CHEMICAL COMPOSITION AND NUTRITIVE VALUE OF YELLOW-SEEDED *BRASSICA NAPUS* CANOLA AND CANOLA-QUALITY *SINAPIS ALBA* MUSTARD FOR POULTRY

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Ping Jiang

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ABSTRACT

Part A: Chemical Composition and Nutritive Value of Yellow-Seeded *Brassica Napus* Canola For Poultry

Plant selection programs directed towards the development of yellow-seeded canola are among approaches undertaken to reduce the fiber content, increase the protein content and to improve nutrient utilization. A relatively new initiative in breeding for yellow-seed coat color has been the development of yellow-seeded *B. napus* canola. The objective of this study was to compare a new yellow-seeded *B. napus* line with its black-seeded counterpart, both types originating from the same genetic background and produced under identical growing conditions in two consecutive years. On average, in comparison to black-seeded, yellow-seeded types contained more protein (46.2% vs 45.2%), more sucrose (9.0% vs 7.8%), less dietary fiber (26.6% vs 30.8%) but similar amounts of oligosaccharides (3.4% vs 3.1%), starch (0.46% vs 0.40%), non-phytate phosphorus (0.71% vs 0.67%) and ash (6.8% vs 6.7%). Although similar in content of non-starch polysaccharides (18.0% vs18.3%), cell wall protein (2.9% vs 3.0%) and minerals associated with the fiber fraction (0.9% vs 1.0%), the overall lower fiber content in yellow-seeded samples was reflected in a lower content of lignin with associated polyphenols (4.9% vs 8.6%). When expressed in g/16g N, no difference in amino acid level was observed. There was a significant difference in the content of glucosinolates which averaged 13.2 and 19.3 μmol/g for yellow- and black-seeded samples, respectively. In a two-week feeding trial, broiler chickens were fed wheat (56%)/canola meal (32%) diets containing 22% crude protein and 3050 kcal/kg metabolizable energy. On average, chickens fed diets containing meals derived from yellow-seeded canola showed significantly higher body weight gain (398 vs 342 g/bird/14 days) and lower feed to gain ratio (1.53 vs 1.60) than those fed the black-seeded type.

Part B: Canola-Quality Yellow Mustard (*Sinapis Alba L.*): The Effect of Water-Soluble Fiber (Mucilage) and Fineness of Grinding on the Nutritive Value of the Seed

The canola-quality *Sinapis alba* (yellow mustard) species has potential as a high protein and high energy alternative to full fat soybean. The objective of the present study was to further investigate the effect of water-soluble fiber (mucilage) on broiler chicken performance and to explore the potential for improved energy utilization using the mucilage depolymerizing or viscosity reducing enzymes. The fineness of grinding of *S.alba* seed was used as a means of investigating an encapsulating effect of the cell walls on energy utilization by poultry. The highest degree of mucilage depolymerization was observed for cellulase preparations indicating that some form of "soluble" cellulose was one of the major components of *S.alba* mucilage. With regard to the viscosity reducing capacity of exogenous enzymes, pectinases B and C were found to be more effective than cellulase preparations. This would indicate that pectic polysaccharides rather than the cellulose-like polymers are responsible for viscous properties of yellow mustard mucilage. Pectinase B showed the greatest effect on viscosity reduction and was chosen, at the dietary inclusion rate of 0.01%, for in vivo studies on the effect of mucilage on broiler chicken performance. Relative to the conventional soybean /canola meal diet (control), there was a significant reduction in body weight gain of broiler chickens fed diets containing micronized soybean seed, raw mustard seed as well as both raw and micronized seeds supplemented with enzyme. In comparison to the raw *S.alba* seed diet, two enzyme supplemented diets, the raw *S.alba* seed and the micronized *S.alba* seed, showed significantly better feed to gain ratios (1.58, 1.52 and 1.54, respectively) and lower, although not statistically significant, intestinal viscosity values (8.2 CPs, 6.6 CPs and 6.8 Cps, respectively). All three yellow mustard diets showed significantly lower dry matter and fat digestibilities and AMEn contents than the control or micronized soybean seed diets. As compared to the raw *S.alba* seed diet, the enzyme supplemented raw and micronized *S. alba* seed diets had higher AMEn contents (2728 kcal/kg, 2778 kcal/kg and

2882 kcal/kg, respectively) and showed statistically significant improvement in dry matter (61.5%, 62.6% and 65.0%, respectively) and fat digestibilities (53.8%, 59.1% and 69.5%, respectively). In a study on the effect of fineness of seed grinding on energy utilization, decreasing the particle size of the seed meal from ≤ 2.0mm to ≤ 0.6mm increased the TMEn content from 2087.1 kcal/kg to 3475.9 kcal/kg for raw *S.alba* seed and from 2891.3 kcal/kg to 3673.6 kcal/kg for micronized *S.alba* seed as determined with adult roosters.

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LIST OF ABBREVIATIONS

ADF	Acid detergent fiber.
AOAC	Association of Official Analytical Chemists.
ANOVA	Analysis of Variance.
AMEn	Nitrogen corrected apparent metabolizable energy.
CF	Crude fiber.
CP	Crude protein.
DF	Dietary fiber.
DM	Dry matter.
NDF	Neutral detergent fiber.
NRC	National Research Council.
NSP	Non-starch polysaccharides.
SAS	Statistical analysis system.
TDF	Total dietary fiber.
TMEn	Nitrogen corrected true metabolizable energy.

1. LITERATURE REVIEW

1.1. Part A: Yellow-seeded Canola/Rapeseed

1.1.1. Introduction

Canola meal is commonly accepted and used as a valuable ingredient in swine and poultry rations. It is a result of plant selection programs in which erucic acid content of canola oil and glucosinolate content of the meal have been largely reduced as compared to its parent rapeseed. The term "canola" describes the rapeseed of *Brassica napus* and *Brassica rapa* (formerly *campestris*) species yielding oil of less than 2 % of erucic acid and meal of less than 30 μmoles/g of glucosinolates. As a protein supplement, canola meal is still less competitive in the market place than soybean meal. When compared to conventional soybean meal, canola meal contains less metabolizable energy (2,640 kcal/kg vs 3,180 kcal/kg for swine and 2,000 kcal/kg vs 2,491 kcal /kg for poultry) (NRC, 1998 and Leeson and Summers,1991), less crude protein (38.3 vs 44.0%) but more fiber (21.5 vs 7.1% neutral detergent fiber; 17.5 vs 5.0% acid detergent fiber). However, it is a richer source of most of the B-vitamins and essential minerals (Bell, 1993). The fiber of canola meal consists of two fractions: the hull and the endosperm cell walls. In addition to being poorly digested by monogastric animals, fiber components affect the quality of canola meal by lowering nutrient density and diluting available energy , protein and amino acid contents. A negative relationship (r = - 0.71) between dietary fiber and crude protein contents of canola meal has been well documented (Simbaya *et al.*, 1995, Slominski, 1997). Total dietary fiber has also been found to be negatively correlated with AME_n and ileal protein digestibility for poultry (Newkirk *et al.*, 1997). In addition, dietary fiber may hinder nutrient digestion through an encapsulating effect of the cell walls which, in turn, would prevent the animal's own digestive enzymes from accessing the nutrients contained within the cells. Fiber would also induce passage rate, more or less resulting in reduction in nutrient digestion (Imbeah and Sauer,

1991). Increasing the dietary level of canola hulls was found to reduce digestibility of dry matter, crude protein, energy and ether extract in pigs (Bell and Shires, 1982). Moreover, fiber is known to depress the bioavailability of some minerals. Both cell wall and hull fibers from canola lowered the apparent availability of Cu, Fe, Ca , P and protein when 12 % cell wall fiber or 12 % hull fiber-containing semi-purified diets were fed to rats (Ward and Reichert, 1986). Therefore, high fiber and low energy content has been considered as the main factor limiting the use of canola meal in poultry and swine rations.

Various approaches have been undertaken to reduce the fiber content and to improve the nutritive value of canola meal. These included dehulling, exogenous enzyme application or genetic selection. Dehulling is an effective means of removing fiber. It was found that the lysine availability in broiler chickens fed a diet containing dehulled canola meal was significantly higher than that of the hulled meal (78.3% and 72.8%, respectively; Zuprizal *et al.*, 1991). The dehulled rapeseed meal, relative to a regular rapeseed meal, increased the digestibilities of both energy and protein for pigs from 73 % and 81 % to 86% and 86%, respectively (Bourdon, 1989). However, it has been speculated that, due to limited levels at which canola meal is used in poultry rations, the economics of dehulling are questionable (Shires *et al.*, 1983). Enzyme supplementation of a semi-purified diet containing 40% commercial canola meal increased non-starch polysaccharides (NSP) digestibility by laying hens from 3% to 37% (Slominski and Campbell, 1990). Although some enzyme preparations were found to be effective in hydrolysis of the cell wall polysaccharides in vitro, the improvement in broiler chicken performance was not statistically significant when the same enzyme preparations were added to the semi-purified canola meal diets at the low and more economically feasible inclusion rates (Simbaya, 1996; Slominski,1997). Further research is needed to effectively target dietary fiber components of canola meal with exogenous enzymes. Since the first report by Jonsson and Bengtsson in 1970, showing yellow-seeded forms of *Brassica rapa* to have high oil, high protein and low fiber contents,

research on yellow-seeded canola has been carried out extensively. In 1974 Stringam et al. further reported yellow-seeded rapeseed to have thinner hulls and a lower percentage of seed coat (hull) than the brown-seeded type. Subsequent research has shown many potentially positive characteristics associated with the yellow-seeded strains of canola/rapeseed (Bell and Shires, 1982; Mitaru *et al.*, 1984; Daun and DeClercq, 1988; Slominski and Campbell, 1990; Slominski and Campbell, 1991; Slominski *et al.*, 1994a; Slominski et al., 1994b; Simbaya *et al.*, 1995; Slominski, 1997).

1.1.2. Chemical Composition of Yellow-seeded Canola/Rapeseed

Chemical composition of yellow-seeded canola/rapeseed in comparison to brown-seeded type is shown in Table 1. Based on examination of yellow and brown seeds from three cultures of *Brassica rapa*, Stringam et al.(1974) reported that yellow seeds contained more oil and protein and less crude fiber than the brown seed with the differences in oil, protein and crude fiber contents between yellow and brown seeds being statistically significant (Stringam *et al.*, 1974). The study on yellow-seeded *Brassica rapa* and brown-seeded *Brassica napus* canola confirmed the lower fiber content in yellow seeds as compared to brown seeds, whether expressed as crude fiber (6.3% vs 8.1%), neutral detergent fiber (21.9% vs 23.6%) or as acid detergent fiber (13.4% vs 17.7%)(Bell and Shires, 1982). Detailed studies on 26 yellow-seeded and 7 brown-seeded *Brassica* samples indicated that, when compared with brown-seeded forms, the yellow-seeded types had higher sucrose (8.7% vs 7.5%) and protein (44.5% vs 42.7%) contents, were lower in dietary fiber (27.7% vs 33.6%) but contained similar amounts of other components including oligosaccharides, ash and fat (Simbaya *et al.*, 1995). It can be concluded from data accumulated to date that yellow-seeded canola/rapeseed has some advantage over the brown-seeded type with regard to nutritive content.

Table 1. Chemical composition of seed and meal samples derived from yellow- and brown-seeded *Brassica* species

Species/Component	Seed coat color	
	Yellow	Brown
Brassica rapa [1]		
Oil (% DM, full fat basis)	40.4	38.4
Crude protein (% DM, fat free basis)	42.0	40.9
Crude fiber (% DM, fat free basis)	7.1	11.5
Brassica species [2] (% DM, fat free basis)		
Sucrose	8.7	7.5
Oligosaccharides	2.3	2.5
Total fiber	27.7	33.6
Crude protein	44.5	42.7
Ash	6.9	7.0
Fat	2.7	2.9

[1] Stringam *et al.*,1974; [2] Included yellow- and brown-seeded *B. napus*, *B. rapa*, *B. juncea* and *B. carinata* canola/rapeseed, Simbaya *et al.*, 1995.

1.1.3. Quality Characteristics of Yellow-seeded Canola/Rapeseed

In recent years, several studies have focused on dietary fiber, carbohydrates, protein and amino acids content of yellow-seeded canola/rapeseed with the objective of exploring any advantage of yellow-seeded type over its brown-seeded counterpart. It is apparent that dietary fiber, carbohydrates, protein and amino acids are key quality characteristics of future canola meal.

1.1.3.1. Dietary Fiber

Dietary fiber is used to describe a variety of plant polysaccharides which are not digested by the alimentary enzymes of man. It is composed of cellulose, hemicellulose, pectins, gums, oligosaccharides and lignin (Trowell *et al.*, 1976). Among the methods used to measure fiber content are crude fiber (CF; AOAC, 1990), acid detergent fiber (ADF; AOAC, 1990), neutral detergent fiber (NDF; AOAC, 1990), non-starch polysaccharide (NSP; Englyst and Cummings, 1984; Englyst and Cummings, 1988; Slominski and Campbell, 1990) and total dietary fiber (DF). The components of plant cell wall polymers analysed by these methods are shown in Table 2.

The content and composition of dietary fiber of yellow- and brown-seeded canola is shown in Table 3. Yellow-seeded canola contained less neutral detergent fiber (NDF) than the brown- seeded type. This is in agreement with the report of lower NDF (21.9 % vs 23.6 %), ADF (13.4 % vs 17.7 %) and CF (6.3 % vs 8.1 %) contents in yellow-seeded canola (rapeseed) as compared to the brown-seeded type (Bell and Shires, 1982). The total dietary fiber, lignin and polyphenol contents of yellow-seeded *B. rapa* canola were lower than those of brown-seeded *B. napus* samples, while the non-starch polysaccharide (NSP) value for yellow-seeded type was higher than that of the brown-seeded type. It should be pointed out that, although the total non-starch polysaccharide content of yellow-seeded

Table 2. The components of plant cell wall polymers and evaluation fractions by Various methods

Class	Type	Evaluation fractions				
		CF	ADF	NDF	NSP	DF
Structural polysacchaides	Pectin/ pectic substance				X	X
(NSP)	Hemicellulose			X	X	X
	Cellulose	X	X	X	X	X
Structural non-carbohydrate	Lignin and polyphenols	X	X	X		X
Protein	Glycoproteins		X	X		

Table 3. Composition of dietary fiber in defatted meals derived from yellow- and brown- seeded lines/varieties of *B. rapa* canola (% of dry matter)[1]

Component	Seed coat color	
	Yellow (n = 14)	Brown (n = 4)
Neutral detergent fiber	18.8	25.7
Total fiber	27.3	30.2
Nonstarch polysaccharides	21.5	17.8
Protein	2.2	3.5
Ash	0.4	1.0
Lignin and polyphenols	3.2	8.0
Soluble fiber	2.0	1.5
Insoluble fiber	19.4	16.4
NSP component sugars (% of total)		
rhamnose	1.0	1.1
fucose	1.2	1.2
arabinose	25.7	25.2
xylose	9.1	9.0
mannose	2.1	2.2
galactose	8.6	9.3
glucose	28.5	27.8
uronic acids	23.8	24.2

[1] Slominski et al., 1994a.

canola is higher than that of the brown-seeded type, there are no qualitative differences in the component sugar profile between both types of canola. It is worth noting that although both types had similar and small amounts of soluble NSP, total NSP digestibility by laying hens was significantly higher for yellow-seeded than for the brown-seeded type (8.6% vs 3.4%)(Slominski et al., 1994b). Relatively little lignification of yellow-seeded canola could be taken into account in explaining the different NSP digestibility values.

Based on numerous reports, it became evident that the difference in the total dietary fiber content between yellow- and brown-seeded canola was attributed to different lignin and polyphenol contents. Comprehensive research on dietary fiber components of yellow- and brown-seeded canola by Simbaya *et al.* (1995) demonstrated somewhat similar results for *B. napus*, *B. juncea*, *B. carinata* canola although yellow-seeded *B. napus* was found to contain less NSP than the yellow-seeded type of *B. rapa*, canola.

It is well known that the hulls in canola/rapeseed are the richest source of fiber and account for 16 - 19 % of the seed weight and about 30 % of the meal weight (Bell, 1993). As early as 1970, Jonsson and Bengtsson suggested that the difference in fiber content between yellow- and brown- seeded rapeseeds resulted mainly from differences in the percentage of the seed coat between these two types. This was further confirmed by Stringam et al. (1974), who reported that brown seed coats made up 15.5 % of the dry seed weight while yellow seed coats accounted for 12 %. Histological studies indicated that yellow seed coats were thinner and the palisade cells, containing much fiber but little oil or protein, were smaller in size as compared to the brown seed coats (Stringam et al., 1974). On the other hand, the research on fiber components of yellow and brown hulls showed that the fiber content of yellow hulls was lower than that of brown hulls, whether expressed as crude fiber, neutral

detergent fiber, acid detergent fiber, or as acid detergent lignin (Table 4; Mitaru *et al.*, 1984). This is in agreement with the report by Bell and Shires (1982). With regard to lignin and polyphenol analysis it has been shown that the method of oxidative degradation could provide a better estimate of lignin content. Examination of rapeseed hulls with oxidative characterization indicated that yellow hulls had a lower polyphenol content than the brown hulls, but their true lignin contents were similar (Theander *et al.*, 1977). In this regard, most of the condensed tannins in rapeseed were found in the hull fraction (Durkee, 1971: Leung *et al.*, 1979). The research on tannin and fiber content of canola/rapeseed hulls also indicated that most of the tannins in hulls were highly polymerized (Mitaruet al., 1982). Therefore, the major difference in fiber components between yellow and brown hulls is in polyphenol (condensed type tannin) content.

Table 4. Fiber components of yellow and brown rapeseed hulls (% of dry matter)[1]

Component	Hull color	
	Yellow	Brown
Crude fiber	20.0	39.4
Neutral detergent fiber	39.9	63.1
Acid detergent fiber	34.1	57.3
Acid detergent lignin	5.5	28.9
Cellulose	28.6	28.4
Hemicellulose	5.7	5.8

[1] Mitaru *et al.*, 1984.

1.1.3.2. Carbohydrates

The carbohydrate content of yellow- and brown-seeded canola is shown in Table 5. Sucrose content of meals derived from yellow-seeded samples was higher than that of brown-seeded canola (9.8 % vs 7.7 %). This value is in agreement with more recent studies conducted on a large number of yellow-seeded samples (Simbaya *et al.*, 1995). Higher sucrose content in yellow - seeded canola would increase the digestible energy content of the meal since sucrose is highly digested and absorbed by the chicken or pig. On the other hand, the oligosaccharides raffinose, stachyose and verbascose are not digested in the small intestine and are believed to depress digestibility of energy by increasing the rate of feed passage through the gut (Leske *et al.*, 1993). The yellow- and brown-seeded canola have been found to contain similar amounts of oligosaccharides (2.4 % vs 2.5 %)(Slominski *et al.*, 1994a; Simbaya *et al.*, 1995). There was no difference in the amount of low-molecular weight carbohydrates present in hulls from yellow- and brown-seeded rapeseed (3.4 % vs 3.7 %), with sucrose content averaging 2.81 % and 2.85 % for yellow and brown hulls, respectively (Theander, 1977).

1.1.3.3. Protein

Yellow-seeded canola/rapeseed tends to have a higher level of protein than that of the brown type. Since the embryos contain much more protein than the seed coats, higher protein content in yellow-seeded canola could result from a greater proportion of cotyledones in the seed. On the other hand, the crude protein content of yellow hulls is, on average, higher than that of brown hulls (17.8 % vs 14.0 %)(Stringam et al.,1974; Bell and Shires, 1982; Mitaru et al.,1984). With regard to protein

Table 5. Carbohydrate content of defatted meals derived from yellow- and brown-seeded canola (% of dry matter)

Species/Seed Coat Color	Sucrose	Oligosaccharides
B. rapa [1] - yellow	9.8	2.4
- brown	7.7	2.5
B. rapa [2] - yellow	9.9	2.6
- brown	7.1	2.5
B. napus [2] - yellow	9.7	3.3
- brown	8.3	3.0

[1] Slominski *et al.*, 1994a; [2] Simbaya *et al.*, 1995.

quality, the results of in vitro protein digestibility of 4 yellow-seeded and 2 brown-seeded samples demonstrated no major difference between the samples (Simbaya, 1996). It has been recognized that the protein in the hull fraction would be highly resistant to degradation in the digestive tract (Finlayson, 1974). The study with pigs indicated that the digestibility values for crude protein in yellow and brown hulls were 20 % and 0 % respectively (Bell and Shires, 1982). Since tannins are known to have an adverse effect on the nutritive value and protein utilization of rapeseed meal (Durkee, 1971; Mitaru et al., 1982), the lower level of tannins in yellow hulls, as compared to brown hulls, would explain the difference in protein digestibility between the two types of canola/rapeseed.

The essential amino acids content of commercial meal derived from yellow-seeded *B. rapa* canola and the conventional meal originating, for the most part, from brown-seeded *B. napus* canola is shown in Table 6. Among the limiting amino acids for non-ruminants, lysine, methionine, arginine or threonine contents for both meals were similar, while the commercial meal "yellow" contained less cystine than the brown type. Studies with cockerels indicated that the true available amino acid content of the commercial meal derived from yellow-seeded *B. rapa* canola was slightly higher than that of the conventional meal from brown-seeded canola (Simbaya, 1996).

1.1.4. Feeding Quality of Yellow-seeded Canola/Rapeseed

With regard to in vivo evaluation of yellow-seeded canola/rapeseed with poultry, published reports now available are very limited. The study with laying hens showed yellow-seeded canola meal to have higher digestible matter and amino acid contents than the brown-seeded type (Slominski et al., 1994b). In an experiment with broiler chickens fed diets containing meals derived from yellow-seeded *B. rapa* and *B. napus* and brown-seeded *B. napus* canola, no differences in weight gain of

Table 6. Essential amino acids content of commercial meals derived from yellow- and brown-seeded canola (g/16g N) [1]

Amino acid	Commercial meal "Yellow"	Commercial meal "Brown"
Arginine	5.51	5.50
Histidine	2.95	3.00
Isoleucine	4.05	4.11
Leucine	6.79	6.72
Lysine	5.61	5.53
Methionine	1.90	1.92
Cystine	2.17[b]	2.61[a]
Phenylalanine	5.89	5.65
Tyrosine	3.04	2.98
Threonine	4.01	3.82
Valine	4.88	4.86

[1] Slominski *et al.*, 1999; [ab] $P \leq 0.05$

broiler chickens was observed although the yellow-seeded *B. napus* had the best feed efficiency value. In addition, yellow-seeded *B. napus* canola showed significantly higher TMEn content (9.71 MJ/kg) as compared to yellow-seeded *B. rapa* and brown-seeded *B. napus* (9.26 and 9.18 MJ/kg, respectively)(Simbaya, 1996).

To date, little has been reported on the performance of pigs fed the yellow-seeded type of canola. Since the major difference between yellow- and brown-seeded types exists in fiber content and composition, some studies on the effect of hulls on nutrient digestibility by pigs have been carried out. Bell and Shires (1982) reported the digestible energy content of yellow versus brown hulls to be 30% and 2%, respectively. When yellow and brown hulls were fed to pigs at dietary levels of 0, 15, and 30 %, the digestibility of dry matter, energy, crude fiber and ether extract decreased with increased hull level for both types of hulls, although the yellow hulls had less of an adverse effect on nutrient digestibility (Bell and Shires, 1982). Furthermore, growing pigs fed diets containing 10 % of yellow hulls digested more protein at the terminal ileum level than pigs fed 10 % of brown hulls (Mitaru et al.,1984). As lignin has a detrimental effect on protein digestibility due to hydrophobic binding of amino acids (Murray *et al.*, 1977; Nomani and Stansberry, 1982), little lignification of yellow hulls relative to brown hulls could explain an improvement in protein utilization.

In conclusion, yellow-seeded canola with the advantage of lower fiber content accompanied by increased digestible energy and protein contents, provides the opportunity for further improvement to the quality of the meal. However, further evaluation of yellow-seeded *B. napus* canola currently under development is needed to ensure a continuous improvement to this type of canola.

1.2. Part B: Canola-quality Yellow Mustard (*Sinapis alba* L.)

1.2.1. Introduction

Yellow or white mustard (*Sinapis alba*), has been known to man for many years and grown primarily as a condiment crop for the spice trade. In Canada, *Sinapis alba* is produced mainly in the Prairie Provinces of Manitoba, Saskatchewan and Alberta. It has superior heat and drought tolerance as compared to *B. napus* and *B. rapa* canola and is highly resistant to blackleg disease (*Lestophaeria maculans*) and flea beetle attacks (Rakow, 1995). In addition, it is highly shatter resistant and has a large bright yellow seed. The condiment *S. alba* seed has never been considered for usage as animal feed because of its high glucosinolate (up to 200 μmol/g oil free meal) and erucic acid (about 25 - 45 % of the fatty acid profile) contents (Hemingway, 1995). In recent years, canola-quality cultivars of *Sinapis alba* with low contents of erucic acid and glucosinolate have been developed by plant breeders (Raney *et al.*, 1995a; Raney et al., 1995b; Krzymanski *et al.*, 1991). It is believed that with the proper processing conditions and the use of dietary enzymes the canola-quality *Sinapis alba* could become a valuable and interesting alternative to the existing protein and energy supplements of animal feeds (Kienzle, 1998).

1.2.2. Chemical Composition

Little is known on the chemical composition of condiment *Sinapis alba* seed. The study by DeClercq and Daun(1997) showed *S. alba* to contain 31.5 % oil and 31.2 % protein. Chemical composition of canola-quality *S. alba* white mustard is shown in Table 7 (Kienzle, 1998). It is apparent that the oil, protein and carbohydrates (i.e., sucrose, starch, oligosaccharides) are the major

Table 7. Chemical Composition of Canola-quality *Sinapis alba* Mustard [1]

Component	% DM (full fat basis)
Protein	37.5
Oil	26.4
Sucrose	3.3
Oligosaccharides	3.4
Starch	1.3
Total fiber	22.2
Water-soluble fiber	1.7
Ash	5.4

[1]Kienzle, 1998.

components of *S. alba* seed. In comparison to full fat soybean, canola type *S. alba* contains more oil (26.4 vs 20.2%) but less protein (37.5 vs 41.4%)(Kienzle , 1998).

As early as 60 years ago, the outer seed coat of mustard seeds was first reported to be rich in mucilaginous material (Bailey and Norris, 1932) which was later attributed by Weber *et al.* (1974) to the consistency of prepared mustard products. Cui *et al.* (1994) reported that crude mucilage of yellow mustard seed made up 5 % of the seed weight with water-soluble and water-insoluble fractions accounting for 55.6 % and 38.8 %, respectively. In the study by Kienzle (1998), the canola type *S. alba* was found to contain 1.7 % water-soluble fiber (i.e., mucilage) which was higher than that present in soybean or *B. napus* canola. Although mustard mucilage is widely used by the food industry (i.e., salad dressings, food pastes) its viscous characteristic would appear to be a limiting factor in monogastric animal feeding. Viscous polysaccharides including mucilage, gums, pectins and some hemicelluloses (i.e., arabinoxylan, β-glucan) have been reported to affect nutrient absorption by interfering with bulk movement, preventing mixing of nutrients with digestive enzymes and eliminating transport of nutrients to intestinal mucosa (Kritchevsky, 1988; Johnson and Gee, 1981; Campbell and Bedford, 1992).

1.2.3. Nutritive Value

Available energy content is of great importance in assessing the nutritive value of feedstuffs. True metabolizable energy (TME) or TME corrected to zero nitrogen balance (TMEn) is gaining wide acceptance in feed evaluation since both TME and TMEn values are obtained by making corrections for metabolic and endogenous losses. The metabolizable energy content of feedstuffs is often influenced by factors associated with fat or carbohydrate digestibility and, as reported for

rapeseed/canola, age of animals, processing conditions and the level of antinutritional factors may all contribute to energy utilization. Antinutritional factors of rapeseed such as glucosinolates and fiber are also present in *S. alba* seed. Kienzle (1998) reported that the canola type *S. alba* contained more dietary fiber than *B. napus* canola (22.2 % vs 20.4 %, full fat basis). The new *S. alba* cultivar was also shown to contain 35.3 μmol/g of glucosinolates. Both dietary fiber and glucosinolates of canola meal were found to have a negative effect on AMEn and ileal protein digestibility in broiler chickens (Classen *et al.*, 1991; Newkirk *et al.*, 1997). Therefore, the viscosity resulting from the mucilaginous material would appear to be a limiting factor in utilization of *S. alba* seed. It has been well documented that the depression in fat digestion in broiler chickens fed diets rich in viscous polysaccharides of rye, barley or oats is a function of intestinal viscosity they create (Campbell *et al.*, 1983; Campbell and Bedford, 1992; Chesson, 1993). Kienzle (1998) suggested that a relatively high soluble fiber content of *S. alba* seed may contribute to its low TMEn value which, in comparison to full fat soybean, was found to be lower by 1330 kcal/kg DM. In addition, three trypsin inhibitors have been identified in the condiment *S. alba* seed. Due to a relatively low level, however, their potentially antinutritive effects would be considered minimal (West and Norton, 1991).

Heat processing is an effective method of improving the nutritive value of feedstuffs for monogastric animals. The classical ways of applying heat include steam pelleting, roasting, toasting or extruding (McNab, 1982). In recent years, micronization - a heat process utilizing infrared electromagnetic short waves has become an interesting meansof improving the nutritive value of animal feeds. In protein supplements, heating alters the tertiary structure of the plant protein and improves its digestibility by monogastric animals (Sunde, 1982). In addition, heat processing has been proven to decrease activities of some antinutritional factors (ie., trypsin inhibitors). In

rapeseed/canola seed processing, heat treatment can effectively inactivate the myrosinase enzyme and lower the glucosinolate content of the meal (Fenwick *et al.*, 1986; Smithard and Eyre, 1986; Slominski et al., 1987; Campbell and Slominski, 1990). In addition, most of the trypsin inhibitor activity present in the original product could be destroyed during various heat processes. It is generally agreed that the effectiveness of heat treatment on the nutritive value of feedstuffs depends on a combination of heating time, temperature, initial moisture content and particle size. Tempering the canola type *S. alba* seed to 20 % moisture content and micronization at temperatures of up to 140°C was found to increase protein digestibility *in vitro* and to lower the activity of myrosinase enzyme (Kienzle, 1998). In addition, micronization and autoclaving of *S. alba* seed improved true metabolizable energy (TMEn) content for cockerels (Kienzle, 1998).

It should be pointed out that the extent of grinding during seed processing would influence the nutritive value of feedstuffs. In general, " the more finely ground fat-containing raw materials are, the more highly digestible the fat fraction and the higher the material's ME value " (McNab, 1982). In studies on the effect of particle size on apparent digestibility of organic matter, crude protein, fat and apparent metabolizable energy (AMEn) content of rapeseed, it was shown that a reduction in particle size significantly increased nutrient utilization by broiler chickens and laying hens (Danicke *et al.*, 1998). Therefore, it would appear that fine grinding improves the nutritive value of feeds as a result of a more complete destruction of the cell wall structure and release of nutrients otherwise encapsulated within the cells.

2. Manuscript One

Chemical Composition and Nutritive Value of Yellow-seeded *Brassica napus* Canola for Poultry

2.1. INTRODUCTION

Plant selection programs directed toward development of yellow-seeded *Brassica rapa* (formerly *Brassica campestris*) rapeseed/canola have been underway for quite some time in Canada. Seeds of yellow-seeded varieties of *B. rapa* have been shown to be significantly higher in oil and protein contents. Thinner hulls were reported to be directly responsible for the lower fiber content of yellow-seeded *B. rapa* (Stringam *et al.*, 1974). In addition, the yellow hulls have been found to be low in polyphenols and lignin (Theander *et al.*, 1977), crude fiber (Daun and DeClercq, 1988) and have been shown to contain less neutral detergent fiber than brown hulls (Bell and Shires, 1982). Further research on *B. rapa* canola showed limited advantage of the yellow-seeded characteristic with regard to dietary fiber content and nutritive value of the meal (Slominski and Campbell, 1990; Slominski *et al.*, 1994a). Total fiber content was found to be only slightly lower than that estimated for black-seeded canola. Although, in comparison to black-seeded type, the *B. rapa* canola was shown to contain much less lignin and polyphenols, the cell wall polysacchride content was found to be much higher in this type of canola. As was recently documented in this laboratory, the small size of the cells in the cotyledons of yellow-seeded *B. rapa* canola was responsible for the overall high fiber content in this type of canola. The first commercially available meal from yellow-seeded *B. rapa* was found to contain comparable to black-seeded *B. napus* amounts of available energy and amino acids (Slominski *et al.*, 1994a; Slominski et al., 1994b). Further evaluation of *Brassica* seed meals conducted on a large number of *B.napus*, *B.juncea*, *B.carinata* and *B. rapa* samples demonstrated more favorable characteristics associated with the yellow seed coat color (Simbaya *et al.*, 1995). On average, when compared to black-seeded type, yellow-seeded canola contained more sucrose (8.7 % vs 7.5 %), more protein (44.5 % vs 42.7) and less dietary fiber (27.7% vs 33.6%). Further in vivo

evaluation has shown yellow-seeded *B. napus* canola to be superior to its black-seeded counterpart or other yellow- or black-seeded species with regard to true metabolizable energy content (9.71 MJ/kg DM) and the overall performance of broiler chickens (Slominski, 1997). Birds fed the yellow-seeded *B. napus* canola showed the lowest feed to gain ratio which averaged 1.51 and differed significantly from that of 1.59 and 1.61 for the commercial yellow-seeded *B. rapa* and the laboratory prepared black-seeded *B. napus* canola, respectively.

The objective of this investigation, which is a continuation of our research on the nutritive characteristics of yellow-seeded canola, was to compare a new yellow-seeded *B. napus* line with its black-seeded counterpart, both types originating from the same genetic background and produced in the same field under identical growing conditions in two consecutive years.

2.2. MATERIALS AND METHODS

2.2.1. Materials

The seed samples representing composited near-isogenic lines of yellow- and black-seeded *B. napus* canola grown in 1996 and 1997 crop years were provided by Agriculture and Agri-Food Canada, Research Station, Saskatoon, Saskatchewan. The "yellowness" of the 1996 and 1997 composites had an average Colorimeter reading of -22. For comparison, the seed of white mustard *Sinapis alba* can be viewed as having a -34 reading and a black-seeded cultivar of *B. napus*, AC Excel, having a reading of +1. The 1996 and 1997 black-seeded samples used in the current study averaged a reading of -1.7.

In preparation for analyses and in vivo evaluation, the seeds were crushed to pass through a 2 mm sieve and were extracted with n-hexane for 8 hours in a Kontes Macro Soxhlet apparatus (Fisher

Scientific, Edmonton, Alberta, Canada). Following drying under a fume-hood, the meals were re-ground to pass through a 1 mm sieve and were re-extracted with *n*-hexane for an additional 8 hours. The dried meals were moist heat treated at 108 ±1°C for 20 minutes in a laboratory cyclomatic thermal sterilizer (autoclave) and then dried overnight at 40°C. All of our meals showed the myrosinase activity below 0.05 unit/g (see section below for unit definition). For chemical analyses, the meals were re-ground to pass through a 1 mm sieve using a Tecator Cyclotec 1093 sample mill (Fisher Scientific).

2.2.2. Chemical Analysis

The meal samples were analyzed in duplicate for dry matter, crude protein (Kjeldahl N x 6.25), ether extract, ash and phosphorus using established methods of analysis (AOAC, 1990). Phytate phosphorus was determined by the method of Hang and Lantzsch (1983). Non-phytate phosphorus was calculated by difference between total and phytate phosphorus. Dietary fiber was determined by a combination of neutral detergent fiber (NDF) and neutral detergent-soluble nonstarch polysaccharide measurements and was calculated as the sum of NDF and detergent soluble NSP (Slominski *et al.*, 1994a). The method of Goering and Van Soest (1970) was used for the determination of NDF, except that the procedure was modified to exclude the use of decalin and sodium sulfite (Mascharanhas Ferreira et al. 1983). Non-starch polysaccharides (NSP) were measured 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 NSP content was determined in both the meals and the NDF residues. Neutral detergent-soluble NSP was calculated as total sample NSP minus NSP present in the NDF residue. Sucrose and galactooligosaccharides (raffinose and stachyose) were determined

by gas-liquid chromatography according to the procedure described by Slominski *et al.* (1994a). Starch was measured using the NSP procedure in which the treatment with dimethylsulfoxide to gelatinize starch was substituted by boiling the samples with water for 30 min. Starch hydrolyzing enzymes including α-amylase, pullulanase and amyloglucosidase were not added to the sample and the starch content was calculated as total sample glucose (no enzyme added) minus NSP glucose. Amino acids were analyzed by ion-exchange chromatography with the aid of a LKB Biochrom 4151 Alpha Plus amino acid analyzer (Biochrom, Science Park, Cambridge, UK) following hydrolysis of the samples with 6 N HCl at 110 C for 24 hours (Andrews and Baldar, 1985). Methionine and cystine were determined after 20 hours of oxidation with performic acid (Moore, 1963). Glucosinolates were analyzed by gas-liquid chromatography using the procedure of Thies (1977) with some modifications (Slominski and Campbell, 1987). Myrosinase activity of the meals was determined using the procedure for glucosinolate analysis and was calculated from difference between total sample glucosinolate and the glucosinolates remaining following incubation of the meal with distilled water at 40 °C for a defined period of time. One unit of myrosinase activity was expressed as the amount of enzyme that catalyses the hydrolysis of 1 *u*mol of glucosinolate per 1 min. Except for ether extract, phytate and NDF contents which were determined in triplicate, all other chemical analyses were performed in duplicate.

2.2.3. Animal Experiment

The nutritive value of the meals was evaluated in a two-week feeding trial with broiler chickens. One-day old male Arbor Acre broiler chickens, vaccinated against Marek's disease, were obtained from a local commercial hatchery. During the first 4 days, the birds were housed in thermally controlled Jamesway brooder batteries and had free access to water and a commercial chick starter

diet. On day 4, the birds were fasted for 4 hours before being individually weighted 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 6 replicates (pens). From Day 4 to Day 18, the birds were fed experimental diets and had free access to water and feed and were provided with continuous light. Prior to the 10- and 18-day weighing, the birds were fasted for 4 h. Feed consumption was recorded for Weeks 1 and 2 in order to calculate weekly and overall feed intakes and feed efficiency values.

Composition and calculated analyses of experimental diets are shown in Table 8. In diet formulation, the energy values assigned to all canola meals and wheat were 2000 kcal/kg and 3150 kcal/kg, respectively (National Research Council, 1994). Crude protein, amino acids and available phosphorus contents of the canola meals were based on the determined values.

2.2.4. Statistical Analysis

All collected data were subjected to analysis of variance (ANOVA) using the general linear models of statistical analysis system (SAS Institute, 1985). Student-Newman-Keuls (SNK) test was used to compare and separate treatment means. The model was a completely randomized design with six pens of five birds in each of four treatments and each pen was the experimental unit.

2.3. RESULTS AND DISCUSSION

The chemical composition of the *Brassica napus* meal samples obtained from 1996 and 1997 crop years is shown in Table 9. In comparison to black-seeded samples, both yellow *B. napus* samples contained more protein, more sucrose and less dietary fiber. The difference in protein content between yellow- and black-seeded samples was not as pronounced as it was observed in our earlier

Table 8. Composition and calculated analyses of *Brassica napus* seed meal diets used in the broiler chicken growth trial (4-18 days of age)

Ingredient	*B. napus* seed meal diet			
	Yellow - 96	Black - 96	Yellow - 97	Black - 97
Wheat (15.5%)	54.85	52.69	58.27	57.40
Brassica Meal	34.10	36.00	31.10	31.90
Vegetable oil	7.00	7.30	6.50	6.60
Limestone[1]	1.45	1.43	1.49	1.48
Biophosphate[2]	1.10	1.08	1.14	1.12
Vitamin premix[3]	1.00	1.00	1.00	1.00
Mineral premix[4]	0.50	0.50	0.50	0.50
Total	100.00	100.00	100.00	100.00
Calculated Composition[5]				
CP, %	22.00	22.00	22.00	22.00
ME, kcal/kg	3049	3047	3050	3048
Calcium, %	1.00	1.00	1.00	1.00
Available P., %	0.45	0.45	0.45	0.45
Lysine, %	0.969	1.007	0.946	0.990
Methionine, %	0.448	0.452	0.443	0.448
Cystine, %	0.520	0.506	0.521	0.503

[1] Contained 380 g calcium per kilogram; [2] Contained 180 g calcium and 210 g phosphorus per kg; [3] Vitamin premix provided per kg of diet: vitamin A, 8250 IU; vitamin D_3, 1000IU; vitamin E, 11IU; thiamin, 0.012 mg; vitamin B_{12}, 0.012mg; vitamin K, 1.1 mg; niacin, 53 mg; choline, 1020 mg; folic acid, 0.75 mg; biotin, 0.25 mg, riboflavin, 5.5 mg; [4] Mineral premix provided per kilogram of diet: manganese, 55 mg; zinc, 50 mg; iron, 80 mg; copper, 5 mg; selenium, 0.1 mg; iodine, 0.36 mg; sodium 1.6 g.; [5] Crude protein, amino acids and available phosphorus were calculated based on analysed data; NRC (1994) feed composition data were used to calculate energy and calcium contents.

Table 9. Chemical composition of meals derived from yellow- and black-seeded *Brassica napus* canola from 1996 and 1997 crop years (% of dry matter)

Component	*B. napus* seed meal			
	Yellow - 96	Black - 96	Yellow - 97	Black - 97
Crude protein	44.9 ± 0.09[1 c]	43.6 ± 0.11 [d]	47.4 ± 0.31[a]	46.8 ± 0.23 [b]
Sucrose	9.3 ± 0.03 [a]	8.0 ± 0.17 [c]	8.7 ± 0.16 [b]	7.5 ± 0.08 [d]
Oligosaccharide	3.4 ± 0.07[a]	3.0 ± 0.11[a]	3.4 ± 0.23 [a]	3.2 ± 0.18 [a]
Starch	0.3 ± 0.24 [a]	0.6 ± 0.30 [a]	0.6 ± 0.41 [a]	0.2 ± 0.24 [a]
Dietary fiber	27.3 ± 0.9 [c]	32.0 ± 0.3 [a]	25.8 ± 0.4 [d]	29.5 ± 0.3 [b]
Ash	7.3 ± 0.01[a]	7.2 ± 0.04 [b]	6.2 ± 0.03 [c]	6.1 ± 0.04 [c]
Fat	3.2 ± 0.16 [ab]	2.9 ± 0.03 [b]	3.6 ± 0.25 [ab]	3.9 ± 0.32 [a]

[1] Mean ±SD, [a,b,c,d] Values within rows with no common superscripts differ significantly ($P \leq 0.05$).

studies (Simbaya *et al.*, 1995), although similar to that reported recently for yellow- and brown-seeded B. napus canola (Slominski et al., 1999). In the current study, the Yellow-97 sample with the lowest dietary fiber level had the highest protein content (47.4 %), while the Black-96 sample with the highest dietary fiber level had the lowest protein content (43.6 %). This agrees with the finding of protein being negatively correlated with the fiber content (Simbaya *et al.*, 1995). Sucrose content was higher in yellow-seeded samples by 1.3 percentage points and was almost identical with that reported for yellow-seeded *B. napus* and *B. juncea* canola (Simbaya *et al.*, 1995, Slominski *et al.*, 1999) and differed significantly from our earlier data showing 3-4 percentage points higher sucrose content in yellow-seeded *B. rapa* canola (Slominski *et al.*, 1994a). Since sucrose is a highly digestible carbohydrate, relatively more sucrose in yellow-seeded canola would increase the digestible energy content of the meal. The oligosaccharide content of the meals from both yellow- and back-seeded samples were similar. Oligosaccharides, including raffinose, stachyose and verbascose, were found to be poorly digested in the small intestine of poultry and were reported to interfere with energy digestibility by increasing the rate of feed passage (Coon *et al.*, 1990; Leske *et al.*, 1993). However, recent studies indicated a minimal effect of canola meal oligosaccharides on energy digestibility in poultry (Slominski *et al.*, 1994b). Dietary fiber content in yellow-seeded samples differed, on average, by 4.2 percentage points from that of the black-seeded type. The difference between the highest fiber level in the black sample from 1996 (32 %) and the lowest fiber level in the yellow sample from 1997 (25.8 %) was up to 6.2 percentage points and was identical with that reported for a number of yellow- and brown-seeded samples of canola (Simbaya *et al.*, 1995). Although the non-starch polysaccharide content in yellow-seeded samples was found to be similar to that of the black type, other fiber components, which based on our earlier research constitute lignin with associated

Table 10. Fiber composition of meals derived from yellow- and black-seeded *Brassica napus* canola from 1996 and 1997 crop years (% of dry matter)

Component	*B. napus* seed meal			
	Yellow - 96	Black - 96	Yellow - 97	Black - 97
NDF [1]	20.3 ± 0.14 [2c]	25.3 ± 0.36 [a]	19.5 ● 0.16 [d]	23.2 ● 0.20 [b]
NDF-S NSP [3]	7.2 ● 0.35 [a]	6.9 ± 0.00 [a]	6.7 ± 0.07 [a]	6.4 ± 0.14 [a]
Total fiber	27.3 ± 0.90 [c]	32.0 ± 0.31 [a]	25.8 ± 0.42 [d]	29.5 ± 0.27 [b]
- NSP	18.5 ±1.56 [a]	18.7 ● 0.49 [a]	17.5 ● 0.71 [a]	17.8 ● 0.42 [a]
- Protein	3.07	3.17	2.77	2.81
- Ash	1.34	1.30	0.52	0.76
- Lignin and polyphenols	4.52 ± 0.10 [d]	9.05 ± 0.13 [a]	5.18 ● 0.04 [c]	8.16 ● 0.07 [b]
NSP component sugars (%)				
rhamnose	1.0 ± 0.16	1.2 ± 0.17	1.3 ± 0.16	1.4 ± 0.04
fucose	0.6 ± 0.08	1.1 ± 0.18	1.2 ± 0.21	1.3 ± 0.04
arabinose	28.2 ± 2.32	28.1 ± 1.57	30.0 ± 1.99	28.2 ± 0.68
xylose	11.2 ± 1.04	10.2 ± 0.37	12.0 ± 0.63	10.5 ± 0.00
mannose	2.9 ± 0.12	2.8 ± 0.07	3.2 ± 0.05	2.9 ± 0.07
galactose	10.2 ± 0.76	10.1 ± 0.28	10.4 ± 0.43	10.1 ± 0.08
glucose	26.7 ± 3.51	29.3 ± 1.29	26.5 ± 1.75	28.3 ± 0.76
uronic acids	19.5 ± 0.73	17.2 ± 0.57	15.7 ± 0.97	17.8 ± 1.24

[1] Neutral detergent fiber; [2] Mean ● SD; [3] Neutral detergent-soluble non-starch polysaccharides;

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

polyphenols, cell wall protein and minerals, showed a major difference between yellow- and black-seeded *B. napus* samples (Table 10). The NSP component sugar profiles for both types of canola were similar. It become evident that the lower content of lignin with associated polyphenols in yellow-seeded canola meals could be attributed to the smaller amount of fiber present in this type of canola and is in agreement with some earlier work on fiber components of yellow-seeded canola (Theander *et al.*, 1977; Slominski *et al.*, 1994a; Simbaya *et al.*, 1995). Lignin and condensed polyphenols (tannins) may have an adverse effect on protein digestibility due to hydrophobic binding to amino acids (Murray et al., 1977; Nomani and Stansberry, 1982). In addition, the relatively low degree of lignification and/or saturation of the cell walls with tannins would be expected to improve the nutritive value of yellow-seeded canola.

Total glucosinolate content of *B. napus* seed meals was well below 30 μmol/g meal, which is the upper limit to qualify for canola status (Table 11). Although the seed samples were of the same genetic background, there was a significant difference in glucosinolate content between the yellow- and black-seeded types, regardless of the crop year. The Black 97 sample showed the highest glucosinolate value of 21.8 μmol/g meal, while the lowest value of 11.1 μmol/g meal was found in the Yellow-96 sample. The indole glucosinolates including glucobrassicin (3-indolylmethyl) and hydroxylucobrassicin (4-OH-3-indolylmethyl) are known to be susceptible to thermal degradation during processing (Campbell and Slominski, 1990). Their similar amounts in all meals indicates that the heat conditions applied for processing of the seed were comparable.

The results of individual amino acid analysis, as expressed in g per 16 g N, are presented in Table 12. All samples had similar lysine values and Yellow-97 sample showed higher cystine value than other meals.

Table 11. Glucosinolate content of meals derived from yellow- and black-seeded *Brassica napus* canola from 1996 and 1997 crop years (μmol/g meal)

Glucosinolate	*B. napus* seed meal			
	Yellow - 96	Black - 96	Yellow - 97	Black - 97
3-butenyl	1.95	2.94	3.39	4.46
4-pentenyl	0.42	1.03	0.86	1.48
2-OH-3-butenyl	4.05	6.82	7.37	10.59
2-OH-4-pentenyl	0.06	0.19	0.13	0.24
3-indolylmethyl	0.61	0.59	0.66	0.55
4-OH-3-indolylmethyl	4.00	3.76	4.42	4.43
Total	11.1 ± 0.2^{1d}	15.3 ± 0.5^{c}	16.8 ± 0.4^{b}	21.8 ± 0.3^{a}

[1] Mean ± SD; [a,b,c,d] Values within rows with no common superscripts differ significantly ($P \leq 0.05$).

Table 12. Amino acid composition of meals derived from yellow- and black-seeded *Brassica napus* canola from 1996 and 1997 crop years (g/16g N)

Amino acid	*B. napus* seed meal			
	Yellow - 96	Black - 96	Yellow - 97	Black - 97
Alanine	4.18 [a]	4.27 [a]	4.26 [a]	4.04 [a]
Arginine	5.07 [a]	5.26 [a]	4.99 [a]	4.97 [a]
Aspartic acid	7.91 [a]	7.97 [a]	7.60 [a]	6.98 [b]
Cystine	2.18 [c]	2.12 [d]	2.43 [a]	2.27 [b]
Glutamic acid	16.75 [b]	17.62 [b]	17.45 [b]	19.23 [a]
Glycine	5.04 [ab]	5.21 [ab]	5.41 [a]	4.88 [b]
Histidine	2.20 [a]	2.28 [a]	2.43 [a]	2.21 [a]
Isoleucine	2.65 [a]	2.99 [a]	3.34 [a]	2.35 [a]
Leucine	6.37 [a]	6.62 [a]	6.21 [a]	6.21 [a]
Lysine	5.46 [a]	5.53 [a]	5.26 [a]	5.30 [a]
Methionine	1.54 [b]	1.57 [ab]	1.62 [a]	1.57 [ab]
NH3	2.12 [ab]	2.13 [ab]	1.93 [b]	2.24 [a]
Phenylalanine	3.43 [a]	3.59 [a]	3.60 [a]	3.24 [a]
Proline	5.96 [ab]	5.74 [b]	6.19 [a]	6.15 [a]
Serine	4.58 [ab]	4.56 [ab]	4.69 [a]	4.45 [b]
Threonine	4.26 [a]	4.33 [a]	4.31 [a]	3.86 [a]
Tyrosine	2.22 [a]	2.24 [a]	2.19 [a]	2.05 [b]
Tryptophan	ND [1]	ND [1]	ND [1]	ND [1]
Valine	3.68 [a]	3.67 [a]	3.41 [ab]	3.27 [b]
Total	85.6 ± 0.86 [2]	87.7 ± 2.14	87.3 ± 1.10	85.4 ± 0.53

[1] Not detected; [2] Mean ± SD; [a,b,c,d] Values within rows with no common superscripts differ significantly ($P \leq 0.05$).

There was no significant difference in phytate phosphorus content between yellow- and black- seeded samples. However, Yellow-96 sample showed higher non-phytate phosporus value than that of Black-96 sample (Table 13).

There was a significant effect of environment on meal quality. As indicated for both yellow- and black-seeded *B. napus* canola grown in 1996 and 1997 (Table 9), some negative relationship between the protein and dietary fiber contents can exist regardless of the genetic makeup. Similar results were reported by Slominski (1997) for a well established canola variety grown under different environmental conditions. In his study the protein content of the seed produced at three different locations was 41.8, 43.8 and 46.4 % (fat free basis) and was reflected, at least to some extent, in the amount of fiber which averaged 32.1, 30.5 and 29.9%, respectively. In the current study, the effect of environment was also evident for other components with the seed samples produced in 1996, regardless of the seed coat color, showing significantly higher total and available phosphorus and lower glucosinolate contents (Tables 11 and 13). In this regard, factors such as water stress, nitrogen and sulphur fertilization, temperature, soil acidity and others may influence the composition of canola seed.

In the current study, the difference in fiber content between yellow- and black-seeded samples (ie., 4% on average) could not be accounted for by differences in the content of protein, sucrose or other components (ie., 2.5%). To investigate this further, the seed samples were subjected to analyses for oil and protein content with the assumption that the higher oil content in the yellow-seeded samples may be responsible for such a discrepancy. As indicated in Table 14, however, the oil content tended to be higher in the black-seeded samples. Some difference in oil content was somewhat offset by the protein content which, on average, was higher by 2.5% percentage points in

Table 13. Phosphorus content of meals derived from yellow- and black-seeded *Brassica napus* canola from 1996 and 1997 crop years (% of dry matter)

Component	*B. napus* seed meal			
	Yellow - 96	Black - 96	Yellow - 97	Black - 97
Total phosphorus	1.20 ± 0.07[1a]	1.21 ± 0.01[a]	0.84 ± 0.04[b]	0.92 ± 0.05[b]
Phytate phosphorus	0.41 ● 0.01[a]	0.48 ± 0.05[a]	0.20 ● 0.05[b]	0.30 ± 0.02[b]
Non-phytate phosphorus	0.78 ± 0.04[a]	0.72 ● 0.03[b]	0.64 ± 0.05[c]	0.62 ± 0.04[c]

[1] Mean ● SD; [a,b,c] Values within rows with no common superscripts differ significantly (P≤0.05).

Table 14. Fat and protein contents of *B. napus* seed of different color produced in 1996 and 1997 crop years (% DM, full fat basis)

Component	Yellow - 96	Black - 96	Yellow - 97	Black - 97
Fat	45.4 ± 1.1 [1a]	49.8 ± 1.1 [a]	44.4 ± 3.6 [a]	48.7 ± 1.3 [a]
Protein	24.1 ± 0.36 [b]	21.3 ± 0.40 [c]	25.9 ± 0.37 [a]	23.4 ± 0.37 [b]

1 Mean ± SD; [a,b,c] Values within rows with no common superscripts differ significantly ($P \leq 0.05$).

yellow-seeded samples. In general, the difference in protein content between yellow and black seeds was more pronounced than that seen in the corresponding meals (Table 9). This finding clearly indicates that following processing the yellow seed would yield more meal but less oil which may have some negative repercussions with regard to the economics of canola crushing. The pattern of protein distribution between the seed and meal samples may suggest that the difference in the content of some other components (ie., carbohydrates, minerals, glucosinolates) between the yellow and black seeds may be even more pronounced. On the other hand, such speculation may not be true since it is difficult to predict the distribution of such components between the cotyledons and the embryo or hull fractions and what the ratio of such fractions in yellow and black seeds would be.

The results of the two-week performance of broiler chickens fed *B. napus* seed meal diets are shown in Table 15. The chickens fed meals derived from yellow seeds had a significantly higher body weight gain and lower feed to gain ratio than those fed the meals from black-seeded samples. This is in agreement with earlier studies on yellow-seeded *B. napus* canola showing a feed to gain ratio of 1.51 being significantly different from that of 1.61 for the black-seeded type of *B. napus* canola (Simbaya, 1996). Higher sucrose, lower fiber and glucosinolate contents in yellow-seeded canola as compared to black-seeded type would appear to have a positive effect on nutrient utilization and broiler chicken performance. With the exception of the Yellow-97 seed meal diet demonstrating significantly higher feed intake value, there was no significant differences between the Yellow-96, Black-96 and Black-97 seed meal diets. It should be pointed out that in this study the Black-97 seed meal diet showed the poorest feed intake and body weight gain values. High aliphatic glucosinolate content in this particular meal might have had a detrimental effect on broiler chicken performance.

Table 15. Growth performance of broiler chickens (4-18 days of age) fed diets containing meals derived from yellow- and black-seeded *Bassica napus* canola from 1996 and 1997 crop years

Parameter	*B. napus* seed meal diet			
	Yellow - 96	Black - 96	Yellow - 97	Black - 97
Feed intake (g/bird)	589 ± 29.3 [1 ab]	561 ± 36.7 [bc]	624 ± 21.0 [a]	528 ± 30.6 [c]
Weight gain (g/bird)	384 ± 20.2 [a]	354 ± 31.2 [b]	411 ± 19.1 [a]	330 ± 18.3 [b]
Feed to gain ratio	1.54 ± 0.01 [a]	1.59 ± 0.04 [b]	1.52 ± 0.03 [a]	1.60 ± 0.03 [b]

[1] Mean ± SD; [a,b,c] Values within rows with no common superscripts differ significantly ($P<0.05$).

2.4. CONCLUSIONS

1. Detailed chemical evaluation of the meals derived from yellow- and black-seeded canola showed a new yellow-seeded *B. napus* line to contain more protein, more sucrose, less fiber and less glucosinolate than its black-seeded counterpart.

2. In comparison to the black-seeded type, improved weight gain and feed conversion ratio of broiler chickens fed yellow-seeded *B. napus* canola was observed.

3. There was a significant effect of environment (crop year) on meal quality as demonstrated by protein, sucrose, dietary fiber, ash, glucosinolate, phytate phosphorus and non-phytate phosphorus contents.

3. Manuscript Two

Canola-quality Yellow Mustard *(Sinapis alba* L.): The Effect of Water-soluble Fiber (mucilage) and Fineness of Grinding on the Nutritive Value of the Seed

3.1. INTRODUCTION

The *Sinapis alba* yellow mustard has potential as a high protein and high energy alternative to full fat soybean. Earlier research from this laboratory demonstrated that, in comparison to full fat soybean, low-glucosinolate, low-erucic acid *S. alba* seed contained more oil (26.4% vs 20.2%), less protein (37.5% vs 41.4%), more methionine and cystine (3.60 vs 3.33 g/16g N), less lysine (5.78 vs 6.49 g/16g N) and a similar amount of carbohydrate (ie., sucrose, starch)(4.6% vs 5.2%)(Kienzle, 1998). A lower content of oligosaccharides (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 positive characteristics associated with the *S. alba* crop. Micronization of the *S. alba* seed decreased glucosinolate content, inactivated the myrosinase enzyme, and increased *in vitro* digestible protein content. Micronized *S.alba* seed showed a higher TME_n (true metabolizable energy) value than the raw sample (2460 vs 2270 kcal/kg). The highest TME_n value (3790 kcal/kg) was observed for micronized soybean seed. Broiler chickens fed rations containing 15% of raw and micronized *S. alba* seed showed lower weight gain and higher feed to gain ratio to those fed micronized soybean seed. Glucosinolates and total and water-soluble fiber were identified as potentially antinutritive components of *S. alba* seed. Although substantially lower than that present in current condiment varieties, the glucosinolate content of the new *S. alba* cultivar was shown to be 35.3 μmol/g. In addition, Kienzle (1998) reported that on a full fat basis, the canola-quality yellow mustard contained more dietary fiber than *B. napus* canola and soybean (23.7%, 20.4% and19.8%, respectively). In the same study, the canola-quality *S. alba* was found to contain 1.7% water-soluble fiber (ie., mucilage) which was higher than that present in soybean (0.5%) or canola (0.5%). In studies by Cui *et al.* (1994), a crude mucilage was found to average 5 % with water-soluble and water-insoluble fractions

accounting for 55.6 % and 38.8 % respectively. Although mustard mucilage is widely used by the food industry (ie., salad dressings, food pastes), its viscous properties would be considered to be a limiting factor in monogastric animal feeding. It has been suggested that the relatively high soluble fiber content of *S. alba* seed may contribute to its low available energy value (TME) which, in comparison to full fat soybean, was found to be lower by 1330 kcal /kg DM (Kienzle, 1998).

The objectives of this research was to investigate any potential negative effect of water-soluble fiber (ie., mucilage) on broiler chicken performance. A viscosity reducing enzyme complex was employed to investigate the effect of *S. alba* mucilage on fat and energy utilization. Fineness of grinding of *S. alba* seed was used as a means of investigating the encapsulating effect of the cell walls on energy utilization by poultry.

3.2. MATERIALS AND METHODS

3.2.1. Materials

A seed sample of canola-quality *Sinapis alba* white mustard, a mixture composed of 12.7 % 1996 Scott Isolation, 32.7 % 1997 Scott Isolation and 54.6 % 1997 Saskatoon Isolation, was provided by Agriculture and Agri-Food Canada Research Station (Saskatoon, Saskatchewan). A micronized soybean seed was provided by InfraReady Products Inc. (Saskatoon, Saskatchewan). Commercial soybean meal was obtained from Feed-Rite (Winnipeg, Manitoba) and canola meal was obtained from a local crushing firm (CanAmera Foods, Altona, Manitoba). Wheat (cv. Katepwa) was acquired from the Glenlea Research Station, University of Manitoba.

Carbohydrase enzyme preparations A, B, C, D, E, G, I and Tc were provided by Finnfeeds (Marlborough, UK) while Biocellulase A20, ACH Conc., Biocellulase 2A, MAPase and TR cellulase

were from Quest International Ireland Ltd.(Kilnagleary, Carrigaline, Co. Cork, Ireland).

The sample of *S.alba* seed was micronized by Infra-Ready Products Inc. at 140 °C with enzyme added at 0.067% on tempering to 20% moisture content. For the animal experiment, samples of raw and micronized *S.alba* seed and micronized soybean seed were crushed to pass through a 2 mm sieve using a Wiley mill standard model No. 3 grinder (Arthur H. Thomas Company, Philadelphia, USA).

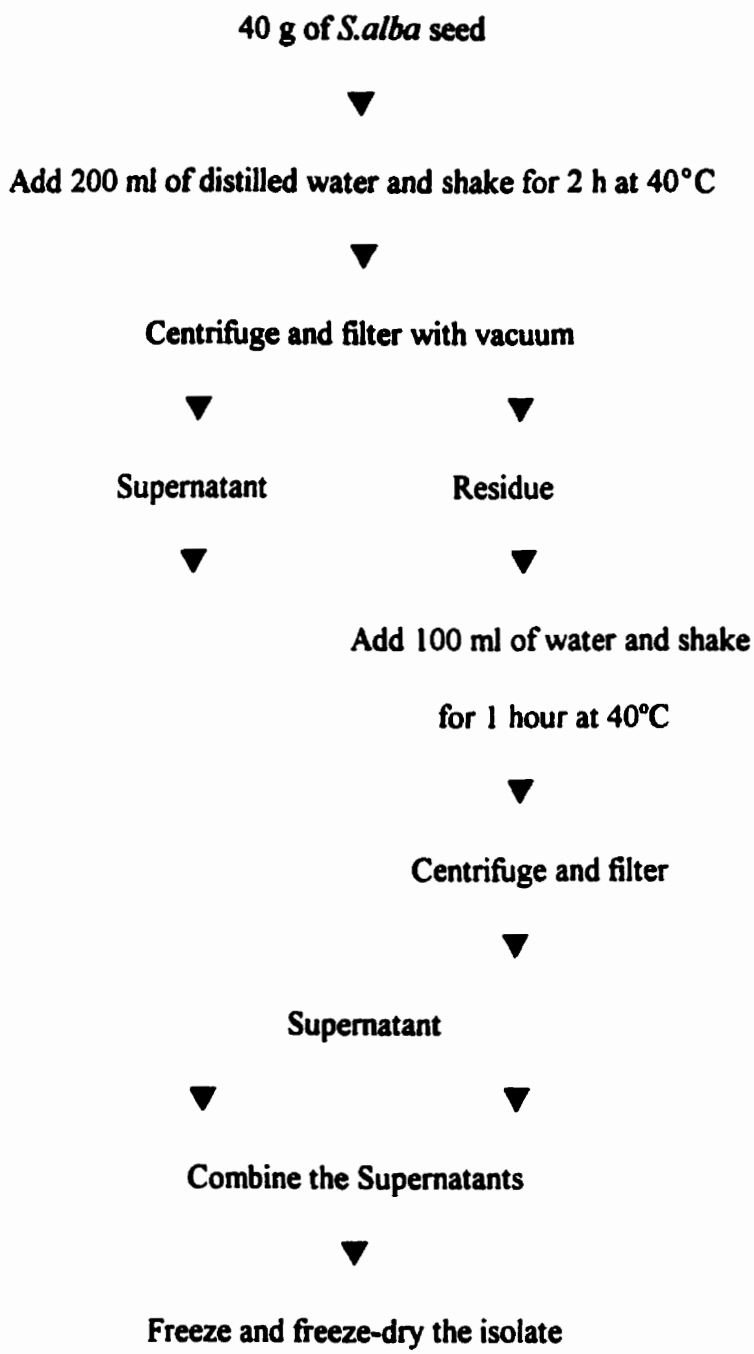
Yellow mustard mucilage was isolated from the whole *S.alba* seeds using the procedure summarized in Figure 1.

3.2.2. Chemical Analyses

The samples of raw and micronized *S.alba* seed, micronized soybean seed, commercial soybean and canola meals, and wheat were analyzed for crude protein content using the established standard method of analysis (AOAC,1990). Fat contents of micronized *S.alba* seed and soybean seed were determined by the AOAC method recommended for animal feed with the exception that the ether was substituted with n-hexane and the extraction time was extended to 6 hours. Diets and excreta samples from the broiler chicken trial were analyzed for chromium oxide using the procedure described by Williams et al.(1963). Gross energy was determined with the aid of an adiabatic bomb calorimeter (Parr Instrument Co., Moline, IL, USA).

Non-starch polysaccharides were determined by gas-liquid chromatography (component neutral sugars) and by colorimetry (uronic acids) using the procedures described by Englyst and Cummings (1984, 1988) with some modifications (Slominski and Campbell, 1990). To investigate the effect of H_2SO_4 concentration on the release of component sugars from *S. alba* mucilage, 40 mg of mucilage isolate was incubated for one hour, under occasional mixing, with 1 ml of 8, 9, 10, 11, 12, 13, 14 and 15 M H_2SO_4 at 35 °C. Six ml of water and 5 ml of myo-inositol (internal standard) solution were then

Figure 1. Flow Chart of Yellow Mustard Mucilage Isolation



added and the mixture was boiled for 2 hrs. One ml and 0.05 ml of the hydrolysate were then taken, respectively for neutral sugars and uronic acids determination by the NSP procedure. To evaluate the effect of H_2SO_4 concentration on recovery of sugar standards, 5 mg of each of rhamnose, arabinose, xylose, mannose, galactose, glucose and glucoronic acid were incubated for one hour, under occasional mixing, with 8, 9, 10, 11, 12, 13, 14 and 15 M H_2SO_4 at 35 °C, and were further processed using the NSP methodology.

3.2.3. In Vitro Evaluation of Carbohydrase Enzymes

To evaluate the mucilage depolimerizing enzymes, 40 mg of mucilage isolate was incubated for one hour, under occasional mixing, with 5 mg of enzyme preparation in 8 ml of sodium acetate buffer (pH 5.2) at 40 °C. Forty three ml of 95% EtOH was then added, the mixture left for 1 h, centrifuged at 3000 rpm for 20 minutes, the supernatants discarded and the residue dried and further processed using NSP methodology.

To evaluate the effect of enzyme addition on viscosity, 40 mg of mucilage isolate was incubated for one hour, under occasional mixing, with 5 mg of enzyme preparation in 8 ml of sodium acetate buffer (pH 5.2) at 40 °C. The solutions were cooled down and the viscosity determined using a Brookfield digital viscometer (Model DV-II+, Brookfield Engineering Laboratiries, Stoughton, MA, USA).

3.2.4. Animal Experiments

The nutritive value of raw and micronized *S. alba* seed with and without enzyme supplementation was evaluated in a two-week feeding trial with broiler chickens. One-day old male Arbor Acre broiler chickens, vaccinated against Marek's disease, were obtained from a local commercial hatchery. During the first 5 days, the birds were housed in thermally controlled Jamesway brooder batteries and

had free access to water and a commercial chick starter diet (Feed-Rite, Winnipeg, Canada). On Day 5, the birds were fasted for 4 hours before being individually weighted 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 5 to Day 19, the birds were fed experimental diets and had free access to water and feed and were provided with continuous light. Prior to the 12- and 19-day weighing, the birds were fasted for 4 h. Feed consumption was recorded for Weeks 1 and 2 in order to calculate weekly and overall feed intakes and feed efficiency values.

The experimental diets included two control and three test diets. Composition and calculated analyses of experimental diets are shown in Table 16. The control diets consisted of a conventional soybean meal/canola meal and a micronized full fat soybean seed. Three test diets included raw *S.alba* seed with and without carbohydrase B supplementation (0.01 %) and the micronized *S.alba* seed supplemented with the same enzyme at the same enzyme to seed ratio. In the formulation of experimental diets, the amounts of metabolizable energy assigned to the raw *S.alba* seed, micronized *S.alba* seed and micronized soybean seed were 3400, 3500 and 3500 kcal/kg, respectively. Crude protein contents of commercial soybean and canola meal, wheat, and *S.alba* seed were based on the determined values.

On Day 19 of the experiment, the excreta samples from each pen were collected, frozen, freeze-dried and pooled to obtain 4 samples per treatment. Both diets and excreta samples were analyzed for chromic oxide (internal marker), gross energy(GE), nitrogen (Kjeldahl) and fat content. Apparent dry matter and fat digestibilities and metabolizable energy (AME_n) contents were calculated as follows (Hill *et al.*, 1960):

Table 16. Composition and Calculated Analysis of Diets Used in the Broiler Chicken Trial (5-19 days)

	Diet				
Ingredient, %	1	2	3	4	5
Soybean meal (48%)	19.72	9.7	14.4	14.49	14.14
Canola meal (36.4%)	11.10	5.50	8.10	8.10	7.60
Wheat (15.5%)	59.34	62.90	54.53	54.53	55.78
Raw S.alba seed [1]			15.00	12.62	
Micronized S.alba seed [1]					15.00
Micronized soybean seed [1]		15.00			
Limestone [2]	1.62	1.65	1.50	1.50	1.50
Biophosphate [2]	1.28	1.32	1.27	1.27	1.27
DL-Methionine	0.08	0.09	0.07	0.07	0.07
Lysine	0.06	0.04	0.04	0.04	0.05
Vitamin [2]	1.00	1.00	1.00	1.00	1.00
Mineral [2]	0.05	0.05	0.05	0.05	0.05
Vegetable oil	5.00	2.00	3.20	3.20	2.80
Cr_2O_3	0.30	0.30	0.30	0.30	0.30
Enzyme premix [3]				2.38	
Total	100.00	100.00	100.00	100.00	100.00
Calculated composition					
ME, kcal/kg	3054.00	3050.00	3054.00	3054.00	3053.00
Crude protein, %	22.00	22.00	22.00	22.00	22.00
P available, %	0.45	0.45	0.45	0.45	0.45
Calcium, %	1.01	1.01	1.01	1.01	1.01
Lysine, %	1.06	1.06	1.07	1.07	1.06
Methionine, %	0.48	0.48	0.48	0.48	0.48
Meth.+Cyst., %	0.90	0.89	0.89	0.89	0.89

[1]The amounts of available energy assigned to raw *S.alba* seed, micronized *S.alba* seed and micronized soybean seed were 3400, 3500 and 3500 kcal/kg, respectively; [2] See Table 10 for detailed composition; [3] Containing 0.42% of carbohydrase B.

Dry matter (%) = [1 - (Cr_2O_3 % feed /Cr_2O_3 % excreta)] x 100

$$\text{Fat digestibility (\%)} = \frac{[\text{fat \% feed} - \text{fat \% excreta} \times (\text{Cr2O3 \% feed/Cr2O3 \% excreta})]}{\text{fat \% feed}}$$

AMEn (kcal/kg) = GE kcal/kg feed [GE kcal/kg excreta x (Cr2O3% feed/Cr2O3%excreta)]

-8.22 x {N kg/kg feed [N kg/kg excreta x (Cr2O3% feed / Cr2O3 % excreta)] }

At termination of the experiment, 10 birds per treatment were randomly selected for intestinal viscosity measurement. Each bird was killed by cervical dislocation and the abdominal cavity was exposed. The contents of small intestine form the Meckel's diverticulum to 1.5 cm prior to the ileal-cecal junction was collected into two 2 ml microcentrifuge tubes and centrifuged at 9000 rpm for 5 minutes. Viscosity was determined using the Brookfield digital viscometer (Model DV- II +, Brookfield Engineering Laboratories, Stoughton, MA.).

To investigate the effect of grinding on feeding quality of *S. alba*, the raw and micronized seed was ground to different particle size and was subjected to the true metabolizable energy (TMEn) determination using the assay described by Sibbald (1986) with modification (Zhang *et al.*, 1994). The coarse (≤ 2.0 mm with 21% of the sample past through 0.6mm sieve) samples were obtained by crushing the raw and micronized *S. alba* seeds to pass through a 2 mm sieve using a Wiley Mill Standard Model No. 3 grinder (Arhtur H. Thomas Company, Philadelphia, USA). To obtain the fine (≤ 0.6mm) samples, the coarse samples were further ground with the aid of a coffee grinder to pass through a 0.6 mm sieve. As compared to micronized *S. alba* seeds, raw *S. alba* seeds needed twice grinding time to reach the fine particle size. Each sample was precision-fed (30 g per bird) to 20 mature single comb white leghorn cockerels following a 28 hours fasting period. During the next 48 hours, the excreta from each bird was collected. The excreta samples were then frozen, freeze-dried,

ground in a coffee grinder and pooled for analysis of gross energy and nitrogen (Kjeldahl). The excreta from unfed birds (pooled excreta from 30 individual birds) was used to determine the endogenous excretion of energy and nitrogen.

3.2.5. Statistical Analysis

All collected data were subjected to analysis of variance (ANOVA) using the general linear models of statistical analysis system (SAS Institute, 1985). Student-Newman-Keuls (SNK) test was used to compare and separate treatment means.

3.3. RESULTS AND DISCUSSION

Figure 1 shows the procedure for water-soluble mucilage isolation from the whole seeds of *S. alba* mustard. Using this method, the yield of soluble mucilage was 2.03 % of the seed weight and was lower than that reported by Cui *et al.* (1994). In their study, the water-soluble fraction of crude mucilage was found to be 2.8 % of the seed weight. Such discrepancy may be attributed to varietal differences as well as the extraction methods. In a preliminary trial it was found that the yield of component sugars on hydrolysis of the mucilage isolate with 1 M H_2SO_4 was very low and averaged 14.7% (Table 17). Since crude protein accounted for only 6% of the isolate, it became apparent that the hydrolysis conditions were insufficient for the complete release of component sugars. Therefore, the effect of H_2SO_4 concentration (from 8M to 15M) on the yield of component sugars on hydrolysis of mucilage isolate was investigated. It should be pointed out that the hydrolysis of water-soluble polysaccharides in 1 M H_2SO_4 is a common practice in non-starch polysaccharide (NSP) analysis and

Table 17. Nonstarch polysaccharide (NSP) content of *S. alba* mucilage isolate[1] (%)

Hydrolysis Conditions	Total NSP
1M H_2SO_4	14.7
12M H_2SO_4	53.7
Optimal conditions (after correction)[2]	64.8

[1] Contains 6.0% crude protein. [2] Conditions under which the recovery of component sugars was maximal.

pre-treatment of the sample with H_2SO_4 of higher molarity (ie., 12 M) is primarily used to solubilize the cellulose molecule. Therefore, in the classical method of NSP determination (Englyst and Cummings, 1984 and 1988), pre-treatment with 12 M H_2SO_4 serves to further divide the NSPs into cellulose and non-cellulosic polysaccharides. From the data presented in Table 18, it became evident that the release of xylose, mannose, galactose and in particular glucose from polysaccharide molecules was incomplete in 8 M H_2SO_4 and increased substantially as the molarity of sulphuric acid increased up to 11-12. A further increase of sulphuric acid concentration resulted in substantial decomposition of all component sugars. The uronic acids were among components showing the highest degree of decomposition at relatively low concentrations of H_2SO_4 (ie., 9-10). This was further substantiated in a study on the effect of H_2SO_4 concentration on sugar standards recovery. As evidenced by data presented in Table 19, a significant decomposition of free sugars occurred in 12 M H_2SO_4. Therefore, incomplete hydrolysis of polysaccharides at low acid concentration (ie., 8M) and decomposition of monosaccharides at higher acid concentration (ie., 12 M) both contributed to underestimation of the total component sugar (or total NSP) content of mucilage isolate. Except for 8M H_2SO_4, glucose appeared to be the predominant sugar component of *S. alba* mucilage and was followed by galatose, uronic acid, mannose, arabinose, rhamnose and xylose. The sugar profile obtained in this study is in agreement with the result of earlier research on water-soluble mucilage by Cui *et al.* (1993a). The same group of researchers also reported that the total component sugar content of mustard mucilage accounted for 66.1 % and indicated that pre-treatment of the samples with 72% H_2SO_4 (=12 M) was essential for the complete release of glucose (Cui *et al.*, 1993a). In the current study, the total NSP content of mucilage isolate subjected to hydrolysis with 12M H_2SO_4 was 53.7 %, while the value calculated based on the maximal yield of component sugars (see Table 18 for details) was found to

Table 18. Effect of H_2SO_4 concentration on component sugar content of *Sinapis alba* mucilage isolate (mg/g)[1]

H_2SO_4 molarity	Rhamnose	Arabinose	Xylose	Mannose	Galactose	Glucose	Uronic acid	Total
8 M	23.5[2]	30.5	13.2	39.6	129.3	53.5	179.3	468.9
9 M	21.2	31.1	13.9	49.3	130.6	179.5	129.1	554.7
10 M	17.7	31.8	14.7	49.9	137.2	193.6	116.0	560.9
11 M	17.8	34.7	16.3	52.1	148.1	193.4	113.1	575.5
12 M	12.4	33.3	12.3	45.2	141.5	194.2	98.4	537.3
13 M	14.7	32.7	10.3	41.3	131.9	183.9	91.9	506.7
14 M	12.5	28.1	4.4	32.9	119.4	174.1	87.7	459.1
15 M	6.9	21.5	0.6	21.5	97.7	154.2	62.9	365.3

[1] The samples were hydrolyzed at 35 °C for one hour; [2] Underlined numbers represent the values used in making final calculations for total NSP content of *S. alba* mucilage.

Table 19. Effect of H_2SO_4 concentration on percent recovery of sugar standards

H_2SO_4 Molarity	Rhamnose	Arabinose	Xylose	Mannose	Galactose	Glucose	Uronic acids
8 M	95	93	96	100	101	102	95
9 M	99	94	95	102	103	104	92
10 M	98	93	96	102	103	105	87
11 M	93	91	91	99	100	102	71
12 M	84	86	84	93	97	99	70
13 M	70	79	70	87	94	98	57
14 M	50	70	44	77	90	93	52
15 M	33	55	28	70	85	91	50

be 64.8 % (Table 17). This allowed us to re-evaluate our earlier data on water-soluble fiber content of *S. alba* seed. When extracted under physiological conditions of the GI tract and subjected to hydrolysis with 12 M H_2SO_4, the mucilage content averaged 3.35% (fat free basis) and was significantly higher than that reported earlier (ie., 2.6%; Kienzle, 1998).

The effect of various enzyme preparations on depolymerization of *S.alba* mucilage is shown in Table 20. The highest degree of depolymerization was observed for Biocellulase 2A enzyme (22.3 %) and was followed by Carbohydrase D (20.2 %), Carbohydrase Tc (18.9 %) and TR cellulase (17.1 %). All four preparations were claimed by the manufacturers to contain cellulase as the main activity. Biocellulase 2A in combination with TR cellulase (1:1 w/w) was found to be the best enzyme cocktail and showed the highest degree of mucilage depolymerization (25.4 %)(Table 20). The fact that all four cellulases showed a substantial degree of mucilage depolymerization, with glucose being a predominant component sugar disappearing from the incubation medium, indicated that some form of "soluble" cellulose is one of the major components of *S. alba* mucilage. In this regard, the glucose content as determined under optimal conditions of acid hydrolysis (see Table 18) accounted for 30% of the total NSP content. Another indication of cellulose presence in mustard mucilage could be derived from earlier studies by Cui *et al.* (1993b) who reported 1,4-linked β-D-glucose polymer to be a predominant component of the water- and 5% CTAB (hexadecyltrimethylammonium bromide)-soluble fraction of mustard mucilage.

The enzymes and the combinations of enzymes showing over 10 % degree of mucilage depolymerization were evaluated further for their ability to reduce the viscosity of *S.alba* mucilage (Table 21). Carbohydrases B and C were found to be more effective in reducing the viscosity than the cellulase preparation. This would indicate that pectic polysaccharides rather than the cellulose-

Table 20. Screening for Mucilage Depolymerizing Enzymes

Enzyme [1]	Degree of mucilage depolymerization, % [2]
Carbohydrase A	4.5
Carbohydrase B	12.1
Carbohydrase C	12.4
Carbohydrase D (cellulase)	20.2
Carbohydrase E	4.7
Carbohydrase G	6.0
Carbohydrase I	11.2
Carbohydrase Tc (cellulase)	18.9
Carbohydrase D + C (1:1 w/w)	18.1
Carbohydrase D + B (1:1 w/w)	21.9
Carbohydrase D + I (1:1 w/w)	20.3
Biocellulase A20	7.3
ACH Conc. (cellulase)	12.7
Biocellulase 2A (cellulase)	22.3
MAPase	8.2
TR Cellulase (cellulase)	17.1
Biocellulase 2A + TR Cellulase (1:1 w/w)	25.4

[1] Added at 12.5%; [2] Represents the amount (% of control) of low-molecular weight polysaccharides soluble in 80% EtOH.

Table 21. Screening for viscosity reducing enzymes

Enzyme	Viscosity of *S. alba* mucilage, CPs
None	8.1
Carbohydrase B	3.1
Carbohydrase C	3.2
Carbohydrase D	8.1
Carbohydrase I	6.5
Carbohydrase TC	6.8
Carbohydrase D + B (1:1 w/w)	4.8
Carbohydrase D + I (1:1 w/w)	6.4
Carbohydrase D + TC (1:1 w/w)	5.5
Biocellulase 2A	7.2
TR Cellulase	5.7
Biocellulase 2A + TR Cellulase (1:1 w/w)	5.3

Table 22. The effect of enzyme concentration on the viscosity of *S. alba* mucilage

Enzyme	Enzyme concentration, %	Viscosity, CPs
None	-	10.7
Carbohydrase B	12.0	4.3
Carbohydrase B	6.0	3.0
Carbohydrase B	2.4	3.2
Carbohydrase C	12.0	3.5
Carbohydrase C	6.0	3.8
Carbohydrase C	2.4	4.0
Carbohydrase B + C (1:1 w/w)	12.0	4.1
Carbohydrase B + C (1:1 w/w)	6.0	3.6
Carbohydrase B + C (1:1 w/w)	2.4	3.0

like polymers are responsible for viscous properties of yellow mustard mucilage. Carbohydrases B and C and their combination were further evaluated to determine the most effective enzyme to substrate ratio and were used at 2.4%, 6.0% and 12.0 % of the mucilage isolate. The results presented in Table 22 indicated that carbohydrase B had the greatest effect on viscosity reduction at 2.4 % and 6.0 % inclusion rates. Thus, carbohydrase B at the dietary inclusion rate of 0.01% was chosen for further in vivo studies on the effect of *S. alba* mucilage on broiler chicken performance.

The results of the two-week growth trial with broiler chickens fed diets containing raw and micronized *S.alba* seeds are shown in Table 23. Relative to the control diet, there was a significant reduction in the body weight gain of chickens fed diets containing micronized soybean seed, raw mustard seed as well as both raw and micronized seeds supplemented with the enzyme. As compared to the raw *S.alba* seed diet, two enzyme supplemented diets showed significantly better feed to gain ratios indicating that viscosity reduction may have had a positive effect on nutrient utilization. However, this was not substantiated by the intestinal viscosity values (Table 24). Although both enzyme supplemented diets demonstrated lower viscosity values, the difference was not statistically significant. In addition, all three yellow mustard diets showed significantly lower values for dry matter (DM) and fat digestibilities and AME_n content than the control or micronized soybean seed diets. As compared to the raw *S.alba* seed diet, both enzyme supplemented diets showed the reduction in digesta viscosity and improvement in dry matter digestibility and AME_n content. However, only the differences in dry matter digestibility and AMEn content between micronized *S.alba* seed diet supplemented with enzyme and raw *S.alba* seed diet were statistically significant. Both enzyme supplemented diets had significantly higher fat digestibilities than raw *S.alba* seed diet. Both control and micronized soybean diets were superior with regard to fat digestibility and AME_n

Table 23. The effect of micronization and enzyme supplementation on broiler chicken performance (5-19 days)

Treatment	Body weight gain (g/bird)	Feed intake (g/bird)	Feed to gain ratio
Control	496 ± 19.95 [a]	713 ± 27.19 [a]	1.44 ± 0.02 [d]
Micronized soybean seed (15%)	456 ± 28.20 [b]	683 ± 43.69 [ab]	1.50 ± 0.03 [c]
Raw *S.alba* seed (15%)	425 ± 23.50 [c]	672 ± 27.47 [b]	1.58 ± 0.04 [a]
Raw *S.alba* seed (15%) + Enzyme[1]	448 ± 23.48 [bc]	680 ± 29.20 [ab]	1.52 ± 0.05 [bc]
Micronized *S.alba* seed (15%) + Enzyme[1]	446 ± 21.77 [bc]	688 ± 23.58[ab]	1.54 ± 0.03 [b]

[1] Carbohydrase B added at 0.01%; [abcd] Values within columns with no common superscripts differ significantly ($P \leq 0.05$).

Table 24. The effect of micronization and enzyme supplementation on digesta viscosity, dry matter (DM) and fat digestibilities, and AMEn content of experimental diets fed to broiler chickens

Treatment	Viscosity,	Digestibility, %		AME_n
	CPs	DM	Fat	kcal/kg diet
Control	4.9 ± 1.59 [b]	70.2 ± 0.68 [a]	84.7 ± 1.71 [a]	3047 ± 29.2 [a]
Micronized soybean seed (15%)	5.2 ± 0.93 [b]	70.5 ± 2.59 [a]	78.5 ± 0.28 [b]	3027 ± 108.0 [a]
Raw *S.alba* seed (15%)	8.2 ± 2.55 [a]	61.5 ± 1.93 [c]	53.8 ± 2.98 [e]	2728 ± 66.5 [c]
Raw *S.alba* seed (15%) + Enzyme[1]	6.6 ± 1.78 [ab]	62.6 ± 1.73 [bc]	59.1 ± 1.86 [d]	2778 ± 80.7 [bc]
Micronized *S.alba* seed (15%) + Enzyme[1]	6.8 ± 2.49 [ab]	65.0 ± 1.76 [b]	69.5 ± 0.84 [c]	2882 ± 72.2 [b]

[1] Carbohydrase B added at 0.01%; [abcd] Values within columns with no common superscripts differ significantly ($P \leq 0.05$).

content. It is a well known fact that glucosinolates may have a negative effect on broiler chicken performance. The level of glucosinolates has been shown to be negatively correlated with AMEn content and ileal protein digestibility in broiler chickens fed rapeseed meal-based diets (Classen *et al.*, 1991; Newkirk *et al.*, 1997). The viscosity may also affect nutrients, especially protein and fat digestibilities (Marquardt, 1997; Graham et al., 1993). The combination of a relatively high glucosinolate and viscous polysaccharide content of raw *S.alba* seed diet could be responsible for the low AME_n value, fat digestibility and consequently body weight gain and feed conversion ratio.

Micronization, a dry-heat process utilizing infrared electromagnetic short waves, has been reported to improve the nutritive value of cereal grains, full fat soybeans and faba beans for growing pigs and chickens (Hutton and Foxcroft, 1975; McNab and Wilson, 1974). In the current study, micronization, combined with enzyme supplementation, showed the greatest positive effect on AME_n content and fat digestibility. However, it is unclear what positive effect the heat treatment had on various components of mustard seed. In an earlier study from this laboratory, it was demonstrated that micronization of *S. alba* seed decreased glucosinolate content, effectively inactivated the myrosinase enzyme, and increased digestible protein content *in vitro* (Kienzle, 1998). The micronized *S. alba* seed, however, showed significantly lower TMEn value than the same sample autoclaved under optimal heat-moisture conditions (ie., 3031 vs 2460 kcal/kg). In the current study, the difference in fat and energy utilization by broiler chickens fed raw and micronized seed was observed, although both diets were supplemented with the same enzyme preparation. All these facts indicate that the low available energy content of *S. alba* seed may result from an ineffective crushing of the seed and insufficient disruption of the oil containing cells in the gizzard with part of the oil escaping digestion in the small intestine. In addition, various heat treatments employed in processing of *S. alba*

seed (Kienzle, 1998; current study) may have influenced the degree of cell wall disruption on grinding and resulted in different energy and, to some extent, amino acid utilization. As suggested by Kienzle (1998), it is possible that with no heat the cell walls withstand the physical force of grinding to a much greater extent than the heat treated material. Since the micronized soybean seed used in the current study was ground under identical conditions, the small cell size within the cotyledon fraction may have been a factor influencing the effectiveness of grinding. To investigate these hypotheses further, both raw and micronized mustard seed were subjected to two grinding procedures, each resulting in different fineness of the seed meal. The TME_n values of variously ground (ie., fine and coarse) *S. alba* seed are shown in Table 25. A distinct increase in TME_n content was observed when the particle size of the seed decreased from ≤2.0 mm to ≤0.6 mm. In addition, the overall higher TME_n values for micronized seed, regardless of grinding procedure, also indicated that heat treatment may have facilitated the effectiveness of grinding. Some cell fracture may have also occurred during the micronization process in a manner similar to that documented in earlier studies on the micronized canola seed (M. Pickard, InfraReady Products Inc., personal communication). A highly significant effect of particle size on apparent digestibility of nutrients by broiler chickens and laying hens fed full fat rapeseed has been reported in the course of the current study (Danicke *et al.*, 1998).

3.4. CONCLUSIONS

1. Incomplete hydrolysis of polysaccharides at low H_2SO_4 concentration (ie., 1M) and decomposition of monosaccharides at high H_2SO_4 concentration (ie., 12M) both contributed to significant underestimation of the total component sugar (or total NSP) content of *S. alba* mucilage.
2. Some form of "soluble" cellulose was identified as the major component of *S. alba* mucilage.

Table 25. Effect of grinding on TMEn of *Sinapis alba* seed

Sample	Particle size	TMEn (kcal / kg)
Raw seed (fine)	≤ 0.6 mm	3475.9 ± 152.8 [1 b]
Raw seed (coarse)	≤ 2.0 mm [2]	2087.1 ± 230.4 [d]
Micronized seed (fine)	≤ 0.6 mm	3673.6 ± 208.3 [a]
Micronized seed (coarse)	≤ 2.0 mm	2891.3 ± 167.1 [c]

[1] Mean ± SD; [a,b,c,d] Values within a column with different superscripts differ significantly (P≤0.05);

[2] 21% of the sample passed through 0.6mm sieve.

However, pectic polysaccharides rather than the cellulose-like polymers appeared to be responsible for the viscous properties of yellow mustard mucilage.

3. In comparison to the conventional soybean /canola meal diet (control) or micronized soybean seed diet, feeding the raw *S. alba* seed to broiler chickens resulted in overall poorer performance and lower nutrient digestibility. However, the enzyme supplemented raw and micronized *S. alba* seed diets showed improved energy utilization and significantly better feed to gain ratio.

4. Fine grinding (ie., particle size ≤ 0.6mm) increased the TMEn content of *S. alba* seed presumably due to effective disruption of the oil containing cells. In addition, the overall higher TMEn values for micronized seed indicated that heat treatment may have facilitated the effectiveness of grinding.

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