POTENTIAL FOR DEVELOPMENT OF A NEW HIGH PROTEIN AND HIGH ENERGY FEEDSTUFF: CANOLA-QUALITY *SINAPIS ALBA* MUSTARD

A Thesis

Submitted to the Faculty

of

Graduate Studies

The University of Manitoba

by Heather D. Kienzle

In Partial Fulfilment of the

Requirements for the Degree

of

Master of Science

Department of Animal Science

THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES

COPYRIGHT PERMISSION

POTENTIAL FOR DEVELOPMENT OF A NEW HIGH PROTEIN AND HIGH ENERGY FEEDSTUFF: CANOLA-QUALITY *SINAPIS ALBA* MUSTARD

BY

HEATHER D. KIENZLE

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

MASTER OF SCIENCE

ABSTRACT

The *Sinapis alba* (white mustard) species holds great potential as a high protein and high energy alternative to full fat soybean. It has superior heat and drought tolerance in comparison to conventional canola, and is therefore well suited to production in dryland areas. Although it has been grown for sometime as a condiment crop, it is only recently that plant breeders have developed a *S. alba* cultivar with low contents of glucosinolates and erucic acid. The objective of these studies was to evaluate the nutritive profile of *S. alba* and to explore the potential for use of the full fat seed in monogastric animal nutrition. Detailed chemical characterization of *S. alba* in comparison with selected feedstuffs (ie., soybean, canola, lupin and peas) was done. Digestible protein content in defatted and heat-treated *S. alba* seed averaged 70.8% of total protein which is equal to or higher than values for other feedstuffs. In comparison to the full fat soybean, *S. alba* seed contained more oil (26.4% vs 20.2%) but less protein (37.5% vs 41.4%) with the content of the two major nutrients in favour of the *S. alba* sample (63.9% vs 61.6%). *S. alba* seed contained slightly more methionine and cystine (3.60 vs 3.33 g/16g N) but less lysine (5.78 vs 6.49 g/16g N) than soybean. The sucrose content was lower in *S. alba* than in soybean (3.3% vs 5.2%) although this was offset by the presence of starch (1.3%) which was not detected in soybean. Considering the remarkable difference in seed size, *S. alba* having a 20-fold smaller seed than soybean, only a small difference in the total dietary fibre content was observed (22.2% vs 18.8% for *S. alba* and soybean, respectively). Soluble dietary fibre content (ie., mucilage), as determined under simulated conditions of the gastrointestinal tract, was found to be 1.7%. A lower content of oligosaccharide (3.4% vs 5.1%) and higher contents of calcium (0.66% vs

0.39%) and available (non-phytate) phosphorus (0.27% vs 0.12%) were among other positive characteristics associated with the *S. alba* crop. Although still relatively high and potentially a limiting factor, the glucosinolate content of the new *S. alba* cultivar was shown to be 35.3 μmol/g which is substantially lower than that present in current condiment varieties. Raw, micronized (140°C) and autoclaved (108°C) *S. alba* seeds were evaluated for digestible protein content, myrosinase activity, glucosinolate content, true metabolizable energy (TME_n), true amino acid availability (TAAA) and broiler chicken performance. Heat treatment of the *S. alba* seed decreased glucosinolate content, effectively inactivated the myrosinase enzyme, and increased digestible protein content *in vitro*. The autoclaved *S alba* seed showed higher TME_n value than the raw and micronized samples (3031 vs 2270 and 2460 kcal/kg). The micronized soybean seed was found to contain the highest TME_n value (3790 kcal/kg). Regardless of heat treatment employed, the TAAA of the *S. alba* seed was lower than that of micronized soybean seed, commercial soybean meal and commercial canola meal. Broiler chickens fed rations containing 10% of the raw, micronized and autoclaved *S. alba* seed showed similar feed intake, weight gain and feed to gain ratio to those fed micronized soybean seed. There was no difference in chicken performance between diets containing 10% or 20% of micronized *S. alba* seed. As compared to other treatments, feeding of the ration containing 20% of the autoclaved *S. alba* seed resulted in poorer feed intake, weight gains and feed efficiency.

FOREWORD

This thesis was prepared in manuscript form according to the Department of Animal Science guidelines. The manuscripts will be submitted for publication as follows:

MANUSCRIPT 1. Kienzle, H.D., Slominski, B.A., Rakow, G. and Pickard, M. 1998. Canola type *Sinapis alba* white mustard: Chemical composition and the effect of micronization on seed quality. J. Agric. Food Chem. (to be submitted).

MANUSCRIPT 2. Kienzle, H.D., Slominski, B.A. and Campbell, L.D. 1998. Canola type *Sinapis alba* white mustard: Nutritive value and the effect of heat treatment on amino acid and energy availabilities for poultry. Anim. Feed Sci. Tech. (to be submitted).

ACKNOWLEDGEMENTS

I am forever grateful to my advisor, Dr. B.A. Slominski, for his continuous encouragement, support and guidance throughout this endeavor. A heartfelt thanks goes out to the members of my examining committee, Drs.L.D. Campbell, W. Guenter and M. Scanlon, for their assistance and constructive input. Help received from the nutrition laboratory personnel, in particular Peter Mills, Alan Tarr and Pauline Robinson, has been greatly appreciated, as has that received from the secretarial staff. I would like to express my gratitude to Harry Muc for his technical assistance throughout my program, and to Brad Sedor for his help with laboratory analysis and with the in vivo trials. The statistical expertise of Drs. G.H. Crow and L.A. Onischuk has been of great assistance. A special thanks is due to Dr. Onischuk whose assistance went beyond the statistical, and whose friendship and encouragement was valued greatly throughout my time at the University of Manitoba.

I would like to pass an extra special thanks on to my fellow graduate students, as well as the undergraduate students and those not directly involved with the university whom I have interacted with as this thesis was completed. Many of these people have become near-and-dear to my heart, and they have made my time in Winnipeg unforgettable.

Lastly, I would like to thank my family and friends at home who were with me throughout the trials and tribulations of my graduate work. Your unwavering confidence in me and your patience and understanding has meant more to me than you will ever know.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AME	Apparent metabolizable energy.
AME_n	Nitrogen corrected apparent metabolizable energy.
ANOVA	Analysis of variance.
AOAC	Association of Official Analytical Chemists.
CP	Crude protein.
DM	Dry matter.
GE	Gross energy.
IS	Internal standard.
IU	International unit.
ND	Not detected.
NDF	Neutral detergent fibre.
NRC	National Research Council.
NSP	Non-starch polysaccharide.
SAS	Statistical Analysis System.
T	Traces.
TAAA	True amino acid availability.
TI	Trypsin inhibitor.
TME_n	Nitrogen corrected true metabolizable energy.

INTRODUCTION

Mustard has been known to man for thousands of years (Man and Weir, 1988). The term "mustard" is thought to have originated from the use of the seeds as a condiment; the crushed seeds were mixed into a paste with the sweet must of old wine and called "mustum ardens" or hot must. Mustard has been described in Sumerian and Sanskirt texts as early as 3000 B.C., in Egyptian texts around 2000 B.C. and in Chinese writings before 1000 B.C. Seed fragments have been discovered in excavations of Indus valley dwellings and from Egyptian tombs at Thebes, both from around 2000 B.C. The Bible, Alexander the Great and Charlemagne all referred to mustard. As well, there are various quotes from medieval times onward referring to trading of the manufactured condiment in the days of Avignon Popes, Dumas and Shakespeare (Colmans, 1985).

Over half (60%) of the mustard seed in today's world market is *Sinapis alba*, commonly known as "white" or "yellow" mustard. *Brassica juncea*, also known as "brown" or "oriental" mustard, makes up most of the remaining 40%. Similarly to earlier times, the principal use of the *S. alba* crop is as a source of condiment for the spice trade. Mustard is also used as leaf and stem vegetables and as a salad crop in the Far East and Southeast Asia, and for green manuring or as a fodder crop in Western Europe (Vaughan and Hemingway, 1959; Rosengarten, 1969).

S. alba production within Canada occurs predominantly in the Prairie provinces of Manitoba, Saskatchewan and Alberta. It is particularly well suited to production in these areas due to its superior heat and drought tolerance in comparison to *Brassica napus* and *Brassica rapa* canola. *S. alba* has a high resistance to blackleg disease (*Lestosphaeria maculans*) and flea beetle

attacks. Further advantages include a highly shatter resistant seed pod and a large bright yellow seed. The condiment *S. alba* grown contains a significant amount of erucic acid (25-45% of the fatty acid profile) and has a high glucosinolate content (approximately 200 μmol/g oil free meal) (Hemingway, 1995). However, due to the goitrogenic effect of glucosinolates (Bell, 1984) and the negative implications related to erucic acid (Kramer *et al.*, 1977), the condiment *S. alba* seed is unsuitable for use in monogastric feeds.

In an attempt to reduce the glucosinolate and erucic acid levels in *Sinapis alba* seed, various plant breeding approaches have been undertaken. Development of a low glucosinolate line in Poland (Krzymanski *et al.*, 1991) and a low erucic acid line in Canada (Raney *et al.*, 1995) offers some potential for future development of a low glucosinolate, low erucic acid cultivar which may be suitable for monogastric feeding. It is thought that this new "canola quality" *S. alba* would hold great potential for Canadian farmers as a high protein and high energy feedstuff. However, further plant breeding efforts are necessary to combine the low glucosinolate and low erucic acid characteristics in order to improve that overall nutritive quality of the seed.

Little is known about newly developed lines of *S. alba* seed. Information needs to be gathered on the chemical composition and any anti-nutritive factors present before the feeding value *in vivo* can be determined. Depending on the outcome of these inquiries, future areas of research may include determining optimum heat treatment to control anti-nutritive factors and increase protein availability, and the use of dietary enzymes to enhance utilization of various seed components.

LITERATURE REVIEW

Economics and Production

In 1996, 237.5 tonnes of mustard seed were produced in Canada. The seeded area covered approximately 241.0 hectares, predominately in the Prairie provinces. Saskatchewan was the leading producer of mustard seed, producing over 81% of the total. Seventy-eight percent of this crop was expected to grade No. 1 Canada (Statistics Canada, 1996; DeClercq, D.R. and Daun, J.K., 1997). According to 1996 values, producing one tonne of mustard in Manitoba costs $293. The gross income from this tonne at harvest time (September), receiving contract prices, would be $440, giving a profit of $147/tonne. The price received for a pound of condiment mustard may vary depending on when the seed is sold, as mid-November non-contract prices in Manitoba may be higher than the contract price received at harvest time.

The profit received from one tonne of mustard is somewhat lower than that which would be obtained from one tonne of canola. Producing one tonne of canola costs $184, according to 1996 values, with a gross income of $441/tonne. This results in a profit of $257/tonne. The difference in profit between the two crops is due to a difference in production level. The mustard crop produces less kg of seeds per acre of production, therefore requiring a larger area, more plants and greater amounts of fertilizer and herbicides/pesticides.

Chemical Composition

Although little is reported on the specific nutritient composition of *Sinapis alba*, some general information has been reported in the literature, applying predominantly to the condiment crop. A small amount of data has been published on the low glucosinolate, low erucic acid *S. alba*.

Lipids

Sinapis alba contains approximately 31.5% oil (DeClercq and Daun, 1997). The oil content of seeds is under genetic control, as well as being also strongly affected by climatic conditions (Grami and Stefansson, 1977). Appelqvist (1972) reported that erucic acid made up 41.1% of the fatty acid profile of condiment *S. alba*. The low glucosinolate, low erucic acid line produced by Raney and collegues (1995) was found to contain only 1% erucic acid (Table 1). In the past there has been concern in regards to the fatty acid composition of high oil seeds, in particular, the level of erucic acid (22:1 n-9). This concern has focused largely on rapeseed. Early studies with rats indicated that feeding high erucic acid rapeseed depressed growth rate which was attributed to the presence of erucic acid (Thomasson and Boldingh, 1955). Effects on the myocardium have been reported. Myocardial lipid accumulation has been demonstrated shortly in a variety of species and was found to occur after feeding high erucic acid rapeseed. However, this effect appears to be short-lived (Kramer *et al.*, 1973; Rocquelin *et al.*, 1973;

Table 1. Fatty acid composition of various *Sinapis alba* seeds

Fatty Acid Composition (%)	*S. alba* cv. Seco[1]	Yellow mustard No.1[2]	High erucic acid, low glucosinolate[3]	Low erucic acid, low glucosinolate[4]
16:0	2.7	2.62	2.2	4.9
16:1	0.3	0.21		
18:0	0.9	1.04	0.4	1.7
18:1	22.8	24.66	10.6	56.6
18:2	8.6	9.25	9.1	17.7
18:3	10.5	10.34	14.8	13.8
20:0	0.6	0.67		
20:1	8.4	10.98	2.3	3.0
20:2		0.31		
22:0	0.5	0.54		
22:1	41.1	35.98	58.7	1.0
22:2		0.26		
24:0	0.2	0.34		
24:1	2.7	2.20		

[1] Appelqvist (1972); [2] DeClercq, D.R. and Daun, J.K. (1997); [3] Raney *et al.* (1995a); [4] Raney *et al.* (1995b)

Kramer *et al.*, 1977). Prolonged feeding of high erucic acid rapeseed oils has been seen to cause myocardial necrosis and fibrosis, primarily in male and, to a lesser extent and severity, female rats (Rocquelin and Cluzan, 1968; Kramer *et al.* 1973; Hulan *et al.*, 1977). It has been found that the composition of the diet may play a role in the occurrence of these detrimental effects. Beare *et al.* (1963) reported that the growth-depressing effect of erucic acid was overcome by increasing the saturated fatty acid content of dietary rapeseed oil. This was supported by a chick study in which the growth-depression was eliminated by the feeding of rapeseed oil blended with a low level of saturated beef tallow (Salmon, 1969). A synergism was suggested by chick studies when rapeseed oil was blended with a low level of animal tallow (Lall and Slinger, 1974). Furthermore, in a study with 180 day old White Leghorn cockerels, it was concluded that the high linoleic acid content in sunflower oil may have been the contributing factor in alleviating the adverse effect of erucic acid (Sim *et al.*, 1985). These findings suggest that the growth depressing effect of dietary erucic acid may be due to metabolic interactions among fatty acids, and that certain fatty acids may reduce the negative effect of erucic acid.

Protein

The protein content of *S. alba* is approximately 31.2% (DeClercq, D.R. and Daun, J.K., 1997). The amino acid profile has yet to be published in the literature. As with oil, these components have been found to vary in seeds due to environment, and, to a lesser extent, genetics. It has been shown that, generally, an inverse relationship exists between protein and oil; protein levels being higher and oil content lower when growing conditions are warm and dry, and vice

versa with a cool, moist growing season (Grami and Stefansson, 1977).

Dietary Fibre

Dietary fibre is defined as "the remnants of plant cells resistant to hydrolysis by the alimentary enzymes of man" (Trowell *et al.*, 1976). This includes structural polysaccharides (non-starch polysaccharides), lignin, polyphenols, protein/carbohydrate complexes, fructans and others (Table 2). Although a vast amount of research has been done on dietary fibre, its effect on the utilization of various nutrients is not fully understood. High dietary fibre levels have been found to have a negative effect on the nutritive quality of rapeseed meal (Bjergegaard *et al.*, 1991; Mitaru and Blair, 1984). Dietary fibre may modify, usually decreasing, the digestibility of proteins, along with lipids and certain minerals (Kritchevsky, 1988; Nwokola and Bragg, 1977), and is known to reduce transit time significantly (Raczynski *et al.*, 1982).

Little research has been conducted on the composition of the fibre component of *S. alba*. The seed coat of *S. alba* constitutes 18 to 24% of the seed (Hemingway, 1976). Over 60 years ago, it was first reported that the seed coat of mustard seeds was rich in mucilage material (Bailey and Norris, 1932). Cui *et al.* (1993) reported that 5% of the condiment seed weight is mucilage. Glucose (22-35%) was the major monosaccharide present, followed by galactose (11-15%), mannose (6.0-6.4%), rhamnose (1.6-4.0%), arabinose (2.8-3.2%) and xylose (1.1-2.0%). While high mucilage levels are important for the spice trade, the viscous characteristic makes mucilage an undesirable component of monogastric feeds. Consumption of purified viscous polysaccharides such as gums, mucilage and pectins may interfere with bulk movement and mixing of digestive

Table 2. Components considered part of dietary fibre

Class	Type
Structural polysaccharides (non-starch polysaccharides)	cellulose, pectins/pectic substances, hemicelluloses
Structural non-carbohydrate	lignin, polyphenols
Protein/carbohydrate	glycoproteins, Maillard products
Non-structural polysaccharides	"resistant starch", mucilages, gums
Fructans	inulin, small polysaccharides
Oligosaccharides	α-galactosides
Other	cutin, waxes, minerals

Slominski (unpublished)

enzymes and nutrients in the intestinal lumen (Kritchevsky, 1988). In combination with an enhanced mucin production, this may increase the resistance of the unstirred water layer at the intestinal surface to nutrient absorption (Johnson and Gee, 1981).

Anti-Nutritive Factors of *Sinapis alba* Seed

Glucosinolates

Glucosinolates are anti-nutritive compounds which limit the use of various feedstuffs in livestock and poultry rations (Slominski and Campbell, 1987). They are generally limited to certain families of dicotyledonous angiosperms, predominantly within the order Capparales *sensu* Cronquist or Taktajan, including the families Capparaceae, Cruciferae, Moringaceae, Resedaceae and Tovariaceae (Kjaer, 1974). In human foods and animal feedstuffs, members of the family Cruciferae have the most importance, including oilseeds and forage crops, condiments, relishes and vegetables (Crisp, 1976).

Glucosinolates have a common skeletal structure (Fig. 1) differing only in the nature of the side chain. This side chain may comprise aliphatic (saturated and unsaturated), aromatic or heteroaromatic groupings. Common substituents include hydroxyl groups (occasionally glycosylated), terminal methylthio groups (and oxidized analogues), esters and ketones. It is the R group which is the most reactive part of the molecule, determining the chemical nature of hydrolysis products and, thus, their biological effects and potencies (Fenwick *et al.*, 1983).

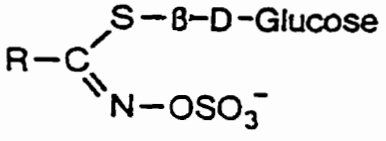


Figure 1. The general structure of glucosinolates (from Fenwick *et al.*, 1983)

Intact glucosinolates themselves are not perceived as harmful. It is the concentration of their hydrolytic products which moderates the toxic and anti-nutritive effects (Hill, 1979; Fenwick and Curtis, 1980). Disruption of plant tissue, in the presence of moisture, results in a loss of glucosinolates due to hydrolysis by the myrosinase enzyme (thioglucoside glucohydrolase, EC 3.2.3.1) (Fenwick and Curtis, 1980) and production of a wide variety of hydrolytic products. Depending on a number of factors (pH, presence of co-factors, structure of parent glucosinolate), these hydrolytic products include the non-toxic glucose and bisulfate and the toxic compounds isothiocyanates, oxazolidinethiones, thiocyanates, epithionitriles and nitriles as well as some other minor compounds (Tookey *et al.*, 1980; Fenwick *et al.*, 1983). To reduce the concentration of the hydrolytic compounds, the myrosinase activity may be inactivated via application of heat. Research conducted by Fenwick *et al.* (1986) indicated that dry extrusion of a rapeseed/soyabean mixture at 150°C effectively inactivated the myrosinase. Smithard and Eyre (1986) suggest that such inactivation can also occur during extrusion at 135°C. Generally, heat is applied to assist in oil extraction during the commercial processing of canola meal. Temperature, time of heating and amount of moisture applied in the crushing process all play a role in the extent of myrosinase inactivation (Slominski *et al.*, 1985).

In addition to being broken down by myrosinase, glucosinolates are susceptible to thermal degradation. Indole glucosinolates are particularly susceptible, yielding 3-indoleacetonitrile, 4-hydroxy-3-indoleacetonitrile and free thiocyanate ion upon degradation (Slominski and Campbell, 1989). The glucosinolate content of canola screenings was reduced from approximately 40 μmol/g to 15 μmol/g via wet cooking (Keith and Bell, 1983). Jensen and collegues (1995) reported that after toasting canola at 100°C for 15, 30, 60 and 120 minutes, the total glucosinolate content

decreased 24%, 46%, 70% and 95%, respectively. Liu *et al.* (1994) had previously reported a similar reduction pattern of glucosinolates for Crambe seed.

Hydrolysis of glucosinolates within the gastrointestinal tract via intestinal microflora metabolism (Greer and Deeney, 1974) has been reported. Cecum and colon microflora of the rat (Greer, 1962) and of the fowl (Marangos and Hill, 1974; Oginsky *et al.*, 1965) have been found to demonstrate hydrolytic properties *in vitro* and *in vivo* (Slominski *et al.*, 1987; Slominski *et al.*, 1988). Nugon-Baudon *et al.* (1988), using germ-free rats and chickens, reported that the intestinal microflora was responsible for growth depression and hypertrophy of glucosinolate targets organs. Intestinal *Lactobacillus* was found to be responsible for a dramatic goitrogenicity in animals fed rapeseed meal (Nugon-Baudon *et al.*, 1990).

The content and composition of glucosinolates of the low and high glucosinolate varieties of *S. alba* are shown in Table 3. While the major glucosinolate in the condiment variety is OH-benzyl glucosinolate, no OH-benzyl glucosinolate was detected any of the low glucosinolate varieties. The anti-nutritional effects attributed to glucosinolates include reduced performance, enlarged thyroid, reduced levels of circulating thyroid hormones and organ toxicity (Vermoral *et al.*, 1986; Fenwick *et al.*, 1983; VanEtten and Tookey, 1983). Much of the research conducted on glucosinolates has involved rapeseed and canola meals.

Negative effects of high glucosinolate intake on growth performance were noted in poultry following the feeding of rapeseed meal (Ibrahim, 1978; Clandinin and Robblee, 1981; Fenwick and Curtis, 1980) and mustard products (Marangos *et al.*, 1974), and in rats fed specially prepared rapeseed diets (Hill, 1976). Leg abnormalities increased in broilers with the feeding of high glucosinolate rapeseed (Timms, 1983; Bell, 1984; Hill, 1979), but was reduced when low

Table 3. Glucosinolate contents of various *Sinapis alba* samples (μmol/g oil extracted meal)

	Glucosinolate Content (μmol/g oil extracted meal)			
Type of sample	hydroxy-butenyl	total alkenyl	hydroxy-benzyl	total indolyl
High glucosinolate[1]	5	-[4]	210	-
Low glucosinolate[1]	5	-	1	-
High erucic acid, low glucosinolate[2]	4.4	4.6	0.0	5.6
Low erucic acid, low glucosinolate[3]	4.3	4.5	0.0	4.7

[1] Uppström (1995); [2] Raney *et al.* (1995a); [3] Raney *et al.* (1995b); [4] not determined

glucosinolate or extracted rapeseed meal was fed (Moody *et al.*, 1978; Holmes and Roberts, 1963). Martland *et al.* (1984) found egg production to be decreased by feeding high glucosinolate meal, supporting work done previously by Marangos and Hill (1976) and Ibrahim (1978).

The capability of glucosinolates to depress iodine uptake and iodification, and affect T_3:T_4 ratios and thyroid histology has been well documented (Bell, 1984; Fenwick *et al.*, 1983). Mouse and rat studies indicated that various *Brassica* meals were goitrogenic and demonstrated that myrosinase enhanced the toxicity of rapeseed meal (Bell *et al.*, 1972; Lo and Bell, 1972; Belzile *et al.*, 1963). Klain *et al.* (1956) indicated that the goitrogenic effects could not be prevented by a high level of dietary iodine. However, Greer (1962) and Langer and Greer (1977) reported that iodine supplementation has proved an effective solution to the effects of the thiocyanate ion. Thiocyanate acts by blocking or reducing iodine capture by the thyroid gland, thus the alleviation of effects with iodine supplementation (Langer, 1966). The goitrogenic effect of glucosinolates is generally associated with an increased weight of the thyroid gland (Marangos *et al.*, 1974; Ibrahim, 1978). Incidence of liver enlargement was found to be related to glucosinolate content and was reduced when low glucosinolate rapeseed meal was fed (Elwinger, 1986). Rapeseed meal is associated with progressive liver damage in certain strains of laying hens, resulting in liver haemorrhage and increased mortality (Ibrahim *et al.*, 1980; Fenwick *et al.*, 1983; Martland *et al.*, 1984). Conversion from rapeseed meal to the lower glucosinolate canola meal reduced, but didn't eliminate, liver haemorrhage (Slominski and Campbell, 1991).

Swine diets can contain 5 μmoles glucosinolates per gram of ration without producing adverse effects, leading to an inclusion rate of 20 to 30% (Canola Council of Canada, 1989). For laying hen, however, a "no-effect" level of glucosinolates of approximately 1.5 μmoles/g has been

recommended, because of their association with mortality due to haemorrhagic liver syndrome (Campbell and Slominski, 1991).

Phytic Acid

Phytic acid (myoinositol 1,2,3,4,5,6-hexakis-dihydrogen phosphate) (Fig. 2) is widely distributed among cereal grains and oilseeds (Ward and Reickert, 1986) and is the main storage pool of phosphorus in the seed. Phosphorus in this form is, for the most part, not available to monogastrics, as they lack the appropriate enzyme to cleave the phytate molecule (Taylor, 1965; Nelson, 1967). Phytic acid is highly reactive with positively charged molecules and binds with Ca, Mg, Zn, Cu, etc., forming complexes that are insoluble in the intestine. Phytic acid may also form complexes with protein, thereby inhibiting enzymatic digestion (Thompson, 1990).

Bell and Rakow (1996) found the high glucosinolate oil-extracted meal of *S. alba* to contain 1.44% phytic acid, while the low glucosinolate meal contained 3.06%. As no feeding trials have been carried out using the low glucosinolate *S. alba,* the effect of phytates in this seed is not known. There has been much reported in the literature on the phytic acid content of other feedstuffs of plant origin (Table 4).

The bound phosphorus in monogastric diets is excreted, posing an environmental concern. Excess phosphorus not utilized by plants is converted to water-insoluble forms which are absorbed by soil particles, potentially contaminating fresh water resources. While currently no regulations exist in North America limiting phosphorus content of faeces, the likelihood of regulations in this area is high.

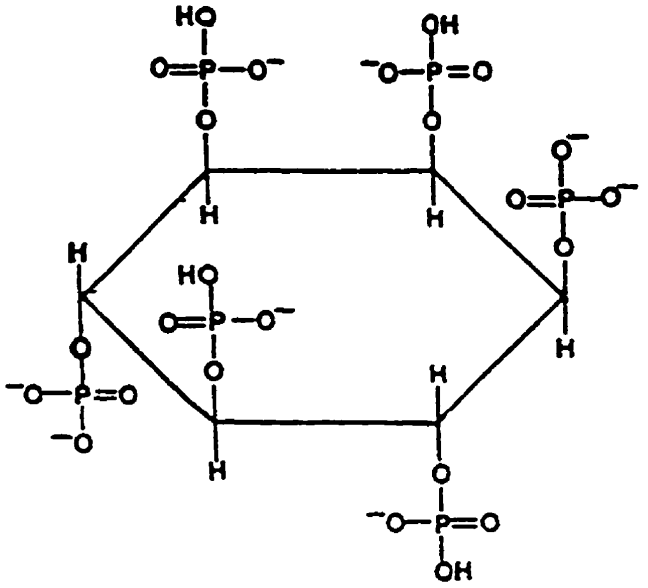


Figure 2. Structure of phytic acid (from Cheryan, 1980)

Table 4. Phytate content of some common feedstuffs[1]

Feedstuff	Total phosphorus (%)	Phytate, % of total phosphorus
Corn	0.26	69
Grain sorghum	0.31	68
Barley	0.34	56
Wheat	0.30	67
Oats	0.34	56
Soybean meal	0.61	61
Canola meal[2]	1.17	74
Cottonseed meal	1.07	70
Sesame meal	1.27	81
Wheat bran	1.37	70

[1] Adapted from Nelson *et al.* (1968); [2] NRC (1994)

The effect of heat treatment on phytic acid content of feedstuffs has been investigated. The phytic acid content of raw African oil bean seeds was found to be significantly reduced by cooking (Kingsley, 1995). Work done by Khan and colleagues (1991) indicated that boiling and roasting of both fresh and dry maize ears/grains resulted in a reduction of phytic acid content.

In an attempt to increase the amount of phosphorus available to the animal, work has been done on the addition of phytase enzyme to monogastric diets. Results have shown that by adding phytase, the phosphorus excreted can be decreased, as can the amount of supplemental inorganic phosphorus added to the diet (Simons *et al.*, 1990; Cromwell, 1991).

Trypsin Inhibitor

Proteinase inhibitors can be found in the seeds of most plants, particularly in the graminaceae and leguminosae, and in the tubers of solanaceae. These inhibitors may exist to regulate endogenous proteinase enzyme activity during seed dormancy, and may play a role in the protection against phytophagous insects and pathogenic fungi (Green and Ryan, 1972).

There have been three trypsin inhibitors identified in the condiment *S. alba* . The predominant inhibitor shares several characteristics, such as thermolability, with the soybean trypsin inhibitor, Kunitz (Iori *et al.*, 1991; Menegatti *et al.*, 1985). Iori *et al.* (1991) found a trypsin inhibitor activity of 0.9 trypsin inhibitor (TI) units/g defatted *Sinapis alba*. In 1996, Bell and Rakow found 1.65 TI units/g high glucosinolate *S. alba* and 2.11 TI units/g low glucosinolate *S. alba*, both oil-free and non-heated. These values are well below those of certain legumes and are thought to be of little nutritional consequence (West and Norton, 1991), particularly if heat

treatment is to be applied. Herkelman *et al.* (1993) reduced the trypsin inhibitor activity of both conventional soybeans and low trypsin inhibitor soybeans with application of heat via autoclaving at 110°C for 20 minutes. As well, Kingsley (1995) found the trypsin inhibitor activity of African oil bean seeds to be decreased during cooking by 98%. If heat were to be applied to *S. alba*, it seems likely that the trypsin inhibitor levels would be minimal.

Heat Treatment

It is well known that heat processing is an effective method of improving the nutritive value of feedstuffs for monogastric animals (McNab, 1982). Nordheim and Coon (1984) reported that some form of heat treatment is necessary to alter that tertiary structure of plant protein to allow the protein to become more susceptible to hydrolysis. Effectiveness of heat treatment on nutritional quality is a function of processing temperature, duration of heating, particle size, moisture content and type of feedstuff (Liener, 1983; Poel, 1989). The effect of heat processing on phytic acid, trypsin inhibitor, glucosinolates and myrosinase enzyme has been discussed in previous sections.

Proper heat treatment is of great importance, as either underheating or overheating will produce a product of inferior nutritional value. While underheating may not inactivate toxic components (Alumot and Nitsan, 1961) or denature proteins to a point where they can be completely digested (Fisher and Johnson, 1958), overheating can damage proteins and amino acids and render them unavailable to the animal (McGinnis and Evans, 1947). The mechanism responsible for the overheating damage in known as the Maillard reaction, where the epsilon

amino acid group reacts with free reducing sugar to form a complex (Moran *et al.*, 1963). Accurate control of heating is therefore necessary to maximize the benefit of heat processing.

Micronization

Micronization is a dry-heat process where industrial propane is burned over ceramic tile or nichrome wire elements to produce infrared electromagnetic short waves (McNab and Wilson, 1974). While there are no documented reports indicating the effect of micronization on the nutritive value of *S. alba*, it has been shown to improve the nutritive quality of cereal grain for growing pigs (Lawrence, 1973) and chickens (Douglas *et al.*, 1991). Positive effects of micronization on nutrient utilization have also been reported for full fat soybeans (Hutton and Foxcroft, 1975) and faba beans (*Vicia faba*) (McNab and Wilson, 1974).

MANUSCRIPT 1

CANOLA-QUALITY *SINAPIS ALBA* MUSTARD: CHEMICAL COMPOSITION AND THE EFFECT OF MICRONIZATION ON SEED QUALITY

ABSTRACT

The *Sinapis alba* (white mustard) species holds great potential as a high protein and high energy alternative to full fat soybean. It has superior heat and drought tolerance in comparison to conventional canola, and is therefore well suited to production in dryland areas. Although it has been grown for sometime as a condiment crop, it is only recently that plant breeders have developed a *S. alba* cultivar with low contents of glucosinolates and erucic acid. The objective of this study was to evaluate the nutritive profile of *S. alba* and to explore the potential for use of the full fat seed in monogastric animal nutrition. Detailed chemical characterization of *S. alba* in comparison with selected feedstuffs (ie., soybean, canola, lupin and peas) was carried out. Digestible protein content in defatted and heat-treated *S. alba* seed averaged 70.8% of total protein which is equal to or higher than values for other feedstuffs. In comparison to the full fat soybean, *S. alba* seed contained more oil (26.4% vs 20.2%) but less protein (37.5% vs 41.4%) with the content of the two major nutrients in favour of the *S. alba* sample (63.9% vs 61.6%). *S. alba* seed contained slightly more methionine and cystine (3.60 vs 3.33 g/16g N) but less lysine (5.78 vs 6.49 g/16g N) than soybean. The sucrose content was lower in *S. alba* than in soybean (3.3% vs 5.2%) although this was offset by the presence of starch (1.3%) which was not detected in soybean. Considering the remarkable difference in seed size, *S. alba* having a 20-fold smaller seed than soybean, only a small difference in the total dietary fibre content was observed (22.2% vs 18.8% for *S. alba* and soybean, respectively). Soluble dietary fibre content (ie., mucilage), as determined under simulated conditions of the gastrointestinal tract, was found to be 1.7%. A lower content of oligosaccharide (3.4% vs 5.1%) and higher contents of calcium (0.66% vs

0.39%) and available (non-phytate) phosphorus (0.27% vs 0.12%) were among other positive characteristics associated with the *S. alba* crop. Although still relatively high and potentially a limiting factor, the glucosinolate content of the new *S. alba* cultivar was shown to be 35.3 μmol/g which is substantially lower than that present in current condiment varieties. Tempering the *S. alba* seed to 20% moisture content and micronization at temperatures up to 140°C (temperature setting on the micronizer) improved protein digestibility *in vitro* and substantially lowered the activity of myrosinase enzyme.

INTRODUCTION

It has been recognized in this laboratory that the canola type *Sinapis alba* white mustard holds great potential as a high protein and high energy alternative to full fat soybean. Although it has been grown for sometime as a condiment crop, it is only recently that plant breeders have developed a "canola quality" cultivar with low contents of glucosinolates and erucic acid (Krzymanski *et al.*, 1991; Raney *et al.*, 1995b). *Sinapis alba* has superior heat and drought tolerance in comparison to conventional *Brassica napus* and *B. rapa* canola, and is therefore well suited to production in dryland areas. It has a high resistance to blackleg disease (*Lestophaeria maculans*) and flea beetle attacks. Further advantages include a highly shatter resistant seed pod and a large bright yellow seed.

In Canada, the production of condiment *S. alba* occurs predominantly in the Prairie provinces of Manitoba, Saskatchewan and Alberta. The condiment variety, however, contains a significant amount of erucic acid (25-45% of the fatty acid profile) and has a high glucosinolate content (approximately 200 μmol/g oil free meal) (Hemingway, 1995). Due to the goitrogenic and antinutritive effects of glucosinolates (Bell, 1984) and potentially negative implications related to erucic acid consumption (Kramer *et al.*, 1977) leading to reductions in oil/meal quality, condiment *S. alba* is unsuitable for use in monogastric animal feeding. Various plant breeding approaches have been undertaken in an attempt to reduce glucosinolate and erucic contents in *Sinapis alba* seed. Development of a low glucosinolate line in Poland (Krzymanski *et al.*, 1991) and a low erucic acid line in Canada (Raney *et al.*, 1995b) offers some potential for further development of an improved low glucosinolate, low erucic acid cultivar better suited for

monogastric animal feeding. However, further plant breeding efforts are necessary to combine the low glucosinolate and low erucic acid characteristics to further improve the nutritive quality of the *S. alba* seed.

It is well known that heat processing is an effective method of improving the nutritive value of feedstuffs for monogastric animals (McNab, 1982). Nordheim and Coon (1984) reported that some form of heat treatment is necessary to alter the tertiary structure of plant protein to allow the protein to become more susceptible to protein hydrolysis. Micronization is a dry-heat process where industrial propane is burned over ceramic tile or nichrome wire elements to produce infrared electromagnetic short waves (McNab and Wilson, 1974). While there are no documented reports indicating the effect of micronization on the nutritive value of *S. alba*, the process has been shown to improve the nutritive quality of cereal grain for growing pigs (Lawrence, 1973) and chickens (Douglas *et al.*, 1991). Positive effects of micronization on nutrient utilization have also been reported for full fat soybeans (Hutton and Foxcroft, 1975) and faba beans (*Vicia faba*) (McNab and Wilson, 1974).

Little is known regarding the nutritive make-up of the canola type *S. alba* seed. The objective of the present study was to gather detailed information on the chemical composition of the *S. alba* seed in comparison with traditional feedstuffs, i.e., soybean, canola, lupin and peas. Any potential beneficial effect of micronization technology on the nutritive quality of the *S. alba* seed was also investigated.

MATERIALS AND METHODS

Materials

Seed samples of canola type *Sinapis alba* white mustard (1995 crop) and a conventional canola (*Brassica napus* cv. Excel) were provided by Agriculture and Agri-Food Canada, Research Station, Saskatoon, Saskatchewan. Soybean seed was obtained from ADM Animal Health and Nutrition (Des Moines, IA, USA). Seedtech Inc. (Qu'Appelle, Saskatchewan, Canada) provided white lupin seeds and Fisher Feeds (Dauphin, Manitoba, Canada) supplied the pea sample, cv Impala.

The effect of micronization was studied in two experiments. One portion of *S. alba* seed was micronized by Infra-Ready Products Inc. (Saskatoon, Saskatchewan, Canada) using seven different temperatures (100°C and 110 to 135°C in 5°C increments) with no moisture added (experiment one). Another set of samples (experiment two) was obtained by tempering the seed to 20% moisture content and micronizing at 110 to 140°C in 5°C increments.

The raw and micronized *S. alba* seed samples along with soybean and *B. napus* canola seeds were crushed to pass through a 2.0 mm sieve and were extracted with n-hexane for 8 hrs in a Kontes Macro Soxhlet apparatus (Fisher Scientific, Edmonton, Alberta, Canada). Following drying under a fumehood, the meals were re-ground to pass through a 1mm sieve and were re-extracted with n-hexane for an additional 8 hrs. The lupin and peas were ground to pass through a 1 mm sieve using a Tecator Cyclotec 1093 sample mill (Fisher Scientific, Edmonton, Canada). Portions of the samples were moist heat treated at 108 $\pm$ 1°C for 20 minutes in a laboratory

autoclave and were dried overnight at 40°C (Simbaya *et al.*, 1997). For fat analysis, the high-oil seed samples (ie., *S. alba* and *B. napus* canola) were ground in a conventional coffee mill.

Chemical Analyses

Representative samples of the *S. alba*, soybean, *B. napus*, lupin and pea meals were analyzed in duplicate for moisture, crude protein, ash, calcium and phosphorus contents using established standard methods of analysis (AOAC, 1990). Fat content was determined by the AOAC method recommended for animal feed (920.39) with the exception that ether was substituted with n-hexane and the extraction time was 12 hours. Sucrose and oligosaccharides were determined by gas-liquid chromatography according to the procedure described by Slominski (1994). Phytate was determined by the method of Hang and Lantzsch (1983).

Dietary fibre was analyzed by a combination of neutral detergent fibre (NDF) and detergent-soluble non-starch polysaccharides (NSP) measurements and was calculated as the sum of NDF and detergent-soluble NSP (Slominski *et al.*, 1994). Detergent-soluble NSP was calculated as total sample NSP minus NSP present in the NDF residue. The value for lignin and other fibre components (ie., cell wall protein, minerals, etc.) was calculated by difference (total dietary fibre - NSP). The method of Goering and VanSoest (1970) was used for the determination of NDF, except that the procedure was modified to exclude the use of decalin and sodium sulfite (Mascharanhas Ferreira *et al.*, 1983). Non-starch polysaccharides were determined by gas-liquid chromatography (component neutral sugars) and by colorimetry (uronic acids) using the procedure described by Englyst and Cummings (1984, 1988) with minor modifications (Slominski and

Campbell, 1990). The content of NSP was measured in both the meals and the NDF residues.

Starch was determined using the NSP procedure in which starch gelatinization with dimethylsulfoxide was substituted by treating the samples with boiling water for 30 min. Starch hydrolyzing enzymes (ie., α-amylase, pullulanase and amyloglucosidase) were excluded from the procedure and the starch content was calculated by difference between total sample glucose (ie., no enzyme added) and the NSP glucose.

Amino acid content of the meals was determined on an LKB Biochrom 4151 Alphaplus amino acid analyzer (Biochrom, Science Park, Cambridge, UK) following hydrolysis of the samples with 6N hydrochloric acid at 110^{0}C for 24 hrs (Andrews and Baldar, 1985). Methionine and cystine were analyzed following a 20 hr oxidation with performic acid (Moore, 1963). Analysis for tryptophan was not carried out.

Glucosinolates were analyzed by gas-liquid chromatography using the procedure of Thies (1977) as modified by Slominski and Campbell (1987). Benzyl (glucotropaeolin) and allyl (sinigrin) glucosinolates were used as the internal standards. Myrosinase activity in micronized samples of *S. alba* was determined by difference between total sample glucosinolate content and the glucosinolates remaining following incubation of the meal with distilled water (autolysis) at 40°C for a defined period of time. Glucosinolate analysis was conducted using sinigrin as the internal standard. One unit of myrosinase activity was defined as the amount of enzyme that catalyses the hydrolysis of 1 μmol of glucosinolate per 1 min.

Digestible protein and water-soluble fibre contents were determined *in vitro* using the digestion/dialysis unit and the procedure described in detail by Simbaya *et al.* (1997). Lysine availability was determined indirectly using the dye binding capacity procedure outlined by Moran

(1963).This gives an indication of lysine availability since orange G binds to the free amino groups of lysine. The more dye that binds to the meal, the higher the available lysine content.

All chemical analyses were performed in duplicate.

Seed size was determined in triplicate by weighing 100 seeds from each *S. alba*, *B. napus* and soybean samples and was expressed as grams per 1000 seeds.

Statistical Analysis

Data were subjected to analysis of variance (ANOVA) using the general linear models of statistical analysis system (SAS Institute, 1990) and the procedures of Snedecor and Cochran (1980). Tukey's procedure was used to compare and separate treatment means.

RESULTS AND DISCUSSION

The chemical composition of the *Sinapis alba* sample as compared to selected feedstuffs is shown in Table 5. The results for soybean, *B. napus* canola, lupin and peas corresponded with those previously published (NRC, 1994; InfraReady Technical Information, 1995; Mohamed and Rayas-Dutarte, 1995; Canola Council of Canada, 1997; Igbasan and Guenter, 1997), with the exception of the protein and fibre values for soybean, which were lower in the published literature than reported in this study. *S. alba* contained more protein than *B. napus* canola, lupin or peas, but less than soybean. The oil content of *S. alba* was lower than in *B. napus* canola but higher

Table 5. Chemical composition of *Sinapis alba* as compared to selected feedstuffs (% DM, full fat basis)

Component	*S. alba*	Soybean	*B. napus* canola	Lupin	Peas
Protein	37.5±0.75[1]	41.4±1.53	25.7±0.75	31.6±0.88	25.0±0.30
Oil	26.4±0.47	20.2±0.31	41.5±1.32	12.2±0.12	1.7±0.00
Sucrose	3.3±0.01	5.2±0.08	4.1±0.01	1.9±0.05	2.0±0.05
Oligosaccharides	3.4±0.04	5.1±0.06	2.0±0.01	6.8±0.20	2.3±0.13
Starch	1.3±0.06	ND[2]	0.4±0.01	0.6±0.01	40.7±1.18
Total dietary fibre:	22.2±0.04	18.8±0.04	20.4±0.04	38.5±0.23	16.5±0.13
- NSP[3]	15.3±0.04	14.7±0.04	11.8±0.04	32.5±0.23	12.1±0.13
- Lignin & others[4]	6.9±0.04	4.1±0.04	8.6±0.04	6.0±0.23	4.4±0.13
Water-soluble fibre	1.7±0.22	0.5±0.11	0.5±0.04	0.9±0.02	0.8±0.16
Ash	5.4±0.35	5.4±0.01	5.0±0.04	3.9±0.03	3.4±0.03
Calcium	0.66±0.00	0.39±0.02	0.46±0.01	0.42±0.00	0.30±0.00
Total phosphorus	0.89±0.01	0.63±0.02	0.89±0.05	0.47±0.07	0.58±0.02
Phytate phosphorus	0.62±0.00	0.51±0.01	0.55±0.03	0.04±0.00	0.16±0.00
Non-phytate phosphorus	0.27±0.00	0.12±0.01	0.34±0.03	0.43±0.00	0.42±0.00

[1] Mean ±SD; [2] Not detected; [3] Non-starch polysaccharides; [4] Includes lignin with associated polyphenols, cell wall protein and some minerals

than that found in soybean, lupin or peas.

Sucrose, a highly digestible carbohydrate, showed lower value for the *S. alba* sample in comparison to soybean or *B. napus* canola. However, the lower sucrose content was somewhat offset by the starch content.

As compared to other feedstuffs, *S. alba* was found to have an intermediate content of oligosaccharides, raffinose and stachyose. Although previous research (Coon *et al.*, 1990) has indicated oligosaccharides as having a negative effect on energy utilization in soybean, recent studies involving canola meal have shown this effect to be less pronounced (Slominski *et al.*, 1994b). As no work has been undertaken on *S. alba* in this regard, it is uncertain what effect the oligosaccharides would have on its nutritive value.

Dietary fibre content of the *S. alba* seed was found to be higher than in *B. napus* canola, soybean and peas. Seed weights (Table 6) were similar to those reported by Velisek *et al.* (1995), although slightly lower. In agreement with earlier reports (Carré and Brillouet, 1986; Bach Knudsen, 1997) lupin showed the highest fibre content. Although dietary fibre content has been reported to be negatively correlated with the seed weight (Downey, 1983; Jensen *et al.*, 1995; Simbaya *et al.*, 1995; Liu *et al.*, 1995), only a small difference in the total dietary fibre content between *S. alba* and soybean was observed. In this context, soybean was found to have a 20-fold larger seed than *S. alba* (Table 6). The difference between *S. alba* and *B. napus* canola also do not conform to the concept that seed size is inversely related to fibre content. Earlier research on *B. napus* and *B. rapa* canola (Slominski *et al.*, 1994a; Simbaya *et al.*, 1995; Slominski, 1997) have indicated that factors other than seed size ie., the colour of the seed or the embryo/cotyledon cell size may significantly affect the level and nature of dietary fibre. Since *S. alba* has a large

Table 6. Seed weight of *Sinapis alba* as compared to *Brassica napus* canola and soybean

Sample	Seed weight, g/1000 seeds
Sinapis alba	6.5
Brassica napus	3.5
Soybean	138.3

yellow seed, small cotyledon cell size would appear to be the factor contributing to the relatively high fibre content, as compared to *B. napus*. This supposition is confirmed by a much higher content of non-starch polysaccharides (NSP) in the *S. alba* sample as compared to soybean or canola seed. A similar phenomenon was reported for the yellow-seeded *B. rapa* canola which, although having less lignin and polyphenols in the hull fraction, was found to contain significantly more NSP than its *B. napus* counterpart (Slominski *et al.*, 1994a). Further evidence for *S. alba* having a small cotyledon cell size can derive from the component sugar profile presented in Table 7. Although distribution of polymers between cellular tissues is difficult to predict from a total component sugar profile, it appears that an exceptionally high content of rhamnose and uronic acids along with a relatively high content of galactose and arabinose is an indication of pectic polysaccharides (ie, rhamnogalacturonans, arabinogalactans) present in the cotyledon fraction of the *S. alba* seed. It would also appear that pectic substances represented by uronic acids, galactose and arabinose make up a significant fraction of the water-soluble fibre (ie., mucilage, gums) of the *S. alba* seed. Arabinose, galactose and uronic acids accounted for 75% of the soluble fibre content of *S. alba* seed. It has been previously determined that mucilage comprised 5% of the condiment *S. alba* seed (Cui *et al.*, 1993) of which 55% was water-soluble. With the exception of lupin, sugars pertaining to viscous polysaccharides such as mucilage, gums and pectins were at higher levels in *S. alba* than in other feedstuffs. It is a well known fact that high soluble fibre levels may contribute to intestinal viscosity, leading to possible reductions in broiler chicken performance. In this context, viscous polysaccharides may interfere with bulk movement and mixing of digestive enzymes and nutrients in the intestinal lumen and, together with enhanced mucin production, could increase the resistance of the unstirred water layer at the intestinal surface

Table 7. Non-starch polysaccharide profiles of *S. alba*, *B. napus* canola, soybean, lupin and peas (% of total)

Component Sugar	*S. alba*	*B. napus* canola	Soybean	Lupin	Peas
Rhamnose	2.7±0.14[1]	1.1±0.07	0.8±0.00	0.4±0.14	0.3±0.28
Fucose	T[2]	1.1±0.14	1.6±0.14	T	T
Arabinose	21.9±0.35	26.4±0.71	14.4±0.57	12.4±0.21	24.7±0.85
Xylose	7.4±0.07	9.2±0.14	8.2±0.07	12.0±0.14	7.2±0.00
Mannose	3.6±0.07	2.1±0.07	5.6±0.07	1.6±0.07	0.6±0.07
Galactose	16.4±0.21	9.7±0.14	23.8±0.28	35.4±0.35	6.0±0.00
Glucose	25.3±0.00	30.4±0.21	30.2±0.14	28.7±0.14	45.1±0.85
Uronic acids[3]	22.7±0.36	20±0.42	15.4±0.18	9.5±0.28	16.1±0.16

[1] Mean ±SD; [2] traces

to nutrients absorption (Johnson and Gee, 1981; Graham and Åman, 1991). This may result in reduced weight gains, higher feed conversion ratios and lower AME values for poultry (Kritchevsky, 1988; Campbell and Bedford, 1992; Choct, 1992).

Ash, phosphorus and calcium levels are presented in Table 5. Ash level of *S. alba* was similar to soybean and higher than that found in other feedstuffs. *S. alba* seed had the highest calcium level of all feedstuffs analyzed. Total phosphorus content of *S. alba* sample was also higher than that of other feedstuffs, with the exception of *B. napus* canola which had a phosphorus level equal to *S. alba*. In comparison to canola, lupin and peas, the higher phytate phosphorus level in the *S. alba* sample resulted in a somewhat lower content of non-phytate phosphorus. Soybean, however, had the lowest available phosphorus content among the feedstuffs analyzed.

The amino acid profiles of *S. alba*, soybean, *B. napus* canola meal, lupin and peas are presented in Table 8, providing an indication of the protein quality. Arginine levels in *S. alba* sample were similar to *B. napus* canola and lower than in the other feedstuffs. Lupin had lower lysine contents than *S. alba*, while soybean, *B. napus* canola and peas had higher levels. *S. alba* was rich in histidine, threonine, valine and isoleucine. Similarly to soybean, *S. alba* contained less methionine and cystine than canola. Overall amino acid profile of *S. alba* sample supports the concept of *S. alba* seed being a valuable protein supplement.

The glucosinolate content of *S. alba* and *B. napus* canola are shown in Table 9. The glucosinolate content of the *S. alba* sample averaged 7.7 μmol/g and was considerably lower than that of the condiment variety ($\sim$200 μmol/g oil free meal; Hemingway, 1995). When a standard procedure for glucosinolate analysis with benzyl glucosinolate (glucotropaeolin) as an internal standard was employed, the glucosinolate content was also lower than that found in the

Table 8. Amino acid composition of *Sinapis alba* as compared to selected feedstuffs (g/16g N)

Amino Acid	*S. alba*	Soybean	*B. napus* canola	Lupin	Peas
Alanine	4.23^{b}	4.37^{ab}	4.52^{a}	3.50^{c}	4.32^{ab}
Arginine	5.93^{c}	7.22^{b}	6.18^{c}	9.22^{a}	9.30^{a}
Aspartic acid	8.31^{c}	11.57^{ab}	7.55^{c}	10.89^{b}	12.41^{a}
Cystine	1.87^{b}	1.78^{b}	2.45^{a}	1.66^{c}	1.42^{d}
Glutamic acid	16.39^{d}	17.63^{bc}	18.03^{b}	20.02^{a}	17.40^{c}
Glycine	5.59^{a}	4.48^{b}	5.38^{a}	4.23^{c}	4.52^{b}
Histidine	3.02^{b}	2.70^{c}	3.23^{a}	2.59^{c}	2.35^{d}
Isoleucine	3.32^{b}	3.86^{a}	3.27^{b}	3.20^{bc}	2.80^{c}
Leucine	6.90^{bc}	7.49^{a}	6.95^{bc}	7.30^{ab}	6.82^{c}
Lysine	5.78^{c}	6.49^{b}	6.22^{b}	4.91^{d}	7.02^{a}
Methionine	1.73^{b}	1.55^{b}	2.04^{a}	0.84^{c}	0.97^{c}
Phenylalanine	3.99^{b}	5.06^{a}	4.17^{b}	4.01^{b}	4.31^{b}
Proline	6.47^{ab}	6.07^{b}	7.14^{a}	4.85^{c}	4.36^{c}
Serine	5.25^{d}	6.34^{b}	5.44^{d}	6.58^{a}	6.09^{c}
Threonine	3.99^{ab}	3.46^{bc}	4.11^{a}	3.48^{bc}	3.42^{c}
Tryptophan	1.02^{a}	1.05^{a}	0.98^{a}	0.63^{b}	0.78^{b}
Tyrosine	2.96^{c}	3.38^{b}	3.02^{c}	3.89^{a}	2.64^{d}
Valine	4.27^{a}	4.09^{ab}	4.30^{a}	3.59^{b}	3.82^{ab}
TOTAL	91.02^{b}	98.59^{a}	94.98^{a}	95.39^{a}	94.75^{a}

^{a,b,c,d} Values within rows with no common superscripts differ significantly ($P \leq 0.05$)

conventional *B. napus* canola (see Table 9). This is in agreement with studies by Raney *et al.* (1995b) who ascertained that low glucosinolate, low erucic acid *S. alba* seed contained 13.5 μmol/g oil free meal. A glucosinolate content of 6 μmol/g oil extracted meal was reported by Uppström (1995) for another low glucosinolate sample of *S. alba* white mustard. Careful examination of the gas-liquid chromatograms, however, indicated that the peak of benzyl glucosinolate, the internal standard added to the sample, was larger than that normally seen in the conventional glucosinolate analysis performed on the canola meal. When the internal standard was changed to allyl glucosinolate (sinigrin) it appeared evident that the sample of *S. alba* contained significant amount of benzyl glucosinolate (Fig. 3). As a consequence, the use of sinigrin as an internal standard resulted in much higher glucosinolate content than that originally determined. This finding was further confirmed by the laboratories of Agriculture Canada in Saskatoon and the Plant Breeding Institute in Poland (Rakow and Krzymanski, personal communication). When using sinigrin as an internal standard, both laboratories showed much higher glucosinolate content than was initially detected in several *S. alba* samples of similar genetic background. Since, in general, benzyl glucosinolate is not present in the condiment mustard, it could be speculated that somewhere in the selection programs the synthesis of aromatic glucosinolates has been impaired resulting in incomplete hydroxylation of benzyl to OH-benzyl glucosinolate. This finding was further substantiated by the rather uncommon profile of indole glucosinolates found in the *S. alba* sample. From data presented in Table 9, it would appear evident that the hydroxylation of indolylmethyl to OH-indolylmethyl glucosinolate was also incomplete in the *S. alba* sample. The nutritional implications of such changes in the glucosinolate profile, however, will more likely be minimal as there is no evidence of different antinutritive responses to either form of aromatic

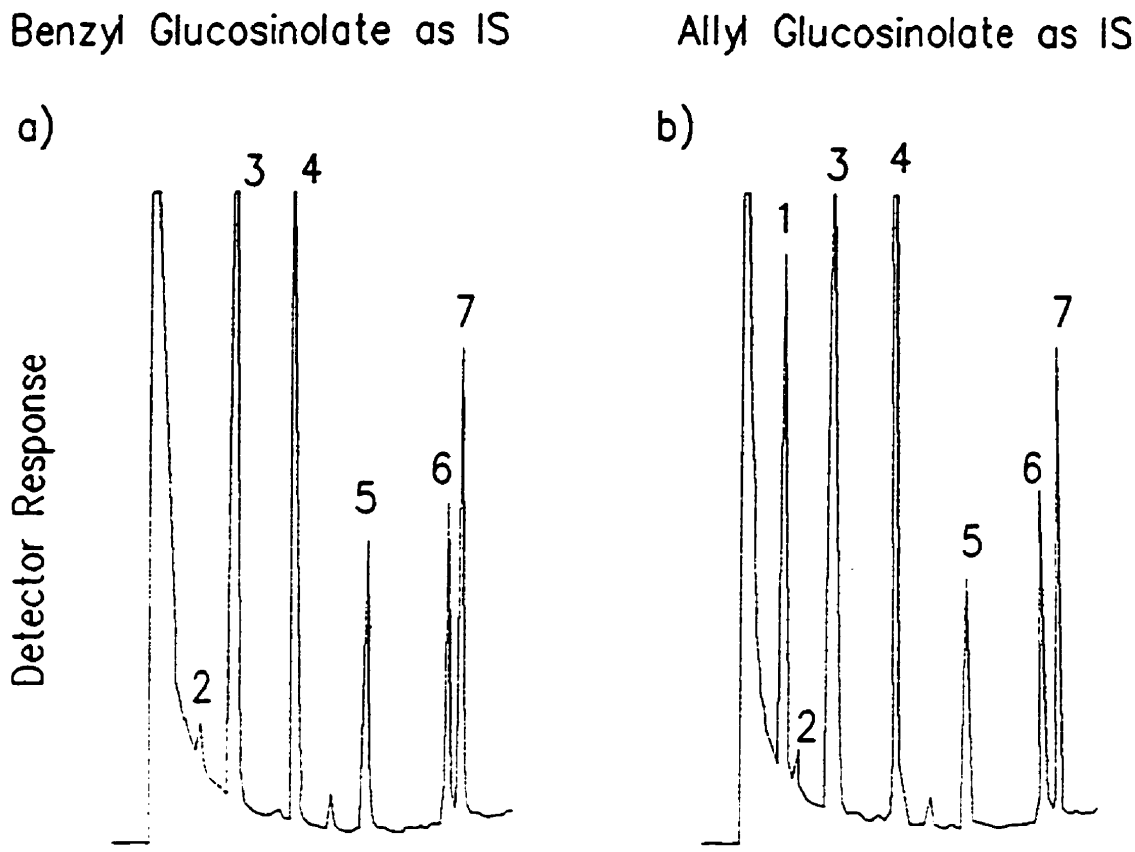


Figure 3. Chromatographic profiles of glucosinolates in a) *S. alba* seed using benzyl glucosinolate as interal standard (IS) (7.3 μmol/g oil free meal) and b) *S. alba* seed using allyl glucosinolate as IS (35.3 μmol/g oil free meal). Peak identification as follows: 1, allyl; 2, 3-butenyl; 3, 2-OH-3-butenyl; 4, benzyl; 5, 4-OH-benzyl; 6, 3-indolylmethyl; 7, 4-OH-3-indolylmethyl.

Table 9. Glucosinolate content of non-heat treated and heat-treated *Sinapis alba* and *Brassica napus* canola (μmol/g oil free meal)

Glucosinolate	*Sinapis alba* (benzyl G as IS[1])		*Sinapis alba* (allyl G as IS[1])		*Brassica napus* (benzyl G as IS[1])	
	non-heat treated	heat treated[2]	non-heat treated	heat treated[2]	non-heat treated	heat treated[2]
3-butenyl	0.1	0.1	0.4	0.3	3.5	2.8
4-pentenyl	ND[3]	ND[3]	ND[3]	ND[3]	0.6	0.4
2-OH-3-butenyl	4.1	4	12.1	11.1	5.7	4.6
2-OH-4-pentenyl	ND[3]	ND[3]	ND[3]	ND[3]	0.1	ND[3]
benzyl	-	-	12	9.8	ND[3]	ND[3]
4-OH-benzyl	0.9	0.9	2.7	2.7	ND[3]	ND[3]
3-indolylmethyl	1	0.5	3.4	1.8	0.5	0.3
4-OH-3-indolylmethyl	1.7	0.3	4.9	0.9	10.1	4.1
TOTAL	7.7	5.8	35.3	26.5	20.5	12.3

[1] internal standard; [2] 108°C ± 1°C for 20 minutes; [3] not detected

glucosinolates. Application of heat treatment, however, may result in the formation of different amounts of breakdown products since benzyl glucosinolate appears to be less stable than its hydroxy derivative while the 3-indolylmethyl glucosinolate withstands higher temperatures than the 4-OH-3-indolylmethyl glucosinolate (Table 9). High stability of OH-benzyl glucosinolate at elevated temperatures and susceptibility of OH-indole glucosinolate to any form of heat treatment is in agreement with earlier data from this laboratory (Slominski and Campbell, 1989; Campbell and Slominski, 1990).

High glucosinolate content encountered in the current study necessitated further research on the potentially positive effect of heat treatment on *S. alba* seed quality. Such studies were undertaken in response to earlier reports indicating beneficial effect of heat treatment on digestible protein contents in soybean (Qin *et al.*, 1996) and canola (Slominski *et al.*, 1998). The micronization technology has been chosen for these studies. The effect of micronization on digestible protein content and myrosinase enzyme activity were studied in two experiments. In experiment 1, the seed was subjected to different micronization temperatures without moisture addition, while in experiment 2, the seed was tempered to 20% moisture content and then micronized. It must be stressed herein that the micronization temperatures represented the instrument settings and did not reflect the temperatures inside the micronized seed.

It is important to remember that heat treatment of feedstuffs may result in the browning or Maillard reaction in which the epsilon amino acid groups react with free reducing sugar (eg. glucose) to form complexes including Amadori products and advanced glycation end products (Friedman, 1996) which, for the most part, are unavailable to the animal. The production of Maillard reaction products is often evidenced by an increase in neutral detergent fibre (NDF)

content. Increased NDF with increased processing temperatures was observed by Keith and Bell (1983) in canola meal. Underheating the seed sample, on the other hand, may not effectively modify the tertiary structure of the protein thereby resulting in improved protein digestion. In an attempt to establish optimum conditions for processing of canola seed, Slominski *et al.* (1998) documented that digestible protein increased substantially with increased temperature of moist heat treatment up to 108°C. Heat treatment below 105°C was not effective in promoting protein digestibility. In the same study, the reduction in digestible protein content for samples heat treated at temperatures above 110°C was substantial and the extent of protein damage was reflected by high NDF content and a high protein content in the NDF residue. Therefore the parameters chosen in the current study to monitor any potential alterations of the protein molecules included protein digestibility *in vitro*, and the NDF and dye binding capacity measurements.

The effect of temperature of micronization on selected quality parameters of the *S. alba* seed is shown in Table 10. There was no difference in digestible protein content as the heating temperature increased and, as compared to the non-heat treated meal, the protein digestibility increased only slightly and remained below the 71 % value found for the sample heat treated under more optimal conditions. The lack of improvement in digestible protein content suggests that the optimal processing conditions may not have been attained (Slominski *et al.*, 1998), resulting in somewhat inferior protein digestibility. This may be due to the fact that no moisture was added prior to micronization. Neither NDF nor dye binding capacity measurements indicated any protein damage. In addition, the myrosinase activity of samples micronized at 125-135°C was still very high with the level of activity sufficient for hydrolysis of all sample glucosinolates within 3 minutes.

Table 10. Effect of *Sinapis alba* seed micronization (no moisture added) on dye binding capacity, NDF, digestible protein content and myrosinase activity in the oil free meal

Micronization temperature (°C)[1]	Orange G Bound (mg/g meal)	NDF (%)	Digestible Protein (%)	Myrosinase Activity[2]
100	51.6^{b}	21.8	50.4^{bc}	33.0^{a}
110	53.0^{b}	20.8	50.6^{bc}	33.6^{a}
115	52.9^{b}	22.3	48.0^{c}	33.4^{a}
120	56.1^{a}	21.2	50.1^{bc}	30.9^{a}
125	56.1^{a}	22.9	52.4^{b}	12.8^{b}
130	55.7^{a}	20.7	48.2^{bc}	8.6^{b}
135	56.8^{a}	23.2	48.7^{bc}	11.1^{b}
Non-heat treated sample	-	-	46.7^{c}	-
Sample heat treated at 108°C for 20 minutes	-	-	70.8^{a}	-

[1] Represents temperature setting on the micronizer; [2] One unit of activity is defined as the amount of enzyme that catalyses the hydrolysis of 1 μmol of glucosinolate per 1 minute

^{a,b,c} Values within columns with no common superscripts differ significantly ($P \leq 0.05$)

Tempering the seed to 20% moisture content prior to micronization (Table 11) resulted in improved protein digestibility, with the digestibility approaching the level detected for the laboratory prepared meal. In comparison to experiment one (Table 10), some increase in dye binding capacity measurement was noted. There was slight improvement in the amount of orange G bound to the micronized *S. alba* as the temperature increased. Although the myrosinase was inactivated to a greater extent than that seen in experiment one, the detectable level of 1.0 unit/g oil free seed of enzyme activity was still present in the seed micronized at the highest temperature (i.e., 140°C).

Although the digestible protein content approached the level of the seed treated under optimal conditions, the heat treatment employed in the current study may have not been optimized. This is substantiated by the presence of myrosinase activity in the seed micronized at the highest temperature (ie., 140°C). The protein digestibility and NDF results indicate that the temperature settings on the micronizer may have exceeded the temperature achieved within the seed by approximately 35-40°C. It also appears that the optimal temperature for micronization of *S. alba* seed may be in the range of 140-150°C.

The chemical composition of the canola type *Sinapis alba* seed indicates that it holds potential as a high protein and high energy feedstuff. Further research is needed to evaluate the nutritive value of the seed *in vivo*.

Table 11. Effect of *Sinapis alba* seed micronization (tempered to 20% moisture content) on dye binding capacity, digestible protein and myrosinase activity in the oil free meal

Micronization temperature (^{0}C)[1]	Orange G Bound (mg/g meal)	Digestible Protein (%)	Myrosinase Activity[2]
110	60.8^{b}	48.0^{d}	22.7^{a}
115	61.1^{ab}	52.9^{cd}	3.2^{bc}
120	62.0^{ab}	59.0^{bc}	4.7^{b}
125	61.5^{ab}	64.0^{ab}	3.7^{bc}
130	63.0^{ab}	64.3^{ab}	2.0^{bc}
135	63.1^{a}	65.8^{ab}	2.4^{bc}
140	63.1^{a}	63.7^{ab}	1.0^{c}
Non-heat treated sample	-	46.7^{d}	-
Sample heat treated at 108^{0}C for 20 minutes	-	70.8^{a}	-

[1] Represents temperature setting on the micronizer; [2] One unit of activity is defined as the amount of enzyme that catalyses the hydrolysis of 1 μmol of glucosinolate per 1 minute

^{a,b,c,d} Values within columns with no common superscripts differ significantly ($P \leq 0.05$)

No missing data on page 45.

MANUSCRIPT 2

CANOLA-QUALITY *SINAPIS ALBA* MUSTARD: NUTRITIVE VALUE AND THE EFFECT OF HEAT TREATMENT ON AMINO ACID AND ENERGY AVAILABILITIES FOR POULTRY

ABSTRACT

This study was conducted to investigate the feeding value of the full fat *Sinapis alba* seed in comparison to selected feedstuffs (ie., micronized soybean seed and commercial soybean and canola meals). Raw, micronized (140°C) and autoclaved (108°C) *S. alba* seeds were evaluated for digestible protein content, myrosinase activity, glucosinolate content, true metabolizable energy (TME_n), true amino acid availability (TAAA) and broiler chicken performance. Heat treatment of the *S. alba* seed decreased glucosinolate content, effectively inactivated the myrosinase enzyme, and increased digestible protein content *in vitro*. The autoclaved *S. alba* seed showed higher TME_n value than the raw and micronized samples (3031 vs 2270 and 2460 kcal/kg). The micronized soybean seed was found to contain the highest TME_n value (3790 kcal/kg). Regardless of heat treatment employed, the TAAA of the *S. alba* seed was lower than that of micronized soybean seed, commercial soybean meal and commercial canola meal. Broiler chickens fed rations containing 10% of the raw, micronized and autoclaved *S. alba* seed showed similar feed intake, weight gain and feed to gain ratio to those fed micronized soybean seed. There was no difference in chicken performance between diets containing 10% or 20% of micronized *S. alba* seed. As compared to other treatments, feeding of the ration containing 20% of the autoclaved *S. alba* seed resulted in poorer feed intake, weight gains and feed efficiency.

INTRODUCTION

It has been recognized in this laboratory that the canola type *Sinapis alba* white mustard holds great potential as a high protein and energy feedstuff for the Canadian Prairies. Although it has been grown for sometime as a condiment crop, it is only recently that plant breeders have developed a "canola quality" cultivar with low contents of glucosinolates and erucic acid (Krzymanski *et al.*, 1991; Raney *et al.*, 1995b).

Detailed chemical characterization of canola type *S. alba* in comparison to selected feedstuffs (ie., soybean, canola, lupin and peas) has been completed in this laboratory (Manuscript 1). In comparison to the full fat soybean, *S. alba* seed contained more oil (26.4% vs 20.2%) but less protein (37.5% vs 41.4%) with the content of the two major nutrients in favour of the *S. alba* sample (63.9% vs 61.3%). Soluble dietary fiber content (ie., mucilage), as determined under simulated conditions of the gastrointestinal tract, was found to be 1.7%, relatively high as compared to the other feedstuffs analyzed. This is of concern, as high soluble fibre levels may contribute to intestinal viscosity, leading to possible reductions in bird performance (Kritchevsky, 1988; Campbell and Bedford, 1992; Choct, 1992). Although still relatively high and potentially a limiting factor, the glucosinolate content of the new *S. alba* cultivar was shown to be 35.3 μmol/g which is substantially lower than that present in current condiment varieties ($^{-}$200 μmol/g oil free meal; Hemingway, 1995) .

It is well known that heat processing is an effective method of improving the nutritive value of feedstuffs for monogastric animals (McNab, 1982). Nordheim and Coon (1984) reported that some form of heat treatment is necessary to alter that tertiary structure of plant protein to

allow the protein to become more susceptible to protein hydrolysis. Micronization is a dry-heat process where industrial propane is burned over ceramic tile or nichrome wire elements to produce infrared electromagnetic short waves (McNab and Wilson, 1974). While there are no documented reports indicating the effect of micronization on the nutritive value of *S. alba in vivo*, it has been shown to have improved the nutritive quality of cereal grain for growing pigs (Lawrence, 1973) and chickens (Douglas *et al.*, 1991). As well, positive effects have been reported for full fat soybeans (Hutton and Foxcroft, 1975) and faba beans (*Vicia faba*) (McNab and Wilson, 1974). *In vitro*, micronization of the *S. alba* seed at temperatures up to 140°C (set temperature on the micronizer), after tempering to 20% moisture, improved protein digestibility, lowered the activity of myrosinase enzyme and did not affect the available lysine content.

The objective of this study was to evaluate the nutritive value of canola type *S. alba* white mustard by examining productive performance of broiler chickens, and energy and amino acid availabilities using an adult rooster TME_n assay. The effect of heat treatment on energy availability and amino acid digestibilities was also investigated.

MATERIALS AND METHODS

Materials

Seed sample of canola type *Sinapis alba* white mustard (1996 crop) was obtained from the Agriculture and Agri-Food Canada Research Station (Saskatoon, Saskatchewan). A micronized soybean seed was provided by InfraReady Products Inc. (Saskatoon, Saskatchewan). Commercial soybean meal (48%CP) was acquired from Feed-Rite (Winnipeg, Manitoba). Canola meal was obtained from a local crushing firm (Altona, Manitoba) while wheat (cv. Katepwa) was provided by the Glenlea Research Station, University of Manitoba.

A portion of *S. alba* seed was tempered to a 20% moisture content and was micronized at 140^{0}C by InfraReady Products Inc. A laboratory heat-treated sample of *S. alba* seed was obtained following tempering the seed to a 35% moisture content and autoclaving in a laboratory autoclave (American Sterilizer Company of Canada, Brampton, Ontario) for 20 minutes at 108^{0}C $\pm$ 1^{0}C (timing began after desired temperature was attained). The autoclaved seed was then dried in an oven at 60°C for 45 hours.

For animal experiments, the raw, micronized and autoclaved *S. alba* seed samples and the micronized soybean seeds were crushed to pass through a 2.0 mm sieve using a Wiley mill standard model No. 3 grinder (Arthur H. Thomas Company, Philadelphia, USA). For chemical analyses the full fat meals were re-ground in a conventional coffee mill. The commercial soybean and canola meal samples were ground to pass through a 1mm sieve using a Tecator Cyclotec 1093 sample mill.

Chemical Analyses

Representative samples of feedstuffs, diets and excreta were analyzed in duplicate for moisture and nitrogen contents using established standard methods of analysis (AOAC, 1990). Fat content was determined by the AOAC method recommended for animal feed (920.39) with the exception that ether was substituted with n-hexane and the extraction time was 12 hours.

Glucosinolate contents of raw, micronized and autoclaved *S. alba* seed and the commercial canola meal were analyzed by gas-liquid chromatography using the procedure of Thies (1977) as modified by Slominski and Campbell (1987). Benzyl (glucotropaeolin) and allyl (sinigrin) glucosinolates were used as the internal standards for the canola and *S. alba* samples, respectively. Myrosinase activity in samples of *S. alba* was determined by difference between total sample glucosinolate content and the glucosinolates remaining after incubation of the meal with distilled water (autolysis) at 40°C for a defined period of time. One unit of myrosinase activity was defined as the amount of enzyme that catalyses the hydrolysis of 1 μmol of glucosinolate per 1 min.

Digestible protein and water-soluble fiber contents were determined *in vitro* using the digestion/dialysis unit and the procedure described in detail by Simbaya *et al.* (1997).

Amino acid contents of the feed and excreta samples from the TME_n assay were determined on an LKB Biochrom 4151 Alphaplus amino acid analyzer (Biochrom, Science Park, Cambridge, UK) following hydrolysis of the samples with 6N hydrochloric acid at 110°C for 24 hrs (Andrews and Baldar, 1985). Methionine and cystine were analyzed following a 20 hr oxidation with performic acid (Moore, 1963). Prior to analyses, the excreta samples were ground

in a coffee mill and equilibrated to atmospheric conditions for 24 hrs.

Gross energy was determined using an adiabatic bomb calorimeter (Parr Instrument Co., Moline, IL). Diets and excreta samples from the broiler study were analyzed for chromium oxide using the procedure described by Williams *et al.* (1963).

Animal Experiments

True metabolizable energy (TME_n) and true amino acid digestibilities were determined using the assay described by Sibbald (1986) with some modifications of Zhang *et al.* (1994). Each sample was precision-fed (25g per bird) to two groups of 11 mature single comb white leghorn cockerels (45 weeks old) following a 28 hr fasting period. During the next 48 hrs, the excreta from each bird was collected. The excreta samples were then frozen, freeze-dried, ground to pass through a 1 mm sieve and pooled for analysis of gross energy, nitrogen (Kjeldahl) and amino acid contents. The excreta from two groups of unfed birds (n=30) was used to determine the endogenous excretion of energy, nitrogen and amino acids. All birds were housed in individual wire metabolic cages, were exposed to 14 hrs of light per day and had free access to fresh water throughout the experimental period. The treatments consisted of raw, autoclaved and micronized *S. alba* seed, micronized soybean seed and commercial soybean and canola meals.

The nutritive value of *Sinapis alba* seed was further evaluated in a two-week feeding trial with broiler chickens. One-day-old vaccinated (Marek's) male, Arbor Acre x Peterson broiler chickens were obtained from a local commercial hatchery. From Day 1 to Day 4, the birds were housed in thermally controlled Jamesway brooder batteries with raised wire floors. The chicks

were exposed to 24 hrs of light per day and fed a standard broiler chick starter diet (Feed-Rite) *ad libitum*. On Day 4, birds were fasted for 4 hrs before being individually weighed and placed into narrow weight classes. Groups of five birds were then assigned to pens in Petersime brooder batteries such that all pens had a similar initial weight. Each treatment was randomly assigned to 10 replicates (pens). From Day 4 to Day 18, the birds were fed experimental diets that were in a mash form. The birds had free access to water and feed and were provided with continuous light. Birds were weighed on days 1, 7 and 14, and feed consumption was recorded after 7,10 and 14 days of experiment. Prior to the 14-day weighing, the birds were fasted for 4 hrs.

The experimental diets included two control diets consisting of a commercial soybean meal/canola meal (control A) and a micronized full fat soybean meal (control B). The treatment diets contained the raw *S. alba* seed (10% inclusion rate), micronized *S. alba* seed (10% and 20% inclusion rates) and autoclaved *S. alba* seed (10% and 20% inclusion rates).

In formulation of experimental diets, the energy contents assigned to the commercial soybean and canola meals as well as the micronized soybean seed were 2520, 2140 and 3440 kcal/kg (as fed basis), respectively. All energy values were based on the preliminary TME_n measurements from this study and were similar to those reported earlier (NRC, 1994; Leeson and Summers, 1991; Canola Council of Canada, 1997; InfraReady Technical Information, 1995). In the case of *S. alba* seed, the value of 3040 kcal/kg representative of the autoclaved seed was used. This value was used to formulate all three *S. alba* diets, including those containing raw and micronized seed. Since the raw and micronized *S. alba* seeds showed lower energy contents by approximately 25 and 20%, respectively, it was assumed that the difference in energy availability would also be reflected in the broiler chicken performance and AME_n values. Composition and

calculated analyses of experimental diets are shown in Table 12.

On Day 15 of the experiment, the excreta samples were collected over a 3 hr period, pooled into 4 samples per treatment and then frozen and freeze-dried. Diets and excreta samples were analyzed for chromic oxide (Cr_2O_3; internal marker), gross energy (GE) and nitrogen (Kjeldahl) contents.

Digestibility of experimental diets and apparent metabolizable energy (AME_n) contents were calculated as follows:

Diet digestibility (%) = $[1-(Cr_2O_{3\ \%\ feed}/Cr_2O_{3\ \%\ excreta}] \times 100$

$$AME_n\ (kcal/kg) = GE_{kcal/kg\ diet} - [GE_{kcal/kg\ excreta} \times (Cr_2O_{3\ \%\ feed}/\ Cr_2O_{3\ \%\ excreta})] - 8.22 \times \{N_{kg/kg\ diet} - [N_{kg/kg\ excreta} \times (Cr_2O_{3\ \%\ feed}/Cr_2O_{3\ \%\ excreta})]\}$$

(Hill *et al.*, 1960)

On Day 15 of the experiment, 8 birds per treatment were randomly selected for intestinal viscosity measurement. The birds were killed by cervical dislocation and the abdominal cavity was exposed. The small intestine was severed at the Meckel's diverticulum and 1.5 cm prior to the ileal-cecal junction, and the contents were collected into a 10 ml test tube. The samples were centrifuged at 3000 rpm for 10 minutes. The supernatants were then decanted into a 2 ml microcentrifuge tubes and centrifuged again at 9000 rpm for 5 minutes. Viscosity was determined using a Brookfield digital viscometer (Model DV-II+, Brookfield Engineering Laboratories, Stoughton, MA).

Table 12. Composition and calculated analysis of experimental rations fed in broiler study

	RATION						
Ingredient (%)	1	2	3	4	5	6	7
Soybean meal, conventional	14.8	7.3	12.0	12.0	8.9	12.0	8.9
Canola meal, conventional	15.0	7.4	11.5	11.5	8.6	11.5	8.6
Soybean meal, micronized	-	20.0	-	-	-	-	-
S. alba, raw	-	-	10.0	-	-	-	-
S. alba, micronized	-	-	-	10.0	20.0	-	-
S. alba, autoclaved	-	-	-	-	-	10.0	20.0
Wheat	60.24	58.42	57.41	57.41	54.19	57.41	54.19
Vegetable oil	5.2	2.1	4.4	4.4	3.7	4.4	3.7
Limestone	1.49	1.55	1.43	1.43	1.36	1.43	1.36
Biophos	1.34	1.35	1.34	1.34	1.35	1.34	1.35
Vitamin premix[1]	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Mineral premix[2]	0.5	0.5	0.5	0.5	0.5	0.5	0.5
DL-methionine	0.07	0.08	0.07	0.07	0.06	0.07	0.06
Lysine	0.06	-	0.05	0.05	0.04	0.05	0.04
Cr_2O_3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Total	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**
Calculated composition							
Protein (N x 6.25), %	22.0	22.0	22.0	22.0	22.0	22.0	22.0
ME_n, kcal/kg	3069	3062	3065	3065	3063	3065	3063
Available P, %	0.45	0.45	0.45	0.45	0.45	0.45	0.45
Calcium, %	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Lysine, %	1.06	1.06	1.06	1.06	1.06	1.06	1.06
Methionine, %	0.48	0.48	0.48	0.48	0.48	0.48	0.48
Meth. + Cys., %	0.94	0.93	0.94	0.94	0.93	0.94	0.93

[1] Supplied vitamins (per kg diet): A, 8250 IU; D_3, 1000 IU; E, 10.9 IU; B_{12}, 0.0115mg; K, 1.1mg; niacin, 53.3mg; choline, 1019.9mg; folic acid, 0.75mg; biotin, 0.25mg; riboflavin, 5.5mg; [2] Supplied minerals (mg/kg diet): manganese, 55; zinc, 50; iron, 80; copper, 5; selenium, 0.1; calcium iodate, 0.08; iodized salt, 0.28

Statistical Analysis

All collected data were subjected to analysis of variance (ANOVA) using the general linear models of statistical analysis system (SAS Institute, 1990) and the procedures of Snedecor and Cochran (1980). Tukey's procedure was used to compare and separate treatment means. The model for digestible protein data was a completely randomized design with two replicates of six treatments. The model for the TME_n was a completely randomized design with two replicates of eleven birds for each of six treatments. Bird was the experimental unit. The model for the broiler performance trial was a completely randomized design with ten pens of five birds in each of seven treatments. Pen was the experimental unit. Intestinal viscosity statistics were performed in a completely randomized design with eight birds in each of seven treatments. Bird was the experimental unit.

RESULTS AND DISCUSSION

The protein and oil contents of the *S. alba* seed from 1995 and 1996 crop year as compared to condiment variety and micronized soybean seed are shown in Table 13. The 1996 crop had the same protein and oil contents as the condiment variety, while the 1995 crop was higher in protein and lower in oil contents. This difference may be attributed to the normal year-to-year variation in growing conditions. Factors such as water stress, temperature or soil conditions may significantly affect the oil or protein contents. It has been indicated that the chemical composition of the 1996 *S. alba* crop better reflects the genetic background of the low-glucosinolate line than the 1995 crop (Rakow, personal communication). In this context, the wet

Table 13. Protein and oil contents of full fat *Sinapis alba* (non-heat treated) from the 1995 and 1996 crop years and full fat micronized soybean (% DM)

	Condiment[1]	1995 crop year	1996 crop year	micronized soybean
Protein	31.2	38.3	31.3	34.1
Oil	31.5	26.4	31.0	21.2
Total	62.7	64.7	62.3	56.3

[1] DeClercq, D.R. and Daun, J.K. (1997)

growing conditions are always referred to as a "good oil year" while the dry conditions often favour higher accumulation of protein at the expense of the oil content. It should be emphasized that the sample used in the current study was from the 1996 crop year and, with regard to the protein to oil ratio, was different from the 1995 *S. alba* crop (Table 13).

The digestible protein contents of raw, micronized and autoclaved *S. alba*, micronized soybean, conventional canola meal and the conventional soybean meal are shown in Table 14. Both micronized and autoclaved *S. alba* samples showed higher protein digestibility values than the raw seed. This is in agreement with earlier research in this laboratory on digestible protein content in the canola seed subjected to various heat treatment conditions (Simbaya *et al.*, 1997). The micronized *S. alba* showed improved protein digestibility as compared to the sample treated under the same conditions in earlier experiments (see manuscript 1 of this thesis). This indicates that the processing conditions used to micronize the current *S. alba* sample were somewhat different and closer to optimal. The autoclaved *S. alba* seed showed higher protein digestibility value than the micronized sample. The micronized soybean seed, however, had digestible protein content similar to micronized and autoclaved *S. alba* samples.

Table 14 indicates the glucosinolate content and myrosinase activity of raw, micronized and autoclaved *S. alba*. No myrosinase enzyme activity was detected in the heat treated samples regardless of the process employed. Similarly to the difference in digestible protein content, the current micronization process appeared to be more effective in enzyme inactivation than that used in the earlier research (see manuscript 1). While myrosinase activity was at the level of 1.0 unit/g

Table 14. Digestible protein, myrosinase activity and glucosinolate content of raw, micronized and autoclaved *S. alba*, micronized soybean and commercial canola and soybean meals

Feedstuff	Digestible Protein (%)	Myrosinase Activity (units[1]/g oil free seed)	Glucosinolate Content (μmol/g oil free meal)
Raw *S. alba* seed	54.9[c]	128.4	35.0
Micronized *S. alba* seed	69.3[b]	ND[2]	25.0
Autoclaved *S. alba* seed	73.7[a]	ND	14.5
Micronized soybean seed	71.9[ab]	-	-
Commercial canola meal	69.2[b]	ND	-
Commercial soybean meal	70.6[b]	-	-

[1] One unit of activity is defined as the amount of enzyme that catalyses the hydrolysis of 1 μmol of glucosinolate per 1 minute; [2] Not detected (activity below 0.05 U)

[a,b,c] Values with no common superscripts differ significantly ($P \leq 0.05$)

in the oil free seed micronized at 140°C (20% moisture) in the first manuscript, in the current study no myrosinase activity was detected in the seed micronized in the same manner. This difference may be attributed to variation in processing conditions.

The effect of the heat treatment on glucosinolate content in variously treated *S. alba* seed is shown in Table 14. The glucosinolate content of raw *S. alba* corresponded well with that detected in the 1995 crop (see manuscript 1). The autoclaved *S. alba* seed showed the highest reduction in glucosinolate content. This could be attributed to the tempering process since high (ie, 35%) moisture content would improve heat conductivity within the seed and thus facilitate glucosinolate decomposition.

The TME_n values of variously treated *S. alba* seed as compared to micronized soybean and commercial canola and soybean meals are shown in Table 15. As expected, the commercial canola and soybean meals differed significantly in energy content. The micronized soybean seed had the highest TME_n content. Among the *S. alba* samples, the autoclaved seed had a TME_n value significantly higher than both the raw and micronized *S. alba* samples while the micronized sample had a significantly higher TME_n value than the raw *S. alba* seed. Sklan *et al.* (1973) indicated that heat treatment may influence energy digestion and reported lower absorption of fatty acids from raw soybean meal as compared to heat treated meal. The same group of researchers (Sklan *et al.*, 1975) concluded that raw soybean meal interfered with the absorption of fatty acids and bile acids in the lower jejunum and ileum. The mechanism of this interference is not known. It would appear that the type of heat applied significantly influenced the availability of energy from the *S. alba* seed. The higher TME_n value of the autoclaved *S. alba* seed as compared to the raw and micronized *S. alba* seeds may be partially attributed to a more effective heat treatment,

Table 15. True metabolizable energy values of variously treated *Sinapis alba* seed in comparison to other feedstuffs

Feedstuff	TME_n (kcal/kg DM)
Raw *S. alba* seed	2270.2
Micronized *S. alba* seed	2460.3[d]
Autoclaved *S. alba* seed	3031.4[b]
Micronized soybean seed	3790.4[a]
Commercial canola meal	2624.9[c]
Commercial soybean meal	2949.3[b]

[a,b,c,d,e] Values with no common superscripts differ significantly ($P \leq 0.05$)

due to the tempering process applied prior to micronization.

In the current study, the TME_n for micronized full-fat soybean was higher than that for the autoclaved *S. alba*, although the *S. alba* sample contained more oil (Table 13). The soluble fibre content of the *S. alba* seed may contribute to the difference in the TME_n values between the *S. alba* and soybean seed. It has been noted that fat digestion is particularly susceptible to increased digesta viscosity associated with feeding rye (Campbell *et al.*, 1983). As *S. alba* seed has a higher soluble fibre content than (1.7 vs 0.5%; see manuscript 1) and, thus, a greater potential for intestinal viscosity, it is possible that fat digestion was hindered with the feeding of the *S. alba* seed, resulting in a lower TME_n value. The higher oil content of *S. alba* would only intensify this effect.

Research has shown that, generally, birds are able to physiologically adapt to changes in fat level in the diet. Hulan and Bird (1972) indicated that switching from a low fat to a high fat diet caused a slow, gradual increase in specific lipase activity. Bucko and Kopec (1979) found similar results. Lindsay *et al.* (1969) noted an increase in bile salt concentration after an inclusion of 15% corn or herring oil in the diet. It was shown by Mateos and Sell (1981a, b) and Mateos *et al.* (1982) that passage rate decreased as fat content in the diet increased. While the birds involved in the TME_n assay may have been able to physiologically adapt to the high oil level supplied by the micronized soybean, they may not have been able to adjust to the additional 10% of oil in the *S. alba* seed. This, however, does not explain the difference between the variously treated *S. alba* samples as all three samples contained the same amount of oil.

It would appear that the problem encountered with the *S. alba* samples and TME_n assay was related to the grinding process and the degree of cell wall disruption. InfraReady Technical

Information (1995) states that the disruption of cell walls during flaking significantly improves nutrient digestibility of both oil and protein in soybeans. It is possible that with no heat or moderate heat (ie., micronized sample) the cell walls withstood the physical force of grinding to a much greater extent than the autoclaved material. Since the micronized soybean seed was ground under identical conditions, the cell size within the cotyledons fraction of *S. alba* may have also been a factor influencing the effectiveness of sample grinding. In this context, earlier research from this laboratory has shown substantial difference in the cotyledon cell size within the *Brassica* species, *B. rapa* canola having much smaller cells than its *B. napus* counterpart (Slominski, 1997). Incomplete cell opening on grinding of *S. alba* seed was also reflected in the results of true amino acid digestibility (Table 16). The micronized full fat soybean and commercial soybean and canola meals all had similar availabilities of all amino acids, with the exception of cystine which was significantly higher in the commercial soybean meal than in the micronized soybean seed. Equal availability of amino acids from these three feedstuffs corresponded well with the *in vitro* protein digestibility figures reported in Table 14. However, the difference in *in vitro* protein digestibility between the raw and heat treated samples of *S. alba* was not reflected in the amino acid digestibility values determined *in vivo* using the TME_n assay (Table 16). In general, all three samples of *S. alba* seed showed significantly lower amino acid digestibility than the commercial meals or micronized soybean seed. Similarly to differences in available energy content between the *S. alba* samples, the autoclaved seed showed the highest amino acid digestibility. While alanine, arginine, glycine, histidine, isoleucine, lysine, methionine, proline and serine availability in autoclaved *S. alba* was equal to that of the

Table 16. True amino acid availability (%) of feedstuffs using the TME_n assay

	raw *S. alba*	micronized *S. alba*	autoclaved *S. alba*	micronized soybean	commercial soybean meal	commercial canola meal
alanine	71.05^{b}	73.49^{b}	81.72^{ab}	83.76^{ab}	89.12^{a}	88.92^{a}
arginine[1]	74.98^{a}	71.51^{a}	80.62^{a}	84.42^{a}	86.12^{a}	87.75^{a}
aspartic acid	65.94^{c}	64.88^{a}	73.41^{b}	83.72^{a}	89.94^{a}	84.08^{a}
cystine	62.90^{cd}	59.33^{d}	65.58^{c}	73.62^{b}	80.96^{a}	79.15^{ab}
glutamic acid	73.09^{c}	70.78^{c}	79.80^{b}	86.86^{a}	92.10^{a}	91.78^{a}
glycine	68.82^{bc}	67.25^{c}	78.29^{abc}	81.54^{ab}	85.17^{a}	85.54^{a}
histidine	60.15^{c}	58.57^{c}	67.63^{bc}	82.96^{ab}	88.97^{a}	88.18^{a}
isoleucine	64.64^{c}	59.20^{c}	71.50^{bc}	82.43^{ab}	87.86^{a}	83.98^{ab}
leucine	70.19^{bc}	66.90^{c}	76.01^{b}	83.65^{a}	89.39^{a}	88.28^{a}
lysine	65.72^{bc}	58.06^{c}	69.49^{abc}	81.87^{ab}	85.79^{a}	83.09^{ab}
methionine	68.08^{c}	67.38^{c}	75.29^{bc}	80.38^{ab}	86.40^{ab}	88.79^{a}
phenylalanine	66.59^{bc}	62.17^{c}	73.53^{b}	83.87^{a}	90.85^{a}	87.43^{a}
proline	69.45^{b}	63.78^{b}	71.44^{b}	84.60^{a}	88.87^{a}	82.72^{a}
serine	65.74^{c}	63.31^{c}	72.44^{c}	85.19^{ab}	89.42^{a}	84.37^{b}
threonine	62.05^{bc}	59.29^{c}	68.81^{b}	82.58^{a}	84.74^{a}	82.37^{a}
tyrosine	67.00^{bc}	63.85^{c}	74.59^{b}	86.11^{a}	90.40^{a}	88.54^{a}
valine	64.77^{cd}	56.35^{d}	70.56^{bc}	80.17^{ab}	84.43^{a}	83.41^{ab}
Total	68.40^{c}	65.41^{c}	74.93^{b}	83.87^{a}	88.77^{a}	86.65^{a}

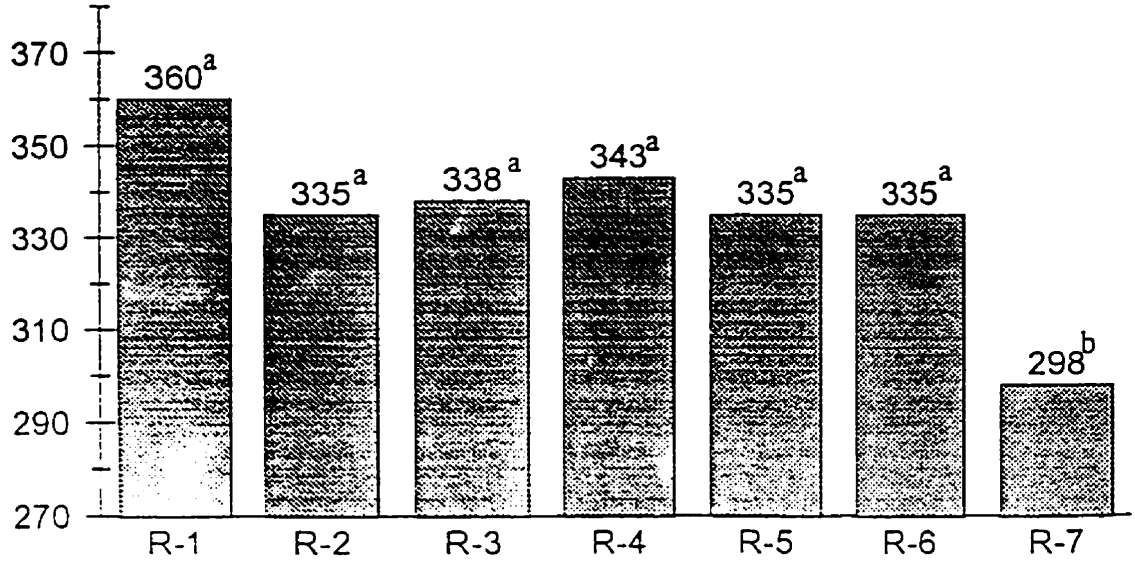
[1] No significant differences were noted using Tukey's Standardized Range, however, the F-test found a significant difference among the means ($P \leq 0.05$)

^{a,b,c,d} Values within rows with no common superscripts differ significantly ($P \leq 0.05$)

micronized and raw *S. alba*, the availability of cystine, leucine, phenylalanine, threonine, tyrosine and valine was significantly higher in the autoclaved *S. alba* seed. As well, aspartic acid and glutamic acid were more available in the autoclaved *S. alba* than in both raw and micronized *S. alba* seed.

The results of two-week performance of broiler chickens fed micronized full fat soybean and variously treated *S. alba* seeds are presented in Figures 4, 5, and 6. There were no significant differences in the weight gain and feed intake of broiler chickens fed the control soybean/canola meal-based diet (R-1), micronized soybean seed at a 20% inclusion rate (R-2), raw *S. alba* seed at 10% inclusion rate (R-3), micronized *S. alba* seed at 10 (R-4) and 20% (R-5) inclusion rate and autoclaved *S. alba* at a 10% inclusion rate (R-6) (Fig. 4 and 5). There was, however, a significant reduction in the feed intake and body weight gain of chickens fed the diet containing 20% of the autoclaved *S. alba* seed (R-7). A similar pattern was followed by the feed to gain ratio, with the autoclaved *S. alba* (R-7) showing the lowest feed efficiency (Fig. 6). It would appear that birds fed the raw, micronized and autoclaved *S. alba* diets at a 10% inclusion rate were not affected by any potentially antinutritive substances present in the seed (ie., glucosinolates, soluble fibre), as feed intake and weight gain were equal to those fed a control diet or a micronized full fat soybean diet. In addition, there was no statistical difference in feed efficiency between the three *S. alba* diets. As opposed to adult roosters in the TME_n assay, it would appear that the chickens utilized a similar amount of energy from the *S. alba* diets regardless of the treatment applied, as performance was similar. This could be further substantiated by a relatively similar AME_n values for the diets containing raw, micronized and

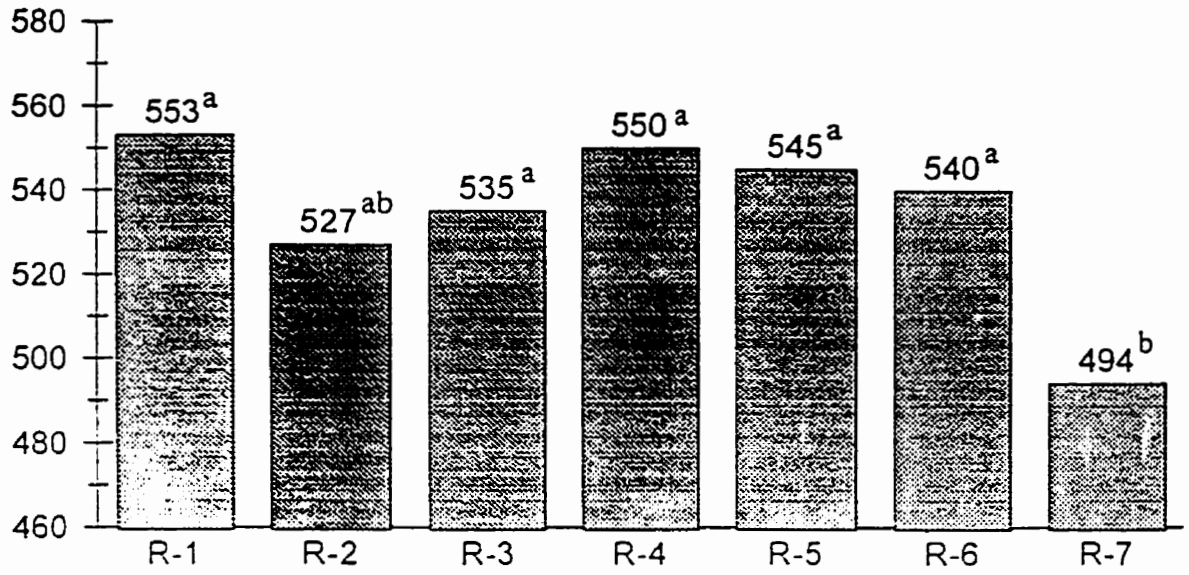
Figure 4. Body weight gain of broiler chickens fed various diets from days 4-18



R-1: Conventional canola meal/soybean meal (control A)
R-2: Micronized full fat soybean (20% inclusion rate)
R-3: Raw *S. alba* seed (10% inclusion rate)
R-4: *S. alba* seed tempered to 20% moisture level and micronized at 140°C (10% inclusion rate)
R-5: *S. alba* seed processed as above (20% inclusion rate)
R-6: *S. alba* seed tempered to 35% moisture content and autoclaved for 20 minutes at 108°C (10% inclusion rate)
R-7: *S. alba* seed processed as above (20% inclusion rate)

[a,b] Values with no common superscripts differ significantly ($P \leq 0.05$)

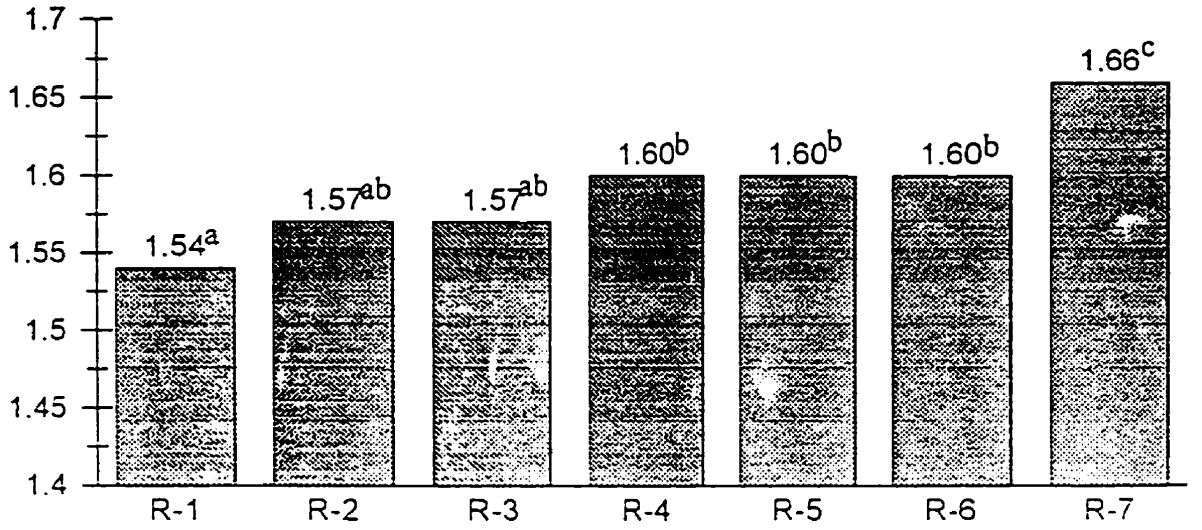
Figure 5. Feed intake of broiler chickens fed various diets from days 4-18



R-1: Conventional canola meal/soybean meal (control A)
R-2: Micronized full fat soybean (20% inclusion rate)
R-3: Raw *S. alba* seed (10% inclusion rate)
R-4: *S. alba* seed tempered to 20% moisture level and micronized at 140°C (10% inclusion rate)
R-5: *S. alba* seed processed as above (20% inclusion rate)
R-6: *S. alba* seed tempered to 35% moisture content and autoclaved for 20 minutes at 108°C (10% inclusion rate)
R-7: *S. alba* seed processed as above (20% inclusion rate)

[a,b] Values with no common superscripts differ significantly ($P \leq 0.05$)

Figure 6. Feed efficiency of broiler chickens fed various diets from days 4-18



R-1: Conventional canola meal/soybean meal (control A)
R-2: Micronized full fat soybean (20% inclusion rate)
R-3: Raw *S. alba* seed (10% inclusion rate)
R-4: *S. alba* seed tempered to 20% moisture level and micronized at 140°C (10% inclusion rate)
R-5: *S. alba* seed processed as above (20% inclusion rate)
R-6: *S. alba* seed tempered to 35% moisture content and autoclaved for 20 minutes at 108°C (10% inclusion rate)
R-7: *S. alba* seed processed as above (20% inclusion rate)

[a,b,c] Values with no common superscripts differ significantly ($P \leq 0.05$)

Table 17. Intestinal viscosity, digestibility and apparent metabolizable energy of various diets fed to broiler chickens from days 4-18

Treatment	Diet Digestibility (%)	Intestinal Viscosity (cPs)	AME_n (kcal/kg diet)
Canola/soybean meal control	$64.9^{b} \pm 1.4$	$6.8^{c} \pm 2.2$	$2816^{b} \pm 65.9$
Micronized soybean seed (20%)	$67.7^{a} \pm 1.3$	$12.3^{bc} \pm 3.9$	$2979^{a} \pm 60.5$
Raw *S. alba* seed (10%)	$64.8^{b} \pm 1.7$	$19.5^{b} \pm 5.2$	$2830^{b} \pm 64.7$
Micronized *S. alba* seed (10%)	$63.5^{bc} \pm 1.2$	$17.5^{b} \pm 5.2$	$2779^{b} \pm 61.6$
Micronized *S. alba* seed (20%)	$59.4^{d} \pm 0.5$	$29.3^{a} \pm 10.3$	$2574^{c} \pm 34.3$
Autoclaved *S. alba* seed (10%)	$62.1^{bcd} \pm 1.4$	$11.3^{bc} \pm 1.7$	$2771^{b} \pm 46.2$
Autoclaved *S. alba* seed (20%)	$61.0^{cd} \pm 0.5$	$19.9^{b} \pm 7.3$	$2713^{b} \pm 23.2$

[a,b,c] Values within columns with no common superscripts differ significantly ($P \leq 0.05$)

autoclaved *S. alba* seed at a 10% inclusion rate (Table 17). Therefore, a relatively low available energy content as determined by the TME_n assay may have resulted from an ineffective crushing of the seed as well as insufficient cell fracture in the gizzard. In this regard, *ad libitum* feeding of the broiler chickens probably resulted in improved motion of the gizzard although part of the oil encapsulated within the cells could have escaped digestion in the small intestine.

It is uncertain why the autoclaved *S. alba* seed at a 20% inclusion rate showed substantial reduction in broiler chicken performance since the micronized sample at the same inclusion rate was equal to other dietary treatments. With regard to certain quality parameters, including protein digestibility *in vitro* or TME_n value, the autoclaved seed appeared to be similar or better than the micronized sample. There was also no indication of any negative effect of water-soluble mucilage on nutrient utilization as the intestinal viscosity for this particular treatment was lower than that of the micronized seed when used at the same inclusion rate (Table 18). However, the micronized seed at a 20% inclusion rate showed a significant reduction in the AME_n value which correlated well with the intestinal viscosity measurement. Direct negative relationship between digesta viscosity and AME_n values has been documented in many research reports (Kritchevsky, 1988; Classen and Bedford, 1991; Campbell and Bedford, 1992; Choct ,1992). It would appear that feeding the micronized sample at a 20% inclusion rate for a prolonged period of time would have resulted in impaired broiler chicken performance. One possible explanation for the difference between autoclaved and micronized seed could be derived from the glucosinolate composition data (Table 14). In this context, the glucosinolates in the autoclaved meal were decomposed to much greater extent than those in the micronized sample. Consequently, the production of various breakdown products may have affected the overall nutritive value of the seed. The breakdown

products, however, would have to be of different nature than those produced on autolysis as the raw *S. alba* diet contained an extremely high activity of myrosinase enzyme.

It appears from the data reported here that the heat treatment of *S. alba* seed did not have an effect on the bird performance, AME_n, diet digestibility or digesta viscosity. It did, however, improve the protein digestibility *in vitro* and lowered myrosinase activity and glucosinolate content. Heat treatment also improved the TME_n value of this feedstuff.

GENERAL DISCUSSION

Detailed chemical evaluation of the canola type *Sinapis alba* seed (Table 5) revealed several positive quality characteristics. In comparison to soybean, the *S. alba* seed contained more oil but less protein than soybean, with the content of the two major nutrients in favour of the *S. alba* sample. Digestible protein content in defatted and heat-treated *S. all.a* seed was equal to or higher than values for the other feedstuffs evaluated. *S. alba* seed contained slightly more methionine and cystine but less lysine than soybean. The sucrose content was lower in *S. alba* than in soybean although this was offset by the presence of starch which was not detected in soybean. Although there was a large difference in seed size between *S. alba* and soybean, only a small difference in the total dietary fibre content was observed. A lower content of oligosaccharides and higher contents of calcium and available (non-phytate) phosphorus were among other positive characteristics associated with the *S. alba* crop.

The seed sample from a 1996 crop year, similarly to the condiment variety, had lower protein and higher oil contents than the 1995 seed (Table 13). This difference may be attributed to normal year-to-year variation in growing conditions. It has been recognized that the 1996 *S. alba* crop better reflected the genetic background of the low glucosinolate line with regard to oil and protein content (Rakow, personal communication).

The glucosinolate content of the seed from 1995 and 1996 crop years corresponded very well with one another and in both cases the heat treatment of the seed resulted in a decrease in glucosinolate content. However, tempering of the 1996 seed prior to autoclaving lowered the glucosinolate content to a greater extent than that seen with the 1995 crop heat treated without

moisture added.

From the chemical composition data it was apparent that the *S. alba* seed had a relatively high soluble fibre content (Table 5). As high soluble fibre levels may negatively affect weight gain, feed conversion and AME by increasing intestinal viscosity (Kritchevsky, 1988; Campbell and Bedford, 1992; Choct, 1992), there was some concern regarding any antinutritive effect of soluble fibre on broiler chicken performance. The potential existed for glucosinolates to be a limiting factor in the *in vivo* studies as well. While the chemical composition results indicated that *S. alba* would be a good candidate as a high protein, high energy supplement for monogastric animals, bird performance in the TME_n assay and broiler chicken trial did not reflect this. From the chemical evaluation, it was thought that the *S. alba* seed would be a suitable alternative to full fat soybean. This theory was not fully supported by the *in vivo* trials. Although *S. alba* seed contained higher levels of oil than the soybean seed, the available energy value was lower for the *S. alba* seed than the soybean seed, regardless of treatment. As well, available amino acid values were lower for the *S. alba* than for the soybean. Birds fed the diets containing raw, micronized and autoclaved *S. alba* at a 10% inclusion rate were not affected by any potentially antinutritive substances present in the seed, as weight gain and feed intake were equal to those fed a soybean/canola meal control or a micronized full fat soybean diet (20% inclusion rate) (Figs. 4 and 5). There was however some indication of a negative response of broiler chickens to higher inclusion rate (20%) of the *S. alba* seed.

Small cell size within the cotyledon fraction of the *S. alba* seed, as well as heat application, may have influenced the effectiveness of sample grinding, as was evidenced by the results of the TME_n assay (Table 15). Incomplete cell opening on grinding of the seed was also

reflected in the results of the true amino acid availability (Table 17). The supposition that *S. alba* has small cotyledon cell size is supported by the chemical composition data. Previous research on *B. napus* and *B. rapa* canola (Slominski *et al.*, 1994a; Simbaya *et al.*, 1995; Slominski, 1997) has indicated that factors such as the colour of the seed or the embryo/cotyledon cell size may significantly affect the level and nature of dietary fibre. Since *S. alba* has a large yellow seed, small cotyledon cell size would appear to be the factor contributing to its relatively high fibre content (Table 5). The NSP content of *S. alba* as compared to soybean and canola further substantiates this. The yellow-seeded *B. rapa* canola, although having less lignin and polyphenols in the hull fraction, was found to contain significantly more NSP than its *B. napus* counterpart (Slominski *et al.*, 1994a).

The TME_n results were not fully reflected in the broiler study (Tables 15 and 18, respectively). As opposed to the adult roosters, it appeared that the chickens utilized a similar amount of energy from the *S. alba* diets regardless of the heat treatment applied. A relatively low available energy content as determined by the TME_n assay may have resulted from an ineffective crushing of the seed on grinding, as well as insufficient cell fracture in the gizzard. In this regard, *ad libitum* feeding of the broiler chickens may have resulted in an improved motion of the gizzard although part of the oil encapsulated within the cells could have still escaped digestion in the small intestine.

CONCLUSIONS

1. Chemical composition of the canola type *Sinapis alba* white mustard indicates that it holds some potential as a high protein and high energy feedstuff for the Canadian Prairies. Growing conditions, however, may significantly affect the chemical composition with the wet conditions resulting in less desirable protein to oil ratio in the seed.
2. Heat treatment of the *S. alba* seed improved protein digestibility, inactivated myrosinase enzyme and decreased glucosinolate content. Autoclaving of the seed improved availability of energy (TME_n) and amino acids to a greater extent than the micronization process.
3. In comparison to the raw seed, no improvement in bird performance was observed when heat treated *S. alba* sample was fed at a 10% inclusion rate. However, there is a potential for some negative responses of broiler chickens to higher dietary inclusion rates.
4. Cotyledon cell size and grinding of the seed appear critical in *in vivo* evaluation of this new product.

SUGGESTIONS FOR FUTURE RESEARCH

1. Further optimize the micronization process and investigate the effect of heat on seed and cell rupture and energy and amino acids availabilities.
2. Explore the potential for improved energy utilization using the mucilage depolymerizing enzymes.
3. Further decrease the glucosinolate content through plant selection programs.

REFERENCES

Andrews, R.P. and Baldar, N.A. 1985. Amino acid analysis of feed constituents. Science Tools. 32: 44-48.

Appelqvist, L.A. 1972. Chemical constituents of rapeseed. In: Rapeseed: cultivation, composition, processing and utilization. Appelqvist, L.A. and Ohlson, R. (editors). Elsevier Publishing Company. Amsterdam. pp 123-173.

Association of Official Analytical Chemists. 1990. Official methods of analysis. Helrich, K. (editor). AOAC Inc. Arlington, VA, USA.

Bach Knudsen, K.E. 1997. Carbohydrate and lignin contents of plant materials used in animal feeding. Anim. Feed Sci. Technol. 67: 319-338.

Bailey, K. and Norris, F.W. 1932. The nature and composition of the seed of white mustard (*Brassica alba*). Biochem. J. 26: 1609-1623.

Beare, J.L., Campbell, J.A., Young, C.G and Craig, B.M. 1963. Effects of saturated fat in rats fed rapeseed oil. Can. J. Biochem. Physiol. 41: 605-612.

Bell, J.M. 1984. Nutrients and toxicants in rapeseed meal: a review. J. Anim. Sci. 58(4): 996-1010.

Bell, J.M., Benjamin, B.R. and Giovannetti, P.M. 1972. Histopathology of thyroids and livers of rats and mice fed diets containing *Brassica* glucosinolates. Can. J. Anim. Sci. 52: 395-406.

Bell, J.M. and Rakow, G. 1996. Trypsin inhibitors and phytic acid in oil-extracted meals from seeds of several *Brassica* species and of *Sinapis alba*. Can. J. Anim. Sci. 76: 423-425.

Belzile, R., Bell, J.M. and Wetter, L.R. 1963. Growth depressing factors in rapeseed oilmeal. V. Effects of myrosinase activity on the toxicity of the meal. Can. J Anim. Sci. 43: 169-173.

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

Bucko, A. and Kopec, L. 1979. The adaptation of pancreas to the nature of foods. Cah. Nutr. Diet. 14: 71-76.

Campbell, G.L., Classen, H.L. and Goldsmith, K.A. 1983. Effect of fat retention on the rachitogenic effect of rye fed to broiler chicks. Poult. Sci. 62: 2218-2223.

Campbell, L.D. and Bedford, M.R. 1992. Enzyme applications for monogastric feeds: a review. Can. J. Anim. Sci. 72: 449-466.

Campbell, L.D. and Slominski, B.A. 1990. Extent of thermal decomposition of indole glucosinolates during the processing of canola seed. JAOCS. 67(2): 73-75.

Campbell, L.D. and Slominski, B.A. 1991. Nutritive quality of low-glucosinolate canola meal for laying hens. Proceedings of the 8th International Rapeseed Congress. Saskatoon, Saskatchewan. pp 442-447.

Canola Council of Canada. 1989. Canola meal for livestock and poultry. Clandinin, D.R. (editor). Canola Council of Canada. Winnipeg, Manitoba.

Canola Council of Canada. 1997. Canola meal feed industry guide. Hickling, D. (editor). Canola Council of Canada. Winnipeg, Manitoba.

Carré B. and Brillouet, J-M. 1986. Yield and composition of cell wall residues isolated from various feedstuffs used for non-ruminant farm animals. J. Sci. Food Agric. 37: 341-351.

Cheryan, M. 1980. Phytic acid interactions in food systems. CRC Crit. Rev. food Sci. Nutr. 13: 297-335.

Choct, M. 1992. Relationship between soluble arabinoxylan and the nutritive value of wheat for broiler chickens. Proc. 13th Western Nutrition Conference. Saskatoon, Saskatchewan.

Clandinin, D.R. and Robblee, A.R. 1981. Rapeseed meal in animal nutrition. I. Non-ruminants. J. Am. Oil Chem. Soc. 58: 682.

Classen, H.L. and Bedford, M.R. 1991. The use of enzymes to improve the nutritive value of poultry feeds. Recent Adv. Anim. Nutr. 95: 116.

Colmans. 1985. The story of mustard and how it is milled. Colmans of Norwich. Norwich, UK.

Coon, C.N., Leske, K.L., Akakvanichan, O. and Cheng, T.K. 1990. Effect of oligosaccharide-free soybean meal on true metabolizable energy and fibre digestion in adult roosters. Poult. Sci. 69: 787-793.

Crisp, P. 1976. Trends in the breeding and cultivation of cruciferous crops. In: The biology and chemistry of the cruciferae. Vaughan, J.G., MacLeod, A.J. and Jones, B.M.G. (editors). Academic Press. London. pp 69.

Cromwell, G.L. 1991. Phytase appears to reduce phytate phosphorus in feed and manure. Feedstuffs. October 7th. pp 14-16.

Cui, W., Eskin, A.M. and Biliaderis, C.G. 1993. Chemical and physical properties of yellow mustard (*Sinapis alba* L.) mucilage. Food Chem. 46: 169-176.

DeClercq, D.R. and Daun, J.K. 1997. Report on the quality of 1996 western Canadian mustard. Canadian Grain Commission. Winnipeg, Manitoba.

Downey, R.K. 1983. The origin and description of *Brassica* oilseed crops. In: High and low erucic acid rapeseed oils: production, usage, chemistry and toxicological evaluation. Kramer, T.K.G., Sauer, F.D. and Pigden, W.J. (editors). Academic Press. New York. pp 1-20.

Elwinger, K. 1986. Continued experiments with rapeseed meal of a Swedish low glucosinolate type fed to poultry. II. An experiment with laying hens. Swed. J. Agric. Res. 16: 35.

Englyst, H. and Cummings, J.H. 1984. Simplified method for the measurement of total non-starch polysaccharides by gas-liquid chromatography of constituent sugars as alditol acetates. Analyst. 109: 937-942.

Englyst, H. and Cummings, J.H. 1988. Improved method for the measurement of dietary fibre as non-starch polysaccharides in plant foods. JAOAC. 71: 808-814.

Fenwick, G.R. and Curtis, R.F. 1980. Rapeseed meal and its use in poultry diets: a review. Anim. Feed Sci. Tech. 5: 255-298.

Fenwick, G.R., Heaney, R.K. and Mullin, W.J. 1983. Glucosinolates and their breakdown products in food and food plants. CRC Crit. Rev. Food Sci. Nutr. 18(2):123-201.

Fenwick, G.R., Hobson-Frohock, A., Land, D.G. and Curtis, R.F. 1979. Treatment of rapeseed meal to reduce tainting potential. Br. Poult. Sci. 20: 323-329.

Fenwick, G.R., Spinks, E.A., Wilkinson, A.P., Heaney, R.K. and Legoy, M.A. 1986. Effect of processing on the antinutrient content of rapeseed. J. Sci. Food Agric. 37(8): 735-741.

Field Crop Reporting Series No.8. 1996. Statistics Canada.

Friedman, M. 1996. Food browning and its prevention: an overview. J. Agric. Food Chem. 44 (3): 631-653

Goering, H.K. and VanSoest, P.J. 1970. Forage fibre analyses (apparatus, reagents, procedures and some applications). Agric. Handbook No. 379. USDA. Washington, DC.

Grami, B. and Stefansson, B.R. 1977. Gene action for protein and oil content in summer rape. Can. J. Plant Sci. 57:625-631.

Graham, H. and Åman, P. 1991. Nutritional aspects of dietary fibres. Anim. Feed Sci. Tech. 32: 191-158.

Green, T.R. and Ryan, C.A. 1972. Wound-induced proteinase inhibitor in plant leaves: a possible defence mechanism against insects. Science. 175(4023): 776-777.

Greer, M..A. 1962. The natural occurrence of goitrogenic agents. Recent Prog. Horm. Res. 18:187.

Greer, M.A. and Deeney, J.M. 1959. Antithyroid activity elicited by the ingestion of pure progoitrin, a naturally occurring thioglucoside of the turnip family. J. Clin. Invest. 38(2): 1465-1474.

Hang, W. And Lantzsch, H.J. 1983. Sensitive method for the rapid determination of phytate cereals and cereal products. J. Sci. Food Agric. 34: 1423-1426.

Hemingway, J.S. 1976. Mustards. In: Evolution of crops and plants. Simmonds, N.W. (editor). Longman. London. pp 56-59.

Hemingway, J.S. 1995. The mustard species: condiment and food ingredient use and potential as oilseed crops. In: *Brassica* oilseeds: production and utilization. Kimber, D.S. and McGregor, D.I. (editors). CAB International. Wallingford, UK. pp 373-383.

Herkelman, K.L., Cromwell, G.L., Cantor, A.H., Stahly, T.S. and Pfeiffer, T.W. 1993. Effects of heat treatment on the nutritional value of conventional and low trypsin soybean for chickens. Poult. Sci. 72: 1359-1369.

Hill, D.C. 1976. Rapeseed Association of Canada. Publication No. 40. pp 53.

Hill, F.W., Anderson, D.L., Renner, R. and Carew, L.B.Jr. 1960. Studies on the metabolizable energy of grain and grain products for chickens. Poult. Sci. 39(3): 573-579.

Hill, R. 1979. A review of the 'toxic' effects of rapeseed meals with observations on meal from improved varieties. Brit. Vet. J. 135: 1-16.

Holmes, W.B. and Roberts, R. 1963. A perotic syndrome in chicks fed extracted rapeseed meal. Poult. Sci. 42: 803.

Hulan, H.W. and Bird, F.H. 1972. Effect of fat level in isonitrogenous diets on the composition of avian pancreatic juice. J. Nutr. 102: 459-468.

Hulan, H.W., Thompson, B., Kramer, J.K.G., Sauer, F.D. and Corner, A.H. 1977. An evaluation of the incidence of heart lesions in rats fed rapeseed oil. Can. Inst. Food Sci. Technol.

J. 10: 23-26.

Hutton, K. and Foxcroft, P.D. 1975. Effects of processing temperature on some indices of nutritional significance for micronized soyabeans. Proc. Nutr. Soc. 34: 49A.

Ibrahim, I. 1978. Ph.D thesis. University of London.

Ibrahim, I.K., Hodges, R.J.C. and Hill, R. 1980. Haemorrhagic liver syndrome in laying fowls fed diets containing rapeseed meal. Res. Vet. Sci, 29:68.

Igbasan, F.A. and Guenter, W. 1997. The enhancement of the nutritive value of peas for broiler chickens: An evaluation of micronization and dehulling processes. Poult. Sci. 75: 1243-1252.

InfraReady Technical Information. 1995. Production information. InfraReady Products Limited. Saskatoon, Saskatchewan.

Iori, R., Leoni, O., Lazzeri, L., Visentin, M. and Palmieri. 1991. Distribution of protease inhibitory activity in some rapeseed genotypes and other cultivated crucifers. Proceedings of the 8th International Rapeseed Congress. Saskatoon, Saskatchewan. pp 911-916.

Jensen, S.K., Liu, Y.G. and Eggum, B.O. 1995. The effect of heat treatment on glucosinolates and nutritional value of rapeseed meal in rats. Anim. Feed Sci. Tech. 53: 17-28.

Jensen, S.K., Liu, Y.G. and Eggum, B.O. 1995. The influence of seed size and hull content on the composition and digestibility of rapeseeds in rats. Amin. Feed Sci. Tech. 54: 9-19.

Johnson, I.T. and Gee, J.M. 1981. Effect of gel-forming food gums on the intestinal unstirred layer and sugar transport *in vitro*. Gut. 22: 398-403.

Keith, M.O. and Bell, J.M. 1983. Effects of ammonia and steam treatments on the composition and nutritional value of canola (low glucosinolate rapeseed) screenings in diets for growing pigs. Can. J. Anim. Sci. 63: 429-441.

Khan, N., Zaman, R. and Elahi, M. 1991. Effect of heat treatment on the phytic acid content of maize products. J. Sci. Food Agric. 54: 153-156.

Kingsley, M.O. 1995. Effect of processing on some antinutritive and toxic components and on the nutritional composition of the african oil bean seed (*Pentaclethra macrophylla* Benth). J. Sci. Food Agric. 68: 153-158.

Kjaer, A. 1974. The natural distribution of glucosinolates: a uniform class of sulphur-containing glucosides. In: Chemistry in botanical classification. Bendz, G. and Santesson, J. (editors). Academic Press. London. pp 229.

Klain, G.J., Hill, D.C., Branion, H.D. and Gray, J.A. 1956. The value of rapeseed oil meal and sunflower oil meal in chick starter rations. Poult. Sci. 35: 1315-1326.

Kramer, J.K.G., Mahadevan, S., Hunt, J.R., Sauer, F.P., Corner, A.H. and Charlton, K.M. 1973. Growth rate, lipid composition, metabolism and myocardial lesions of rats fed rapeseed oils (*Brassica campestris* var. Arlo, Echo and Span, and *B. napus* var. Oro). J. Nutr. 103: 1696-1708.

Kramer, J.K.G., Mahadevan, S., Sauer, F.P. and Corner, A.H. 1977. Nutritional evaluation of rapeseed oil. In: Rapeseed oil meal and by-product utilization. Bell, J.M. (editor). Rapeseed Association of Canada. No. 45. pp 64-74.

Kritchevsky, D. 1988. Dietary fibre. Annu. Rev. Nutr. 8: 301-328.

Krzymanski, J., Pietka, T., Ratajska, I., Byczanska, B. And Krotka, K. 1991. Development of low glucosinolate white mustard (*Sinapis alba* syn *Brassica hirta*). Proceedings to the 8th International Rapeseed Congress. Saskatoon, Saskatchewan. pp 1545-1548.

Lall, S.P. and Slinger, S.J. 1974. Effect of refining on the nutritional value of rapeseed oils for the chick and rats. J. Sci. Food Agric. 25: 423-432.

Langer, P. Antithyroid activity in rats of small doses of some naturally occurring compounds. Endocrinology. 79: 1117-1122.

Langer, P. and Greer, M.A. 1977. Antithyroid substances and naturally occurring goitrogens. Karger, S. Basel.

Leeson S. and Summers, J.D. 1991. Commercial Poultry Nutrition. University Books, Guelph, Ontario, Canada.

Liener, I.E. 1983. Removal by processing of naturally occurring toxicants and antinutrients. In: Chemistry and worl food supplies: The new frontiers. Shemit, L.W. (editor). Pergamon Press. New York, NY. pp. 453-463.

Lindsay, O.B., Biely, J. and March, B.E. 1969. Excretion of bile acids by cockerals fed different lipids. Poult. Sci. 48: 1216-1222.

Liu, Y.G., Jensen, S.K. and Eggum, B.O. 1995. The influence of seed size on digestibility and growth performance of broiler chickens fed full-fat rapeseed. J. Sci. Food Agric. 67: 135-140.

Liu, Y.G., Steg, A., Smits, B. and Tamminga, S. 1994. Crambe meal: removal of glucosinolates by heating with additives and water extraction. Anim. Feed Sci. Tech. 48: 273-287.

Lo, M.T. and Bell, J.M. 1972. Effects of various dietary glucosinolates on growth, feed intake

and thyroid function of rats. Can. J Anim. Sci. 52: 295-302.

Man, R. and Weir, R. 1988. The compleat mustard. Constable. London.

Marangos, A. and Hill, R. 1974. The hydrolysis and absorption of thioglucosidases of rapeseed meal. Proc. Nutr. Soc. 15: 405-414.

Marangos, A. and Hill, R. 1976. The use of rapeseed meals and a mustard seed meal as a protein source in diets for laying pullets. Br. Poult. Sci. 17: 643-653.

Marangos, A., Hill, R., Laws, B.M. and Muschamp, D. 1974. The influence of three rapeseed meals and a mustard seed meal on egg and broiler production. Br. Poult. Sci. 15:405.

Martland, M.F., Butler, E.J. and Fenwick, G.R. 1984. Rapeseed induced liver haemorrhage, reticulolysis and biochemical changes in laying hens: the effect of feeding high and low glucosinolate meals. Res. Vet. Sci. 36(3): 298-309.

Mascharanhas Ferreira, A., Kersten, J. and Cast, C.H. 1983. The study of several modifications of the neutral detergant fibre procedure. Anim. Feed Sci. Tech. 9: 19-28.

Mateos, G.G and Sell, J.L. 1981a. Nature of the extra metabolic effect of supplemental fat used in semipurified diets for laying hens. Poult. Sci. 60: 1925-1930.

Mateos, G.G. and Sell, J.L. 1981b. Influence of fat and carbohydrate source on rate of food passage of semipurified diets for laying hens. Poult. Sci. 60: 2114-2119.

Mateos, G.G., Sell, J.L. and Eastwood, J.A. 1982. Rate of food passage (transit time) as influenced by level of supplemental fat. Poult. Sci. 61: 94-100.

McDonald, P., Edwards, R.A., Greenhalgh, J.F.D. and Morgan, C.A. 1995. Lipids. In: Animal nutrition, 5th ed. Addison Wesley Longman. Harlow, UK. pp 28.

McNab, J.M. 1982. Effect of processing on the nutritive value of diets for poultry. In: Handbook of nutritive value of processed food: Volume II. Rechcigl, M.Jr. (editor) CRC Press Inc. Boca Raton, FL. pp. 403-424.

McNab, J.M. and Wilson, B.J. 1974. Effects of micronizing on the utilization of field bean (*Vicia faba* L.) By the young chick. J. Sci Food Agric. 25: 395-400.

Menegatti, E., Palmieri, S., Walde, P. and Luisi, P.L.1985. Isolation and characterization of a trypsin inhibitor from white mustard (*Sinapis alba* L.). J. Agric. Food Chem. 33: 784-789.

Mitaru, B.N. and Blair, R. 1984. The influence of dietary fibre sources on growth, feed efficiency

and digestibilities of dry matter and protein in rats. J. Sci. Food Agric. 35: 625-631.

Mohamed, A.A. and Rayas-Duarte, P. 1995. Composition of *Lupinus albus*. Cereal Chem. 72(6): 643-647.

Moody, D.L., Slinger, S.J., Leeson, S. and Summers, J.D. 1978. Utilization of dietary Tower rapeseed products by growing turkeys. Can J. Anim. Sci. 58: 585.

Moore, S. 1963. On the determination of cysteine and cysteic acid. J. Biol. Chem. 38: 235-237.

Moran, E.T., Jensen, L.S.Jr. and McGinnis, J. 1963. Dye binding by soybean and fish meal as an index of quality. J. Nutr. 79: 239-244.

National Research Council. 1994. Nutrient requirements of poultry, 9th revised edition. National Academy Press. Washington, DC.

Nelson, T.S. 1967. The utilization of phytate phosphorus by poultry - a review. Poult. Sci. 46: 801.

Nelson, T.S., Ferrara, L.W. and Storer, N.L. 1968. Phytate phosphorus content of feed ingredients derived from plants. Poult. Sci. 47: 1372.

Nordheim, J.P. and Coon, C.N. 1984. Comparison of four methods for determining available lysine in animal protein meals. Poult. Sci. 63: 1040-1051.

Nugon-Baudon, L., Szylit, O. and Raibaud, P. 1988. Production of toxic glucosinolate derivatives from rapeseed meal by intestinal microflora of rat and chicken. J. Sci. Food Agric. 43: 299-308.

Nugon-Baudon, L., Szylit, O. and Raibaud, P. 1990. Interactions of the intestinal microflora with glucosinolates on rapeseed meal toxicity: first evidence of an intestinal Lactobacillus possessing a myrosinase-like activity *in vivo*. J. Sci. Food Agric. 52: 547-559.

Nwokolo, E.N. and Bragg, D.B. 1977. Influence of phytic acid and crude fibre on the availability of minerals from four protein supplements in growing chicks. Can. J. Anim. Sci. 57: 475-477.

Oginsky, E.L., Stein, A.E. and Greer, M.A. 1965. Myrosinase activity in bacteria as demonstrated by the conversion of progoitin to goitrin. Proc. Soc. Exp. Biol. Med. 119: 360-364.

Poel van der, A.F.B. 1989. Effects of processing on antinutritional factors (ANF) and nutritional value of legume seeds for non-ruminaent feeding. In: Recent advances of research in antinutritional factors in legume seeds. Huisman, J., van der Poel, A.F.B and Leiner, I.E. (editors). PUDOC. Wageningen, The Netherlands. pp. 213-229.

Qin, G., ter Elst, E.R., Bosh, M.W. and van der Poel, A.F.B. 1996. Thermal processing of whole soya beans: Studies on the inactivation of antinutritional factors and effects on ileal digestibility of piglets. Anim. Feed Sci. Technol. 57: 313-324

Raczynski, G., Eggum, B.O. and Chwalibog, A. 1982. Z. Tierphysiol. Tierernährg. u. Futtermittelkde. 47: 160.

Raney, P., Rakow, G. and Olson, T. 1995a. Development of high erucic acid, low glucosinolate *Sinapis alba*. Proceedings to the 9th International Rapeseed Congress. Cambridge, UK. pp 452-454.

Raney, P., Rakow, G. and Olson, T. 1995b. Development of low erucic acid, low glucosinolate *Sinapis alba*. Proceedings to the 9th International Rapeseed Congress. Cambridge, UK. pp 416-418.

Rocquelin, G. and Cluzan, R. 1968. L'huile de colza riche en acide erucique et l'huile de colza sans acide erucique: valeur nutritionelle et effets physiologiques chez le rat. I. Effets sur la croissance, l'efficacite alimentaire et l'etat de differents organes. Ann. Biol. Anim. Bioch. Biophys. 8: 395-406.

Rocquelin, G., Cluzan, R., Vodovar, N. And Levillain, R. 1973a. Recent research into the physiopathological effects of rapeseed and canbre oils on the myocardium and other organs. Cah. Nutr. Diet. 8: 103-116.

Rosengarten, F. 1969. The book of spices. Livingston Publishing Co. Wynnewood, Pennsylvania. pp 297-305.

Salmon, R.E. 1969. The relative values of rapeseed and soybean oils in chick starter diets. Poult. Sci. 48: 1045-1050.

Sibbald, I.R. 1986. The TME system of feed evaluation: methodology, feed composition data and bibliography. Technical Bulletin 1986-4 E. Animal Research Centre, Research Branch. Agriculture Canada. Ottawa.

Sim, J.S., Toy, B., Crick, D.C. and Bragg, D.B. 1985. Effect of dietary erucic acid on the utilization of oils or fats by growing chicks. Poult. Sci. 64: 2150-2154.

Simbaya J., Slominski, B.A., Rakow, G. and Campbell, L.D. 1997. Nutritive value of canola/rapeseed meal: Digestible protein, dietary fibre and the effect of moist heat treatment on meal quality. J. Sci. Food Agric., submitted.

Simbaya, J., Slominski, B.A., Rakow, G., Campbell, L.D., Downey, R.K. and Bell, J.M. 1995.

Quality characteristics of yellow-seeded *Brassica* seed meals: Protein, carbohydrates and dietary fibre components. J. Agric. Food Sci. 43: 2062-2066.

Simons, P.C.M., Versteegh, H.A.J., Jongbloed, A.W., Kemme, P.A., Slump, P., Bos, K.D., Wolters, M.G.E., Beudeker, R.F and Verschoor, G.J. 1990. Improvement of phosphorus availability by microbial phytase in broilers and pigs. Br. J. Nutr. 64 (2): 525-540.

Sklan. D., Budowski, P., Ascarelli, I. and Hurwitz, S. 1973. Lipid absorption and secretion in the chick: effect of raw soybean meal. J. Nutr. 103: 1299-1305.

Sklan, D., Hurwitz, S., Budowski, P. and Ascarelli, I. 1975. Fat digestion and absorption in chicks fed raw or heated soybean meal. J. Nutr. 105: 57-63.

Slominski, B.A. 1994. Hydrolysis of galactooligosaccharides by commercial preparations of α-galactosidase and β-fructofuranosidase: potential for use as dietary additives. J. Sci. Food Agric. 65: 323-330.

Slominski, B.A. 1997. Developments in the breeding of low fibre rapeseed/canola. J. Anim. Feed Sci. 6: 303-317.

Slominski, B.A. and Campbell, L.D. 1987. Gas chromatographic determination of indoleglucosinolates: a re-examination. J. Sci. Food Agric. 40: 131-143.

Slominski, B.A. and Campbell, L.D. 1989. Indoleacetonitriles - thermal degradation products of indoleglucosinolates in commercial rapeseed (*Brassica napus*) meal. J. Sci. Food Agric. 47: 75-84.

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

Slominski, B.A., Campbell, L.D. and Guenter, W. 1994a. Carbohydrates and dietary fibre composition of yellow and brown-seeded canola. J. Agric. Food Chem. 42: 704-707.

Slominski, B.A., Campbell, L.D. and Guenter, W. 1994b. Oligosaccharides in canola meal and their effect on non-starch polysaccharide digestibility and true metabolizable energy in poultry. Poult. Sci. 73: 156-162.

Slominski, B.A., Campbell, L.D. and Stanger, N.E. 1987. Influence of cecectomy and dietary antibiotics on the fate of ingested intact glucosinolates in poultry. Can. J. Anim. Sci. 67: 1117-1124.

Slominski, B.A., Campbell, L.D. and Stanger, N.E. 1988. Extent of hydrolysis in the intestinal

tract and potential absorption of intact glucosinolates in laying hens. J. Sci. Food Agric. 42: 305-314.

Slominski, B.A., Simbaya, J., Campbell, L.D., Rakow, G. and Guenter, W. 1998. Nutritive value of yellow-seeded canola: Chemical composition and feeding quality of meals derived from newly developed varieties. Anim. Feed Sci. Technol., submitted.

Smithard, R.R and Eyre, M.D. 1986. The effects of dry extrusion of rapeseed with other feedstuffs upon its nutritional value and antithyroid activity. J. Sci. Food Agric. 37: 136-140.

Snedecor, G.W. and Cochran, W.G. 1980. Statistical methods (7th edition). Iowa State University Press. Ames, IA.

Statistical Analysis Systems Institute. 1990. SAS Users Guide: Statistics. SAS Institute. Cary, NC.

Taylor, R.G. 1965. The availability of the calcium and phosphorus of plant materials for animals. Nut. Soc. 24: 105.

Thies, W. 1977. Analysis of glucosinolates in seeds of rapeseed (*Brassica napus*): concentration of glucosinolates by ion exchange. Z. pflanzenzuchtg. 79: 331-335.

Thomassen, H.J. and Boldingh, J. 1955. The biological value of oils and fats. II. The growth retarding substance in rapeseed oil. J. Nutr. 56: 469-475.

Thompson, L.U. 1990. Phytates in canola/rapeseed. In: Canola and rapeseed: production, chemistry, nutrition and processing technology. Shahidi, F. (editor). Van Nostrand Reinhold Co., Inc. New York, NY. pp 173-192.

Timms, L.M. 1983. Forms of leg abnormality observed in male broilers fed on a diet containing 12.5 per cent rapeseed meal. Res. Vet. Sci. 35: 182.

Tookey, H.L., VanEtten, C.H. and Daxenbichler, M.E. 1980. Glucosinolates. In: Toxic constituents of plant foodstuffs, 2nd ed. Liener, I.E. (editor). Academic Press. New York. pp 103.

Trowell, H.C., Southgate, D.A.T, Wolever, T.M.S., Leeds, A.R., Gassull, M.A. and Jenkins, D.J.A. 1976. Dietary fibre redefined. Lancet. 1: 967.

Uppström, B. 1995. Seed chemistry. In: *Brassica* oilseeds: production and utilization. Kimber, D.S. and McGregor, D.I. (editors). CAB International. Wallingford, UK. pp 217-242.

VanEtten, C.H. and Tookey, H.L. 1983. Glucosinolates. In: Handbook of naturally occurring

food toxicants. Rechcigl, M.Jr. (editor). CRC Press. Boca Raton, FL. pp 15.

Vaughan, J.G. and Hemingway, J.S. 1959. The utilization of mustards. Economic Botany. 13(3): 196-204.

Velisek, J., Mikulcova, R., Mikova, K., Woldie, K.B., Link, J. and Davidek, J. 1995. Chemometric investigation of mustard seed. Lebensm. Wiss. Technol. 28: 620-624.

Vermoral, M., Heaney, R.K and Fenwick, G.R. 1986. Nutritive value of rapeseed meal: effects of individual glucosinolates. J. Sci. Food Agric. 37: 1197-1202.

Ward, A.T. and Reickert, R.D. 1986. Comparison of the effect of cell wall and hull fibre from canola and soybean on the bioavailability for rats of minerals, protein and lipid. J. Nutr. 116: 233-241.

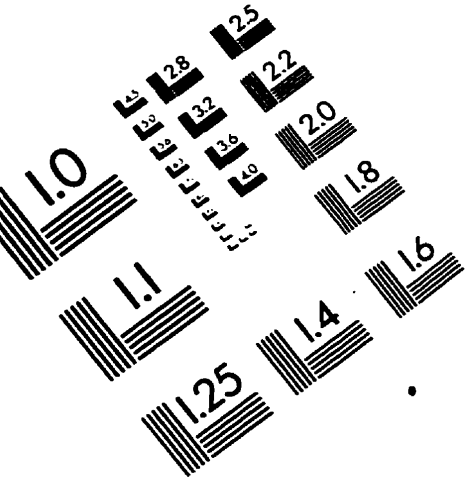
West, G. and Norton, G. 1991. Rapeseed proteinase inhibitors. Proceedings of the 8th International Rapeseed Congress. Saskatoon, Saskatchewan. pp 928-933.

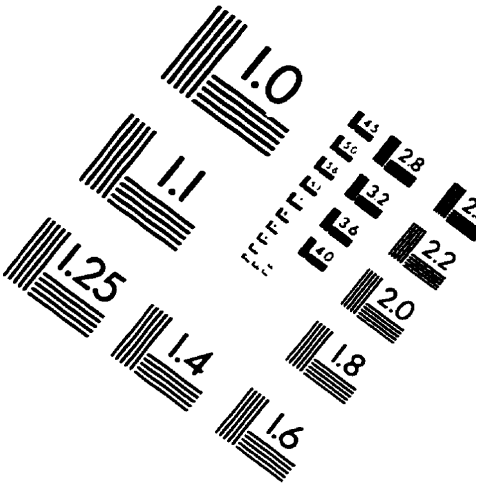
Williams, C.H., David, D.J. and Lismoa, O. 1962. The determination of chromic oxide in fecal samples by atomic absorption spectrophotometry. J. Agr. Sci. 59: 381.

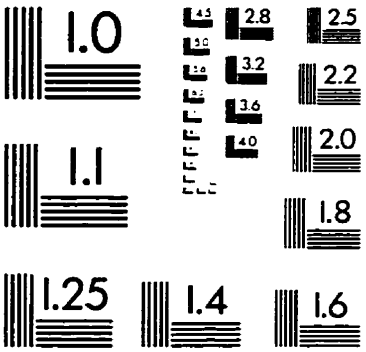
Wiseman, J. and Salvador, F. 1991. The influence of free fatty acid content and degree of saturation on the apparent metabolizable energy value of fats fed to broilers. Poult. Sci. 70: 573-582.

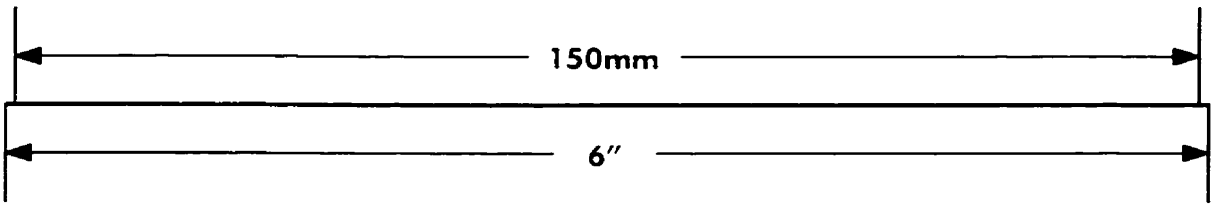
Zhang, W.J., Campbell, L.D. and Stothers, S.C. 1994. An investigation of the feasibility of predicting nitrogen-corrected true metabolizable energy (TME_n) content in barley from chemical composition and physical characteristics. Can. J. Anim. Sci. 74: 355-360.

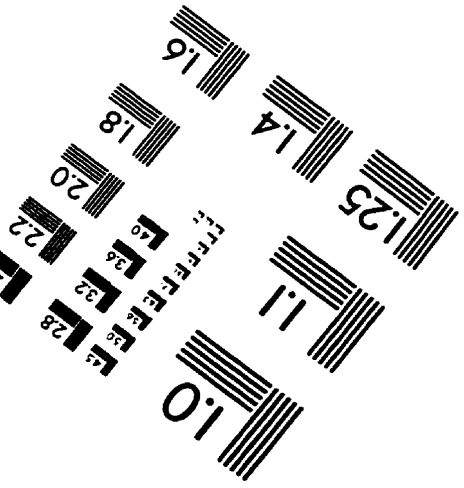
IMAGE EVALUATION TEST TARGET (QA-3)

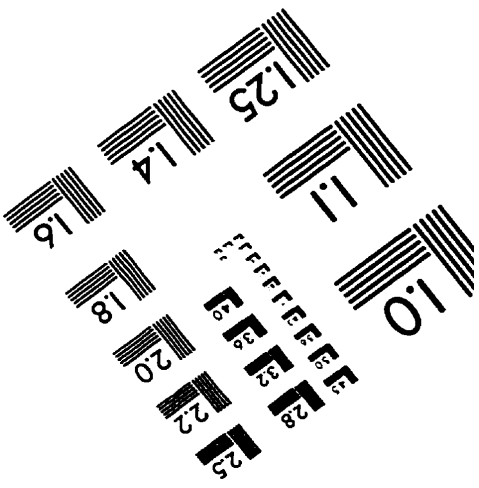












APPLIED IMAGE, Inc
1653 East Main Street
Rochester, NY 14609 USA
Phone: 716/482-0300
Fax: 716/288-5989