EFFECT OF PROCESSING AND MICROBIAL ENZYME TREATMENT ON THE NUTRITIVE VALUE OF CANOLA MEAL FOR RAINBOW TROUT (*Onchorynchus mykiss*)

by

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ABSTRACT

Salmonids (trout, salmon and charr) require high dietary concentrations of protein, and they utilize protein and lipids as their principle sources of dietary energy. In culture conditions the protein is mainly supplied by fish meal and other products from the capture fishery. However, the high cost, and variability in fish meal quality due to processing and storage conditions, dissimilar raw material quality, as well as fluctuations in the supply of fish meal are major concerns. Feed accounts for 40% to 60% of the operating costs of salmon farming. Much of this cost is associated with the inclusion of fish meal as the predominant protein source. Replacement of at least some of this fish meal by cheaper plant protein products is one way of reducing these costs. This study was undertaken to assess the chemical composition and potential nutritive value (digestibility) of canola protein products developed by processing canola meal by substituting fish meal in salmonid diets, with rainbow trout (*Oncorhynchus mykiss*) in fresh water as the test animal. Sieving, methanol/ammonia treatment and the use of three types of enzyme (Phytase, SP-249, Alpha-Gal) treatments, singly or in various combinations, were employed in this study. In addition, a commercially-produced canola protein isolate (CPI) was also evaluated for potential nutritive value. It was hoped that one of these products would be potentially suitable as a replacement for fish meal.

The results indicated that sieving canola meal did not have any significant influence on its chemical composition. Treatment of canola meal with methanol/ammonia however, decreased the amount of total glucosinolates by over 80%, and lowered the levels of phenolic compounds but this protocol increased the concentration of phytate. Application of carbohydrases did not result in any appreciable improvement in the fibre composition of the meals, although it did lead to increases in the protein content and levels of amino acids, and to decreases in the levels of glucosinolates and phytate. Low levels of glucosinolates were detected in all the canola protein products that received the enzyme treatments either singly or in combination. The processing methods used in the

production of canola protein isolate (CPI) significantly improved the nutrient composition of the resultant product. Levels of total glucosinolates, protein, gross energy and phytate of the canola protein isolate (CPI) were 1.95 μmol/g, 90.8% , 24.4 MJ/kg and 4.3 μmol/g, respectively compared to 8.95 μmol/g, 36.3%, 19.6 MJ/kg and 45.5 μmol/g, of commercial canola meal. In a three-week digestibility experiment, the effect of processing canola meal on the digestibility of dry matter, protein and energy by 74 g rainbow trout was assessed using a modified "Guelph system" of faecal collection. The canola products were included at 30% of the diets (70 % reference: 30% test canola protein product). Each of the test diet treatment was assigned to triplicate groups of fish using a completely randomized block design with chromic oxide (0.5%) as an indigestible marker. The fish were hand fed to satiation twice daily in special designed 150 L fibre glass tanks supplied with fresh running water (9.9 °C to 11.0 ° C) at a flow rate of 4-5 L/min.

Processing methods had variable effects on the apparent nutrient (dry matter, protein and energy) digestibility coefficients. All the laboratory processing protocols employed with a view to enhance the nutritional value of canola meal had significant negative effects on dry matter digestibility coefficients and generally negligible effects on protein digestibility. There were however, significant reductions in protein digestibility coefficients in the meals treated with SP-249 (77.4%) alone or in combination with Alpha-Gal (79.5%) relative to untreated canola meal (88.1%). Energy digestibility coefficients and the digestible energy contents of the test canola protein products were the least affected by the processing methods selected for this study. However, treatment of canola meal with either methanol/ammonia or combination of carbohydrate-degrading enzymes had negative effects on the energy digestibility coefficients and digestible energy contents. For instance, these two parameters were lowered by about 14 percentage units and 3 MJ/kg in the ammoniated canola meal and by about 17 percentage units and 2.7 MJ/kg for the meal treated with the two carbohydrate-degrading enzymes. Digestibility coefficients for dry matter, protein and energy for the canola protein isolate (CPI) were 77.1%, 97.6%, and 84.7 %, respectively, and it is noteworthy that all these values were much higher than those values found for the untreated and treated canola meals.

Fish growth performance and feed utilization was not significantly affected by either the type of processing treatment employed or the kind of canola product included in the diet. However, fish fed the diets containing canola protein isolate or those fed the diet containing the alpha-galactosidase-treated canola meal showed non-significant improvements in weight gain, specific growth rate and relative growth. Mean daily dry feed intakes per fish (DFI) were higher for fish receiving the diets containing the laboratory processed canola meals than for those consuming diets containing the canola protein isolate. The effect of processing treatment tended to (decrease utilization) increase the feed to gain ratio (FGR) of rainbow trout fed diets containing the test canola protein products. Fish consuming the diet containing the canola protein isolate, however, exhibited improved FGR value (1.22) relative to those fed the herring meal control diet (1.45). In general, the canola protein isolate (CPI) had a higher content of available (digestible) protein and energy for rainbow trout than fish meal and the laboratory processed canola meals. This advantage was also translated into improvement in fish performance. While the processing methods employed resulted in some improvements in the chemical composition and nutrient digestibility of canola protein products, this did not bring about any enhancement of growth and feed utilization of rainbow trout. However, it must be emphasized that the diets employed in the digestibility study were not balanced with respect to nutrient and energy content. Hence, valid conclusions regarding fish performance (growth) can not be drawn from these results.

On the basis of the digestibility assessment conducted in the present study, it is concluded that some canola protein sources (canola protein isolate) may have potential as substitutes for fish meal in salmonid diets. Also it can be concluded that careful processing of canola meals or seed with a view to reducing or eliminating the antinutritional factors present in the meal or seeds can result in protein products that can be used to potentially replace fish meal in salmonid diets without compromising fish performance. It was also found that although laboratory processing improved the chemical composition of canola meal, it did not have any significant effect in nutrient (dry matter, protein and energy) utilization or fish performance.

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DEDICATION

This thesis is dedicated to my dad for all his hard work in raising and educating me and for encouraging me to always work hard. Unfortunately he did not live long enough to see the completion of this work. To him I owe all my success.

Chapter 1

INTRODUCTION

World aquaculture production of finfish, crustaceans and molluscs has increased about three fold since 1975. By the year 2000, it is estimated that the output will have reached about 21 million metric tonnes (New, 1991). However, by the year 2025 the output will need to be three times as much in order to maintain the 1989 availability of 19.1 kg of aquatic products per person per year. In the past, the world fisheries harvests have kept pace with growing population demands for seafood however, in recent years the wild fishery catches have plateaued at 100 million tonnes (MT) per year (Chamberlain, 1993). Therefore, the gap between supply and demand will have to be filled by aquaculture.

Global farmed salmon production has also shown remarkable temporal changes. For example, in 1985 the production was about 50 thousand MT (Needham, 1990), whereas in 1995 the production is expected to be in excess of 400 thousand MT (Higgs *et al.,* 1995b). Because of this dramatic rise in the production of farmed salmonids and good wild catches, the market prices of farmed salmon have not always been high enough to break even with all the production costs (Higgs *et al.,* 1994). In addition, there has been strong demand on the global supply of fish meals from the pig, poultry and pet food industries. Hence, in order to increase the profitability of salmon farming, new approaches are required, not only to enhance the market value of the farmed product, but to reduce the cost of production as well.

Feed represents the largest single operating cost in commercial salmon farming, accounting for about 40-60% of the total cost. This high proportion is due to reliance on high quality fish meals to meet the high dietary protein needs of salmon. Fish meals are preferred sources of protein because they are highly palatable. Also meals of good quality have high levels of digestible (available) protein and energy as well as excellent essential amino acid profiles relative to the needs of salmonids (Higgs *et al.,* 1995a). However, fish meal quality and price can vary considerably due

to (a) dissimilar composition and freshness of raw materials; (b) suboptimal processing, transport and storage conditions; (c) different ratios of solubles to presscake and fish soluble quality; (d) varying proportions of whole fish, offal and filleting residues resulting in different levels of collagen, ash and other proximate constituents; and (e) inappropriate antioxidant, moisture and chlorinated hydrocarbon levels (Higgs *et al.,* 1994). The improvement of existing protein sources and development of new ones to replace animal protein sources in fish diets is desirable for the development of more economical diets. For this reason, several alternative protein sources to fish meal have been studied as the chief sources of protein in fish diets. These have included animal (Fowler, 1991; Gallagher and Degani, 1988; Mohsen and Lovell, 1990) as well as plant (Eusebio, 1991; Hughes, 1991; Olvera-Novoa *et al.,* 1988, 1990) protein sources.

Plant protein sources, especially some legume and oilseed protein products, appear to offer great potential for inclusion in formulated diets for fish by partial or total replacement of fish meal (De la Higuera *et al.,* 1988; Gomes *et al.,* 1993; Higgs *et al.*, 1988; Rumsey *et al.,* 1994; Tacon *et al.*, 1984). There is also a higher worldwide production of oilseed proteins than of fish meal protein. Greater use of locally produced protein sources as replacements for fish protein and imported plant protein sources could be desirable for economic reasons. Of the two sources (plant and animal), plant materials have received the greatest attention (Gallagher, 1994; Higgs *et al.,* 1982, 1988, 1991; McCurdy and March, 1992; Oliva-Teles *et al.,* 1994; Tacon and Jackson, 1985) because they are less expensive and are more available than animal protein (Higgs *et al.*, 1982). Protein of vegetable origin such as yeast, algae, leaves or grains has been used widely as a partial replacement for fish meal (Jackson *et al.,* 1982; Viola *et al.,* 1982). However, some plant products have limited value as protein sources in animal diets due to improper amino acid balance, the presence of enzyme inhibitors and/or low levels of available energy. The oilseed plants are now regarded to a great extent not only as sources of oil, but also as sources of animal feed protein or protein suitable for human consumption. Leguminous seed and oilseed meals have been used as feed ingredients due to their high protein content, despite their varying levels of antinutritional factors (ANFs) and low levels of sulphur-containing amino acids (Bressani and Ellis, 1979;

Winfree and Stickney, 1984). Coconut (copra), cottonseed, soybean, groundnut, and sunflower meals have all been tested as protein sources in animal feeding trials. The oil from most of these seeds is also important for edible purposes. Oil from plants of the *Brassica* genus (which includes mustard, cabbage, rutabagas, broccoli, kale and rape) especially turnip and rape was once used for oil lamps in Asia and the Mediterranean countries and later as a cooking oil. Its cultivation in Europe did not start until the thirteenth century (Bell, 1982). Use of the oil for edible purposes later decreased because of the high content of eicosenoic and erucic acids (Higgs *et al.*, 1988).

Due to technological difficulties and the inferior sensory properties of rapeseed oil compared to other vegetable oils, the production of rapeseed substantially decreased after the Second World War. However, as a result of plant breeding research, the world production of rapeseed/canola (hence, oil and meal) has increased steadily. Today, rapeseed /canola plays an ever increasing role in the vegetable oil industry, and the utilisation of the meal as a protein rich component in animal feed is still growing. Research on the use of rapeseed-derived protein for direct human consumption is also being carried out. This is directed toward improvement of rapeseed/canola protein by reduction or removal of toxic and antinutritional factors by both genetic selection and novel processing methods.

Interest in rapeseed meal (RSM) as a protein source started in the 1960's when it became generally appreciated that the essential amino acid composition of rapeseed protein compared favorably with that of soybean protein and the FAO/WHO reference protein. The meal also contains about 40% protein and 14% fibre, making it suitable for feeding to animals (McCurdy, 1992). Over 900,000 metric tons were produced in Canada in 1986-87, of which about half was used domestically in cattle, pig and poultry feed. However, this represents under 4% of the total tonnage of manufactured feed in Canada (Downey and Bell, 1990). Soybean meal (SBM) is used extensively in formulated diets and it is the main alternative to rapeseed/canola meal. Canola meal differs from soybean meal in having lower levels of protein and some essential amino acids and a

lower digestible and metabolizable energy content. It also contains more fibre and is richer in several minerals and some vitamins.

By-products of vegetable oil processing are commonly used as livestock feedstuffs, for both ruminants and non-ruminants, though the fibrous nature of the material is a major concern in non-ruminant diets. Rapeseed meal has been evaluated as a non-ruminant feedstuff through chemical analyses and dry matter digestibility experiments (May and Bell, 1971; Saben 1971). The digestible energy (DE) content of RSM was reported by Bayley *et al.* (1969) to be 11.5 MJ/kg when tested as 40% of a corn-soybean diet fed to 45-90 kg gilts. This figure is less than that found for SBM and was attributed to the high fibre content of rapeseed meal. Similarly the digestibility of RSM protein and essential amino acids was found to be less than value obtained for SBM. However, when pelleted and reground, the DE value increased to about 12.6 MJ/kg. Such results indicate that rapeseed /canola meal could be a reasonable source of energy and protein for non-ruminant animals, especially fish, in spite of the high fibre content providing improvements in the processing techniques could be made. A reduction in the carbohydrate content of rapeseed/canola meals should enhance its nutritive value for salmonids and other monogastric animals.

Rapeseed/canola is conventionally processed to oil and feed-grade meal by an extraction process that is an adaptation of soybean technology adjusted to high oil content, small seed size and presence of glucosinolates. The seeds are crushed to break the fibrous coat and then cooked at 75-100 °C for 15-25 minutes to facilitate oil release. The seeds are then pressed to reduce the oil content to about 20% and the cake fragments formed are flaked and then hexane extracted to remove the residual oil. A meal containing about 40% protein is obtained after the extraction process. The intact glucosinolates left in the meal after heating may, however, still produce toxic aglucones in the lower gastrointestinal tract of animals. Other antinutritional constituents such as phenolics and phytic acid also introduce their own complications (Shahidi and Naczk, 1990). Therefore efforts must be made to upgrade the nutritive value of commercial canola meal so that it can be used without any reservations for salmonids. Due to the chemical composition and

reasonable cost of canola meal relative to both soybean meal and fish meal, it was decided to evaluate the effects of different methods of treating commercial canola meal from *Brassica napus* in order to improve its utilization as a protein source replace fishmeal in diets for rainbow trout.

Chapter 2

LITERATURE REVIEW

2. 1 UTILIZATION OF OILSEED MEALS

2.2. Oilseed meals as protein sources

Problems related to dependence on and probable future restrictions in fish meal supplies have stimulated research on a wide array of protein sources. The objective has been to replace as much fish meal as possible in commercial fish diets without compromising fish performance (Dabrowska *et al.,* 1989; De la Higuera and Cardenete, 1987). A large variety of high protein plant seeds have the potential of being utilized as protein sources in fish diets. Oilseed meals are generally regarded as protein supplements for use in animal feeds following appropriate processing. Their chemical composition varies widely. Some contain less than 30% crude protein (CP; % N×6.25) whereas some meals may contain as much as 50% CP. The crude fibre content varies from about 3% to 35 %, with the highest values always being associated with the presence of high levels of hulls, which also affect the CP content and the nutritional value. The usefulness of the oilseed meals is also influenced by antinutritional factors that adversely affect palatability, digestibility, and the nutritional balance of absorbed amino acids. Digestibility coefficients may vary according to the class and age of animal, the level of inclusion of the oilseed meal in the diet, the kind of meal and the processing conditions to which the meal has been subjected. Among the oilseed meals used in practical fish diets, soybean meal has been studied extensively due to its high global availability and protein content (45-50%) and good amino acid profile (Akiyama, 1988; Alexis, 1990; Kaushik, 1990). Studies on rainbow trout (Reinitz *et al.,* 1978; Rumsey *et al.,* 1993; Tacon *et al.,* 1983), tilapia (Shiau *et al.,* 1987), striped bass (Gallagher, 1994), and red sea bream (Reigh and Ellis, 1992) have shown that soybean meal can be used as a dietary protein source with satisfactory results. Sunflower meal protein has been shown to be effectively used by

rainbow trout at low dietary levels (Martinez, 1986; Sanz *et al.,* 1994; Tacon *et al.,* 1984) and by tilapia at high dietary levels (Jackson *et al.,* 1982) when supplemented with methionine. Other oilseed meals that have been tested as protein sources in fish diets include, rapeseed/canola meals (Higgs *et al.,* 1995a), lupin seed meal (Gomez *et al.,* 1989), cottonseed meal (Tacon and Rodrigues, 1984) and coconut (copra) meal (Jackson *et al.,* 1982).

2.3. Limitations to the use of oilseed meals.

The extraction of oil from oilseed produces protein-rich by-products that can be used as protein supplements in animal feeds. Their potential as feedstuffs however, has not been fully exploited because of the presence of interfering factors that reduce protein and energy digestibility and result in poor physicochemical and functional properties of the protein sources (Oomah and Mazza, 1993). Thus, the nutritional value of plant protein sources depends, in addition to chemical composition, on the extent to which their nutrients are digested, absorbed and utilized. The full nutritional potential of some of the oilseed meals has been attained only after an appropriate amount of heat has been applied to inactivate factors present in the oilseed meals that interfere with the utilization of their protein. In addition to those factors that can be destroyed by heat, other factors are known to be present that are not fully inactivated by heat and can likewise affect nutritional value of oilseed meals. According to Huisman and Tolman (1992) the factors that can interfere with digestibility, absorption and utilization of nutrients may be divided into two major classes. The first class contains those that cannot be utilized by the animal due to lack of appropriate enzymes in the gastro-intestinal tract or because the factors hamper digestion, absorption and/or utilization of nutrients. The factors in the second class are called antinutritional factors (ANFs). ANFs can be defined as non-fibrous natural substances that exert negative effects on growth and health of man and animals. This definition excludes fibre because in human food, fibre may be a positive health factor. Further, fibre may have some energy value when digested in the large intestine and the rumen of ruminants. In plants, the ANFs afford natural protection against attack by moulds, bacteria, insects and birds (Bond and Smith 1990) by intrefering with

the digestive processes in these organisms. Because there are similarities between the digestive processes in farm animals, micro-organisms and insects, ANFs can be expected to disturb the digestive processes in farm animals in a similar way. ANFs are classified according to their effects on the nutritional value of feedstuffs and on the biological response in the animal. For instance, they depress protein digestion and utilization, carbohydrates digestion and mineral bioavailability. Alternatively, they may inactivate vitamins, stimulate the immune system or exert a combination of the foregoing effects (Huisman and Tolman, 1992).

2.3.1. Occurrence of ANFs

The presence and effects of various ANFs in seeds have recently been reviewed by Savage and Deo (1989) and Huisman and van der Poel (1988), and their distribution has been outlined by Friedman (1986) and Pusztai (1989). Various types of seeds may contain common (e.g. phytic acid) or different kinds of ANFs. Trypsin and chymotrypsin inhibitors and lectins are important constituents of legume seeds (soya, peas and beans), whereas tannins may be found in some cereal crops, coloured beans and rapeseed/canola. Glucosinolates, are important components of rapeseed and lupins, and may be found in soya. Gossypol is present in cottonseed. In the plant, ANFs serve important functions. Sinapine (polyphenolic compound present in, for example, rapeseed/canola) serves as a reserve for sinapic acid and choline, which are important in the biosynthesis of lignin and in the methylation cycle, respectively (Kozlowska *et al.*, 1990). Phytic acid (PA) is the primary source of phosphorus and myoinositol and is thought to store metal ions such as calcium, magnesium and potassium. In addition PA protects the plant against oxidative damage and moulds attack by binding the zinc required for fungal metabolism (Thompson, 1990). Phytic acid is found in the crystalline globoids inside the protein bodies in the cells of the radicle and the primary cotyledons (Yiu *et al.*, 1983). When any of the preceding seeds are fed to animals, the negative effects that result may be attributed to a combination of the different effects due to the types and levels of ANFs present.

2.3.2 Effects of ANFs in monogastric animals

The effects of ANFs on animals are mainly physiological, and differ with source or species and age of the animal. ANFs adversely affect digestive functions by altering flow of chyme, impairing interactions between nutrients and digestive enzymes, restricting diffusion, altering absorptive surfaces and changing microbial activity (Krogdahl, 1989). For example, piglets have been found to be more sensitive than fattening pigs to trypsin inhibitors (Huisman and Tolman, 1992), while lectins from beans (*Phaseolus vulgari)* are much more toxic than those from peas. Visitpanich *et al.* (1985) found no differences in relative growth depression between rats and piglets fed pigeon peas (*Cajanus cajan*), but they did find differences in response between the two species when fed chick peas (*Cicer arietnum)* were fed. Huisman and van der Poel (1988) found greater antinutritional effects in piglets than in rats and chickens when peas (*Pisum sativum)* were fed.

The levels of ANFs in the diets are also important in determining the extent of animal response. The work of Jansman *et al.,* (1989) showed that broilers were less sensitive to tannins than piglets and that normal dietary inclusion levels did not exert any negative effects in broilers in contrast to pigs. The same authors also showed that although the inclusion of 3 g of tannin per kg of diet decreased the apparent ileal protein digestibility in piglets, it did not produce the same effect in chicks. Rainbow trout and Atlantic and chinook salmon are also known to respond differently to the effects of the antinutritional factors present in soybean (Arnesen *et al.,*1989) and rapeseed meal.

2.3.2.1 Glucosinolates:

Glucosinolates, previously called thioglucosides, are compounds that are generally biosynthetically derived from several amino acids in which the carboxyl group is lost but the nitrogen from the amino acids is retained (Shahidi and Nazck, 1990). They are found in plants of the *Cruciferae* family and other cultivated plants (Kjaer, 1960). Glucosinolates are responsible for the flavors of the condiments, radish and mustard, and they contribute to the characteristic flavour

of turnip, cabbage and other related vegetables. They are anions and usually occur as salts in plants, although sinalbin occurs in association with the complex organic cation sinapine. Glucosinolates *per se* seem to be non-toxic but metabolites present after hydrolysis by the natural enzyme, myrosinase (also known as glucosinolase or thioglucosidase) have antinutritional effects. There is ample evidence that the enzyme is separated from its glucosinolate substrate in the intact plant, but when this separation is breached, as happens during germination or when raw plant material is crushed, the glucosinolates may be hydrolyzed.

Depending on the conditions of hydrolysis and the substrate type, isothiocyanates, nitriles, thiocyanates or oxazolidine-2-thione (in addition to glucose and acid sulphate ion) may be produced by intramolecular rearrangement of the organic aglucon portion (Tookey *et al.*, 1980). The presence of glucosinolates in the meal or seeds may exert deleterious effects on poultry and swine, depending on the dietary levels of rapeseed/canola meal. Although there is less experimental work done on ruminants, they are reported to be less susceptible to the toxic effects of rapeseed/canola meals. Bush *et al.* (1980), for example, concluded that commercial rapeseed meal could include 30% of the total diet of cattle with no ill effects. Some of the glucosinolate decomposition products such as nitriles have special harmful influences. The deleterious effects of glucosinolate hydrolytic products in animals have been well documented (Bell, 1993; Liu *et al.*, 1993). The adverse effects include a depression of feed intake, growth and feed conversion efficiency and, in poultry, a decrease in egg production. Though the precise way in which these various glucosinolates exert their negative effects is not entirely clear, the detrimental effects of glucosinolate hydrolysis products on animals are thought to be produced by different mechanisms. Isothiocyanates (ITC) and uncleaved glucosinolates compete with iodine uptake into the thyroid gland and liberate the iodine already accumulated, but do not affect thyroid function (Niewiadomski, 1990). The goitrogenic effects of these compounds can therefore be counteracted by dietary iodine supplementation. By contrast, vinyloxazolodinethione (VOT or goitrin) acts via a different mechanism. It impairs thyroid hormone synthesis. This suppression of thyroid function is characterized by thyroid hypertrophy and hyperplasia, reduced levels of intrathyroidal

iodothyronines, depressed thyroid hormone titres and altered affinity of serum albumin for thyroxine (Higgs *et al.,* 1979; Teskeredzic *et al.,*1995). There may also be reduced conversion of thyroxine (T_4) to 3, 5, 3'-triiodo-L-thyronine (T_3) in the liver and the kidney. These effects cannot be overcome by increased dietary iodine supplementation. The effect of nitriles from glucosinolate decomposition has been shown to lead to poor growth and liver and kidney lesions in animals (Tookey *et al.,* 1980; Van Etten and Tookey, 1983). Acute toxicities due to 3-hydroxypent-4-enenitrile and 3-hydroxy-4,5-epithiopentane nitrile (threo) have also been shown to be greater than those from oxazolidine-2-thione. Although canola meals contain reduced levels of glucosinolates (<30 μmoles/g air dried oil-free meal) these low concentrations can still exert antithyroid effects in some fish species, particularly salmonids. This may lead to reduced growth and protein utilization when the dietary levels of the glucosinolates exceed the tolerance level for a particular fish species (Higgs *et al.,* 1988). The adverse effects of glucosinolates on thyroid function and overall fish performance have been demonstrated in trout (Gomes *et al.,* 1993; Hardy and Sullivan, 1983; Hilton and Slinger, 1986; Leatherland and Hilton, 1988), coho salmon (Higgs *et al.,* 1979), chinook salmon (Higgs *et al.,* 1982; 1983), carp (Hossain and Jauncey, 1989) and tilapia (Higgs *et al.,* 1989; Davies *et al.,* 1990). Yurkowski *et al.* (1978) found that for a given glucosinolate concentration, the form (meal, flour or concentrate) in which the rapeseed/canola protein was added to the diet of rainbow trout appeared to influence its toxicity.

2.3.2.2 Phytates

Phytate occurs naturally in many foods derived from plants in the form of mineral salts of phytic acid. Its principle function in plants is the storage of phosphorus that is used gradually during dormancy and germination. Phytic acid (PA) is a cyclohexane compound with six phosphate groups (myoinisitol 1,2,3,4,5,6-hexakis-dihydrogen phosphate) that are capable of forming complexes with cations, resulting in their reduced availability and solubility (Nwokolo and Bragg, 1977; Liener, 1994). In most seed types, the phytic acid is associated with specific components within the seeds, for example, the germ and aleurone layers of cells and hulls in wheat

and rice kernels, but only the germ in corn. In oilseeds, the phytates are distributed throughout the kernel within the aleurone grains or protein bodies and crystalloids or globoids within the protein body membranes. Phytates are considered to be the chief storage form of phosphorus and inositol in almost all seeds. During the ripening process there is active transport of phosphorus from the leaves and roots to the seeds. Most of the phosphorus is eventually found in the form of phytic acid. As phytic acid accumulates in various storage sites in seeds and tubers, other minerals apparently bind to it and thus form the complex salt, phytate. In feed ingredients of vegetable origin about 2/3 of phosphorus is present as phytate phosphorus (Lall, 1991). The interaction of phytic acid with proteins, vitamins and several minerals is considered one of the primary factors limiting the nutritive value of cereal grains and legume seeds. The interaction mechanism can be explained by the structure of the phytate molecule. Phytic acid is a strong chelating agent that can bind mono- and divalent metal ions to form the complex phytate. It is strongly negatively charged at all pH values normally encountered in foods, and thus it has high potential for complexing or binding with proteins and minerals. Animal feeding trials have revealed poor availability of minerals such as zinc, magnesium, phosphorus and possibly iron when the diets have contained high levels of phytate. High dietary calcium levels appears to exacerbate the effect of phytate on zinc availability. The formation of zinc-calcium-phytate complexes in the upper digestive tract of monogastric animals is the major mechanism by which phytate reduces zinc bioavailability (Richardson *et al.*, 1985).

Phytic acid may interfere with the digestibility of proteins and amino acids. Liener (1994) suggested that the mechanism could involve phytate inhibition of the activity of several digestive enzymes such as trypsin, pepsin, pancreatin and amylase. The inhibition may result from the chelation of calcium ions which are essential for the activity of these enzymes. Phytic acid has also been shown to form salt-like linkages with the terminal alpha of protein or peptides, epsilon amino group of lysine and with the guanidyl amino groups of arginine (Cheryan, 1980). The complexing of proteins with PA, either directly or indirectly via mineral ions, may alter the protein structure, leading to reduced solubility, functionality and digestibility of the proteins (Cosgrove, 1980).

Phytic acid may also bind with starch either directly through the formation of phosphate linkages, or indirectly through its association with proteins (Thompson, 1990), leading to reduced starch digestibility and lowered blood glucose levels (Yoon *et al.,* 1983).

Phytate phosphorus is either unavailable or only slightly availability in simple stomached animals (Andrews *et al.,* 1973; Nelson *et al.,* 1968; Simons *et al.,* 1990). This is because these animals lack the enzyme phytase, which is required for the hydrolysis of phytate. Fish, like terrestrial monogastric animals, also do not secret phytase in their digestive systems (Hastings, 1976; Lall, 1990) and therefore are unable to utilize phytate phosphorus. Indeed, phytate phosphorus has been shown to be unavailable to channel catfish (Andrews *et al.,* 1973), trout and salmon (Ketola, 1982, 1985, 1992), red sea bream (NRC, 1993) and carp (Ogino *et al.,* 1979) and most of it is excreted in the faeces. Zinc utilization has also been shown to be affected negatively by phytic acid in channel catfish (Gatlin and Phillips, 1989; Satoh *et al.,* 1989). In rainbow trout, Riche (1993) reported that about 75% of faecal phosphorus was in the form of phytate. Spinelli *et al.* (1983) found that rainbow trout fed diets containing 0.5% PA had a 10% reduction in growth and feed conversion compared to control fish fed diets without PA. The work of Richardson *et al.,* (1985) also revealed that high dietary phytic acid content not only reduced growth, feed utilization and protein conversion in juvenile chinook salmon, but also caused hypertrophy and vacuolization of the pyloric caecal epithelial cells. They speculated that this effect could have impaired nutrient absorption resulting in poor fish performance. Apart from being an antinutritional factor, phytate also poses special problems in the formulation of aquaculture feeds since it is necessary to supplement the diets with inorganic phosphorus. This leads to increased excretion of phosphorus in the faeces which have important implications on allowable fish stocking densities in countries where stocking densities are determined by the total nutrient output in the faeces.

2.3.2.3. Phenolic compounds:

Phenolics can be defined as substances having a molecular weight greater than 500 (consist of flavoid units linked by carbon-to-carbon bonds) and where the degree of hydroxylation

and the molecular size is sufficient to form complexes with proteins and other polymers at a suitable pH and concentration (Goldstein and Swain, 1953). They are widely distributed in foods and feeds of plant origin. Some phenolic compounds are essential metabolites while others have unknown functions and may be unique to particular plant families or genera. In rapeseed, phenolics occur as free acids, or are esterified and insolubly bound. Phenolic esters and free phenolic acids make up to 80% and 16%, respectively, of total phenolics, with sinapine being the dominant phenolic acid (Kozlowska *et al.,* 1990). Phenolic acids may be responsible for the reduced diet palatability and poor feed intake in animals due to their bitter taste and astringent flavors. Also in poultry, sinapines are converted to trimethylamine (TMA) in the large intestine and this is responsible for the fishy odour in brown shelled eggs from Rhode Island Red hens. Phenolic compounds and their oxidation products form complexes with essential amino acids and other substances and hence lower their nutritional value (Milic *et al.,* 1968; Martin-Tanguy *et al.,* 1977; Kumar and Singh, 1984). These complexes may be produced through the formation of hydrogen bonds. Enzyme-catalyzed oxidation of phenols in seeds result in quinoidal production and hydrogen peroxide formation, both of which are destructive to labile amino acids. Also they denature proteins and inhibit enzymes such as indole acetic acid oxidase, trypsin and lipase by binding covalently with amino, thiol and methylene groups. The epsilon amino group (ε-NH_2) of lysine and the thioether (CH_3S) group of methionine readily react with oxidized phenols to form complexes that render them nutritionally unavailable to the monogastric animal (Sosulski, 1979).

Tannins occur either as hydrolyzable tannins, so called because they may be readily hydrolysed into mixtures of carbohydrates and phenols (gallic or ellagic acids), or condensed tannins which are complex flavanoid polymers. Generally, hydrolyzable tannins are more reactive than the condensed tannins, and their oxidized derivatives form complexes with proteins that are resistant to the digestive enzymes of the monogastric animal. The relative affinity of tannins for different proteins varies as a function of protein molecular weight, open or closed structure, presence of hydrophobic amino acids, and proline content. The latter is due to proline's inability to fit into the α-helix which leads to a loose, open structure readily accessible to tannins and the

formation of hydrogen bonds with phenolic groups of tannins (Haggerman and Butler, 1981).

The tannin content of legumes ranges from a high of 20 mg/g in faba beans to a low value of 0.45 mg/g in soybeans. Shahidi and Naczk (1980) found that canola varieties contain 0.68% to 0.77% condensed tannins, with hulls accounting for only 0.02-0.22% of the extractable tannins (Mitaru *et al.,* 1982). Cyanidin, pelargonodin, leucocyanidin and an n-butyl derivative of cyanidin have been identified as the major types of tannins in rapeseed (Leung *et al.,* 1979). The presence of tannins in a feedstuff will significantly reduce protein and dry matter digestibility. This is because both condensed and hydrolysable tannins react with terminal amino acids of proteins in the gut to produce indigestible complexes. They may also bind endogenous enzymes such as trypsin and lipase, but this is not thought to interfere with nutrient digestibility (Butler *et al.,* 1982). Tannins also react with methionine, possibly with other essential amino acids and proline, thus making them unavailable, which in turn lowers the utilization of dietary proteins (Amstrong *et al.,* 1974). Other antinutritional effects associated with tannins include damage to the intestinal mucosa, and the inherent toxicity of any of the tannins or their metabolites that may be absorbed from the intestine. Also they may interfere with the absorption of iron, glucose and vitamin B12 . While the actual mechanism of protein binding by tannins has not been fully clarified, some workers have suggested several ways that it could happen (Haslam, 1974; Hagerman and Butler, 1980; Loomis, 1974). Tainting of eggs may also be due to the formation of a tannin-TMA oxidase complex making the enzyme unable to breakdown TMA to TMA oxide (Butler, 1982; Fenwick *et al.,* 1979, 1984). Although there is little information on the effect of tannins on fish performance, Hossain and Jauncey (1989) suggested that tannins may have had partially contributed to the poor growth responses that they observed when carp were fed diets containing oilseed meals.

In conclusion, research on oilseeds protein sources has demonstrated that phenolic compounds may be as important as non-enzymatic browning or oxidation of unsaturated lipids in the development of adverse flavours, colours, odours and antinutritional effects on proteins and vitamins (Sosulski, 1979).

2.3.2.4. Carbohydrates:

Protein represents about two-thirds of the cost of formulated diets for salmon. Part of the high cost is due to the high relative needs of salmon and other salmonids for protein due to their inherent propensity to catabolize amino acids rather than carbohydrates (glucose) for energy. In recent years much work has been devoted to the protein-sparing effects of carbohydrates in fish, and although carbohydrates are the least expensive form of dietary energy for both man and domestic animals their utilization by fish varies and remains somewhat obscure (Wilson, 1994). The utilization of carbohydrates is generally poor due to deficiencies in both digestive and metabolic capacity. However, it is important to provide the appropriate level and type of carbohydrate in the diet for fish because, if carbohydrates are not provided, other energy yielding nutrients such as protein and lipids will be catabolized for energy and for provision of metabolic intermediates for synthesis of other biologically important compounds.

In fish, the digestibility of carbohydrates varies with the species and also with source and level of carbohydrates in the diet. Processing methods (cooking, extrusion) also affect the digestibility of carbohydrates in fish. According to Singh and Nose (1967), the digestibility of raw starch by rainbow trout is low and its digestibility decreases as the level of starch in the diet is increased. Other workers have reported higher digestibility values for cooked starch than raw starch for rainbow trout (Smith, 1976; Smith *et al.,* 1980). Generally, warm water fish are able to utilize higher dietary levels of carbohydrates than cold water or marine fish. This may be related to the differences in relative amounts of amylase present in the digestive system of the various species of fish (Hoffer and Sturmbauer, 1985; Shimano *et al.,* 1977). Kaushik and Oliva-Teles (1985) have reported increased protein and energy utilization and a concomitant decrease in the nitrogenous metabolic losses associated with protein deamination for energy provision in rainbow trout fed diets containing gelatinized starch.

The optimal or recommended dietary level of digestible carbohydrate varies among fish species (Ellis and Reigh, 1991; El-Sayed and Garling, 1988; Furuichi and Yone, 1980; Garling and Wilson, 1976, 1977; Hilton and Atkinson, 1982; Nimatipour *et al.,* 1992). Generally, levels

of about 20% digestible carbohydrate is acceptable for marine and cold water fish whereas higher levels (as high as 40%) are used by warm water fish (Wilson, 1994). In certain situations, Kaushik *et al.,* (1989) reported that diets containing digestible carbohydrate levels as high as 38% may be used efficiently by salmonids. The variation in relative utilization of dietary carbohydrates by fish appears to be related to the complexity and source of the carbohydrate (Akiyama *et al.,* 1982; Buhler and Halver, 1971; Furuichi and Yone, 1982; Wilson and Poe, 1987), fish species, water temperature and the life history stage of the fish. Increase in frequency of feeding has also been shown to improve utilization of simple sugars in tilapia and carp (Murai *et al.,* 1983). High levels of digestible carbohydrate in salmonid diets however, have been reported to result in increased blood glucose (hyperglycemia), liver size and glycogen content proportional to the dietary carbohydrate level (Buhler and Halver, 1961; Pieper and Pfeffer, 1979). High contents of digestible carbohydrates in salmonid fish diets have also been shown to cause liver abnormalities due to accumulation of glycogen. Similar effects have also been reported in sea bream (Furuichi and Yone, 1971), plaice (Cowey *et al.,* 1975) and yellow tail (Shimeno *et al.,* 1977). The relative inability of salmonids to utilize high dietary levels of carbohydrates has been thought to be due to suboptimal secretion of endogenous insulin (Palmer and Ryman, 1972; Wilson and Poe, 1987). However, the work of Plisetskaya (1990) and Mommsen and Plisetskaya (1991) has shown similar or even higher levels of plasma insulin in fish than in mammals. This relative intolerance of salmonids to high glucose doses despite their high levels of circulating insulin suggests non-insulin-dependent diabetes mellitus rather than insulin-dependent diabetes (Hertz *et al.,* 1989; Hilton *et al.,* 1987). The lack of an inducible glucokinase and the low activity of hexokinase, and the possible saturation and subsequent decrease in the activity of the hexokinase enzyme by glucose have been suggested as other causative agents for the poor utilization of complex carbohydrates by salmonids (Tung and Shiau, 1991; Wilson, 1994). In addition, the possible inhibition of insulin release by somatostatins due to high blood glucose levels and the relatively low potency of glucose compared to certain amino acids as a stimulant for insulin release may be other factors as well. Enhanced hexokinase activity due to feedback-inhibition by glucose-6-phosphate may explain why

carbohydrate utilization is improved as feeding frequency is increased. Excessive amounts of digestible carbohydrates in salmonid diets depress growth rate and increase mortality (Hilton and Slinger, 1982).

Oligosaccharides are formed by the successive addition of the galactosyl moiety to a sucrose primer and they accumulate in many leguminous species during seed development (Saini, 1988). Monogastric animals also lack the digestive enzyme for the hydrolysis of oligosaccharide sugars (raffinose and stachyose), also referred to as alcohol soluble carbohydrates (ASC). These sugars are fermented by intestinal bacteria in the large intestine resulting in the production of volatile fatty acids (VFAs), carbon dioxide, hydrogen and methane (Saini, 1989). When present in high concentrations in the small intestine these sugars also increase the osmotic pressure of luminal contents, which in turn may cause osmotic diarrhoea. Although oligosaccharides containing stachyose and raffinose have been implicated as the principle causes of flatulence in human and animal studies this should not be a source of concern in fish where hind gut fermentation would be expected to be absent. In fish, alcohol-soluble carbohydrates have been shown to have a negative effect on lipid digestibility (Arnensen *et al.,* 1989). Several mechanisms have been suggested to explain this effect. These include the alteration of feed passage rate, inhibition of digestive enzymes, and alteration of micelle formation of absorptive surfaces. The same authors, while working with rainbow trout and Atlantic salmon, also found that these substances have negative effects on dry matter digestibility and they reported that the presence of ASCs may be one of the most important factors depressing nutrient absorption.

Carbohydrates also react with proteins in the presence of heat, forming indigestible protein-carbohydrate complexes (Maillard non-enzymic browning products). Maillard reaction products are the result of the reaction between the amino groups of proteins, peptides and amino acids and the glycosidic hydroxyl groups of reducing sugars. These complexes impair the digestibility of the protein product which passes undigested in the faeces. The formation of Maillard products is also a function of temperature and the presence of moisture. The extraction of oil from oilseeds entails the production of considerable heat which can be detrimental to the

nutritive value of the protein. Because of the use of heat in the processing of canola meal there is the possibility that the protein could sustain some damage. Therefore, although analytical data may give an adequate amino acid profile in the meal, the amino acids may not be totally biologically available to the animal because of losses or formation of complexes with reducing sugars. While all amino acids are susceptible to heat damage, lysine appears to be preferentially complexed by reducing sugars.

2.3.2.5. Fibre:

Dietary fibre is a heterogeneous group of compounds. Fibre can be classified as soluble (some hemicelluloses, pectins, gums, mucilages) and insoluble (most hemicelluloses, cellulose and lignin) fibre, the latter passing through the gastrointestinal tract without major alteration. In fact, Hilton *et al.* (1983) defined fibre as all that part of plant matter in animal diets such as cellulose, lignin and other complex carbohydrates that is indigestible. The various components of fibre, including cellulose, hemicellulose and lignin, are variably available to non-ruminants. Cutin which is a waxy polymer of hydroxy fatty acids found on leaves and seed hulls, is also indigestible in non-ruminants, as are other associated waxes (Theander and Aman, 1984). Release of energy-yielding molecules through microbial fermentation of the main fibre components, cellulose and hemicellulose in the colon and caecum of monogastric animals although important, occurs at a very low efficiency (Low, 1985). In addition, the presence of indigestible and unfermentable lignin complicates the issue by co-bonding with cellulose reducing its fermentation even more (Theander and Aman, 1984). High dietary fibre (>10%) in the diet of fish has been reported to depress mineral availability due to the ion exchange characteristics of some of the fibres. Fibre has also been shown to decrease the transit time of intestinal contents in addition to adsorbing amino acids and peptides thereby withholding these from absorption in monogastric animals (Krogdahl, 1989). These effects may be influenced by the degree of lignification.

Soluble fibres have been shown to inhibit the activity of digestive enzymes *in vitro* (Dutta and Hlasko, 1985; Isakson *et al.*, 1982) due to the fibre absorbing the enzymes, leading to

increased enzyme secretion. Fibre also has the ability to bind bile salts (Vahouny *et al.*, 1980, 1981) and thereby decreasing their activity. This causes the liver and gallbladder to respond by increasing bile secretion. This may be part of the reason for decreased lipid absorption. High dietary fibre levels may also cause increases in faecal nitrogen and lipid excretion. The inclusion of fibre in the diets of monogastric animals has also been shown to increase sloughing of intestinal mucosal cells and to enhance mucus production, leading to high losses of endogenous amino acids. The gelling effect of soluble fibres restricts diffusion of hydrolysis products (intestinal absorption) by covering the absorptive lining of the intestine leading to increased thickness of the unstirred water layer (Green and Greene, 1984). Soluble fibres also slow down the intestinal transit time, change the microflora environment and increase the time of exposure to substrates. These responses influence the digestion of dietary components and may result in the generation of toxic substances, such as the deconjugation and dehydroxylation of bile salts to secondary bile salts which have negative effects on lipid absorption (Cole and Fuller, 1984). Fibres also affect the receptors that are responsible for regulating the flow of chyme, secreta and ingested feed as well as those that mediate signals that influence the internal metabolism of nutrients. These effects can depress protein and energy availability. The natural diet of salmonid fishes generally contains little dietary fibre, but formulated diets contain varying amounts of complex carbohydrates depending upon the inclusion level of plant ingredients in the formulation (Hilton *et al.*, 1983). Salmonids are unable to utilize cellulose and other fibre components. The small capacity of the digestive system, the short alimentary tract, short food retention time and the low body temperature in poikilothermic salmonids allow little opportunity for microbial action necessary for utilization of non-starch carbohydrates. Rainbow trout fed guar gum and alginate have been found to excrete increasing amounts of water, nitrogen and fat with increasing levels of fibre. Studies of the effects of soybean hulls and wheat bran in diets for rainbow trout have also only shown moderate effects on nutrient absorption and water retention. The effects on the absorption of lipids, especially the long-chain saturated and monounsaturated fatty acids, were most pronounced. Buhler and Halver (1961) observed depressed growth rates in chinook salmon fed diets with increasing levels of

alpha-cellulose. While the feeding of high fibre diets to rainbow trout has not been reported to affect blood parameters (protein, glucose, haematocrit and haemoglobin), body mineral content or proximate composition, significant decreases in diet dry matter digestibility, and increases in the stomach weights to accommodate the increased bulk of the diets have been noted (Hilton *et al.*, 1982). Salmonids are unable to digest cellulose or metabolically utilize high dietary levels of digestible carbohydrate effectively. Therefore, the reduction of these constituents not only improves the nutritive value of plant protein sources but also increases their protein concentration. According to the National Research Council (NRC, 1993) the fibre content of salmonid diets should not exceed 8%.

2.4. Elimination/Reduction of ANFs

The nutritive value of many plant proteins is lower than expected on the basis of their chemical composition. This is because of chemical and physical properties that result in reduced biological availability (digestibility) of one or more of their nutrients (van der Poel, 1989). The role of legume seeds in non-ruminant diets could be much greater than at present if the negative effects of several common and unique limiting factors (ANFs) were adequately and economically eliminated. This goal can likely be achieved by one or a combination of the different strategies outlined below.

2.4.1. Plant breeding:

Plant breeding (genetic manipulation) is a long term strategy for removal or reduction of ANFs (van der Poel, 1989). There is evidence for genetic variation in most of the ANFs of legumes and it should therefore be possible to reduce in their levels through breeding programs. This is well illustrated by the production of canola from rapeseed. Due to the presence of ANFs and the widespread use of rapeseed, plant breeding research has been undertaken to improve the chemical composition and seed yield. The objectives of this breeding program have been to produce varieties with high yields of oil and protein and reduced levels of erucic, eicosenoic and

linolenic fatty acids, glucosinolates, fibre, and sinapine (Niewiadomski, 1990). In 1974, the efforts of Canadian plant breeders led to the development of the world's first *Brassica napus* canola variety of rapeseed and three years later this was again done with the summer variety *B. campestris*. Canola is the registered name given to genetically selected varieties of *Brassica napus* and *B. campestris* that are low in both glucosinolates (<30 μmol/g) and erucic acid (<2% of total fatty acids in the oil) (Bell, 1993). Canola varieties increase the profitability of cultivation, and the oils and meals have an improved nutritional value. The reduced amount of linolenic acid in the oil improves the oil's sensory properties and the stability. However, it should be mentioned that improvement in one characteristic is often accompanied by a deterioration of another. For example, the oil content of Canadian seeds increased by 5% between 1956 and 1976, but the protein content declined by about 5.5% during the same period. A decrease in the erucic acid content of the oil has also been shown to be accompanied by an increase in the oleic acid content (Niewiadomski, 1990). This is due to a deficiency of the enzyme responsible for the processing of carbon chain elongation during the formation of erucic acid. The genetic manipulation of the fatty acid composition of *Brassica* oils is an active research area, although nutritionists contend that the present fatty acid composition of canola oil is highly desirable for human health (Downey and Bell, 1990). The presence of as little as 8-10% short chain fatty acids reduces the tendency for canola margarines to form undesirable crystals when held in storage. Another concern of the plant breeder relates to the fact that most ANFs have important functions in the plant either as nutrient reservoirs or in protecting the plant against pests or diseases. Therefore reduction in the levels of ANFs may increase disease susceptibility. Damage to low-tannin varieties by birds and the concern about the possible susceptibility of low-glucosinolate canola to small mammals are examples. Current breeding research underway in Canada is aimed at producing canola varieties that offer many advantages, including higher seed yield, less pod shattering, excellent disease resistance, early maturity, and bright yellow seed with thinner hull (lowered fibre content).

2.4.2. Mechanical processing

Several different methods of processing have been attempted to obtain oilseed meals of improved nutritional quality. Front-end dehulling of rapeseed/canola is considered to be uneconomical because the hulls contain about 20% of the seed oil. Also, there are difficulties in the process of dehulling seeds of uneven size in a solvent extractor. There is also the problem of marketing the hulls. Air classification of defatted meal into fine and coarse fractions can increase the protein of the fine fraction by about 6% and reduce the fibre content by 7-10%. However, the small improvements may not be worth the cost (McCurdy, 1990). The excessive fineness of the meal is also another concern. The most promising strategy for economically reducing the fibre content is by plant breeding. The new yellow seeds have a thinner hull, and hence the fibre content is reduced and the oil- and protein-rich embryo comprises a large proportion of the seed. Meals from these varieties therefore, have a higher digestible protein and energy content.

Since phytic acid is associated with specific components and is found within certain locations in the seeds, mechanical processes such as milling and grinding should be able to selectively separate phytic acid-containing fractions from the rest of the seed (Cheryan, 1980). This strategy may not, however, improve the nutritive value of the products that are produced because the PA content in cotyledons represents about 89% of the total PA in the seed. Dehulling would therefore, increase the PA content of dehulled canola products.

2.4.3. Heat and Chemical Processing

Rapeseed/canola products are not suitable for human and animal consumption unless the enzyme myrosinase has been inactivated and the glucosinolate levels are greatly reduced. Special processing of rapeseed or canola seed or of rapeseed/canola meals themselves can result in products that contain protein and energy contents comparable to those of fish meal (Jones 1979; McCurdy and March, 1992). Heat treatment, irradiation, soaking, fermentation, enzyme treatment or supplementation of ingredients have shown encouraging results. Detoxification (removal of glucosinolates) of rapeseed and canola meals has been achieved by inactivation of myrosinase and

the destruction of glucosinolates and their degradation products by the use of heat. The effect of heat is to inactivate the proteinaceous ANFs and this results in increased accessibility of protein to enzymatic attack.. Although heat is effective in inactivating myrosinase, excessive heat may damage the protein. Further, because of the presence of myrosinase in intestinal bacteria, this is not an appropriate way to totally eliminate the antinutritional effects of residual levels of glucosinolates. Various chemical treatments have been found to facilitate ANF inactivation at low temperatures. Dehulled seeds may be subjected to boiling water to inactivate myrosinase and remove problem sugars (raffinose and stachyose) and sinapine. Crushed and fibre- reduced seeds can also be exposed to a two-phase solvent system comprised of methanol, ammonia and water. Alkanol or alkanol/ammonia solutions have been reported by Schlingman and coworkers (Schlingman and Praeve, 1978; Schlingman and von Rymon-Lipinski, 1980; 1982) to be effective in removing undesirable constituents, including glucosinolates. The combined effect of the right ammonia concentration and the polarity of the alkanol determines the actual extent of removal of antinutritional factors. The extraction efficiency has been improved further by the addition of water or the use of ammonium hydroxide (Schlingman and von Rymon-Lipinski, 1980; 1982). Methanolic solutions containing 10% ammonia in the presence of 5% water have been found to be highly effective (Shahidi *et al.*, 1988). Large amounts of water, although improving the removal of glucosinolates, have resulted in a sticky and dark-coloured meal. Reduction in levels of phenolics, carbohydrates, glycosides, nucleic acids and non-protein nitrogen has also been achieved. Ammoniated methanol extracts either intact glucosinolates or their degradation products. The breakdown pathway involves the release of thioglucose from the glucosinolate molecule to yield nitriles or hydroxynitrite or the formation of isothiocyanates and epithionitriles (Shahidi and Gabon, 1989). No oxazolidinethione is produced in the process. McCurdy and March (1992) further processed fibre-reduced canola meals by washing them with selected solvents such as acidified water (pH 4.5), 80% aqueous ethanol, ammoniated aqueous methanol, or acidified water and ethanol to decrease the levels of antinutritional factors and concurrently increase the protein

content.

2.4.4 Enzyme Treatment

The benefits gained by the destruction of antinutritional factors in feedstuffs using enzymes have been reviewed by Chesson (1987). The addition of pectic enzyme preparations to diets containing significant amounts of plant protein improved feed utilization in farm animals. Endo-β-glucanase has been demonstrated to be very effective against the mixed-linked glucans found in relatively high concentrations in the endosperm walls of barley. Enzyme addition has also been used to eliminate the antinutritional effects of glucosinolates (Mukherejee *et al.,* 1976; Vaccarino, 1977). This latter method, however, may have the disadvantage of leaving the organic aglucons in the oil.

Monogastric animals have little or no intestinal enzymes for the hydrolysis of phytate and cell wall polysaccharides that are found in feedstuffs of vegetable origin. Pretreatment of these protein sources with microbial phytases and cell wall polysaccharide enzyme mixtures causes the degradation of these ANFs and the release of nutrients that can be utilized by animals. Several other studies have been conducted to remove the adverse effects of antinutritional factors by using enzyme treatment (Frølich *et al.,* 1986; Jongbloed *et al.,* 1992; McCurdy and March, 1992; Prendergast *et al.,* 1994; Sugimoto and van Baren, 1970). Stone *et al.* (1984) also reported improvement of the nutritive value of canola meal by reducing its high phytate content through blending canola meal with acidified fish wastes and wheat as a result of dephosphorylation of phytate by phytase from wheat bran.

2.4.5 Dialysis and Membrane Processing

Dialysis is another technique that can be used to eliminate ANFs, particularly phytate, as long as the pH and cation concentrations are closely monitored. If phytic acid or its salt are not complexed with the protein and are in soluble form, it becomes feasible to separate them using methods based on differences in molecular size. Using this principle, Smith and Rackis (1957)

were able to remove most of the phytate from a soybean meal suspension with minimum loss of protein at pH 7. Extraction with a saturated solution of ammonium sulphate followed by dialysis to remove salt was also effective in lowering phytate content. Although dialysis is an effective way of removing phytate, it is not practical on a large commercial scale.

Ultrafiltration (UF) is another separation process that is based on the selectivity of membranes to discriminate between molecules of different sizes and shapes. UF is a pressure-activated process that causes greater rates of removal of solution. It is a rapidly developing technology and a wide variety of applications have been reported (Porter and Michaels, 1971). Phytate has a molecular weight of 1000 or less, hence a membrane with a "molecular weight cut" of 10,000 to 50000 is suitable for the production of low phytate protein products (Cheryan, 1980). Mild operating conditions are used during UF, thus resulting in a product with functional properties superior in some cases to those produced by other methods. This method has been used to produce low phytate soybean protein isolate and concentrate from defatted soybean meal (Okubo *et al.,* 1975). A reduction of phytate by 90% from full-fat soybean meal extracted with water has been reported by Omasaiye and Cheryan (1979) when UF was carried out at pH 6.7. The advantage of this method is that no manipulation of pH or chemical treatment is necessary to produce low phytate protein products from oilseed meals.

2. 5 NUTRITIONAL EVALUATION OF CANOLA MEAL

2.5.1 Commercial processing of canola

Currently, three types of commercial processing systems are employed for producing oil and meals from oilseed. In the expeller process the oil is mechanically squeezed from the seed. In the prepress solvent extraction process, a portion of the oil is removed by expellers and the remainder is then extracted with organic solvents. The solvent is then removed from the oil by a solvent recovery system which ensures a solvent-free oil. The direct solvent method removes all the oil directly from the conditioned seeds with an organic solvent. The extracted oil must be refined (physical, steam or alkali refining) to remove phosphatides and free fatty acids or to reduce

these constituents to very low levels in order for bleaching, hydrogenation and deodorization to be carried out. The cake fragments which remain after oil extraction are injected with live steam to remove residual solvent and the resulting meal is dried in kettles. The meal is granulated after cooling, pelletized or stored directly for subsequent use as a feed ingredient.

Regardless of the method of oil extraction used, it is necessary to maintain a high quality of meal. High temperature treatment and residual oil in the meal due to poor rupture of cell walls reduces the quality of the meal and the rate of oil recovery. Several approaches have been attempted in order to obtain meals of the highest quality. Proteolytic and cell-wall degrading enzymes have in some cases been employed to improve the release of oil from finely ground slurries of melon, soybean and rapeseed (Fullbrook, 1983). Sosulski and Sosulski (1988, 1990) used carbohydrase enzymes to pretreat canola seeds in order to modify cell walls and cotyledons. This led to increased extraction of oil and improved quality of the meal. Enzymatic processing without the use of organic solvents has also been used by Jensen *et al.* (1990) to produce canola meal that is rich in protein (53%) but low in phenolic compounds and glucosinolates. The cell-wall degrading enzymes increase the permeability of the cell wall, allowing the low molecular weight compounds to be washed out and separated from the meal.

2.5.2. Production and cost of rapeseed protein

In addition to nutritive value, the overall assessment of the potential use of rapeseed/canola products or any other novel protein source in fish diets should include consideration of supply and cost. Rapeseed/canola ranks third in the world production of oilseeds (11.6%) and contains 21-23% protein, thus making its protein potentially more available for inclusion in animal diets than the global quantity of protein produced annually from fish meal (Higgs *et al.*, 1994). The worldwide production of *Brassica* oilseeds is now expanding faster than that of any other annual oilseed crop. More than thirty countries now produce rapeseed, making *Brassica* oilseeds the third most important world source of edible oil after soybean and palm. The production of rapeseed/canola oil and meal has increased in step with the overall production of rapeseed

(Niewiadomski, 1990). The largest rate of increase has occurred in Canada, where in 1994 the annual area devoted to canola is about 14 million acres. In Canada, rapeseed is the dominant oilseed crop and the summer varieties of this oilseed are grown extensively in the Prairie provinces and in British Columbia (Higgs *et al.,* 1982). The two main species grown are, *Brassica napus* (Argentine type) and *B. campestris* (Polish type). Development of rapeseed varieties with low levels of glucosinolates (canola) suitable for the short growing season and the climatic conditions has helped to make this increase in production and utilization possible. Canola is now Canada's main source of edible oil, with 61% of the market (Downey and Bell 1990). This high consumption in Canada can be attributed to the conversion to low erucic acid rapeseed (LEAR) varieties of which the "double low" cultivars account for 87 % of all deodorized vegetable oil consumption (Vaisey-Genser and Eskin, 1987). In recent years there has also been a growing interest in the cultivation of rapeseed in the U.S.A. after the USDA granted GRAS (generally recognized as safe) status to low erucic acid rapeseed (canola) oil in 1985 (Ohlson, 1992). Growers predict a potential area of 5 million acres, mainly in the Eastern and Southeastern states.

The cost of canola meal expressed in U.S. $/kg protein is presently 46.1% of that of South American anchovy meal (0.365 versus 0.791 U.S. $/kg protein) and below that of soybean meal (0.419 U.S. $/kg) according to the estimates of Higgs *et al.* (1995). Therefore extensive replacement of fish meal with rapeseed/canola meals would reduce the production cost of salmon farming considerably. More than a decade ago Hardy and Sullivan (1983) estimated that feed costs could be reduced by $18.70 U.S. per metric ton when canola meal constituted 20% of the diet.

2.5.3 Nutritional composition of canola meal

2.5.3.1 Proximate composition

The proximate composition of canola seed varies widely because of both genetic and environmental factors. Indeed, several studies have shown considerable variation in protein content between species, cultivars and samples of certain cultivars (Bell, 1984; Salunkhe *et al.,* 1992).

The crude protein (CP) content of canola meal may range from 33 to 40%, considerably less than soybean meal (45-50%). The majority of these proteins are classified as albumins and globulins (70%). Some of the protein is unavailable due to binding by lignin, tannins and fibre. Like most processing by-products of plant origin, canola meal contains a substantial amount of fibre. The high content of hulls and indigestible polysaccharides are among the most important factors limiting the utilization of canola meals in animal diets. The dark seed coat contains a polyphenol complex as a component of the fibre in commercial rapeseed meal (Jones, 1979). The crude fibre content of canola/rapeseed meal is about 12%. McCurdy and March (1992) reported acid detergent (ADF) and neutral detergent fibre (NDF) values of about 20% and 24% of dry matter (DM) respectively. The high fibre content of rapeseed/canola meals contributes to their reduced levels of gross energy, about 17.7 MJ/kg and available energy in animals (Higgs *et al.,* 1995b). Low levels of residual lipid in the meal (<4.0%) and protein are also partly responsible.

2.5.3.2 Fatty acids and triacylglycerols

Unlike many vegetable crops, the fatty acid composition of rapeseed varies between cultivars, mainly due to their dissimilar erucic acid (C22:1) content. However, due to selective breeding of rapeseed, major changes in the fatty acid composition of the oil have occurred. Rapeseed/canola contains a substantial amount of long-chain fatty acids (C18) in the oil. New low erucic acid varieties are characterized by very high levels of oleic, linoleic and linolenic acids and a substantial level of eicosenoic acid (C20:1). The composition of minor fatty acids in both high and low erucic acid varieties has also been studied (Sebedio & Ackman, 1979). Triacylglycerol composition of canola/rapeseed is characterized by the occurrence of C20 and C24 fatty acids exclusively in the 1 and 3 positions, C18 fatty acids, linoleic and linolenic acids in position 2 (Appelqvist, 1972). The positioning of the polyunsaturated fatty acids gives canola/rapeseed oil a greater oxidative stability than that of soybean oil. However, these changes have little effect on the nutritional value of the meal which contains 4 to 6% oil.

2.5.3.3 Amino acid composition

The available levels of the ten essential (indispensable) amino acids in plant and animal protein sources influence their nutritive value. The amino acid composition of canola/rapeseed meal has been shown to compare favorably with or to be even superior to that of soybean meal (Bell, 1884; Niewiadomski, 1990). The amino acid composition of commercial canola meal is influenced by the way the seeds are processed. For example, the content of basic amino acids, especially that of lysine, is greater in meals produced by the prepress-solvent extraction procedure or by solvent extraction alone than in meals produced by the expeller procedure. Although the lysine content of canola/rapeseed meal is lower than that of soybean meal, the former contains higher sulphur amino acid levels, which improves its nutritional value (Niewiadomski, 1990). Higgs *et al.* (1988) examined the potential quality of rapeseed meal and canola/rapeseed protein concentrates for rainbow trout using the essential amino acid index (EAAI) procedure of Oser (1959) and found that they had quality equal to that of herring meal protein and superior to that of soybean meal protein.

2.5.3.4 Mineral and vitamin composition

Canola/rapeseed meal contains 4-7% ash and the mineral composition depends strongly on the soil on which the plant is grown (Salunkhe *et al.*, 1992). The mineral content of canola/rapeseed has not been regarded as a special problem area. Niewiadomski (1990) reported that rapeseed meal was a better source of calcium, phosphorus, zinc, iron, manganese, magnesium and selenium than soybean meal. The last element is important in the prevention of exudative diathesis in chicks. However, as with cereal grains and oilseed meals in general, the phytin content of rapeseed/canola meals influences the availability of phosphorus, calcium and zinc and this effect requires attention when formulating diets with high levels of canola/rapeseed protein products (Bell, 1984). Even greater care must be taken when formulating diets for fish because they lack the enzyme phytase, which is required for the hydrolysis of phytate. About 60-90% of total oilseed phosphorus is in the form of phytic acid, the hexaphosphate of myoinositol. This is

present in the form of a complex salt of calcium, magnesium and potassium. When compared to fish meals, canola/rapeseed protein products contain less calcium and more magnesium, and either lower (meals) or similar (concentrates) levels of phosphorus.

The vitamin composition of canola/rapeseed has been reported by several workers (Finlayson, 1977; Josefsson, 1972). Canola/rapeseed meal contains more niacin, folic acid and biotin than soybean meal, while the amounts of pantothenic acid, riboflavin and thiamine are similar. Canola/rapeseed meals also contain more choline and vitamin E than soybean meals. In fact, only cottonseed meal and sunflower meal contain more vitamin E than canola/rapeseed meal among the oilseed meals. Vitamin E is known to have antioxidative properties. Although vitamins in canola/rapeseed meal have not been associated negatively with the feeding value of the meal it is noteworthy that sinapine is an ester of sinapic acid and choline. The bitter taste of sinapine may cause feed palatability problems (Blair and Reichert, 1984) and fishy odour in brown shelled eggs (Fenwick *et al.*, 1979).

2.5.3.5 Canola/rapeseed hulls

Canola/rapeseed hulls may form 27% to 30% of the seed after removal of the oil. It is difficult to assemble a meaningful composition table for canola/rapeseed hulls due to uncertainty about the amounts of lignin and polyphenols in hulls, as well as disagreement between values obtained by different analytical methods. The composition also differs between hulls from brown and yellow seeded varieties, with the latter containing less fibre. Rapeseed hulls are high in crude fibre, and in acid and neutral detergent fibre (60% and 71%, respectively, on an oil-free basis (Downey and Bell, 1990). Cellulose is the dominant carbohydrate, with the rest being pentosans (14.5%). Pectin has also been reported in rapeseed hulls (Aspinell and Jiang, 1974). The tannin content of rapeseed hulls has been reported by several workers (Leung *et al.*, 1979; Naczk *et al.*, 1994) to be about 1.5%, with most of it in nonextractable form. Because of the carbohydrate composition of rapeseed hulls and the amount of lignin, the digestibility of rapeseed hull carbohydrates by monogastric animals is low. Digestibility coefficients for protein and energy in

animals have been found to be higher for yellow hulls than for brown hulls due to the differences in their composition (Downey and Bell, 1990).

2.5.3.6 Carbohydrates

Carbohydrates constitute a relatively small proportion of the seeds of rape and canola. They are found in both the cotyledon and hulls. The high molecular weight polysaccharides include cellulose, hemicellulose, pentosans and pectins and they make up a large proportion of the hulls. Low molecular weight carbohydrates are more abundant in the cotyledons and embryo. In general, soluble sugars, cellulose, hemicellulose and starch comprise respectively 10%, 5%, 3% and less than 1% of dry weight of canola/rapeseed meals (Nazck and Shahidi, 1990). Pectins and amyloid and arabinose-based carbohydrates are also dominant. These are not regarded as being readily digested by enzymes secreted into or by the gastrointestinal tract (Bell, 1984). The polysaccharide fraction of canola consists of 3.5% arabinogalactan, 6.9% arabinan, 15.5% amyloid, 24.1% cellulose and 50% pectin. The low values for digestible and metabolizable energy content of canola meals for animals is due to the effect of these high molecular weight carbohydrates. The low-molecular-weight carbohydrates make up approximately 10-15% of canola and rapeseed meals (Blair and Reichert, 1984). However, the recorded range in value may vary depending on the method of analysis used (Naczk and Shahidi, 1990). The composition of the soluble sugars has also been reported (Theander *et al.,* 1977). These soluble sugars consists of galactose, glucose, rhamnose, ribose, sucrose, raffinose, stachyose and traces of xylose. Shahidi and Naczk (1990) reported that rapeseed meals contain 3.93-5.73% sucrose, 0.27-0.62% raffinose and 0.83-1.61% stachyose as their major soluble sugars and that canola meals contain over 50% more raffinose than rapeseed meals. Raffinose and stachyose are indigestible to the monogastric animal and it is noteworthy that they are not included as part of the crude fibre fraction. Due to the undesirable effects of raffinose and stachyose, several strategies have been suggested for their removal. These include enzyme degradation (Sugimoto and Van Buren, 1970), soaking, germination and resoaking (Kim *et al.,* 1973) and autolysis at pH 5 and 55 °C (Becker *et al.,*

1974).

2.5.4 Digestibility of canola/rapeseed

2.5.4.1 Dry matter, protein and energy digestibility

Many studies have been carried out on the digestibility of canola/rapeseed meal in monogastric animals (Bell *et al.,* 1981; Sauer *et al.,* 1982). These studies have found depressed values for protein and energy digestibility as the dietary concentrations of canola/rapeseed meals were increased. The values were, in addition to those of amino acid digestibility, also found to be lower than those in soybean meals. Studies on swine fed diets containing rapeseed meals by May and Bell (1971) and Saben *et al.* (1971) found a DE value of about 3210 kcal/kg of dry matter whereas the value of Bell *et al.* (1981) was slightly higher (3370 kcal/kg dry matter). The energy and organic matter digestibility coefficients for swine were found to be 71% and 76% respectively. The protein in canola and rapeseed meal is about 81% digestible for pigs (Bell *et al.,* 1981; Sauer *et al.,* 1982). In fish the digestibility coefficients of plant proteins for both energy and protein are highly variable. This has been attributed to the methods used to assess feedstuff digestibility (Cho *et al.,* 1982) in addition to the presence of antinutritional factors. However, as pointed out by Bell (1984), there may be important differences between animal species in these values.

All fish studies aimed at the assessment of the availability of protein and energy in canola/rapeseed protein products have been carried out using salmon and rainbow trout but none has been undertaken for warm water species. Salmonids' *in vivo* digestibility coefficients of plant proteins tend to be lower than those for fish meal and most other animal proteins. The crude protein and energy digestibility coefficients and digestible energy values have been noted to vary with size, species of fish, water conditions and also with type and source of canola product. Hajen *et al.* (1993) while working with chinook salmon in sea water found that the crude protein digestibility coefficients for glucosinolate-free canola meal, glucosinolate-extracted meal and rapeseed protein concentrate were 87.9%, 78.6% and 95.6% respectively. Canola protein also appears to be more digestible in diets for Atlantic and chinook salmon than for rainbow trout. In

trout, crude protein digestibility coefficients of rapeseed meal range from 63.8% to 92.9%, whereas the range in salmon is between 74.1% to 87.9%. The high availability of protein in rapeseed protein concentrate is because of the minimal levels of ANFs in these products. Protein digestibility coefficients for rapeseed protein concentrate in trout have been found to be almost identical to those for fish meal (Prendergast *et al.,* 1994). The apparent amino acid digestibility coefficients for canola meal using rainbow trout in fresh water and Atlantic salmon in sea water appear to be similar (Anderson *et al.,* 1992; Hilton and Slinger, 1986).

2.5.4.2 *In vitro* digestibility of canola/rapeseed

Feeding trials undertaken to determine the response of fish to diets are long, expensive, tedious and time consuming. The relativey slow growth of cold water fish is also a concern. Hence, different *in vitro* digestibility methods have been developed. Rutkowski and Kozlowska (quoted in Josefson, 1972) reported that the *in vitro* protein digestibility coefficients of canola/rapeseed meal protein were similar to those of other oilseed meals and that processing methods that favor the formation of melanoids (yellow pigments) lower the digestibility coefficients. Recently, McCurdy and March (1992) found that, although the total solubility of canola products obtained through different treatment methods was similar (80-90%), the proportion of protein solubilized by acid alone and that requiring the assistance of pepsin varied considerably among the canola products. Differences also exist between *in vivo* and *in vitro* digestibility coefficients. The digestibility coefficient of rapeseed meal protein has been found to be approximately 10% lower in poultry than that determined by *in vitro* methods, possibly due to the adverse effects of glucosinolates in poultry Rutkowski *et al.* (quoted in Josefson, 1972). Several workers have studied the solubility of rapeseed/canola protein (Gillberg and Tornell, 1976; McCurdy, 1990). They found that it varies with the processing treatments used as well as with the method applied to recover the protein. Low protein solubilities have been observed for canola/rapeseed protein sources that had previously been heated to inactivate myrosinase.

2.6.. Improved canola products

2.6.1. Upgraded canola meals

The minimization or elimination of the adverse effects of the antinutritional factors present in whole rape/canola seed or meal on fish and animal performance (depression of growth, appetite, feed and protein utilization and thyroid function) has been achieved by treatment of seeds or meal with different solvents (water, methanol, ammonia followed by hexane). This has led to the production of nutritionally improved canola products. These products are referred to as upgraded canola meals. McCurdy and March (1992) produced upgraded canola meals by milling and sieving the meals over a 70-mesh screen. Subsequently the fibre-reduced meals were washed with different combinations of solvents. The products resulting from this processing had higher levels of protein and phytate and lower levels of glucosinolates and sinapine than the starting meals. The protein contents of these upgraded canola products (>58% of dry matter), were however, less than that of canola protein concentrate (CPC) produced by the process outlined by Jones (1979).

2.6.2 Rapeseed/canola protein concentrates (RPC/CPC)

These are protein products prepared from high quality, sound, clean dehulled rape or canola seeds by removal of the oil and water-soluble non-protein constituents and contain not less than 60% protein on a moisture-free basis. The basic principle involves the extraction of seeds or defeated meal with solvents that dissolve the polysaccharides, ash and other minor constituents but not the proteins. Aqueous ethanol is the most commonly used solvent, but in a recent modification, defeated flakes are extracted with a combination of hexane and ethanol to remove residual lipid prior to alcohol extraction. Alternatively, crushed and fiber-reduced seeds may be exposed to a two-phase solvent system comprised of methanol, ammonia and water followed by hexane to almost completely remove glucosinolates and decrease carbohydrate, phenolic compounds, non-protein nitrogen and lipid (Shahidi and Naczk, 1990). Subsequent washing with water removes the soluble constituents and a concentrate is obtained by drying the washed product. This yields a full-fat canola concentrate. Another method (FRI -71) was outlined by Jones (1979). In this method

rapeseed or canola seeds are first dehulled, then myrosinase is inactivated with boiling water and the meats are washed with water to remove glucosinolates, problem sugars such as raffinose and stachyose and phenol-like compounds such as sinapine. The resulting cotyledons are then dried and hexane-extracted to yield a fat-free protein concentrate containing about 65-70% protein and 8% lipid. Acceptable fibre levels (<5%) are also achieved. Rapeseed/canola protein concentrates typically have improved levels of protein, lipid, fibre and nitrogen-free extract relative to those of conventional rapeseed/canola meals (Prendergast *et al.*, 1994). Further, unlike the meals, RPC/CPC contains levels of available (digestible) energy and protein similar to those found in fish meal.

2.6.3. Canola protein isolates

Protein isolates are derived from other canola products by dissolution of the protein and generally they contain more than 90% protein (N×6.25) on a moisture-free basis. Extraction and recovery of protein from rapeseed/canola may be conducted by various methods using seeds, meal, flour and concentrates that have received a variety of pre-processing treatments. Protein extraction has been accomplished using dilute alkali (Blaicher *et al.*, 1983; Sosulski *et al.*, 1976), sodium chloride (Lo and Hill, 1971) and sodium hexametaphosphate (Thompson *et al.*, 1977). In some cases, more than one extractant has been used. The preparation of protein isolates from canola meal has been used by several workers as a means of eliminating some of the ANFs (Blaicher *et al.*, 1983). The method is based entirely on the dissolution of the protein using one or more of the aforementioned extraction techniques from the oilseed meal and subsequent recovery of the protein isolate by precipitation through adjustment of pH, heat treatment (Thompson *et al.*, 1977), use of acidic polymers (Gillberg and Tornell, 1977), and ultrafiltration (Diosady *et al.*, 1984). However, despite extensive dissolution of the meal protein at certain pH values, the resulting extracts do not always give the highest yield of isolates. This has been attributed to the presence of phytic acid (PA) which is co-extracted with the protein as a complex (Gillberg and Tornell, 1976). Difference in the solubility of phytic acid and protein contained in rapeseed meal at various pH values can

also form the basis for the removal of phytic acid from the meal and the preparation of a low phytate protein isolate. At pH 11, PA has minimal solubility whereas proteins have maximum solubility. Hence, protein extracted at this pH followed by precipitation at the isoelectric pH with or without sodium hexametaphosphate (SHMP) as a polyelectrolyte, produces isolates with low PA content (0.004%).

Unfortunately, under the conditions described above, the product has a brown colour and a bitter taste even after extraction with alcohol. This is because of the large amount of phenolics co-extracted with the proteins (Thompson *et al.*, 1982). Therefore, additional steps have been included in the procedure to remove the undesirable compounds. These include the use of activated carbon for the removal of glucosinolates (Woyewoda *et al.*, 1978), acylation or dialysis of protein to reduce phytate and glucosinolates (Okubo *et al.*, 1975) and alcohol washing for the partial removal of phenolics (Thompson *et al.*, 1982). Extraction with 2 or 2.5% SHMP at milder pH followed by isoelectric precipitation and alcohol washing produces a lighter coloured product with a slightly higher PA (1.26%) content. At present, there are no data available on the digestibility assessment and actual nutritive values of canola protein isolates for fish species. Further, although the products from these processes show improved chemical profiles compared to the meals and concentrates, the procedures used to prepare them may be impractical for commercial production of isolates at a cost comparable to fish meal on an equivalent protein basis.

2.6.4 Canola Protein in Monogastric Diets

2.6.4.1 Rodents

Early studies on the utilization of rapeseed meals by rodents are often difficult to interpret because of paucity of information on the origin of seed used for meal manufacture. The literature however, contains many reports on the adverse effects of including rapeseed meals in animals diets. Experiments by Tookey *et al.* (1980) showed that rats fed diets containing 45% rapeseed meal had marked increases in thyroid weights, depressed growth rates and delayed egg development in females. Thyroid follicle hypertrophy and histological changes in the pituitary, adrenals and livers

were also observed. Doses of potassium iodide (1.3 mg daily) partially alleviated the thyroid problem but not the follicular hypertophy and hyperplasia, indicating that isthiocyanates and goitrin were the causative agents. Similar results have been reported by other workers (Sauer and Kramer, 1983; Kramer and Sauer, 1983). Working with crambe (*Crambe abbysinica*), another member of Cruciferae, van Etten *et al.* (1969) found that rats fed diets containing 5% dehulled and defeated seeds gained 90% as much weight as the control rats. All the rats however, died when the dietary level of dehulled and defeated seed was increased to 15%. This may be attributed to the presence of high levels of glucosinolate in crambe meal.

Protein-rich fractions of excellent nutritional quality can be obtained from rapeseed meals through removal of ANFs (Lo and Hill, 1971). These products have been shown to be better utilized by animals than the original materials from which they were derived. The inclusion of expeller-processed meal at 18% of a mouse diet depressed growth but gave marked improvement in weight gains when the meal was washed with hot-water or alcohol (Liu *et al.*, 1994). Sarwar *et al.*, (1975) found weight gains and PER values for mice fed a diet based on a glucosinolate-free rapeseed protein isolate were superior to those of mice fed a casein-based diet when the adverse effects of phytate in the isolate were eliminated by zinc supplementation. A similar product produced by aqueous acetone extraction gave weight gains in rats that were 94% of those of controls when included at 23% of the diet (van Etten, 1969).

2.6.4.2 Poultry

Studies on the effect of including rapeseed meal in poultry diets, have generated considerable literature on the subject. Many of these studies have shown that in addition to growth depression and enlargement of the thyroid gland, the feeding of rapeseed meal to poultry also causes lowered egg production, off-flavors in eggs and liver damage because of the presence of the antinutritional factors in the meal. Excessive heat treatment during the processing of canola may also lead to increased lysine supplementation in poultry. These effects vary between different breeds of laying hens. This prompted investigations into the utilization and improvement of

rapeseed meals to enable higher inclusion levels in poultry feeds (Blair *et al.*, 1987; March, 1987; Muztar *et al,* 1980; Shires *et al.*, 1983). Robblee (1987) found that canola meal can comprise 20% of the diet of broiler chicken and 10% of the diet of laying hens without any adverse effects on the productive performance. High levels of rapeseed meal in poultry diets have been reported to cause liver hemorrhage and reticulosis and no difference in effect was noted between high and low glucosinolate varieties (Clandinin and Milne, 1977). Nitriles in the meal were thought to be the causative agents. Further, when rapeseed/canola meal was included in diets of brown-egg layers, the birds produced eggs with a fishy odour due to the presence of trimethylamine (TMA) that was derived from sinapine present in the meal (Goh *et al.*, 1987).

Rapeseed/canola protein products have also been evaluated as protein sources in diets for turkeys. Salmon and co-workers (1987) fed turkeys diets containing 20% canola meal and found that growth rates and feed efficiencies were comparable to those of turkeys fed a soybean meal control diet. Full- fat canola seeds at 16.5% of the diet supported good growth. However, doubling the inclusion level resulted in reduced weight gains. The same authors also reported that canola prepress expeller cake included at a dietary level of 12% gave final weights equal to those of birds fed the wheat-soybean-fishmeal control diet although the feed efficiency was slightly reduced. Canola expeller cake, because of its residual oil content, is an attractive ingredient in poultry feedstuff due to its high energy content.

Improved rapeseed/canola protein products obtained by novel processing methods to remove ANFs have enhanced nutritive value for poultry, and accordingly can be incorporated into diets at higher levels without compromising performance. Goh *et al.* (1987) observed improved growth and no structural abnormalities in body organs when ammonia-treated crambe meal comprised 20% of the diet of chick. Although the nitrile content of the meal was not reported, nearly all the glucosinolates were removed. Similar results have also been reported by Goh *et al.* (1987) with chickens fed diets containing hydrous ammonia-treated canola meals. In addition to reduced sinapine content, these authors also noted a 50% reduction in the egg TMA content as well as a reduced incidence of fishy odour in the egg. Experiments with turkeys conducted by Salmon

et al. (1987) revealed that cooking at 80 °C for 30 minutes failed to improve the nutritive value of canola meal. However, steam pelleting and flaking resulted in higher liveweights but a lower feed efficiency relative to turkeys fed the whole, unprocessed seeds.

2.6.4.3 Swine

Early feeding trials involving traditional rapeseed meals in the diets for pigs had little success. Inclusion of 10-20% rapeseed meal in swine diets caused symptoms similar to those observed in rats fed diets containing rapeseed meals. Reduction in litter size and weight at weaning as well as difficulty in conception and enlarged livers also occur. Liver and pancreatic zinc concentrations in pigs have also been shown to be lowered by inclusion of canola meal in the diets (Bell and Keith, 1987). These problems have been ascribed to the high levels fibre (Bjeergegaard *et al.,* 1991), phytate and poor palatability of the rapeseed meals (Baidoo *et al.,* 1987). Bowland and Hardin (1973) found that rapeseed meal could comprise only about 6% of the diet for sows without detrimental effects, while finishing diets for swine may contain up to 11% rapeseed meal if they are concurrently supplemented with lysine and methionine (Bayley *et al.,* 1969). The amount of rapeseed meal that might successfully be included in diets for various classes of swine has been reported by Josefsson (1972). Most recent work on nutritional evaluation of rapeseed meal for swine has centred on low-glucosinolate rapeseed (canola) meals. Canola meal is an excellent protein source for growing swine and may be included at higher dietary levels. However, even these low levels of glucosinolates in these meals are still of some concern.

Young pigs are sensitive to high dietary levels of canola meal. Indeed, they exhibit depressed feed intakes and weight gains relative to those fed diets based on soybean meal. The palatability of very low glucosinolate canola meal for weaning pigs is known to be similar to that of soybean meal in when included in formulated diets for weanling pigs. However because of the reduced digestibility of the former product it was not found to be a desirable ingredient in such diets. The development of double low rapeseed varieties coupled with novel processing methods for the reduction of fibre and other antinutrients have resulted in meals that can be used potentially

as the principle sources of protein in swine diets. Bell (1993) found that pigs fed diets with regular canola meal performed just as well as those fed diets containing very low glucosinolate meals. This suggests that glucosinolates may not be the most important factors affecting the growth of pigs. Increasing the protein and available energy levels in canola meals through dehulling resulted in improved protein and energy digestibility coefficients for the meal (Bourdon *et al.,* 1985) although this strategy did not improve pig performance. It was also found that dehulled canola meal could comprise levels up to 24% of the diet without compromising pig performance.

Feeding experiments with sows fed high glucosinolate rapeseed (RSM) meals have demonstrated that conception and litter size are adversely affected when RSM constitutes more than 8% of the diet. Lewis *et al.* (1980) found no evidence of detrimental effects on reproductive performance when pigs were fed diets containing 50% or 100% of protein from canola meal (low glucosinolate) throughout two reproductive cycles. Several different methods have been attempted to improve the nutritive value of canola meal for swine including dehulling, wet cooking (Bell and Keith, 1983), ammoniation (Keith and Bell, 1982), extrusion (Ladbrooke, 1987), amino acid and iodine supplementation (Aherne and Lewis, 1980), aqueous ethanol extraction (van Megen, 1983) and combining canola with field peas (Bell and Keith, 1983). Recently, Danielsen *et al.* (1994) fed pigs diets containing "dehulled protein-rich rapeseed meal" (DPR-meal) produced by aqueous enzymatic extraction and found that the amount of nitrogen deposited compared well with that of animals fed diets with skim milk powder and fish meal as the protein sources. The residual levels of glucosinolates in the DPR-meal did not exert any negative effects on protein utilization although the insoluble dietary fibre content of this product depressed both protein and energy digestibility coefficients.

2.6.4.4 Warm water fish

The feasibility of rapeseed/canola protein products as a practical ingredient in fish diets has already been reported by several workers (Dabrowski and Kozlowska, 1981; Davies *et al.,* 1990; Hardy and Sullivan, 1983; Higgs *et al.,* 1979, 1982, 1983, 1988, 1991, 1995b; Gomez *et*

al., 1993; Yurkowski *et al.*, 1978). The acceptable dietary levels of canola/rapeseed protein products for finfish vary according to whether they are warm or cold water (or marine versus fresh water) fish and is also influenced by the type of canola/rapeseed product (rapeseed or canola meal, upgraded canola meal, concentrate). Fish size and species also are important factors that can influence the acceptable dietary level of canola/rapeseed protein products. The ability of rapeseed/canola meal to replace either fish meal or soybean meal in practical fish diets is supported by the findings of many researchers. On the basis of growth and feed utilization, rapeseed/canola meals have been reported to have higher acceptability in diets for warm water fish than in those of cold water fish (salmonids) species (Higgs *et al.*, 1995a).

Although studies on warm water fish are few, the results from these studies are quite promising. Dabrowski and Kozlowska (1981) successfully replaced up to 50% of fishmeal with a steam-treated rapeseed meal in feeding experiments with common carp (*Cypinus carpio*). Different workers have reported varying acceptable dietary levels of rapeseed/canola meal in tilapia. Davies *et al.* (1990) indicated a practical inclusion limit of 15% rapeseed meal in diets for juvenile tilapia (*Oreochromis mossambicus*). Higher levels than this were associated with erosion of the colloid region and follicular lumen of the thyroid as a result of compensatory increases in thyroid activity. In preliminary studies with fingerling tilapia, *Oreochromis mossambicus,* Jackson *et al.* (1982) obtained favorable growth performance when rapeseed meal of high glucosinolate content (7.5 mg/g) contributed up to about 50% of total protein in the diet. This was attributed to the relatively good amino acid profile of rapeseed meals. However, inclusion of 75% of protein from rapeseed meal resulted in growth depression, mainly because of the effects of glucosinolates. Higgs *et al.* (1989) working with hybrid tilapia (*Oreochromis mossambicus* × *O. aureus*) in fresh water found that complete replacement of soybean meal protein with that of canola meal did not significantly affect weight gain, growth rates or feed efficiency. However, tilapia hybrids fed diets containing over 25% of the dietary protein from canola meal resulted in depressed energy conversion efficiency and protein utilization.

2.6.4.5 Cold water fish

The inadequate supply of West Coast herring meal and the low cost and high availability of rapeseed/canola meal make the latter an economically attractive constituent for salmon diets. This has prompted the evaluation of rapeseed/canola protein as a replacement for fish meal in salmonid (Pacific salmon and trout) diets in the past decade by several workers (Fagerland *et al.*, 1987; Gomez *et al.*, 1993; Higgs *et al.*, 1982, 1983, 1988, 1990; Hilton and Slinger, 1986; McCurdy and March, 1990; Teskeredzic *et al.*, 1995; Yurkowski *et al.*, 1978). Complete replacement of fish meal protein in salmonid feeds with rapeseed/canola meal protein has not been achieved. In fact, high dietary levels of rapeseed/canola meal have been associated with negative fish performance. Salmonid species differ in their response to the inclusion of canola meal in the diet. Generally, rainbow trout have been found to be more sensitive than salmon to the antinutritional factors present in canola meals, and frequently they exhibit poor feed intake and growth, especially when canola meal exceeds 10% of the diet and the diet does not contain a palatability enhancer. Small rainbow trout (~2 g) appear to be especially sensitive to the residual levels of glucosinolates in canola meals. Total dietary glucosinolate levels of 172 μg/g of 3-butenyl isothiocyanate were found by Hilton and Slinger (1986) to be deleterious to trout, whereas juvenile chinook salmon can tolerate as high as 300 μg/g as 3-butenyl isothiocyanate. Higgs *et al.* (1983) reported that a dietary level of canola meal of 23% (22% of dietary protein) could successfully be used for rearing chinook salmon. However, Higgs *et al.* (1983), Leatherland *et al.* (1987), and Fagerland *et al.* (1987) working with both trout and salmon discovered that the acceptable dietary level of canola meal could be raised to 24% to 27% of dietary protein if T_3 was simultaneously given in the diets to counteract impairment of thyroid function due to the effect of glucosinolates, although thyroid follicle epithelial cell hypertrophy was still noted. Glucosinolates have been considered to be the major limiting factor in the use of rapeseed/canola meals in fish diets. However, other constituents such as fibre, phytic acid and phenolic compounds are important as well. Further enhancement of the nutritive value of rapeseed/canola meals may be achieved by additional processing in order to remove these antinutritional constituents. Experiments conducted

by McCurdy and March (1992), for instance have shown that upgraded canola meals produced by solvent washing of fibre-reduced meals have high acceptability in trout and salmon diets (40% and 25% of dietary protein, respectively) owing to their reduced levels of glucosinolates and sinapine (a phenolic compound). Rapeseed/canola protein concentrates have also been shown to have excellent protein quality for both chinook salmon and rainbow trout, even when included at levels of 25% of dietary protein (Higgs *et al.*, 1982, 1991). However, attempts byYurkowski *et al.* (1978) to incorporate more than 30% of dietary protein as rapeseed protein concentrate for rainbow trout resulted in poor growth, feed intake and utilization. This was, in part, due to the presence of a high level of phytic acid (5.3-7.5%) which can potentially reduce the availability of both proteins and minerals, as well as causing depression of thyroid function. In fact, phytic acid is the only major antinutritional factor present in rapeseed/canola protein concentrates. Teskeredzic *et al.* (1992) replaced 66% of herring meal protein in a basal diet for trout with dephytinized or undephytinized rapeseed protein concentrate without affecting growth, appetite, feed efficiency, mortality or health. Total replacement of herring meal protein, however, depressed growth and feed efficiency. Recently, Prendergast *et al.* (1994) employing an improved dephytinization procedure for RPC (Alko Ltd, Rajamaki. Finland), were able to entirely replace high quality fish meal (steam-dried whole herring meal or Norse LT-94 comprising 59% of dietary protein) with dephytinized RPC in trout diets without adversely affecting fish performance, as long as the diets were simultaneously supplemented with an appetite enhancer (Finnstim®) and careful attention was paid to supplemental dietary minerals.

2. 7 APPLICATION OF ENZYME BIOTECHNOLOGY

2.7.1 Enzymes In Animal Diets

Enzymes are specialized proteins that control the rates of specific reactions and/or have the ability to degrade complex chemical substances into more simple forms. In the animal feed industry (mainly swine and poultry) the utilization of enzymes to pre-digest various compounds has been made possible by the development of suitable sources of low-cost enzyme preparations

(Chesson, 1987). The addition of enzymes to food has been reported to enhance growth in some cases and to afford animals some resistance against diseases in other cases. Enzyme supplementation of animal diets has been studied widely, especially in Europe, where encouraging results have been reported for pigs and poultry. At present, the application of enzymes in aquaculture feeds is not widespread, although there is a growing potential for their use.

Canola meal is an abundant source of protein but its utilization in fish diets is limited by the presence of ANFs. Although advances in plant breeding have reduced the glucosinolate content of canola meal to very low levels, phytic acid (4.5%) and fibre (~12%) contents are still of great concern. These antinutritional factors have deleterious effects on digestion and on the morphology and physiology of the digestive tract (van den Ingh *et al.*, 1991). Further, the presence of oligosaccharide sugars in canola meal may hinder the realization of the maximum potential of canola protein in fish diets. The addition of enzymes to diets containing plant protein sources or the pretreatment of these sources with enzymes before diet incorporation would therefore modify composition, and thus expected to increase nutrient utilization and growth due to increased digestibility as a result of the reduction or elimination of ANFs.

A wide range of enzymes has been investigated in animal feeding trials. These studies have involved the use of phytases (Higgs *et al.*, 1991; Prendergast *et al.*, 1994; Spinelli, 1979; Teskeridzic *et al.*, 1995) and polysaccharidases (Jensen *et al.*, 1990). Enzyme blends have been shown to improve liveweight gains and feed efficiencies of early weaned pigs and broiler chicks (Collier and Hardy, 1986; Inborr, 1990). The improved performance observed in animals fed diets supplemented with enzymes has resulted from better feed utilization because of improved release of nutrients through hydrolysis or by removal of barriers that wrap around nutrient reserves. The benefits of enzyme supplementation can be related directly to the improvement of the digestive capability of the animal. Young animals (chicks, piglets, calves and larval fish) have underdeveloped digestive enzyme systems or capabilities compared to adult animals. Adult animals may also lack enzymes for the hydrolysis of particular food classes. For example, no animal can synthesize the enzyme responsible for breaking down cellulose and other cell wall

components of plant cells. Moreover, the digestive enzyme capability of an animal is made up of a combination of indigenous enzymes and those originating from the microorganisms that inhabit the gut. Hence, there are potential benefits to be obtained from supplemention of diets for both young and adult animals with enzymes. Enzyme additives can be used to introduce digestive capabilities into animals either earlier than they normally occur or they may give an animal an entirely new range of enzyme capabilities.

2.7.1.1 Augmentation of Host Digestive Enzymes

Alternative protein sources for salmonids must contain at least 35% protein in order to be potentially valuable for inclusion in salmonid diets (Hardy, 1995). This limits the choices to just a few ingredients. Several plant protein sources have been shown to have the desired levels of protein. However, many of these sources have common or unique undesirable characteristics which limit their level of inclusion because they impair energy and protein utilization. Increasing use of plant protein sources in diets for cold water fish requires that further improvements in their nutritive value be investigated. Chesson (1987) has presented an excellent review on the subject of enzyme utilization in monogastric nutrition.

Monogastric animals utilize plant non-starch polysaccharides (NSP) poorly. These eventually pass through the digestive tract largely unaffected until they are exposed to the microflora in the hind gut. NSPs are important nutritionally because they are potential sources of energy. However, they can block enzyme access to other important nutrients (proteins, starch) and thus, in their macromolecular form, they act as antinutritional factors because of the physical effects that they exert. Polysaccharidases are enzymes that break down complex polysaccharides. Consequently the introduction of these enzymes into the feed ingredients of plant origin can facilitate the degradation of cell wall material earlier in the gut. This strategy would mimic one of the advantages of ruminants without the penalty of losing nutrients through microbial activity. Many kinds of polysaccharidases have been used to maximize the nutritive value of feedstuffs while at the same time minimizing the antinutritional properties of plant protein sources with

varying results. The addition of amylolytic enzyme preparations to grain-based feeds has been shown to improve weight gain and feed conversion in turkeys (Parkany-Gyarfas, 1975), broiler chicken (Parkany-Gyarfas and Toth, 1978) and layers (Gleaves and Dewan, 1970). Reese *et al.* (1983) however, could not confirm these results. These differences in the results have been attributed to variations in the purity of the enzyme preparations used in these studies. The application of pectinases, xylanases and cellulases singly or in combination also produces significant improvements in the digestibility of nutrients in both poultry and pigs. It has been suggested that the improvements in feed utilization after supplementation with polysaccharide-degrading enzymes is due to improvements in nitrogen or starch utilization rather than any enhancement of fibre utilization. These effects may be attributed to the presence of non-carbohydrate components (pectin, lignin or condensed tannins) in the cell wall which may influence the degradation of specific polysaccharides. In addition, although monogastrics are capable of degrading α-linked bonds in disaccharides (sucrose and lactose), their enzymes are inactive against β-linked oligomers produced by the action of polyssacharidases on plant non-starch polysaccharides (Chesson, 1987).

2.7.1.2 Enzymatic Destruction of ANFs

Experiments on the pretreatment with β-glucanase of poultry diets containing barley have shown that there is improved growth, metabolizable energy content, apparent protein retention and feed conversion and reduced incidence of sticky droppings (Newman and Newman, 1987). The improvement in performance was thought to result primarily from enhanced utilization of starch because of partial degradation of β-glucans, and not so much from better utilization of the glucose residues resulting from the break down of β-glucans. Linseed meal, guar gum and other legume seed meals (Vohra and Kratzer, 1964), and rye (Day and Thomas, 1980) have been shown to depress growth and feed intake and to cause sticky dropping problems in poultry due to the presence of specific gel-forming polysaccharides. However, the specific enzymatic destruction of these polysaccharides has been shown to solve these problems (Anderson and Warnick, 1970;

Newman and Newman, 1987). The antinutritional factors from plant protein sources that have been considered in salmonid diets fall under two broad categories, viz. those that bind to nutrients thereby restricting their availability to animals (e.g. phytate, tannins) and those that interfere with the digestive cabability of the animal (e.g. glucosinolates, fibre, protease inhibitors). Digestible carbohydrates are poorly utilized by salmonids if they exceed 15-20% of the diet. Hence, the addition of polysaccharidases into salmonid diets based on plant protein sources may be of little benefit because the resulting digestible carbohydrates would be of little or no use to the fish. With regard to the use of plant protein sources in fish diets, enzyme pretreatment before incorporation into diets or dietary enzyme supplementation may be beneficial, depending on the type of ANFs present.

2.7.1.3 Application of Enzymes In Fish Feed

Much of the research in fish nutrition is directed towards the production of the most efficient growth at a minimum cost by improving feed utilization. Feed intake is greatly affected by the chemical nature of the diet and is the major determinant of fish growth. As a result, many attempts have been made to improve the utilization of feed in aquaculture. There has been a growing interest in recent years on the subject of enzyme utilization in fish nutrition. Several studies on the use of enzymes in both larval and adult fish diets have been carried out with variable results. Most of these studies have focused on the actions of enzymes in the digestive tract with a view to improving dietary utilization by increasing digestion efficiency. Supplementary enzymes in fish diets have received little attention. Most studies have concentrated on larval fish diets (Dabrowska *et al.*, 1979; Munilla-Moran *et al.*, 1990; Uys *et al.*, 1987). Difficulties in rearing fish larvae on formulated diets indicate that there is low efficiency of digestion and enzyme production at this stage of life history due to the simple morphology of the digestive tract. Indeed the digestive capability of some larval fish have been shown to be improved by the supply of exogenous enzymes present in their natural food (Lauff and Hofer, 1984). Dabrowski and Glogowski, (1977) studied the effect of adding bovine trypsin to the diets of carp and noted an

increase in total digestive enzyme activity. However, the proteolytic activity of the hepatopancreas was significantly lowered. Dabrowska *et al.* (1979) found that carp larvae had improved growth and survival when enzyme extracts from adult carp hepatopancreas and intestines were added to their diet. Growth however, was not as good as when the fish received natural food.

Supplementary enzymes have rarely been used to increase the digestibility of feed ingredients included in the diets of post-larval or adult fish. In the few studies reported, the addition of enzymes to fish diets has met with little success. Reinitz (1983) fed rainbow trout fry diets containing a commercial protease enzyme preparation. He reported no effect on growth or feed conversion efficiency. Similar results were also reported by Carter *et al.* (1992) although they did obtain an increase in nitrogen retention when a commercial amylase preparation was included in diets for Atlantic salmon parr. Probably the poor responses in these studies resulted from the application of a single enzyme to diets containing high proportions of fishmeal protein. Recently, Carter *et al.* (1994) showed that the addition of a multi-enzyme mixture (proteolytic enzymes and carbohydrases) to a soybean meal-based diet improved feed consumption, growth rate, weight gain and feed conversion efficiency of Atlantic salmon reared in sea water, although there were no improvements in digestibility of protein or organic matter.

While the direct addition of enzymes to salmonid feeds may be the most practical approach, it may not be the most efficacious strategy. The thermostability of enzymes may be critical, especially during feed milling and pelleting, since the enzyme would probably have to be added to the feed mix before the pelleting process (Collier and Hardy, 1986). The application of heat during pelleting may affect enzyme action. These above authors reported recoveries of 52-77% and 34-65% of amylase and protease activities respectively after pelleting, but these values were highly dependent on the composition of the diet. Enzymes might also be added to the diets by spraying after pelleting. However, feeds treated this way may be more prone to the growth of moulds as a result of release of fermentable carbohydrates (Vohra and Kratzer, 1964) in poor storage conditions. Further, added enzymes are exposed to a variety of proteolytic activities in the animal's gut. Although most microbial polysaccharidases are resistant to microbial proteases, this

may not be the case with proteases from animals (Chesson, 1987). Also, while temperature conditions remain relatively constant in the gut, pH values show considerable variation, depending on the section of the gastro-intestinal tract and the nature of the feed ingested (Chesson, 1987).

Therefore, pH stability and optimum pH for activity are important factors to consider. Supplemental enzymes, therefore, have to withstand the acidic conditions of the stomach without undue loss of activity in order to be effective in the lower sections of the gut. Enzymes of fungal origin generally show pH optima in the range of 4 to 5.5, whereas those of bacterial origin have optimum activity at pH values near neutrality. In this regard, Chesson (1987) suggested that a mixture of enzymes of both fungal and microbial origin with the same activity may be more effective under these varying pH conditions than an enzyme with the same activity from a single source.

In recent years, enzyme treatment of individual feed ingredients before incorporation in fish diet has also been used, particularly to minimize the effects of polysaccharides and other ANFs. McCurdy and March (1992) while working with specially processed canola meals, concluded that enzymatic reduction of fibre did not significantly improve the response of either trout or salmon to the test canola protein product as assessed by specific growth rate or feed conversion efficiency. Improved values for each of these parameters were found by the same authors when solvent-washed fibre-reduced meals provided 40% of the total protein in the diet rainbow trout. This was attributed to the reduced levels of both glucosinolates and sinapine in the upgraded canola meals. Rapeseed/canola protein concentrates (RPC/CPC) have been shown to be valuable substitutes for fish meal in salmonid diets on the basis of their higher protein content, quality and digestibility (Prendergast *et al.*, 1994), especially when the negative influence of the high phytic acid content in such products was removed. The content of this compound is increased from 3.1-3.7% in meals (Cheryan, 1980; McCurdy and March, 1992) to 5.3% to 7.5% in the concentrates (Jones, 1979) as a result of the removal of other constituents by the processing procedures that are employed. A significant amount of phytate can be removed by processing techniques. Alternatively, there is potential for using treatment of diets or feed ingredients with

exogenous sources of phytase in order to reduce the level of phytate. A number of studies have been conducted to investigate the effect of dephytinization (removal of phytic acid) by phytase treatment on the utilization of canola protein products. Teskeredzic *et al.* (1995) found that pretreatment of RPC with Finase S 2X (a commercial source of phytase) to reduce phytic acid content did not markedly improve its nutritive value for trout. These authors attributed this finding to problems in the dephytinization procedure that was employed, since it was shown to significantly reduce the quality of the RPC protein. Consequently, the benefits of phytate removal on the nutritive value of RPC were less than expected. By contrast, Prendergast *et al.* (1994) found improved weight gains and feed efficiency of rainbow trout fed diets containing canola RPC pretreated with a commercial microbial phytase (Finase FP 500) compared to those fed diets containing undephytinized RPC. In addition, this study also showed that dephytinized RPC could comprise 59% of the dietary protein by replacement of high quality fish meal (steam dried herring meal or Norse LT-94) without compromising any aspect of trout performance. Further, phytase pretreatment of diets containing canola meal has also been shown to increase weight gain, phosphorus availability and bone ash, as well as to decrease faecal phosphorus excretion (Cain and Garling, 1993; Riche, 1993). Hence, improved products can be obtained by enzyme treatment of canola protein products that are more comparable or equivalent in nutritive value to fish meal for trout. Possibly this may be the case for other salmonids and finfish species as well.

Chapter 3

EXPERIMENTAL

The research reported in this thesis was carried out in two parts. In the first part, commercial canola meal was processed to reduce fibre by grinding and mechanical sieving. The sieved product was then treated with methanol-ammonia-water solution before being treated with phytase alone or together with carbohydrate-degrading enzymes singly or in combination. Altogether seven test canola protein products were produced. In addition a commercial canola protein isolate (CPI) was included in the study to asses the quality of canola protein characterised by the presence of low levels of antinutritional factors. Determination of chemical composition of the eight products through laboratory analyses was carried out. The effect of grinding and sieving through different screen sizes on the chemical composition of canola meal was investigated.

Standard analytical methods were used for the determination of protein, amino acids, lipid, dry matter and energy (AOAC, 1990). Total dietary fibre methodology (Prosky *et al.,* 1984) and detergent forage fibre methods (Goering and Van Soest, 1970) were used to partition the fibre components of the canola products. Detailed carbohydrate characterization of the canola products was undertaken to assess the effect of the different processing methods on the carbohydrate fraction of commercial canola meal. Levels of glucosinolates, phytate and phenolic compounds in these products were also determined by the methods of Daun and McGregor (1981), Latta and Eskin (1981) and Reed et al. (1985), respectively. The aim was to ascertain the amount of readily available nutrients as well as the proportion and compositions of the unavailable fibrous portion of these products. In the second part of the project the feeding value of the canola products was determined. This was aimed at assessing the potential nutritive value of the test canola protein products for trout. Accordingly, a digestibility trial was designed following the procedure of Hajen *et al.* (1993). Diets containing 30% of anyone canola product and 70 % reference diet with 0.5% chromic oxide as the indigestible marker were formulated. The test diets and a reference diet were

each fed to triplicate groups of trout and feaces were collected using a modified "Guelph system". Subsequently, the dry matter, protein, and energy digestibility coefficients of the canola products were determined for trout. Mortality, weight gain, specific growth rate (SGR), feed conversion ratio (FCR) and protein efficiency ratio (PER) were also determined to gain some insight of the effect of diet on fish performance and to provide a greater understanding of the relative importance of the antinutritional factors present in different canola products on the quality of the protein. meal in terms of how they prevent the full expression of the high quality protein of canola.

Chapter 4

PROCESSING AND CHEMICAL ANALYSIS OF CANOLA MEALS

4. 1 INTRODUCTION

Feedstuffs of plant origin (including oilseed meals) are important sources of protein for man and animals although they generally have lower protein content than those of animal origin. Seeds of many legumes and by- products from other seeds (oilseeds) are used as feed ingredients in animal production. However the carbohydrate fraction is a concern when oilseed meals are included in salmonid diets because both indigestible and digestible carbohydrates are poorly utilized by salmonids (Krogdahl, 1989). Further, nearly all oilseed meals have deleterious constituents that make some additional processing necessary to improve nutritive value. These constituents, if not eliminated, can markedly influence the digestion and utilization of many nutrients (Huisman and van der Poel, 1988). Most oil seed meals contain high levels of fibre which is of no value to salmonids and is only partially digested by other monogastric animals. These meals also contain varying levels of carbohydrates (poly- and oligosaccharides) which are poorly utilized by salmonids (Arnesen *et al.*, 1989; Murai *et al.*, 1983). Two types of carbohydrates are present in rapeseed meals, these being soluble mono and oligosaccharides and the insoluble polysaccharides. A major concern is the high level of galactosylsucrose oligosaccharides also known as α-galactosides of sucrose (raffinose, stachyose, verbacose and ajucose). The enzyme α-galactosidase is absent in man, fish and other monogastric animals. Consequently these oligosaccharides present in oilseed meals escape digestion in the digestive tract. Further salmonids are known to utilize high levels of digestible carbohydrates poorly. It is therefore desirable that the levels of these sugars is reduced in any potential ingredient for salmonids.

It is therefore important not only to quantify and characterize those components which are generally of medium to high availability to monogastric animals (protein, fat, simple

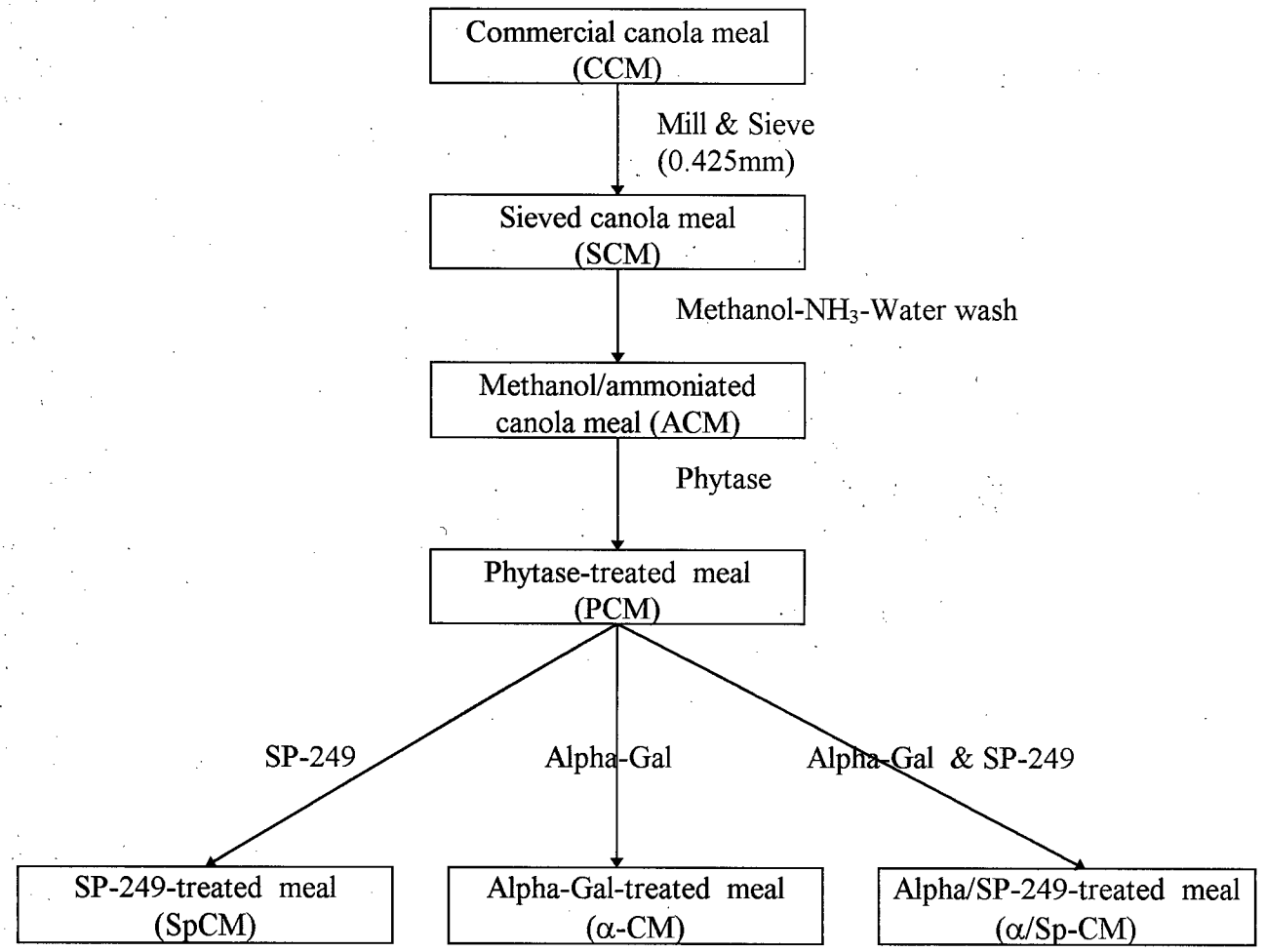
carbohydrates) but also to characterize those components of low and variable availability that influence the availability of other nutrients as well. Many of the analyses in this work were undertaken to quantify the fibre and carbohydrate fraction. Two methods for measuring fibre were used; the total dietary fibre method (Prosky *et al.*, 1984) and the detergent fibre method (Goering and van Soest, 1970). Cellulose, hemicellulose, lignin and phenolics were determined.

4. 2 MATERIALS AND METHODS

4.2.1 Canola meal processing

Enzymes were obtained from commercial enzyme manufacturers. Oligosaccharide degrading enzyme (Alpha-Gal) and a plant cell wall-degrading enzyme (SP-249) were kindly donated by Novo Nordisk Bioindustrials, Inc. (Danbury, North CT). Finase S 2X, a commercial phytase of microbial origin, was supplied by Alko Ltd (Rajamaki, Finland). The canola protein isolate, (CPI) was obtained from a private supplier. Commercial canola meal (CCM) from *Brassica napus* was obtained from CanAmera, Manitoba in late 1993. The meal was thoroughly mixed for one hour for measurement of mass distribution. Portions of the mixed samlpe were ground through a series of screens of diffferent mesh sizes in a Ro-Tap shaker for the determination of mass distribution (see Appendix 1) and effect of screen size on proximate composition (Appendix 2). The results of the mass distribution were then used to estimate the amount of canola meal to be ground and to set the mesh size to be used in the rest of the experiment. A schematic representation of the laboratory procedure used in the production of the test canola protein products from commercial canola meal is shown in Figure 4.1. The fibre content of 60 kg of commercial canola meal was reduced by milling in a hammer mill equipped with a 0.5 mm screen. The screen unders were then sieved manually through a 0.425 mm sieve to reduce the fibre content further. The resulting product (sieved canola meal, SCM) was then subjected to methanol/ammonia wash and a series of enzyme treatments.

Figure 4.1 Schematic representation of the laboratory procedures used to produce the test canola protein products



For the methanol/ammonia treatment, ammoniated aqueous methanol was produced by mixing ammonium hydroxide, water and absolute methanol in the ratio 10:5:85 (v/v/v). Fifty kg of the fibre-reduced meal were treated with stirring in 300 g portions in 4L beakers, each with 10 parts by weight of the ammoniated methanol at room temperature for 2 hr. This procedure was repeated three times for each 300 g portion (see Appendix 3 for methanol recovery). The meal was then separated by vacuum filtration on Whatman No. 41 filter paper and then air dried in a fume hood for 48 hr. pending further treatment. Four-fifths of the ammonia/methanol treated meal (ACM) was then subjected to the Alko Ltd (Finland) method of dephytinization (Teskeredzic *et al.*, 1995). Samples (5 kg) of solvent washed canola meal were suspended in 50L of water, pH was adjusted to 5.0 and Finase S 2X (a commercial phytase) was added at the manufacturers' recommended dosage of 5000 PU/g of canola meal (One PU is the amount of enzyme that liberates from sodium phytate 1nmol of inorganic phosphorus in one minute under standard conditions, pH 5.0, temperature 37° C). The suspension was incubated at 55° C with continuous shaking for 4 hr., vacuum filtered and the resulting product then stored in a freezer at -20 °C. For further treatment, phytase-treated meal (PCM) was thawed and then divided into three equal batches. The first batch of canola meal was treated with a carbohydrate-degrading enzyme (Alpha-Gal) by incubating in water at the manufacturer's recommended dosage of 0.5 L/ton of feed component at a temperature of 50° C and pH 5.0 with shaking for 6 hr. The slurry was then vacuum filtered and the residue collected as α-CM. To produce SpCM (SP-249-treated canola meal) the remaining 2/3 of meal (PCM) was treated with 0.1% v/w of SP-249 (a cell wall degrading enzyme) and vacuum filtered, thereafter half of this meal was further treated with Alpha-Gal as described previously to produce a meal treated with two carbohydrases (α/SpCM). The SP 249 enzyme hydrolysis was carried out for 6 hr at 50° C and pH 5.0. All the enzyme treated products were stored in a freezer (-20° C) immediately after filtration and then freeze dried at Agriculture Canada Research Station, Summerland, B.C before feed formulation.

4.2.2 Chemical analyses of canola products

4.2.2.1 Proximate, phytate and glucosinolate analyses

Dry matter contents were determined by drying 2 g samples of each product in crucibles in an oven at 105° C for 20 hr. Ash was assayed as the residue after charring a dried, ground sample in a muffle furnace at 600° C for 2 hr. Nitrogen (N) content of the products was determined by the AOAC method (1993) by first digesting 2 g sample with a solution of concentrated sulphuric acid and hydrogen peroxide and then analysing for N using a Technicon auto analyser. The crude protein content was then determined as N× 6.25. Crude lipid content of samples was determined as described by Bligh and Dyer (1959). Gross energy was determined with an automatic bomb calorimeter (AC 300 Leco Corp., Michigan) following the method outlined in the technical manual. Amino acid compositions were determined by A A A Laboratories, Seattle, WA using a Beckman Spinco Analyser 120B. Plasma emission spectroscopy (Quanta Trace Laboratories Inc. (Burnaby, B.C.) was used to analyze the mineral composition. Determinations of phytate and total phenolic content of dried samples were carried out according to the methods of Latta and Eskin (1980) and Reed *et al.,* (1985), respectively. The individual glucosinolate content was determined at Agriculture & Agri-Food Canada (Saskatoon) by the method of Daun and McGregor (1981) and the total amount of glucosinolate was taken as the sum of gluconapin, glucobrassicanapin, progoitrin, gluconasturtiin, glucobrassicin and 4-Hydroxyglucobrassicin.

4.2.2.2 Van Soest Forage Fibre Analysis

Fibre components of the canola products were determined using the forage fibre method of Goering and Van Soest (1970). Samples (0.35 g) were initially refluxed with acid detergent solution to determine acid detergent fibre (ADF) and also to prepare for further analysis. The residue from this procedure was then used for sequential lignin analysis by treatment with saturated permanganate solution. Cellulose was determined as that part of ADF that was solubilised by 72% sulphuric acid. Neutral detergent fibre (NDF) content was measured by refluxing 0.35 g samples

of each product in neutral detergent solution for 1 hr followed by ashing of residue. Hemicellulose was measured as the difference between NDF and ADF.

4.2.2.3. Total Dietary Fibre Method

Total dietary fibre can be defined as the polysaccharide and lignin resistant to mammalian digestive enzymes, and thus is relevant to most monogastric animals with hindgut fermentation. Part of the fibre fraction including some legitimate cell wall components, such as β-glucans, gums, mucilages and pectins, is not recovered in NDF but is resistant to monogastric enzymes. This fraction forms the water soluble non-starch polysaccharides (NSP). In this regard fibre was partitioned into soluble and insoluble dietary fibre fractions using the method of Prosky *et al.* (1984).

Triplicate groups of finely ground defeated samples (1g) were suspended with 50 ml of phosphate buffer in tall beakers and 0.1 ml of heat-stable alpha-amylase (Termamyl), covered with aluminium foil and placed in boiling water for 15 min. with occassional shaking in order to gelatinize the starch. This was followed by sequential digestion in a 60° C bath with a protease for 30 min. and finally with amyloglucosidase for another 30 min. Samples were agitated at all times and the pH of the solution was adjusted before each digestion to ensure maximum enzyme activity. Immediately after the last digestion, the hot solution was filtered through a dried, weighed fritted glass crucible containing celite as a filtering aid. The crucible containing the insoluble fibre component was then dried at 70° C in an oven overnight. This residue contains cellulose, hemicellulose, lignin, insoluble ash and indigestible protein. For the determination of the insoluble ash fraction and indigestible protein one of the triplicate samples was ashed at 500° C for 12 hr. and the remaining two samples transferred to filter papers and then digested in a solution of concentrated sulphuric acid and hydrogen peroxide, catalysed by lithium sulphate in order to determine the N content using the auto analyser procedure. Insoluble fibre was then calculated as the difference after subtracting insoluble protein and ash content and correcting for fat, weight of blank sample and the loss of celite upon ashing. The filtrate from above contains the soluble

components of fibre (pectins, gums, mucilages) and a substantial amount of ash. The filtrate was collected and precipitated by the addition of four volumes of 95% pre-heated ethanol in the original beaker. Precipitation was allowed to continue overnight. The solution was then filtered through a weighed fritted glass crucible, dried overnight and weighed. Soluble fibre was also corrected for ash, weight of blank and loss of celite on ashing.

4.3. RESULTS

4.3.1 Proximate composition of canola products.

Descriptions of the various laboratory processes employed are shown in Table 4.1 and the results of the proximate composition of commercial canola meal, laboratory processed canola meals and the canola protein isolate are given in Table 4.2. The protein levels for the commercial and laboratory processed canola meals ranged from 36.32 % to 49.73 % of dry matter. The increase in crude protein content of the laboratory processed meals was due to the dissolution of the meal solids containing carbohydrates and other soluble constituents. There were small reductions in the protein content of the alpha-galactosidase- and alpha-galactosidase/SP-249 treated canola meals. The reason for this depression is not clear. Sieving of canola meal did not lead to any appreciable increase in the protein level of commercial canola meal. Canola protein isolate (CPI) had a substantially higher protein content (90.76 %) but had the lowest lipid content (3.32 %) than all the meals. This high protein content of the canola protein isolate (CPI) is due to removal of other components (fibre, lipid and carbohydrates) during its preparation. Among the laboratory processed canola meals, the ammonia/methanol- and SP-249- treated meals had the lowest and highest protein levels respectively. The protein levels in the commercial canola meal used in the present study was lower than that found by McCurdy and March (1992). Except for the meal treated with the two carbohydrate-degrading enzymes (α/SpCM), all the canola products had lower lipid levels relative to the unprocessed meal. Within the laboratory processed meals the ammonia/methanol treated canola meal had the lowest lipid level (3.84%) due possibly to some lipid dissolution by the alcohol. Levels of lipid were higher in all the enzyme treated canola meals

than the methanol/ammonia treated canola meal as a result of removal of water soluble materials from the meal. Ash contents in the meals did not show large differences, although the carbohydrate-degrading enzyme treated meals had lower ash levels than the commercial canola meal. The mineral composition of these two meals reveals a substantial lowering of readily water soluble minerals (sodium, potassium, phosphorus, and calcium) which could possibly account for the observed reduction in ash content. Ash levels were highest (12.7 %) in the sieved meal and lowest (5.47 %) in the canola protein isolate. Indeed the canola protein isolate had an improved proximate composition than the other test canola protein sources. Energy evaluation of the commercial canola meal and the laboratory processed canola meals revealed little differences in the gross energy content. The lowest and highest gross energy values differed by about 1.5 MJ/kg. Gross energy value for commercial canola meal reported in the present experiment was close to that reported by Bell and Keith (1991) and Higgs *et al.* (1995). These results indicate that the processing techniques employed in this study did not result in any appreciable improvement in the gross energy content of the meals. By contrast, a high gross energy value (24.4 MJ/kg) relative to that of unprocessed canola meal (19.6 MJ/kg) was exhibited by the canola protein isolate (CPI). Indeed, this value is higher than the 20.5 MJ/kg reported by Higgs *et al.* (1995a) for herring meal.

Table 4.1 Description of processing treatments with designated abbreviations used in the laboratory to produce test canola products from canola meal[1]

Treatment	Abbreviation
Unprocessed commercial canola meal	CCM
Sieved canola meal	SCM
Sieved, methanol/ammonia treated meal	ACM
Sieved, methanol/ammonia and phytase treated meal	PCM
Sieved, methanol/ammonia, phytase, SP-249 treated meal	SpCM
Sieved, methanol/ammonia, phytase, Alpha-Gal treated meal	α-CM
Sieved, methanol/ammonia, phytase, SP 249, Alpha-Gal treated meal	α/SpCM
Commercial canola protein isolate	CPI

[1] Abbreviations (acronyms) are derived from the last treatments applied to the meal except α/SpCM where the last two treatments were used to give the product its acronym. Refer to Materials and Methods for conditions of each treatment.

Table 4.2 Proximate composition and gross energy content of test canola products derived by different processing methods

Ingredient		Proximate constituents (DM basis)				
Name	Code	Moisture (%)	Protein (%)	Lipid (%)	Ash (%)	GE (MJ/kg)
Commercial canola meal	CCM	10.8	36.3	6.85	11.0	19.6
Sieved canola meal	SCM	10.5	37.1	6.75	11.2	19.5
Ammoniated canola meal	ACM	11.8	39.1	3.84	12.7	19.2
Phytase treated canola meal	PCM	0.98	48.2	4.74	11.5	19.6
SP-249-treated canola meal	SpCM	0.75	49.7	5.59	10.2	19.8
a-galactosidase treated canola meal	α-CM	1.86	43.8	5.39	9.81	20.1
SP-249 & a-galacto-sidase treated canola meal	α/SpCM	0.93	41.3	6.53	9.06	20.7
Canola protein isolate	CPI	2.86	90.8	3.32	5.47	24.4

4.3.2 Fibre composition

Table 4.3 shows the different fibre components of unprocessed commercial as well as the laboratory processed canola meals. Substantial variations were noted in the content of different fibre components. The crude fibre content of the commercial canola meal (10.6 %) was similar to that reported by Higgs *et al.* (1995) but lower than that found by McCurdy and March (1992). Acid detergent fibre, neutral detergent fibre and acid detergent lignin values for the unprocessed meal were lower than those reported by McCurdy and March (1992) and all fibre components except ADF and TDF were further lowered by the sieving technique. Methanol/ammonia treatment of canola meal increased the crude fibre concentration in relation to that of sieved canola meal. Crude fibre concentration was highest in canola meal treated with phytase and the two carbohydrate-degrading enzymes (α/SpCM). Solubilization of polar lipids by the alcohol and removal of other constituents (soluble carbohydrates) by water may have been the reason for this concentrating effect. With the exception of the sieved canola meal, there was little variation in the concentration of canola meal hemicellulose. Dietary fibre concentrations of the meals ranged from 32.2% to 46.2%. Acid detergent fibre values ranged from 22.8 % to 32.3% while those for neutral detergent fibre ranged between 27.8 % and 37.1 %. Solvent washing and the application of different enzyme treatments to canola meal resulted in a concentrating of these fibre components. Canola meal treated with both carbohydrate reducing enzymes showed the highest ADF, NDF and lignin values. This was completely opposite to what was expected. Sieved canola meal had lower hemicellulose, lignin and cellulose values than the unprocessed canola meal. The highest values cellulose and hemicellulose were observed in the phytase SP-249 (cell-wall degrading enzyme) treated canola meal. Canola protein isolate (CPI) showed a much improved fibre composition in comparison to the rest of the test canola protein products. Value of 2.06 %, 2.36%, 2.55% and 1.30 % were observed for crude fibre, acid detergent fibre, neutral detergent fibre and cellulose respectively.

Table 4.3 Crude fibre, acid detergent fibre (ADF), neutral detergent fibre (NDF), Total dietary fibre (TDF), cellulose, hemicellulose and acid detergent lignin (ADL) content of test canola products.

	Fibre composition[1] (% DM)							
Ingredient[2]	CF	ADF	NDF	CL	HC*	ADL	IDF	TDF[3]
CCM	10.6	22.8	27.8	14.5	5.04	8.27	29.8	34.2
SCM	7.25	22.9	25.1	12.1	2.20	7.92	27 6	35.6
ACM	8.33	23.9	31.1	13.6	7.16	8.67	33.9	40.1
PCM	12.2	27.4	33.0	15.1	5.65	10.7	37.6	43.0
SpCM	10.4	27.2	35.0	19.0	7.89	10.5	38.3	44.1
α/CM	11.3	29.1	34.8	17.8	5.68	11.8	36.4	41.4
α/SpCM	13.2	32.3	37.1	18.6	4.81	12.3	40.5	46.2
CPI	2.06	2.36	2.55	1.30	0.29	0.63	ND[4]	ND

[1] CF: crude fibre, ADF: acid detergent fibre, NDF: neutral detergent fibre, CL: cellulose, HC: hemicellulose (*calculated as NDF-ADF), ADL: acid detergent lignin, IDF: insoluble dietary fibre, TDF: Total dietary fibre

[2] CCM, unprocessed canola meal; SCM, sieved canola meal; ACM, sieved, methanol/ammonia treated meal; sieved, methanol/ammonia, phytase treated meal; sieved, methanol/ammonia, phytase, SP-249 treated meal; sieved, methanol/ammonia, phytase, Alpha-Gal treated meal; sieved, methanol/ammonia, phytase, SP-249, Alpha-Gal treated meal; CPI, canola protein isolate. Refer to Table 4.1 for description of treatments.

[3] Total dietary fibre by enzymatic digestion method of Prosky *et al.* (1984)

[4] ND=Not determined

4.3.3 Inositol phosphate (phytate) composition

Results of the analyses of unprocessed, and processed canola meals and protein isolate for inositol phosphates are shown in Table 4.4. Significant variations in the individual inositol phosphates were evident as a result of the processing procedures applied to commercial canola meal. These results show that inositol hexa- and penta-phosphate were, respectively, the most and least dominant inositol phosphate species in canola meal. High levels of inositol hexaphosphate were found in the ammonia/methanol treated canola meal and the lowest in the meal treated with phytase the two carbohydrate-degrading enzymes. Ammonia/methanol treatment of canola meal seems to elevate inositol hexaphosphate concentration, maybe due to the removal of canola meal constituents soluble in this solution, possibly glucosinolates, phenolics, soluble proteins and some carbohydrates. Canola meal treated with all the processing treatments (α/SpCM) exhibited the lowest level of inositol hexaphosphate. The inositol hexaphosphate value in α/SpCM (1.8 μmol/g) was even lower than that of the canola protein isolate (2.9 *u*mol/g). SP-249 treated canola meal exhibited the highest levels of the lower inositol phosphates (inositol tri-, tetra- and penta-phosphate) whereas the canola protein isolate (CPI) had the lowest values. Inositol hexaphosphate is a major antinutritional factor in upgraded canola meals and protein concentrates because the procedures used to improve the nutritional value of canola seeds or meals have a concentrating effect on the level of inositol phosphate (phytate). Although most inositol phosphate compounds were detected in all canola protein products, inositol tetraphosphate (IP4) was only detected in the meals treated with the carbohydrate-reducing enzymes (SpCM, α-CM, α/SpCM). There was a general trend towards decreased values of inositol hexaphosphate and increased values of inositol triphosphate as the number of enzyme treatments was increased from one (PCM) to all of the four different types of enzymes (α/SpCM). Over 90 % of phytate was broken down in the test canola protein sources receiving all the enzyme treatments. Phytase treatment is known to result in a more-or-less stepwise breakdown of phytate resulting in the production of the different species of inositol phosphate (Fløllich *et al.*, 1986). The reason for the incomplete hydrilysis of phytate in

PCM are unclear. On the basis of the findings of Teskeredzic et al. (1995) the dephytinization protocol used in this study should have been more efficaciuos.

Table 4.4 The inositol tri- (IP3), tetra- (IP4), penta- (IP5) and hexaphosphates (IP6) composition (μmol/g DM) of the test canola protein sources.

Ingredient[1]	IP6	IP5	IP4	IP3	IP3-IP6[2]
CCM	38.8	2.9	nd[3]	3.8	45.5
SCM	41.2	3.1	nd	4.5	48.8
ACM	49.4	3.4	nd	4.2	57.0
PCM	25.7	2.6	nd	2.7	31.0
SpCM	15.8	4.2	4.0	7.1	31.1
α-CM	11.6	2.5	2.1	6.3	22.5
α/SpCM	1.8	1.5	2.4	7.2	12.9
CPI	2.9	0.5	nd	0.9	4.3

[1] CCM, unprocessed canola meal; SCM, sieved canola meal; ACM, sieved, methanol/ammonia treated meal; sieved, methanol/ammonia, phytase treated meal; sieved, methanol/ammonia, phytase, SP-249 treated meal; sieved, methanol/ammonia, phytase, Alpha-Gal treated meal; sieved, methanol/ammonia, phytase, SP-249, Alpha-Gal treated meal; CPI, canola protein isolate. Refer to Table 4.1 for description of treatments

[2] Total inositol containing 3 to 6 phosphate molecules per inositol residue

[3] nd=not detected

4.3.4 Glucosinolates and phenolics

Table 4.5 shows the effects of processing on the glucosinolate and ytterbium-precipitated (Yt-ppt) phenolic concentrations of canola protein products. The content of Yt -ppt phenolics was significantly lowered by methanol/ammonia treatment. Phenolic concentration in the methanol/ammonia treated meal (1.1%) was close to that found in the canola protein isolate (0.98%) and lower than that of the unprocessed canola meal. Similar results in phenolic reduction have also been reported by Naczk and Shahidi (1989). Methanol/ammonia treatment has also been shown to reduce the concentration of condensed tannins from canola seeds (Shahidi and Naczk, 1989). Additionally, treatment with enzymes singly or in combination further reduced the content of phenolics. Soaking in water or salt solution has also been shown to affect phenolic content in legumes (Brassani and Ellis, 1979), which may explain the reduction in the enzyme treated meals.

Significant changes were observed in the concentrations of individual glucosinolates as a result of the various treatments applied to canola meal. Progoitrin (2-hydroxybut-3-enylglucosinolate), gluconapin (But-3-enylglucosinalote) and 4-hydroxyglucobrassicin (4-hydroxyindol-3-ylmethylglucosinolate) were found to be the dominant types glucosinolate present in the canola protein products. Canola protein isolate (CPI) had the lowest levels of glucosinolates (0.45 μmol/g aliphatic and 1.5 μmol/g indole-glucosinolates) compared to 6.55 μmol/g and 2.7 μ mol/g in commercial canola meal. In the laboratory processed meals sieved canola meal had slightly higher levels of each of the glucosinolates analysed than any other product. Sieving appeared to cause a small concentrating effect on the level of these compounds. The methanol/ammonia treated canola meal had glucosinolate levels comparable to those of the canola protein isolate and substantially lower than the levels encountered in the commercial canola meal. No glucosinolates were detected in the rest of the laboratory processed canola products. This significant reduction in the glucosinolate levels is largely due to ammonia/methanol treatment. Ammonia has been reported to be very destructive to some glucosinolates and their hydrolysed products in both meals and canola seeds (McCurdy and March, 1992). In addition, because

glucosinolates and their degradation products are water soluble as well as heat degradable, the large quantities of water and the mild heat used in this study during the enzyme treatments may have lowered the glucosinolate levels even further. Although high water to sample ratio results in removal of large proportions of glucosinolates, substantial loss of dry matter occurs due to disappearance of some constituents in solubilized or suspended form. Among the three main glucosinolate present (two aliphatic and one with the indole side chain) in canola meal, the indole glucosinolate 4-hydroxy-glucobrassicin is known to be most sensitive to heat. Indeed heat treatment has been reported to degrade 40-60 % of aliphatic glucosinolates (Daun, 1986) and up to 100 % degradation of the indole glucosinolates as a result of heat treatment (Campbell and Cansfield, 1983). Care should, however, be taken to avoid excessive heating as this reduces the protein quality of the meal due to decreased lysine availability by way of forming complexes with carbohydrates.

Table 4.5 The glucosinolate[1] and phenolic[2] composition of the canola protein products resulting from the different processing techniques.

Ingredient[3]	CCM	SCM	ACM	PCM	SpCM	α–CM	α/SpCM	CPI
Gluconapin	1.95	2.1	0.25	nd[4]	nd	nd	nd	0.2
Glucobrassicanapin	0.35	0.45	nd	nd	nd	nd	nd	0.1
Progoitrin	3.65	3.95	0.45	nd	nd	nd	nd	0.2
Gluconasturtiin	0.20	0.20	nd	nd	nd	nd	nd	0.0
Glucobrassicin	0.50	0.60	0.1	nd	nd	nd	nd	0.0
4-OH-glucobrassicin	2.20	2.35	0.25	nd	nd	nd	nd	1.5
Total aliphatic	6.05	6.5	0.7	0	0	0	0	0.45
Total indole	2.7	2.95	0.3	0	0	0	0	1.5
Total GSL[5]	8.75	9.00	1.0	0	0	0	0	1.95
Yt-ppt phenolics	2.7	2.4	1.1	0.5	>0.3	>0.3	>0.3	0.98

[1] Expressed as μmol/g

[2] Ytterbium precipitated phenolics including tannins expressed as a percentage of DM

[3] CCM, unprocessed canola meal; SCM, sieved canola meal; ACM, sieved, methanol/ammonia treated meal; sieved, methanol/ammonia, phytase treated meal; sieved, methanol/ammonia, phytase, SP-249 treated meal; sieved, methanol/ammonia, phytase, Alpha-Gal treated meal; sieved, methanol/ammonia, phytase, SP-249, Alpha-Gal treated meal; CPI, canola protein isolate. Refer to Table 4.1 for description of treatments.

[4] nd=not detected

[5] GSL= Glucosinolates

4.3.5 Mineral concentration

Mineral content of the test canola products is provided in Table 4.6. The mineral composition varied considerably between the canola protein products. The levels of cobalt and chromium were affected least by the processing methods. Chromium, cobalt, copper and iron levels were positively affected by processing; so were those of manganese and zinc. Levels of these minerals increased with increase in the number of treatments applied to the commercial canola meal. Among the minerals analysed copper, sodium and sulphur had higher concentrations in the canola protein isolate compared to unprocessed canola meal. Magnesium, phosphorus, sodium and sulphur were lowered by all the combinations of the treatments applied during the laboratory processing of canola meal, the only exception being sulphur in the ammoniated canola meal. This element did not show large variations in concentration among the laboratory prepared canola protein sources. There was a trend toward an increase of all the mineral concentrations with sieving. Thereafter the levels of the soluble minerals (calcium, magnesium, phosphorus, potassium and sodium) declined with subsequent treatments.

Table 4.6 Mineral composition of test canola protein sources[1]

Mineral	CCM	SCM	ACM	PCM	SpCM	α-CM	α/SpCM	CPI
Calcium	7280	7370	7200	7550	6680	6840	6320	977
Chromium	1.3	1.1	1.3	1.6	2.3	2	2.2	1.5
Cobalt	0.3	0.3	0.4	0.3	0.4	1.1	0.7	0.1
Copper	8.8	9.2	8.2	12.7	17.6	27.4	67.9	24.5
Iron	513	574	688	528	854	741	1260	47.2
Magnesium	5540	5890	5480	5000	4040	3760	2230	482
Manganese	57.1	65.2	70.4	80.7	76.5	77.6	65	11.2
Phosphorus	11400	12000	11500	10500	8020	7500	5200	10200
Potassium	14100	14500	12800	6370	3300	2980	1450	350
Selenium	<2	<2	<2	<2	<2	<2	<2	<2
Sodium	1290	1350	1190	520	347	270	420	3130
Sulphur	7500	8100	5800	5600	5500	5700	7100	11400
Zinc	55.3	62.5	67.5	84.3	85.9	88.1	93.3	13.3

[1] Expressed as mg/kg DM as determined by Quanta Trace Laboratories Inc., Burnaby, B.C., CCM, unprocessed canola meal; SCM, sieved canola meal; ACM, sieved, methanol/ammonia treated meal; sieved, methanol/ammonia, phytase treated meal; sieved, methanol/ammonia, phytase, SP-249 treated meal; sieved, methanol/ammonia, phytase, Alpha-Gal treated meal; sieved, methanol/ammonia, phytase, SP-249, Alpha-Gal treated meal; CPI, canola protein isolate. Refer to Table 4.1 for description of treatments.

4.3.6 Amino acid profile

The amino acid composition of the test canola protein sources are given in Tables 4.7 and 4.8. The amino acid levels were strongly affected by the processing treatments since the concentrations of all the amino acids were increased by all the treatments. Levels of all amino acids in the commercial canola meal used in the present study were lower than the averages reported by Bell and Keith (1991) for canola meal obtained from Western Canadian crushing plants. The canola meal used in this study contained 1.9 % lysine and 0.36 % methionine compared to 2.49 % lysine and 0.86 % methionine measured by Bell and Keith (1991). The lower protein content of the meal used in this study may explain the inferior amino acid content observed. Canola protein isolate showed the highest concentrations of amino acids compared to either the unprocessed meal or any of the laboratory processed canola meals. Additional processes performed to reduce the levels of antinutritional factors during the preparation of protein isolate could possibly be the reason for the improved amino acid profile of the canola protein isolate. Among the laboratory processed canola meals methanol/ammonia treated canola meal exhibited the lowest amino acid concentrations whereas the canola meal treated with the two carbohydrases (α/SpCM) showed the highest values (except methionine). Highest methionine concentrations (0.61 %) were found in the phytase treated canola meal. These changes in the amino acid concentrations of canola meals can be related to the effect of treatment on the protein content of the meals. In relation to the known amino acid needs of rainbow trout (NRC, 1993) all the canola products contained sufficient levels of arginine and tryptophan and all except CPI had adequate levels of lysine. However, the levels of methionine in all products except ACM and CPI were below the required ranges.

Table 4.7 The amino acid composition of unprocessed, laboratory processed canola meals as well as the canola protein isolate (% DM)[1]

Amino acid[2]	CCM	SCM	ACM	PCM	SpCM	α-CM	α/SpCM	CPI
Alanine	1.38	1.47	1.63	1.91	1.85	1.88	1.94	2.96
Arginine	2.24	2.18	2.46	2.71	2.62	2.71	2.59	6.06
Aspartic acid	2.30	2.35	2.53	2.90	2.94	2.99	3.09	6.03
1/2 Cystine[3]	0.63	0.59	0.57	0.77	0.87	0.86	0.88	1.12
Glutamic acid	5.21	5.50	6.04	6.63	6.35	6.56	6.32	14.9
Glycine	1.54	1.62	1.80	2.05	1.99	2.03	2.08	4.13
Histidine	0.99	0.96	0.96	1.14	1.16	1.18	1.20	2.17
Isoleucine	1.34	1.38	1.53	1.80	1.84	1.86	1.96	3.26
Leucine	2.20	2.25	2.52	2.96	3.06	3.10	3.30	5.67
Lysine	1.90	1.87	2.03	2.38	2.49	2.57	2.61	3.19
Methionine	0.36	0.47	0.58	0.61	0.48	0.53	0.38	1.30
Phenylalanine	1.30	1.40	1.53	1.79	1.82	1.84	1.99	3.29
Proline	2.14	2.30	2.6	2.98	2.77	2.82	2.89	5.32
Serine	1.35	1.42	1.61	1.84	1.83	1.88	1.96	2.97
Threonine	1.41	1.47	1.64	1.91	1.88	1.92	1.97	2.86
Tryptophan[4]	0.33	0.43	0.52	0.62	0.61	0.59	0.65	1.37
Tyrosine	0.85	0.98	1.14	1.31	1.31	1.34	1.38	1.90
Valine	1.74	1.73	1.92	2.25	2.34	2.37	2.58	3.95

[1] CCM, unprocessed canola meal; SCM, sieved canola meal; ACM, sieved, methanol/ammonia treated meal; sieved, methanol/ammonia, phytase treated meal; sieved, methanol/ammonia, phytase, SP-249 treated meal; sieved, methanol/ammonia, phytase, Alpha-Gal treated meal; sieved, methanol/ammonia, phytase, SP-249, Alpha-Gal treated meal; CPI, canola protein isolate. Refer to Table 4.1 for description of treatments.

[2] Amino acids concentrations were determined by AAA Laboratory, Mercer Island, WA., 20-hr. 6N HCl/ 0.05% mercaptoethanol/ 0.02% phenol hydrolysis at 115 °C. Serine increased by 10% and threonine by 5% to compensate for destruction by acid.

[3] Performic acid oxidized prior to acid hydrolysis. Calculated from cysteic/alanine ratio

[4] 48-hr. alkaline hydrolysis at 135 degrees C.

Table 4.8 The amino acid composition of unprocessed, laboratory processed canola meals as well as the canola protein isolate (g/16 g N)[1]

Amino acid[2]	CCM	SCM	ACM	PCM	SpCM	α-CM	α/SpCM	CPI
Alanine	3.79	3.96	4.16	3.96	3.78	4.21	4.69	3.26
Arginine	6.16	5.87	6.28	5.62	5.44	5.97	6.26	6.67
Aspartic acid	6.33	6.32	6.46	6.02	6.01	6.70	7.47	6.64
1/2 Cystine[3]	1.73	1.60	1.46	1.59	1.73	1.97	2.12	1.23
Glutamic acid	14.3	14.8	15.4	13.76	13.2	14.4	15.3	16.4
Glysine	4.24	4.36	4.60	4.25	4.08	4.53	5.03	4.55
Histidine	2.75	2.58	2.54	2.36	2.37	2.64	2.90	2.39
Isoleucine	3.68	3.71	3.91	3.73	3.74	4.19	4.74	3.59
Leucine	6.05	6.05	6.44	6.14	6.23	6.97	7.98	6.24
Lysine	5.23	5.03	5.18	4.94	5.16	5.67	6.31	3.51
Methionine	0.99	1.27	1.48	1.26	0.96	1.20	0.91	1.43
Phenylalanine	3.57	3.77	3.91	3.71	3.69	4.15	4.81	3.62
Proline	5.89	6.19	6.64	6.18	5.67	6.31	6.99	5.86
Serine	3.71	3.82	4.11	3.82	3.78	4.17	4.74	3.27
Threonine	3.88	3.95	4.19	3.96	3.86	4.28	4.76	3.15
Tryptophan[4]	0.91	1.15	1.31	1.27	1.20	1.39	1.56	1.50
Tyrosine	2.34	2.64	2.91	2.72	2.69	2.98	3.33	2.09
Valine	4.79	4.65	4.90	4.67	4.76	5.33	6.24	4.35

[1] CCM, unprocessed canola meal; SCM, sieved canola meal; ACM, sieved, methanol/ammonia treated meal; sieved, methanol/ammonia, phytase treated meal; sieved, methanol/ammonia, phytase, SP-249 treated meal; sieved, methanol/ammonia, phytase, Alpha-Gal treated meal; sieved, methanol/ammonia, phytase, SP-249, Alpha-Gal treated meal; CPI, canola protein isolate. Refer to Table 4.1 for description of treatments.

[2] Amino acids concentrations were determined by AAA Laboratory, Mercer Island, WA., 20-hr. 6N HCl/ 0.05% mercaptoethanol/ 0.02% phenol hydrolysis at 115 °C. Serine increased by 10% and threonine by 5% to compensate for destruction by acid.

[3]Performic acid oxidized prior to acid hydrolysis. Calculated from cysteic/alanine ratio

[4] 48-hr. alkaline hydrolysis at 135 °C.

4.4. DISCUSSION

4.4.1 Effects of mechanical and chemical processing.

Fibre, protein and energy are three of the most important factors to consider when including plant protein sources in formulated diets for salmonids. There is potential for using canola meal in salmonid diets, however, high fibre content and the presence of toxic constituents are primary concerns. With respect to elevation of dietary levels of crude fibre when using canola meal in salmonid diets as a substitute for fish meal it may not be desirable for optimum fish performance. In order to use this product as a partial or complete replacement for fish meal in salmonid diets there is need to improve its chemical composition. To enhance the utilization of canola meal more efficient processing techniques are needed so that products with higher levels of protein and lower levels of deleterious components are produced. This goal may be attained by both physical and chemical processing of canola meal. High fibre content (>10%) restricts growth, impairs feed and protein conversion and decreases the intestinal transit time and diet digestibility. Hull and cotyledon material tend to become tightly bound during the processing of canola seeds to produce oil. Hence, milling should enhance the separation of fibre from protein of the meal. McCurdy and March (1992) reported that sieving canola meal elevated its protein levels, contrary to the results of the present study although this treatment did less in the reduction of the fibre content of canola meal. The reasons for differences in these results are not clear but could be partly due to differences in the mesh sizes of the sieves used in these two trials. Smaller sieve size would be much more effective in sifting off much of the hull fraction thus increasing the protein content, while at the same time reducing the fibre content. Sieving and roller milling has also been shown to .affect nutrient composition of canola meals. Although grinding did not have any influence on most crude nutrients it has been found that fine grinding does reduce the crude fibre content of canola meal (Kracht *et al.*, 1995). However smaller sieve sizes result in large reductions in yields of the fibre-reduced meal on a dry matter basis. The relatively high fibre (non-starch polysaccharides (NSP) and lignin) content of canola meal due to its large proportion of hulls (30%)

has been implicated as being responsible for the lowered digestible protein and energy content. Highest proportions of NSP and lignin are present in the hulls, and these constituents are responsible for the low digestibility coefficients of both protein and energy found in isolated hull fractions given to different animal species (Bell, 1993; Jensen *et al.*, 1990). Attempts to improve the nutritional value of canola meal have included among other things, increasing the digestibility of the hulls and/or reducing the hull proportion by reducing the fibre content of the meal . Additionally, because yellow-seeded varieties of canola are lower in fibre content than brown-seeded type, plant selection programs have been directed towards increasing the production of the yellow-seeded canola varieties. Dehulling of both brown- and yellow-seeded varieties of canola meal has been shown to lower the total dietary fibre level considerably, relative to that found in commercial canola meal (Campbell *et al.*, 1995) as was large seed size (Jensen *et al.*, 1994), because the larger the seeds are, the smaller the proportion of hulls that constitutes the whole canola seed.

Chemical composition of oilseed meals after processing may provide important insight into nutritional value, especially in regard to the possible effects of toxic constituents. Antinutritional factors play a major role in adversely affecting the feed quality of oilseed meals, canola meal being no exception. The adverse effects of glucosinolates on salmonids have been well documented (Higgs *et al.*, 1995; Jensen *et al.*, 1994) and, by a variety of mechanisms, cellulose, hemicellulose, pentosans and lignin adversely affect mineral availability. While the soluble carbohydrate fraction of plant protein sources appears to be well utilized as an energy source by warm water fish, this may not the case with salmonids, especially rainbow trout. Soluble carbohydrates, especially oligosaccharide sugars, are responsible for interfering with nutrient absorption and increasing the intestinal transit time. Monogastric animals are unable to utilize oligosaccharides as energy sources and can therefore be considered as antinutrients with regard to salmonids. Removal of toxic constituents through the application of chemical processing techniques has been shown to improve the chemical as well as the nutritional quality of oilseed meals (Liu *et al.*, 1994). Results of the analyses of the canola protein products from the laboratory processing in this study indicate

substantial changes in the chemical composition with respect to the processing techniques. Laboratory processing of canola meal significantly influenced the levels of the various antinutritional factors present in canola meals, as well as some proximate constituents. Chemical processing resulted in an increase in the protein concentration of canola meal, consistent with the results of Sosulski *et al.* (1993). The increases in protein content can be attributed to the removal of other soluble constituents from the meal as a consequence of the processing treatments applied. The removal of glucosinolates by methanol/ammonia treatment and the possible dissolution of other soluble constituents during the enzyme treatment may have been major factors in the elevation of the levels of canola meal protein. Indeed Diosady *et al.* (1984) reported that the increase in protein content of meals after methanol/ammonia treatment was a direct result of the dissolution of non-protein components from the meal. This may also explain the increase in most of the fibre constituents observed in methanol/ammonia treated canola meal (ACM) relative to the sieved canola meal because fibre constituents are not soluble in ammoniated methanol. Methanol/ammonia treatment of canola meal has been shown by many workers (Keith and Bell, 1982; Shahidi and Gabon, 1989) to be a very effective treatment for the reducing some of the antinutritional factors present in canola meal. Similar findings were observed in this study. For instance, washing of canola meal with methanol/ammonia solution reduced the total glucosinolate content by almost 90% but increased the phytate content by 26%, because phytate is not soluble in alcohol. Glucosinolates are important since high glucosinolate content in canola meal causes enlargement of the thyroid in animals. This thyroid enlarging effect is mainly due to vinyloxazolidinethione (VOT) which is a breakdown product of hydroxybutenyl glucosinolate (progoitrin), which is a major component in rapeseed, by interfering with the synthesis of thyroxine in the thyroid gland. This compound impairs thyroid hormone synthesis and perhaps of extrathyroidal convertion of T_4 to T_3 (Higgs *et al.*, 1995a). No studies on the thyroid physiology were conducted in the present experiment.

Ammonia treatment, like other alkali treatments, is also very destructive towards glucosinolates, and this decomposition is thought to occur via two distinct mechanisms. The major

pathway involves the release of thioglucose from the glucosinolate molecule to yield a stable nitrile or hydroxynitrile (Shahidi and Gabon, 1989), whereas the other pathway results in the formation of minor amounts of isothiocyanates and epithionitriles, but no oxazolidinethione is produced in this process. These substances can be removed by washing with water because of their high solubility. Methanol/ammonia treatment of meal in this study also resulted in elevation of the concentration of amino acids. This is consistent with the results of Wanusandara and Shahidi (1994) for linseed meal treated in a similar manner. These authors suggested that the observed results were due to removal of polar compounds, namely, phenolic acids, cynogenic glycosides, sugars and phospholipids from the meal by the alkanol.

It is well established that phytic acid in oilseeds is present in association with storage proteins (Thompson, 1990). Therefore, the removal of polar substances from the meal resulted in concentration of proteins and a similar enhancement in the content of phytic acid. Canola meal contains a high quality protein, but its use in monogastric diets has been limited by relatively low availability of phosphorus (due to phytate presence), energy and amino acids. Phytic acid is strongly negatively charged in foods and has a high affinity toward protein and cations, especially zinc, at intestinal pH. Complexing of zinc with phytic acid is further enhanced in the presence of excess calcium ions. The removal of phytic acid from soy has been shown to improve growth and feed conversion in rainbow trout (Spinelli *et al.,* 1979).

4.4.2 Enzyme treatment

The use of enzyme treatment in order to modify the chemical composition and subsequently the nutritive value of canola meal has been reported (Guenter *et al.*, 1995; Sosulski *et al.,* 1993). In the present study the use of enzyme treatment to modify the meal for use in rainbow trout diets produced some very interesting observations. All the enzyme treatments had significant effect on the protein content of the meal (49.7% in SP-249- and 48.2% in phytase-treated canola meals). Dissolution of soluble materials from canola meal by the substantial amount of water used in these treatments may explain this. It was expected that this trend would continue

in the rest of the enzyme treated meals (α-CM and α/SpCM). However, this was not the case. Although the protein contents of the Alpha-Gal and the SP-249/Alpha-Gal treated canola meals were higher than those of untreated canola meal, they were lower than the levels found in the two canola meals treated with either phytase or phytase and SP-249 (PCM and SpCM). These results differ from those reported by Sosulski *et al.* (1993). The reason for this reduction in protein content is, however, not clear. Enzyme treatments also decreased the levels of glucosinolates further. This can not, however, be attributed to the action of the enzyme *per se.* Glucosinolates and their hydrolysed products are water soluble and most of these can be removed by water washing and in this way non-toxic canola meal may be produced. Complete removal of residual glucosinolates was achieved in this study because of the methanol/ammonia treatment coupled with the presence of large quantities of water used during the enzyme treatment. Water extraction has been shown to remove nearly all the glucosinolates as well as their derivatives and increased the crude protein content of canola meal (Liu *et al.,* 1994).

Application of enzymes (except SP-249) significantly raised the levels of most of the fibre constituents of canola meal. SP-249 is a cell wall degrading enzyme with mixed activity that has previously been shown to be effective in reducing the fibre content of canola meal (Sosulski *et al.,* 1993). Application of SP-249 in this trial slightly reduced the fibre content as measured by the AOAC (1990) crude fibre method but resulted in increased values for ADF, NDF, cellulose, hemicellulose and lignin. These findings are contrary to the results of Sosulski *et al.* (1993) who reported the use of this enzyme for the reduction of fibre content of canola meals. No logical explanation can be offered for this discrepancy in results. Removal of canola soluble constituents (glucosinolates, phenolics and soluble carbohydrates) by methanol/ammonia treatment may have possibly masked any positive effects, if any, of the fibre-reducing enzyme treatment employed. Aqueous enzymatic hydrolysis of rapeseed cell walls has also been shown to considerably modify the carbohydrate profile, especially, the levels of oligosaccharides as well other low molecular weight sugars (Jensen *et al.*, 1990). Low molecular weight sugars including oligosaccharides were however, not measured in this study.

Processing techniques can remove a significant amount of phytate from the meal (Reddy *et al.,* 1989) or alternatively there is potential for treatment of diet or dietary ingredients with exogenous sources of phytase to reduce the amount of phytate, and hence increase the amount of available phosphorus. The use of wheat bran phytase has been shown to improve the nutritional value of wheat bran/canola meal/fish silage blend (Stone *et al.,* 1984). Indeed these authors reported no presence of phytic acid, organic phosphorus or lower inositol phosphates after 35 days. Phytase, Alpha-Gal and Alpha-Gal/SP-249 treatment in this trial elevated the fibre content of canola meal at the same time reducing the level of inositol hexaphosphate. Breakdown of phytate by phytase and the possible removal of oligosaccharide sugars by α-galactosidase may explain the increased concentration of fibre components, similar to results reported by McCurdy and March (1992) for acid washed fibre-reduced canola meals. Phytase treatment of canola meal reduced the phytate content from 45.5 μmol/g to 31.0 μmol/g. This is in good agreement with the results of Riche (1993) but not with those of Teskeredzic *et al.* (1995). Fløtich *et al.* (1986) have also shown that incubation of whole wheat flour with microbial phytase resulted in over 80% hydrolysis of total phosphate to inorganic phosphate after 4 hr. Further, potentiation of endogenous phytase has also been shown to aid in the hydrolysis of phytate of plant origin (Ruth *et al.*, 1977). Phytase causes the dephosphorylation of phytate first resulting in an increase and subsequently decline in the lower inositol phosphate content as phytate is converted to inositol monophosphate. Complete dephosphorylation of inositol monophosphate to inositol and phosphate can be achieved with the use of phosphatases (Ruth *et al.,* 1977; Stone *et al.,* 1984). In the present study treatment of canola meal with carbohydrate-reducing enzymes (SP-249 and Alpha-Gal) reduced the amount of phytate further. No logical explanation can be offered for this observation since no endogenous phytase activity has been reported in canola meal and none was reported as being present in the carbohydrate-reducing enzymes. Phytate activity remaining from from the initial phytase treatment could be a possible explanation. According to the findings of this present study it can be concluded that a considerable amount of phytate is hydrolysed by treating canola meal with

exogenous phytase.

4.4.3. Canola protein isolate (CPI)

The chemical composition of the canola protein isolate tested in this study is superior to that of the other test canola protein sources and to fish meal with respect to protein content. The canola protein isolate contained high levels of protein (90.8%), gross energy (24.4 MJ/kg) and a low level of fibre. The gross energy content of CPI was much higher than that noted in soybean protein isolate reported Hajen (1990) but the protein concentrations were comparable. The improved chemical composition of CPI relative to upgraded canola meals and concentrates can be attributed to the successful reduction of antinutritional factors, namely phenolic compounds, carbohydrates (indigestible and digestible) as well as glucosinolates through processing. Crude fibre, ADF and NDF values for the canola protein isolate were less than 3% (2.06%, 2.36% and 2.55%, respectively) and the values for hemicellulose and acid detergent lignin were almost negligible. All of these values were significantly lower than the corresponding values in any of the laboratory processed meals. In addition, the levels of other ANFs were also very low. For instance, in CPI total glucosinolates (1.95 μmol/g) and ytterbium-precipitated phenolic (0.98%) levels were lower than the corresponding values noted for commercial canola meal. Thus, on the basis of chemical composition, improved canola protein products (upgraded canola meals and isolates) can de obtained by processing of canola meals. Canola protein isolate contains higher levels of protein and energy than any of the other improved canola products.

Chapter 5

THE POTENTIAL NUTRITIVE VALUE OF CANOLA PRODUCTS FOR RAINBOW TROUT (*Oncorhynchus mykiss)*

5.1 INTRODUCTION

Evaluation of the nutritive value of ingredients to be used in animal feeds is a very important aspect of nutrition research. Although chemical analysis is the first step in evaluating the nutritive value of feedstuffs for animals, it is an imperfect standard since the value of a feedstuff cannot be accurately defined by its chemical composition, although changes in chemical composition will influence the nutritive value. Digestibility is a major determinant of the nutritional value of feed. The value of a feedstuff depends upon the amounts of these nutrients that the animal can digest and utilize. Hence measurement of digestibility gives a good indication of the nutritional quality of feedstuffs. A biological assessment of feed ingredients, which incorporates not only digestibility but a range of other variables involves the feeding of these ingredients to animals and the analysis of some aspects of animal performance (growth, feed efficiency, and carcass nutrient and energy retention). High quality fish meals are well recognized as the best sources of protein for salmonids (Pike *et al.,* 1990), although they are the most expensive. Digestibility can be measured by one of the following two methods: (a) quantitative, or total collection method which is time consuming and is adversely affected by errors in measuring ingested feed and in collecting and measuring of faeces and (b) the indirect or indicator method where accurate measurement of feed intake and faecal output are not necessary. However, errors can occur through leaching of nutrients from faeces collected in water and inaccuracies in measuring indicator concentration.

Several kinds of indicators have been used in digestibility experiments. They can all be divided into two classes based on their origin. External or exogenous indicators are added to the diet and include such diverse substances as glass beads, ferric oxide, radioactive metals such as chromium, lanthanum, and yttrium (Kotb and Luckey, 1972), polyethylene, chromic oxide, acid

washed-sand (Tacon and Rodrigues, 1984) and barium carbonate (Riche, 1993). Natural internal (indigenous) indicators are those that occur in the diet naturally. Crude fibre (De Silva and Perera, 1983; Tacon *et al.,* 1983) and acid insoluble ash (Bowen, 1981) have been used as internal indigestible markers in animal digestibility experiments. External indicators added to the diets for the estimation of nutrient digestibility in fish include magnesium ferrite (Barashi *et al.,* 1984; Fadley, *et al.,* 1990*)*, crude fibre (De Silva *et al.,* 1990), silica, cellulose and hydrolysis-resistant ash (De Silva and Perera, 1983) and radiolabelled phosphorus (Austreng, 1978). At present, chromic oxide is the most commonly used external indicator and was, therefore, used in the present experiment.

Faecal collection has been done by manual stripping (Nose, 1960), anal suction (Windell *et al.,* 1978) and intestinal dissection (Austreng, 1978; Hajen *et al.,* 1993; Hilton *et al.,* 1983). The drawbacks of these collection methods include contamination of faeces with undigested feed, sloughed off intestinal epithelial cells, mucus, blood and sexual products. Induced sudden defecation and accelerated transit of intestinal contents (Spyridakis *et al.,* 1989) and the problem of stress as a result of fish handling is also a concern. Some researchers have tried to overcome these problems by collecting fish faeces voided into the water. Despite the absence of fish handling in this technique, nutrient leaching is the major concern. Smith (1971) confined fish in metabolism chambers that allowed him to separate and collect faeces, urine and gill excretions, permitting the determination of digestibility coefficient and metabolizable energy. The disadvantages of this method are that the fish are confined, force fed, catheterized and are under considerable stress, which are not ideal culture conditions. Ogino *et al. (*1973) collected faeces by passing the effluent water from the fish tanks through a filtration column. Other investigators have used fine mesh nets (Windell *et al.,* 1978) and mechanically rotating screen devices (Choubert *et al.,* 1982; De la Noue and Choubert, 1986) to filter out and collect the faecal material from the outflow water. The disadvantage with these mechanical methods is that faecal pellet disruption increases the chances of nutrient leaching. The "Guelph system" of fecal collection developed by Cho *et al.* (1982) has been shown to be highly reliable. In this procedure the effluent water from the fish tanks passes

into a settling column that is equipped with an outlet at the bottom, where settled faecal material rests in undisturbed water before collection. This allows fish to feed normally, without any need to handle them, it also allows repeated sampling so that digestibility evaluation of different diets can be carried out simultaneously with observations of growth rate. One concern about this method is nutrient leaching, but the close agreement between digestibility values obtained by intestinal dissection and collecting of faecal material by suction (anal suction) with results from "Guelph system" (Cho and Slinger, 1979) indicate that leaching is not a major source of error. As long as faecal pellets do not "breakup" and that they are removed from the water swiftly, leaching is kept at a minimum (Cho and Slinger, 1979; Choubert *et al.*, 1982). The "Guelph system" was chosen the technique for faecal collection in this study.

No single feed ingredient can be used as the sole component in formulating a diet, hence it is common practice when determining the digestibility of feed ingredients to combine them with a reference diet. The digestibility of a test material is then determined by comparing the digestibility of the test ingredient plus reference diet with that of a reference diet only; both the test and the reference diet containing an indigestible marker. The use of a reference diet assumes that there is no interaction between the components of the diet during the process of digestion. This experiment was designed to study the influence of different processing methods outlined in Chapter 4 on the potential nutritive values (digestibility) of canola meal by measuring digestibility by rainbow trout (*Oncorhynchus mykiss*) in fresh water.

5.2. MATERIALS AND METHODS

5.2.1. Aquarium facility and fish history

The experiment was conducted at the Department of Fisheries and Oceans, West Vancouver Laboratory. Each of the twenty-seven specially designed (Hajen, 1990) digestibility tanks was supplied with filtered well water at a flow rate of 4-5 litres per minute in a flow-through system. This was the highest flow rate that could be attained at the time of the experiment without affecting the water supply to other on-going experiments. Nevertheless this flow rate was enough

to ensure swift removal and gentle settling of faecal material in the collection column. The digestibility system used in the experiment was a modification of the "Guelph system" of Cho and Slinger (1979) but with modifications as described by Hajen (1990). A central filtering system however, was used in this experiment to filter the water before flowing into the tanks. Air flow rate into each tank was through aeration tubing and was adjusted to maintain oxygen levels close to saturation at the water outflow. Water temperature, dissolved oxygen levels, were monitored daily. The conditions of the experiment are presented in Table 5.1. Seven hundred rainbow trout (average weight 70 g) were obtained from Spring Valley Trout Farm (Langley, B.C) in December, 1994 and were transported to the D.F.O, West Vancouver Laboratory where they were held in a 4000 L stock tank in the outdoor aquarium supplied with fresh creek water. Fish were fed the basal diet twice daily for one week and were then sampled for uniform weight before being transferred to the digestibility tanks. Fish were anesthetized with Marinil (1g in 4000L of water in stock tank) and MS 222 (0.25 g in buffered fresh water) and then distributed in groups of seven until each of the twenty-seven tanks had twenty-one fish in it. Thereafter one fish was added to each tank to make the total number of fish in a tank equal to twenty-two. The fish were fed the basal diet and after three days were then switched to the experimental diets.

5.2.2 Diet preparation and composition

The composition of the basal diet is provided in Table 5.2 and the description and proximate analysis of the experimental diets are given in Tables 5.3 and 5.4, respectively. All the test diets were composed of 30% test material and 70% of basal (control) diet on an air-dry basis. All diet components were obtained commercially and, where necessary, ground in a Fitz mill (The Fitzpatrick Company, Elmhurst, Illinois) equipped with a size 30 U.S. screen.

The mineral supplements were ground to a fine powder in a coffee grinder before weighing them individually. Each pre-weighed mineral was added to half of carrier (α-cellulose) with mixing after each addition, using the small bowl of Hobart mixer. After all the minerals were added the remainder of carrier was added and mixed for 10 minutes before being transferred to a

V-shaped mixer (Patterson-Kelley Co., Division of Harsco Corp, Pennsylvania) and further mixed for 30 minutes. The same procedure was followed for the vitamin supplements with Whetstar 3 (unmodified wheat starch , Ogilvie mills) as the carrier and the final mixing in the V-shaped mixer was carried out for 60 minutes.

Preparation of the basal diet involved the mixing of all the ingredients in a Hobert commercial mixer for 30 minutes. The reference diet (basal mix and 0.5% chromic oxide) and the test diets (70% basal mix: 30% test canola product and 0.5% chromic oxide) were then prepared by using the procedure described above. To ensure uniform mixing of chromic oxide with the diets, chromic oxide was combined in small amounts with a small portion of either the basal mix or the canola test material and ground in a mortar and pestle until there were no lumps. The remainder of the test material was added, and mixed for 5 minutes in a small bowl of Hobert mixer before being mixed again in a V-shaped mixer for 20 minutes. This chromic oxide-containing test materials and basal mix were then combined with the remaining portion of basal mix (70% for test diets), moisture adjusted to 9.0 %, mixed in large bowl of a Hobart mixer for 30 minutes before pelleting. The diets were cold-pelleted in a California model CL-type 2 laboratory pellet mill (California Pellet Mill Co., San Fransisco, CA) equipped with a 2.38 mm die. Immediately after pelleting the diets were placed in a vertical drying tower to cool. Fines were then removed by screening and oil was added by using an electric spray gun as the pellets tumbled in a cement mixer. The diets were then bagged and stored in a refrigerator (4° C) throughout the experiment.

5.3.2 Experimental procedure and sampling

The row of twenty seven tanks was divided into three blocks of nine tanks each. On January 6, 1995 the nine prepared diets were randomly assigned to triplicate groups of fish such that each diet was allocated once in each block. The fish were fed their prescribed diets twice daily (0900 and 1500 hr) for three days before faecal collection commenced. To avoid wastage, feed was offered as long as the fish were feeding actively. Each day after the afternoon feeding, the drain pipe and the faecal settling column affixed to each tank were brushed out to remove feed

residues and faeces from the system and a third of the water was drained out to ensure complete removal of uneaten feed and faeces. The following morning the settled faeces and surrounding water were gently withdrawn from the settling column of each tank into 250 ml centrifuge tubes. The faeces were centrifuged (10000 g for 20 min at 5° C) in a Sorval RC5 refrigerated centrifuge as described by Hajen *et al.,* (1993). The supernatant was discarded and each pellet was stored in a sealed container at -40° C. The fish were fed immediately after each faecal collection, thus allowing repeated sampling over the next twenty-one days. At the end of the experimental period the fish in each tank were again anesthetised and their individual weights recorded.

The pooled faeces from each tank were first freeze-dried and then ground in a coffee grinder (Braun) due to the large volume. Faeces and feed were analysed for proximate composition. Moisture was measured by drying a 1 g sample in an oven for 16 hr, ash by incinerating a sample in a furnace at 600 °C and protein according to the procedure of Higgs *et al.* (1979). The mineral composition of the basal diet was determined by plasma emission spectroscopy (ICP) by Quanta Trace Laboratories Inc. (Burnaby, B.C.).

5.2.4 Chromic oxide determination

Chromic oxide concentrations in the samples of feed and faeces were determined using the dry ashing colorimetric method of Fenton and Fenton (1979). Feed (6 g) or faeces (2 g) samples were ashed at 450 °C for 20 hr, then heated with 15 ml of digestion mixture (10 g sodium molybdate dihydride in a solution of distilled water, sulphuric acid and perchloric acid in a 150:150:200 v/v/v ratio) on a hot plate until the solution turned yellow. Distilled water (5-10 ml) was added to the cool solution before transferring the contents into a 200 ml volumetric flask and making to volume with distilled water. Portions of solution (10 ml) were centrifuged at 700 g for 5 min and the absorbance read at 440 nm using distilled water to zero the spectrophotometer. Chromic oxide standards were prepared by treating portions of pure Cr_2O_3 (5-60 mg) in a similar manner throughout the procedure (see Appendix 4 for details).

Table 5.1 Experimental conditions of digestibility trial

Parameter	
Experimental fish	Rainbow trout
Initial mean wt (g)	74.1
Final mean wt (g)	98.7
Number of fish/tank	22
Stocking density (kg/m^3)	10.9
Number of groups/diet	3
Type of tank	Fiberglass
Water volume (L)	150
Water source	Well water
Temperature (° C)	9.9-11.0
Water flow rate (L/min)	4-5
Dissolved oxygen (mg/L)	10.8-11.5
Feeding frequency/day	2
Duration of experiment (days)	21
Photoperiod[1]	Natural

[1] Photocell to Vitalite Durotest light

Table 5.2 Composition of basal diet used for preparation of test diets (70% basal diet : 30% test ingredient air dry basis) for digestibility experiment

Ingredients	Concentration (g/kg dry diet)
B.C. Packers herring meal	580
Spray dried blood flour	40
Dried whey	75
Freeze-dried euphausids	60
Gelatinized wheat starch	67
Vitamin supplement[1]	20
Mineral supplement[2]	20
Sardine oil[3]	111
Soybean lecithin	10
Choline chloride (60%)	5
Vitamin C	2
Permapel	10

[1] The vitamin supplement provided the following (mg/kg dry diet): pantothenate (as DL-calcium pantothenate), 165; pyrodoxine (as pyridoxine-HCl), 40; riboflavin, 60; folic acid, 15; thiamine (as thiamine mononitrate), 50; biotin, 1.5; vitamin B_{12}; 0.09, vitamin K (as menadione sodium bisulfite), 18; vitamin E, 300 IU; vitamin D_3, 2400 IU; vitamin A, 5000 IU; inositol, 400; niacin, 300; BHT, 22 with 17.766 g of Whetstar 3 (modified wheat starch, Ogilvie mills, as carrier)

[2] The mineral supplement provided the following amounts (mg/kg dry diet): manganese (as $MnSO_4 . H_2O$), 75; zinc (as $ZnSO_4 . 7H_2O$), 90 cobalt (as $CoCl_2 . 6H_2O$), 2; copper (as $CuSO_4 . 5H_2O$), 6; iron (as $FeSO_4 . 7H_2O$), 100; iodine (as KIO_3), 10; fluorine (as NAF), 10; selenium (as Na_2SeO_3), 0.1; sodium (as NaCl), 1000; manganese (as $MgSO_4 . 7H_2O$), 350 and 12.717 g of α-cellulose (as a carrier)

[3] Stabilized with 500 ppm ethoxyquin.

Table 5.3 Description of diets used in the evaluation of apparent digestibility coefficients for dry matter, protein and energy

Diet description	Diet Number
Basal (Reference) diet	1
Basal + unprocessed canola meal	2
Basal + sieved canola meal	3
Basal + sieved, methanol/ammonia-treated canola meal	4
Basal + sieved, methanol/ammonia-, phytase-treated meal	5
Basal + sieved, methanol/ammonia-, phytase-, SP-249-treated canola meal	6
Basal + sieved, methanol/ammonia-, phytase-, Alpha-Gal-treated canola meal	7
Basal + sieved, methanol/ammonia-, phytase-, SP-249-, Alpha-Gal-treated canola meal	8
Basal + canola protein isolate	9

Table 5.4 The proximate composition of the experimental diets (Moisture as % air dry, other constituents as % DM)[1]

Content (%)	Diet[2]								
	Control	2	3	4	5	6	7	8	9
Moisture	8.49	8.96	8.23	8.25	8.39	8.12	8.78	8.71	8.47
Crude protein	51.9	47.1	46.8	48.7	48.7	49.3	49.6	48.2	61.1
Lipid	18.3	17.6	18.0	18.1	17.74	15.6	16.5	18.0	18.2
Ash	15.4	13.8	13.9	14.2	13.4	13.2	13.4	12.7	10.9
GE (MJ/kg)	21.5	20.9	20.9	20.8	20.9	20.9	21.1	21.4	22.3
Chromic oxide	0.55	0.55	0.60	0.55	0.55	0.56	0.55	0.55	0.56

[1] Diets contained 70% reference diet and 30% of the following: 2, commercial canola meal; 3, sieved canola meal; 4 , ammoniated canola meal; 5, phytase treated canola meal; 6, SP-249 treated canola meal; 7, α-galactosidase treated canola meal; 8, α-galactosidase and SP-249 treated canola meal; 9, canola isolate containing diet and 1 was the control diet. (Refer to Table 4.1 for proximate composition of canola materials)

[2] Refer to Table 5.3 for description of diets

5.2.5 Calculation of digestibility and fish performance

The apparent digestibility coefficients (ADC) for dry matter, protein and energy for each diet were calculated using the indicator method according to the equation of Maynard and Loosli (1969).

ADC (%) = 100- 100 (%Cr_2O_3 in feed/ %Cr_2O_3 in feces)×(% nutrient in feces/ % nutrient in feed)

The digestible energy content was calculated using the apparent digestibility coefficient of energy and the gross energy data according to Hajen (1990). The apparent digestibility coefficients for the test ingredients were calculated from the apparent digestibility coefficients of the reference and the test diets by the method of difference on the basis of the level of inclusion (30%) of the test ingredient in the test diets according to the equation of Cho *et al.* (1982):

ADC of test ingredient = 100/30 (ADC of test diet - 0.7 ADC of reference diet)

From the results obtained using the above calculations the digestibility coefficients of the nutrients in the canola test materials were calculated by difference. At the end of the experiment, fish performance was evaluated by calculating the following parameters; feed conversion ratio, feed to gain ratio, protein efficiency ratio, specific growth rate, relative growth, absolute growth rate. However the results of fish performance should be interpreted with caution due the diet being neither isocaloric nor isonitrogenous; also, the duration of the experiment was short (21 days).

5.2.6 Statistical analyses.

The results of the experiment were analysed as a completely randomized block design using analysis of variance (ANOVA) after testing for homogeneity of variance of the data with Bartlett's test (Systat, 1990). The effect of processing on the apparent digestibility of dry matter,

organic matter and protein of the diets and ingredients (test materials) was compared using ANOVA. Where necessary the data were transformed by arcsine square root (Steel and Torrie, 1960). When significant differences between mean digestibility values were detected they were tested by the Bonferroni test with $p=0.05$.

5.3. RESULTS

5.3.1 Diet digestibility

The apparent dry matter, protein and energy digestibility coefficients by rainbow trout of the experimental diets as well as the digestible energy content are shown in Table 5.5. There were significant differences in nutrient digestibility between the diets tested. The dry matter and energy digestibility coefficients for the reference diet (diet 1) in which all the protein originated from high quality animal protein were always higher than the diets containing 30% of the various laboratory-prepared canola protein sources. Dry matter digestibility (DMD) of the diet containing protein isolate (diet 9) was 79.6%, which was not significantly different from that of the reference diet (herring meal diet, 80.7%), but was higher than the DMD of the diets containing the various laboratory-processed canola meals. There were no significant differences in DMD and energy digestibility between the diets containing the laboratory-processed canola products except the values for DMD and energy availability of the ACM- and the a/SpCM-containing diets (diets 3 and 4) were significantly lower than those of the other diets ($p<0.05$) . The energy digestibility of the diets showed the same trend as that of DMD. Protein digestibility coefficients of the diet containing CPI was higher (93.2%) than that of the control diet (91.3%) although this difference was not significant ($p>0.05$). The diet containing α/SpCM and the α-CM had the lowest protein digestibility values. The digestible energy (DE) contents of the diets containing canola meals were not significantly different from each other. The DE value of the reference diet (18.6 MJ/kg) was lower but not significantly different from the diet containing the canola protein isolate (19.1 MJ/kg). In general the diets containing the laboratory-prepared canola products had nutrient digestibilities inferior to both the reference diet and the diet containing CPI

Table 5.5 Nutrient digestibility (%) and digestible energy content (MJ/kg) of whole diets containing 30% test canola products[1]

Diet[2]	Dry matter	Protein	Energy	DE (MJ/kg)
1	80.7[a]	91.3[a]	86.4[a]	18.6[a]
2	71.4[b]	90.3[a]	77.2[b]	16.2[b]
3	70.4[b]	89.7[a]	76.2[b]	15.9[b]
4	65.4[c]	89.1[a]	72.9[c]	15.2[b]
5	68.7[b]	89.3[a]	75.9[b]	15.9[b]
6	69.7[b]	87.1[c]	76.3[b]	15.9[b]
7	71.0[b]	89.2[a]	77.3[b]	16.3[b]
8	65.7[c]	87.8[b]	72.2[c]	15.4[b]
9	79.7[a]	93.2[a]	85.9[a]	19.1[a]
sem[3]	1.33	0.97	1.07	0.23

[1] Means in each column without a common superscript letter are significantly different ($p<0.05$)
[2] Refer to Table 5.3 for diet descriptions
[3] Pooled standard error of the mean

5.3.2. Effect of diet on fish performance

No mortalities were observed during the entire experimental period. Tables 5.6 and 5.7 show the influence of the different diets on feed intake, feed to gain ratio, protein efficiency ratio and growth performance of rainbow trout. There were no significant differences in the above parameters observed in these performance parameters for rainbow trout fed the experimental diets. Fish growth (specific growth rate, SGR; absolute growth rate, AGR and relative growth, RG) was neither affected by the type of ingredient included or the kind of processing applied to the tested canola products. However, there were small non-significant differences in all these three growth parameters for fish fed the diets containing the α-galactosidase- treated canola meal and canola protein isolate (CPI). Fish receiving these two diets showed improved SGR and AGR values relative to those fish fed the reference diet. Fish fed the diet containing the sieved canola meal exhibited the lowest SGR, AGR and RG values relative to all the other diets, although these differences were not significant ($p>0.05$).

Mean daily dry feed intake per fish were higher for all groups ingesting diets containing the test canola products than noted for the fish fed the reference diet, but the results were not significantly different ($p>0.05$). Except for the fish fed the diet containing the canola protein isolate, feed utilization data reveals increased values of feed to gain ratio (FGR) for fish consuming diets containing laboratory processed canola products when compared to fish on the reference diet. Fish consuming the diet containing the protein isolate had a non-significant improvement in FE (0.82) relative to the fish fed the reference diet (0.69). Fish fed the diets containing the laboratory processed canola protein products had poor protein efficiency ratios compared to those fed the herring meal reference diet. However none of these differences were significant. There was no significant difference in either the average daily feed intake or the mean daily dry feed intake per fish (DFI) receiving the experimental diets, but these values were slightly lower in the fish receiving the control diet and those fed the canola protein isolate-containing diet. However, interpretation of data on fish performance should be made with caution as the growth and feed

utilization parameters were not intended to be used for feedstuff evaluation. The test diets were not balanced for either energy or protein content. Also the differences in nutrient composition, especially the protein content, between some of the diets, in addition to the short duration of the trial, do not permit valid conclusions to be drawn regarding the effects of diets (hence ingredients) on the performance of the fish. The positive growth of the experimental fish indicates that they were in a positive nitrogen balance during the period of the experiment. For those diets with similar composition, the growth and feed utilization parameters can be used as indicators for utilization potential of the feedstuffs. Definite and valid conclusions regarding the potential utilization of feedstuffs as fish meal substitutes would only be reached if long term feeding trials using balanced diets were conducted.

Table 5.6 Mean daily feed intake per fish (DFI), feed to gain ratio (FGR), average daily gain (ADG) and protein efficiency ratio (PER) of rainbow trout fed the experimental diets[1]

Diet[2]	ADG (g)	DFI (g)	FGR (g/g)	PER (g/g)
1	1.09[3]	1.31	1.45	1.55
2	1.07	1.63	1.75	1.34
3	0.99	1.36	1.59	1.48
4	1.10	1.50	1.56	1.47
5	1.16	1.56	1.51	1.51
6	1.04	1.48	1.65	1.39
7	1.22	1.55	1.47	1.53
8	1.14	1.54	1.66	1.44
9	1.32	1.40	1.22	1.48
sem[4]	0.09	0.08	0.17	0.16

[1] DFI= daily dry feed intake/fish; FGR= dry feed intake/weight gain; PER= weight gain/protein intake

[2] Refer to Table 5.3 for description of diets

[3] There was no significant differences between treatments in any of these parameters ($p>0.05$)

[4] Pooled stadard error of the mean.

Table 5.7 Initial (W1) and final weight (W2), weight gain, specific growth rate (SGR), absolute growth rate (AGR) and relative growth (RG) of rainbow trout fed canola containing diets[1]

Diet[2]	W1 (g)	W2 (g)	Gain (g)	SGR (%/d)	AGR (g/d)	RG (g/g)
1	73.4[3]	96.2	22.9	1.30	1.09	0.32
2	72.5	95.1	22.5	1.29	1.07	0.31
3	74.5	95.3	20.8	1.17	0.99	0.28
4	72.1	94.4	22.3	1.34	1.10	0.33
5	75.5	99.8	24.3	1.32	1.16	0.32
6	74.9	96.8	21.8	1.22	1.04	0.29
7	74.9	100.6	25.7	1.40	1.22	0.34
8	73.4	96.8	23.4	1.34	1.14	0.33
9	75.3	103.0	27.8	1.50	1.32	0.37
sem[4]			2.04	0.11	0.09	0.03

[1] SGR=100 (ln w1-ln w2)/days of experiment; RG= weight gain/mean initial weight; Gain= Mean final wt-Mean initial wt, AGR=w2-w1/days of experiment.

[2] Refer to Table 5.3 for description of diets

[3] There were no significant differences between treatments in any of these parameters($p>0.05$)

[4] Pooled standard error of the mean

5.3.3. Apparent nutrient and energy digestibility of test canola products

The mean apparent digestibility coefficients for dry matter, crude protein and gross energy, in addition to the digestible energy content of all the test canola ingredients are listed in Table 5.8. There were significant differences in nutrient digestibilities of the canola meals ($p<0.05$). The dry matter, protein and energy digestibilities as well as digestible energy values of the canola protein isolate (CPI) were consistently higher than values for the laboratory-processed canola products. Within the laboratory-processed products the methanol/ammonia treated canola meal (ACM) and the triple enzyme treated meal (α/SpCM) exhibited significantly lower dry matter and energy digestibility coefficients as well as digestible energy values. The protein digestibility of the isolate was significantly higher than that of the rest of the canola products tested (97.6%). The α-galactosidase- treated canola meal (α-CM) had superior energy digestibility coefficients (56.1%) and digestible energy (11.3 MJ/kg) in relation to commercial canola meal and the other laboratory prepared products but these differences were not always significant ($p>0.05$). The SP-249- and α/SP-249-treated canola meals showed significantly lower protein digestibility coefficients (77.4% and 79.5%) relative to the other canola protein sources. Interestingly, the different laboratory processing methods employed in this study did not significantly improve the nutrient digestibility of canola meal, in fact there was actual reduction in nutrient (dry matter, protein and energy) digestibility in two of the treatments (ACM and α/SpCM). Solvent extraction (ammonia in methanol) and treatment with Alpha-Gal and SP-249 (α/SpCM) of canola meal resulted in significant lowering of the dry matter and energy digestibility coefficients, as well as a reduction in the content of digestible energy. Treatment with SP-249 alone (SpCM) and in combination with oligosaccharide-reducing enzyme (α/SpCM) resulted in a significant decrease in protein digestibility of canola meals (relative to that of commercial canola meal). Apparent protein digestibility coefficients were 77.4 % for SpCM and 79.5 % for α/SpCM compare to 88.1 % in unprocessed canola meal. Whereas the laboratory processes employed in the reduction of ANFs in this study did not improve the nutritive value of canola meal for rainbow trout, techniques used in

the reduction of the content of glucosinolate and fibre in canola protein isolate significantly improved the nutrient digestibility coefficients of for rainbow trout. These results suggests that there is potential for including canola protein sources in rainbow trout diets as long as proper processing methods are employed for the reduction of ANFs.

Table 5.8 Mean percent apparent dry matter, crude protein and gross energy digestibility coefficients (%) and digestible energy values of canola meals fed to rainbow trout.

Ingredient[1]	Dry matter	Protein	Energy	DE (MJ/kg)
CCM	49.8[a2]	88.1[a]	55.6[a]	10.9[a]
SCM	46.3[a]	85.8[a]	52.3[a]	10.2[a]
ACM	29.8[b]	83.8[a]	41.4[b]	7.92[b]
PCM	40.8[c]	84.4[a]	51.5[a]	10.1[a]
SpCM	44.0[c]	77.4[b]	52.6[a]	10.4[a]
α-CM	48.5[a]	84.2[a]	56.1[a]	11.3[a]
α/SpCM	30.9[b]	79.5[b]	38.9[b]	8.10[b]
CPI	77.1[d]	97.6[c]	84.7[c]	20.6[c]
sem[3]	3.91	2.11	3.35	0.71

[1] CCM, commercial canola meal; SCM, sieved canola meal; ACM, sieved canola, methanol/ammonia treated canola meal, PCM, sieved canola, methanol/ammonia, phytase treated canola meal; SpCM, sieved canola, methanol/ammonia, phytase, SP-249 treated canola meal; α-CM, sieved canola, methanol/ammonia, phytase, SP-249, Alpha-Gal treated canola meal; α/SpCM, sieved canola, methanol/ammonia, phytase, alphagalactosidase and SP-249 treated canola meal; CPI, canola protein isolate. Refer to Table 4.1 for description of ingredients and treatments

[2] Means in each column without a common superscript letter are significantly different ($p<0.05$).

[3] Pooled standard error of the mean

5.4 DISCUSSION

5.4.1 Nutrient digestibility of canola protein isolate

Evaluation of alternative cheap and readily available protein sources as potential fish meal substitutes was attempted in this thesis. A variety of laboratory processing techniques were applied to commercial canola meal with the view of enhancing its nutrient and energy digestibility. In addition, a canola protein isolate was also evaluated as a potential ingredient in diets for trout. With regard to the canola products tested, only the diet containing canola protein isolate had nutrient and energy digestibility values close to those of the reference diet. The canola protein isolate (CPI) was extremely well digested and its apparent nutrient digestibility coefficients exceeded those of commercial canola meal, as well as those found for the other laboratory-processed canola products tested. Digestible energy content (20.6 MJ/kg) of the isolate was about twice that of commercial canola meal and was significantly higher than the values found for laboratory processed canola products. Protein digestibility of the isolate (97.6%) was higher than the value reported for herring meal (92.0%) by Cho *et al.* (1982) with chinook salmon. Although the energy digestibility of the isolate was lower than that reported by Cho *et al.* (1982) for herring meal (84.7% compared to 91.0%), the digestible energy values did not show much difference. This improvement in the nutrient digestibility of canola protein isolate (CPI) stems from the low levels of ANFs (glucosinolates, phenolics and carbohydrates) present in this product. Similar results in improved nutrient availability were reported by Hajen *et al.* (1993) for soybean protein isolate fed to chinook salmon. Other similar canola protein products have been shown to result in satisfactory fish performance when used to partially replace fish meal in rainbow trout diets (Teskeredzic *et al.*, 1995; Yurkowski *et al.*, 1978).

5.4.2 Effect of sieving on canola meal digestibility coefficients

Dry matter digestibility coefficients of the laboratory processed canola products were lower than those for the commercial canola meal. The apparent dry matter and energy digestibility

coefficients of canola meal obtained in this experiment were lower than those reported by Hilton and Slinger (1986) for rainbow trout. This low nutrient and energy availability of commercial canola meal in relation to the canola protein isolate would have largely resulted from the higher levels of fibre and high levels of complex carbohydrates (Hilton and Slinger, 1986; Jones, 1979) present in unprocessed canola meal. Sieving of commercial canola meal to reduce its fibre content had no significant improvement in the nutrient digestibility coefficients and did not also lead to any significant improvement in fish performance. Fibre reduction by enzyme treatment of canola meal also did not result in any improvement in the nutrient or energy digestibility, fish growth or feed utilization. These observations are in agreement with those of McCurdy and March (1992), who observed that fibre reduction processing by sieving and enzyme treatment of canola meal did not significantly improve the response of either salmon or rainbow trout to canola protein as measured by specific growth rate and feed conversion. No data on nutrient absorptions were however reported in that study. Fibre in the diet has a negative effect on nutrient digestibility by reducing gut transit time and blocking enzyme substrates, and is also capable of adsorbing amino acid and peptides as well as increasing the endogenous faecal nitrogen losses as a result of increased sloughing of intestinal mucosal cells and mucus production (Krogdahl *et al.,* 1989), the result of which is the underestimation of the true nutrient digestibility. Although the crude fibre content of the sieved canola meal was lower than that of the commercial canola meal, it is difficult to explain why there was no improvement in the digestible energy content of the former product. Perhaps the level of other fibre components were not sufficiently different between the two products. With respect to reduced protein availability in SCM, it should also be borne in mind that fine grinding of oilseed meals may lead to the release of tannins that are bound to the fibre, which then form complexes with protein and amino acids in the gastrointestinal tract, thereby reducing protein availability.

5.4.3 Effect of ANF removal on nutrient digestibility of canola meal

The toxic constituents in canola meal are a primary concern as regards the utilization of canola meal by fish. Solvent washing of canola meal is required for the reduction of the antinutrient content of canola meal. Growth depression and deformities in rainbow trout caused by presence of glucosinolates and the lowered palatability due to the bitterness of phenolic compounds are major concerns when using canola meal in fish diets. Minimization of these antinutritional factors should therefore be expected to enhance the nutritive value of canola meal. However washing of canola meal with methanol/ammonia to reduce glucosinolates and phenolic compounds neither improved the nutrient digestibility of the unprocessed canola meal or the performance of fish as measured by weight gain and specific growth rate. This closely agrees with the findings of Hajen *et al.* (1993) who reported low apparent protein and energy digestibility coefficients for glucosinolate-extracted canola meal (78.6% and 51.2%) in chinook salmon relative to those of unprocessed canola meal (84.5% and 64.5%). The coefficients for energy digestibility and digestible energy content of the methanol/ammonia-treated canola meal in this experiment (41.4% and 7.92 MJ/kg) were lower than those reported by Hajen *et al.* (1993) for glucosinolate extracted canola meal (51.2% and 9.93 MJ/kg). Growth performance of salmonids has been shown to be improved by washing canola meal with methanol/ammonia (McCurdy and March, 1992). Differences in results of the present and these two studies may be explained by the dissimilar screen sizes used in sieving the canola meals, as well as by the dissolution and removal of some potentially useful feed substances during the glucosinolate/phenolic extraction process. In the present experiment a 40 mesh screen was used as opposed to a 70 mesh screen in the trial referred to above, which may have screened out more fibrous particles than the larger screen used in the present study. Percent recovery of the material passing through the 70 and 40 screen sizes were 34-39% and 56-60%, respectively (see Appendix 1). The apparent dry matter, protein and energy digestibility coefficients of the methanol/ammonia treated-meal (ACM) were significantly lower (by 20, 4, 14 percentage units) than the corresponding values for commercial canola meal (CCM). The lower nutrient digestibility coefficients have resulted from loss of some readily soluble components (carbohydrates, lipid and

proteins) during the washing process, leading to an increase in the proportion of more indigestible constituents. Indeed ammonia/methanol washing of fibre-reduced canola meals has been shown to reduce the proportion of protein soluble in dilute acid (McCurdy and March, 1992). This may also account for the lack of improvement in performance of the fish receiving the ammoniated canola meal (ACM). This is contrary to improved weight gain and specific growth rates observed by Hajen (1990) when glucosinolate reduced canola meal was fed to chinook salmon. However this comparison should be viewed with caution due to the differences in the species, and also, the canola meal in the referred (Hajen, 1990) trial received additional extractions that were not reported. Alkali treatment has also been reported to cause protein polymerization as well as Maillard-type reactions which adversely affect protein utilization (Bell and Keith, 1983) as a result of amino acid damage.

The objective of using enzymes in animal feed is to improve the nutrient and energy digestibility and utilization as a result of the reduction in the levels of antinutritional factors present in some feedstuffs. Phytic acid ties up minerals, especially phosphorus, and lowers their availability. This leads to poor feed utilization by animals. Treatment of canola products with phytase (to reduce phytate) and oligosaccharide-reducing enzymes did not improve the nutrient and energy digestibility or feed utilization of canola meal for rainbow trout. The combined effect of the cell-wall degrading enzyme and the oligosaccharide reducing enzyme was to lower dry matter, protein and energy digestibility as well as the levels of digestible energy. Large quantities of water used in the enzyme processing procedures may have contributed to the negative effect on nutrient and energy digestibility. The use of microbial phytase to reduce the level of phytate in animal diets has had variable results. Pretreatment of diets with microbial phytase has been shown to improve the digestibility of dry matter by pigs (Jongbloed *et al.,* 1990; Jongbloed *et al.,* 1992), and broilers (Sosulski *et al.,* 1993) and the growth performance and feed utilization of rainbow trout (Cain and Garling, 1995; Riche, 1993). By contrast by Nasi (1990) was unable to demonstrate any effect while working with pigs. Teskeredzic *et al.* (1995) also observed that the pretreatment of rapeseed protein concentrate with microbial phytase did not markedly improve its nutritive value for

rainbow trout. This is in agreement with results from this study. Reduced protein quality as a result of the dephytinization procedure was likely the cause of the findings in the present and the latter-mentioned study. Indeed the same method for dephytinization was employed in these two trials. Further, protein denaturation may have occurred due to the prolonged heating of canola products at 50° C during the dephytinization process. Properly dephytinized rapeseed protein concentrate has been shown to be utilized by rainbow trout without compromising fish performance (Prendergast *et al.*, 1994). Further, the addition of microbial phytase into fish diets has also been shown to improve digestibility and utilization of phosphorus by trout (Riche, 1993) and to improve weight gain and total body ash in carp (Schäfer, 1994). Improved phosphorus utilization eliminates the need for adding supplemental phosphorus to salmonid diets, hence there is reduced phosphorus released into the effluent water. Cain and Garling (1995) and Ketola and Richmond (1994) have suggested that dietary phosphorus level has a greater effect on body ash and phosphorus content than on growth of rainbow trout, suggesting another explanation for the lack of improved performance of fish consuming the diet containing phytase-treated canola meal. Phosphorus utilization and total body ash levels were not measured in the present experiment.

Leske *et al.* (1991) observed that materials removed from soybean meal by alcohol extraction were directly responsible for the reduced energy value of the meal for poultry, and that addition of raffinose had similar effect. This suggests that raffinose and the raffinose family of oligosaccharides can lower the nutritive value of feedstuffs. In this study the Alpha-Gal (oligosaccharide-reducing enzyme) treatment of canola meal resulted in higher DE value (11.3 MJ/kg) than for unprocessed canola (10.9 MJ/kg), although this difference was not significant. Similarly, no significant effect was observed on the dry matter and protein digestibility. The present results differ from those of Coon *et al.* (1990) who noticed improved dry matter, fibre, carbohydrate and energy digestibility of oligosaccharide-extracted soybean meal for poultry. Use of ethanol in extraction of the sugars in the above experiment may explain the difference in these results. Further, differences in experimental animals and oilseed types may also be other factors as well. Removal of oligosaccharides from oilseed meals results in a less acidic intestinal

environment and a longer transit time for the diet, providing optimal conditions for microbial hydrolysis of polysaccharides, hence increased nutritive value. However Arnesen *et al.* (1989) found that oligosaccharides not only had a negative effect on dry matter digestibility of soybean meal fed to rainbow trout but also exerted negative effects on the digestibility of dry matter, lipid and protein for Atlantic salmon. This effect has usually been attributed to an increase in the amount of undigested nutrients passing through the hindgut and/or to an increase in the water content of the faeces.

The lack of significant improvement in the digestibility of canola meal after removal of oligosaccharides in this experiment may be explained by several reasons. In part, it may be due to the short colon of carnivorous salmonids and absence of a fibre fermentation site because oligosaccharide removal stimulates increased fibre (cellulose and hemicellulose) digestion in most monogastric animals. However, it is yet to be established whether oligosaccharide-degrading microflora are present in salmonids. Further, based on chemical and biological evaluation, Rumsey *et al.* (1993) concluded that soybean oligosaccharides do not have to be removed or modified for maximum growth or nitrogen utilization by rainbow trout. Thus oligosaccharide sugars may not be the most important factors affecting nutrient absorption in rainbow trout. According to Coon *et al.* (1990), gut anatomical differences between animals may also account for differences in the nutritive value of feedstuffs containing oligosaccharide sugars. The experiments of Arnesen *et al.* (1989) established that the negative effects of oligosaccharides on nutrient digestibility were close to being significant when the fish adaptation period was increased to three months from the initial two weeks, indicating that the effect of these factors on nutrient digestibility may be pronounced with time. The short duration of the present experiment may not have been long enough for the effects of oligosaccharides and/or of the treatment to be pronounced. In addition, Murai *et al.* (1983) while working with rainbow trout with mean body weights of 0.7, 5.1 and 8.9 g, showed that only the smallest fish benefited from alcohol extraction of oligosaccharides.

5.4.4 Influence of canola processing on fish performance

Apparent digestibility coefficients for organic matter, crude protein and energy as well as the digestible energy content were higher for the canola protein isolate than for commercial canola meal. This improved nutrient digestibility was translated into higher weight gain and growth rate as well as better feed utilization by rainbow trout. Although a valid comparison can not be made between this treatment and the rest, owing to the differences in nutrient and energy densities of the diets, this positive effect on nutrient digestibility and fish performance can be attributed to the low levels of carbohydrates (1.2 %), phenolics (0.93 %), phytates (0.35 %) as well as glucosinolates (2.16 μmol/g). The scarcity of studies conducted on the utilization of canola protein isolate by animals makes it even difficult to make any further conclusions on the potential of this product as an ingredient in salmonid diets. While no research was found in which canola protein isolate has been evaluated for inclusion in fish diets, one study conducted on the utilization of soybean protein isolate by Hajen *et al.* (1993) found high proportions of endogenous faecal losses and poor feed intake of chinook salmon fed a diet containing 30% soybean protein isolate. Protein and energy digestibility of the soybean protein isolate for chinook salmon were lower than the digestibility of fish meal and the canola protein isolate found in the present experiment, the values of which were comparable to or even better than those of fish meal. The low soybean isolate nutrient digestibility values may have been due to the small size of the fish, as juvenile fish are known to be more sensitive to any residual antinutritional factors present in oilseed meals and other products derived from them. Moreover, soybean meal is reported to be poorly utilized by chinook salmon (Fowler, 1980; Akiyama *et al.*, 1988). Canola protein isolate may be nutritionally superior to soybean protein isolate for trout and other salmonids.

Since the diets containing commercial, (CCM) sieved (SCM), ammoniated (ACM), phytase-(PCM) and SP-249-(SpCM) treated canola meals as the test ingredients were isonitrogenous as well as isoenergetic, some valid comparisons on the fish performance can be made between these dietary treatments. Although there were no significant differences in the digestible energy and protein contents of these diets there were non-significant improvements in the

feed to gain ratio (FGR) for these treatments in relation to the unprocessed canola meal (CCM). Despite the absence of significant differences these results would suggests that the levels of some of the antinutritional factors (fibre, glucosinolate, phenolics and phytate) present in canola meal can affect the growth and feed utilization of rainbow trout. The values for feed efficiency and protein efficiency ratio (PER) of trout fed the diet containing the dephytinized canola meal in this trial were close to those reported by Teskeredzic *et al.* (1995) for trout fed diets with dephytinized rapeseed protein concentrate. However, the specific growth rates of the fish in the present experiment were inferior. Similar trends for improved growth performance and feed utilization were also observed by Hajen *et al.* (1993) for chinook salmon held in sea water that were fed a diet containing methanol/ammonia extracted canola meal. Sparging of canola meals with ammonia, however, has been shown to have little effect on the feeding value of canola meal for chick growth but it did reduce the fishy odour development in brown shelled eggs (Goh *et al.,* 1987). Treatment of canola seeds and meals with methanol/ammonia reduces the concentration of phenolic compounds and glucosinolates which have been implicated in causing poor diet palatability, impaired thyroid function, and reduced growth and feed utilization in salmonids and other animals. Higgs *et al.* (1995) note that pretreatment of canola protein concentrate with phytase is probably impractical and uneconomical and they suggested high temperature extrusion processing of canola protein products as an alternative. Blending canola meal with hydrolysed marine products and wheat bran as the source of natural phytase (Stone *et al.,* 1984), oral administration of phytase (Rumsey, 1993) and incorporation into fish diets of ground transgenic seeds that are rich in phytase (Pen *et al.,*1993) are other options.

Although the processing techniques employed in the reduction of canola meal antinutritional factors in this experiment did not produce appreciable improvement in nutrient utilization or fish performance, these results serves to confirm results of other findings that canola meal and products derived from it can be included up to levels of 30% of diet without affecting the performance of rainbow trout. The results of the present experiment also indicate that processing techniques can remove or reduce the levels of antinutritional factors present in canola meal, and

that products resulting from careful processing of canola meal to reduce the levels of antinutritional factors (canola protein isolate) can have equal or even better protein availability than fish meal. Coupled with its cost and supply, canola meal has potential in the aquaculture feed industry as long as the adverse effects of its ANFs are eliminated through either breeding or economical processing techniques. The supply of canola meal, unlike that of fish meal, can easily be increased in North America and elsewhere. Moreover canola products can be produced that are more nutritionally stable and have a consistent nutrient composition. Therefore successful replacement of fish meal with inexpensive canola protein products could lead to a significant cost saving in salmonid production and so contribute to future salmon farming profitability

Chapter 6

SUMMARY AND CONCLUSIONS

This study was undertaken to assess the effects of mechanical, chemical and enzymatic processing on the chemical composition and potential nutritional value of canola meal for rainbow trout (*Oncorhynchus mykiss*). In addition, a new product, namely a canola protein isolate was tested as a potential substitute for fish meal in trout diets. The objective of the study was to determine the chemical profile of canola protein products and the apparent digestibility coefficients for dry matter, protein and energy in order to identify new canola protein products that are palatable, have a high nutrient availability and potential for replacing fish meal in diets for salmonids.

In evaluating new feedstuffs for animals, the first fundamental step is a detailed description of the chemical and nutritional properties. With regards to canola meal, like many members of the *Cruciferae* family of plants, investigation of the antinutritional factors is important as well. It is also essential to assess the acceptability and utilization by animals through the determination of palatability and digestion by the target animals. This thesis consisted of two experiments, one in which the effect of a variety of processing methods on the chemical and toxic composition of canola meal were investigated. The effects of sieving, methanol/ammonia treatment, phytase, SP-249 and a-galactosidase enzymes on the proximate composition and toxic constituents such as glucosinolates, phytic acid, fibre and phenolic compounds in canola meal were assessed in the first experiment with a view to obtain more knowledge about the suitability of canola protein products for inclusion in rainbow trout diets. In the second part of the study the resultant canola protein products were evaluated as potential substitutes for fish meal in diets for rainbow trout (*Oncorhynchus mykiss*) by measurement of the apparent digestibility coefficient for dry matter, protein and energy. Unprocessed canola meal contained 36.3% protein, 6.9% lipid, 11.0% ash, 10.6% crude protein and 19.6 MJ/kg of energy. Milling and sieving resulted in the reduction of

crude fibre (7.3%) due to removal of portions of the hull fraction, while at the same time increasing the content of protein (37.1%), amino acids, glucosinolate and phytate. This treatment also had some significant effects on other fibre components (NDF, cellulose, hemicellulose and lignin) as well, but had little or no effect on the mineral content of the meal. Levels of neutral detergent fibre, cellulose and hemicellulose in unprocessed meal were 27.8%, 14.5%, and 5.04% respectively compared to 25.1%, 12.2%, 2.20% in the sieved canola meal. Levels of acid detergent lignin (ADL) which also contains tannins and heat-damaged protein-carbohydrate complexes, were 8.27% and 7.92% in unprocessed and sieved canola meals, respectively. In regard to utilization of canola meal by rainbow trout, sieving did not cause any significant improvement in the apparent digestibility coefficients of dry matter, protein or energy. Thus, although mechanical processing slightly increased the protein content of canola meal, it did not appear to have any significant influence on protein quality. The yield of fibre-reduced meal was 56-60% on a dry matter basis, but yields decreased with a decrease in the sieve size.

Methanol/ammonia treatment resulted in a significant improvement in the chemical composition of canola meal. Not only did this treatment cause a reduction in the glucosinolate and lipid contents of the meal but it also increased the protein and amino acid contents as well. Protein content was increased from 36.3% in unprocessed canola meal to 39.1% in methanol/ammonia treated meal. Concurrently, there was a 90% reduction in the concentration of glucosinolates. Among the glucosinolates measured, progoitrin and 4-hydroxyglucobrassicin were most affected (over 85% reduction). The total levels of aliphatic and indole glucosinolates were decreased to trace levels (0.7 and 0.3 μmol/g). Crude fibre, acid detergent, neutral detergent fibre, cellulose, hemicellulose and lignin concentrations were all elevated by the use of the methanol/ammonia treatment. Methanol/ammonia treatment of canola meal also resulted in a 27% increase in the content of inositol hexaphosphate (phytate). The concentration of phytate was lowered by all the enzyme treatments. Phytase, SP-249 and Alpha-Gal enzymes reduced the phytate content of canola meal by 50%, 40% and 26%, respectively, and the combination of all these three enzymes resulted in an 84% reduction in the content of phytate. These enzymes, whether administered

singly or in combination, resulted in complete removal of all the residual glucosinolates remaining after methanol/ammonia treatment .

In the digestibility trial, it was clear that the dry matter, protein and energy in canola protein isolate were more efficiently digested and absorbed by rainbow trout than the other canola protein products tested. The improved digestibility of nutrient and energy is due to the presence of low levels of fibre and toxic constituents in the canola protein isolate. None of the laboratory treatments treatments applied to canola meal in this study resulted in any significant improvement in the dry matter digestibility. In fact with the exception of sieving and Alpha-Gal treatment, the rest of the treatments (methanol/ammonia, phytase, SP-249, Alpha-Gal and SP-249) significantly lowered the dry matter digestibility. Methanol/ammonia treated canola meal dry matter was only 29.8% digested by rainbow trout. The crude protein digestibility of feedstuffs, especially that of plant origin, can be influenced by the kind of technical processing procedures that were employed. In this thesis, crude protein digestibility of canola meal was significantly lowered by treatment with SP-249 and the combined effect of the two carbohydrate-reducing enzymes (Alpha-Gal and SP-249). Methanol/ammonia also had the same effect, although this reduction was not significant. Canola meal has a low energy value for salmonids because of the presence of high levels of fibre and unavailable carbohydrates. Energy digestibility and the digestible energy (DE) content of canola meal was significantly lowered by methanol/ammonia treatment and by the combined effect of the two carbohydrate-reducing enzymes (Alpha-Gal and SP-249).Collectively, the processing treatments employed in this thesis resulted in no improvement in the dry matter, protein or energy digestibility coefficients. Optimal utilization of canola protein requires more efficient processing methods to avoid problems when canola meal is used as a protein source. Based on the digestibility assessments, novel feed ingredients such as canola protein isolates (CPI) are highly promising as partial or complete substitutes for fish meal in salmonid diets because the toxic constituents that cause lowered palatability problems can be overcome through innovative processing methods. This is translated in improved nutrient digestibility. However the final assessment of the suitability of new protein products as a replacements for fish meal in salmonid

diets should also include observations of weight gain, feed efficiency and body composition of fish receiving isonitrogenous and isoenergetic diets under good culture conditions over an extended period.

The results of this study demonstrate that chemical and enzymatic processing can effectively remove antinutritional factors present in canola meal resulting in canola products with a high protein content. Also, chemical analyses coupled with digestibility evaluations can allow reasonable inferences to be drawn on the nutritive value of novel plant protein products for rainbow trout in fresh water. It can also be concluded from this study that although the chemical profile of canola meal was modified through various processing procedures, these changes were not reflected in the nutrient digestibility by rainbow trout. Further based on the digestibility values, canola protein isolates seem to have potential in substituting fish meal in diets for rainbow trout and perhaps other salmonids as well. Finally, the results of the present experiment also serve to illustrate that there is need to investigate other alternative processing methods for the improvement of the nutritional value of canola meal. These should include the production of careful processed upgraded canola meals, canola protein concentrates as well as canola protein isolates. Significant progress is currently underway through novel processing of canola meals and seeds to achieve this objective.

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APPENDIX 1.

Information on the mass distribution trial of canola meal sieved through different sieve sizes in a Ro-Tap shaker for 5 minutes. The sample weights given refer to the sample weight passing through the sieve.

sieve size (mm)	**sample wt (g)**	**%**
0.125	51.85	2.15
0.15	82.43	3.42
0.25	6.72	6.72
0.425	577.40	23.93
1.00	1539.49	63.79

The following information represents the result of using the Ro-Tap shaker for 10 min. with a 0.425 (Sieve No. 40, US screen size) mm screen.

Replicate	Weight (g)			
	Above 0.425	%	Below 0.425	%
1	65.53	43.60	84.47	56.41
2	60.13	40.31	89.03	59.63
3	59.26	39.96	89.04	60.03
4	61.54	41.15	88.00	58.88
5	61.64	42.08	88.40	58.92
6	67.27	45.32	80.99	54.62
Mean	**62.06**	**41.90**	**86.71**	**58.07**

APPENDIX 2.

Preliminary trial to investigate the effects of sieving canola meal through different screen sizes on its chemical composition (% air dry).

sieve size (mm)	**Moisture**	**Protein**	**Ash**	**Lipid**	**ADF**	**NDF**
1	12.12	31.33	10.04	2.81	29.60	25.76
0.45	12.91	36.50	11.08	3.25	28.83	23.81
0.25	12.50	37.30	12.15	3.41	26.01	23.82
0.15	10.37	37.77	12.53	3.93	25.64	23.01
0.125	11.36	37.24	14.16	4.50	23.09	22.72

Each value in the table above is the mean of three replicates. Lipid determination was done by the Soxhet procedure.

APPENDIX 3

Recovery of methanol from the solution of methanol, ammonia and water used in removal of glucosinolates and phenolics from canola meal through distillation.

Trial No.	Vol (soln.)	Meth. vol.	% Recovery
1	800 mls	650 mls	81
2	800 mls	600 mls	75
3	800 mls	610 mls	76
4	800 mls	600 mls	75
Mean	**800 mls**	**640 mls**	**74.2**

Recoveries of more than 80 % are achievable but such high recoveries were associated with a yellowish colour suggesting the presence of impurities. Also, the presence of ammonia was noticed. Although the laboratory ditillation process carried out did recover some of the methanol used, it was a very slow process hence, time consuming. Distillaton was therefore abandoned and fresh methanol was used throughout the experiment.

APPENDIX 4

Procedure followed in the spectrophotometric determination of chromic oxide by perchloric acid digestion.

Prepare digestion mixture by adding 150 mls of concentrated sulphuric acid to an equal volume (150 mls) of distilled water in a volumetric flask.

Add 200 mls of perchloric acid and 10 g of sodium molybdate dihydride and dissolve. Warn if necessary to allow quick and complete dissolution of molybdate.

Prepare standards by weighing portions of pure chromic oxide from 5 to 60 mg and treat them similar to the samples of faeces or feed. From this data a slope (M) and intercept (b) can be determined by regression of absorbance on chromic oxide concentration.

Weigh duplicate samples of faeces of feed (6 g) or faeces (2 g) into a 50 mls pyrex beaker and ash in a muffle furnace at 450 °C for 20 hours.

Add 15 mls of digestion mixture to the cooled ashed samples and heat on a hot plate until a yellow colour develops (about 300 °C). If black colour persists remove the sample from the heat as an explosive mixture may form.

Heat for a further 10-15 minutes, cool and add 5-10mls of distilled water.

Transfer contents of beakers to 200 mls volumetric flasks and make to volume with distilled water and shake well.

Shake again and add 10 mls of sample solution to a 15 mls centrifuge tube and centrifuge for 5 minutes at 1000 rpm (700 g)and then determine the absorbance at 440nm using distilled water as a blank to zero the spectrophotometer.

Although not always necessary, blanks (samples of feed or faeces containing no Cr_2O_3 should be included. If an effect on absorbance is detected, calculate slope (Mo) and intercept (bo should equal b) by regression.

Calculate the mg of chromic oxide per g of sample (x) as:

$$\text{mg } Cr_2O_3/\text{g of sample} = A\text{-}b/M\ (w)$$

A = absorbance of sample
b = intercept of regression of standards
M = slope of regression of standards
w = original sample weight in g

When blanks are found to be necessary the following equation should be used:

mg Cr_2O_3/g of sample = A- Mo (w)-2b/M (w)

A = absorbance of sample
Mo = slope of regression with interference
b = intercept of regression of standards
M = slope of regression of standards
w = original sample weight in g