

of the flakes while cooking (Pickard, 1993). The cooking temperatures in the cooker are usually adjusted to reach 85-90°C at the top kettle. Residence times tend to vary from plant to plant, but generally they range from 30 to 60 minutes and moisture content is adjusted to fall within 6 to 10%. These cooking conditions are required to increase oil coalescing and fluidity and to rapture the remaining oil cells in order to facilitate oil extraction (Beach, 1983; Unger, 1990).

2.2.4. Screw Pressing

After cooking, the cake is conveyed on to the expeller for screw pressing in rotating screw shafts contained in cylindrical barrels. The pressure in the barrel is developed by rotating screws working against the adjustable choke which is constricted to discharge the cake from one end of the barrel. As the oil is expelled from the cake, it passes through the slots of the barrel cages to be collected for screening and filtration. Upon oil filtering, the remaining residue (foots) are collected to be recycled back to the cooker/conditioner or may be repressed in a foots press expeller, which is a special version of the main screw press (Beach, 1983). The pre-pressed cake containing 14-20% residual oil is conveyed to the extruder where it is steam heated and mixed by breaker screws as it is forced against small die openings at the end of the plate (Pickard, 1993). As the cake is passed through the openings, the sudden release in pressure, expands the meal to form a series of rope-like segments which are ideal for solvent extraction.

2.2.5. Solvent Extraction

The cooked and extruded meal is placed into a mechanical oil extractor where hexane heated to 50 - 60°C is passed in a repeated counter-current process to extract oil from the meal. When oil concentration in the solvent is increased to about 30%, the "marc" as the oil-hexane mixture is called, is taken to the distillation unit for oil refining.

2.2.6. Desolventizing

The oil extracted meal containing 1.0 to 1.5% residual oil is transferred to the desolventizer and toaster unit where the hexane is removed from the meal by direct steam application (Unger, 1990). The added steam provides heat to vaporize the solvent while condensing to replace the liquid solvent in the meal. The applied heat raises the temperature in the desolventizer to 103-107°C and moisture content to 15 - 18%. In processing plants with canola oil refining, gums and/or acidulated soap stocks from the oil refinery may be added to the meal at this stage. The desolventized meal is toasted on heated metal plates before being granulated and moisture adjusted to about 10%. The meal is then packaged for storage or shipping, though a small portion destined for the export market may be pelleted for easy handling.

2.3. QUALITY CHARACTERISTICS OF CANOLA MEAL

2.3.1 Chemical Composition

The value of CM as a protein supplement for livestock and poultry has increased considerably in the last few years mostly as a result of reduced meal glucosinolate levels (Bell, 1993b). The nutritional composition of canola meal in comparison to that of soybean meal is presented in Table 1. The main components include crude protein, carbohydrates (sucrose, oligosaccharides and starch), dietary fibre, ether extract and minerals (Downey and Bell, 1990). On comparative basis, soybean meal contains more protein and true metabolizable energy than canola meal. On the other hand, CM tends to have more dietary fibre, minerals and ether extract than soybean meal. The high fibre content in CM is mostly attributed to the smaller seed size with the proportion of hulls, the main source of canola fibre, to be substantially higher than that of SBM. Canola meal also contains more lignin and associated polyphenols in its dietary fibre fraction.

Starch content in rapeseed/canola varies from almost 50% during early seed development to about 2-3% at harvest as most of it is converted into canola oil. The amount of ether extract in CM depends on the extent of canola oil extraction and the amount of gums added back to the meal during canola processing. This is the main reason for the higher ether extract levels in CM as compared to soybean meal. The gums are obtained from canola oil refining and are composed of phospholipids, glycolipids and variable amounts of triglycerides, sterols and free fatty acids (Bell, 1984).

TABLE 1. Chemical composition of canola and soybean meals (% as fed basis).

Component[1]	Canola meal	Soybean meal
Protein	40.2	50.7
Sugars		
Starch	2.5	0.7
Sucrose	7.7	6.9
Oligosaccharides	2.5	5.3
Fat	4.1	0.9
Ash	8.1	6.5
Dietary fibre		
Non-starch polysaccharides	17.9	20.3
Lignin and polyphenols	8.0	1.0
Glucosinolates	0.8	-
Sinapine	1.0	-
Phytate	2.9	1.6
Tannins	2.4	-
Total	98.1	93.9

[1] Source: Adapted from Clandinin *et al.*, 1989 and University of Manitoba data.

Gums assist in increasing the meal metabolizable energy, palatability and reducing its dustiness. Canola meal is also a better source of many minerals (calcium, iron, manganese, phosphorus, selenium) (Nwokolo *et al.*, 1976). However, the availability of most minerals in CM is less than that of soybean meal (Nwokolo and Bragg, 1977;). The reduced availability of minerals is mostly attributed to high dietary fibre and phytic acid contents in CM. The mineral content in various samples of CM tend to be influenced by regional and climatic growing conditions in addition to soil fertility and fertilizer applications (Bell and Keith, 1991). Canola meal is a better source of most B-complex vitamins (choline, biotin, folic acid, niacin, riboflavin, and thiamine) than soybean meal (Bell, 1984; Clandinin *et al.*, 1989). There are no problems associated with vitamins in CM, except for choline which has been associated with the development of fishy flavours in some strains of brown egg laying hens (Hobson-Frohock *et al.*, 1975; 1977).

2.3.2. Protein and Amino Acids

There appear to be variations in CM protein content of samples derived from different locations and processing plants in Western Canada. Most of the variations have been attributed to the cultivars grown and the processing conditions applied by different plants (Bell *et al.*, 1991). Differences due to environmental growing conditions have also been associated with observed year to year variations in CM protein and amino acid compositions (Beste *et al.*, 1992). However, on comparative basis, variations among CM samples tend to be relatively lower than those of other protein supplements (Deggusa

Study, 1992). The amino acid content in CM is lower than that of soybean meal, which is more a reflection of differences in crude protein content rather than protein quality. This becomes apparent when the amino acid content of the two meals are compared on crude protein basis. Under such conditions, the amino acid composition of canola meal is very similar to that of soybean meal, with the only notable differences being in the content of lysine and the sulphur amino acids which are higher and lower than in soybean, respectively (Clandinin *et al.*, 1981; 1986). Because of these differences, the two meals tend to complement each other when used together in poultry and swine rations (Bell, 1984).

The quality of any protein supplement does not only depend on protein and amino acid composition of the meal but more so on their digestibility and subsequent utilization in farm animals. Table 2. gives the amount of available protein and amino acids in different canola meal samples in comparison to that of soybean meal. Crude protein digestibility is lower in brown- and yellow-seeded canola as compared to dehulled canola meal and soybean meal. Thus, dehulling of canola meal brings the digestibility of canola protein closer to that of soybean meal (Bell, 1993b). The amino acid availabilities are lowest in the brown commercial meal. The total amino acid availabilities in the yellow-seeded canola are relatively similar to that of the dehulled meal and that of soybean meal. It appears that dehulling of canola seed or changing to yellow-seeded varieties has an improving effect on meal quality. It has also been demonstrated that the processing conditions have significant effects on protein and amino acid availability. Although heat treatment is required to facilitate oil extraction and

TABLE 2. Protein and amino acids availability of different canola meals and soybean meal in poultry (% of total in the meal).

Component	Commercial[1]	Dehulled[1]	Yellow-seeded[2]	Soybean meal[2]
Protein	73.4	82.3	74.1	84.5
Amino acids				
Aspartic acid	81.1	85.8	83.4	90.2
Threonine	75.2	83.6	83.4	87.8
Serine	76.2	85.0	85.5	91.1
Glutamic acid	84.2	90.4	92.8	92.4
Alanine	81.9	86.6	84.5	87.2
Valine	78.6	85.7	82.9	88.8
Isoleucine	79.7	86.4	89.8	89.8
Leucine	82.1	87.6	87.4	89.8
Tyrosine	75.0	84.8	89.2	89.2
Phenylalanine	82.2	88.4	90.0	90.0
Lysine	76.9	81.4	88.4	87.5
Arginine	81.2	84.3	79.3	85.2
Cystine	73.4	88.1	80.5	80.5
Methionine	94.5	95.0	87.7	95.6
Total	80.2	85.9	87.9	88.9

Source: [1] Zuprizal *et al.*, 1991b; [2] University of Manitoba data.

removal of solvent from the meal, an excess or prolonged heat have negative effects on canola protein and amino acids. Excess heat may lead to the formation of Maillard reaction products which are indicative of protein damage. Protein damage results in reduced availability of amino acids as was demonstrated for canola (Anderson-Herfamann *et al.*, 1993) and soybean (Parsons *et al.*,1991; 1992) meals. Apparently, there are two types of protein damage. The first one results in some form of protein binding such that the amino acids are not liberated *in vivo* or by enzyme hydrolysis *in vitro* but are liberated by acid hydrolysis (Youngs *et al.*, 1972). In the second instance, the amino acids are irreversibly bound in such a way that they cannot be recovered even by acid hydrolysis. The extent of heat damage depends on temperature, time, moisture content and reducing sugar content. The most susceptible amino acid is lysine which suffers both a reduction in content and availability of the remaining fraction (Jensen *et al.*, 1995). Apart from arginine and cystine, which may be affected to a limited extent, excess heat appears to be of little effect on the content and availability of other amino acids (Cave, 1988).

2.3.3. Metabolizable Energy

The economic value of canola meal is influenced to a great extent by its metabolizable energy content. The amount of metabolizable energy in the meal is dictated by many factors including type of seed being processed, processing conditions, species, age and type of animals being fed. In diet formulation, apparent metabolizable energy (AMEn) is usually used for growing chickens, while true metabolizable energy

(TMEn), determined with adult roosters, is preferred for laying hens (Bell, 1993a). These values range from 7.41 to 10.90 Mj kg^{-1} (AMEn) and from 8.60 to 9.51 Mj kg^{-1} (TMEn) for broiler chickens and laying hens, respectively, and correspond well with European data for low glucosinolate rapeseed meal (Bell, 1993a). In swine, the available energy is often determined by its digestibility at ileal or faecal levels (Sauer *et al.*, 1989) and is reported to range from 69 to 72% of the total energy content (Bell *et al.*, 1991; Bell and Keith, 1989; Bourdon and Aumaitre, 1990). As in poultry, the digestible energy tends to be influenced by age and weight of the animal and, on average, increases by about 0.2% units per kg increase in animal body weight (Bell and Keith, 1988). Apparently, changing from rapeseed to canola improved the utilization of energy mainly as a result of reduced toxicity of glucosinolates. The metabolizable energy content in CM is still less than that of soybean meal (Mutzar and Slinger, 1980).

2.3.4. Low-molecular Weight Carbohydrates

Among the major energy sources in rapeseed/canola meals are the low-molecular weight carbohydrates which account for approximately 8-10% of dry matter (Bach Knudsen, 1994; Slominski *et al.*, 1994). The low molecular weight carbohydrates (LMWC) in canola/rapeseed meal are subdivided into 3 groups with the neutral sugars (ie., glucose, fructose, sucrose, raffinose, stachyose) constituting the largest group (Theander and Åman, 1976). The alcohol sugar components (galactinol, digalactosyl and myo-inositol) make up the second group and galactoglycerol which is derived from phospholipids the third and smallest group (Siddiqui *et al.*, 1973). The alcohol sugar

components function more as intermediate biosynthetic precursors than energy reserves.

There appears to be substantial variation among CM samples in the content of LMWC (3 to 15%) most of which is attributed to method of analysis and/or growing or processing conditions of processed seed (Rao and Clandinin, 1972). The LMWC content may also be influenced by seed-coat colour as yellow-seeded varieties tend to have more sucrose (9.8 vs 7.7%) than brown-seeded varieties. Since LMWC tend to be lower in heat processed than in unheated oil extracted meals, it seems possible that processing temperature may have a destructive effect on such carbohydrates as sucrose or oligosaccharides. Dehulling of the seed results in increased content of simple sugars as a result of removing the diluting effect of the hulls (Theander and Åman, 1974; Theander *et al.*, 1977).

While sucrose is readily digested by sucrase enzyme, the oligosaccharides (raffinose, stachyose and verbascose) escape digestion in the small intestine and are fermented by anaerobic bacteria of the lower gut to produce short-chain fatty acids (SCFA) and flatulent gases (carbon dioxide, methane and hydrogen) (Rackis, 1975). The SCFA yield less energy than would be derived from the digestion of monosaccharides and the flatulent gases tend to cause discomfort and increase rate of feed passage through the gut which results in reduced digestibility of energy as was demonstrated for soybean meal (Coon *et al.*, 1990; Leske *et al.*, 1993). Alcohol extraction of soybean meal reduced the level of LMWC in the meal by about 97.5% and increased the digestibility of non-starch polysaccharides and TMEn.

2.3.5. Dietary Fibre

Dietary fibre is among the most important factors limiting unrestricted use of CM in rations for poultry and other rapidly growing monogastric animals (Sarwar *et al.*, 1981; Bell and Shires, 1982). Canola meal contains approximately 30% of dietary fibre. The fibre components include lignin with associated polyphenols, non-starch polysaccharides, cell wall protein (glycoprotein) and minerals associated with the fibre components (Table 3). Non-starch polysaccharides are composed of rhamnose, fucose, arabinose, xylose, mannose, galactose, glucose and galacturonic and glucuronic acids. The major component sugar is glucose, which was shown to originate mainly (87%) from cellulose (Slominski and Campbell, 1990). High concentration of uronic acid residues suggests that pectic substances are the major non-cellulosic polysaccharides present in CM. Pectic polysaccharides are characterized by having arabinogalactan and rhamnogalacturonan chains, both of which contain arabinose, galactose, rhamnose and xylose units covalently linked to the galacturonan side chains. Arabinose, galactose and xylose units found in canola could also be derived from the arabinans, arabinogalactans, xylans and xyloglucans not associated with pectic polysaccharides (Siddiqui and Wood, 1974; 1977).

The fibre components of CM are not digested in the small intestine of poultry but are degraded to some degree by the microflora of the lower gut. Microbial fermentation generates some short-chain fatty acids (SCFA) but the contribution in net energy to the host animal is minimal since NSP digestibility in poultry is very low (2.3%) (Slominski

TABLE 3. Dietary fibre composition of different canola meal samples as compared to that of soybean meal (% DM).

Component	Treatment meal samples				
	Brown commercial meal	Brown dehulled meal	Yellow commercial meal	Yellow dehulled meal	Soybean dehulled meal
Lignin	7.98	5.87	4.59	3.65	1.38
Ash	3.58	1.56	1.02	0.60	0.43
Protein	3.54	5.16	3.90	2.30	1.44
Non-starch polysaccharides (mg g^{-1} meal)					
Rhamnose	1.94	1.40	2.62	1.64	1.62
Fucose	2.21	2.34	2.62	2.90	3.61
Arabinose	46.75	44.45	47.58	47.39	25.80
Xylose	20.01	19.07	21.24	22.82	10.90
Mannose	4.50	3.70	4.66	4.27	7.18
Galactose	18.05	15.12	19.23	18.67	49.23
Glucose	65.01	55.14	74.69	66.04	31.64
Uronic acids	51.24	38.76	64.18	50.82	30.65
Total NSP (%)	19.37	17.99	23.68	21.46	16.06
Total fibre (%)	34.47	30.58	33.19	28.01	19.31

Source: University of Manitoba unpublished data.

and Campbell, 1990). Low digestibility of dietary fibre also impairs nutrient digestion due to encapsulating effect of the cell walls.

2.3.6. Glucosinolates

Despite recent reductions in glucosinolate content through plant breeding, the glucosinolates are still the main factor limiting CM utilization in swine and poultry diets (Bell, 1984; 1993a; Fenwick and Heaney, 1983; Shahidi, 1990a). Glucosinolates are a group of structurally related sulphur containing substances that are characterized by a common **R**-group in the side chain and yield a range of aglucone and sulphur containing compounds upon hydrolysis. To date, more than 100 glucosinolates have been isolated from rapeseed and other cruciferous crops and nearly all of them have been associated with specific flavours and anti-nutritive or toxic effects in animals (Bjerg *et al.*, 1989). Canola cultivars contain only 10 to 12% of the original 110-150 μmoles g^{-1} meal of aliphatic glucosinolates found in earlier rapeseed varieties (Bell, 1984). With the exception of laying hens and young growing animals, the change over to canola varieties resulted in increased usage of CM in animal feeds (Campbell, 1987; 1988), and the recent development of ultra-low glucosinolate varieties offers a potential for further increase of CM in swine and poultry rations (Bell *et al.*, 1991). It should be pointed out that although the major reductions in glucosinolate content occurred with the change over to canola, the reduction was not consistent with all the glucosinolates. This is because the original methods of analysis emphasized aliphatic glucosinolates with little regard for the indole glucosinolates, despite the fact that these make up to almost half of

the total glucosinolates found in canola (Slominski and Campbell, 1987; McGregor, 1978; Thies, 1977). Because of this discrepancy, genetic selections to reduce glucosinolate levels in rapeseed did not include the indole glucosinolates.

Canola/rapeseed contains four main aliphatic (alkenyl) glucosinolates including gluconapin (3-butenyl-), glucobrassicanapin (4-pentenyl-), progoitrin (2-OH-3-butenyl-) and napoleiferin (2-OH-4-pentenyl-) (Table 4). In the presence of moisture and following rapture of the cells the glucosinolates are hydrolysed by myrosinase to yield a range of products including isothiocyanates, goitrin (oxazolidinethione) and nitriles (Bell, 1984).

In addition to myrosinase catalyzed reaction, thermal degradation during canola seed processing and microbial fermentation in the lower gut of monogastric animals are two additional means of glucosinolate hydrolysis. Therefore, the anti-nutritive effects of glucosinolates depend on the nature and concentration of hydrolysis products. The pungency of isothiocyanates, antithyroid activity and bitterness of goitrin or a combination of their deleterious properties are usually responsible for reduced feed intake and poor performance of monogastric animals fed high glucosinolate rapeseed meal. Studies on the nitrile 1-cyano-3-hydroxybutene, the major thermal degradation product of progoitrin, have not confirmed a common perception with regard to its toxic character (Papas *et al.*, 1979; Slominski *et al.*, 1983).

TABLE 4. Glucosinolates content of rapeseed and canola meal samples (μmoles g^{-1} of oil extracted meal).

Glucosinolate	Rapeseed meal	Canola meal	
		Standard	Ultra low
Aliphatics			
Gluconapin	21.4	3.1	0.2
Glucobrassicanapin	5.9	0.4	Trace
Progoitrin	73.3	6.4	0.6
Gluconapoleiferin	4.6	0.7	Trace
Indoles			
Glucobrassicin	0.3	0.2	0.1
Hydroxyglucobrassicin	2.7	2.5	1.4
Contaminants			
Glucosinalbin	3.0	2.8	2.3
Total - μmol g^{-1}	111.2	15.6	4.6
- %	4.9	0.7	0.2

Source: Slominski, 1993.

Hydroxyglucobrassicin (4-hydroxy-3-indolylmethyl) and glucobrassicin (3-indolylmethyl) are the two indole glucosinolates found in rapeseed and canola meals (Slominski and Campbell, 1988; Tookey *et al.*, 1980). In the presence of moisture the indoles are hydrolysed by myrosinase to indolemethanols and thiocyanate ion (SCN). Both indole glucosinolates are markedly susceptible to thermal degradation with indolemethanols, SCN and indoleacetonitriles being among the major degradation products (Slominski and Campbell, 1987, 1989). When fed to poultry and rats the indoleacetonitriles and SCN had no effect on performance or target organ weights with normal levels of glutathione in the liver and selected enzymes and nitrogen constituents in the blood (Slominski and Campbell, 1991b). Indole glucosinolates are said to have beneficial effects when used in human and animal diets including inhibition of chemically induced carcinogenesis and stimulation of enzymes involved in cellular detoxification reactions (Wattenberg and Loub, 1982). There are some reports indicating reduced feed intake and overall performance of laboratory animals fed large quantities of indole glucosinolates (Darroch *et al.*, 1991; Darroch and Bell, 1991). Some negative effect of 4-hydroxyglucobrassicin degradation products on protein quality was reported by Jensen *et al.* (1991). In the latter case, however, the production of degradation products from pure 4-hydroxyglucobrassicin was performed at 140°C which is rather unusual for processing of canola seed and might have resulted in the formation of a variety of unknown breakdown products.

Total glucosinolate content in CM may also be influenced by weed seed contamination. The presence of contaminating seed is exhibited by sinigrin (allyl-) and

glucosinalbin (hydroxy benzyl-) glucosinolates derived from wild mustard (*Sinapis arvensis*) and stink weed (*Thlaspi arvense*) or brown mustard (*B. juncea*) seeds (Bell and Keith, 1991). It is currently not possible to separate contaminating seeds from canola during processing and their presence in the meal is more a reflection of farm weed control efficiency than inadequate seed cleaning. The presence of contaminating seeds is widespread and levels of up to 5% contamination have been reported (Bell *et al.*, 1991). Destruction of sinigrin during canola processing has been reported but the content of glucosinalbin remains unchanged even at extreme temperatures (Bell and Keith, 1991). Both allyl and hydroxy benzyl glucosinolates have been suggested to affect thyroid function (Langer and Stolc, 1965; Bell *et al.*, 1987) and palatability of CM based rations. Feeding of isolated glucosinalbin to laying hens, however, showed no negative effect on feed intake and egg production at the level corresponding or exceeding that found in commercial CM (Slominski and Campbell, 1991b).

Various approaches have been undertaken in order to reduce toxic effects of glucosinolates. Some adverse effects associated with the small amounts of glucosinolates still present in CM may be minimized by proper processing or the use of various detoxification methods (Fenwick and Curtis, 1980). The most common physical method employed in commercial crushing operation involves inactivation of myrosinase by moist heat treatment of the meal at 105-107°C. Glucosinolates may also be eliminated from the meal by ammoniation. Kirk *et al.*, (1966) used ammonia to reduce the concentration of epigroitin and sinapine which resulted in improved palatability and nutritional quality of crambe meal. Coxworth and McGregor (1980) reported an almost complete removal

of strong flavour from mustard seed after ammoniation, though a combination of steam and ammonia treatment removed only half of the glucosinolates contained in meal and did not have any significant effect in promoting chick growth (Keith and Bell, 1982). Effective removal of glucosinolates by alcohol ammonia solutions was achieved only when methanol was used (Shahidi, 1991). So far research has shown that the most promising method of removing glucosinolates from the meal is through breeding for very low (ultra-low) glucosinolate varieties (Bell *et al.,* 1991; Sorensen, 1985; 1990).

2.3.7. Sinapine

The main phenolic compounds found in canola include sinapine and tannins (Fenwick *et al.*, 1984). Sinapine is a choline ester of sinapic acid and accounts for 0.6-1.8 % of the meal (Buttler *et al.*, 1982). Apart from the bitter taste which may limit feed intake when consumed in large amounts, the most common negative effect of sinapine is the production of a fishy taint in brown-shelled eggs. It has been found that the taint is due to the presence of trimethylamine (TMA) in the yolk which results from a genetic defect among certain strains of laying hens. Under normal circumstances, sinapine is hydrolysed to choline which is further broken down to TMA by enteric bacteria. TMA is then converted to odourless nitrous oxide by liver or kidney TMA-oxidase. In genetically defective birds (i.e. Rhode Island Red breeding), there is a reduced ability to synthesize TMA-oxidase enzyme in its active form and the TMA accumulates in the blood and passes on to the developing ova (Mueller *et al*, 1978). Sinapine may be hydrolysed under alkaline conditions to ensure choline absorbtion from

the upper part of the gastrointestinal tract of the bird (Tayaranian and Henkel, 1991). However, the cost of such innovations and environmental regulations prohibit routine use of alkaline hydrolysis and reductions through genetic selection of low sinapine containing canola varieties seem to be far fetched at the moment. It appears the only feasible way of avoiding a fishy taint would be the elimination of the genetic defect by animal breeding programs. This is justified by the fact that the TMA-oxidase deficiency is not linked to the shell colour, as has sometimes been supposed, and it was found in Brown Leghorns whose eggs have white shell but was not present in a New Hampshire red hybrid which lays brown eggs. Nevertheless, no taints or off flavours have been reported in eggs of white egg laying hens and have not been detected in broiler carcasses (Butler and Fenwick, 1984).

2.3.8. Tannins

Tannins account for 1.6-3.1% of canola/rapeseed meal and are subdivided into hydrolysable and condensed portions (Yapar and Clandinin, 1972). The condensed tannins are mostly found in seed coat with the brown hulls containing more than the yellow hulls (Durkee *et al.*, 1971; Theander *et al.*, 1977). In addition to giving the meal a dark unattractive colour, tannins form complexes with proteins and proteolytic enzymes in the gastrointestinal tract, thereby affecting protein digestion (Naczk and Shahidi, 1991). Astringency and bitter taste are additional negative effects associated with high tannin contents in canola meal. Tannins have also been reported to bind other enzymes but Mitaru *et al.* (1982; 1983) and Yapar and Clandinin (1972) failed to

demonstrate α-amylase binding by rapeseed tannins. However, removal of tannins from the meal significantly increased the metabolizable energy of the meal, probably due to increased activities of endogenous enzymes. The hydrolysable tannins constitute most of the cotyledonous phenolic acids and these have not been associated with any negative effects.

2.3.9. Phytic acid

Phytic acid [*myo*-inositol 1,2,3,5/4,6-Hexakis (dihydrogen phosphate)] accounts for 3-6% of CM dry matter and is mostly found in the seed embryo. Phytic acid is the storage form of about 80-87% of 1.2% phosphorus found in canola meal (March, 1991). Phytic acid plays a protective role against oxidative damage and fungal pathogens during seed storage but forms insoluble complexes with proteins and a number of minerals to render them biologically unavailable (Mills and Chong, 1977). In addition to binding to dietary proteins, phytic acid also forms complexes with digestive enzymes thereby reducing the availability of proteins and amino acids (Lott, 1985; Thompson, 1990). Efforts are currently underway to reduce the negative effects of phytic acids through the use of phytase enzyme in swine and poultry diets (Simmons *et al.*, 1990).

2.4. IMPROVEMENTS TO THE NUTRITIVE VALUE OF CANOLA MEAL

2.4.1. Breeding for Yellow-Seeded Canola

Selection for the yellow seed coat colour is among recent approaches undertaken in an attempt to reduce the fibre content, increase the protein content and to improve nutrient utilization. Interest in yellow-seeded characteristic began when Jonsson and Bengtsson (1970) noticed differences in oil and protein contents between yellow- and brown-seeded types of rapeseed. This was confirmed later by Stringam *et al.*, (1974) who reported thinner hulls and larger oil and protein containing embryos in yellow-seeded as opposed to brown-seeded rapeseed. Since seed coats are the main source of dietary fibre, it was concluded that yellow-seeded rapeseed should have less fibre than brown-seeded type (Finlayson, 1974; Theander *et al.*, 1977). It was also established that the content of polysaccharides and lignin in the hull fraction were similar for both types, though the polyphenols tended to be higher in the dark than in the yellow-hulled seeds. With regard to consumer perception, the yellow-seeded characteristic had another advantage in that the meal from yellow-seeded type was lighter and more attractive (Theander *et al.*, 1977). On the basis of these results indicating a negative effect of brown hulls on protein and energy digestibility (Sarwar *et al.*, 1981; Bell and Shires, 1982), it was suggested that selection for yellow-seeded characteristic would likely improve the nutritive value of the meal.

In more recent years, the value of yellow seed canola/rapeseed has been extensively evaluated to exploit any potential positive characteristics associated with the

yellow seed coat colour. Working with partially and fully yellow-seeded samples, Slominski *et al.* (1994a) established that yellow-seeded canola is composed of 8-10% sucrose, 2-3% oligosaccharides and 27% dietary fibre. The sucrose content was positively correlated with the percentage of yellow seeds in the samples and was 3 to 4 percentage points higher in fully yellow-seeded as opposed to brown-seeded samples. The fibre profiles of brown- and yellow-seeded canola differed considerably with the latter showing 2, 2 and 3-4 fold lower concentration of cell wall protein, lignin with associated polyphenols and minerals, respectively. Non-starch polysaccharide (NSP) values, however, tended to be higher for yellow-seeded canola (22 vs 18%). In addition, the meal derived from yellow-seeded canola showed a higher content of water-soluble NSP and improved polysaccharide digestibility (9 vs 3%). The total fibre content of the two canola types, however, was only slightly lower in yellow-seeded canola than brown-seeded canola (27% vs 30%) due to increased NSP content at the expense of reduced lignin and polyphenols. This was supported by the findings of Bell (1993a) who reported higher NDF content in the cotyledons from yellow- than brown-seeded canola and concluded that the beneficial effects of thinner hulls in yellow-seeded type has been offset by increased fibre content in the seed embryos. Due to high solubility of NSP in neutral detergent solution, however, yellow-seed canola was shown to contain only 19% NDF which was much lower than the 26% NDF value recorded for the brown-seeded type (Slominski and Campbell, 1990). Comparative results were reported by Eriksson *et al.* (1994) and showed yellow-seeded varieties to have less dietary fibre due to lower Klason lignin content.

2.4.2. Dehulling of Canola Seed

Since the hulls are the main source of dietary fibre, one approach in reducing nutrient diluting effect of fibre and enhancing the nutritive value of canola meal is through dehulling of the seed prior to or after oil extraction. Dehulling of rapeseed after oil extraction by applying fine milling and air classification was employed in the 1970s (Leslie *et al.*, 1973; Bayley and Hill, 1975; Jones and Sibbald, 1979; Sarwar *et al.*, 1981). Despite the increase in protein and energy contents and their improved digestibility, feeding of dehulled meals to growing chickens and pigs did not improve growth rates and feed efficiencies. The lack of positive response was attributed to the fine texture of the resulting meal and the increase in glucosinolate content which are known to be present in the cotyledons at much higher concentration than in the hulls (Röbbelen and Thies, 1980). Fine milling and air classification of rapeseed meal also had short falls with regard to the efficiency of separating hulls from embryos and the lack of an outlet market for the separated hulls (Downey and Bell, 1990).

Recently, dehulling was also employed to newly developed low glucosinolate varieties (Bell, 1991; Zuprizal *et al.*, 1991a). As opposed to air classification and fine milling, the recent technique was based on dehulling of the seed prior to oil extraction and tended to increase the oil loses due to incomplete separation of cotyledons from the hulls. Dehulling of brown-seeded canola increased protein and energy content from 40% and 9.123 Mj/kg to 47% and 12.000 Mj/kg, respectively (Bell, 1991). Dehulling of canola seed also increased the concentration of most amino acids except for lysine, phenylalanine and tyrosine which were reduced in content (Zuprizal *et al.*, 1991a) due

to their association with the hull fraction (Finlayson, 1974; Sarwar *et al.*, 1981; Bell, 1991). It was concluded that the reduced lysine content in dehulled meal should not compromise the meal nutritive value since the lysine present in the hull fraction is likely to be poorly utilized by animals (Finlayson, 1974; Zuprizal *et al.*, 1991b). The increase in the amount of amino acids in dehulled canola meal did not equal that of 48% soybean meal, except for methionine, threonine, glycine and cystine which were higher in canola meal. The content of histidine and alanine were similar in both meals. Dehulling of the seed also reduced the amount of crude fibre and acid detergent fibre by about 50%, though the meal still contained 26 and 33% of fibre in a form of neutral detergent fibre and total dietary fibre, respectively (Simbaya, 1992).

Digestibility trials with growing swine showed the dehulled canola to have increased amount of digestible protein and metabolizable energy (Bell, 1993a). However, the positive effect of dehulling on feeding quality of canola meal in poultry and swine still remains to be substantiated. In this regard, feeding of dehulled canola meal to weanling and growing pigs resulted in poor growth rates as a result of inferior energy digestibility values (Christison and Bell, 1993). Also Bourdon and Aumaitre (1990) reported inferior performance of pigs fed dehulled canola meals.

3.4.3. Use of Exogenous Enzymes

Another approach in improving the nutritive value of canola meal is through the use of exogenous enzymes. Enzymes were used as early as the late 1950s and early 1960s to enhance protein and starch utilization in cereal-based non-ruminant diets (Jensen

et al., 1957; Fry *et al.*, 1958; Burnett, 1962). Current interest in enzyme application is related to the use of lower cost feedstuffs while maintaining high level of production. It has been established that the increase in fat, starch, nitrogen and energy digestibilities in enzyme supplemented diets is brought about by elimination of anti-nutritive properties of viscous polysaccharides and by the release of nutrients encapsulated by the cell walls (Moran, 1982; Hesselman and Åman, 1986; Pettersson and Åman,, 1989; Bedford and Classen, 1992). The main cell wall polysaccharides in cereal grains include pentosans (arabinoxylans) in wheat, rye and triticale and the mixed-linked β-glucans in barley and oats (Pettersson *et al.*, 1990; Bedford and Classen, 1992). Gel-forming properties of soluble polysaccharides reduce transit time of the digesta (Jenkin *et al.*, 1978) which tends to cause satiety in birds and result in reduced feed intake and subsequent poor performance (Blackburn *et al.*, 1984). Viscous conditions in the gut also increase the thickness of the unstirred layer adjacent to the gut epithelium and thereby impend nutrient absorption. Viscosity in the intestinal contents also causes wet and sticky litter in which infections may be spread to cause detrimental effects on the growth of birds. Chemical characterization of cell wall components appears necessary to select appropriate enzyme preparations for specific cell wall polysaccharides in a given feedstuff. Based on dietary fibre profiles of canola meal, Slominski and Campbell (1990) were able to increase the *in vivo* solubilization of cell wall polysaccharides from 3 to 37% by supplementing laying hen diets with pectinase preparation. On the same basis, inclusion of cellulase and hemicellulase enzymes in CM based broiler diets enhanced weight gain, feed consumption, improved feed conversion ratios and increased apparent retention of

minerals (Fe, Mn, Ca, and P) (Ward *et al.*, 1991). The increase in mineral retention may be associated with cell wall degradation and the release of additional nutrients (Campbell and Bedford, 1992). Supplementation of CM based diets with protease, however, did not have any effect on broiler chickens performance (Ward *et al.*, 1991) as was also reported by Sebastian *et al.* (1994) when SBM was supplemented with protease preparations.

Another enzyme with the potential for application in CM based diets is α-galactosidase (Chesson, 1993). The enzyme is required to hydrolyse the α-galactosides raffinose, stachyose and verbascose to component sugars glucose, fructose and galactose. The oligosaccharides are not digested in the small intestine of monogastric animals but are degraded to some degree by the microflora of the lower gut (Cristofaro *et al.*, 1974; Rackis, 1975). The microbial fermentation of oligosaccharides generates short- chain fatty acids but the contribution in net energy from SCFA is lower than from the direct absorption of monosaccharides. Fermentation of galactooligosaccharides is also associated with the production of flatulent gases such as carbon dioxide, hydrogen, and methane which increase the rate of feed passage and result in reduced nutrient digestibility. Removal of galactooligosaccharides by alcohol extraction has been reported to increase transit time and to enhance non-starch polysaccharide and energy availabilities in soybean meal (Leske *et al.*,1991). Application of α-galactosidase for hydrolysis of oligosaccharides in canola and soybean meal was demonstrated in vitro (Slominski, 1994) and *in vivo* (Slominski *et al.*, 1994b) and showed the level of activity to be too low to warrant the use of current preparations of α-galactosidase in practical feeding.

Another enzyme with the potential to be included in the bulk of supplemental enzymes for monogastric rations is phytase. Phytic acid is known to have complexing effects on minerals (calcium, potassium, zinc and iron), dietary proteins and intestinal enzymes like ∝-amylases, trypsin, tyrosine and pepsin (Nair *et al.*, 1991; Caldwell, 1992). Application of phytase from *Aspergillus ficum* or *niger* either as a pretreatment (Nair *et al.*, 1991) or as a supplement (Simmons *et al.*, 1990; Newman, 1991) allows for the formulation of a diet with reduced supplemental inorganic phosphorus. Phytase application to a low phosphorus corn/soybean diet was shown to improve broiler performance through the increase in weight gain and feed consumption (Sebastian *et al.*, 1994). In countries with strict environment regulation, phytase is used to increase phytic phosphorus utilization to reduce the impact of excreted phosphorus on the environment (Anon, 1991). It should be pointed out that at present the cost of production still restrict a wide use of phytase preparation and it is hoped that if more economical methods of manufacture are developed and the premiums are still tied to its use, more and more farmers are likely going to adopt its use.

2.5. *IN VITRO* EVALUATION OF FEEDSTUFFS

Feed formulations for monogastric animals are based on chemical composition and estimated digestibility values of individual nutrient constituents. Nutrient digestibilities are normally determined by conducting *in vivo* feeding trials which are often expensive

and time consuming (Sauer *et al.*, 1989; Boisen and Eggum, 1991). Animal digestibility trials may have limitations due to sample size and availability of intact or surgically modified animals which require large quantities of feed and long adaptation periods (Lowgren *et al.*, 1993). To alleviate these problems, simple, rapid and accurate *in vitro* techniques need to be developed for the study of digestion and prediction of nutrient digestibilities in monogastric animals (Graham *et al.*, 1988; Lowgren *et al.*, 1989; 1993). Even though development of such techniques may be purely dictated by time and economical reasons, increasing public awareness on animal welfare and animal rights issues will probably accelerate the search for such methods.

To date, a number of *in vitro* techniques have been developed all of which involve one- or two-step enzymatic digestion of the sample under simulated environment of the gastrointestinal tract. Pre-caecal digestion of proteins, carbohydrates and lipids is usually estimated from consecutive incubation of feed samples with pepsin and pancreatin form porcine stomach and pancreas, respectively (Savoie and Gauthier, 1986). Since *in vitro* enzyme digestion is usually limited by end product inhibition, it is recommended that released products be continuously removed from the assay by use of dialysis membranes or other chromatographic techniques (Mouron, 1973; Gauthier *et al.*, 1982). A semi-in vivo technique involve the use of mobile nylon bag technique which allows for rapid measurement of apparent nutrient digestibility at either faecal or ileal levels (Dierick *et al.*, 1985; Metz *et al.*, 1985. The technique has some draw backs as it requires cannulated animals and the results tend to be more variable than those obtained with conventional digestibility techniques (Sauer *et al.*, 1989). Post caecal digestion of

undigested protein and dietary fibre components can be further estimated by incubation of the sample with ruminal or hind gut fluids (Faruya *et al.*, 1979). Even though these methods have proven effective in ranking different feedstuffs, all have short comings on standardization and reproducibility within and among the laboratories. This is because the ruminal and ileal fluids may contain the microorganisms producing often unwanted or unexpected enzymes activities. To avoid such problems, industrial cell wall degrading enzymes have been proposed as a better alternative. Multi-enzyme preparations from *Aspergillus* species with a wide range of carbohydrases, have proved reliable in degrading dietary fibre components of different feedstuffs (Boisen and Eggum, 1991).

3. MANUSCRIPT ONE

Quality Characteristics of Yellow-Seeded *Brassica* Seed Meals: Protein, Carbohydrates and Dietary Fibre Components.

ABSTRACT

A comprehensive evaluation of the nutritive profiles of the meals derived from 26 *Brassica* seed meals of yellow-seeded *Brassica napus*, *B. rapa*, *B. juncea* and *B. carinata* genotypes and 7 samples of conventional brown-seeded canola was undertaken. The evaluation involved the analyses for sucrose, galactooligosaccharides, protein, total dietary fibre, ash and residual fat. The fibre components determined included: non-starch polysaccharides, lignin and polyphenols, cell wall protein and minerals. On average, in comparison to brown-seeded, yellow-seeded types contained more sucrose (8.7% vs 7.5%) and protein (44.5% vs 42.7%) but similar amounts of oligosaccharides (2.3% vs 2.5%), ash (6.9% vs 7.0%) and non-starch polysaccharides (20.4 vs 19.7%). Dietary fibre averaged 28% for yellow-seeded samples (Min 22%, Max 32%) and 33% for brown-seeded samples (Min 28%, Max 36%) and was negatively correlated (r=-0.71) with protein content. Lower dietary fibre content in yellow-seeded samples as compared to brown-seeded samples was reflected in a lower content of lignin with associated polyphenols (4.3% vs 8.2%) and less wall-inserted protein (2.3% vs 3.3%) and minerals (0.7% vs 2.4%) associated with the fibre fraction. It may be surmised that future cultivars of yellow-seeded *Brassica* oilseed crops will have improved nutritive value.

Key words: *Brassica* meal, protein, carbohydrates, dietary fibre, seed colour

3.1. INTRODUCTION

It is generally agreed that canola meal could be more competitive in the market place if it had more protein, more digestible energy and less dietary fibre (Bell, 1993a). The selection for yellow-seed coat colour, a characteristic related to low fibre content, has been one of several approaches undertaken to improve the nutritive value of canola meal. In addition to containing less fibre, seeds of yellow-seeded strains in comparison to brown-seeded strains of *Brassica rapa* have been shown to be significantly higher in oil and protein contents (Stringam *et al.*, 1974). Thinner hulls were reported to be directly responsible for the lower fibre content in yellow-seeded *B. rapa*. Specifically, yellow hulls have been found to be low in lignin (Theander *et al.*, 1977) and crude fibre (Daun and DeClercq, 1988) and have been shown to contain less neutral detergent fibre than brown hulls (Bell and Shires, 1982). Recent results from our laboratory indicated a total dietary fibre content for yellow-seeded *B. rapa* of 27% on average. The fibre level was a reflection of a relatively high content of non-starch polysaccharides (NSP) at the expense of lignin and polyphenols with the overall dietary fibre level being 3 percentage units below that for brown-seeded samples (Slominski and Campbell, 1990; Slominski *et al.*, 1994a). This fibre profile, however, is characteristic of *B. rapa* canola since in most of the earlier studies this species was the only source of yellow-seeded samples. It is only recently that plant breeders have been able to incorporate the yellow-seed character into the agronomically important *B. napus* canola. Also, plant breeders have recently developed canola quality forms of *B. juncea*, a species known for its pure

yellow seed coat. Under western Canadian conditions *B. juncea* suffers less from heat and drought stress and matures earlier than *B. napus*. Such characteristics are the basis for high yields of oil and low chlorophyll content in the seed (Rakow and Raney, 1993).

The objective of this study was to evaluate protein, carbohydrates and dietary fibre components of seed meal of yellow- and brown-seeded strains of *B. napus*, *B. rapa*, *B. juncea* and *B. carinata*.

3.2. MATERIALS AND METHODS

3.2.1. Materials

The seed samples represented brown- and yellow-seeded strains/cultivars of *B. napus* (3 brown and 3 yellow), *B. rapa* (2 brown, 6 yellow), *B. juncea* (1 brown, 16 yellow) and *B. carinata* (1 brown, 1 yellow). *B. napus* and *B. rapa* strains tested were of canola quality type and by definition contained less than 30 μmole/g of aliphatic glucosinolates. Ten of the 16 yellow-seeded *B. juncea* strains were of canola quality; the remaining six *B. juncea* strains and *B. carinata* were high in glucosinolate content. All plant materials were grown in field plots at the Agriculture and Agri-Food Canada research farm at Saskatoon, Saskatchewan, Canada. In preparation for analysis, the seeds were crushed and extracted with *n*-hexane for 2 hours in a Soxhlet apparatus. Following drying under a fume-hood, the meals were ground to pass through a 1 mm sieve and were re-extracted with hexane for 8 h.

3.2.2. Analytical procedures

Meal samples were analyzed for protein (Kjeldahl N X 6.25), ash and ether extract using established standard methods of analysis (AOAC, 1990). Sucrose and galactooligosaccharides were determined by gas-liquid chromatography according to the procedure described by Slominski *et al.* (1994a).

Dietary fibre was determined by a combination of neutral detergent fibre (NDF) and detergent-soluble NSP measurements and was calculated as the sum of NDF and detergent-soluble NSP (Slominski *et al.*, 1994a). The method of Goering and Van Soest (1970) was used for the determination of NDF, except that the procedure was modified to exclude the use of decalin and sodium sulfite (Mascharenhas Ferreira *et al.*, 1983). Non-starch polysaccharides were determined by gas liquid chromatography (component neutral sugars) and by colorimetry (uronic acids) using the procedure described by Englyst and Cummings (1984, 1988) with minor modifications (Slominski and Campbell, 1990). The content of NSP was measured in both the meals and the NDF residues. Detergent-soluble NSP was calculated as total sample NSP minus NSP present in the NDF residue. The contents of cellular protein (Kjeldahl nitrogen) and ash in NDF residue were also measured. The value for lignin and associated polyphenols was calculated by difference [NDF - (NSP + protein + ash)].

Seed size was determined in triplicate by weighing 100 seeds from each canola/rapeseed sample and was expressed as g/1000 seeds.

3.3. RESULTS AND DISCUSSION

The chemical composition of the *Brassica* meal samples is shown in Table 5. On average, in comparison to brown-seeded, yellow-seeded samples contained more sucrose, more protein, less fibre and similar amounts of oligosaccharides and ash. These components accounted for approximately 90% of the dry matter of the meal in both types. The remaining (approximately 10%) dry matter may be assumed to consist of free glucose and fructose, sinapine (0.6 - 1.8%), soluble tannins (1.5 - 3.0%), phenolic acids, phytate (3 - 6%), glucosinolates (0.5 - 0.7%) and other minor components as reviewed by Bell (1993a).

The sucrose and oligosaccharide contents in the meals were similar to those reported earlier for brown-seeded rapeseed (Theander and Åman, 1976; Finlayson, 1977) and yellow-seeded canola (Slominski *et al.*, 1994a). The difference in sucrose content between the yellow- and brown-seeded samples was not as pronounced for the *B. napus*, *B. juncea* or *B. carinata* samples as it was for the *B. rapa* samples (Table 6). This is in agreement with our earlier data showing 3-4 percentage points higher sucrose content in yellow-seeded *B. rapa* as compared to brown-seeded canola (Slominski *et al.*, 1994a). As sucrose is a highly digestible carbohydrate, its increased content in yellow-seeded canola would be expected to have a positive impact on the digestible energy content of the meal. This is not necessarily the case for galactooligosaccharides which were reported in a recent study to have minimal effect on energy digestibility in poultry (Slominski *et al.*, 1994b). Consequently, it is uncertain to what extent the relatively high

amount of oligosaccharides such as that observed for *B. napus* canola (Table 6) would have on the nutritive value of the meal.

Dietary fibre content in yellow-seeded samples was found to be significantly lower, differing by 6 percentage points from that of the brown-seeded types (Tables 5 and 6). This relatively large difference is in contradiction to our earlier work (Slominski and Campbell, 1990; Slominski *et al.*, 1994a), however, in comparing the two data sets the yellow-seeded samples were of similar fibre content while the brown-seeded canola samples for the earlier studies had lower fibre values of approximately 3 percentage points. Such a discrepancy could be explained by environmental conditions, genetic differences or location as the canola samples used in our earlier studies were collected from different canola breeding stations in Canada and Sweden. Values for dietary fibre of rapeseed which were determined as the sum of non-starch polysaccharides and Klason lignin have recently been reported (Eriksson *et al.*, 1994). The dietary fibre values were similar to those obtained in the current study and in addition also indicated variation between rapeseed types with winter varieties showing higher dietary fibre values than summer varieties (34.4 vs 31.5%, respectively).

The protein content of the canola/rapeseed samples was shown to be related to seed colour and, on average, was higher by 2 percentage points in yellow-seeded samples. The difference in protein content due to seed colour was less evident for *B. rapa* samples than for *B. napus*, *B. juncea* and *B. carinata* samples in which the increase in protein content averaged 3.8, 3.5 and 3.8 percentage points, respectively (Table 6). The relatively small increase in mean protein value for *B. rapa* canola may have been,

in part, due to an exceptionally low (33.6%) protein content in one of the yellow-seeded samples. In general, the protein content was negatively correlated with dietary fibre level for all analyzed samples, regardless of seed coat colour (Fig. 2). The regressions of protein and fibre content for *B. napus* (Fig. 3) and *B. juncea* samples (Fig. 4) showed relatively high correlation coefficients of -0.88 and -0.82, respectively, while the coefficient for *B. rapa* samples was only -0.59 (Fig. 5). In this regard, the former samples had a large variation in protein and fibre contents among the samples while the latter samples had average protein contents for both yellow- and brown-seeded types. It can be suggested from the results that the diluting effect of dietary fibre on protein content in *B. rapa* canola may not be as significant as was earlier portrayed (Sarwar *et al.*, 1981; Stringam *et al.*, 1974). This result agrees with other work from our laboratory in which the nutritive quality of yellow-seeded *B. rapa* canola was shown to be similar to that for brown-seeded *B. napus* canola (Simbaya, 1992; Slominski *et al.*, 1994a). Apart from the brown-seeded samples which were limited in number in the current study, the yellow-seeded samples, including *B. juncea* (n=16), *B. rapa* (n=6) and to some extent *B. napus* (n=3), showed a large variation in protein and fibre contents (Figs. 3, 4 and 5). Such variation may reflect the differences in the seed size, oil content and the cotyledon cell size. Such factors should be taken into account when breeding for new, improved varieties of yellow-seeded canola/rapeseed. Since only two samples of *B. carinata* were included in this study, the apparent differences in dietary fibre and protein contents between yellow- and brown-seeded types can only be considered as trends (Table 6). The extremely low fibre content in the yellow-seeded *B.*

carinata sample is of particular interest as the protein content by consequence was elevated above 50%, a value higher than that reported for dehulled canola meal (45.9%) (Simbaya, 1992). This was also the case for selected samples of *Brassica napus* canola (i.e., line YN90-1018; 48.4% protein, 26.2% fibre) or *B. juncea* lines J90-2741 (47.0% protein, 22.6% fibre), J90-2736 (47.9% protein, 26.1% fibre) and J90-4316 (46.3% protein, 27.5% fibre) (specific data not shown).

The composition of dietary fibre present in brown- and yellow-seeded samples is shown in Table 7. Yellow-seeded *B. rapa* showed the highest NSP content within the yellow-seeded samples and this value differed substantially from that of brown- and yellow-seeded *B. napus* canola which were not different. A similar difference between brown-seeded *B. napus* and yellow-seeded *B. rapa* samples was noted in earlier work (Slominski *et al.*, 1994a). Yellow-seeded *B. juncea* showed an intermediate NSP values with an average of 20.7% and a range from 17.7% to 22.6%. Very low NSP and thus dietary fibre values were characteristic of *B. carinata*.

As indicated in Table 8. a similar NSP component sugar profile was evident for both brown- and yellow-seeded samples which indicates that changes in NSP for yellow-seeded canola are quantitative rather than qualitative in nature. This response has been shown in previous work (Slominski and Campbell, 1990; Slominski *et al.*, 1994a) and since NSP accounts for a major portion of the dietary fibre of *Brassica* seed meals future studies on the influence of dietary fibre on the nutritive quality of the meal should include an evaluation of potential differences in the total content of NSP among species and strains within species of canola.

Fibre components other than NSP which included cell wall protein and ash and lignin with associated polyphenols showed major differences (Table 7). The pronounced difference in lignin and polyphenol content between the brown- and yellow-seeded samples confirms earlier work from this laboratory (Slominski and Campbell, 1990; Slominski *et al.*, 1994a). This fraction appears to be directly responsible for the seed colour and as indicated by Theander *et al.* (1977) polyphenols rather than lignin are predominant in brown-seeded rapeseed. However, samples of yellow-seeded *B. juncea* revealed a wide range in the content of lignin and polyphenols (Min 2.5%, Max 5.4%) without any major change in seed colour as the samples were all of the fully yellow-seeded type. In this regard, relatively low lignin and polyphenol content in yellow-seeded canola may have important repercussions with regard to the nutritive worth of the meal. While the digestibility of the meal may be improved due to lower cell wall lignification, there is a potential for increased solubility of structural polysaccharides. Soluble polysaccharides in cereal grains (i.e., ß-glucan, arabinoxylan) have been shown to result in altered nutrient utilization (Graham and Åman, 1991). A weak negative correlation between seed weight and fibre content in yellow-seeded samples (Fig. 6) further elucidates this relationship and indicates the importance of canola cotyledons in determining the level and nature of dietary fibre. Thus, in contrast to the concept of the fibre in the hull fraction being the sole factor affecting nutrient utilization, the fibre associated with the seed cotyledons may also be of importance. Further work is needed to determine water solubility of fibre components and to investigate the nutritive properties of the soluble components of canola fibre including both structural (i.e., pectic

substances, hemicelluloses) and non-structural (i.e. mucilages, gums) polysaccharides. The association of carbohydrates with proteins in canola is also a factor that requires further study relative to the nutritive quality of the meal.

TABLE 5. Chemical Composition of Meals Derived from Brown- and Yellow-Seeded *Brassica* Species (% of Dry Matter).

	Type of Sample	
Component	Brown-seeded (n=7)[1]	Yellow-seeded (n=26)[2]
Sucrose	7.5 ± 1.0[3b]	8.7 ± 1.3[a]
Oligosaccharides[4]	2.5 ± 0.5[a]	2.3 ± 0.6[a]
Dietary fibre[5]	33.6 ± 2.8[a]	27.7 ± 2.3[b]
Protein	42.7 ± 3.1[a]	44.5 ± 3.5[a]
Ash	7.0 ± 0.5[a]	6.9 ± 1.1[a]
Fat	2.9 ± 0.3[a]	2.7 ± 0.2[a]
Total[6]	90.5 ± 1.4	89.7 ± 2.0

[1] Includes samples of *Brassica napus* (n=3), *B. rapa* (n=2), *B. juncea* (n=1) and *B. carinata* (n=1). [2]Includes samples of *B. napus* (n=3), *B. rapa* (n=6), *B. juncea* (n=16) and *B. carinata* (n=1). [3]Mean ± SD; [a,b]Values within a row with no common superscript differ significantly ($P<0.05$). [4]Includes raffinose and stachyose. [5]Includes non-starch polysaccharides, lignin with associated polyphenols, cell wall protein and cell wall minerals. [6]Corrected for cell wall protein and minerals present in the dietary fibre fraction.

TABLE 6. Protein, carbohydrate and dietary fibre content of meals derived from brown- and yellow-seeded *Brassica* species (% of Dry Matter).

Species/ seed colour	Number of samples	Protein[3]	Sucrose	Oligo-saccharides[1]	Dietary Fiber[2]
Brassica napus					
Brown	3	42.6[b]	8.3[a]	3.0[a]	34.1[a]
Yellow	3	46.3[a]	9.7[a]	3.3[a]	27.5[b]
Brassica rapa					
Brown	2	40.5[a]	7.1[b]	2.5[a]	35.0[a]
Yellow	6	41.1[a]	9.9[a]	2.6[a]	28.5[b]
Brassica juncea					
Brown	1	41.4	7.0	2.0	35.1
Yellow	16	44.9	8.3	2.0	27.8
Brassica carinata					
Brown	1	48.8	6.1	1.7	27.6
Yellow	1	52.6	6.8	1.6	21.9

[1] Includes raffinose and stachyose. [2] Includes non-starch polysaccharides, lignin with associated polyphenols, cell wall protein and cell wall minerals. [3]N x 6.25. [a,b]Values within *B. napus* or *B. rapa* species with no common superscripts differ significantly ($P \leq 0.05$).

TABLE 7. Composition of dietary fibre of meals derived from brown- and yellow-seeded *Brassica* species (% of Dry Matter).

Species/ seed colour	Number of samples	NSP	Protein	Ash	Lignin[1]
Brassica napus					
Brown	3	18.6[a]	4.4[a]	1.9[a]	9.1[a]
Yellow	3	18.6[a]	3.5[b]	0.7[b]	4.7[b]
Brassica rapa					
Brown	2	20.1[a]	2.6[a]	3.0[a]	9.3[a]
Yellow	6	21.3[a]	2.1[a]	0.6[b]	4.5[b]
Brassica juncea					
Brown	1	23.3	2.9	1.2	7.6
Yellow	16	20.7	2.2	0.8	4.1
Brassica carinata					
Brown	1	18.3	2.1	5.0	2.8
Yellow	1	15.0	1.6	2.3	3.0

[1] Includes lignin with associated polyphenols. [a,b]Values within *B. napus* and *B. rapa* species with no common superscripts differ significantly ($P \leq 0.05$).

TABLE 8. Non-starch polysaccharide profiles of meals derived from brown- and yellow-seeded *Brassica* species (mg g^{-1} of Dry Matter).

	Type of Sample	
Component sugar	Brown-seeded (n=7)[1]	Yellow-seeded (n=26)[1]
Rhamnose	0.9	0.8
Fucose	1.1	1.1
Arabinose	21.8	22.9
Xylose	8.0	8.6
Mannose	1.9	1.9
Galactose	9.5	9.0
Glucose	30.5	30.0
Uronic acids	26.3	25.7

[1] See Table 5 for type of samples.

FIGURE 2. Relationship between the protein and dietary fibre contents of meals derived from strains/cultivars of *Brassica napus, B. rapa, B. juncea,* and *B. carinata.*

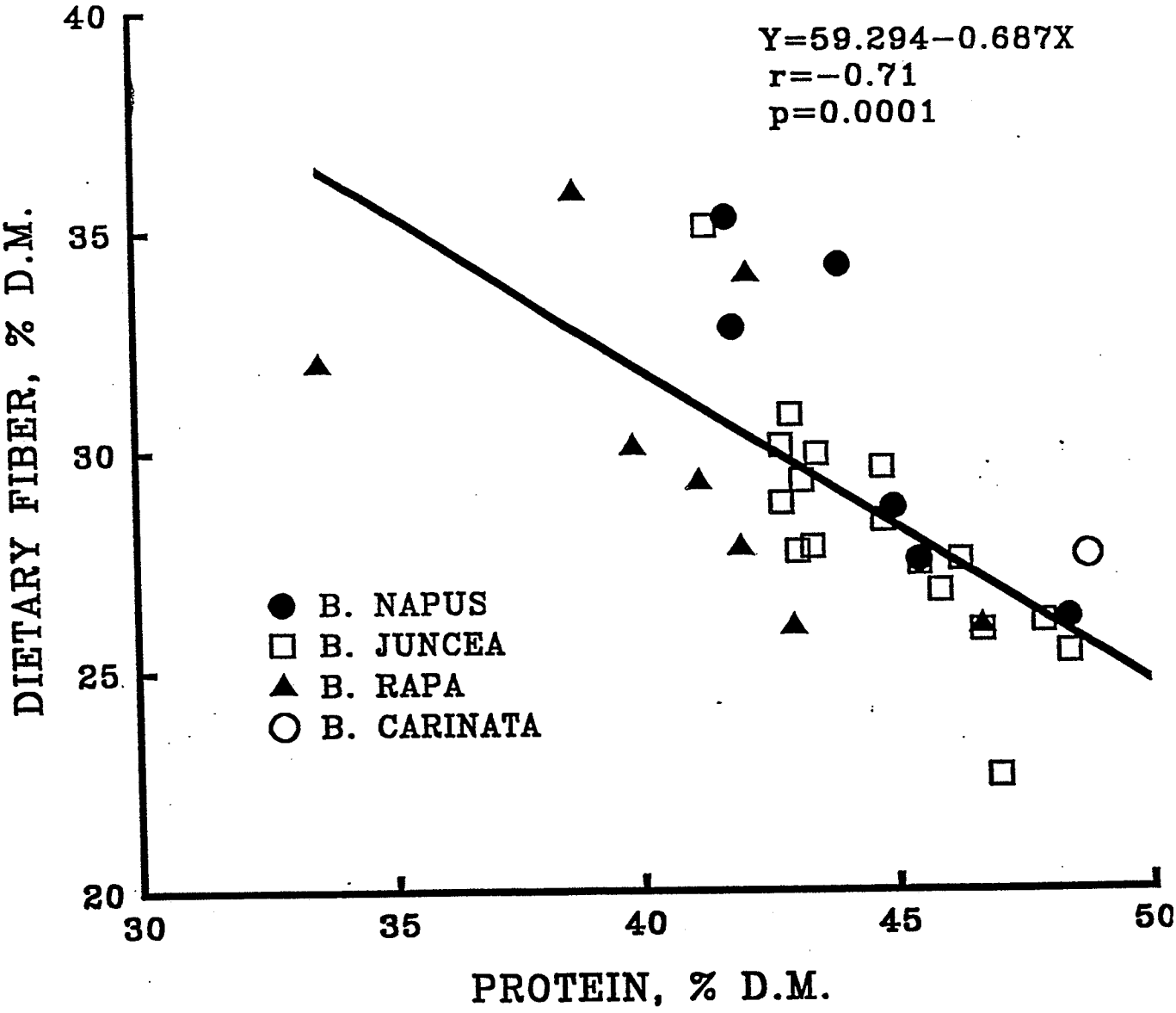

Y=59.294−0.687X
r=−0.71
p=0.0001
B. NAPUS
B. JUNCEA
B. RAPA
B. CARINATA
DIETARY FIBER, % D.M.
PROTEIN, % D.M.
40
35
30
25
20
30
35
40
45
50

FIGURE 3. Relationship between the protein and dietary fibre contents of meals derived from brown- and yellow-seeded *Brassica napus*.

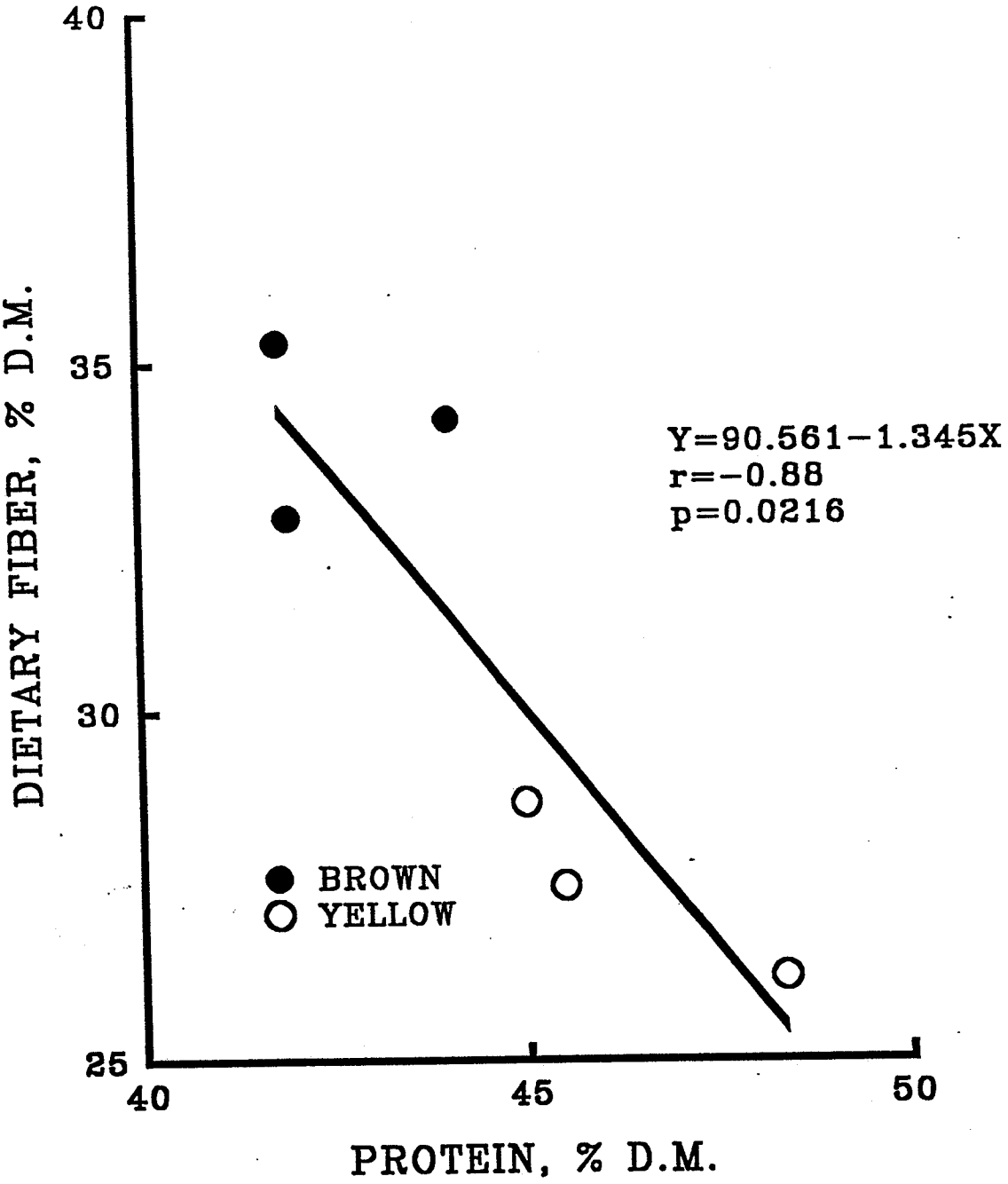

40
35
30
25
DIETARY FIBER, % D.M.
Y=90.561−1.345X
r=−0.88
p=0.0216
BROWN
YELLOW
40
45
50
PROTEIN, % D.M.

FIGURE 4. Relationship between protein and dietary fibre contents of meals derived from brown- and yellow-seeded *Brassica juncea*.

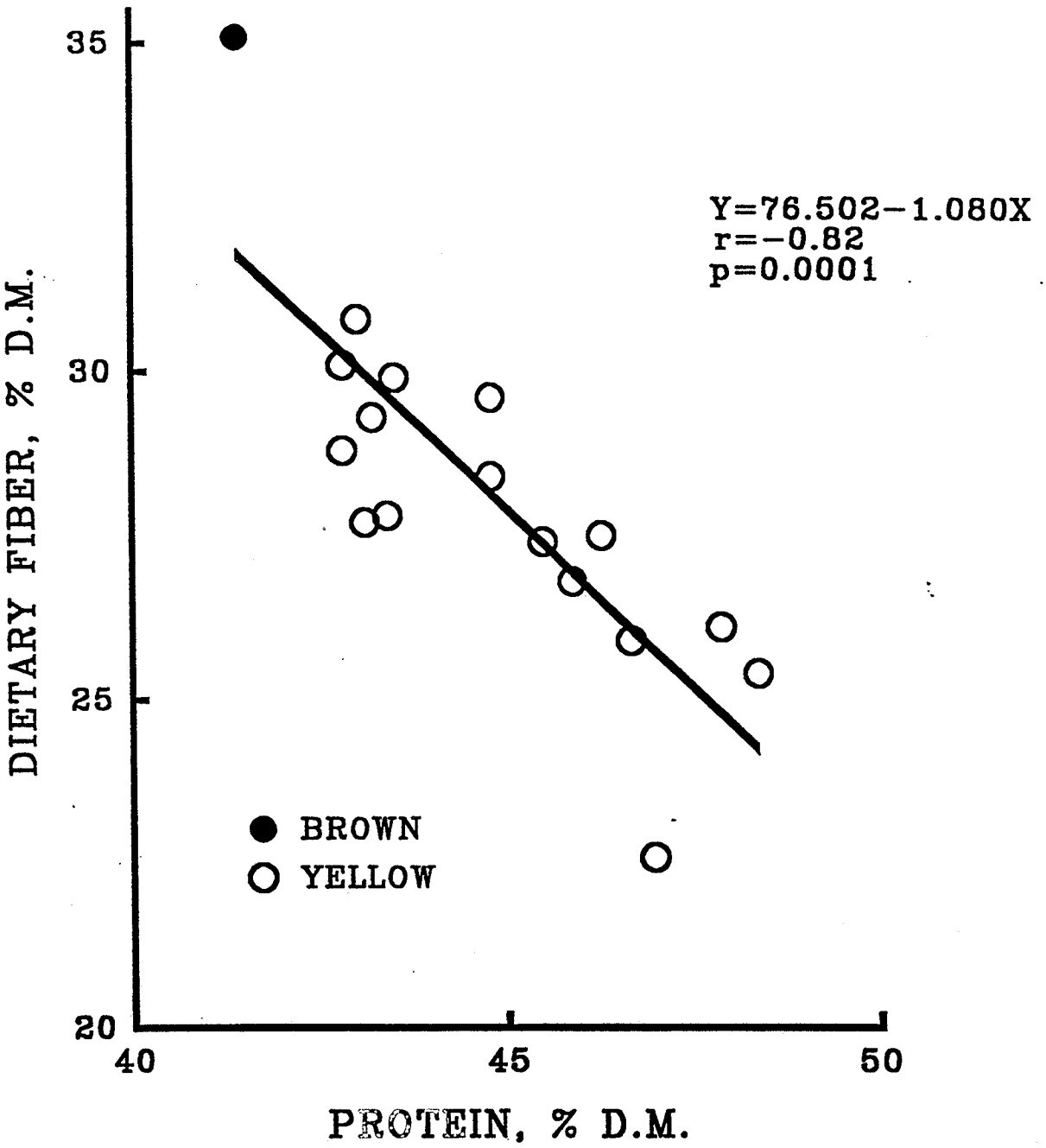

Y=76.502−1.080X
r=−0.82
p=0.0001
DIETARY FIBER, % D.M.
35
30
25
20
40
45
50
PROTEIN, % D.M.
BROWN
YELLOW

FIGURE 5. Relationship between protein and dietary fibre contents of meals derived from brown- and yellow-seeded *Brassica rapa*.

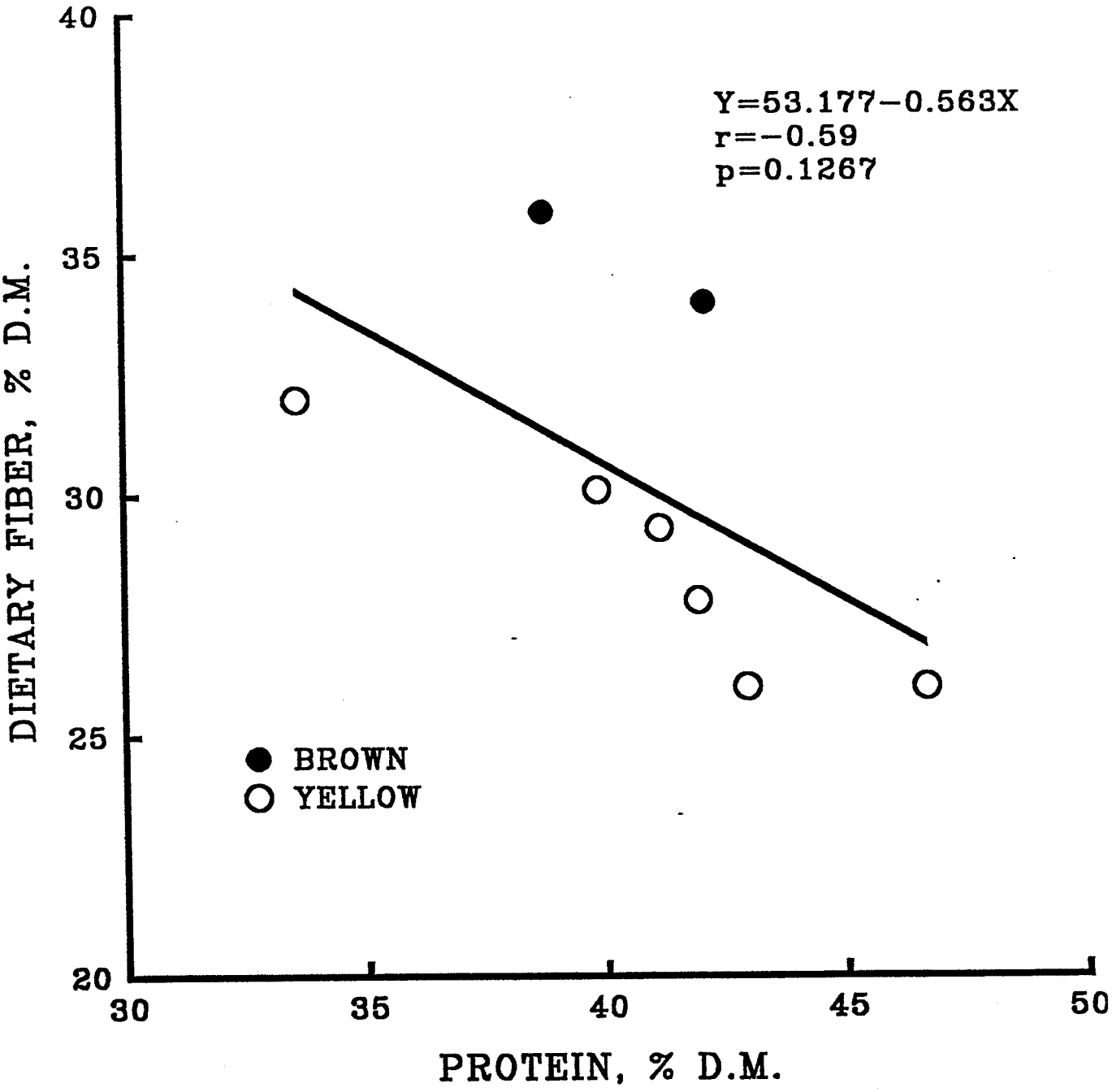
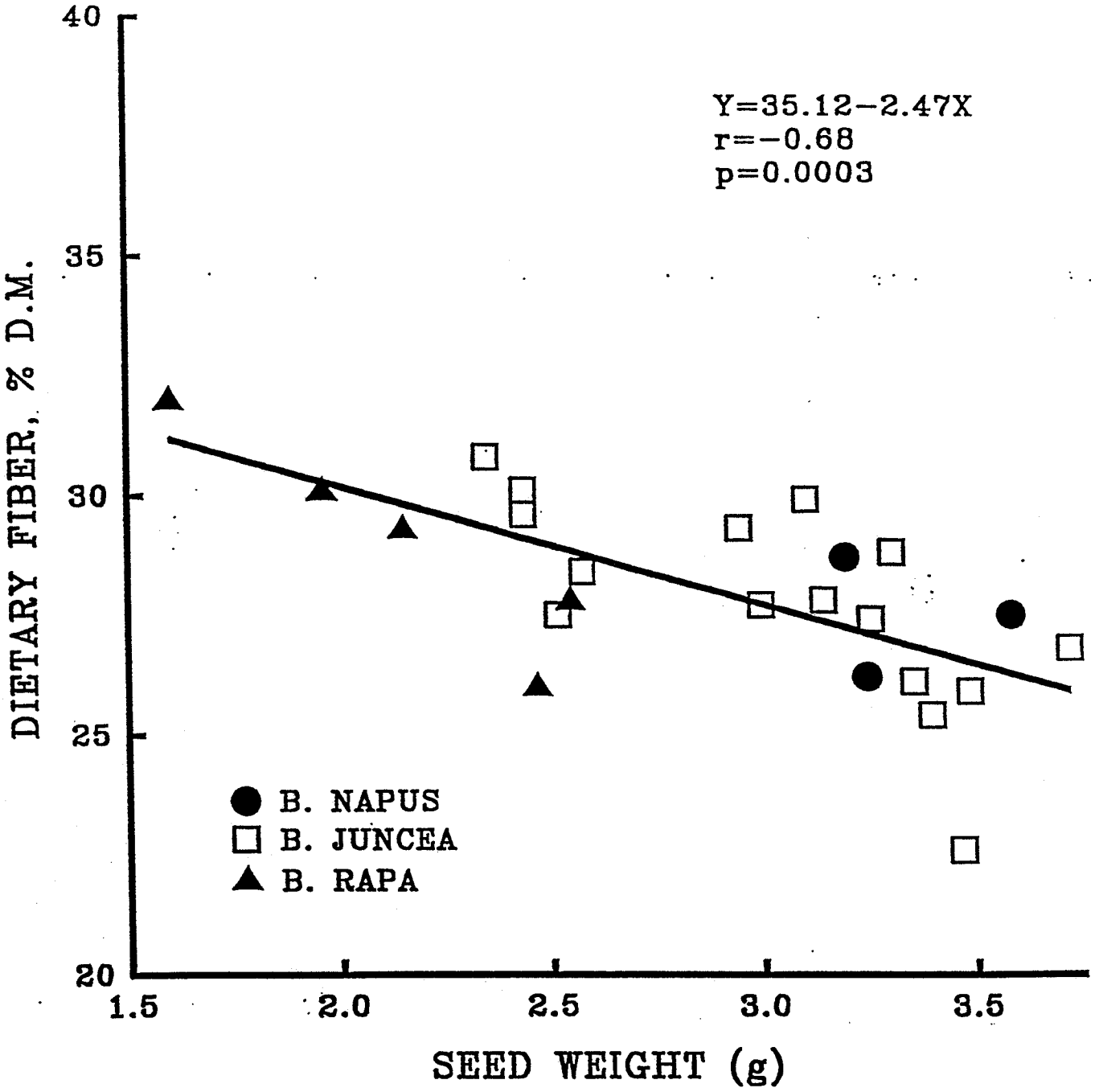

Y=53.177−0.563X
r=−0.59
p=0.1267
DIETARY FIBER, % D.M.
40
35
30
25
20
30
35
40
45
50
PROTEIN, % D.M.
BROWN
YELLOW

FIGURE 6. Relationship between dietary fibre content in the meal and seed size of selected strains/cultivars of *Brassica napus, B. rapa* and *B. juncea* (expressed as grams per 1000 seeds).


Y=35.12−2.47X
r=−0.68
p=0.0003
DIETARY FIBER, % D.M.
40
35
30
25
20
1.5
2.0
2.5
3.0
3.5
SEED WEIGHT (g)
B. NAPUS
B. JUNCEA
B. RAPA

4. MANUSCRIPT TWO

Nutritive Value of Yellow-seeded Canola. Part I. Digestible Protein, Dietary Fibre and the Effect of Moist Heat Treatment on Meal Quality.

ABSTRACT

The study was conducted to determine digestible protein, soluble fibre, soluble phenolics and extract viscosity of selected yellow- and brown-seeded *Brassica* seed meals. The effects of total and soluble dietary fibre as well as that of soluble phenolics and extract viscosity on *in vitro* protein digestibility were also determined. Digestible protein content was determined by using a two step pepsin-pancreatin system. There were no major differences in the content of soluble fibre, soluble phenolics and extract viscosity between *Brassica* seed species. There were, however, some differences in digestible protein content with *B. rapa* and *B. napus* showing the highest values. Digestible protein was poorly correlated with total and soluble fibre as well as soluble phenolics. There was no relationship between soluble dietary fibre and extract viscosity. An attempt was made to establish optimum conditions for processing of canola seed. Digestible protein of three oil free seed samples increased substantially with increased temperature of moist heat treatment up to 108 ± 1°C. Heat treatment below 105°C was not effective in promoting protein digestibility. The reduction in digestible protein content for samples heat treated at temperatures above 110°C was associated with increased neutral detergent fibre (NDF) content. The extent of protein damage was reflected by high protein content in the NDF residue. Time of heat treatment did not have any positive effect on protein digestibility.

Key words: yellow-seeded canola, soluble fibre, *in vitro* protein digestibility, heat treatment.

4.1. INTRODUCTION

Canola meal could be more competitive in the market place if it had more digestible energy, more protein and less fibre (Bell, 1993a). Among the approaches undertaken in order to produce a meal of superior nutritive value is breeding for canola of yellow-seeded type (Stringam *et al.*, 1974; Bell and Shires, 1982). Such efforts have led to the development of partially yellow- and fully yellow-seeded *Brassica rapa* canola and are justified as a means to improve the meal quality by increasing seed protein and decreasing seed fibre, with a resultant potential improvement in the available energy and amino acid contents of the meal. Earlier research which involved a number of *B. rapa* (*campestris*) canola varieties showed limited advantage of the yellow-seeded characteristic with regard to dietary fibre content (Slominski *et al.*, 1994). Total dietary fibre was found to be only slightly lower than that estimated for brown-seeded canola. Although, in comparison to brown-seeded type, the yellow-seeded *B. rapa* canola was shown to contain much less lignin and polyphenols, the cell wall polysaccharide content was found to be much higher in this type of canola. As was recently investigated in this laboratory, the high content of cell wall polysaccharides was found to result from significantly smaller cells in the cotyledon fraction of *B. rapa* canola (unpublished). The sucrose content, on the other hand, was higher by 3-4 percentage points in fully yellow-seeded cultivars as opposed to brown-seeded varieties (Slominski *et al.*, 1994a). However, the first commercially available meal from fully yellow-seeded *B. rapa* (cv. Parkland)

contained similar metabolizable energy and amino acid digestibilities in comparison to conventional canola meal (Slominski *et al.*, 1994a).

A relatively new initiative in plant breeding has been the development of canola quality forms of *B. juncea*, a species known for its pure yellow seed coat. Only recently have plant breeders been able to incorporate the yellow-seeded character into *B. napus* canola and in a recent study yellow-seeded *B. juncea* and *B. napus* seed meals along with selected *B. rapa* and *B. carinata* samples were shown to contain more protein (44.5% vs 42.7) and less fibre (28% vs 33) than their brown-seeded counterparts (Simbaya *et al.*, 1995). In these samples, total dietary fibre was negatively correlated with protein content, with *B. napus* and *B. juncea* showing the highest correlation coefficients. A relatively weak negative correlation between seed size and fibre content in yellow-seeded samples further indicated the importance of canola cotyledons in determining the level and nature of dietary fibre (Simbaya *et al.*, 1995). These and some earlier data on *B. rapa* canola (Stringam *et al.*, 1974; Theander *et al.*, 1977; Bell and Shires, 1982; Slominski and Campbell, 1990) indicate that there are at least three factors affecting the fibre content: the colour of the seed (the higher proportion of yellow seeds in the sample, the lower content of lignin and polyphenols); the seed size (the smaller the seed, the more fibre in the resulting meal), and the cotyledon cell size (the smaller the cells, the more non-starch polysaccharides and thus dietary fibre in the meal). Since any of the factors may significantly affect the nutritive value of the meal, all three should be given consideration in developing new varieties of canola/rapeseed.

The research reported in this paper is a continuation of our earlier work on quality evaluation of the meals derived from the new, yellow-seeded types of canola (Simbaya *et al.*, 1995). The objective of this study was to determine protein digestibility as well as soluble dietary fibre and soluble phenolic (tannins) contents as a means of estimating the nutritive worth of the meals from newly developed yellow-seeded *Brassica* varieties. The effect of moist heat treatment on protein digestibility was also examined in order to define the optimal conditions necessary for the production of meal of high nutritive value.

4.2. EXPERIMENTAL

4.2.1. Materials

Brassica seed samples represented two yellow- (Y-1016, Y-1018) and two brown-seeded (Westar, Elect) strains/cultivars of *Brassica napus*, two yellow- (Parkland, R 500) and two brown-seeded (Echo, Torch) samples of *B. rapa*, six yellow- (Cutlass, ZEHO, J-4253, J-4316, J-2741, J-2776) and one brown-seeded ("Commercial") samples of *B. juncea*, one yellow- (Dodolla) and one brown-seeded (Cv-s-67) samples of *B. carinata* and one yellow-seeded sample of *Sinapis alba* (Ochre). All plant materials were grown in field plots at the Agriculture and Agri-Food Canada Research farm at Saskatoon, Saskatchewan, Canada. In preparation for analysis, the seeds were crushed to pass through a 2 mm sieve and were extracted with *n*-hexane for 2 h in a Soxhlet apparatus.

Following drying under a fume-hood at room temperature, the meals were ground to pass through a 1 mm sieve and were moist-heat treated at 105°C for 20 minutes in a cyclomatic laboratory autoclave (American Sterilizer Co., Brampton, Ontario. Canada). The processed meals were then re-defatted with *n*-hexane for 8 h. Commercial canola meal was purchased from a local crushing plant.

4.2.2. In vitro protein digestibility and soluble fibre content

Digestible protein and soluble dietary fibre contents were determined following digestion of the sample with pepsin and pancreatin enzymes as recommended by Gauthier *et al.* (1982), Savoie and Gauthier (1986), Kennedy *et al.* (1989) and Boisen and Eggum (1991). The system developed for the current study is summarized in Fig. 7 and is described in detail below.

Five grams of sample was weighed into a 100 ml erlenmeyer flask containing 500 mg of pepsin (P 7000, Sigma, St. Louis, MO, U.S.A.). Fifty millilitres of 0.1 M HCl/54 mM NaCl solution were then added, and the contents were shaken for 1 h at 40°C in an environmentally controlled incubator shaker (New Brunswick Scientific Co., Inc. Edison, NJ. U.S.A.). Following digestion with pepsin, the pH was adjusted to approximately 7.0 with 2.5 ml of 2.0 M NaOH. The pH was stabilized by adding 20 ml of 0.1 M phosphate buffer containing 0.05% sodium azide. The contents were then transferred into pre-soaked dialysis tubes (Spectrum, Houston, TX, U.S.A.) with a molecular weight cut off value of 12000 -14000. One ml of buffer solution containing 50 mg of pancreatin (P 1750, 4 x U.S.P.; Sigma Chemical Co., St. Louis, MO, U.S.A.)

was then added and the tubes were closed allowing for a small air gap in the tube to facilitate continued mixing of the contents (Kennedy *et al.*, 1989).

To simulate the environment of the small intestine and to minimize the effect of end product inhibition, protein digestion with pancreatin was performed with the aid of the digestion/dialysis unit which was constructed by mounting a speed reducer motor (Bodine Electric Co., Chicago, Illinois, U.S.A.) onto a thermally controlled water bath (Blue M. Electric Co., Blue Island, U.S.A.) filled with 0.05 M phosphate buffer pH 7.0. The motor was used to rotate a rectangular aluminum frame (32 x 18 cm) at a speed of 20 rpm. Attached to the metal frame were 6 dialysis tubes, each containing a sample of test material.

Protein digestion with pancreatin was conducted for 6 h at 40°C. After incubation, enzyme activities were terminated by replacing the buffer with ice-cold distilled water. To ensure removal of the hydrolysis products, the contents were subjected to dialysis against ice-cold distilled water for 72 h with continuous rotation of the tubes. The water was changed regularly at 8 h intervals. Following dialysis, the samples were centrifuged for 15 minutes at 3000 x g in a Centra GP8 centrifuge (International Equipment Co. Needham, MA., U.S.A.). The insoluble (pellet) and soluble (supernatant) residues were frozen, freeze-dried and analyzed for crude protein (Kjeldahl N x 6.25). Digestible protein content was calculated as total sample protein minus protein retained in the soluble and insoluble residues. Soluble dietary fibre was defined as non-starch polysaccharides present in the soluble (supernatant) residue.

Several modifications to the *in vitro* procedure described above were employed to establish the optimum conditions required to accomplish maximal hydrolysis of canola protein.

As opposed to pepsin concentration which was chosen based on the literature data reviewed by Boisen and Eggum (1991), the ratio of pancreatin to the substrate was investigated by subjecting 5 g of commercial canola meal to digestion with increased concentration of this enzyme (Fig. 8). Pronase, the protease from *Streptomyces griseus* (P 6911, Sigma), was used as a reference protease. Both enzymes were further tested at the optimal concentrations to study the effect of Tris-HCl buffer on *in vitro* protein digestibility. The addition of sodium dodecyl sulphate (SDS) to facilitate protein digestion as recommended by Brillouet *et al.* (1988) was also investigated (Table 9). The effect of dialysis time on the removal of the digestive products was assessed by monitoring the disappearance of soluble protein following 24, 48 and 72 h of dialysis (Table 10).

4.2.3. Evaluation of *Brassica* seed meals

The digestion/dialysis unit was used to evaluate the *Brassica* seed meals for digestible protein and soluble dietary fibre contents. The samples were also analyzed for content of phenolic substances (tannins) and extract viscosity.

The digestion unit was also used to study the effect of moist heat treatment on *in vitro* protein digestibility. The effect of temperature was evaluated by heating each of three different samples of defatted seed at 90, 100, 105, 110, 120 and 126°C for 20

minutes. In a study on the effect of heating time, the samples were autoclaved at 105°C for 20, 40, 60 and 90 minutes. A cyclomatic laboratory autoclave (American Sterilized Co., Brampton, Ontario, Canada) was used with the "steam to jacket" valve closed in order to achieve desirable temperatures. Time of heat treatment was monitored after the set temperature inside the autoclave was attained (about 18-20 minutes). The processed meals were dried overnight under a fume-hood and were subjected to analysis for digestible protein and neutral detergent fibre (NDF) contents. The unheated samples of defatted seed served as the control meals.

4.2.4. **Analytical procedures**

Crude protein was determined with the aid of a Kjeltec Auto 1030 Protein Analyzer (Tecator AB, S-26321, Hoganos, Sweden). Total dietary fibre was determined by a combination of neutral detergent fibre (NDF) and detergent soluble non-starch polysaccharides (Slominski *et al.*, 1994a). Soluble dietary fibre analysis utilized the procedure for non-starch polysaccharides as described by Englyst and Cummings (1984) with minor modifications (Slominski and Campbell, 1990). The method of Goering and Van Soest (1970) was used to determine neutral detergent fibre (NDF). The NDF procedure was modified to exclude the use of decalin and sodium sulfite (Mascharenhas Ferreira *et al.*, 1983). Soluble phenolics were analyzed according to the Follin-Denis procedure (Swain and Hillis, 1959) as outlined by AOAC (1990). Extract viscosity measurements were made on a Wells-Brookfield Cone/Plate Digital Viscometer (Brookfield Engineering Laboratories Inc., Stoughton, MA, U.S.A.) using the procedures

of Bedford and Classen (1993) and Boros *et al.* (1993). Viscosity measurements were done with and without pancreatin addition.

5.2.5. Statistical analysis

Data was analyzed by ANOVA and linear regression using the general linear models (SAS Institute, 1985) and the procedures of Snedecor and Cochran, 1980). Treatment means were compared and separated by Duncan's (1955) multiple range test.

4.3. RESULTS AND DISCUSSIONS

4.3.1. In vitro protein digestibility procedure

The effect of enzyme to substrate ratio on protein digestibility is shown in Figure 2. The *in vitro* protein digestibility increased with increased enzyme concentration up to 5 mg g^{-1} meal. On the protein basis, a smaller concentration of pancreatin and pronase were reported to be optimal for the proteolysis of casein (Gauthier *et al.*, 1986) and bovine serum albumin (Brillouet *et al.*, 1988), respectively. Incubation of canola meal with a combination of pronase and pancreatin resulted in slightly higher protein digestion than when either enzyme was used separately (Fig. 8). As with individual enzymes, the hydrolysis of protein by a 1:1 w/w combination of pronase and pancreatin reached the plateau at 5 mg g^{-1} meal. In general the pronase enzyme was more effective in protein hydrolysis than pancreatin. Similar results were reported by Gauthier *et al.*

(1986), Morrison (1973) and Brillouet *et al.* (1988). In this regard, the pronase preparation was found to contain a combination of exo- and endopeptidases and was shown to be effective in hydrolysing plant proteins up to the amino acid level (Selvendran, 1975). Pancreatin, on the other hand, is composed of trypsin, chymotrypsin, elastase and carboxypeptidases A and B which are only effective in breaking the basic and aromatic peptide bonds (Brulé and Savoie, 1988; Boisen and Eggum, 1991). In the latter case, however, it is presumed that some of the polypeptides are likely to be digested by the brush-border peptidases in the small intestine of monogastric animals (Silk, 1980). According to the current results, this fraction would not exceed 5% of the total protein content as judged from the difference in digestible protein content between the pronase and pancreatin treated samples (Fig. 8).

The effect of Tris-HCl and phosphate buffer on activities of the pronase and pancreatin preparations (Table 9) indicated a negative effect of Tris-HCl buffer on protein digestibility. The difference between the two buffers could not be associated with different pH values as both solutions had a relatively similar pH (7.0 vs 7.1) which was within the recommended range for optimum pancreatin (Savoie *et al.*, 1986) and pronase (Brillouet *et al.*, 1988) activities. It may be speculated that the observed variations in protein digestibility was due to the overall ability of phosphorus to promote the enzyme reaction. While addition of sodium lauryl sulphate (SDS) to the phosphate buffer had a slightly positive effect on pronase activity, a significant negative effect was observed on pancreatin activity (Table 9). Of particular interest was the extent to which SDS enhanced solubilization of dry matter (data not shown). This may be a reason why SDS,

along with the pronase enzyme, is so effective in non-cellular protein solubilization (Brillouet *et al.*, 1988). The reason for the negative effect of SDS on pancreatin activity was not apparent.

The effect of time on protein remaining in the dialysis tube (Table 10) showed a continuous loss of nitrogenous matter throughout the 72 hrs dialysis period, with no significant differences between the two canola meal samples. Although the dialysis did not reach a plateau after 72 hrs, it could not be extended due to the potential for microbial contamination of the sample over the prolonged period of time. Therefore, dialysis for 72 hrs was chosen for the *in vitro* procedure.

4.3.2. Quality characteristics of *Brassica* seed meals

Selected quality parameters of meals derived from yellow- and brown-seeded Brassica seed samples are shown in Table 11. Since there were no significant differences between brown- and yellow-seeded samples within each species, only the mean values are reported. With regard to digestible protein content, *B. rapa* canola was shown to have significantly higher values than *B. juncea, B. carinata* and *S. alba*. Protein digestibility for *B. napus* canola was only statistically different from that of *S. alba,* which had the lowest value. The low digestible protein content in *S. alba*, however, may not be representative of the species since only one sample was available for comparison. Although earlier results from this laboratory showed a negative correlation between the protein and total dietary fibre contents (Simbaya *et al.*, 1995), there was no relationship between total dietary fibre and *in vitro* protein digestibility in the current study (Fig. 9).

Therefore, it may be inferred that any potential adverse effect of dietary fibre is solely due to nutrient dilution rather than to any anti-nutritive properties associated with the fibre components. This also applies to the soluble dietary fibre fraction, although as determined under the environment of the gastrointestinal tract, averaged only 1.9% (Min 1.2%, Max 2.6) and represented only a small proportion (4-8%) of the total dietary fibre content present in canola samples (Table 11). It should be noted that the soluble fibre content determined in the current study is similar to that of wheat (2.2%) and is much lower than that of barley or rye (5.2%), both known to contain large amounts of water-soluble and viscous polysaccharides (Rakowska *et al.*, 1989; Thomke *et al.*, 1987). Soluble fibre, however, was not correlated with digestible protein (Fig. 10) or extract viscosity (Fig. 11). The viscosity values were highest for *S. alba* and lowest for *B. napua* and *B. rapa* canola, with no statistical differences between *B. juncea* and *B. carinata* (Table 11). Extract viscosity was negatively correlated with digestible protein content (Figure 12) and it was unclear which component(s) of canola meal contributed to this relationship since, with the exception of *S. alba* variety Ochre, the viscosity was substantially reduced by treatment of the samples with pancreatin (Fig. 13). The association of digestible protein with extract viscosity is difficult to explain. It may be related to the degree of protein solubility with lesser contribution of well soluble and thus digestible protein to extract viscosity. It should be reiterated, however, that the viscosity values for canola meal are much lower than those reported for cereal grains as indicated here and the viscosity of canola extracts should not be considered detrimental with regard to the quality of the meal.

With the exception of one sample of *Sinapis alba*, there were no major differences within the *Brassica* species with regard to content of soluble phenolics (Table 11), although all values were slightly higher than those reported for free phenolics present in canola/rapeseed (1.5 to 1.8% of oil free meal) (Kozlowska *et al.*, 1990). However, the current values were similar to those reported by Fenwick and Hoggan (1976). The differences may be attributed to either different extraction conditions, as was demonstrated by Naczk and Shahidi (1989), or different methods used for the detection of phenolic substances. In this regard, the vanillin method, commonly used for tannin determination, was found to be relatively ineffective in detecting the phenolic substances in the water-soluble fraction of canola meal (data not shown). Therefore, it was concluded that the low soluble tannin content, as judged by the difference between the vanillin and the Swain and Hillis (1959) method for total phenolics, would have positive implications with regard to the quality of canola meal. This was further substantiated by a lack of correlation between the digestible protein and the content of total soluble phenolics (Fig. 14).

4.3.4. Effect of moist heat treatment on meal quality

There was a profound effect of moist heat treatment on meal quality as measured by digestible protein content in three samples of defatted canola seed processed under different conditions. As shown in Figure 15, *in vitro* protein digestibility increased substantially with increased temperature up to 107-108°C. Application of higher temperatures resulted in a significant decline in protein digestibility. The increase in

digestible protein content in moderately heat treated samples is in agreement with earlier reports which demonstrated that some form of heat treatment is required to alter the three-dimensional structure of plant proteins in order to make the protein more susceptible to proteolysis (Nordheim and Coon, 1984). While heat treatment is widely used in the commercial crushing operation of canola seed, the rationale behind its application is more related to aspects other than the manufacture of meal of high protein quality. These include the prepress cooking at 75-85°C to enhance coalescence of the oil droplets and the reduction of oil viscosity to facilitate screw pressing; steam sparging at 103-107°C (at the discharge) in a desolventizer-toaster to remove hexane and to improve the nutritive quality of the meal by myrosinase enzyme inactivation and partial destruction of undesirable glucosinolates; drying of the desolventized meal which involves some additional heat treatment (Unger, 1990). In modern crushing plants, the heat treatment that is routinely applied would appear to lead to the production of high quality canola meal. This is supported by the results of the current study which showed an excellent *in vitro* protein digestibility figure for the commercial sample of canola meal (Table 12). The digestible protein content of 71.8% agrees very well with recently reported 70% and 72% values determined *in vivo* at the ileal level of the pig for Canadian commercial canola meal (Grala *et al.*, 1993) and the low glucosinolate rapeseed meal from Finland (Nystrom *at al.*, 1995), respectively. In the current study lower, but only slightly, values for digestible protein content were obtained for laboratory prepared meals heat treated at 110°C. The unheated samples, however, showed very poor protein digestibility values which in essence were similar to those obtained following heat

treatment of the samples at 90°C or 100°C (Fig. 15). This is in agreement with some earlier *in vivo* studies showing the unheated or heated under mild conditions meal to have a lower nutritive value and lower digestible protein and/or amino acids contents as compared to that of the commercial meal (Slominski *et al.*, 1985, Nystrom *et al.*, 1995, Jensen *et al.*, 1995). Therefore, as indicated in Fig. 15, it appears that the optimal temperature for processing of canola seed is within a range of 107 - 108°C. Any further increase in temperature, however, may result in substantial destruction of the protein as evidenced by low protein digestibility values for samples processed at 120 and 126°C. Protein damage at high temperatures is well known and results from the formation of Maillard reaction products which are condensation and polymerization products of amino acids such as lysine, histidine or arginine with sugar residues. Maillard products in essence should be considered dietary fibre as evidenced by a substantial increase in neutral detergent fibre (NDF) content in samples processed at 120 and 126°C (Fig. 16). Protein damage at high temperatures was further reflected in a substantial increase of total protein content in the NDF residues (Fig. 17). Similar findings were reported by Keith and Bell (1983) who observed increased NDF content in the meals with increased processing temperatures. A slight increase in NDF content at temperatures below or equal to 110°C did not have any negative effect on *in vitro* protein digestibility. Therefore, the reduction in protein solubility rather than protein damage appear to be responsible for the increase in NDF content in the moderately heat treated meal.

The effect of varying times of moist heat treatment (105°C) on digestible protein and NDF contents are shown in Figs. 18 and 19, respectively. Protein digestibility did

not reach the level observed in an earlier experiment and declined slightly over time. This was followed by an increase in NDF content which, after 90 min of heat treatment, significantly exceeded the NDF values for samples processed at high temperature (ie., 110°C) but for a shorter period of time (ie., 20 min) (Fig. 16 vs Fig. 19). Only a slight decline in digestible protein content over a 90 min period of heat treatment is in contrast to the findings of Anderson-Herfarmann *et al.* (1993) and Jensen *et al.* (1995) for canola meal and Parsons *et al.* (1991) for soybean meal who reported the adverse effect of prolonged time of heat treatment on protein quality. It should be noted, however, that the temperatures applied in most of these studies varied substantially (100 - 121°C) and the meals, which were derived from processing plants, had already undergone some form of heat treatment.

The results of the current study indicate the importance of moist heat treatment in the processing of canola seed with temperatures of 108 ± 1°C applied for a short period of time being beneficial to the meal quality.

TABLE 9. Effect of buffer and sodium dedocyl sulphate (SDS) addition on *in vitro* protein digestion by pancreatin and pronase enzymes (% of control).

	Type of buffer		
Enzyme	Phosphate (pH 7.0)	Tris-HCl (pH 7.1)	Phosphate + SDS (1%)
Pancreatin[1]	100.0	84.8 ± 0.8	34.6 ± 1.0
Pronase[2]	100.0	86.9 ± 0.0	105.3 ± 0.1

[1] Used at 10 mg g^{-1} meal; [2] used at 5 mg g^{-1} meal.

TABLE 10. Effect of time of dialysis on soluble nitrogenous matter remaining in the dialysis tube following digestion of two samples of canola meal with pepsin and pancreatin (% of total protein).

Dialysis time (hrs)	Canola meal A[1]	Canola meal B[1]
24	13.7 ± 0.2[2]	13.4 ± 0.3
48	10.5 ± 0.4	8.5 ± 0.3
72	7.2 ± 0.1	7.0 ± 0.4

[1] Canola meals were two representative samples of commercial meal from different sources. [2] Mean ± SD.

TABLE 11. Digestible protein, soluble dietary fibre, soluble phenolics and extract viscosity of meals derived from *Brassica* and *Sinapis* species.

Species/ samples number[1]	Digestible protein (% of total)	Soluble fibre (% DM)	Soluble phenolics (% DM)	Extract viscosity (cps)
B. napus (n=4)	65.2[2]±3.7^{ab}	2.05±0.27^{ab}	2.27±0.09^{ab}	1.06±0.06^{c}
B. rapa (n=4)	67.3±4.6^{a}	2.41±0.26^{a}	2.15±0.26^{ab}	1.15±0.08^{bc}
B. juncea (n=7)	57.3±5.4^{bc}	1.81±0.49^{ab}	2.43±0.16^{a}	1.35±0.18^{b}
B. carinata (n=2)	56.9±1.6^{bc}	1.47±0.04^{b}	2.45±0.20^{a}	1.36±0.02^{b}
S. alba (n=1)	47.8±0.6^{c}	1.81±0.02^{ab}	1.92±0.00^{b}	1.66±0.00^{a}

[1] See Materials and Methods Section for number of brown- and yellow-seeded samples within species; [2] Mean ± SD; $^{a, b, c}$ Values within a column with different superscripts differ significantly ($P \leq 0.05$).

TABLE 12. Effect of moist heat treatment (110°C, 20 min) on digestible protein content in three samples of defatted *Brassica* seeds as compared to that of the commercial meal (% of total).

Defatted seed[1]	Unheated	Heated
Sample A	42.4 ± 1.8	68.9 ± 1.5
Sample B	40.6 ± 2.4	64.1 ± 0.7
Sample C	36.7 ± 0.4	68.7 ± 0.7
Commercial meal	71.8 ± 0.4	

[1] Represented three different varieties of canola.

FIGURE 7. Procedure used for digestible protein and soluble dietary fibre determinations.

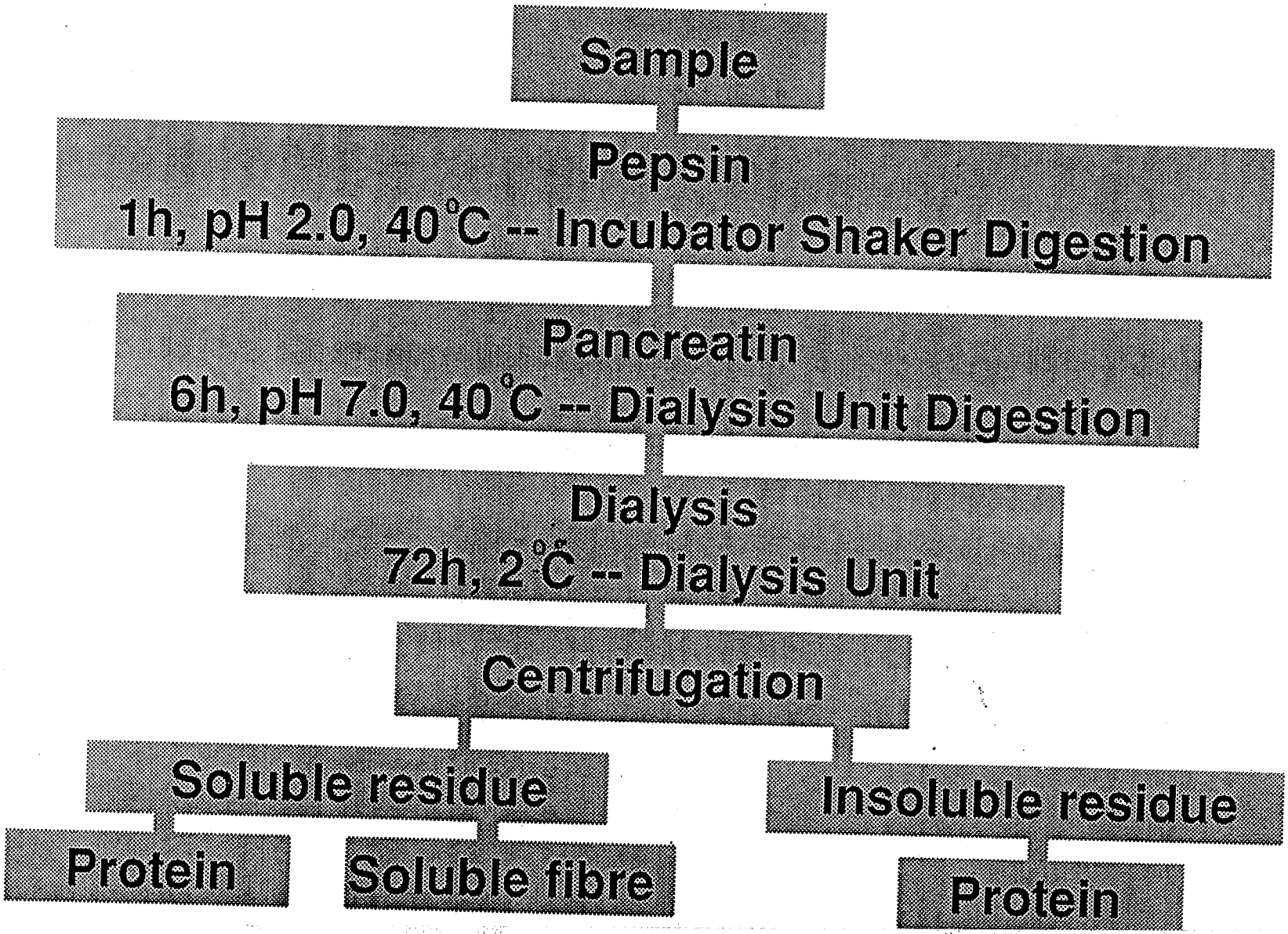

Sample
Pepsin
1h, pH 2.0, 40°C -- Incubator Shaker Digestion
Pancreatin
6h, pH 7.0, 40°C -- Dialysis Unit Digestion
Dialysis
72h, 2°C -- Dialysis Unit
Centrifugation
Soluble residue
Insoluble residue
Protein
Soluble fibre
Protein

FIGURE 8. Effect of enzyme concentration on *in vitro* protein digestibility in canola meal using pronase, pancreatin or a 1:1 (w/w) combination of both.

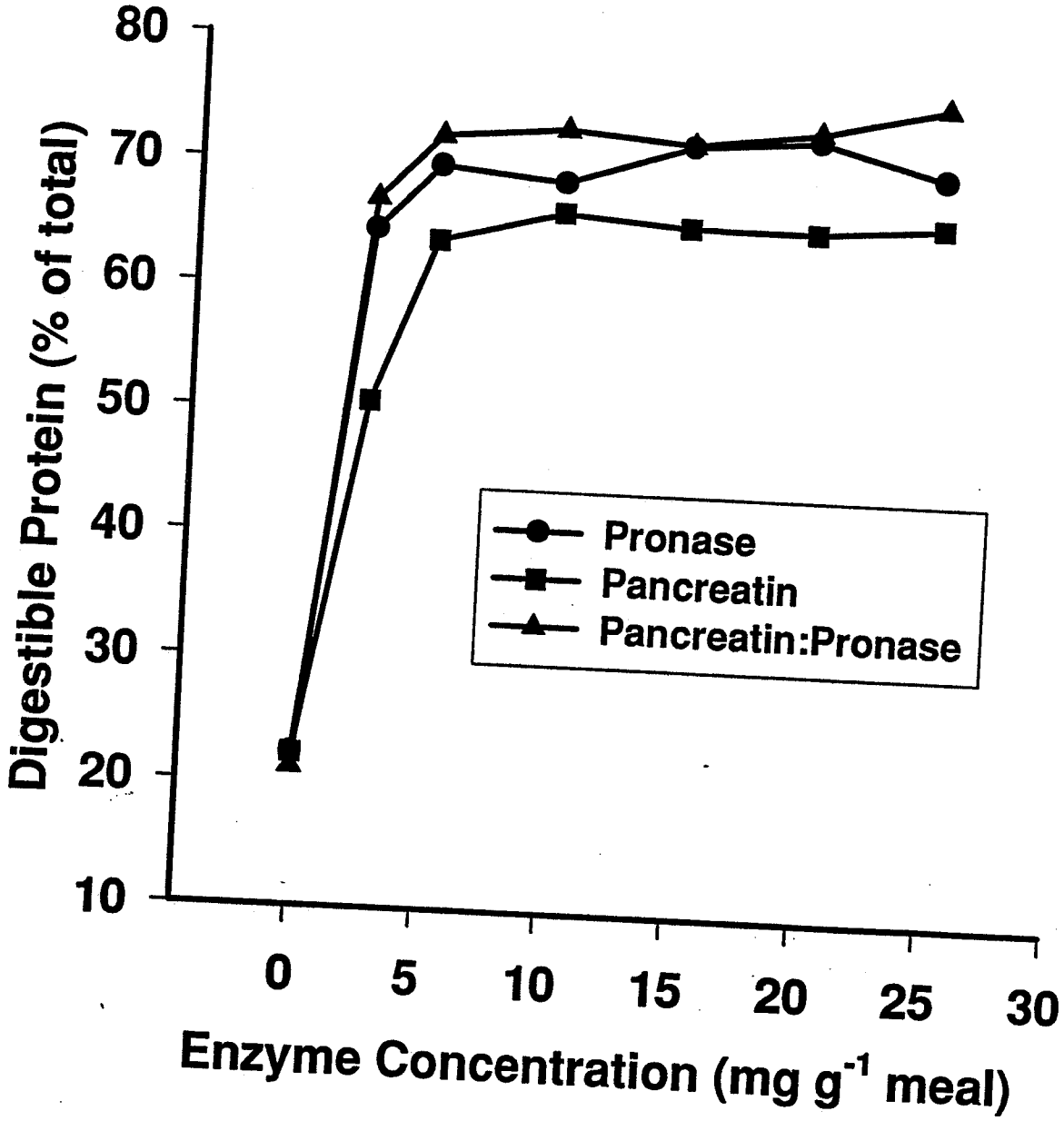

80
70
60
50
40
30
20
10
Digestible Protein (% of total)
0
5
10
15
20
25
30
Enzyme Concentration (mg g-1 meal)
Pronase
Pancreatin
Pancreatin:Pronase

FIGURE 9. Relationship between the total dietary fibre and digestible protein contents of meals derived from selected stains/cultivars of *B. napus, B. rapa, B. juncea,B. carinata and Sinapis alba.*

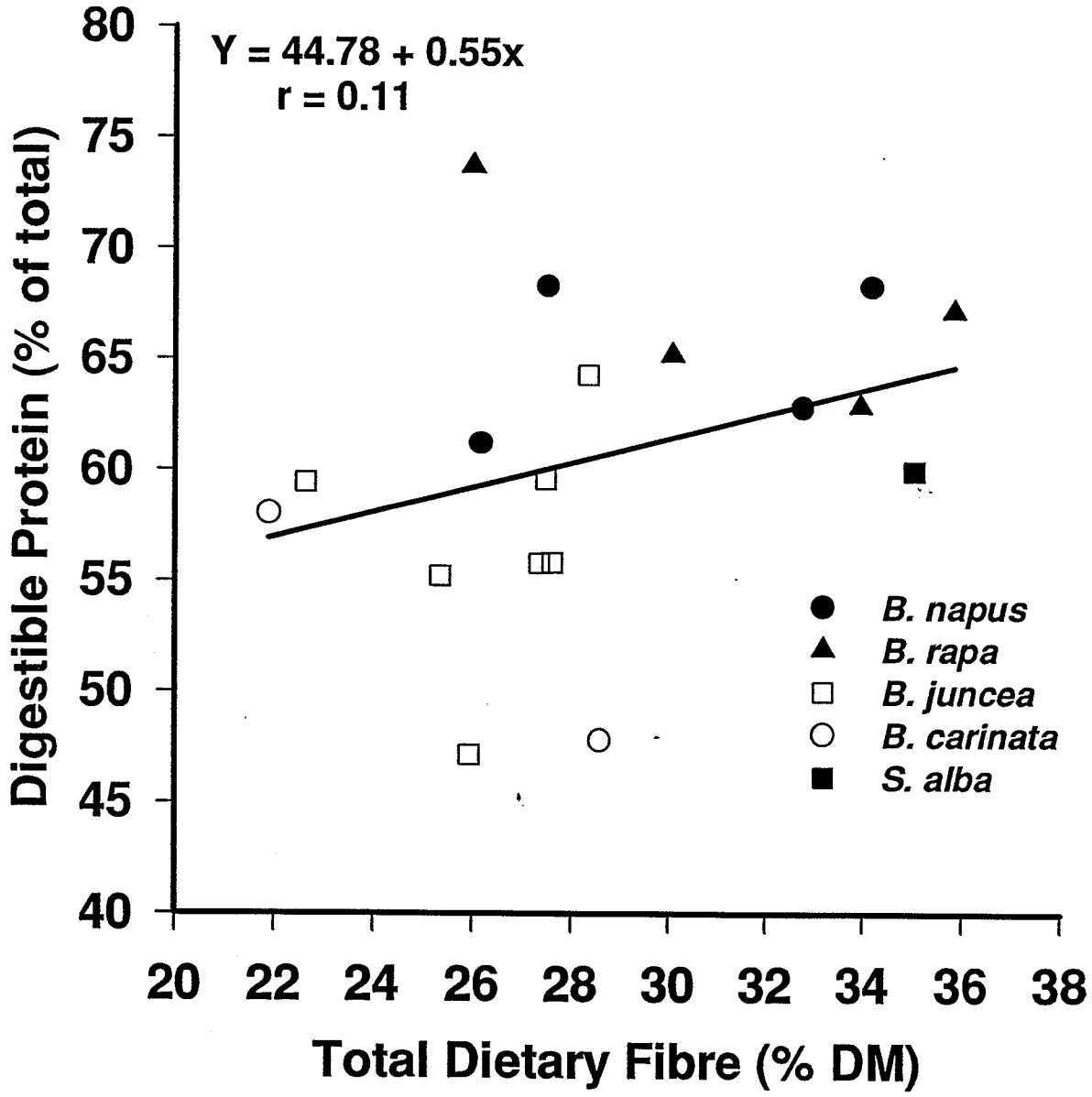

Y = 44.78 + 0.55x
r = 0.11
Digestible Protein (% of total)
80
75
70
65
60
55
50
45
40
20
22
24
26
28
30
32
34
36
38
Total Dietary Fibre (% DM)
B. napus
B. rapa
B. juncea
B. carinata
S. alba

FIGURE 10. Relationship between the soluble dietary fibre and digestible protein contents of meals derived from selected stains/cultivars of *B. napus, B. rapa, B. juncea,B. carinata and Sinapis alba.*

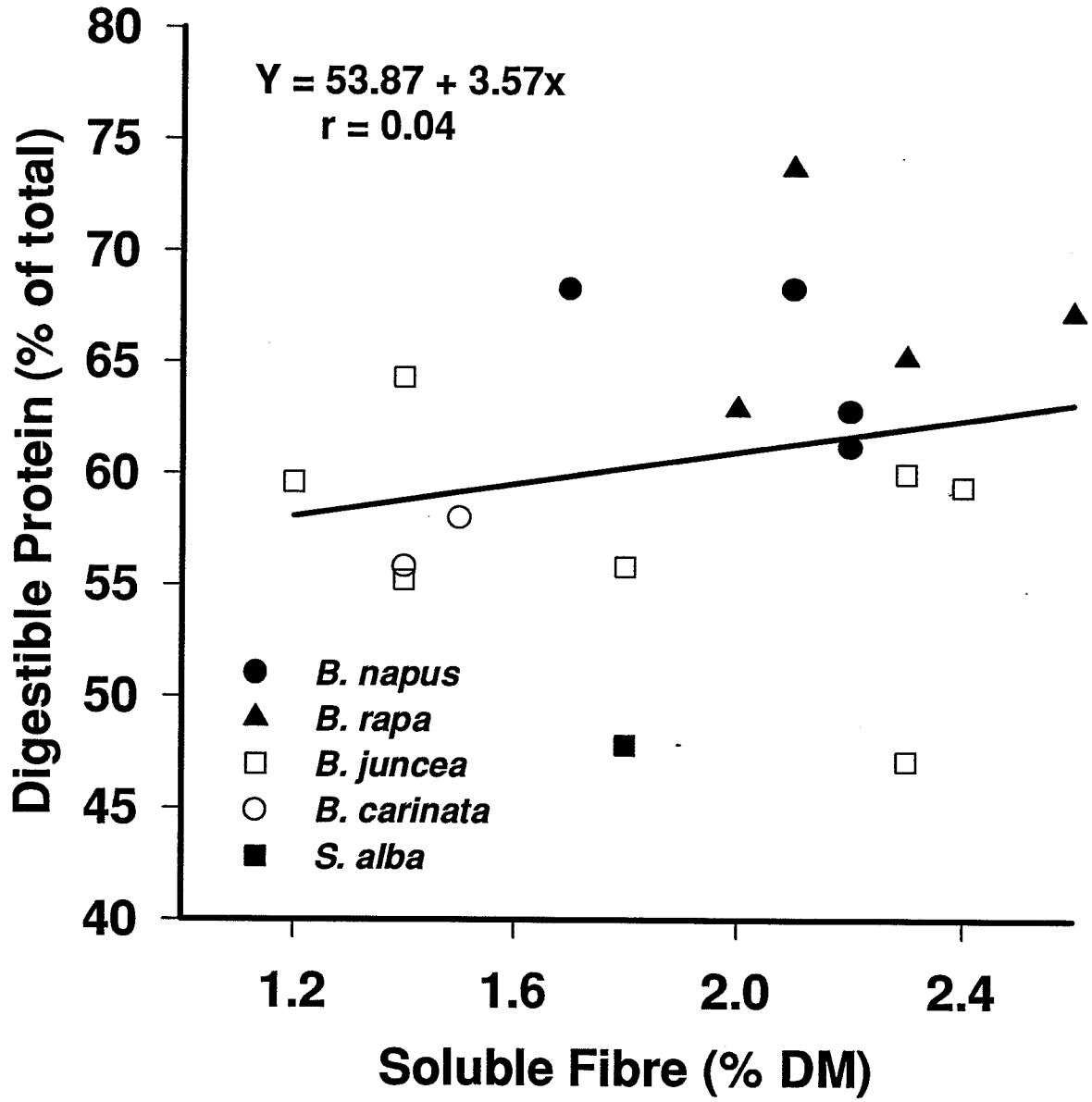

Y = 53.87 + 3.57x
r = 0.04
Digestible Protein (% of total)
80
75
70
65
60
55
50
45
40
1.2
1.6
2.0
2.4
Soluble Fibre (% DM)
B. napus
B. rapa
B. juncea
B. carinata
S. alba

FIGURE 11. Relationship between the extract viscosity and soluble dietary fibre content of meals derived from selected stains/cultivars of *B. napus, B. rapa, B. juncea,B. carinata and Sinapis alba.*

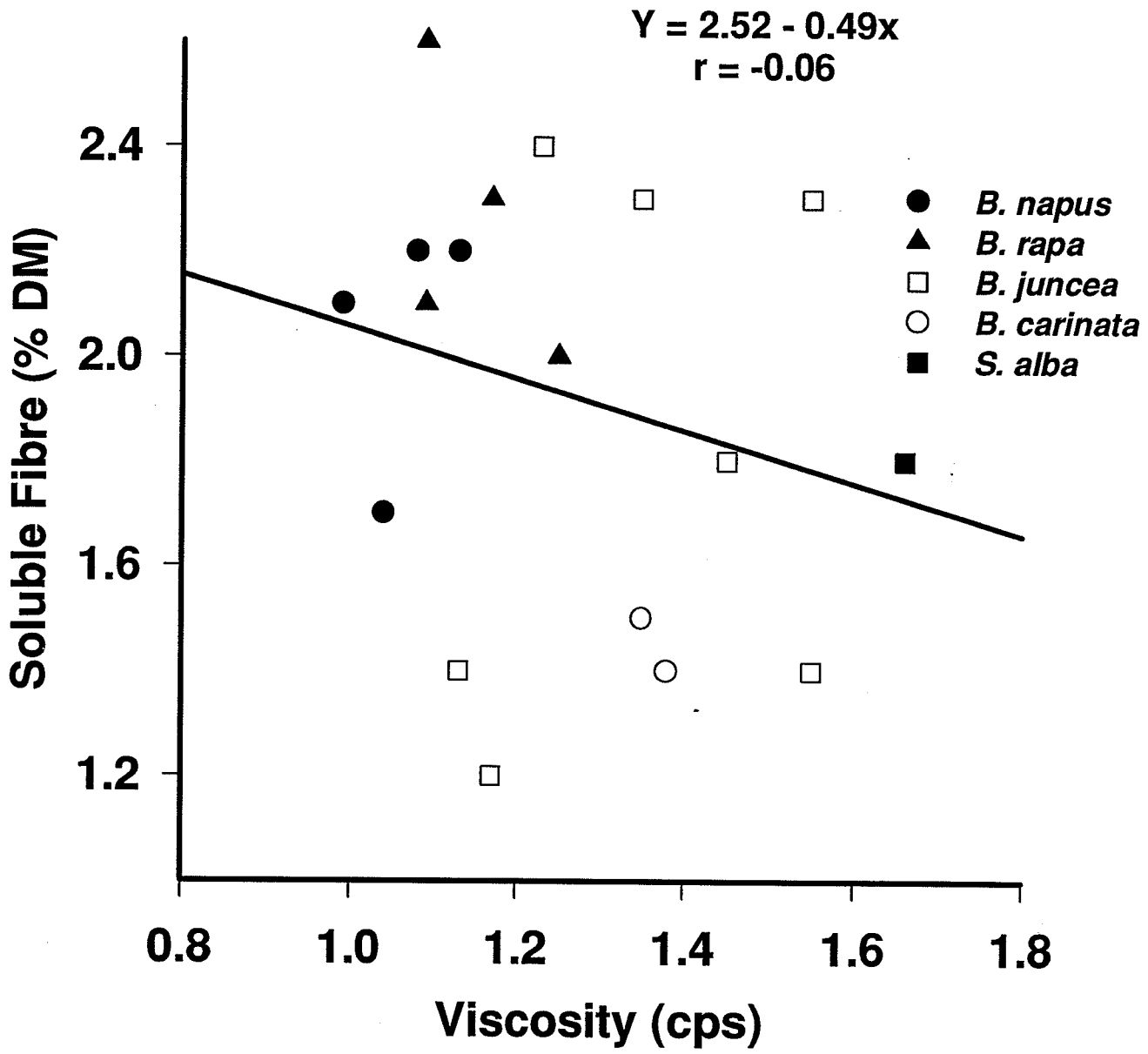

Y = 2.52 - 0.49x
r = -0.06
Soluble Fibre (% DM)
2.4
2.0
1.6
1.2
0.8
1.0
1.2
1.4
1.6
1.8
Viscosity (cps)
B. napus
B. rapa
B. juncea
B. carinata
S. alba

FIGURE 12. Relationship between the extract viscosity and digestible protein contents of meals derived from selected stains/cultivars of *B. napus, B. rapa, B. juncea, B. carinata and Sinapis alba.*

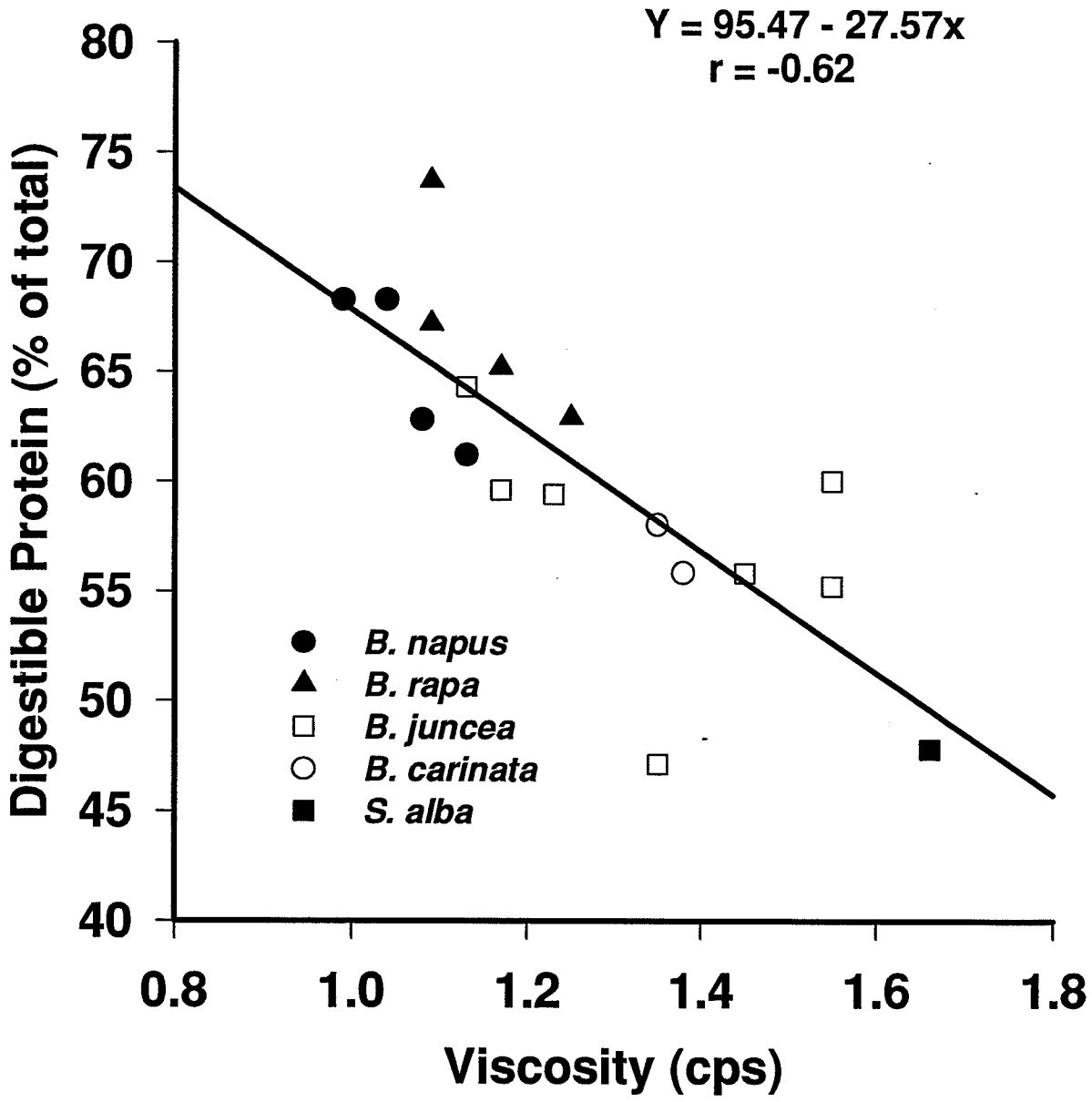

Y = 95.47 - 27.57x
r = -0.62
Digestible Protein (% of total)
80
75
70
65
60
55
50
45
40
0.8
1.0
1.2
1.4
1.6
1.8
Viscosity (cps)
B. napus
B. rapa
B. juncea
B. carinata
S. alba

FIGURE 13. Effect of Pancreatin addition on extract viscosity in selected samples of *Brassica* and *Sinapis alba* (Ochre) seed meals.

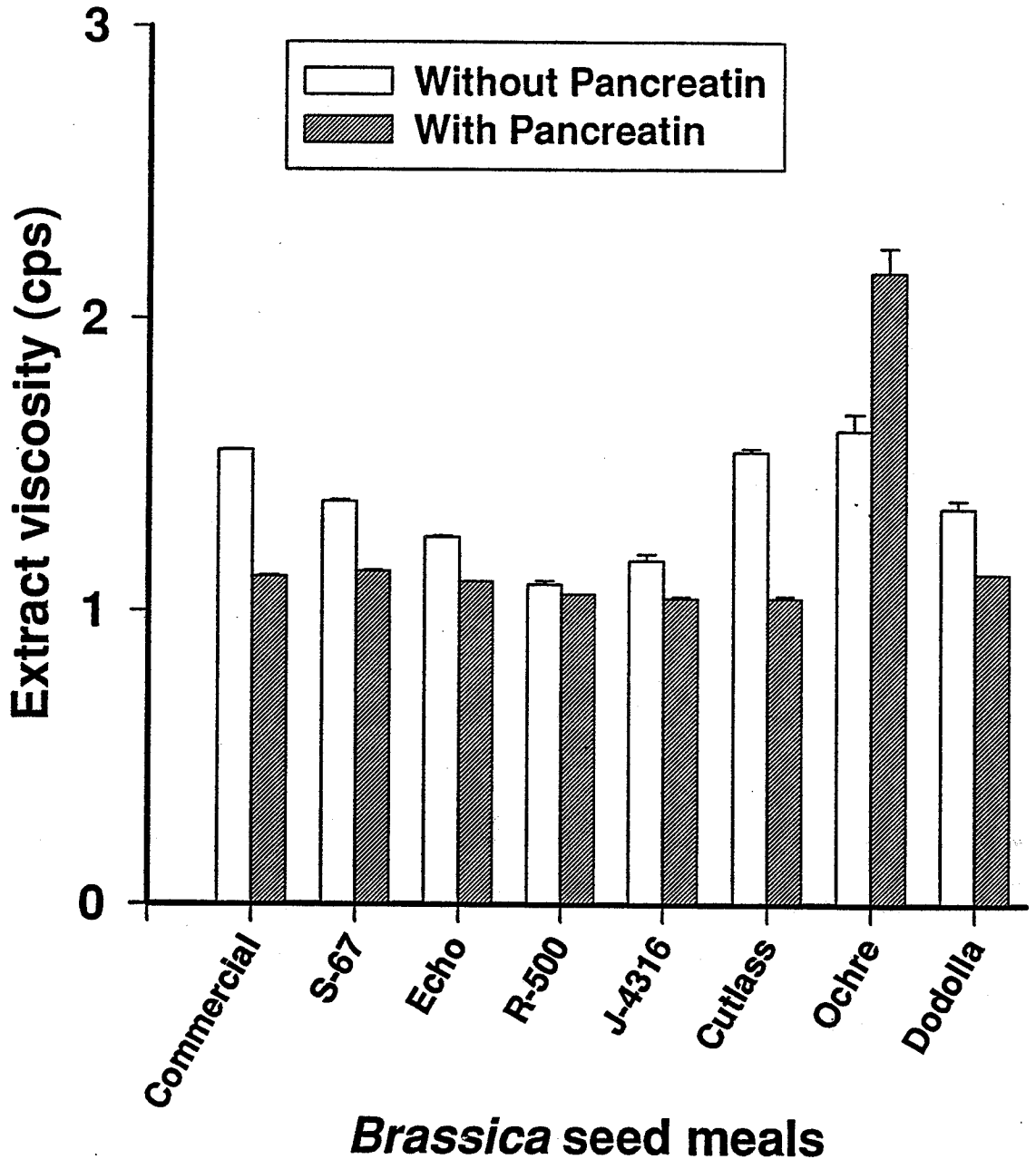

Without Pancreatin
With Pancreatin
Extract viscosity (cps)
0
1
2
3
Commercial
S-67
Echo
R-500
J-4316
Cutlass
Ochre
Dodolla
Brassica seed meals

FIGURE 15. Effect of varying temperatures on digestible protein contents in defatted seed samples from three *Brassica* cultivars subjected to moist heat treatment for 20 min.

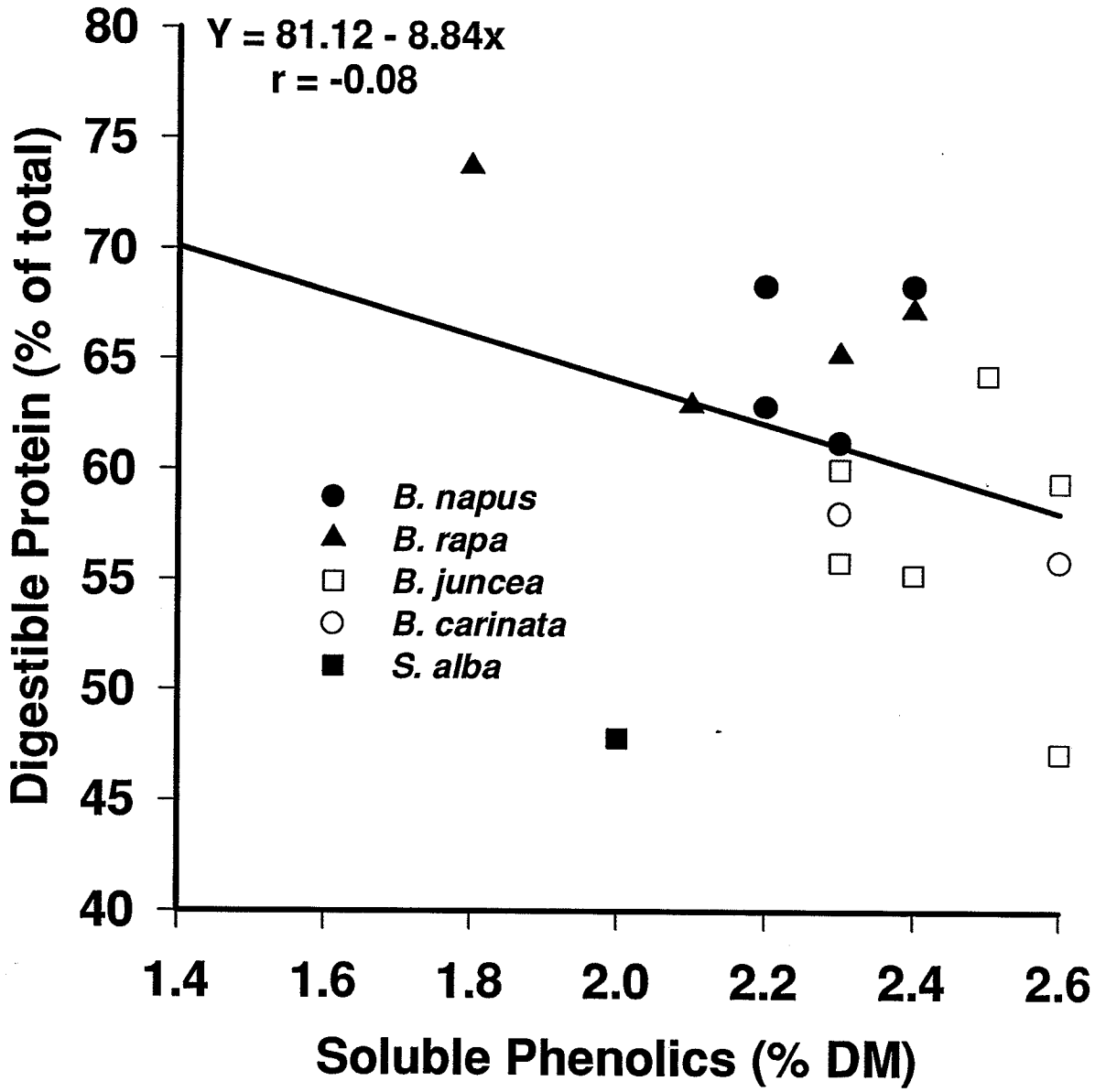

Y = 81.12 - 8.84x
r = -0.08
Digestible Protein (% of total)
80
75
70
65
60
55
50
45
40
1.4
1.6
1.8
2.0
2.2
2.4
2.6
Soluble Phenolics (% DM)
B. napus
B. rapa
B. juncea
B. carinata
S. alba

FIGURE 15. Effect of varying temperatures on digestible protein contents in defatted seed samples from three *Brassica* cultivars subjected to moist heat treatment for 20 min.

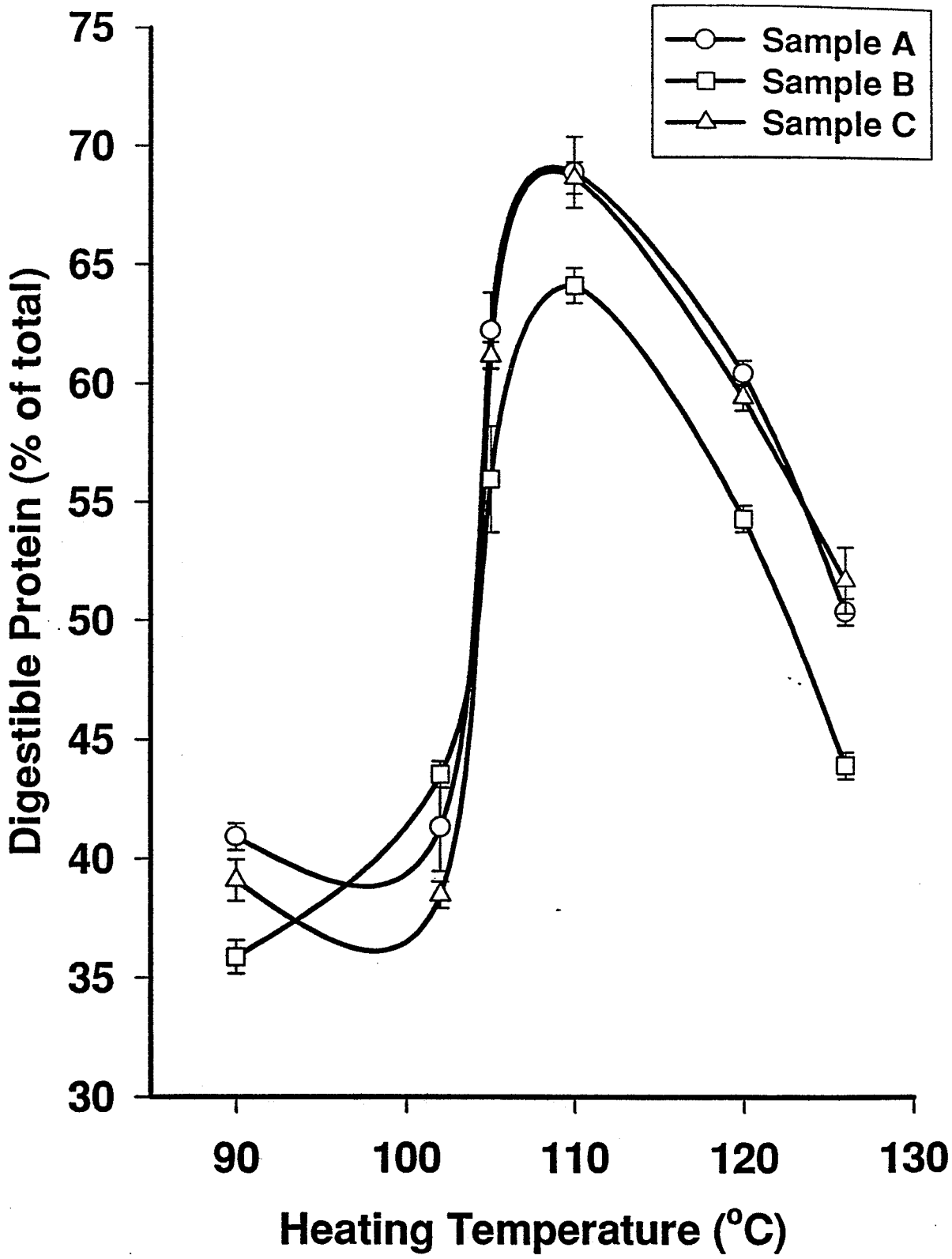

Sample A
Sample B
Sample C
Digestible Protein (% of total)
75
70
65
60
55
50
45
40
35
30
90
100
110
120
130
Heating Temperature (°C)

FIGURE 16. Effect of varying temperatures on neutral detergent fibre (NDF) content in defatted seed samples from three *Brassica* cultivars subjected to moist heat treatment for 20 min.

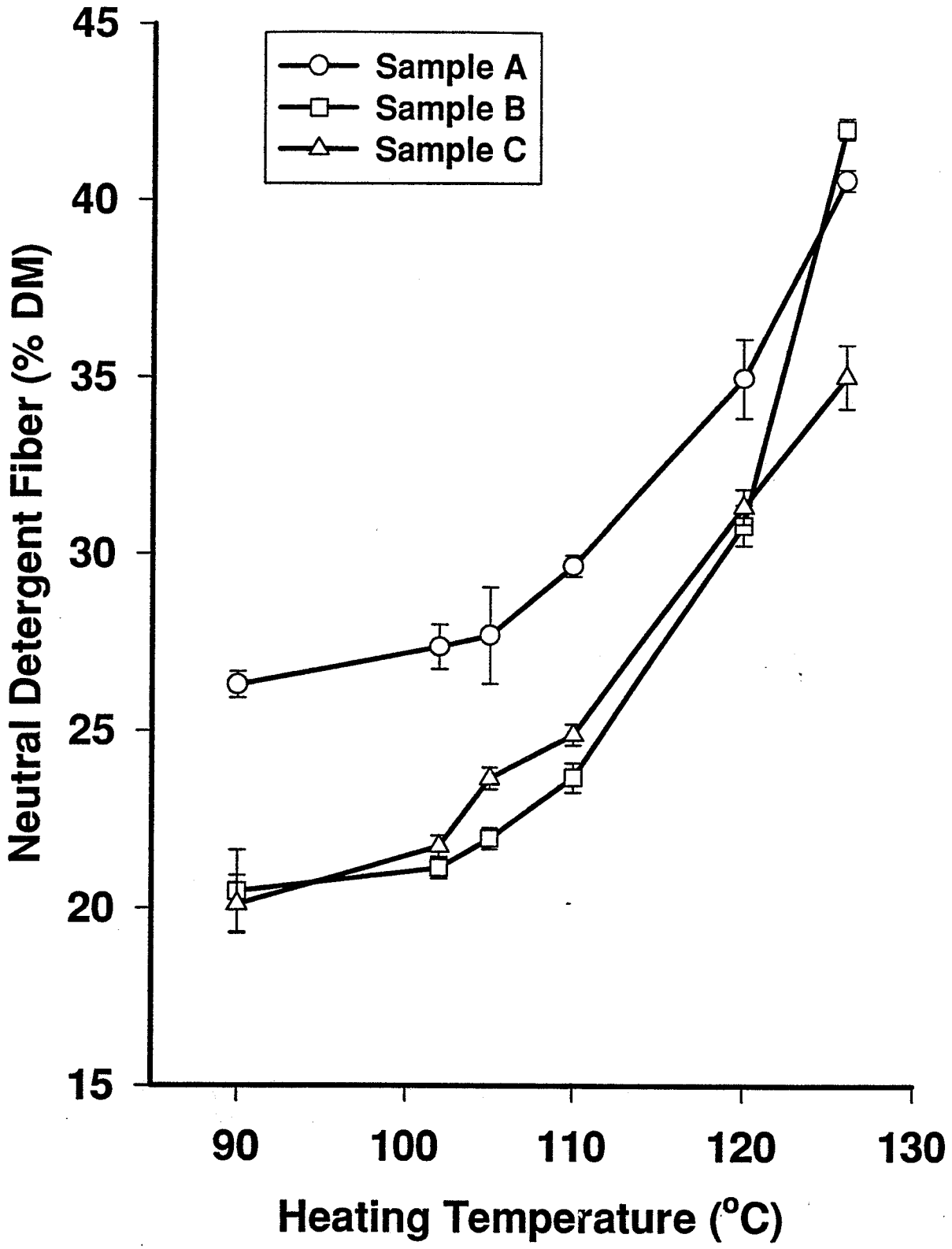

Sample A
Sample B
Sample C
Neutral Detergent Fiber (% DM)
45
40
35
30
25
20
15
90
100
110
120
130
Heating Temperature (°C)

FIGURE 17. Protein content in the neutral detergent fibre (NDF) residues of deffated seed samples from three *Brassica* cultivars as affected by various temperatures of moist heat treatment.

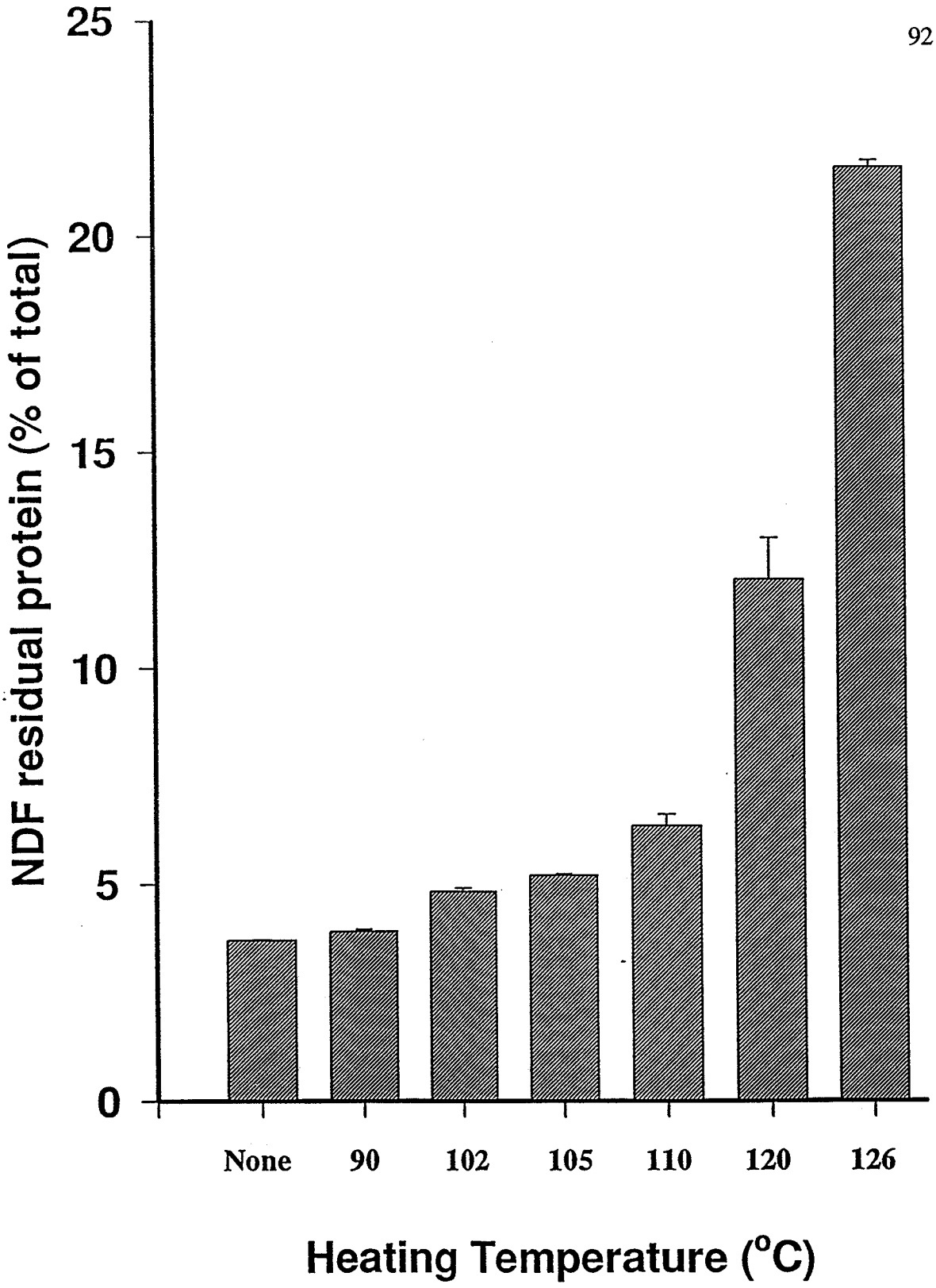

25
20
15
10
5
0
NDF residual protein (% of total)
None
90
102
105
110
120
126
Heating Temperature (°C)

FIGURE 18. Effect of varying times of moist heat treatment at 105°C on digestible protein content of defatted seed samples from three *Brassica* cultivars.

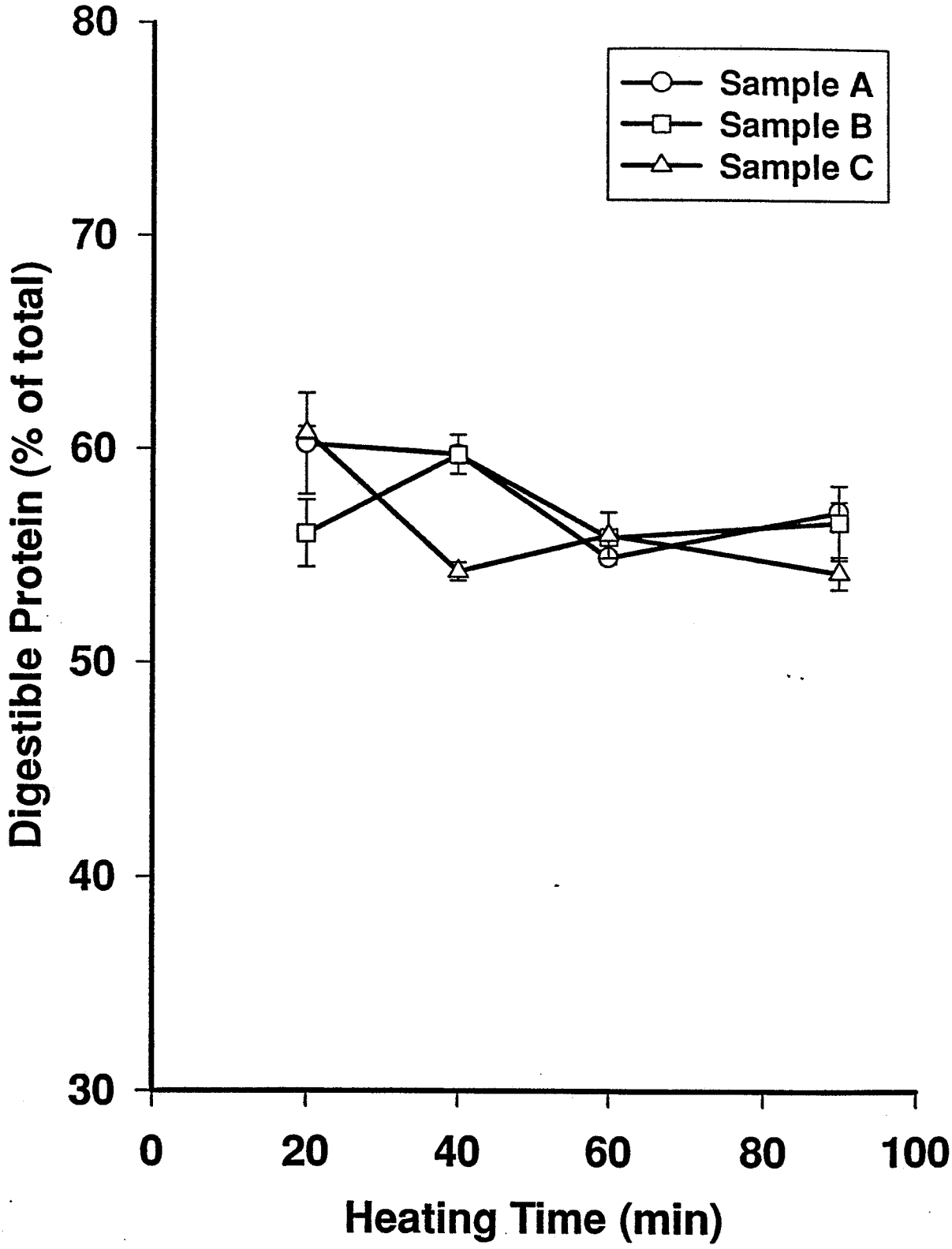
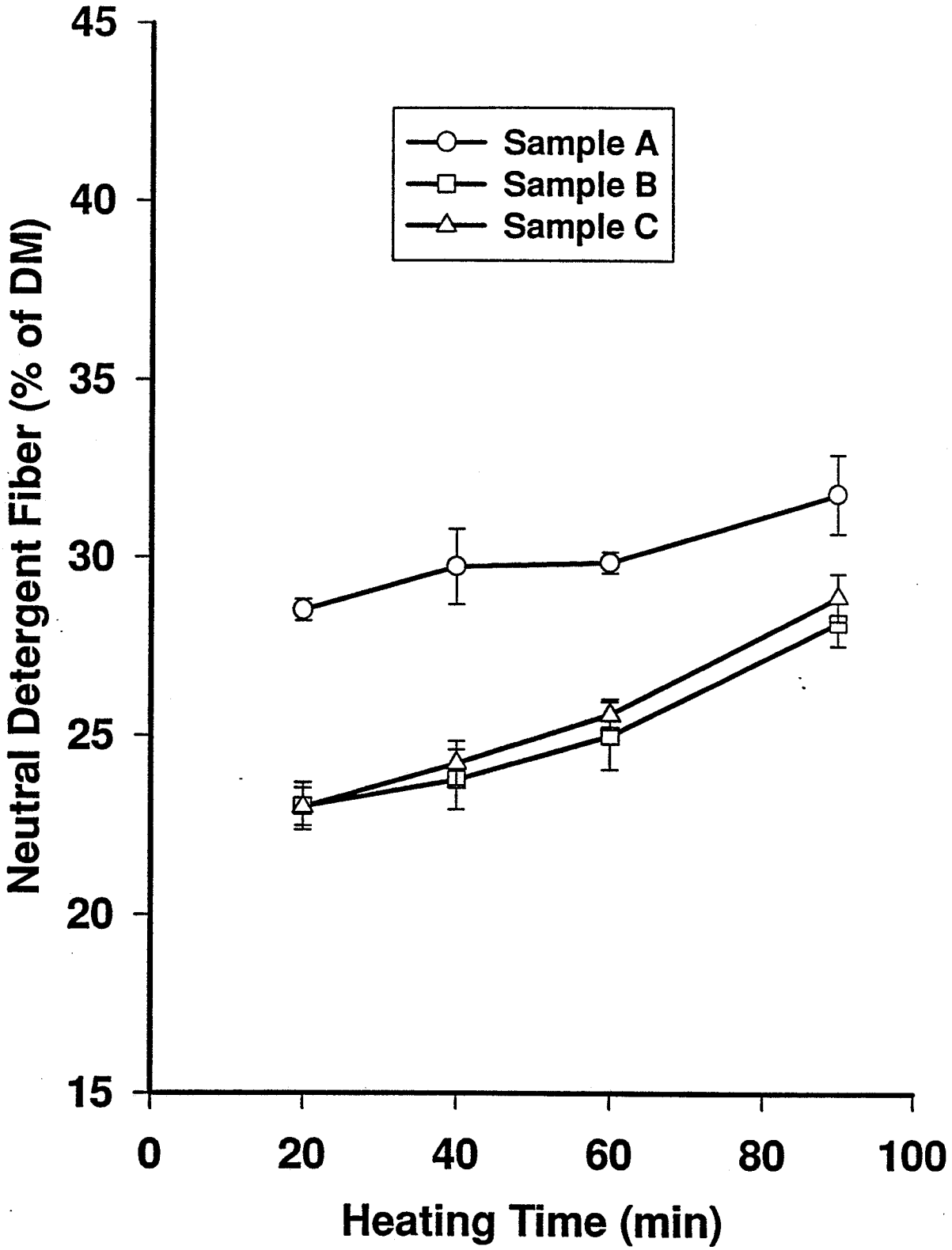

Sample A
Sample B
Sample C
Digestible Protein (% of total)
80
70
60
50
40
30
0
20
40
60
80
100
Heating Time (min)

FIGURE 19. Effect of varying times of moist heat treatment at 105°C on neutral detergent fibre content in of defatted seed samples from three *Brassica* cultivars.


Sample A
Sample B
Sample C
Neutral Detergent Fiber (% of DM)
45
40
35
30
25
20
15
0
20
40
60
80
100
Heating Time (min)

5. MANUSCRIPT THREE

Nutritive Value of Yellow-seeded Canola. Part II. Chemical Composition and Feeding Quality of Meals Derived from Newly Selected Varieties.

ABSTRACT

This study was conducted to investigate the quality characteristics of meals derived from newly developed cultivars/varieties of canola. Meals from yellow-seeded *Brassica rapa* (cv. Parkland), *B. napus* (cv. Y1016) and *B. juncea* (cv. J4316) and from brown-seeded *B. napus* (cv. Excel) were evaluated for chemical composition, in vitro protein digestibility, available energy and amino acid contents and overall feeding quality. All samples were of canola quality and apart from *B. juncea* which contained a relatively high content of gluconapin, the meals had glucosinolate levels comparable to those of commercial meals from yellow- and brown-seeded canola. Except for *B. rapa* cv. Parkland, all cultivars had significantly higher protein contents than the commercial meals. Sucrose content varied from 6.2 to 8.0% with yellow-seeded cultivars having slightly higher values than the brown-seeded cultivars. The content of oligosaccharides ranged from 2.6 to 3.1% with no notable differences between yellow- and brown-seeded samples. Dietary fibre varied only to a limited extent with the yellow-seeded *B. napus* and *B. juncea* samples having significantly lower values. The levels of amino acids reflected differences in protein content and, when calculated as percentage of crude protein, there were no major differences among meals. Results of in vitro protein digestibility showed minor variations among meals and confirmed moist heat treatment at 108 + 1°C for 20 minutes to be optimal for processing of canola seed. Availability of amino acids averaged 84.1% with only minor differences among samples. True metabolizable energy content was highest in the yellow-seeded *B. napus* sample. There

were no differences in weight gain of broiler chickens fed the commercial or laboratory prepared *Brassica rapa* and the yellow- and brown-seeded *B. napus* meals. Chickens fed *B. juncea* meal showed significantly lower feed intake and body weight gain which appeared to be attributed to a relatively high content of aliphatic glucosinolates in this meal. Birds fed the yellow-seeded *B. napus* canola showed the highest feed efficiency value.

Key words: Yellow-seeded canola, chemical composition, nutritive value

5.1. INTRODUCTION

Recent evaluation of the meals derived from a number of strains/cultivars of *Brassica* showed some positive quality characteristics for the yellow-seeded type of canola/rapeseed (Slominski *et al.*, 1994; Simbaya *et al.*, 1995). In comparison to brown-seeded, yellow-seeded canola was found to contain more sucrose and protein, less dietary fibre and similar amounts of oligosaccharides, minerals and residual fat. The dietary fibre was negatively correlated with protein content which indicated the potential for further quality improvements within the yellow-seeded type of canola. Detailed evaluation of *Brassica* seed meals, reported in part I of this publication, showed only minor differences between the two types of canola with regard to contents of digestible protein, soluble dietary fibre or soluble phenolics. Due to the small size of the samples,

the improved nutrient content in newly selected strains/varieties of canola has not been substantiated in practical feeding trials. However, detailed chemical composition of *Brassica* seed meals assisted in ranking the samples and, along with the agronomic characteristics, served to select the most promising cultivars for further *in vivo* evaluation. Therefore, the objective of this investigation was to determine the nutritive value of yellow- and brown-seeded *Brassica* seed meals by using the TMEn assay and broiler feeding trial. Since for high quality meal, the processing conditions were found to be as essential as nutrient content *per se,* the crushing of experimental meals was performed in accordance with the optimal parameters established and reported in part I of this study.

5.2. EXPERIMENTAL

5.2.1. Materials

Brassica seed samples consisting of yellow-seeded *B. rapa* (cv. Parkland), *B. napus* (cv. Y1016), *B. juncea* (cv. J4316) and brown-seeded *B. napus* (cv. Excel) were provided by Agriculture Canada Research Station, Saskatoon, Saskatchewan. The seeds (21 kg) were crushed to pass through a 2 mm sieve and were extracted with *n*-hexane for 8 hrs in a Kontes Macro Soxhlet apparatus (Fisher Scientific, Edmonton, Alberta, Canada). Following drying under a fume-hood at room temperature, the meals were re-ground to pass through a 1 mm sieve and were re-extracted with *n*-hexane for an

additional 8 hrs. The dried meals were moist heat treated at 108 ± 1°C for 20 minutes in a laboratory cyclomatic thermal sterilizer (American Sterilizer Co., Brampton, Ontario, Canada). To achieve the desired temperature, the "steam to jacket" valve was kept closed and the time was monitored after attaining the set temperature inside the meal. The meals were dried overnight at 40°C. Two commercial meals from the yellow-seeded variety Parkland (*B. rapa)* and from a mixture of brown-seeded varieties grown in Western Canada were obtained from a local crushing plant (Altona, Manitoba, Canada).

5.2.2. Chemical Analyses

The meals were analyzed in duplicate for dry matter, crude protein (Kjeldahl N x 6.25), ash, and ether extract using established methods of analysis (AOAC, 1990). Amino acids were analyzed by ion-exchange chromatography with the aid of a LKB Biochrom 4151 Alpha Plus amino acid analyzer (Biochrom, Science Park, Cambridge, U.K.) following hydrolysis of the samples with 6 N HCl at 110°C for 24 h (Andrews and Baldar, 1985). Methionine and cystine were determined according to the procedure of Moore (1963). Glucosinolates were analyzed using the method of Thies (1977) with some modifications (Slominski and Campbell, 1987). Sucrose, oligosaccharides (raffinose and stachyose) and dietary fibre with its components were determined according to the procedures described in detail by Slominski *et al.* (1994).

Digestible protein content in *Brassica* seed meals was determined *in vitro* using the digestion/dialysis unit and the procedure described in Part I (Manuscript two) of this study.

5.2.3. Animal Experiments

True metabolizable energy (TMEn) and amino acid availabilities were determined using the assay described by Sibbald (1986) with some modifications (Zhang *et al.*, 1994). Briefly, each *Brassica* seed meal sample was precision-fed (25 g per bird) to a group of 12 mature single comb white leghorn cockerels (45 weeks old) following a 28 hrs fasting period. During the next 48 hrs, the excreta from each bird was collected. The excreta samples were then frozen, freeze-dried, ground to pass through a 1 mm sieve and analyzed for gross energy, nitrogen (Kjeldahl) and amino acid content. Gross energy was determined with the aid of an adiabatic bomb calorimeter (Parr Instrument Co. Moline, Illinois, U.S.A.). The excreta from two groups of unfed birds was used to determine the endogenous excretion of nitrogen and amino acids.

The nutritive value of *Brassica* seed meals was further evaluated in a two-week feeding trial with broiler chickens. One-day old Arbor Acres broiler chicks were purchased from a local commercial hatchery and were placed in Jamesway brooder batteries where they had free access to water and a commercial chick starter diet. On day 4, the birds were fasted for 4 hrs before being individually weighed and placed in five narrow-weight classes. Groups of five birds were then assigned to pens in Petersime brooder batteries in such a way that all pens had a similar initial weight. Each treatment

was randomly assigned to 7 replicates. From day 4 to day 18, the birds were fed experimental diets. The birds had free access to water, feed and light throughout the experiment. Prior to the 18-day weighings, the birds were fasted for 4 h. Feed consumption was recorded at the end of the experiment to calculate feed-to-gain rations.

In formulation of the experimental diets, a concept of evaluating the biological value of protein along with the overall quality of the meal was followed. Therefore, the inclusion rate of wheat in the experimental diets was kept constant at 54% and the contribution of *Brassica* seed meals to the total dietary protein content of 22% was from 30 to 34% (Table 13). No amino acids were added to the experimental diets and the fat (energy) supplement was kept constant so as to reflect the differences in TMEn contents. Two diets containing the commercial meal from yellow-seeded canola variety Parkland served as controls each with and without supplemental lysine and methionine. The commercial brown-seeded canola was excluded from the broiler trial since the protein content was too low to formulate a ration comparable to the other meals.

5.2.4. Statistical Analysis

All data were subjected to analysis of variance (ANOVA) using the general linear models of statistical analysis system (SAS Institute, 1985) and the procedures of Snedecor and Cochran (1980). Duncan's multiple range test was used to compare and separate treatment means (Duncan, 1955).

5.3. RESULTS AND DISCUSSIONS

Total glucosinolate content in all *Brassica* seed meals was well below the 30 μmol g^{-1} meal upper limit and consequently all meals qualified for canola status (Table 14). The glucosinolate profiles of the laboratory prepared meals were comparable to those of the commercial meals with the exception of *B. juncea* cv. J4316 which showed a relatively high proportion of 3-butenyl (gluconapin) and allyl (sinigrin) glucosinolates. The indole glucosinolate contents in laboratory prepared *B. rapa* and *B. napus* meals was almost identical with the levels present in the commercial meals obtained from the same species. It is well known that indole glucosinolates are susceptible to thermal degradation during processing (Campbell and Slominski, 1990) and in this context the degree of degradation in the laboratory processed samples was similar to that of commercially crushed canola. This indicates that the heat applied for the processing of laboratory meals was comparable with that used in the commercial crushing operation.

Crude protein, carbohydrate, dietary fibre, minerals and fat contents of *Brassica* seed meals are shown in Table 15. The high protein content in the meals derived from *B. napus* cv. Y1016 and cv. Excel, and *B. juncea* cv. J4316 is in agreement with our earlier data and was one of the quality characteristics upon which the samples were selected for this study. The protein content in the laboratory prepared meal from *B. rapa* cv. Parkland was similar to that of the commercial meal obtained from the same variety. The commercial meal from brown-seeded canola had the lowest protein content. In a survey of chemical composition of commercial meals produced in Western Canadian

crushing plants, Bell and Keith (1991) reported crude protein to vary from 38.0 to 43.5% (dry matter basis). Such variation was mostly attributed to changes in soil and seasonal environmental conditions. Variation in crude protein content due to varietal differences was indicated by Finlayson *et al.* (1974), and similarly, Goh *et al.* (1980) reported crude protein content to vary from 36.2 to 40.0% (as is basis) in different canola/rapeseed cultivars. It is of particular interest to note that the level of crude protein in the commercial canola meal has been on a gradual decrease in the last few decades (Bell, 1993a) which may be attributed to the on going selection of canola varieties towards high oil content in the seed.

The sucrose content in *Brassica* seed meals ranged from 6.2 to 8.0% and is comparable with that reported by Theander and Åman (1976) for rapeseed (*B. napus*) and turnip (*B. rapa*) meals. The current results are also in agreement with those reported by Slominski *et al.* (1994a) for commercial meals from yellow- (8.7%) and brown-seeded (6.7%) canola. The yellow-seeded *B. napus* and *B. rapa* meals were found to have the highest sucrose content and with the exception of *B. juncea* cv. J4316, the yellow-seeded samples had more sucrose than brown-seeded cultivars. The contents of oligosaccharides was similar for all samples with only minor differences between yellow- and brown-seeded canola.

There was some variation in dietary fibre content among the samples with yellow-seeded *B. napus* cv. Y1016 showing significantly lower values. Small differences in the total fibre content between brown-seeded *B. napus* (33.0%) and yellow-seeded *B. rapa* canola (32.1%) is in agreement with earlier reports from this laboratory (Slominski *et*

al., 1994a). In this regard, a higher content of NSP at the expense of other fibre components (ie., lignin and polyphenols, cell wall protein and minerals) appears responsible for small differences in the fibre content between yellow-seeded *B. rapa* and brown-seeded *B. napus* canola. The yellow-seeded *B. napus* cv. Y1016, however, showed lower NSP content than its brown-seeded counterpart. The *B. juncea* sample showed a similar high content of NSP to *B. rapa,* which may be indicative of the smaller size of the cells in the cotyledons. This was further substantiated by similar contents of glucose and uronic acids (Table 16) in the NSP profiles of *B. rapa* and *B. juncea* as both sugars are known to be the major components of the cell wall polysaccharides (Slominski and Campbell, 1990).

The ash content was highest in the two commercial meals and within the range commonly found in commercial canola meal (Bell and Keith 1987; Bell *et al.*, 1991). The high ash content in the commercial meals may reflect the presence of various admixtures (i.e. sand, dust particles and other foreign materials) common for the crushing operation of canola seed (Unger, 1990) rather than the mineral content *per se*. Fat levels varied among the samples and the amounts found in the commercial meals were a reflection of gums being added back to the meal following oil refining (Bell, 1984) while in the laboratory prepared meals the fat levels were an indication of incomplete oil extraction.

To eliminate the influence of crude protein when evaluating protein quality of the meals, the amino acid composition was expressed in grams per 16 grams of total nitrogen (Table 17). The results on individual amino acids showed no statistical differences

among meals with regard to valine, methionine, isoleucine, leucine and histidine contents. The composition of other amino acids, however, differed variably among meals with no defined pattern. A closer look at individual amino acids indicated a trend toward lower amino acid values in *B. juncea* and the brown-seeded commercial meal which may be responsible for the numerically lower total amino acid content in these meals.

The results of in vitro protein digestibility are shown in Table 18 and indicate no major differences among the samples. In general, the digestible protein content was slightly higher than that reported in part I of this study. Differences in the results of the two studies may be explained by variations in the processing conditions, especially that samples utilised in this study were moist heat treated at 108 $\pm$ 1°C for 20 minutes, the conditions established as optimal for improved meal quality. Since the total protein content was significantly higher for *B. napus* (cv. Excel and Y1016) and *B. juncea* (cv. J4316) canola, the digestible protein, as expressed on a meal dry matter basis, was significantly higher for the two *B. napus* samples, regardless of the seed coat colour, and for the *B. juncea* sample. On the same basis, the true available total amino acid contents were also highest for the three samples with a less pronounced difference between the *B. juncea* sample, the two commercial meals and the *B. rapa* sample. The true total amino acid availabilities, as expressed in % of total protein content, ranged from 82.2 to 86.0% with a mean value of 84.2%. On average, these values were slightly higher than the true amino acid availability figures reported by Zuprizal *et al.* (1991b) for oil free meal, ranging from 73.1 to 96.7% with an average of 80.1%. On the other hand,

the results of the current study were considerably lower than those of Barbour and Sim (1991) who reported true available amino acid content ranging from 79 to 98% with a mean value of 91%. The results on true availability of individual amino acids (Table 19) corroborate those of amino acid composition since they also indicate only minor differences among meals in the true availability of most amino acids. The relatively high digestible protein and available amino acid contents in the *B. juncea* sample do not confirm the poor protein digestibility in this species as earlier determined at the terminal ileum of the pig by Bell (1994, personal communication). In contrast, the results of this study indicate the potential usefulness of *B. juncea* as a protein source for monogastric animals. As, to date, *B. juncea* is not available as a commercial meal, the low quality meal reported by Bell would have been a laboratory-prepared meal and as such could have been processed under the mild heat treatment conditions.

In general, the true metabolizable energy (TMEn) values for laboratory prepared meals were higher than those for the commercial meals with the yellow-seeded *B. napus* sample showing the highest energy content (Table 18). Among the commercial meals, the yellow-seeded sample had a significantly higher TMEn value. This is in contrast to our earlier report which indicated the yellow- and brown-seeded commercial meals to have similar TMEn contents (9.71 vs 9.57 Mj kg^{-1}) (Campbell *et al.*, 1995). The current results for the two commercial meals, however, were higher than the 8.22 Mj kg^{-1} value reported by Barbour and Sim (1991). Differences in the processing of canola seed and more specifically the extent of oil extraction may be in part responsible for such variation. In the current study, however, the residual oil content in the laboratory

prepared yellow-seeded *B. napus* cv.Y1016 was lower than in its brown-seeded counterpart and yet the available energy content was significantly higher. Since the protein contents in both *B. napus* samples were identical, the higher sucrose content and more digestible fibre may explain the improved energy availability for this particular meal.

The results of the two-week performance of broiler chickens fed *Brassica* seed meal-based diets are shown in Fig. 20. There were no significant differences in the weight gain of broiler chickens fed the commercial yellow-seeded canola (Control B), *B. rapa* meal cv. Parkland and two *B. napus* samples cv. Y1016 and cv. Excel. There was, however, a significant reduction in the body weight gain of chickens fed the *B. juncea* meal. A similar pattern was not necessarily followed by the feed-to-gain ratio, with the yellow-seeded *B. napus* cv. Y1016 canola showing the best feed efficiency value. This was followed by *B. rapa* cv. Parkland, with the *B. juncea* sample showing a similar feed-to-gain ratio to that of the brown-seeded *B. napus* canola or the control meal without supplemental amino acids. Since in this study the *B. juncea* meal was found to be high in protein and showed a good protein digestibility value and true available energy and amino acid contents, the only factor related to the poor performance of broiler chickens appeared to be a relatively high content of aliphatic glucosinolates in this particular meal. The chickens fed the commercial meal from yellow-seeded *B. rapa* canola supplemented with lysine and methionine (Control A) had a significantly higher weight gain and feed-to-gain ratio than broilers fed the same meal without supplemental amino acids (Control B). This is in support of earlier findings which demonstrated the amino acid imbalance

being responsible for the overall poor performance of chickens fed canola meal-based diets (Leslie and Summers, 1975; Leslie *et al.*, 1976). The use of the two control diets also served to demonstrate that the experimental design effectively reflected differences in diet quality.

TABLE 13. Composition and calculated analyses of *Brassica* seed meal diets used in the broiler chicken growth trial (4-18 days).

Ingredients (%)	Diet					
	Commercial meal "yellow" control A	Commercial meal "yellow" Control B	*B. rapa* "yellow" cv.Parkland	*B.napus* "brown" cv.Excel	*B. napus* "yellow" cv.Y1016	*B. juncea* "yellow" cv.J4316
Wheat	55.80	54.00	54.00	54.00	54.00	54.00
Brassica meal	32.00	33.70	33.70	29.70	29.50	30.10
Vegetable oil	7.65	7.90	7.90	6.30	6.20	6.50
Limestone	1.41	1.40	1.40	1.45	1.45	1.45
Biophosphate	1.20	1.15	1.15	1.25	1.25	1.23
Vitamin premix[1]	1.00	1.00	1.00	1.00	1.00	1.00
Mineral premix[2]	0.50	0.50	0.50	0.50	0.50	0.50
Starch	-	0.35	0.35	5.80	6.10	5.22
Lysine	0.37	-	-	-	-	-
DL-methionine	0.07	-	-	-	-	-
Total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
Calculated composition						
Energy, Mj kg^{-1}	12.97	12.97	13.15	13.10	13.26	13.08
Protein, %	22.00	22.00	22.00	22.00	22.00	22.00
Lysine, %	1.11	0.91	0.89	0.89	0.97	0.88
Methionine, %	0.50	0.43	0.40	0.40	0.37	0.42
Cystine, %	0.46	0.47	0.46	0.51	0.45	0.49
Calcium, %	1.00	1.00	1.00	1.01	1.00	1.00
Available P., %	0.45	0.45	0.45	0.45	0.45	0.45

[1] Provided per kg diet: vitamin A, 8250 IU; vitamin D_3, 1000IU; vitamin E, 11 IU; vitamin B1, 0.012 mg; vitamin K, 1.1 mg; niacin, 53 mg; choline, 1020 mg; folic acid, 0.75 mg; biotin, 0.25 mg; riboflavin, 5.5 mg.

[2] Provided per kg diet: manganese, 55 mg; zinc, 50 mg; iron, 80 mg; copper, 5 mg; selenium, 0.1 mg; iodine, 0.36 mg and sodium, 1.6g.

TABLE 14. Glucosinolate content of *Brassica* seed meals (μmol g^{-1} dry matter).

Glucosinolate	*Brassica* seed meal					
	Commercial meal "yellow"	*B. rapa* "yellow" cv.Parkland	Commercial meal "brown"	*B. napus* "brown" cv.Excel"	*B. napus* "yellow" cv.Y1016	*B. juncea* "yellow" cv.J4316
allyl	0.1[1]	nd[2]	nd	nd	nd	1.2
3-butenyl	3.0	3.0	2.3	2.3	1.8	16.1
4-pentenyl	1.8	2.7	0.5	0.5	0.5	1.2
2-OH-3-butenyl	4.9	5.5	4.8	4.6	4.6	1.8
2-OH-4-pentenyl	0.7	0.9	0.2	0.1	0.2	0.1
3-indolylmethyl	nd	nd	0.6	0.3	0.5	nd
4-OH-indolylmethyl	2.3	2.3	4.2	3.5	3.8	1.3
4-OH-benzyl	5.8	nd	2.0	nd	nd	nd
Total[3]	18.6[b]	14.5[c]	14.6[c]	11.4[d]	11.4[d]	21.7[a]

[1] Mean of duplicate determinations; [2] Not detected; [3] Means with different superscripts are significantly different ($P \leq 0.05$).

TABLE 15. Chemical composition of *Brassica* seed meals (% of dry matter).

Component	*Brassica* seed meal					
	Commercial meal "yellow"	*B. rapa* "yellow" cv.Parkland	Commercial meal "brown"	*B. napus* "brown" cv.Excel	*B. napus* "yellow" cv.Y1016	*B. juncea* "yellow" cv.J4316
Crude protein	41.0^{b}	40.2^{bc}	38.4^{c}	46.1^{a}	46.1^{a}	45.3^{a}
Sucrose	7.9^{a}	7.4^{b}	7.0^{c}	6.2^{e}	8.0^{a}	6.6^{d}
Oligosaccharides	3.1^{a}	3.0^{ab}	2.8b^{c}	2.8^{bc}	2.9^{ab}	2.6^{c}
Dietary fibre						
NSP[1]	22.0^{a}	21.8^{a}	17.8^{bc}	16.9^{c}	19.1^{b}	21.2^{a}
Other[2]	9.4^{c}	10.3^{bc}	15.1^{a}	16.1^{a}	10.2^{bc}	10.7^{b}
Ash	8.0^{b}	7.2^{c}	8.4^{a}	6.6^{d}	7.2^{c}	7.3^{c}
Fat	3.2^{c}	3.9^{b}	4.0^{b}	5.3^{a}	4.0^{b}	3.2^{c}
Total[3]	90.0^{c}	89.8^{c}	89.0^{c}	94.7^{a}	92.8^{b}	92.6^{b}

[1] Non-starch polysaccharides; [2] Includes lignin, polyphenols, cell wall protein and minerals present in the neutral detergent fibre residue; [3] Corrected for cell wall protein and minerals present in the dietary fibre fraction. [a-d] Means with different superscripts within rows are significantly different ($P \leq 0.05$).

TABLE 16. Non-starch polysaccharide profiles of *Brassica* seed meals (mg g^{-1} meal).

Component sugar	Brassica seed meals					
	Commercial meal "yellow"	*B. rapa* "yellow" cv.Parkland	Commercial meal "yellow"	*B. napus* "brown" cv. Excel	*B. napus* "yellow" cv.Y1016	*B. juncea* "yellow" cv.J4316
Rhamnose	1.7^{ab}	1.53^{b}	1.48^{b}	1.40^{b}	1.40^{b}	1.80^{a}
Fucose	2.7^{a}	2.53^{a}	2.11^{b}	2.05^{b}	1.99^{b}	1.81^{b}
Arabinose	45.8^{a}	45.60^{a}	45.05^{a}	37.30^{b}	40.05^{b}	45.30^{a}
Xylose	20.8^{a}	18.15^{ab}	16.73^{bc}	15.85^{bc}	13.78^{c}	14.93^{c}
Mannose	5.0^{a}	4.30^{b}	4.10^{bc}	3.68^{d}	3.48^{d}	3.80^{cd}
Galactose	18.9^{a}	17.48^{b}	15.63^{c}	15.03^{c}	14.80^{c}	18.43^{a}
Glucose	65.9^{a}	64.83^{ab}	56.58^{bc}	52.30^{c}	47.97^{c}	66.35^{a}
Uronic acids	59.0^{ab}	63.45^{a}	48.92^{c}	51.62^{bc}	45.10^{c}	59.26^{ab}

[a-d] Means with different superscripts within rows are significantly different ($P \leq 0.05$).

TABLE 17. Amino acid composition of *Brassica* seed meal (g 16 g N^{-1}).

Amino acid	Brassica seed meal					
	Commercial meal "yellow"	*B. rapa* "yellow" cv.Parkland	Commercial meal "brown"	*B. napus* "brown" cv.Excel	*B.napus* "yellow" cv.Y1016	*B. juncea* "yellow" cv.J4316
Aspartic acid	6.76^{a}	6.83^{a}	6.10^{c}	6.84^{a}	6.66^{a}	6.37^{b}
Threonine	3.53^{a}	3.58^{a}	3.37^{ab}	3.52^{a}	3.39^{ab}	3.25^{b}
Serine	4.78^{bc}	4.87^{ab}	4.69^{c}	4.89^{a}	4.75^{c}	4.56^{d}
Glutamic acid	14.24^{c}	14.82^{ab}	14.61^{b}	15.02^{a}	14.04^{c}	14.19^{c}
Proline	5.83^{b}	6.09^{ab}	6.22^{a}	5.82^{b}	5.99^{ab}	5.83^{b}
Glycine	4.65^{ab}	4.72^{a}	4.38^{c}	4.60^{b}	4.58^{b}	4.44^{c}
Alanine	4.23^{b}	4.30^{ab}	4.22^{b}	4.34^{a}	4.24^{ab}	4.04^{c}
Cystine	2.09^{d}	2.16^{cd}	2.47^{a}	2.26^{bc}	2.29^{b}	2.32^{b}
Valine	3.21	3.27	3.20	3.38	3.16	3.00
Methionine	1.88	2.04	1.90	1.91	2.04	1.78
Isoleucine	2.47	2.38	2.51	2.57	2.46	2.46
Leucine	5.90	6.02	5.83	6.05	5.88	5.71
Tyrosine	3.04^{a}	3.12^{a}	2.98^{ab}	3.04^{a}	3.04^{a}	2.85^{b}
Phenylanine	4.89^{ab}	4.95^{a}	4.65^{c}	4.80^{abc}	4.55^{c}	4.70^{abc}
Histidine	2.95	3.42	3.00	3.46	2.65	2.96
Lysine	4.83^{abc}	4.97^{a}	4.76^{bc}	4.95^{ab}	4.65^{c}	4.39^{d}
Arginine	5.05^{c}	5.11^{bc}	5.03^{c}	5.45^{ab}	5.25^{ab}	5.60^{a}
Total	80.56	83.83	79.89	80.20	79.59	78.44

$^{a-d}$ Means with different superscripts within rows are significantly different ($P \leq 0.05$).

TABLE 18. Digestible protein and true available amino acids and metabolizable energy contents of *Brassica* seed meals.

Item	*Brassica* seed meals					
	Commercial meal "yellow"	*B. rapa* "yellow" cv.Parkland	Commercial meal "brown"	*B. napus* "brown" cv.Excel	*B. napus* "yellow" cv.Y1016	*B. juncea* "yellow" cv.J4316
Digestible protein[1]						
- % of total	75.8	73.8	73.5	76.5	73.4	75.8
- % of dry matter	33.5^{b}	31.4^{c}	31.6^{c}	36.6^{a}	36.7^{a}	36.6^{a}
Available amino acids[2]						
- % of total	84.6^{abc}	82.2^{d}	83.2^{cd}	86.0^{a}	84.8^{ab}	84.0^{bc}
- % of dry matter	30.1^{c}	29.3^{e}	28.7^{f}	33.6^{a}	33.0^{ab}	31.6^{c}
Metabolizable energy[2]						
- Mj kg^{-1}	8.83^{c}	9.26^{b}	8.50^{d}	9.18^{b}	9.71^{a}	9.01^{bc}

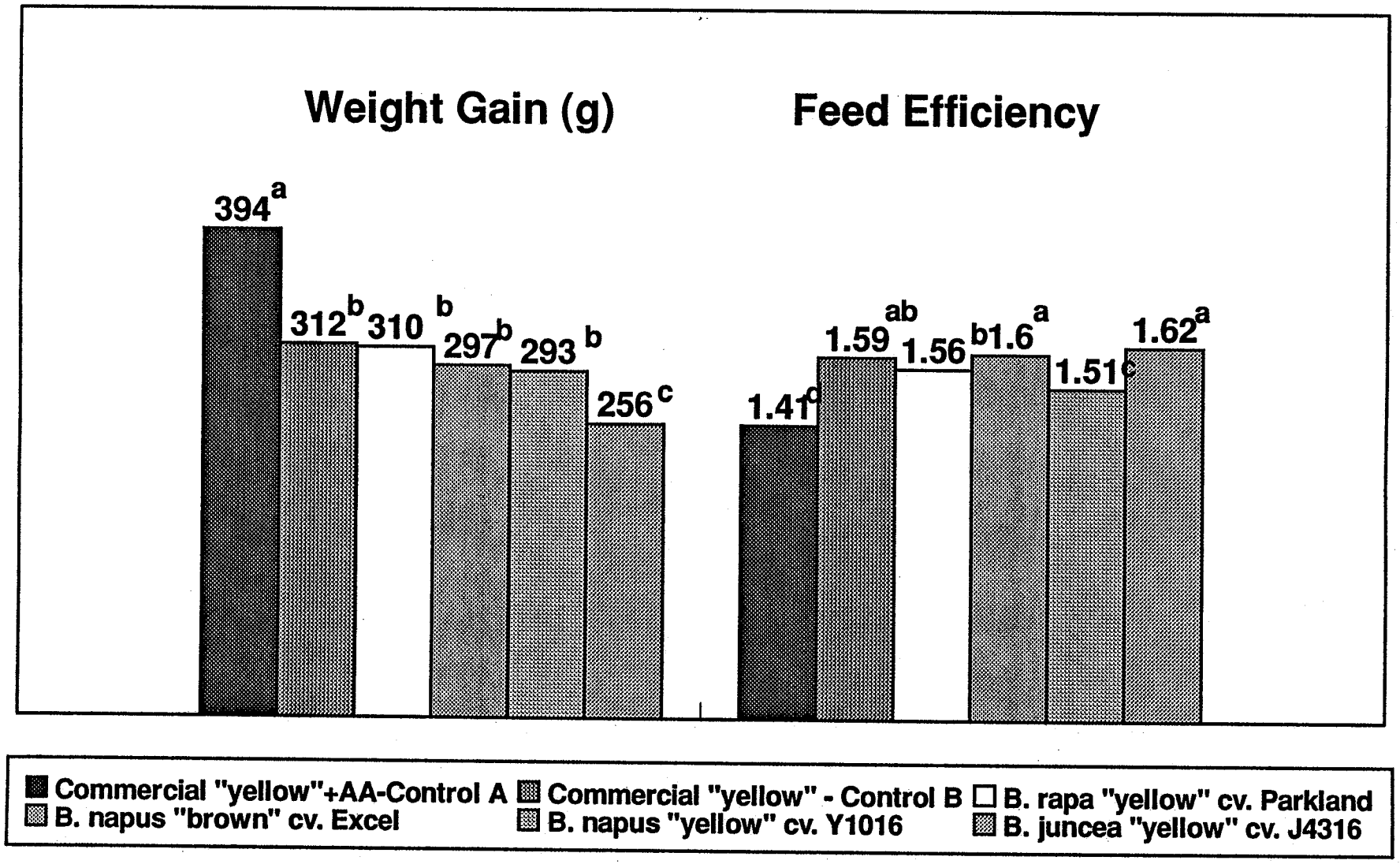
1 Determined *in vitro;* 2 Determined *in vivo.*

TABLE 19. True amino acid availabilities of *Brassica* seed meals (%)

Amino acid	Brassica seed meal					
	Commercial meal "yellow"	*B. rapa* "yellow" cv.Parkland	Commercial meal "brown"	*B. napus* "brown" cv.Excel	*B. napus* "yellow" cv.Y1016	*B. juncea* "yellow" cv.J4316
Aspartic acid	80.09	82.48	82.69	81.85	82.59	80.57
Threonine	79.41	77.80	79.88	78.24	80.25	77.79
Serine	80.08^{ab}	78.51^{b}	81.80^{a}	79.78^{ab}	81.72^{a}	79.32^{b}
Glutamic acid	90.36	89.59	90.87	89.91	90.85	89.91
Proline	81.30	84.08	82.33	83.33	85.34	84.25
Glycine	81.83^{ab}	80.93^{b}	82.98^{ab}	81.46^{b}	84.69^{a}	80.01^{b}
Alanine	85.82^{ab}	84.16^{b}	86.06^{ab}	85.81^{ab}	87.32^{a}	84.40^{ab}
Cystine	73.47^{b}	74.76^{b}	76.99^{ab}	73.55^{b}	81.98^{a}	76.78^{ab}
Valine	81.85^{b}	78.43^{b}	81.18^{b}	80.26^{b}	85.80^{a}	78.69^{b}
Methionine	96.11	95.44	95.33	97.53	95.86	97.22
Isoleucine	85.62^{b}	81.30^{c}	84.67^{b}	83.47^{cd}	89.03^{a}	83.25^{bc}
Leucine	88.28^{ab}	84.44^{e}	87.35^{bc}	86.43^{cd}	89.03^{a}	85.62^{d}
Tyrosine	83.71	82.29	82.95	80.77	84.26	83.55
Phenylanine	88.78^{a}	85.79^{c}	87.48^{abc}	86.18^{bc}	88.17^{abc}	88.42^{ab}
Histidine	81.21	81.96	75.84	70.27	72.13	79.66
Lysine	77.67^{ab}	78.18^{ab}	78.81^{ab}	76.23^{b}	80.75^{a}	77.32^{ab}
Arginine	88.01	86.92	88.92	82.82	88.97	87.48

[1]Total true available amino acid values were as shown in Table 18. $^{a-d}$Means with different superscripts within rows are significantly different (P $\pm$ 0.05).

FIGURE 20. Overall weight gain and feed efficiency of broiler chickens fed wheat/*Brassica* seed meal based diets from 4-18 days of age.


Weight Gain (g)
Feed Efficiency
394a
312b
310b
297b
293b
256c
1.41c
1.59ab
1.56
1.6a
1.51c
1.62a
Commercial "yellow"+AA-Control A
Commercial "yellow" - Control B
B. rapa "yellow" cv. Parkland
B. napus "brown" cv. Excel
B. napus "yellow" cv. Y1016
B. juncea "yellow" cv. J4316

6. MANUSCRIPT FOUR

The Effects of Protease and Carbohydrase Supplementation on the Nutritive Value of Canola Meal for Poultry: *in Vitro* and *in Vivo* Studies.

ABSTRACT

An *in vitro* incubation system was used to assess a variety of enzyme preparations regarding activities toward protein and carbohydrate components in canola meal with the objective of identifying those preparations that possessed potential for improvement of the nutritive value of canola meal. Promising preparations were evaluated further in 2-week growth trials utilizing 4-day old broiler chickens. Enhanced protein hydrolysis was demonstrated for several of the protease enzyme preparations studied in the *in vitro* incubation system. Protein hydrolysis was most effective when either pancreatin or pronase were included in the incubation medium along with the protease enzyme and for the most effective protease preparations values for percent of total protein hydrolysed exceeded those for pancreatin or pronase acting alone. The effective protease preparations also resulted in improved broiler chick growth performance when added to semi-purified or conventional canola meal diets. While some carbohydrase enzyme preparations were shown to be effective in the *in vitro* test of cell wall polysaccharide solubilization, only a trend toward improved growth performance was noted when broiler chickens were fed semi-purified canola meal diets containing these enzyme preparations. A synergistic response in growth of young (4-11 days of age) broiler chickens was noted when phytase, carbohydrase and protease enzymes were added to a wheat/canola meal-based diet deficient in available phosphorus.

Key words: Canola meal, protease and carbohydrase enzymes, protein digestibility, dietary fibre.

6.1. INTRODUCTION

The unrestricted use of canola meal as a high quality protein supplement in poultry and other rapidly growing monogastric animals is limited by low available energy and protein content. The metabolizable energy content of canola meal ranges from 2000 kcal/kg for poultry to 2400 and 2700 kcal/kg, respectively for ruminants and swine and, on average, is 280 kcal/kg lower (as fed basis) than that of 44% soybean meal. Excreta apparent protein digestibility in canola meal based diets averages 74% in poultry (Thomke *et al.*, 1983; Bell and Keith, 1987; Zuprizal *et al.*, 1991) and 78% in growing pigs (Bell et al., 1991; Sauer *et al.*, 1991).

Inversely related to metabolizable energy and protein contents are fibre components of canola meal which include cellulose (4-6%), non-cellulosic polysaccharides (13-16%), lignin with associated polyphenols (8%), and protein (3.5%) and minerals (1%) associated with the cell walls (Slominski and Campbell, 1990; Slominski *et al.*, 1994). Selection for a yellow seed coat colour, dehulling of canola seed or the use of exogenous enzymes as feed additives are among recent approaches undertaken in an attempt to reduce the fibre content, increase the protein content and improve nutrient utilization.

Application of cell wall degrading enzymes has been proven beneficial in improving digestibility of canola polysaccharides in poultry (Slominski and Campbell, 1990) and in facilitating the extraction of canola oil in the aqueous extraction process (Olsen, 1987; Sosulski *et al.*, 1988). It is believed that the use of carbohydrase enzymes

in animal nutrition would reduce the nutrient encapsulating effect of the cell walls (Bedford and Classen, 1992; Graham and Pettersson, 1992) and could further increase the nutritive value of feedstuffs by rendering the cell wall polysaccharides available for hindgut fermentation (Chesson, 1993).

Although the use of supplementary enzymes in animal nutrition has increased considerably in recent years, most of the investigations on this subject have been on cereal based diets, where bacterial and fungal enzyme preparations are used to alleviate mixed-linked β-glucan and arabinoxylan anti-nutritive effects (Petterson and Åman, 1989; Classen *et al.*, 1991; Campbell and Bedford, 1992). The use of phytase preparations has also increased in recent years in an attempt to reduce the negative effects of phytic acid and to improve the availability of phosphorus and to reduce phosphorus excretion (Chesson, 1993). Some consideration has also been given to the application of α-galactosidase (Slominski, 1994). The use of proteases to further improve the productive value of feedstuffs by complementing the animal's own digestive system and to hydrolyse certain types of protein which are resistant to endogenous secretory enzymes has also been proposed (Inborr, 1990; Classen *et al.*, 1991).

The objective of this study was to evaluate a wide range of enzymes for their ability to hydrolyse canola meal protein and carbohydrates *in vitro* and to explore the potential for improving the nutritive worth of the meal by supplementing broiler chicken diets with the most active preparations. The effect of protease and carbohydrase enzymes, acting in concert with phytase, was also investigated.

6.2. MATERIALS AND METHODS

6.2.1. Materials

Commercial protease preparations A, B, D, E, F, G, H, J, K, L, M, N and O were obtained from a number of different sources including Finnfeeds International Ltd., Marlborough, U.K., Novo Industri A/S, Bagsvaerd, Denmark, Enzyme Development Corp., New York, U.S.A. and Alltech Inc., Nicholasville, KY, U.S.A. A pancreatin preparation (P-1750) from porcine pancreas and pronase (P-6911) from *Streptomyces griseus* were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A. and were used as reference proteases. Eight carbohydrase preparations (Carbohydrases A to H) were obtained from Finnfeeds International Ltd., Marlborough, U.K. Energex, a commercial carbohydrase from Novo Industri A/S, Bagsvaerd, Denmark, was used as a reference preparation in the *in vitro* evaluation of cell wall degrading enzymes. Natuphos, a phytase preparation was supplied by Gist-brocades, Delft, Holland. Xylanase premix from Finnfeeds International Ltd contained xylanase from *Trichoderma longibrachiatum*. Samples of conventional canola meal were obtained from a local crushing plant (CanAmera Foods, Altona, MB, Canada).

6.2.2. In vitro evaluation of protease enzymes

The evaluation involved the incubation of canola meal with an enzyme preparation in the presence or absence of pancreatin or pronase enzymes.

In the standard assay procedure, 5 g of canola meal was incubated with 25 mg of the protease under study in 150 ml of phosphate buffer (0.1 M, pH 7.0) containing 0.02% sodium azide. The assay was performed under continuous mixing in a controlled-environment shaker (New Brunswick Scientific Co. Edison, NJ, U.S.A.) for 6 h at 40°C. At the end of incubation period, the samples were centrifuged at 7000 x g for 30 minutes at 2°C. The supernatants were decanted and filtered. The meal residues were then washed with 50 ml of distilled water and the contents were centrifuged again. The supernatants were pooled and placed in pre-soaked dialysis tubes (Spectrum, Houston, TX, U.S.A.) with a molecular weight cut-off value of 12000 - 14000. The sealed membrane tubes were subjected to dialysis against distilled water for 72 h at 2°C on continuous stirring. The water was changed nine times at regular intervals. The meal residues and the retentates from the dialysis tubes were then frozen, freeze-dried and analyzed for crude protein using the Kjeldahl procedure (Procedure 7.021) as described in Association of Official Analytical Chemists (1990). *In vitro* protein hydrolysis was calculated by subtracting the amount of protein remaining in the meal residue and the dialysis tube (retentate) from that present in the original meal.

Additional evaluation involved incubation of canola meal with an appropriate commercial protease in the presence of pancreatin or pronase added to the incubation medium at 5 mg/g of meal. The inclusion rates were justified based on preliminary studies in which both pancreatin and pronase showed maximal hydrolysis of canola protein under the assay conditions described above.

6.2.3. In vitro evaluation of carbohydrase enzymes

The evaluation involved the incubation of canola meal with carbohydrase enzyme preparation in the presence of pancreatin and the determination of the total water-soluble carbohydrate content of the final incubation medium with and without added carbohydrase. Pancreatin was included in the assay to facilitate polysaccharide solubilization as recommended by Slominski *et al.* (1993).

In the assay, 5 g of canola meal was incubated for 5 h, under continuous mixing, with 25 mg of carbohydrase preparation and 25 mg of pancreatin in 100 ml of either distilled water (pH 5.3) or phosphate buffer (0.1 M, pH 7.0) at 40 °C. Following centrifugation (7000 x g, 30 min), the solutions were filtered through MSI filters (0.22 μm). Soluble carbohydrates were determined by high-performance size exclusion chromatography on a TosoHaas TSK-GEL G5000-PWLX column. The column was maintained at 40°C and eluted with either deionized water or phosphate buffer (pH 7.0, 0.1 M) at a flow rate of 0.5 ml/min. Sample volumes of 10 μl were injected onto the column through a Rheodyne injection valve. The eluate was monitored using a Shimadzu RID-6A refractive index detector and the carbohydrates were quantified using a Shimadzu C-R4A Chromatopac integrator. Pure pectin, pullulan, arabinogalactan, stachyose and sucrose (Sigma Chemical Co.) were used as reference compounds. Enzyme activity was expressed as miligrams of solubilized carbohydrate per milligram of enzyme preparation.

6.2.4. In vivo enzyme evaluation

Two-week feeding trials with 4-day-old broiler chicks were employed to study the effect of adding exogenous protease, carbohydrase and phytase enzymes on the nutritive value of canola meal diets.

One-day-old vaccinated (Marek's) male, Arbor Acres broiler chicks were obtained from a local commercial hatchery. From Day 1 to 4, the birds were housed in Jamesway chick batteries and fed a commercial chick starter diet containing 20% protein. On Day 4, birds were individually weighed and placed into narrow weight classes. Groups of five birds were then assigned to pens in Petersime brooder batteries such that all pens had a similar initial weight. Each treatment was randomly assigned to 7 replicates (pens). From Day 4 to 18, the birds were fed experimental diets that were in a mash form. The birds had free access to water, feed, and light throughout the experiments. Prior to the 11- and 18-day weighings, the birds were fasted for 4 h. Feed consumption was recorded for Weeks 1 and 2 in order to calculate weekly and overall feed intakes and feed efficiency values.

In formulation of experimental diets, some consideration was given to the nitrogen-to-protein conversion factor for canola meal. The practice of calculating protein content by multiplying the nitrogen content by 6.25 is widely used in ration formulation and derives from early research on protein of animal origin known to contain around 16% nitrogen. Later work by Tkachuk (1969), however, indicated the potential for substantial overestimation of protein content in many feedstuffs when using the 6.25 factor. As the objective of the current study was to enhance the nutritive value of canola

meal by enzyme supplementation, it appeared essential to formulate the diets based on a true protein value. Thus, five different samples of canola meal were analyzed for amino acid content (Andrews and Baldar, 1985) and the values were compared with those of nitrogen content as determined by a standard Kjeldahl procedure. A nitrogen-to-protein factor of 5.83 (Table 20) was determined and this factor was used in the formulation of the canola meal diets.

6.2.4.1. Experiment 1

An experiment was conducted to determine the effect of supplemental proteases on the performance of broiler chicks fed a semi-purified canola meal-based diet containing a sub-optimal amount of total protein (Table 21, Diet 1). Based on *in vitro* data, Proteases G, D, M, N and O were selected for this trial as being the most potent preparations.

6.2.4.2. Experiment 2

An experiment was conducted to determine the effect of proteases G and M supplementation on broiler chick performance when fed either an optimal protein (22%) diet (Table 21, Diet 2) or a low protein (17%) diet (Table 21, Diet 3). Proteases G and M were chosen for this study and were incorporated into the semi-purified canola meal diets at levels of 0.02% and 0.05%, respectively.

6.2.4.3. Experiment 3

The performance of broiler chicks fed a semi-purified canola meal-based diet containing a sub-optimal amount of protein was studied in an experiment involving the use of carbohydrase enzymes. Carbohydrases G, F, and H were selected for this study since the highest activity toward canola cell wall polysaccharides was demonstrated for these enzyme preparations. The enzymes were supplemented at the 0.05% level to the same diet as that used in Experiment 1 (Table 21, Diet 1).

6.2.4.4. Experiment 4

An experiment was conducted to determine the effect of dietary protease supplementation on the performance of broiler chicks fed a practical wheat/soybean-based diet (Table 22, Diet 4) and a wheat/canola-based diet (Table 22, Diet 5). Protease G inclusion rates were 0.02% for the wheat/soybean diet, and 0.02% and 0.05% for the wheat/canola-based diets. A xylanase enzyme preparation (Finnfeeds International Inc.) was included in all diets to eliminate any potential anti-nutritive effects of the viscous polysaccharides present in wheat.

6.2.4.5. Experiment 5

The synergistic effect of enzyme preparations was studied in an experiment involving the use of both protease G and carbohydrase G preparations acting in concert with a phytase enzyme preparation. The enzymes were added to a phosphorus deficient (0.35% available P) negative control wheat/canola-based practical diet (Table 22, Diet

6). Inclusion rates for protease and carbohydrase enzymes were 0.01% and 0.05%, respectively. The dietary level of 0.01% of phytase enzyme was justified based on preliminary studies conducted in this laboratory. A wheat/canola meal-based diet containing 0.45% available phosphorus (Table 22, Diet 6) served as a positive control.

6.2.5. Statistical analysis

Data were subjected to ANOVA (SAS Institute, 1985) and treatment means separated using Duncan's multiple comparison test (Snedecor and Cochran, 1980).

6.3. RESULTS AND DISCUSSION

6.3.1. In vitro evaluation of protease enzymes

Incubation of canola meal with various commercial proteases resulted in a varying degree of protein hydrolysis (Table 23). Among the enzymes studied, Proteases G, N and O showed the highest digestibility values which were equal to or higher than that for pancreatin acting alone. Other proteases that showed some potential for *in vitro* protein hydrolysis included the preparations D, J, and M. When evaluated in combination with pancreatin, Proteases G, D, M and N exhibited the highest activities towards canola protein. The same preparations were also superior when evaluated in combination with pronase. The relatively high rate of hydrolysis observed for the latter combination was,

in part, due to a high protein hydrolysis by pronase alone. In this regard, the pronase preparation consists of a combination of exo- and endopeptidases and has been shown to effectively hydrolyse plant protein to constituent amino acids (Brillouet *et al.*, 1988). In contrast, pancreatin has been shown to be unable to hydrolyse protein to free amino acids or to di- and tripeptides due to the lack of presence in the preparation of a variety of peptidases (e.g. aminopeptidase N) (Boisen and Eggum, 1991). The lack of ability of pancreatin to completely hydrolyse protein was evident in the current study since following incubation of canola meal with pancreatin the amount of soluble protein and/or polypeptides remaining in the dialysis tube (12000-14000 MWCO) averaged 23% of the total protein content. Given that adequate peptidases are produced in the brush border intestinal cells of animals it is reasonable to assume that the soluble protein and polypeptides produced in the *in vitro* assay would be completely hydrolysed to constituent amino acids *in vivo*. With this assumption the extent of protein digestion with the pancreatin preparation becomes 67.6% (ie., 44.6% + 23.0%) and is similar to protein digestion determined *in vivo* at the ileal level (Grala *et al.*, 1993). When calculated in a similar manner the protease preparations (G, D, M, N and O) chosen for further *in vivo* study showed the highest protein digestibility values (75% on average).

6.3.2. In vitro evaluation of carbohydrase-like enzymes

Carbohydrase activities as determined by monitoring the increase in water-soluble carbohydrate content following incubation of canola meal with various enzyme preparations is shown in Table 24. Hydrolysis and/or solubilization of cell wall polysaccharides is assumed to be responsible for an increase in the soluble carbohydrate fraction. The overall low activity levels, however, are in agreement with the suggestion that cell wall degrading enzymes exert their effects by a relatively slow mechanism of surface peeling (Hotten, 1991). Thus, as indicated in our earlier research (Slominski *et al.*, 1994a), a high degree of cell wall lignification would appear to be a limiting factor to effective cell wall disruption in canola. Among the preparations tested in the current study, three carbohydrases (F, G, and H) were found to be more active than Energex, the reference carbohydrase. The latter preparation has been reported to contain a complex of cell wall degrading enzymes (Massiot *et al.*, 1989; Slominski and Campbell, 1990), and is recommended by the manufacturer for the enhancement of the nutritive value of soybean meal. In the current study, the Energex preparation showed a level of activity comparable to that determined earlier (Slominski *et al.*, 1993). Similarly to our earlier research, the carbohydrase activities were lower at pH 7.0 than at pH 5.3, which is in agreement with the fact that many fungal enzyme preparations have optimum activity at a low pH. Thus, it is uncertain to what extent the carbohydrase enzymes would exert their effects under the gastrointestinal tract environment since the residence time of digesta in such compartments as the crop, proventriculus and gizzard is relatively short (Shires *et al.*, 1987). In this regard, three of the enzymes (carbohydrases F, G and

H) with the highest activity toward cell wall polysaccharides were chosen for further testing *in vivo*.

6.3.3. In vivo enzyme evaluation

The results of the two-week performance of broiler chicks fed semi-purified canola meal-based diets supplemented with protease preparations (Experiment 1) are given in Table 25. There were no differences in chick performance between the first and second weeks of the experiment and hence, only overall data are reported. Protease supplementation had only a slight effect on feed intake with only Protease N showing a significant ($P \leq 0.05$) reduction in feed consumption as compared to Protease G (0.002%) and Protease O (0.05%). There was no significant effect of protease enzymes on body weight gain although supplementation of the diet with Protease G at 0.02% inclusion rate numerically increased body weight gain which then appeared to account for an improvement in feed efficiency associated with this enzyme. A similar, although not significant, increase in weight gain was noted for the diet supplemented with 0.05% of Protease M. Supplementation of canola meal diets with Protease G at the 0.002% dietary level tended to enhance feed intake but there was no effect on weight gain or feed efficiency.

The results of the current study are inconclusive in that some proteases showed a response and others did not. It is unclear whether the positive effects from Protease G supplementation were related to augmentation of the animal's own secretory capacity

or due to the hydrolysis of certain types of protein not digested by endogenous enzymes. As speculated by Chesson (1993), it is uncertain as to whether the newly hatched chick is deficient in proteolytic enzymes, but some recent data (Noy and Sklan, 1995) indicated a limited proteolysis in the young broiler chicks up to 10 d of age. It is also possible that some of the positive effects of protease supplementation may be related to initiation of protein digestion in certain compartments of the upper gut (i.e., crop) where normally protein hydrolysis is minimal. This response could then result in more effective hydrolysis of protein and hence improved amino acid absorption from the duodenum and the small intestine as a whole.

To explain the mode of action of protease enzymes, Protease G and Protease M were added to canola meal-based diets containing an optimal (22%) or low (17%) level of protein (Experiment 2). It should be remembered that in the diet formulation, the factor of 5.8 was used to convert nitrogen to protein content. Therefore, by conventional standards, the so-called optimal-protein diet contained 23.3% protein and exceeded the protein requirement of growing chicks as recommended by NRC (1994). The performance from 4-18 days of age of broiler chicks fed diets containing the different levels of protein with and without Protease G or Protease M supplementation is shown in Table 26. There were no significant differences among treatment groups, although positive tendencies, due to enzyme addition, were observed. Chicks fed enzyme supplemented diets tended to have numerically higher gains than those fed the control diets. For the 22% protein diet, both proteases tended to have a lowering effect on feed intake with chicks fed Protease G showing some improvement in feed efficiency, though

not statistically significant. There were no differences with respect to feed intake, body weight gain or feed efficiency between either of the enzyme treatments and the control diet at the low (17%) dietary protein level. An overall more effective protein digestion throughout the whole gastrointestinal tract, rather than the digestion of a specific type of protein, would appear to be responsible for the slight difference ($P<0.1$) in chick performance following Protease G supplementation.

The effects of dietary supplementation with Carbohydrases F, G and H on body weight gain, feed intake and feed efficiency of broiler chicks fed a semi-purified canola meal-based diet from 4-18 days of age are shown in Table 27. There was no improvement in chick performance when the canola meal-based diet was supplemented with either Carbohydrases F or H. Carbohydrase G, however, resulted in slightly positive ($P>0.05$) effects on feed intake, weight gain and feed efficiency. Since in the *in vitro* studies all three carbohydrases exerted similar activities toward canola cell wall polysaccharides with Carbohydrase F showing the highest level of activity at both pH 5.3 and 7.0, it is unclear why Carbohydrase G showed the most consistent positive trend *in vivo*. As many commercial enzymes are in fact crude preparations, it is possible that Carbohydrase G contains some potentially beneficial activities other than pectinase or α-galactosidase. Both of these enzyme activities are present in the Carbohydrase G preparation.

The performance from 4-18 days of age of broiler chicks fed conventional wheat/soybean- and wheat/canola-based diets with and without protease supplementation (Experiment 4) is shown in Table 28. In general, the response of chicks depended on

the type of protein supplement, with soybean meal being superior to that of canola meal. The total replacement of soybean meal by canola meal resulted in reduced body weight gain of 7% which, however, was less pronounced (3% reduction) and not significant when 0.02% Protease G was incorporated into the canola diet. At this level, however, Protease G showed a significant negative effect on feed consumption when soybean meal was used as the main protein supplement. Although the reduction in feed intake resulted in a lower body weight gain, there was a trend ($P>0.05$) to an increase in feed efficiency for this particular diet. A similar, significant reduction in feed intake was observed for chicks fed a wheat/canola meal-based diet supplemented with Protease G at the high (0.05%) inclusion rate. This finding is in agreement with our earlier research in which a substantial reduction in feed intake was observed in laying hens fed canola meal diets containing a 0.05% protease supplement. Similar results have been reported recently by Sebastian *et al.* (1994) for a protease supplemented corn/soybean meal-based diet.

While it is unclear what mechanism is involved in feed intake reduction following protease supplementation, it seems probable that the same mechanism may have been responsible for the decreased feed consumption associated with increased dietary protein level demonstrated in Experiment 2. In both high dietary protein or protease supplemented diets the surplus supply of amino acids in the blood stream could result in a reduced appetite and consequently a depression in feed intake. As dietary energy is a more important regulator of feed intake than protein (Sturkie, 1986), it is probable that the excessive supply of amino acids results in an elevated utilization of energy. A

distinct difference between soybean meal and canola meal with respect to nutrient density and dietary fibre content would suggest that soybean meal contains more protein which is available for immediate digestion. This could explain why the reduction in feed consumption occurred at a low (0.02%) level of supplementary protease for soybean meal while for the canola meal diet a high inclusion rate (0.05%) was necessary to significantly affect feed intake.

The synergistic effects of protease, carbohydrase and phytase enzymes were investigated in an experiment (Experiment 5) in which a wheat/canola meal-based diet deficient in available phosphorus was fed to broiler chicks. As chick performance tended to differ between weeks 1 and 2, both sets of data are presented (Table 29). During week 1 there was a significant difference in body weight gain and feed efficiency between the phosphorus deficient negative control diet (0.35% avail. P) and the positive control (0.45% avail. P). Addition of phytase enzyme followed by phytase acting in concert with either Protease G or Carbohydrase G and finally a combination of all three preparations resulted in a progressive improvement in feed efficiency. In this regard, the feed efficiency value along with body weight gain for the phytase, protease and carbohydrase supplemented diet, were equal to values for the positive control and differed significantly from the negative control. As the effect of phytase on chick performance tended to be intermediate between the two control diets, both protease and carbohydrase preparations appeared beneficial in promoting chick growth and feed utilization. Improved performance of broiler chicks fed a blend of phytase, protease and carbohydrase enzymes confirms earlier findings of a more effective use of exogenous

enzymes when applied in combination rather than individually (Massiot *et al.*, 1989; Classen *et al.*, 1991; Chesson, 1993). The overall performance of broiler chicks, however, was different from that of week 1, with the enzyme supplements showing less pronounced effects when added to the phosphorous deficient diet. This could be attributed to the lower phosphorus requirement of broiler chicks at 11- to 18-days of age which, in the current study, was reflected in a similar overall performance of chicks fed the two control diets (0.35% and 0.45% available phosphorus). Similar results were reported by Simons *et al.* (1990) and Jongleod and Kemme (1990) who found a decline in positive response to phytase supplementation in two-week- as opposed to one-week-old birds.

A potential for the development of an effective "cocktail" of enzymes for use with canola meal diets in practical poultry feeding is indicated by the results of this study. However, more research is needed to develop an effective carbohydrase preparation and to determine the synergistic effects and dose responses of protease, carbohydrase and phytase enzymes.

TABLE 20. Calculation of nitrogen-to-protein conversion factors for samples of commercial canola meal

Canola meal	Amino acid content (%)	Nitrogen (%)	Conversion factor
A	38.44	6.581	5.84
B	38.34	6.646	5.77
C	38.56	6.523	5.91
D	34.78	6.064	5.74
E	34.54	5.877	5.88
Mean	36.93	6.338	5.83

TABLE 21. Composition and calculated analysis of semi-purified canola meal diets used in Experiment 1 (Diet 1), Experiment 2 (Diet 2 and 3) and Experiment 3 (Diet 1).

Ingredients and Composition	Diet		
	1	2	3
	---------- (%) ----------		
Canola meal	54.8	51.0	51.0
Sucrose	32.4	32.8	38.1
Casein	-	6.0	-
Vegetable oil	9.0	6.5	7.2
Limestone	1.2	1.2	1.2
Biophosphate	1.0	1.0	1.0
Vitamin premix[1]	1.0	1.0	1.0
Mineral premix[2]	0.5	0.5	0.5
Methionine	0.1	-	-
TOTAL	**100.0**	**100.0**	**100.0**
Calculated composition			
Metabolizable energy, kcal kg^{-1}	3050	3050	3050
Crude protein, % (N x 5.8)	18.9	22.0	17.0
Lysine, %	1.02	1.34	0.95
Methionine, %	0.47	0.52	0.37
Calcium, %	1.00	1.02	1.02
Available phosphorus, %	0.47	0.45	0.45

[1] Vitamin premix provided per kilogram of diet: vitamin A, 8250 IU; vitamin D_3 1000 IU; vitamin E, 11 IU; vitamin B_1, 0.012 mg; vitamin K, 1.1 mg; niacin, 53 mg; choline, 1020 mg; folic acid, 0.75 mg; biotin, 0.25 mg, riboflavin, 5.5 mg.

[2] Mineral premix provided per kilogram of diet: manganese, 55 mg; zinc, 50 mg; iron, 80 mg; copper, 5 mg; selenium, 0.1 mg; iodine, 0.36 mg, sodium 1.6g.

TABLE 22. Composition and calculated analysis of wheat/soybean/canola-based diets used in Experiment 4 (Diet 4 and 5) and Experiment 5 (Diet 6 and 7).

Ingredients and Composition	Diet 4	5	6	7
	(%)			
Wheat	64.6	54.2	54.3	53.9
Canola meal	-	32.0	32.0	32.0
Soybean meal	20.0	-	-	-
Casein	3.0	3.0	3.0	3.0
Vegetable oil	5.0	6.8	6.8	6.9
Limestone	1.9	1.5	1.7	1.5
Biophosphate	1.3	1.0	0.6	1.1
Vitamin premix[1]	1.0	1.0	1.0	1.0
Mineral premix[2]	0.5	0.5	0.5	0.5
Alphacell	2.5	-	-	-
Xylanase premix	0.1	0.1	0.1	0.1
TOTAL	**100.0**	**100.0**	**100.0**	**100.0**
Calculated composition				
Metabolizable energy, kcal kg^{-1}	3050	3050	3050	3050
Crude protein[3], %	22.4	22.0	22.0	21.9
Lysine, %	1.11	1.15	1.15	1.15
Methionine, %	0.50	0.50	0.50	0.50
Calcium, %	1.05	1.01	1.00	1.01
Available phosphorus, %	0.43	0.43	0.35	0.45

[1] Vitamin premix provided per kilogram of diet: vitamin A, 8250 IU; vitamin D_3 1000 IU; vitamin E, 11 IU; vitamin B_1, 0.012 mg; vitamin K, 1.1 mg; niacin, 53 mg; choline, 1020 mg; folic acid, 0.75 mg; biotin, 0.25 mg, riboflavin, 5.5 mg.

[2] Mineral premix provided per kilogram of diet: manganese, 55 mg; zinc, 50 mg; iron, 80 mg; copper, 5 mg; selenium, 0.1 mg; iodine, 0.36 mg, sodium 1.6g.

[3] Determined by using N x 5.7 for wheat, N x 5.8 for canola meal and N x 6.25 for soybean meal

TABLE 23. *In vitro* hydrolysis of canola protein (% of total) by commercial proteases when evaluated alone or in combination with either pancreatin or pronase.

	Assay conditions		
Enzyme evaluated[1]	Alone	+ pancreatin	+ pronase
None (control)	2.2 ± 0.2[2]	44.6 ± 0.4	62.0 ± 0.5
Protease A	1.8 ± 0.2	-	-
Protease B	14.2 ± 0.4	-	-
Protease D	34.7 ± 0.7	63.3 ± 0.8	71.7 ± 2.4
Protease E	19.5 ± 2.4	56.4 ± 0.2	67.9 ± 0.7
Protease F	20.9 ± 0.7	54.3 ± 1.2	68.9 ± 0.5
Protease G	53.7 ± 1.6	68.1 ± 0.9	70.8 ± 0.1
Protease H	13.1 ± 0.5	-	-
Protease J	37.1 ± 1.3	51.0 ± 0.5	59.5 ± 0.4
Protease K	6.3 ± 0.8	-	-
Protease L	4.6 ± 0.9	-	-
Protease M	33.7 ± 1.4	61.4 ± 1.0	66.7 ± 0.2
Protease N	49.0 ± 2.2	62.4 ± 1.4	69.4 ± 1.2
Protease O	42.1 ± 1.0	55.5 ± 1.4	65.9 ± 0.4

[1] See text for enzyme identification

[2] Mean ± SD

TABLE 24. Carbohydrase-like enzyme activities as determined under different pH conditions

Carbohydrase[1]	Enzyme activity[2]	
	pH 5.3	pH 7.0
Energex	4.6 ± 0.2[3]	3.8 ± 0.2
A	3.1 ± 0.3	2.7 ± 0.1
B	3.8 ± 0.2	2.3 ± 0.1
C	3.8 ± 0.2	3.1 ± 0.1
D	4.1 ± 0.1	2.9 ± 0.2
E	4.6 ± 0.2	3.5 ± 0.0
F	7.2 ± 0.2	6.4 ± 0.3
G	6.0 ± 0.2	3.9 ± 0.3
H	6.4 ± 0.2	3.8 ± 0.2

1 See text for enzyme identification

[2] Expressed as mg of solubilized carbohydrate per mg of enzyme

[3] Mean ± SD

TABLE 25. Effect of protease enzyme supplementation on broiler chick performance (4-18 days) when fed semi-purified canola meal diets (Experiment 1).

Enzyme Added[1]	Enzyme level (%)	Feed intake (g)	Weight gain (g)	Feed efficiency
None (control)	-	545.1^{ab}	358.6	1.52^{ab}
Protease G	0.002	565.2^{a}	375.1	1.51^{abc}
Protease G	0.020	544.2^{ab}	382.5	1.42^{c}
Protease D	0.002	550.1^{ab}	359.9	1.53^{ab}
Protease D	0.020	552.8^{ab}	369.7	1.53^{ab}
Protease M	0.050	549.1^{ab}	381.1	1.44^{bc}
Protease N	0.050	531.9^{b}	360.6	1.48^{abc}
Protease O	0.050	565.6^{a}	377.9	1.50^{abc}
SEM		**9.612**	**8.206**	**0.031**

[1] See text for enzyme identification

[a-b] Means within a column with no common superscript are significantly different ($P \leq 0.05$).

TABLE 26. Growth performance of chicks (4-18 days) as influenced by addition of protease enzymes to canola meal diets containing optimal and sub-optimal levels of protein (Experiment 2)

Protein level/ Enzyme	Enzyme level (%)	Feed intake (g)	Weight gain (g)	Feed efficiency
22% protein				
None (control)	-	548.8^{bc}	372.4	1.47^{abc}
Protease G	0.02	543.1^{c}	380.6	1.43^{c}
Protease M	0.05	540.8^{c}	371.2	1.46^{bc}
17% protein				
None (control)	-	570.1^{abc}	370.4	1.54^{a}
Protease G	0.02	581.8^{ab}	382.2	1.52^{ab}
Protease M	0.05	584.0^{a}	378.7	1.54^{a}
SEM		7.816	7.321	0.024

[1] See test for enzyme identification

[a-c] Means within a column with no common superscript are significantly different ($P \leq 0.05$).

TABLE 27. Effect of carbohydrase enzyme supplementation (0.05%) on broiler chick performance (4-18 days) when fed semi-purified canola meal-based diets (Experiment 3).

Carbohydrase added[1]	Feed intake (g)	Weight gain (g)	Feed efficiency
None (control)	551.6	365.6[ab]	1.51
Carbohydrase F	543.6	361.8[ab]	1.50
Carbohydrase G	558.8	381.6[a]	1.46
Carbohydrase H	544.4	356.0[b]	1.53
SEM	9.322	6.552	0.028

[1] See text for enzyme identification

[a,b] Means within a column with no common superscript are significantly different ($P \leq 0.05$).

TABLE 28. Effect of protease enzyme supplementation on broiler chick performance (4-18 days) when fed practical wheat/soybean and wheat/canola diets (Experiment 4).

Protein supplement	Enzyme added[1]	Enzyme level (%)	Feed intake (g)	Weight gain (g)	Feed efficiency
Soybean meal	None	-	576.9[a]	409.3[a]	1.41[ab]
Soybean meal	Protease G	0.02	510.4[c]	379.0[b]	1.35[b]
Canola meal	None	-	528.7[bc]	373.3[b]	1.42[a]
Canola meal	Protease G	0.02	551.4[b]	387.0[ab]	1.43[a]
Canola meal	Protease G	0.05	482.9[d]	343.2[c]	1.41[ab]
SEM			8.097	8.010	0.019

[1] See text for enzyme identification

[a-c] Means within a column with no common superscript are significantly different ($P \leq 0.05$).

TABLE 29. Synergistic effects of enzyme supplementation on broiler chick performance (4-18 days) when fed practical wheat/canola meal-based diets (Experiment 5)

Diet/enzyme[1]	Feed intake(g)	Weight gain(g)	Feed efficiency
	---------- Week one ----------		
Negative control[2]	176.8	116.8[b]	1.52[a]
+ phytase	175.2	118.0[ab]	1.48[ab]
+ phytase, protease G	170.8	116.5[b]	1.47[ab]
+phytase, carbohydrase G	171.1	117.0[b]	1.46[ab]
+ phytase, car. G, protease G	175.6	124.2[a]	1.41[b]
Positive control[3]	176.4	124.8[a]	1.42[b]
SEM	5.049	5.003	4.935
	---------- Overall ----------		
Negative control[2]	544.1	379.0	1.44
+ phytase	545.4	380.6	1.43
+ phytase, protease G	526.7	380.3	1.39
+ phytase, carbohydrase G	529.2	374.3	1.41
+ phytase, car. G, protease G	545.0	387.6	1.41
Positive control[3]	531.0	377.4	1.41
SEM	3.319	3.997	2.640

[1] Enzyme inclusion rates were as follows: phytase, 0.01%; protease g, 0.01%; carbohydrase G, 0.05%.

[2] Wheat/canola diet containing sub-optimal phosphorus (0.35% available P).

[3] Wheat/canola diet containing optimal phosphorus (0.45% available P).

[a,b] Means within a column with no common superscript are significantly different ($P \leq 0.05$).

7. GENERAL DISCUSSION AND CONCLUSIONS

7.1. GENERAL DISCUSSION

The use of canola meal as a high quality protein supplement for livestock and poultry is still limited by high dietary fibre (Bell and Shires, 1982) and low metabolizable energy contents (Bell, 1993a). A number of approaches have been proposed for the elimination or alleviation of the dietary fibre effects to improve canola meal utilization in non-ruminant animals. In the current research, experiments were conducted to investigate the potential for improving the nutritive value of the meal by breeding for yellow-seeded canola and by using supplementary enzymes to reduce the negative effects of dietary fibre so as to enhance canola meal utilization in poultry. In this study, 26 genotypes of yellow-seeded canola were compared with 7 samples of conventional brown-seeded canola for differences in chemical composition and dietary fibre contents. The oil-extracted seed samples were analyzed for protein, carbohydrates (ie., sucrose, oligosaccharides), dietary fibre, ash and residual fat. On average, the yellow-seeded canola was found to contain more protein (44.5 vs 42.7) and sucrose (8.7 vs 7.5%) than its brown-seeded counterpart. Similarly, the total dietary fibre content was lower in yellow-seeded canola (27.7 vs 33.6%). The contents of oligosaccharides, ash and ether extract were similar in both types of canola. The analyzed components accounted for approximately 90% of dry matter in both the yellow- and brown-seeded samples. In general, findings from this study were in agreement with previous reports from this laboratory (Slominski and Campbell, 1990; Slominski *et al.*, 1994a) although the higher number of samples allowed for more detailed comparisons between the yellow- and

brown-seeded types. This was also the first time that the yellow-seeded samples of *B. napus* and *B. juncea* were included in such studies.

Content of the dietary fibre components differed significantly among the species with *B. rapa*, *B. juncea* and *B. carinata* showing higher concentrations of non-starch polysaccharide than *B. napus*. The contents of other fibre components (i.e. lignin and polyphenols, cell wall protein and mineral associated with the fibre fraction) were significantly higher in the brown-seeded canola. This appeared to be the main reason for higher total dietary fibre values reported in some earlier studies on brown-seeded canola (Bell and Shires, 1982; Sarwar *et al.*, 1981; Theander *et al.*, 1977). Total dietary fibre was shown to be negatively correlated with protein content demonstrating the potential for further quality improvements within the yellow-seeded type of canola. A weak relationship between total dietary fibre and seed weight indicated that the seed hulls are not the primary source of dietary fibre as was earlier believed (Strigam *et al.*, 1977; Finlayson, 1974) and demonstrated the importance of cotyledonous cell wall polysaccharides in the overall determination of the level of canola fibre.

On the basis of chemical composition and dietary fibre profiles, selected *Brassica* seed samples were further evaluated for digestible protein, soluble fibre, soluble phenolics and extract viscosity. There were no significant differences between the yellow- and brown-seeded types of canola with regard to most of the quality characteristics studied. However, comparisons among different species showed *B. rapa* and *B. napus* to have a relatively high digestible protein content in comparison to *B. juncea* and *B. carinata*. Regression analyses showed a lack of correlation between total

and soluble dietary fibre and digestible protein content. This indicated a minimal negative effect of dietary fibre on meal utilization per se and pointed to an overall nutrient diluting effect of dietary fibre as the determinant of canola meal quality.

An attempt was made to examine the effect of heat treatment on the nutritive quality of canola meal. To determine optimal processing conditions three samples of oil-extracted canola seed were moist heat treated for 20 minutes at different temperatures. The effect of time was assessed by heating the samples at 105°C for different time periods. From this study, it was shown that *in vitro* protein digestibility increased significantly with increased processing temperature up to 108 ± 1°C after which it declined substantially. It was also demonstrated that moist heat treatment of canola seed at temperatures beyond 110°C results in substantial protein damage as was evidenced by a decrease in *in vitro* protein digestibility and an increase in protein present in the neutral detergent fibre residue. Similar effects of high temperature on protein damage were reported by Andersonn-Harfermann *et al.* (1993) and Jensen *et al.* (1995). Moist heat treatment at temperatures below 105°C did not have any significant effect on *in vitro* protein digestibility and contrary to earlier findings (Jensen *et al.*, 1995; Anderson-Harfermann *et al.*, 1993), time of heat treatment did not appear to have any effect on canola meal quality.

Based on chemical composition and quality parameters, four *Brassica* cultivars were selected for further evaluation *in vivo*. Seed samples were defatted and moist heat treated under the optimal conditions (108 ± 1°C) established in the earlier study. The evaluation involved chemical composition, *in vitro* protein digestibility, true

metabolizable energy (TMEn) content, true amino acid availabilities (TAAA) and feeding quality as determined in a two-week feeding trial with broiler chickens. With regard to chemical composition, the selected samples appeared superior to that of commercial canola meal. The protein content in 3 samples was 4 to 5 percentage points higher than that of the commercial meals. The increase in protein and/or a reduction in dietary fibre content significantly improved the true metabolizable energy (TMEn) content of the meals. The content and availability of amino acids in the new cultivars, however, was not significantly different from that of the commercial meals. *In vitro* protein digestibility in the laboratory prepared meals was comparable to that of the commercial meals and indicated that the processing conditions employed were similar to those commonly used in the crushing operation of canola seed. With the exception of *B. juncea* sample, the performance of broiler chickens fed laboratory prepared meals was similar or higher than that of the commercial meal from the yellow-seeded canola cv. Parkland (control). The performance of broilers fed the yellow-seeded *B. napus* canola (cv.Y1016) was comparable to that of the control diet with regard to body weight gain and was significantly higher with respect to feed to gain ratio. The relatively low performance of broiler chickens fed the *B. juncea* cv.J4316 sample was attributed to the relatively high glucosinolate content in this particular meal.

Information on the chemical composition and dietary fibre profiles of the canola meal assisted in selecting the exogenous protease and carbohydrase enzymes for studies on the enhancement of the nutritive value of canola meal in poultry. Twenty-one protease and 8 carbohydrase preparations were evaluated *in vitro* for their ability to solubilize

canola protein and canola cell wall polysaccharides, respectively. The most effective protease (G, M and O) and carbohydrase (F, G and H) enzymes were selected for in vivo evaluation in broiler chicken feeding trials (14-day). Protease supplementation in broiler rations was inconclusive in that some of the preparations enhanced performance of chickens while others did not and the response tended to decrease with increased dietary protease concentration. The response to dietary protease supplementation was most effective when protein level in the diet was sub-optimal. Supplementation of the diets with selected carbohydrase preparations showed a trend towards improved broiler performance only in the case of one preparation: carbohydrase G. Overall, the most effective was a cocktail of different enzyme activities (ie., protease, carbohydrase and phytase) when supplemented to a phosphorus deficient diet.

7.2. CONCLUSIONS

1. The consistent negative relationships between dietary fibre and protein contents exemplify the potential for improved nutritive content of yellow-seeded forms of *B. rapa*, *B. juncea* and *B. napus* with further reductions in dietary fibre content. To achieve maximum results any such reduction in dietary fibre content should be accompanied by quantitative changes in protein. Due to the relatively similar values obtained for the complex quality characteristics (ie., soluble and insoluble dietary fibre, digestible protein, available amino acids, phenolics, etc.) measured in this study it appears valid to use simple protein analysis as a quality criterion in the early stages of selection programs in the evaluation of canola/mustard materials. Final evaluation, however, of new cultivars would require more detailed chemical analyses (i e., carbohydrates, amino acids and dietary fibre contents) and *in vivo* experimentation. It was also demonstrated in this study that the processing of material for such evaluation is critical and must include optimal moist heat treatment which was shown to be 108 $\pm$ 1°C for 20 minutes. These conditions gave quality results equivalent to that obtained in the commercial processing of canola.

2. Effective introduction of *B. juncea* as a new crop for the Canadian Prairies would require further quality improvement by lowering the content of undesirable aliphatic glucosinolates in the seed.

3. A two step pepsin-pancreatin in vitro system, perfected in the current study, could assist in the evaluation of new protein supplements and could become a useful tool in future research on the effect of heat treatment (ie., live steam injection, cooking, micronization) on nutritive value and protein quality of feedstuffs.

4. There is potential for enhanced utilization of canola meal by monogastric animals through the use of exogenous dietary enzymes. According to the research data reported herein, the use of a cocktail of various enzyme activities (ie., protease, carbohydrases, phytase, etc.) rather than individual preparations appears necessary to achieve effective meal utilization. In this context, further research is needed to develop a blend of carbohydrase and protease enzymes suitable for degradation of cell wall polysaccharides and glycoproteins.

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