THE EFFECTS OF PROCESSING ON THE NUTRITIONAL VALUE OF CANOLA MEAL FOR BROILER CHICKENS

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ABSTRACT

The effect of commercial prepress-solvent extraction of canola on the nutritional value of canola meal was studied in broiler chicks. In addition, methods of determining the digestible amino acid content of canola meal *in vitro*, the effects of canola genotype on susceptibility to heat damage during processing, and the feeding value of non-toasted canola meal were determined. Desolventization/toasting reduced the content and digestibility of amino acids in canola meal, especially Lys. In a survey of canola meals from plants across western Canada, non-toasted meal was shown to be of higher and more consistent nutritional value than toasted meals, suggesting that the toasting process should be avoided. Reducing the temperature and duration of desolventization/toasting also resulted in higher quality meals. Desolventization without added moisture resulted in a light-coloured, non-toasted meal with the highest nutritional value. Feeding this non-toasted meal resulted in improved weight gain and feed conversion than did feeding a toasted canola meal, indicating that the non-toasted meal was superior to the toasted product. Toasting reduced the glucosinolate levels in the meal but resulted in larger livers and poorer performance when fed to broiler chickens suggesting the non-toasted meal was less toxic. Lys digestibility of canola meal in broiler chickens was not accurately predicted by *in vitro* determination of protein solubility in 0.5% KOH, the assay currently used by the canola industry. The neutral detergent insoluble nitrogen (NDIN) content of canola meal was correlated with Lys digestibility, suggesting it could be used as an indicator of the nutritional value of canola meal. Near infrared reflectance spectroscopy was the most accurate predictor of the content and digestibility of the key amino acids in canola meal. Canola genotype affected NDIN content both before and after toasting, suggesting it may be possible to select varieties that are less susceptible to damage during toasting. In conclusion, toasting of canola meal reduces the nutritional value of canola meal and reduces broiler performance and should therefore be eliminated. However, further studies are required to establish methods of producing non-toasted meal commercially and to determine the nutritional value of non-toasted meal for other species.

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LIST OF ABBREVIATIONS

AME, apparent metabolizable energy

AME_n, apparent metabolizable energy, nitrogen corrected

CM, canola meal

CP, crude protein

DT, desolventizer/toaster

g/16 g N, grams of amino acid per 16 grams of nitrogen

NDIN, neutral detergent insoluble nitrogen

NIRS, near infrared spectroscopy

NTCM, non-toasted canola meal

SE, standard error of the mean

SEM, pooled standard error

SBM, soybean meal

TCM, toasted canola meal

T_3, triiodothyronine

T_4, thyroxine

1 INTRODUCTION

Canola, the low glucosinolate, low erucic acid form of rapeseed (*Brassica napus* or *Brassica rapa*) is an important oilseed crop in western Canada. Growing conditions in the region are ideally suited to canola production and, as a result, canola is commonly included in crop rotations. Canola oil has excellent nutritional value, due to its low saturated fatty acid content, making it the oil of choice in many applications. The seed contains approximately 42% oil and 58% meal. As a result of the high content and value of the oil, the primary focus in the past has been to maximize the value of the seed through its oil content. The meal has an excellent balance of amino acids, low levels of glucosinolates, and is used in all species of agricultural animals as a source of protein. However, canola meal sells at a discount relative to soybean meal. Canola meal contains 75% of the protein content of soybean, yet may sell at 55-65% of its price.

In 2000, 7.1 million tonnes of canola were grown in Canada, which, after crushing, would yield approximately 4.1 million tonnes of meal. With an average price of $156/tonne and assuming an average 10% discount relative to its protein content, the product was discounted $64 million dollars in 2000 alone. A significant improvement in the value of the seed could be realized if this discount were eliminated, but this would require an understanding of the reasons for the relatively low value of the meal.

Factors that might affect the value of canola meal in non-ruminant species include the concentration of nutrients (energy and protein), the digestibility of amino acids and the consistency of the meal. The concentration of nutrients in canola meal is affected to a large degree by its fibre content, which is higher than that of soybean meal. However, the reasons for the low digestibility of amino acids and the less consistent nature of the meal are unknown. Seed genotype and growing conditions can affect seed composition and, therefore, affect meal quality. Similarly, oil removal from canola and processing of the meal include numerous steps that could affect meal quality. Both seed and processing effects on meal quality have received little research attention. It is hypothesized that amino acid digestibility and content, as well as meal consistency, can

be affected by seed variety and compromised during the commercial prepress-solvent extraction methods practiced in western Canada. Therefore, the objectives of this thesis were to: 1) review the literature related to canola processing and the reactions that may be reducing protein quality; 2) to study the effect(s) of commercial prepress-solvent extraction on the nutritional quality of the meal; 3) to identify the processing conditions that promote protein damage; 4) to examine ways of producing a higher quality meal; 5) to establish methods of measuring protein quality in the meal; and, 6) to study the relationship (if any) between genotype and *in vitro* protein quality and susceptibility to heat damage.

2 NUTRITIONAL VALUE OF CANOLA MEAL FOR BROILER CHICKENS – A REVIEW

2.1 INTRODUCTION

Rapeseed, the seed from the *Cruciferae* family, *Brassica* genus, was introduced into western Canada in the 1940's to diversify crop production and to reduce the economic dependence on wheat and barley which are prone to cyclical price fluctuations (Anon, 1992). The seed, which contains approximately 42% oil, was originally used as a source of lubricant in steam powered locomotives and marine engines. In the 1950's, the oil started to be used in human food (Anon, 1992). However, rapeseed oil contained high levels of a long chained polyunsaturated fatty acid, erucic acid (24-45% of the oil), which was linked to reduced growth rates, altered endocrine function and heart disease in rats (Caroll, 1951). Therefore, Canadian breeding programs focused on reducing the erucic content of the oil, and in 1960, Dr. Baldur Stefansson in Winnipeg, Manitoba and Dr. Keith Downey in Saskatoon, Saskatchewan reported finding *B. napus* seeds with low levels of the fatty acid. The first low erucic acid variety of rapeseed, "Oro" was registered in 1968 (Anon, 1992).

Rapeseed meal also contained high levels of glucosinolates that upon hydrolysis, form goitrogenic compounds which interfere with iodine metabolism and thyroid function, and reduce animal performance (Mawson et al., 1994). Cooking the seed prior to oil extraction inactivated myrosinase, the enzyme which hydrolyses the glucosinolates, and reduced the toxic effects of the rapeseed meal (Youngs and Wetter, 1969). Heating or toasting the meal at 105 to 110°C also destroyed 40% of the glucosinolate content, further reducing the toxic effects of the meal and increasing animal performance. Through plant breeding, a low erucic and low glucosinolate variety of *B. napus* (Tower) was released in 1974 and a low erucic acid, low glucosinolate variety of *B. rapa* (Candle) was released in 1977 (Anon, 1992). These new low erucic acid, low glucosinolate varieties no longer produced the same toxic

effects as earlier varieties of rapeseed, and consequently, the meal became widely accepted as a source of protein in animal feeds (Anon, 1992).

The new low erucic acid, low glucosinolate rapeseed varieties were given the name "canola" in North America to differentiate them from earlier varieties of rapeseed (Anon, 1992). In Europe, the new low erucic acid, low glucosinolate varieties were referred to as "double low" or "double zero rapeseed". Due to its ability to germinate and grow at low temperatures, canola has become one of the most important crops in western Canada with an average annual production of 6.86 million tonnes between 1990 and 2000 (Statistics Canada, 2001). Canola is crushed to yield approximately 42% oil and 58% meal (Unger, 1990). The majority of the oil is used as vegetable oil for human consumption and the meal is used as animal feed. The meal is rich in protein and is used, therefore, as a source of protein in the diets of most agricultural species (Hickling, 2001). However, the value of canola meal, relative to its protein content is low when compared to other protein sources such as soybean meal. The reason for the discounted value is likely related to its reduced nutrient density and poorer utilization by monogastric species. Amino acid utilization in canola meal is lower than that of other solvent extracted oilseed meals such as soybean (NRC, 1994). The reduced protein quality may be the result of processing as the meal is heated at several stages. The objective of this chapter is to review the feed applications of canola meal, and the composition and utilization of canola meal. Since canola meal is used primarily as a source of protein and its utilization is lower than that of other oilseed protein meals, special emphasis will be placed on factors affecting amino acid content and utilization by poultry.

2.2 FEED APPLICATIONS FOR CANOLA MEAL

After the introduction of canola, the reduction in glucosinolate content resulted in a superior meal that could be used at higher inclusion rates than before. Canola meal is used widely in ruminant diets, especially in dairy rations. The low solubility of the protein combined with an excellent balance of amino acids, especially Cys, His, Lys, Met and Thr, makes the meal a good source of rumen-undegraded protein for high producing dairy cows (NRC, 2001). In addition, numerous studies (Ingalls and Sharma,

1975; Laarveld and Christensen, 1976; Papas et al., 1978; Laarveld et al., 1981; Sanchez and Claypool, 1983; Depeters and Bath, 1986; McClean and Laarveld, 1991; Emanuelson et al., 1993; Vincent et al., 1990 and Dewhurst et al., 1999) reported increases in milk production when canola meal was fed. As a result, large quantities of canola meal are exported from Canada to areas with intensive dairy production, such as California.

Canola meal is used effectively in swine diets, especially in the diets of growing and finishing pigs and during sow gestation. Research from the 1980's showed that swine performance decreased with canola meal inclusion but the diets were formulated on a total amino acid basis. Because true ileal swine digestibilities of Lys, Met, Cys, Thr and Trp are 78 vs 90%, 86 vs 91%, 83 vs 87%, 76 vs 87% and 75 vs 90% for canola meal and soybean meals, respectively (Anon, 1998), canola based diets formulated on total amino acid content will be deficient in digestible amino acids, which will reduce performance (Bell et al., 1988). When diets are formulated on a digestible amino acid basis, reductions in performance are not observed, indicating canola meal can be used successfully in most swine rations (Siljander-Rasi et al., 1996). Due to the reduced digestibility of the amino acids, canola meal sells at a discount relative to soybean meal. Canola meal appears to impair intake of starter pigs (Bourdon and Aumaitre, 1990) so a maximum inclusion level of 5% in these diets is usually recommended (Hickling, 2001).

Canola meal is a good source of protein in poultry diets (Hickling, 2001). However, as is the case for swine, amino acid digestibility of canola meal is lower than that of soybean meal (Table 2.1), making it necessary to formulate on a digestible amino acid basis. The metabolizable energy of canola meal is also approximately 17% lower than that of soybean meal (NRC, 1994). The combination of reduced amino acid digestibility and metabolizable energy of the canola meal reduces its value relative to soybean meal and limits its inclusion in nutrient-dense poultry diets. Not only are the digestibilities of the amino acids in canola meal lower than that in soybean meal, they are also more variable, as indicated by the large relative standard errors of true digestibility (Table 2.1). This, too, reduces the value of canola meal for poultry diets.

Table 2.1 True digestibility (%±SE) of amino acids in canola and soybean meal by poultry (NRC, 1994)

Amino Acid	Canola meal	Soybean meal
Cys	75±9	82±6
Lys	80±6	91±3
Ile	83±4	93±2
Thr	78±5	88±3
Val	82±4	91±3

2.3 COMPOSITION OF CANOLA MEAL

2.3.1 Protein Content

Canola meal is an enriched source of protein with a guaranteed minimum of 34% protein as received (COPA, 1998). Bell and Keith (1991) showed that crude protein content is variable and that part of the variation in protein content is due to differences in growing conditions. In a survey of canola seeds conducted by the Canadian Grain Commission over the past 10 years, the average protein content of the seed has varied from 19.2 to 21.9%, which would have a significant impact on the final protein content of the meal. Protein content may also be affected by the amount of screenings and gums added back to the meal after processing. Gums, phospholipids and waxes removed from the oil during processing are added back to the meal during desolventization to increase the energy content of the meal and to reduce dustiness (Summers and Leeson, 1977). In addition, the screenings produced when canola seed is cleaned at the crushing plant are also typically added back to the meal (Hickling, 2001).

2.3.2 Amino Acid Composition

As shown in Table 2.2, canola meal has a good balance of amino acids. It is slightly lower in Arg, Ile, Leu, Lys, Phe and Trp than soybean meal, but it is enriched in Met, Cys, Thr and Val. However, the seed contains higher levels of amino acids, expressed as a proportion of the protein, than the meal (Lee et al., 1995), indicating that commercial processing is reducing amino acid content.

2.3.3 Fibre Content

Canola meal has a high concentration of fibre relative to soybean meal (Table 2.3) which contributes the most to its lower metabolizable energy (Newkirk et al., 1997). Canola meal contains between 15.0 and 28.6% insoluble fibre, and between 2.0 and 12.6% soluble fibre (Jensen et al., 1995b).

Table 2.2 Amino acid composition (g/16 g N) of canola and soybean meal

Amino acid	Canola meal	Soybean meal
Arg	5.98	7.33
His	2.67	2.69
Ile	3.94	4.46
Leu	7.10	7.87
Lys	5.57	6.23
Meth	2.04	1.41
Cys	2.50	1.52
Phe	4.14	4.93
Thr	4.40	3.94
Trp	1.26	1.56
Val	5.06	4.67

Source: (NRC, 1994)

Table 2.3 Fibre content (%) of meals from *Brassica napus*, *rapa* and *juncea*, and dehulled soybean (*Glycine max*)

Composition	*B. napus*	*B. rapa*	*B. juncea*	Soybean
Acid detergent fibre	20.6	13.2	12.8	6.7
Neutral detergent fibre	25.7	19.6	21.1	9.1
Total dietary fibre	29.5	29.7	27.2	16.8

Source: (Newkirk et al., 1997)

The high fibre content is a function of the hull and oil content of the seed. Canola seed is small (~ 2mm diameter) in comparison to soybean seed (~ 5 mm diameter) and, therefore, has a higher surface area and, therefore, proportionately more hull. As a percentage of the seed, canola contains 10.5 to 17% hull, depending on the size of the seed (Jensen et al., 1995b), with smaller seeds having a higher proportion. In addition, canola seed is much more difficult to dehull than soybean seed, so hull removal is not currently used to increase protein content. Canola seed also contains a higher proportion of oil (~42%) than soybean (~18%) and, therefore, its extraction further concentrates the hull and other fibre components. After solvent extraction of the oil, the hull comprises between 19.6 and 30.4% of the meal (Jensen et al., 1995b).

2.3.4 Anti-nutritional Factors

Canola meal contains several anti-nutritional factors which have been the subject of a prior review (Bell, 1984).

2.3.4.1 Phytate

Phytate (myo-inositol 1,2,3,4,5,6-hexakis-dihydrogen phosphate), the main storage form of phosphorus in plant seeds (Kirby and Nelson, 1988), is comprised of a central ring of myo-inositol with six ester bonds to phosphate groups. Each phosphate group is capable of forming two ionic bonds with positively charged ions and, therefore, can bind minerals and proteins. Canola meal is a rich source of phytate (~ 3%, Zhou et al., 1990) containing approximately twice the level of soybean meal (~1.5%, De Boland et al., 1975). Since poultry lack the ability to hydrolyse the majority of phytate in spite of having endogenous intestinal phytase (Maenz and Classen, 1998), the bound phosphorus is only poorly digested (Nahashon et al., 1994). Similarly, some of the protein bound to phytate is poorly digested, which reduces the availability of some amino acids. However, the effect of phytate on protein utilization in canola meal is minimal (Newkirk and Classen, 2001). Phytate also appears to reduce the metabolizable energy of canola meal, but the reason for the effect is not known (Newkirk and Classen, 2001).

2.3.4.2 Sinapine

Sinapine is the choline ester of sinapic acid (Lajolo et al., 1991) and occurs naturally in the seeds of plants from the *Cruciferae* family, such as canola. Sinapine is thought to impart a bitter taste to canola meal and, therefore, may reduce its palatability (Clandinin, 1961). Part of the sinapine is hydrolysed by intestinal and cecal bacteria of chickens to form trimethylamine, which is absorbed and later degraded by trimethylamine oxidase in the liver. However, some strains of laying hens that produce brown shelled eggs are deficient in trimethylamine oxidase and the trimethylamine is deposited in the eggs, producing an objectionable fishy odour (Hobson-Frohock et al., 1973).

2.3.4.3 Glucosinolates

The seeds of plants from the *Cruciferae* family, including the *Brassica* genus, contain thioglucosides or "glucosinolates". Glucosinolates consist of a β-D- thioglucose group, a sulphonated oxime moiety, and a side chain derived from an amino acid (Figure 2.1). Table 2.4 shows the main glucosinolates found in canola meal. There are three principal types of glucosinolates: aliphatic, aromatic and indolyl. The seed of traditional varieties of rapeseed contained approximately 120 to 150 μmol/g total glucosinolates on an oil free, dry basis. Current varieties of canola meal contain approximately 16 μmol/g of oil free, dry meal, with the aliphatic glucosinolates accounting for approximately 75% of the total (Hickling, 2001). These compounds are degraded in damaged seed by the endogenous enzyme, myrosinase (EC 3:2:3:1), resulting in the production of glucose, an aglycone and a sulphate. The aglycone is unstable and further decomposes to thiocyanates, nitriles and isothiocyanates (Figure 2.2; Mithen et al., 2000). Glucosinolates with hydroxylated side chains form aglucones that cyclise to form oxazolidine-2-thiones. For example, 2-hydroyxy-3-butenyl glucosinolate "progoitrin" forms "goitrin" upon hydrolysis (Mithen et al., 2000). At low pH, the aglucone of many of the glucosinolates is preferentially converted directly to nitrile, with the liberation of sulphur (Mithen et al., 2000).

$$R\text{-}CH_2\text{-}CH_2\text{-}C(\text{-S-Glucose})=N\text{-}OSO_3^-$$

Figure 2.1 The basic chemical structure of glucosinolates (adapted from Mithen et al., 2000).

Table 2.4 The primary glucosinolates found in canola meal (adapted from Bell, 1984)

Glucosinolate	Semi-systematic name	R
Progoitrin	2-OH-3-butenyl-	$CH_2{=}CH{\cdot}CHOH{\cdot}CH_3$
Gluconapin	3-butenyl-	$CH_2{=}CH(CH_2)_2$
Glucobrassicanapin	4-pentenyl-	$CH_2{=}CH(CH_2)_3$
Napoleiferin	2-OH-4- pentenyl-	CH2=CH·CH2·CH(OH)·CH2
Glucobrassicin	3-indolyl-methyl-	indole ring –CH2 (N–H)
Neoglucobrassicin	1-methoxy- 3-indolyl –methyl-	indole ring –CH 3 (N–OCH2)

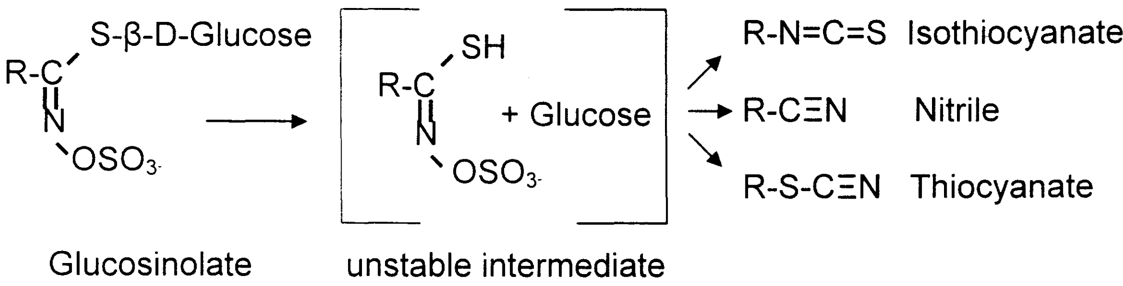

Figure 2.2 Hydrolysis of glucosinolates by myrosinase (adapted from Mithen et al., 2000).

Glucosinolates and particularly their breakdown products, isothiocyanates, thiocyanates and nitriles, have been shown to have toxic effects on animals. Feeding of high glucosinolate rapeseed meal to poultry causes reduced plasma thyroid hormone concentration, enlarged thyroids (goitre; Elwinger, 1986), enlarged livers (Campbell and Smith, 1979; Vermorel et al., 1986), hemorrhagic liver syndrome (Campbell and Slominski, 1991), and reduced growth rate (Campbell and Smith, 1979).

Hypothyroidism is due to the antagonistic effects of glucosinolate breakdown products on iodine organification within the thyroid. Feeding high glucosinolate rapeseed reduces iodine retention by the thyroid (iodine organification) and decreases secretion of triiodothyronine (T3) and thyroxine (T4) into the blood. T3 and T4 normally inhibit anterior pituitary secretion of thyroid stimulating hormone (TSH) via the hypothalamus. However, the reduced plasma levels of T3 and T4 reduce the feedback inhibition, resulting in higher levels of TSH and, therefore, enhanced thyroid activity and size (goitre; Lo and Hill, 1971).

The reasons why or how glucosinolates increase liver weight is not known. Feeding high glucosinolate rapeseed causes elevated levels of cytochrome P450 (Israels et al., 1979), suggesting that the liver is responding to a need to detoxify components of the glucosinolates. In addition, as a result of feeding high glucosinolate rapeseed meal, plasma levels of aspartate and alanine transaminases increase, suggesting that the glucosinolates are causing liver damage (Elwinger, 1986). There have been reports of laying hens dying from hemorrhagic livers when fed high glucosinolate canola meal (Marangos and Hill, 1976; Hulan and Proudfoot, 1981); however, the mechanism of action is not known. Hemorrhagic liver syndrome is less prevalent with low glucosinolate varieties of canola, suggesting glucosinolates may be the cause (Campbell and Slominski, 1991; Campbell and Smith, 1979). However, feeding intact glucosinolates failed to produce hemorrhagic liver syndrome (Vermorel et al., 1986).

Modern low glucosinolate varieties of canola have been shown to have less toxic effects than earlier varieties of high glucosinolate rapeseed (Schone et al., 1993). However, complete elimination of glucosinolates appears to improve chick performance as compared to low glucosinolate meal (Classen et al., 1991), indicating that further reductions in glucosinolate contents would be beneficial. Since that study, however, the

level of glucosinolates in canola has steadily declined, and it is not known whether the levels of glucosinolates in these new varieties are low enough to prevent physiological effects. Although the hydrolysis products of glucosinolates have been shown to reduce bird growth rate, there is no evidence that they affect nutrient utilization in canola meal.

2.4 ENERGY UTILIZATION

Canola meal has a low AME content for poultry (~2000 kcal/kg) relative to soybean meal (~2400 kcal/kg; NRC, 1994) which limits its use in high density diets. The high concentration of fibre in the meal (21.5 vs 7.1% neutral detergent fibre in canola meal and soybean meals, respectively) dilutes the digestible nutrients (Bell, 1993) reducing its metabolizable energy. Canola meal contains low levels of starch (~2.5%) which, therefore, contributes little to the energy content of the meal (Slominski and Campbell, 1991). Canola meal contains approximately 7.7% sucrose (Slominski and Campbell, 1991), which would contribute approximately 255 kcal/kg to the metabolizable energy content of the meal. The majority of the oil is removed during solvent extraction, but gums (phosphatidyl compounds) removed from the oil during refining are added back to the meal (approximately 1.5-2%; McCuaig and Bell, 1981). These added gums increase the ME of canola meal by approximately 150 kcal/kg (March and Soong, 1978).

2.5 PROTEIN AND AMINO ACID UTILIZATION

Due to the high protein content of canola meal, it is used primarily as a protein supplement in animal diets. However, as discussed previously, the digestibility of the protein and amino acids is lower than that of other protein supplements such as soybean meal. For example, true Lys availability of canola meal is approximately 10% lower than that of soybean meal (NRC, 1994). Similarly, other amino acids are also less digestible in canola meal than in soybean meal. Since canola meal is primarily used as a protein source, and the reduced amino acid digestibility reduces its value, the remainder of this review will focus on: a) the factors that may affect amino acid utilization; b) *in vitro* methods of assessing protein quality; and c) areas that require further study.

2.5.1 Factors Affecting Amino Acid Content and Utilization

Canola meal contains large amounts of fibre due to its high proportion of hull material, and this may be a factor in the reduced amino acid digestibility of the meal. True protein digestibility of canola meal is negatively correlated with the hull (r=-0.76) and lignin (r=-0.68) contents (Jensen et al., 1995b), indicating that the protein in the hull is indigestible and, therefore, reduces protein utilization of the meal. However, separating the meal into low and high fibre fractions by tail-end dehulling had little impact on amino acid utilization by poultry (Clark et al., 2001), suggesting that the effect of fibre does not account for the large differences in amino acid availability between canola and soybean meals.

In the presence of divalent minerals, phytate can chelate protein, reducing its solubility and preventing hydrolysis by proteolytic enzymes. However, dephytinization of canola meal only caused a small increase in amino acid availability (Newkirk and Classen, 2001). This would suggest that phytate is only partially responsible for the low amino acid availability of canola meal. In addition, phytate only complexes with protein and does not degrade it so should have no effect on the concentration of amino acids.

Plant genotype may have an impact on protein quality in canola meal. Slominski et al. (1999) showed that yellow-seeded types had less fibre and, therefore, more protein than their brown-seeded counterparts. The Lys content, expressed as a proportion of the protein, was similar for the yellow-seeded *B. rapa* and *B. napus* varieties tested, but the brown-seeded *B. napus* had more Lys than the yellow-seeded *B. napus*. The Lys content of *B. juncea* seed was lower than that of *B. rapa* and *B. napus*. The brown-seeded *B. napus* also had the highest true digestible amino acid content (% of total), while the yellow-seeded *B. napus* has the lowest. However, none of the varieties tested had true Lys digestibility (range 76.2-80.8%) equal to that of soybean meal (~ 90%).

2.5.2 Canola Processing

Processing may affect the quality of protein in canola meal. Amino acids, especially Lys and Met are susceptible to damage during the meal processing (Hurrell, 1984). Rapeseed meal was originally characterized as having lower levels of digestible Lys than current canola meal. However, research in the late 1950's discovered that

processing was reducing the content of Lys (Clandinin et al., 1959) and steps were taken to minimize heat input during processing. Modern varieties of canola meal contain higher levels of Lys than earlier rapeseed meals, but few studies have looked at the effects of current processing methods on the content and availability of amino acids in canola meal. It may be possible that the content and availability of amino acids, particularly Lys, in canola meal is still reduced by processing. Therefore, this review will focus on processing methods employed to extract oil from canola, their effects on amino acid content and availability, the reactions that can occur during processing to reduce protein quality, and the conditions under which these reactions occur.

2.5.2.1 Expelling

The first commercial crushing plants in western Canada expelled the oil under pressure (Bredeson, 1983). Expelling rapeseed involves cleaning to remove debris, conditioning for about five minutes to raise the temperature to 105-108°C, flaking between rollers, cooking at approximately 130°C for 30 minutes, and then feeding into a screw-press extruder (Clandinin and Tajcnar, 1960). The screw-press extruder consists of a screw conveyer with decreasing pitch, which forces the seed against a slotted cage that lets the oil pass out of the expeller. Cooking or expelling at excessively high temperatures results in the loss of Lys content and digestibility. Clandinin and Tajcnar (1960) showed that the Lys content of the expelled meal, as a proportion of the protein ranged from 3.7 to 5.3, with a mean of 4.6 g/16 g N, which is markedly less than the values reported for prepress-solvent extracted meal (5.4 g/16 g/N, NRC, 1994). Expelling resulted in a meal with high residual oil content and, therefore, reduced the yield of valuable oil for human use. As a result, most crushing plants switched to solvent extraction to recover as much oil as possible.

2.5.2.2 Direct Solvent Extraction

Due to the low oil content, solvent extraction had been employed by soybean crushers dating back to the 1930's. However, the first solvent extraction plant in Canada for rapeseed was not built until 1959. Since the solvent extracts the oil, it was not necessary to expel the seed to extract the oil after flaking and cooking (Anon, 1992).

However, due to the tendency of the flaked seed to disintegrate into fine particles, the expeller was reintroduced into the process (Youngs, 1965). There does not appear to be any information available on the amino acid content or digestibility of meals produced in this manner.

2.5.2.3 Prepress-solvent extraction

The process which includes expelling prior to solvent extraction is called "prepress-solvent extraction". The expelling process however, is scaled down from the original method since the solvent will extract most of the oil. Only enough energy is applied to form a cohesive, but porous, particle that remains intact during hexane extraction. The new expelling conditions extract approximately half of the oil prior to solvent extraction. Since the amount of energy input into the expeller is minimized, little heat is generated and, therefore, the potential to damage protein is virtually eliminated. The earliest reports on meals from prepress-solvent extracted meal indicated they contained more Lys than meals directly expelled to 6% oil content (Youngs, 1965). This would indicate that prepress-solvent extraction causes less protein damage than does direct expelling. However, several studies have demonstrated that protein quality can be compromised during prepress-solvent extraction of oilseeds such as soybean (Parsons et al., 1992) and sunflower (Zhang and Parsons, 1994).

Prepress-solvent extraction of canola meal consists of 10 basic stages (Unger, 1990), as shown in Figure 2.3. The residence time and temperatures are approximate, and represent only one example of the conditions used in a modern commercial crushing plant. Each plant in western Canada uses virtually identical equipment. However, there are differences in how the equipment is operated to extract oil. The process consists of the following stages: 1) cleaning the seed to remove debris; 2) drying to approximately 6% moisture; 3) preconditioning (heating) to prevent the seed from shattering during flaking; 4) flaking by passing through a roller mill to form fine, thin flakes; 5) cooking for approximately 1 hour at 75-85°C to deactivate the myrosinase and to coalesce the oil and to prepare the seed for expelling; 6) expelling to reduce the oil content from approximately 42% to 15 - 20%; 7) solvent extraction with hexane to remove additional oil; 8) desolventization in a desolventizer/toaster (DT) to remove the hexane from the

Marc (solvent laden, oil extracted meal); 9) cooling-drying with forced air while adding back the gums and screenings and 10) grinding and in some cases pelleting.

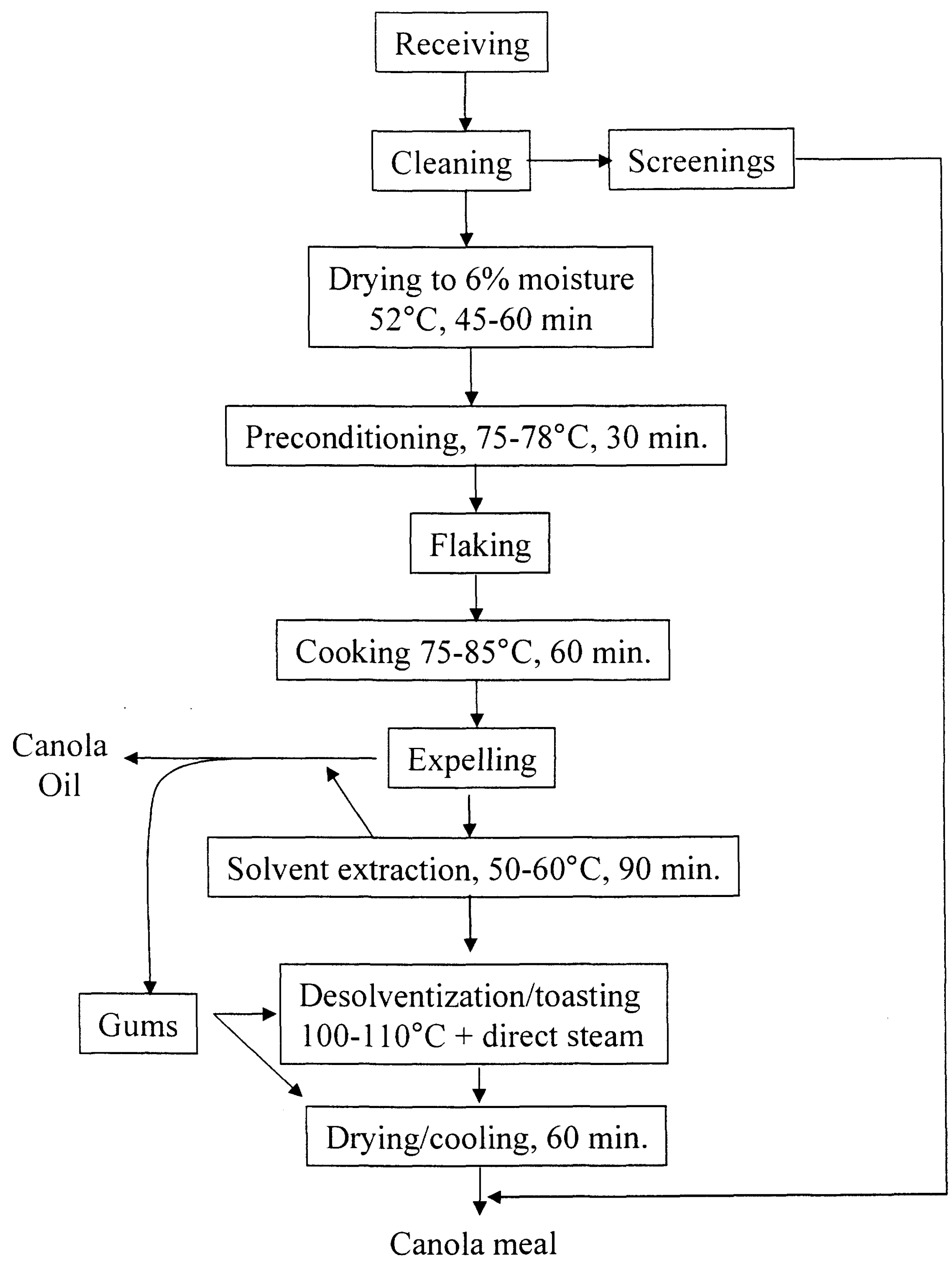

Figure 2.3 Stages of commercial prepress-solvent extraction of canola meal.

Protein damage can occur at any of the stages in which heat is applied to the meal including, drying, preconditioning, cooking, expelling, desolventization/toasting or drying/cooling. During drying, very little heat, if any, is applied so it is unlikely that protein quality will be compromised. Likewise, the seed is heated during preconditioning, but the amount of heat is minimal. Although expelling can generate enough heat to cause significant damage to the protein, in the case of prepress-solvent extraction energy input is intentionally minimized and the risk of over heating greatly reduced. The stages of processing that are most likely to cause protein damage would be cooking, desolventization/toasting and drying/cooling.

Several studies have shown that heating canola meal in an autoclave for extended periods of time will reduce the content and bio-availability of Lys (Goh et al., 1978; Jensen et al., 1995a). However, only one study has examined the effects of processing stage on canola meal (Anderson-Hafermann et al., 1993). In this study, a single set of canola meal samples was collected during four stages of commercial processing. The amino acid availability of the initial seed, the seed after flaking and cooking, the cake after expelling, and the final desolventized meal were determined in cecectomized cockerels. There was no significant effect of processing on true amino acid digestibility, but the desolventized meal had the lowest Lys digestibility value (80%) of the four samples tested. The effect of processing on amino acid concentration was not reported in this study. However, a single set of samples is likely inadequate to assess the effects of processing.

Anderson-Hafermann et al. (1993) also examined the effect of over processing on meal quality by autoclaving canola meal. As expected, autoclave treatment reduced the availability and content of key amino acids, especially Lys, indicating that meal quality would decrease if over processed. This type of heat damage could potentially occur at any stage where heat is applied, but would most likely occur during cooking, desolventization/toasting or drying-cooling. To understand the potential risk of over processing, one must have a basic understanding of each of these processing steps.

Cooking is conducted in either a vertical tube with heated trays or in a horizontal heated tube that rotates. A temperature of 80°C or higher is routinely achieved for periods up to one hour or more, and the product remains at approximately 6% moisture.

After solvent extraction, the residual solvent is removed in a desolventizer/toaster (DT). The DT typically consists of a vertical cylinder with several heated trays. The meal is dropped on the first tray, mixed, and eventually falls through a chute to the second tray, and so on. The heat transferred from the trays to the meal causes the hexane to evaporate, and it is collected and condensed. In the most common design of DT, the Schumacher, the last few trays use not only indirect heat in the form of heated trays but also direct heat in the form of steam injected directly into the meal (sparge steam). As hexane is evaporated by the sparge steam, the steam condenses into the meal increasing the moisture content of the meal. Meal resides in the DT for up to an hour, but in the first few trays the temperature of the meal would not be higher than the boiling point of n-hexane (69°C) until the majority of the hexane was evaporated. However, in the final sparge deck trays, most of the hexane has already evaporated and heat is being applied both directly and indirectly so the meal temperature rises very quickly. The temperature normally reaches approximately 110 to 115°C in the final trays of the DT. The actual residence time at the elevated temperatures has not been determined but if one assumes a total residence time of 60 minutes in a DT equipped with 8 trays, it would be expected to be approximately 10 to 15 minutes at the highest temperatures. In addition to the moisture due to condensed steam, water is often sprayed on the top deck of the DT to reduce dust levels in the vapours leaving the DT. As well, if the gums are added in the DT, they also contain water. Therefore, the moisture content of the meal exiting the DT is typically between 15 and 18% (Unger, 1990).

The dryer/cooler consists of either a horizontal rotating tube or a vertical stacked tray unit that blows air over the meal. The units are designed such that auxiliary heat can be applied if necessary to dry the meal. It is unlikely that heat damage would occur in the dryer/cooler. However, the meal is entering at high temperature and moisture, so if cooling is not effective or is delayed, the reactions started in the DT may be prolonged.

Previous studies have examined the effects of prepress-solvent extraction on soybean meal (Parsons et al., 1992), but little information is available on its effects on canola meal. Therefore, further studies should be conducted to examine the effects of commercial prepress-solvent extraction of canola on amino acid content and availability.

However, based on the data available on the conditions during prepress extraction of canola, one might expect the protein to be involved in some of the reactions typically found to occur during heat processing of foods and feeds. Reactions of proteins during heat processing, especially during food preparation, have been studied extensively. An understanding of these reactions may provide some insight into the reactions that could potentially occur during canola processing.

2.5.3 Reactions That May Reduce Protein Quality During Processing

Proteins are highly reactive molecules and are susceptible to degradation by several reactions during processing (Hurrell, 1984). These reactions include Maillard reactions, protein/polyphenolic interactions and protein/protein interactions. In the next section of this review, the reactions, the conditions under which the reactions are promoted, and the potential for them to occur during canola processing will be discussed.

2.5.3.1 Maillard Reactions

First identified by the French chemist Louis Maillard in 1912, the Maillard reactions involving protein are common during food processing. Maillard reactions are responsible for the formation of brown pigments and several aromas during processing of proteins (Hurrell, 1984). Maillard reactions have been the subject of numerous reviews regarding both food (Hodge, 1953; Adrian, 1974; Mauron, 1981; Friedman, 1992; Martins et al., 2001) and feed (Van Soest and Mason, 1991) processing. The chemistry of Maillard reactions is complex but can be divided into three stages, namely early, advanced and final Maillard reactions. A reaction scheme is shown in Figure 2.4.

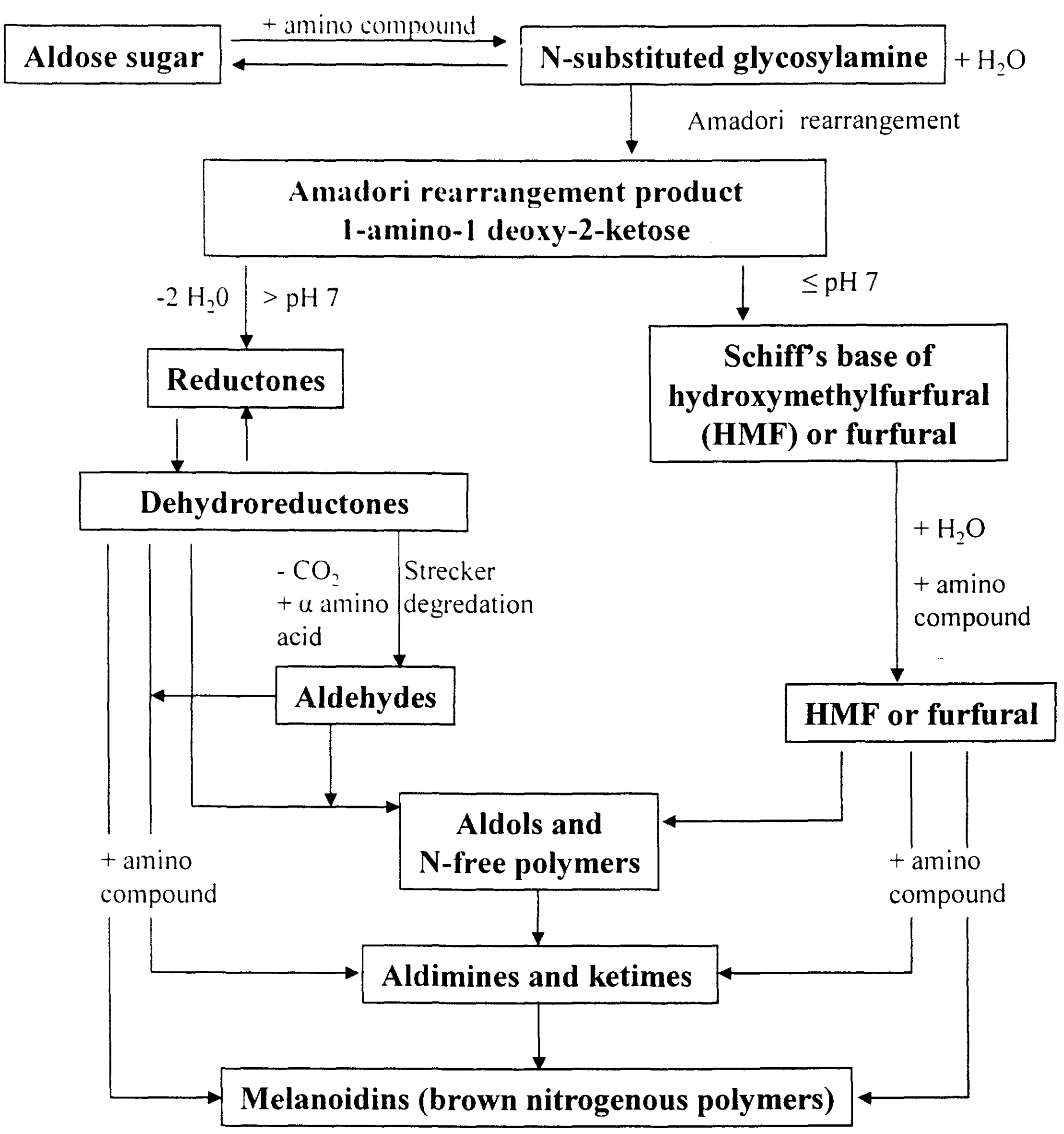

Figure 2.4 Maillard reaction scheme (adapted from Martins et al., 2001).

The early Maillard reactions consist of a condensation reaction between the carbonyl group of a reducing sugar or ketone and a free amino group, loss of a molecule of water to form a Schiff's base, and cyclization to an N-substituted glycosylamine (Figure 2.5). Up to this stage, the reactions are reversible. However, the N-substituted glycosylamine is quickly converted to 1-amino-1-deoxy-2-ketose by Amodori rearrangement, which is catalyzed by weak acids. The formation of the 1-amino-1-deoxy-2-ketose is considered the key reaction in early Maillard reactions and unless the heating conditions are extreme, the reaction stops at this stage (Mauron, 1981). The 1-amino-1-deoxy-2-ketose compound does not impart any colour or flavour changes to the product, but can reduce the digestibility of the amino acids (Hurrell, 1984) involved.

There are several pathways for the advanced Maillard reactions, depending on the pH and/or moisture content of the product in question. However, the acidic pH of canola meal (5.8-6.0; Newkirk unpublished data) and its relatively dry state will likely limit the reaction types.

At acidic pH the 1-amino-1-deoxy-2 ketose is dehydrated to form the Schiff's base of furfurals or 5-hydroxy-methyl-furfural (HMF). Part of the Schiff's base then decomposes to yield the amine and the furfural or HMF (Hodge, 1953). It is important to note that the amine compound in this pathway is acting primarily as a catalyst of the early and advanced Maillard reactions as it is liberated. The intact amine is also liberated with acid hydrolysis of the intermediary products, so the contents of the amino acids are not reduced at this point. These intermediary compounds are only poorly digested (Adrian, 1974). Although essential to the browning process, up to the end of the advanced Maillard reactions, there are no visible changes in colour. Therefore, changes in colour are not a good indicator of nutritional value of the product (Mauron, 1981).

The final Maillard reactions involving the furfurals and HMF are a series of complex polymerizations that incorporate the remaining Shiff's bases that have not been deaminated, as well as additional amine compounds to form insoluble dark-coloured melanoidins (Adrian, 1974). Most of the amino acids involved in the final reactions are decomposed and, therefore, are not detected after acid hydrolysis for analysis (Mauron, 1981).

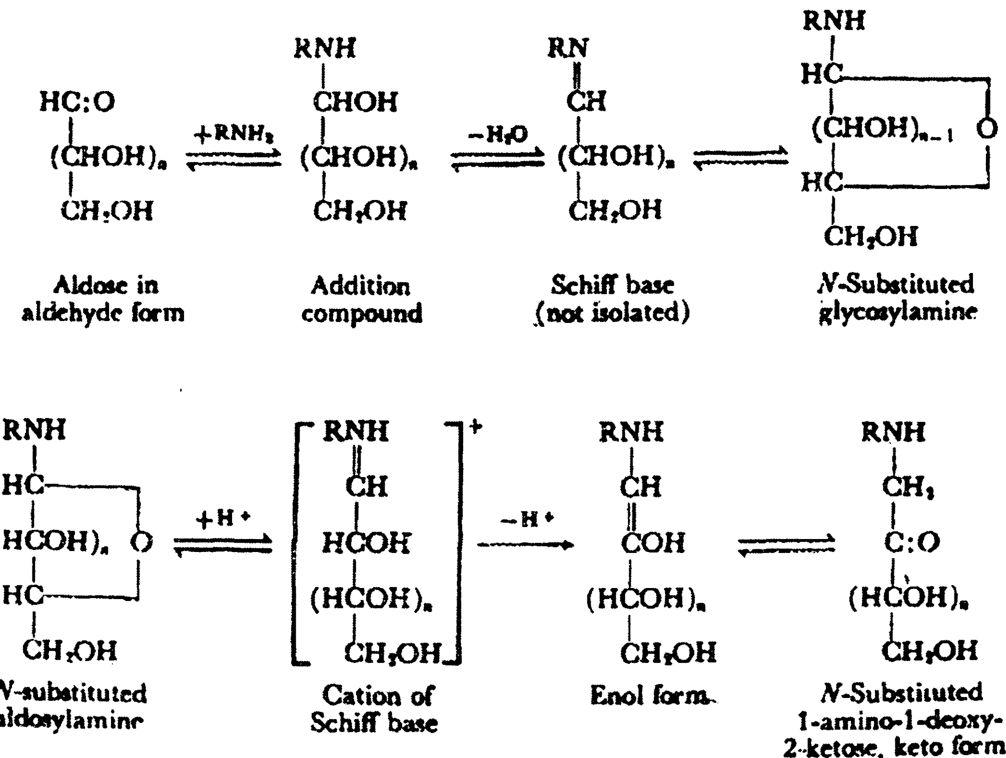

Figure 2.5 Reactions of the early Maillard reactions (Mauron, 1981).

Most Maillard reactions have been studied in solution but additional reactions occur in a semi-dry state as found during the processing of canola meal. Under "dry" conditions dehydration of the 1-amino-1-deoxy-2 ketose also yields "reductones" in addition to the furfurals and HMF. The reductones formed are similar to the furfurals except they do not posses the furan ring and have much higher reducing power. These reducing agents react with intact amino acids and convert them to aldehydes after decarboxylation in a process called Strecker degradation (Adrian, 1974). It is the dehydro form of the reductones and the aldehydes that enter the final stages of the Maillard reactions and cause the formation of brown pigments (Hodge, 1953).

The source of the amino group for Maillard reactions in proteins or peptides is either a terminal α-amino group or the ε-amino of Lys, with the reactions with Lys being most common (Adrian, 1974). Canola contains only minute amounts of free monosaccharides (0.36% of fat-free dry matter, Newkirk unpublished data) that could be involved directly in Maillard reactions. However, canola meal does contain relatively high levels of sucrose (6.9 – 10.4%; Slominski et al., 1994). Sucrose is not a reducing sugar *per se*, but upon hydrolysis, yields a molecule of glucose and fructose which are able to participate in Maillard reactions. Jensen et al. (1995a) showed that the sucrose content decreased with toasting, supporting the hypothesis that sucrose could indirectly be a source of carbonyl groups for the Maillard reactions during processing. Sucrose is susceptible to hydrolysis during heating especially in the presence of Lys. However, the rate of the Maillard reactions could conceivably be limited to the rate of sucrose degradation. This has been demonstrated in experiments with albumin. After heating for one hour at 100°C at pH 5 and 14% moisture, albumin, albumin + sucrose and the albumin + glucose mixtures contained 97, 85 and 13% of the level of reactive Lys in untreated albumin, respectively (Hurrell and Carpenter, 1977). Therefore, the availability of reducing sugars in canola meal may be a factor which limits Maillard reactions in canola meal during processing (Schebor et al., 1999).

The conditions that promote Maillard reactions have not been defined specifically for canola meal, but the conditions in food processing, especially milk products, are well known. Temperature is an important factor contributing to the rate of Maillard reactions. At room temperature, Maillard reactions proceed very slowly. However,

Maillard reactions increase exponentially with temperature and occur commonly during drying when temperatures are over 100°C (Adrian, 1974). Canola meal is heated at several stages of the process, with the desolventization/toasting being the most rigorous with temperatures of 110 to 115°C achieved routinely.

pH is an important factor affecting the rate of the Maillard reactions and the type of reactions that predominate. Alkaline pH promotes Maillard reactions and low pH is inhibitory, but Maillard reactions are known to occur when the pH is above 3. The rate of reactions increases approximately linearly at pH's between 3 and 10 (Adrian, 1974). At neutral and acidic pH, the production of Shiff's bases of HMF or furfural is promoted over fission products or reductones (Martins et al., 2001). pH is also important in the conditions which cause sucrose to be hydrolysed into reducing sugars that react in the Maillard reactions. Sucrose is most heat labile at acid pH so the conditions (Lindberg et al., 1975) during canola processing may be well suited to sucrose hydrolysis and the subsequent Maillard reactions.

The rate of Maillard reactions is highly dependent on the level of moisture in the product being heated. In the anhydrous state, virtually no Maillard reactions are observed but they increase with moisture level. However, moisture eventually inhibits the reactions and very little in the way of Maillard reaction products is formed in dilute solutions (Adrian, 1974). The reasons for this relationship can be explained on the basis of the nature of the reactions that are occurring. High levels of water inhibit the dehydration steps such as the conversion of 1-amino-1-deoxy-2-ketose to furfural or reductones (Adrian, 1974). As a result, food processes that involve heating moist products at high temperatures and low humidity, such as baking, tend to promote higher levels of Maillard reactions than high humidity cooking methods such as pressure cooking (Adrian, 1974). In a mixture of casein and glucose, Maillard reactions occur optimally between 15 and 18% moisture (Lea and Hannan, 1949b). Coincidentally, the moisture content after canola meal desolventization/toasting prior to drying/cooling is approximately 14 to 18% moisture, suggesting the conditions during commercial processing may be optimal for Maillard reactions. Acid hydrolysis of sucrose is also promoted with increasing moisture (Hurrell and Carpenter, 1977). Therefore, the introduction of moisture during canola processing may be promoting Maillard reactions

by both increasing the rate of the reaction and the production of substrate for the reaction. It would seem likely that the content and availability of amino acids in canola meal may be reduced in some, if not in most cases, as a result of Maillard reactions occurring during processing.

2.5.3.2 Protein/Polyphenolic Interactions

Proteins have also been shown to react with phenolic compounds resulting in losses in protein quality and the production of dark-coloured products. Phenolic acids are found in almost all plant materials, including canola, and are easily oxidized, especially under alkaline conditions. The resulting products, *o*-quinones are highly reactive and react with protein-bound Lys to form N-quinones. The N-quinones react to form cross linkages, producing poorly digested, dark-coloured products (Hurrell, 1984). The rate of the reaction is dependent on pH, oxygen level, temperature and duration of the manufacturing process. In the absence of phenolyase activity, the optimal pH for protein/polyphenolic reactions appears to be above pH 10. However, most plant materials have polyphenolyase activity which promotes the reaction below pH 6 or between pH 8-9. Exposure to high temperatures can deactivate these enzymes, thereby limiting the reactions to pHs in excess of 10 (Hurrell, 1984). Unless the polyphenolic enzymes are active during conditioning, one would not expect significant amounts of this type of reaction during canola processing since the pH is acidic and cooking and/or desolventization/toasting should deactivate the enzymes responsible. It is not known if protein quality in canola meal is reduced due to protein/phenolic reactions during processing.

2.5.3.3 Protein/Protein Interactions

In addition to Maillard reactions and protein/phenolic interactions, proteins are capable of cross reacting to form indigestible products. The free carboxyl groups of dicarboxylic amino acids react with the ε-amino group of Lys to form compounds that resist enzymatic digestion. Evans et al. (1961) demonstrated this by autoclaving purified α-proteins from soybean meal. The amount of Lys, aspartic acid and glutamic acid liberated by *in vitro* protein digestion or acid hydrolysis at 40°C was reduced by

autoclaving. This would suggest that the digestibility of these amino acids was reduced by autoclaving as a result of protein/protein reactions. Bjarnason and Carpenter (1970) showed that ε-amino groups of Lys reacted primarily with the amide groups of asparagine and glutamine in bovine plasma albumin to form indigestible proteins when heated at 14% moisture for 27 h at 115°C. This reaction resulted in the deamination of the asparagine and glutamine and the liberation of nitrogen during the reaction. In contrast to Evans et al. (1961), they concluded that the reaction with carboxyl groups was less prevalent than reactions with the amino group of asparagine and glutamine. In either case, it demonstrates that it is possible for proteins to cross react during heat processing to form indigestible compounds, which reduces the biological availability of Lys, asparagine, glutamine, aspartic acid and glutamic acid. Anderson-Hafermann et al. (1993) found that asparagine and glutamine content decreased and aspartic acid and glutamic acid content increased after autoclaving canola meal for 90 min. This would indicate that the ε-amino group may be reacting with the side amino group of asparagine and glutamine. This results in a deamination of those amino acids, suggesting that protein/protein reactions may occur in canola meal if overheated during processing.

2.5.4 *In vitro* Methods of Assessing Amino Acid Availability in Processed Canola Meal

Since it is possible that amino acid content and availability in canola meal can be compromised during prepress-solvent extraction, an *in vitro* method of assessing meal quality is desirable. A number of *in vitro* methods of assessing amino acid availability in poultry have been developed and they have been reviewed previously by Ravindran and Bryden (1999).

2.5.4.1 Reactive Lys Dye Binding Assays

Lys is the amino acid that is most susceptible to heat damage and is first or second limiting in most practical diets; therefore, most of the chemical assays developed measure the reactive Lys directly. One such assay is to react the ε-amino group of Lys with a dye, such as 1- fluoro-2,4-dintrobenzene (FNDB; Hurrell and Carpenter, 1981) which, after protein hydrolysis, is released and measured. The dye only reacts with

reactive amino groups on Lys; therefore, it would be a good indicator of its biological value. Assays of this type have been shown to be highly effective for assessing processed animal products such as meat meal (Nordheim and Coon, 1984). It is not known if these dye binding methods correlate with Lys digestibility in processed canola meal samples, but it would seem likely. However, additional studies are required to correlate dye binding Lys values with *in vivo* Lys digestibility in canola meal prior to commercial application.

2.5.4.2 Pepsin Digest Dialysate Index

Pepsin Digest Dialysate (PDD) Index (Kennedy et al., 1989) has been proposed as a method of assessing protein digestibility. It involves hydrolyzing the protein with pepsin, then dialyzing the solution to remove small fragments of digested protein. The protein remaining after dialysis is considered non-digestible, as pepsin did not reduce its size to a point that it can pass through the dialysis membrane. This method has been used to assess the effects of heating on the digestibility of protein in canola meal (Slominski, 1997). Protein digestibility as measured by PDD initially increased with heat treatment, but beyond 108°C, protein digestibility was reduced, suggesting this assay will predict if the meal has been overheated. However, the PDD index was not compared to *in vivo* digestion so it is not known if they are directly related.

2.5.4.3 Immobilized Digestive Enzyme Assay

The Immobilized Digestive Enzyme Assay (IDEA) system is a relatively new *in vitro* method of assessing protein digestibility in feed products. The assay consists of digesting the test product in the presence of immobilized pepsin, then with immobilized trypsin, chymotrypsin and intestinal mucosal peptidases. Initial tests suggest that the IDEA values correlate with available Lys in a number of food products (Chung et al., 1986) and this assay has been used to assess protein digestibility in chemically-treated proteins (Chang et al., 1990). Novus International Inc.[1] is planning on selling an IDEA enzyme kit that will simplify the assay but it is not known if the assay will be effective for assessing the available content of amino acids in canola meal. Prior to using the

[1] 20 Research Park Drive, St. Charles, MO, 63304

assay to assess amino acid digestibility in canola meal, studies should be conducted to determine if *in vitro* data correlate with the *in vivo* values.

2.5.4.4 Protein Solubility in Potassium Hydroxide

The most commonly used method of assessing meal quality in oilseeds is the protein solubility assay as described by Parsons et al. (1991). It is a relatively simple procedure that consists of adding meal to a solution of KOH. The slurry is mixed for 20 min and the insoluble material is removed by filtration or centrifugation. The protein content of the remaining solution is then analysed and expressed as a percentage of the original protein content of the meal. Protein solubility is based on the principle that heating protein causes it to be denatured, which reduces its solubility in mild alkaline solution.

Protein solubility in canola meal is inherently lower than in soybean meal and therefore, a modified procedure for canola meal has been proposed (Pastuszewska et al., 1998) where 0.5% KOH is used instead of 0.2%. Anderson-Hafermann et al. (1993) reported that autoclaving canola meal reduced both protein solubility in 0.5% KOH and true digestibility of Lys by poultry. Pastuszewska et al. (1998) also found that heating canola meal reduced protein solubility in 0.5% KOH, which tended to be correlated with the biological value of the protein in rats. However, in that study, protein solubility of commercial meals was poorly correlated with total or available Lys. The commercial samples analysed had a relatively narrow range of digestibility values, which may be the reason for the poor correlation coefficient. Additional studies should be conducted to determine if the results of the assay correlate with digestibility values of commercially produced meals, before the assay is used to monitor meal quality after processing.

2.5.4.5 Protein Dispersibility Index

Protein Dispersibility Index (PDI) is used frequently in food applications to assess protein solubility. It is a simple and relatively quick method of analysing processed protein products. The procedure consists of weighing a sample into a blender cup, adding water, blending for 10 min, filtering and measuring the protein in solution. PDI

is expressed as the percentage of total protein solubilized (Anon, 1999). Similar to protein solubility in 0.5% KOH, the assay is based on the principle that heat treatment causes protein to denature and cross react thereby reducing its solubility in water. The higher the temperature, the more effective the denaturation and the more extreme the cross linking and, therefore, the lower the solubility.

Protein dispersibility index has been proposed as a method of determining the level of processing in soybean meal (Batal et al., 2000). Since an alkali is not used to enhance protein solubility, PDI tends to be a more sensitive indicator of heating level, especially under heating. In samples of raw soybean meal autoclaved for 36 min, protein solubility in 0.2% KOH decreased from 97 to 78% whereas PDI decreased from 76 to 24%, demonstrating the greater sensitivity of the latter assay. It is not known, however, if PDI would be an accurate predictor of amino acid availability. One might speculate that PDI would be too sensitive to heat treatment and, therefore, would be reduced prior to irreversible protein damage. In addition, protein solubility in canola meal prior to any heating is lower than that in soybean meal, even after heating (Anderson-Hafermann et al., 1993), and therefore PDI may not be effective.

2.5.4.6 Near Infrared Reflectance Spectroscopy

Near infrared reflectance spectroscopy (NIRS) consists of directing electromagnetic radiation of specific wavelengths in the near infrared region on a sample and measuring how much of the radiation is absorbed. Each type of chemical bond absorbs at a unique wavelength; therefore, the number of bonds present can be quantified in a sample. Near infrared reflectance spectroscopy has been used for almost 30 years to assess the protein and moisture content of feed ingredients (Hymowitz et al., 1974). More recently, NIRS has been used to predict the amino acid contents of processed feed ingredients, including canola meal (Fontaine et al., 2001). In that study, the contents of all of the essential amino acids were predicted based on a calibration set of sixty four commercial canola meals with R^2 values ranging from 0.73 to 0.98. Although it is possible to predict the amino acid content of processed canola meal, digestion of nutrients and the factors that reduce digestibility are markedly more

complex. However, NIRS is calibrated based on reflectance over a large number of wavelengths, and, therefore, it may be possible to predict digestible amino acid content.

A number of assays are available to predict the nutritional value of processed protein meals. However, only protein solubility in 0.5% KOH has been related to amino acid availability. None of the methods described herein have been tested using a sufficiently large set of commercial canola samples to determine if they are strongly correlated to *in vivo* amino acid digestibility. Therefore, further studies should be conducted to determine their suitability as quality control tools in the canola industry.

2.6 CONCLUSIONS

Canola is an economically important crop in western Canada and elsewhere. Genetic improvements in rapeseed which lead to the development of low glucosinolate, low erucic acid canola varieties greatly increased the market acceptance and demand for the oil and the meal. The meal is a good source of protein for animal feeds, but has lower metabolizable energy, Lys content (as a proportion of the protein) and amino acid digestibility than soybean meal. The reduced metabolizable energy content is likely a result of the high fibre content of the meal. The Lys content of the meal is lower than that of the seed, indicating that processing is reducing the level of this highly reactive amino acid. Processing may also be reducing the availability of Lys and other amino acids by promoting Maillard reactions, protein/protein interactions and/or protein/phenolic interactions. Initially, rapeseed was extracted using expelling after cooking and conditioning, but the oil yield and protein quality (especially Lys content and availability) were compromised by the process. Conventional canola processing employs prepress-solvent extraction of the oil and results in higher yields of oil and higher quality meal. However, during prepress-solvent extraction, the meal is heated at several stages and this may compromise meal quality. The Maillard reactions would appear to be the most likely reactions to be occurring during processing, and involve the condensation of a carbonyl group of a sugar and free amino groups. Lys, due to its ε-amino group, is most susceptible to Maillard reactions. Maillard reactions occur optimally at high temperatures and moderate moisture contents. Although the meal is heated at several points in the prepress-solvent process applied to canola, the high

temperatures and moderate moisture content during desolventization/toasting are apparently optimal for Maillard reactions. Therefore, additional studies should be conducted to determine if the conditions of commercial prepress-solvent extraction are compromising meal quality, particularly Lys content and digestibility.

Since canola meal is exposed to heat during processing and the potential to reduce digestible amino acid content exists, the final meal product should be monitored for amino acid digestibility. *In vivo* methods of determining digestible amino acid content are too time consuming and expensive for routine analysis; therefore, *in vitro* methods are required. Several *in vitro* methods of assessing meal amino acid digestibility exist, but none of them has been studied using a large enough set of commercial canola meal samples to determine if the values correlate with *in vivo* digestibility values. Therefore, studies should be conducted to determine if any of the available *in vivo* methods of assessing protein quality are good predictors of amino acid digestibility in canola meal.

3 EFFECTS OF PREPRESS-SOLVENT EXTRACTION ON THE NUTRITIONAL VALUE OF CANOLA MEAL FOR BROILER CHICKENS

3.1 ABSTRACT

The objective of the experiment was to determine if standard prepress-solvent extraction of canola (low glucosinolate, low erucic acid rapeseed) affects the nutritional value of the resulting meal for broiler chickens. Three sets of samples were collected from all stages of prepress-solvent extraction at a commercial crushing plant and examined for nutritional value. Except for cystine and serine, processing stages up to and including solvent extraction did not affect amino acid digestibility. However, the desolventization/toasting process reduced ($P<0.05$) Lys content (6.03 to 5.50% of crude protein), crude protein digestibility (81% to 76.6%), Lys digestibility (87% to 79%) and the digestibility of all the other amino acids except glutamine, leucine, Met and serine. Desolventization/toasting increased the coefficient of variation (CV) for digestibility of Lys (1.4 vs 5.6% before and after desolventization/toasting, respectively) and of all other amino acids except glutamine, histidine, leucine, and serine. As expected, apparent metabolizable energy content decreased ($P<0.05$) with expelling and solvent extraction due to the reduced oil content, but was not affected by other processing stages. In conclusion, commercial desolventization/toasting can reduce the content and digestibility of many amino acids in canola meal and increase the variability of canola meal samples with respect to nutritional value.

3.2 INTRODUCTION

Canola meal (the oil-free residue of low glucosinolate, low erucic acid rapeseed) is a good source of protein for animals and is a particularly rich source of the sulphur amino acids, Met and cystine. Prepress-solvent extracted canola meal is characterized as having lower and less consistent amino acid digestibility than soybean meal (NRC,

1994). The reasons for the reduced digestibility are not known, but may be related to processing conditions. Excessive heating of oilseed meals during processing can lead to losses in the content and digestibility of amino acids (Parsons et al., 1992); however, it is not known if current processing conditions cause this effect in canola meal. In a previous study, Lys digestibility in canola prior to oil extraction was 85%, but in the desolventized meal it was 80% (Anderson-Hafermann et al., 1993). Although the difference was not statistically significant, it suggests a potential negative effect of the desolventization process, which includes meal toasting.

Canola meal is also characterized as having a lower apparent metabolizable energy (AME) level than that of other protein sources such as soybean meal (NRC, 1994). The lower AME is at least partially due to the higher fibre content of the meal, but this does not appear to account for all of the difference. It is not known if prepress-solvent extraction conditions adversely affect the metabolizable energy content of canola meal, but if nutrient retention is reduced then one would expect lower energy utilization as well.

Prepress-solvent extraction of canola involves ten stages, including cleaning, drying, conditioning, flaking, expelling, cooking, solvent extraction with hexane, desolventization/toasting, drying and cooling. The objective of this study was to examine the effects of current commercial processing conditions on amino acid digestibility and energy utilization of canola meal in broiler chickens.

3.3 MATERIALS AND METHODS

3.3.1 Meal Collection

Three separate sets of samples were collected over a three month period from a prepress-solvent extraction canola plant located in western Canada. Samples (10 kg in 25 L plastic pails) were collected from sample ports after flaking, conditioning, expelling (presscake), cooking, solvent extraction (Marc), desolventization/toasting, and drying and cooling. The solvent extracted, but hexane laden, samples were air desolventized for 24 hours in cotton sacks at POS[2]. The presscake and Marc were

[2] POS Pilot Plant Corp., Saskatoon, SK, Canada, S7N 2R4

ground in a hammer mill equipped with a 10 mm screen to reduce particle size and ensure even distribution when mixed into experimental diets. Samples prior to flaking were not analyzed for nutrient retention since the degree of heat processing applied to these samples was minimal and the intact seed would impair digestion independent of any processing effects.

3.3.2 Experimental Design

The three sets of samples were analyzed in three separate digestibility studies, (Experiments 1, 2 and 3). Male broiler chicks were raised to 42 days of age in raised-floor cages and fed commercial diets. Feed and water were provided *ad libitum* at all times. Whole room brooding was practiced where initial temperatures were 34°C at bird level and reduced gradually to 25.5°C by 21 d of age and held at this temperature to the end of the experiment. Care and management of the broilers were in accordance with the Canadian Council of Animal Care Guidelines (Canadian Council on Animal Care, 1993) and were approved by the University of Saskatchewan Animal Care Committee.

At 42 d of age the birds were randomly assigned to one of eight dietary treatments (seven meal types plus a basal reference diet); each treatment was replicated six times with a replicate consisting of a cage with two birds. Celite™[3] was added to all diets as a source of indigestible marker (acid insoluble ash). Diet composition is shown in Table 3.1. The birds were killed by cervical dislocation on day 47 and the contents of the distal ileum (terminal half of ileum less 2 cm of distal section) were collected. Fecal samples were collected at 46 and 47 d of age in experiments 2 and 3 for AME determination as described by Sibbald and Slinger (1963).

[3] Celite Corporation, Lompoc, California, USA, 93436

Table 3.1 Diet composition and calculated nutrient content (%)
(Experiments 1, 2 and 3)

Ingredient	Reference diet	Test diet
Maize	91.39	52.85
Canola oil	3.46	2.0
Celite	1.00	1.00
Chromic oxide	0.50	0.50
Vitamin/mineral premix[a]	0.50	0.50
Choline chloride	0.1	0.1
Dicalcium phosphate	1.81	1.81
Limestone	0.84	0.84
Sodium chloride	0.40	0.40
Test ingredient	0	40.00
Calculated nutrient content		
Crude protein (N* 6.25)	7.7	18.9
AME_n (kcal/kg)	3380	2755
Non-phytate phosphorus	0.42	0.50
Calcium	0.74	1.00
Lys	0.24	0.942
Met + cystine	0.34	0.87

[a] Supplied per kg of diet: 11500 mg vitamin A (retinal acetate + retinal palmitate), 2600 mg vitamin D_3, 40 mg vitamin E, 2.5 mg menadione, 2.5 mg thiamine, 10 mg riboflavin, 84 mg niacin, 4 mg pyridoxine, 0.016 mg vitamin B_{12}, 15.4 mg pantothenic acid, 1.1 mg folic acid, 0.14 mg biotin, 56 mg iron, 77 mg zinc, 94.5 mg manganese, 7 mg copper, 0.91 mg iodine, and 0.21 mg selenium.

The diet, test ingredient and freeze dried ileal samples were analyzed for amino acids (Edney et al., 1992) and apparent ileal digestibility was calculated (Ten Doeschate et al., 1993). Samples were hydrolyzed with performic acid (Llames and Fontane, 1994) for the determination of sulphur amino acids and 6N HCl for the determination of the remaining amino acids prior to analysis on a Beckman 7300 High Performance Amino Acid Analyzer[4]. Fecal samples were dried in a forced air oven at 55°C prior to analysis. Diet, ileal and fecal samples were analyzed for acid insoluble ash using a modification of the method of Vogtmann et al. (1975) by weighing 1-2 g of sample into 16x125 mm disposable borosilicate tubes and ashing at 500°C for 24 hours or until contents were reduced to white ash. Then, 5 ml of 4N HCl was slowly added and vortexed, the tubes were covered with glass marbles, and heated in an oven at 120° for one hour before centrifuging at 2500 × g for 10 min. The supernatant was then removed and samples were washed repeatedly with 5 ml water (with vortex, centrifugation as described above). Samples were then dried at 80°C overnight, followed by ashing at 500°C overnight. The percent acid insoluble ash was calculated as (total ashed wt - tube wt) / (original wt - tube wt). Diet and fecal samples were measured for gross energy (Association of Official Analytical Chemists, 1990) using a Parr Adiabatic Calorimeter Model 1241[5] and crude protein using a Leco model FP-528L[6] nitrogen analyzer. Diet samples were analysed for moisture by heating at 135°C for 2 hours according to the method of AOAC (Association of Official Analytical Chemists, 1990). AAFCO[7] check samples were also analysed for amino acid, crude protein, gross energy and moisture and the values obtained were comparable to those reported by other laboratories participating in the program.

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[4] Beckman Instruments, Inc., Fullerton, CA, USA 92834-3100
[5] Parr Instrument Company, Moline, Il, USA, 61265
[6] Leco Corp., St. Joseph, MI, USA, 49085-2396
[7] Association of American Feed Control Officials, Inc., College Station, TX, USA, 77841-3160

Apparent retention of 17 amino acids was calculated using the method of Ten Doeschate et al. (1993) using the formula:

$$DC_{diet} = 1-[(M_{diet}/M_i)xC_i/C_{diet})]$$

$$DC_{cm}=(DC_{diet}xC_{diet}-DC_{ref}xC_{ref}x0.60)/(C_{diet}-C_{ref}x0.60)$$

Where: DC_{diet}, DC_{cm}, DC_{ref} = digestibility coefficient of an amino acid in the diet, canola meal, and the reference diet: M_{diet}, and M_i = acid insoluble ash content of the feed and the ileal digesta; C_i, C_{diet} and C_{ref} = amino acid content of ileal digesta, of feed, and reference diet. The equation for DC_{diet} was also used to determine DC_{ref}.

3.4 STATISTICAL ANALYSIS

The nutrient digestibility data were analyzed by ANOVA using the General Linear Models (GLM) procedure (SAS Institute, 1989) blocked within sample set. Variables with significant F tests ($P \leq 0.05$) were compared using Duncan's multiple range test (Duncan, 1955). Differences were considered significant when $P<0.05$.

3.5 RESULTS AND DISCUSSION

3.5.1 Amino Acid Content

Prior to desolventization/toasting, processing had no effect on amino acid content. The solvent extracted meal entering the desolventizer/toaster (DT) was yellow in colour with dark particles of hull visible; however, desolventization/toasting changed the colour of the meal to a brownish colour (Figure 3.1) indicating that Maillard browning reactions may have occurred (Hurrell, 1984). The Lys content of the meal was reduced by desolventization/toasting (Table 3.2) which suggests that color change was due to the formation of brown pigments during Maillard reactions. The Maillard reactions were likely promoted by the elevated temperature and moisture conditions during desolventization/toasting (Mauron, 1981). The degree of heating at each of the stages varies from plant to plant, but generally the meal is heated to between 75 and 100°C in the cooker and preconditioner; the moisture content is relatively low (~6-8%), however, so Maillard reactions would only proceed at a slow pace (Mauron, 1981). During

desolventization/toasting, the meal is heated indirectly on heated trays and directly with steam (sparge steam). The sparge steam introduces both heat and moisture and, therefore, has the potential to promote Maillard reactions. Maximal loss of Lys, in the presence of reducing sugars occurs between 15 and 18% moisture (Mauron, 1981), which coincides with the level of moisture in the meal exiting the DT in this study. The Lys content of sunflower meal has also been shown to be reduced during desolventization/toasting (San Juan and Villamide, 2001), indicating that the effect is not limited to canola processing.

The desolventized/toasted meal had a numerically lower Lys content than the final meal, but this was likely an artifact of the method of sample collection. The meal exiting the DT (approximately 15% moisture and 110°C) is normally cooled and dried, which would effectively stop the Maillard reactions. However, in these experiments the samples were collected directly into 20 L plastic pails and sealed, and therefore, would have remained at elevated temperatures and moisture for an extended period. Therefore, it is impossible to separate the effects of desolventization/toasting and the drying/cooling stages. Since, the dryer/cooler consists of passing unheated air over the meal to cool and dry it as quickly as possible, it is unlikely that this stage promotes further loss of amino acids by the Maillard reaction. If additional losses are observed in the drying cooling stage, it is more likely an extension of the effect of the heating process started in the DT.

Figure 3.1 Appearance of canola meal before toasting, after toasting and after drying-cooling (shown on the left, center and right, respectively).

Table 3.2 Crude protein (% fat free, dry basis), and amino acid content (% of protein) of canola samples collected after various stages of prepress-solvent extraction

	Flaked	Cooked	Expelled	Extracted	Desolventized/ toasted	Dried	SEM
CP	21.67[c]	20.58[c]	31.05[b]	39.27[a]	39.12[a]	38.89[a]	1.96
Ala	4.44	4.52	4.47	4.41	4.34	4.40	0.03
Arg	5.85	5.95	5.79	5.89	5.57	5.60	0.05
Asp	7.24	7.54	7.33	7.32	6.95	7.18	0.06
Cys	2.31	2.34	2.23	2.18	2.10	2.12	0.04
Glu	16.58	16.55	16.45	16.46	15.93	16.53	0.11
Gly	11.26	11.84	11.08	13.38	13.21	12.67	0.04
His	2.73	3.03	3.02	3.02	2.96	2.97	0.05
Ile	4.32	4.45	4.31	4.32	4.32	4.33	0.03
Leu	6.97	7.18	6.99	7.02	6.97	7.02	0.04
Lys	6.05[a]	6.04[a]	5.98[a]	6.03[a]	5.28[b]	5.50[b]	0.09
Met	2.08	2.09	2.07	1.92	2.02	2.01	0.02
Phe	3.90	3.99	3.92	3.89	3.81	3.89	0.03
Pro	6.00	6.11	6.00	6.03	5.93	5.99	0.04
Ser	3.93	4.09	3.90	3.98	4.00	3.92	0.04
Thr	4.38	4.54	4.39	4.39	4.36	4.34	0.04
Tyr	2.78	2.82	2.76	2.64	2.71	2.64	0.04
Val	5.47	5.63	5.50	5.50	5.41	5.43	0.04

[a-c]Means within a row without a common superscript differ significantly ($P<0.05$).

N=3.

3.5.2 Amino Acid Digestibility

Prior to desolventization/toasting, processing had no effect on amino acid digestibility, except that Cys and Ser were reduced by solvent extraction. The desolventization process reduced the digestibility of crude protein, and that of all the amino acids except Leu, Met, Ser, and Tyr, as digestibility was lower in the final meal than in the solvent extracted material (Table 3.3). The loss in digestibility is likely due to early Maillard reactions, resulting in the formation of aldose derivatives of amino acids by Amadori rearrangement (Mauron, 1981). The products of early Maillard reactions (hexose amino acids) are not effectively digested, but still yield Lys after acid hydrolysis during the analysis of amino acids (Mauron, 1981). Interestingly, even though the products of the early Maillard reaction (dexoxyketosyl-derivatives) reduce digestibility, they do not result in colour changes (Hurrell, 1984). Therefore, colour may not be a good indicator of Lys digestibility. The fact that both the content and the digestibility of Lys were reduced during desolventization/toasting suggests that products of both early and late stage Maillard reactions were appearing during this phase. Lys is the primary amino acid involved in early stage Maillard reactions between protein and reducing sugars, due to the presence of the highly reactive ε-amino group on Lys (Mauron, 1981), although most of the amino acids are susceptible to advanced Maillard reactions, such as Strecker degradation, which leads to the formation of dark brown pigments (Mauron, 1981).

It would appear that not all of the browning is due to Maillard reactions. If all the colour change observed during desolventization/toasting were due to Maillard reactions alone, one might expect higher losses of Lys. Part of the change in colour and losses in amino acid availability during desolventization/toasting may be the result of protein-polyphenol interactions (Hurrell, 1984), resulting in large molecular weight, brown pigments.

Table 3.3 Apparent ileal digestibility (%) of crude protein and amino acids in canola samples collected after various stages of prepress-solvent extraction

	Flaked	Cooked	Expelled	Extracted	Desolventized-toasted	Dried	SEM
CP	84.4[a]	83.2[a]	80.6[b]	81.4[ab]	73.8[c]	76.6[c]	0.6
Ala	85.8[a]	84.2[a]	84.3[a]	84.2[a]	79.2[ab]	79.5[b]	0.5
Arg	91.0[a]	91.0[a]	89.8[a]	90.0[a]	87.2[b]	87.0[b]	0.4
Asn	79.3[a]	75.9[a]	74.8[a]	77.7[a]	66.8[b]	66.7[b]	0.9
Asp	99.6[a]	99.2[a]	98.4[a]	99.7[a]	91.4[b]	91.7[b]	0.6
Cys	83.2[a]	81.9[ab]	78.8[ab]	77.8[b]	65.4[c]	69.5[c]	0.9
Gln	87.9[a]	86.6[ab]	85.7[abc]	85.6[abc]	83.7[bc]	83.3[c]	0.4
Glu	99.7[a]	99.6[a]	98.3[a]	99.7[a]	91.7[b]	91.9[b]	0.6
Gly	82.0[a]	82.9[a]	81.1[a]	81.4[a]	74.3[b]	73.0[b]	0.8
His	85.6[ab]	84.2[bc]	86.6[ab]	87.7[a]	81.8[c]	81.7[c]	0.5
Ile	84.0[a]	83.6[a]	83.0[a]	82.5[a]	78.9[b]	78.7[b]	0.5
Leu	82.2[a]	82.1[a]	82.1[a]	81.1[a]	77.1[b]	78.7[ab]	0.6
Lys	88.3[a]	87.2[a]	85.9[a]	86.5[a]	79.2[b]	79.3[b]	0.5
Met	91.9[a]	90.8[a]	89.5[ab]	89.7[ab]	85.9[c]	87.9[b]	0.4
Phe	86.7[a]	86.2[a]	85.7[a]	85.5[a]	82.6[b]	82.4[b]	0.5
Pro	79.1[a]	77.3[a]	77.2[a]	76.5[a]	71.7[b]	71.1[b]	0.6
Ser	75.8[a]	75.1[a]	76.1[a]	68.7[b]	67.0[b]	65.4[b]	0.8
Thr	78.1[a]	77.6[a]	77.5[a]	74.9[a]	70.6[b]	69.3[b]	0.7
Tyr	77.1	79.6	78.2	75.9	77.4	74.8	0.9
Val	82.4[a]	82.1[a]	82.1[a]	81.1[a]	77.9[b]	77.3[b]	0.5

[a-c]Means within a row with no superscript differ significantly ($P<0.05$).

n=3.

Earlier studies (Barbour and Sim, 1991; Anderson-Hafermann et al., 1993) observed similar changes in amino acid digestibility of canola meal during desolventization/toasting, but only single samples were analyzed and statistical significance was not observed. Lys digestibility in sunflower meal has also been shown to be reduced from 75 to 68% during solvent extraction and desolventization (San Juan and Villamide, 2001), presumably due to desolventization/toasting. In the current study, the effect of desolventization/toasting was not consistent between sets of samples. Desolventization/toasting reduced Lys digestibility by 11.0, 6.2 and 4.5% in sets 1, 2 and 3, respectively, indicating that part of the variation in the quality of canola meal is due to variable desolventization/toasting conditions. The coefficient of variation of Lys digestibility also increased from 1.4 to 5.6 as a result of desolventization/toasting, further demonstrating the variation introduced by this process. All three sets of samples in this study were collected from a single source, so it is not known if the negative effect of the desolventization process is isolated to this one plant, but the final meal samples were similar to that of canola meal examined in previous studies (Zuprizal et al., 1993), suggesting that desolventization/toasting may be affecting meal quality in other crushing plants as well.

3.5.3 Metabolizable Energy

The metabolizable energy of the samples examined in Experiment 1 was not determined. However, since significant reductions in amino acid availability were observed in this experiment and reductions in metabolizable energy might also be expected, the effects of processing on meal metabolizable energy were determined in experiments 2 and 3. As expected, AME decreased as oil was removed during expelling and solvent extraction (Table 3.4), which is in general agreement with previously published values for canola seed and meal (Lee et al., 1995). In Experiment 2, the final meal had lower metabolizable energy than the solvent extracted meal, which is possibly due to the reduction in protein and amino acid utilization. The same trend towards decreased metabolizable energy was evident in Experiment 3, but the difference was not statistically significant.

3.6 CONCLUSIONS

The desolventization/toasting process reduced Lys content, the digestibility of most amino acids and the metabolizable energy of canola meal products, and also increased meal variability. Prepress-solvent extraction is currently the most effective method of extracting oil from canola meal, so it is unlikely that the processing methods will change in the near future. However, the loss in value of the canola meal during desolventization/toasting would indicate that the process should be modified to prevent destruction of amino acids through Maillard reactions. The elevated moisture content imparted by the sparge steam during desolventization appears to be promoting Maillard browning, so the addition of moisture should be avoided. The sparge steam injected into the meal during desolventization is thought to be an integral part of the process of stripping residual hexane from the meal, so alternatives to direct steam injection should be considered.

Table 3.4 Apparent metabolizable energy (kcal/kg, as received) of canola products after various stages of commercial processing

Stage of processing	Experiment 2	Experiment 3
Flaked	4800^{a}	5171^{a}
Cooked	5018^{a}	5113^{a}
Expelled	3774^{b}	3087^{b}
Extracted	2471^{c}	2171^{c}
Desolventized-Toasted	2347^{cd}	2008^{c}
Dried	2217^{d}	1995^{c}
SEM	210	246

$^{a-d}$Means within a column with no common superscript differ significantly (P<0.05).

4 THE AVAILABILITY AND CONTENT OF AMINO ACIDS IN TOASTED AND NON-TOASTED CANOLA MEALS

4.1 ABSTRACT

The objective of this study was to determine the level and digestibility of amino acids and the level of glucosinolates in toasted and non-toasted canola meal samples produced in commercial crushing plants in western Canada. A total of 26 non-toasted and 31 toasted canola meals samples were analysed. Apparent ileal amino acid digestibility of toasted and non-toasted canola meals samples was determined using broiler chickens between 21 and 28 d of age. Commercially toasted canola meal contained 5.6 g/16 g N (range 5.3-5.9) of Lys with digestibility ranging from 65.5 to 85.7% (mean = 78.0%). Non-toasted canola meal contained 6.0 g/16g N (range 5.7 – 6.3) Lys with digestibility values ranging from 87% to 92% (mean = 89.7%). Toasted canola meal had an average total digestible amino acid content (not including Tyr and Trp) of 69.6g/16 g N (range 56.6-75.6). Non-toasted canola meal had a total digestible amino acid content (not including Tyr and Trp) of 77.6 g/16g N (range 73.1-82.0). The levels of aliphatic glucosinolates in both the toasted (6.16 μmol/g meal, range 1.2-12.5) and non-toasted meals (10.5 μmol/g meal, range 7.8-15.1) were low, and likely of no nutritional consequence. In conclusion, non-toasted canola meal contained higher levels of digestible amino acids than conventionally toasted canola meal, but also contained more glucosinolates.

4.2 INTRODUCTION

Canola meal accounts for 60% of the whole canola seed and is, in most cases, produced by prepress-solvent extraction with hexane. The meal contains approximately 36% CP and 5.4 g Lys/16g N (NRC, 1994) and is, therefore, a good source of protein for poultry diets. The content and availability of amino acids in canola meal are reduced

during desolventization/toasting, and it has been proposed that desolventization practices be changed to produce a non-toasted meal (see section 3). Toasting has not been well defined but is a term used to describe the chemical and physical changes that occur in the meal during standard desolventization. In this thesis, therefore a non-toasted meal is considered to be a meal that has been desolventized but no visible or physical changes to the meal are observed. Only limited studies have examined the nutritional value of non-toasted meal (section 3 and Anderson-Haferman et al., 1993) and as a result, a survey of the nutritional value of non-toasted canola meals is required prior to any changes being made to commercial processing methods. The nutritional value of canola meal has been surveyed previously (Bell and Keith, 1991), but most of the canola crushing plants in western Canada have been upgraded since that time, potentially influencing the nutritional value of the meal. Accordingly, a new survey of the nutritional value of commercially toasted canola meal should be conducted as well.

Glucosinolates are heat labile and, consequently, their levels are reduced during commercial processing (Jensen et al., 1995a). Changing the processing method to produce a non-toasted meal would likely result in a meal with increased levels of glucosinolates, but the level of glucosinolates that would be present is not known. Characterization of the level of glucosinolates in toasted and non-toasted canola meal produced in modern processing plants and using current low glucosinolate canola varieties is warranted.

The objectives of this study were to determine the content and availability of amino acids, and the level of glucosinolates, in toasted and non-toasted canola meals produced in the canola crushing plants located in western Canada.

4.3 MATERIALS AND METHODS

4.3.1 Sample Collection

Conventional toasted meals (TCM) and hexane laden, non-toasted meals (NTCM) were collected from canola crushing plants located in western Canada. Samples were

collected from seven crushing plants belonging to four companies: ADM[8] (Lloydminster AB), CanAmera Foods[9] (Fort Saskatchewan, AB, Nipawin, SK, Harrowby, MB and Altona, MB), Canbra Foods[10] (Lethbridge, AB), and Cargill[11] (Clavet, SK). Plants were randomly assigned plant numbers so as to retain confidentiality of data. Five sets of TCM and NTCM samples were collected from each plant over a two-week period with no more than one set per plant being collected in any given 48 h period. The TCM and NTCM samples were to be collected simultaneously to ensure that they were processed under the same conditions and originated from the same source of seed. Plant seven was only able to provide the one TCM sample and plant six collected five TCM samples and only one NTCM sample. In total 31 TCM and 26 NTCM samples were collected from the seven crushing plants. A sample of canola meal, obtained from a local feed manufacturer was used as a control. The NTCM samples were laden with hexane upon receipt and were desolventized at the POS Pilot Plant Corp.[12]. Desolventization was achieved by placing samples in cotton sacks and allowing the hexane to evaporate at room temperature in an experimental air desolventizer until no residual hexane could be detected by smell.

4.3.2 Nutrient Retention

The 26 NTCM, 31 TCM, and a locally obtained canola meal sample were tested for amino acid digestibility in broiler chickens using a modification of the method of Scott et al. (1998) at the Pacific Agri-Food Research Centre[13]. Due to the large number of samples, the samples were assayed over two experiments. The NTCM and TCM meals were tested in Experiments 1 and 2, respectively. The commercial canola meal served as a control in both experiments. Broiler chicks (6 per 2500 cm^2 cage, replicated 4 times) were raised to 28d of age, and treatment and replication were assigned

[8] Archer Daniels Midland Company, 4805-62 Ave., P.O. Box 1620, Lloydminster, AB/SK, Canada, S9V 1K5.
[9] CanAmera Foods, 2190 South Service RD. W., Oakville, ON, Canada, L6L 5N1.
[10] Canbra Foods, P.O. Box 99, Lethbridge, AB, Canada, T1J 3Y4.
[11] Cargill Limited, P.O. Box 190, Clavet, SK, Canada, S0K 0Y0.
[12] POS Pilot Plant Corp., 118 Veterinary Road, Saskatoon, SK, Canada, S7N 2R4.
[13] Pacific Agri-Food Research Centre, P.O. Box 1000, Agassiz, British Columbia, Canada, V0M 1Z0.

randomly to the cages. Due to limited quantities of NTCM available for the study, the birds in Experiment 1 were provided a starter diet containing 20% locally obtained canola meal for 23 days prior to feeding the test or reference diets up to 28 d (Table 4.1), whereas in Experiment 2, birds were fed a starter diet containing 20% of the test ingredient and the test and reference diets were started on day 21. Water and feed were provided *ad libitum* at all times. The test diet contained 40% of the test ingredient and the reference diet contained none (Table 4.1). All test and reference diets contained 1.1 % Celite™[14] as an indigestible acid insoluble ash marker. The birds were killed by cervical dislocation at 28 d in both experiments and the contents of the distal half of the ileum less the terminal 2 cm were collected. The ileal digesta of the six birds within a cage were pooled, freeze dried, and ground prior to analysis.

Whole room brooding was practiced where initial temperatures were 34°C at bird level and reduced gradually to 25.5°C by 21 d of age and held at this temperature to the end of the experiment. Care and management of the broilers were in accordance with the Canadian Council of Animal Care Guidelines (1993). The Pacific Agri-Food Research Centre Animal Care Committee approved all experimental protocols.

4.3.3 Analytical Procedures

Test ingredients, diets and digesta were analyzed for acid insoluble ash marker using a modification of the method of Vogtmann et al. (1975) using 1-2 g of sample in 16x125 mm disposable borosilicate tubes. The samples were ashed at 500°C for 24 h or until contents were reduced to white ash, then 5 ml of 4N HCl slowly added and vortexed. The tubes were covered with glass marbles and heating in oven at 120° for one h before centrifuging at 2500 × g for 10 min. The supernatant was removed and samples washed repeatedly with 5 ml water (with vortex, centrifugation as described above). Samples were then dried at 80°C overnight, followed by ashing at 500°C overnight. The percent acid insoluble ash was calculated as (total ashed wt - tube wt) / (original wt - tube wt).

[14] Celite Corporation, Lompoc, California, USA, 93436

Table 4.1 Composition of the starter, test and reference diets

Ingredient	Starter	Grower	
		Test diet	Reference diet
	(%)		
Corn	56.4	56.2	91.1
Soybean meal	14.7	0	0
Canola oil	4.3	2.0	3.5
Celite marker	0	1.1	1.1
Vitamin premix[1]	0.5	0.5	0.5
Mineral premix[2]	0.5	0.5	0.5
Choline chloride	0.1	0.1	0.1
Dicalcium phosphate	1.35	1.61	1.61
Limestone	1.57	1.21	1.21
Sodium chloride	0.47	0.40	0.40
Test ingredient	20.0	40.0	0
Calculated composition			
Crude protein (%)	19.2	18.9	7.7
AME (kcal/kg)	3050	2755	3380
Non-phytate P (%)	0.42	0.50	0.42
Calcium (%)	1.00	1.00	0.74
Lys (%)	1.00	0.94	0.24
Met+Cys (%)	0.82	0.87	0.34

[1]Supplied per kilogram of diet: vitamin A, 11,000 IU; cholecalciferol, 2,200 IU; vitamin E, 30 IU; vitamin K, 0.5 mg; vitamin B_{12}; 0.02 mg; thiamine, 1.5 mg; riboflavin, 6 mg; folic acid, 0.6 mg; biotin, 0.15 mg; niacin, 60 mg; pyridoxine, 5 mg; choline chloride, 400 mg.

[2]Supplied per kilogram of diet: chloride, 788 mg; sodium, 511 mg; iron, 80 mg; manganese, 21.8 mg; selenium, 0.1 mg; iodine, 0.35 mg; zinc, 100 mg.

The dried test ingredients, diets and ileal digesta were analyzed for amino acids using a Beckman[15] amino acid analyzer equipped with a Spheroge 1 IEX sodium ion exchange column, System Gold 126AA solvent module, 508 auto-sampler, 166 detector, ninhydrin 232 post column reactor, Mistral[16] column. The samples were hydrolysed using the performic acid oxidation with acid hydrolysis and sodium metabisulfite method as described by Llames and Fontaine (1994, Alternative I) prior to injection on the amino acid analyzer. Feed, meal and digesta samples were analyzed for crude protein (Nx6.25) with a Leco[17] nitrogen analyzer, FP-528L, and moisture (Association of Official Analytical Chemists, 1990). All ingredients and digesta samples were analyzed in duplicate and the feed samples analyzed in quadruplicate except for amino acids. The TCM and NTCM samples were analyzed for glucosinolates as described by Daun et al. (1989). AAFCO[18] check samples were also analysed for amino acid, crude protein and moisture and the values obtained were comparable to those reported by other laboratories participating in the program.

Apparent retention of 17 amino acids was calculated using the method of Ten Doeschate et al. (1993) using the formula:

$$DC_{diet} = 1-[(M_{diet}/M_i)xC_i/C_{diet})]$$

$$DC_{cm}=(DC_{diet}xC_{diet}-DC_{ref}xC_{ref}x0.60)/(C_{diet}-C_{ref}x0.60)$$

Where: DC_{diet}, DC_{cm}, DC_{ref} = digestibility coefficient of an amino acid in the diet, canola meal, and the reference diet: M_{diet}, and M_i = acid insoluble ash content of the feed and the ileal digesta; C_i, C_{diet} and C_{ref} = amino acid content of ileal digesta, of feed, and reference diet. The equation for DC_{diet} was also used to determine DC_{ref}.

[15] Beckman Instruments, Inc., Fullerton, CA, USA, 92834-3100.
[16] Holland Spark, P.O. Box 388, 7800 AJ Emmen, The Netherlands.
[17] Leco Corp., St. Joseph, MI, USA, 49085-2396.
[18] Association of American Feed Control Officials, Inc. College Station, TX, USA, 77841-3160.

4.4 STATISTICAL ANALYSIS

The amino acid and glucosinolate content of the toasted and non-toasted canola meals were compared by analysis of variance using the GLM procedure of the SAS Institute (1989). The two amino acid digestibility studies were analyzed separately by ANOVA using the same procedure. Variables with significant F tests ($P \leq 0.05$) were compared using Duncan's multiple range test (Duncan, 1955). Differences were considered significant when $P<0.05$.

4.5 RESULTS

4.5.1 Amino Acid Level

The content of amino acids, crude protein and moisture of the toasted and non-toasted meals on an as-received basis are shown in Table 4.2. Since the crude protein and moisture content of the meals varied among samples, the remainder of the amino acid results are expressed as a proportion of the protein.

The proportions of amino acids (g/16g nitrogen) in the toasted canola meal (TCM) and non-toasted canola meal (NTCM) samples are shown in Table 4.3. The non-toasted canola meal contained significantly more Lys, Arg, His, Asn, Ser, Val, Leu, Ile, Pro, and Phe than did the toasted meals. The toasted meals contained higher levels of Met, Gln and crude protein (% dry matter basis) than did the non-toasted meals. Desolventization/toasting had no effect on the level of Cys or Gly. There were plant effects on protein and all of the amino acids except Arg, Met and Asp (Table 4.4). Plants 6 and 7 were removed from the Duncan's means separation (Table 4.4) because they did not contain equal numbers of toasted and non-toasted meals and could have biased the results. There were significant plant by processing interactions on the proportions of Lys, Gln, Ser and Thr in meals (Table 4.5).

Table 4.2 The level of amino acids, crude protein and moisture in non-toasted canola meals (NTCM) and toasted canola meal (TCM)

	NTCM			TCM			
Amino Acid	Mean	Min	Max	Mean	Min	Max	SEM
	(% as received)						
Lys	2.35[a]	2.25	2.43	2.16[b]	2.12	2.20	0.02
Arg	2.34[a]	2.22	2.43	2.59[b]	2.18	2.35	0.02
His	1.24[a]	1.18	1.28	1.21[b]	1.17	1.29	0.01
Cys	0.92	0.85	0.98	0.93	0.87	0.98	0.01
Met	0.77	0.73	0.80	0.81	0.77	0.86	0.01
Asn[1]	2.90[a]	2.82	2.95	2.83[b]	2.78	2.89	0.01
Gln[2]	6.45[b]	6.01	6.78	7.13[a]	6.70	7.67	0.06
Gly	1.95[a]	1.88	2.00	1.92[b]	1.88	2.01	0.01
Ser	1.59[a]	1.53	1.68	1.57[b]	1.50	1.68	0.01
Thr	1.74[a]	1.70	1.79	1.71[b]	1.67	1.79	0.01
Ala	1.74[a]	1.68	1.78	1.71[b]	1.67	1.79	0.01
Val	2.18[a]	2.11	2.24	2.14[b]	2.10	2.23	0.01
Leu	2.80[a]	2.70	2.87	2.76[b]	2.69	2.89	0.01
Ile	1.73[a]	1.67	1.77	1.69[b]	1.66	1.75	0.01
Pro	2.39[a]	2.67	2.50	2.34[b]	2.23	2.45	0.01
Phe	1.53[a]	1.48	1.57	1.50[b]	1.46	1.56	0.01
CP	39.53[a]	37.36	41.00	38.89[b]	37.77	40.49	0.19
Moisture	8.73	7.32	12.67	11.13	10.36	12.30	0.24
N	26			31			

[ab]Means within a row without a common superscript differ significantly $P<0.05$.
[1]Includes Asp and Asn.
[2]Includes Gln and Glu.

Table 4.3 The proportions (g/16gN) of amino acids in non-toasted canola meals (NTCM), toasted canola meal (TCM), and the statistical probability of the effect of plant, meal type or an interaction

	NTCM			TCM				Probability		
Amino Acid	Mean	Min	Max	Mean	Min	Max	SEM	Meal	Plant	Interaction
Lys	5.97[a]	5.66	6.30	5.55[b]	5.30	5.87	0.04	***	***	*
Arg	5.94[a]	5.74	6.34	5.78[b]	4.70	6.10	0.03	**	NS	NS
His	3.16[a]	2.98	3.33	3.10[b]	2.96	3.33	0.01	***	***	NS
Cys	2.34	1.17	2.54	2.39	2.09	2.57	0.02	NS	NS	NS
Met	1.97[b]	0.98	2.13	2.06[a]	1.97	2.20	0.02	*	NS	NS
Asn[1]	7.37[a]	7.00	7.71	7.25[b]	6.98	7.71	0.03	***	***	NS
Gln[2]	16.48[b]	15.74	17.73	18.15[a]	17.17	19.06	0.13	***	***	**
Gly	4.95	4.68	5.16	4.91	4.75	5.13	0.02	NS	***	NS
Ser	4.06[a]	3.76	4.34	4.00[b]	3.76	4.23	0.02	*	***	***
Thr	4.32[b]	4.16	4.66	4.38[a]	4.24	4.57	0.02	*	***	**
Ala	4.42	4.23	4.61	4.37	4.21	4.56	0.01	*	***	NS
Val	5.54[a]	5.31	5.76	5.46[b]	5.28	5.73	0.02	**	***	NS
Leu	7.14[a]	6.83	7.45	7.06[b]	6.81	7.34	0.02	*	**	NS
Ile	4.39[a]	4.23	4.60	4.33[b]	4.18	4.54	0.01	**	**	NS
Pro	6.10[a]	5.84	6.53	5.97[b]	5.78	6.29	0.02	***	***	NS
Phe	3.90[a]	3.75	4.08	3.83[b]	3.69	3.97	0.01	***	**	NS
CP (% DM)	43.02[b]	40.06	45.84	43.79[a]	40.69	46.60	0.20	*	*	NS
N	26			31						

[ab]Means within a row without a common superscript differ significantly $P<0.05$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, NS = not significant ($P > 0.05$).

[1]Includes Asp and Asn.

[2]Includes Gln and Glu.

Table 4.4 The levels of amino acids and protein of canola meal samples (average includes both meals before and after desolventization/toasting) from seven crushing plants

Amino Acid	Plant 1	Plant 2	Plant 3	Plant 4	Plant 5	Plant 6[1]	Plant 7[1]
				(g/16 N)			
Lys	5.86^{a}	5.81^{a}	5.85^{a}	5.63^{b}	5.67^{b}	5.63	5.44
His	3.13^{bc}	3.20^{a}	3.18^{ab}	3.10^{cd}	3.05^{d}	3.07	3.18
Asn[2]	7.59^{a}	7.32^{b}	7.39^{b}	7.10^{c}	7.18^{c}	7.21	7.14
Gln[3]	17.05^{c}	17.48^{ab}	17.67^{a}	17.12^{bc}	17.18^{bc}	17.88	18.94
Gly	5.01^{a}	4.98^{a}	4.99^{a}	4.88^{b}	4.79^{c}	4.91	4.97
Ser	4.10^{a}	4.02^{ab}	4.11^{a}	3.95^{bc}	3.89^{c}	4.10	4.16
Thr	4.53^{a}	4.42^{bc}	4.46^{b}	4.36^{c}	4.26^{d}	4.40	4.41
Ala	4.46^{a}	4.43^{a}	4.46^{a}	4.34^{b}	4.30^{b}	4.35	4.43
Val	5.60^{a}	5.52^{ab}	5.58^{a}	5.44^{bc}	5.38^{c}	5.43	5.50
Leu	7.20^{a}	7.10^{ab}	7.19^{a}	7.04^{b}	6.98^{b}	7.02	7.15
Ile	4.44^{a}	4.37^{ab}	4.42^{a}	4.32^{b}	4.30^{b}	4.28	4.31
Pro	5.98^{bc}	6.06^{b}	6.20^{a}	6.03^{bc}	5.94^{c}	5.91	6.06
Phe	3.93^{a}	3.87^{ab}	3.91^{a}	3.80^{b}	3.81^{b}	3.81	3.85
CP (% DM)	41.61^{c}	42.98^{b}	43.12^{b}	44.53^{a}	44.14^{a}	44.48	45.37
N	10	10	10	10	10	6	1

[abcd]Means within a row without a common superscript differ significantly $P<0.05$.

[1]Means were excluded from the Duncan's analysis due to an imbalance between TCM and NTCM samples. Plant 6 contributed 5 TCM and 1 NTCM. Plant 7 contributed 1 TCM and no NTCM samples.

[2]The sum of Asp and Asn.

[3]The sum of Gln and Glu.

Table 4.5 The interaction of toasting and processing plant on the level (g/16g N) of amino acids

Amino Acid	Meal	Plant 1	Plant 2	Plant 3	Plant 4	Plant 5	Plant 6	Plant 7
		(g/16g N)						
Lys	NTCM[1]	6.01[ab]	6.04[ab]	6.10[a]	5.89[bc]	5.79[cd]	6.04[ab]	NA
	TCM[1]	5.70[de]	5.56[ef]	5.59[ef]	5.36[g]	5.54[efg]	5.54[efg]	5.44[fg]
Gln	NTCM	16.35[cd]	16.99[c]	16.97[c]	16.10[d]	16.08[d]	16.01[d]	NA
	TCM	17.75[b]	17.98[b]	18.36[ab]	18.13[b]	18.29[ab]	18.25[ab]	18.94[a]
Ser	NTCM	4.23[a]	4.13[abc]	4.22[a]	3.92[de]	3.80[e]	4.08[abcd]	NA
	TCM	3.97[cde]	3.91[de]	4.01[bcd]	3.99[bcd]	3.98[bcd]	4.11[abc]	4.16[ab]
Thr	NTCM	4.61[a]	4.97[abc]	4.49[abcd]	4.32[efg]	4.23[g]	4.53[ab]	NA
	TCM	4.46[bcde]	4.35[defg]	4.28[bcdef]	4.40[bcdef]	4.29[fg]	4.37[cdef]	4.41[bcdef]

[abcdefg]Means within an amino acid without a common superscript differ significantly $P<0.05$.

[1]NTCM, non-toasted canola meal; TCM, toasted canola meal

4.5.2 Amino Acid Digestibility

Apparent ileal digestibilities (mean plus minimum and maximum) of amino acids and crude protein in the non-toasted canola meals and the toasted canola meal control are shown in Table 4.6. Digestibilities were higher for all amino acids in the non-toasted canola meal than in the toasted canola meal control. The crushing plant used also affected the digestibilities of Lys, His, Met, Gly, Thr, Ile, Phe and crude protein (Table 4.7).

The apparent digestibilities of the amino acids and crude protein in the toasted canola meal samples are shown in Table 4.8. The TCM had higher Phe and Asn digestibility, but values for crude protein and the rest of the amino acids were similar to the control TCM. The crushing plant used had a significant effect on digestibility of all of the amino acids and crude protein (Table 4.9).

4.5.3 Glucosinolate Level

Glucosinolate levels in the TCM and NTCM are shown in Table 4.10. Toasting reduced the levels of all glucosinolates except the 2-hydroxy-4-pentenyl form, which was already low (0.20 μmol/g). The crushing plant had a significant effect on glucosinolate levels (Table 4.11). Meals from crushing plants 6 and 7 were removed from the Duncan's analysis of overall plant means, as previously described, due to an imbalance in the number of TCM and NTCM meals from these two crushing plants. The non-toasted meal from plant 5 contained the least amount of allyl glucosinolate, a glucosinolate from non-canola origin, while the meals from plant 6 contained the most.

Table 4.6 Apparent ileal amino acid digestibility of non-toasted canola meal (NTCM) and control toasted canola meal (TCM, Experiment 1)

	NTCM			TCM control	
Amino Acid	Mean	Min	Max	Mean	SEM
	(%)				
Lys	89.7[a]	87.1	92.3	76.5[b]	0.3
Arg	93.5[a]	92.5	95.2	86.3[b]	0.2
His	90.6[a]	86.3	93.2	83.0[b]	0.2
Cys	86.4[a]	84.2	88.8	69.8[b]	0.4
Met	91.5[a]	80.5	95.0	85.6[b]	0.4
Asn[1]	87.1[a]	84.9	89.9	71.0[b]	0.3
Gln[2]	92.2[a]	90.7	94.0	84.6[b]	0.2
Gly	87.9[a]	85.0	91.1	75.0[b]	0.3
Ser	83.5[a]	80.5	87.3	70.2[b]	0.3
Thr	82.4[a]	78.2	87.2	67.2[b]	0.4
Ala	88.3[a]	85.7	90.6	79.6[b]	0.2
Val	85.2[a]	83.1	88.2	77.1[b]	0.2
Leu	86.6[a]	85.1	88.9	77.4[b]	0.2
Ile	84.0[a]	81.0	87.9	71.6[b]	0.3
Pro	83.3[a]	80.1	86.5	69.6[b]	0.4
Phe	88.5[a]	86.1	90.4	78.6[b]	0.3
CP	81.9[a]	78.8	86.1	66.2[b]	0.3
N	26			1	

[ab]Means within a row without common superscript differ significantly $P<0.05$.

[1]Includes Asp and Asn.

[2]Includes Gln and Glu.

Table 4.7 The apparent ileal digestibility of amino acids in non-toasted canola meal samples from six[1] crushing plants (Experiment 1)

Amino Acid	Plant 1	Plant 2	Plant 3	Plant 4	Plant 5	Plant 6	Probability
				(%)			
Lys	90.1^{ab}	90.3^{a}	90.0^{ab}	89.0^{b}	89.2^{ab}	89.1^{ab}	***
His	91.2^{a}	91.3^{a}	90.8^{a}	90.5^{ab}	89.2^{b}	90.4^{ab}	*
Met	93.7^{a}	93.5^{a}	93.2^{a}	91.2^{a}	87.4^{b}	85.3^{b}	***
Gly	88.7^{a}	88.4^{a}	88.3^{a}	87.3^{b}	87.2^{b}	86.8^{b}	***
Thr	83.8^{a}	83.8^{a}	83.7^{a}	80.9^{b}	80.8^{b}	78.7^{c}	***
Ile	85.4^{a}	84.6^{ab}	84.5^{ab}	82.8^{c}	83.3^{bc}	81.0^{d}	***
Phe	88.7^{ab}	89.3^{a}	89.1^{a}	88.1^{ab}	87.6^{bc}	86.8^{c}	***
CP	82.6^{a}	82.2^{a}	82.5^{a}	81.1^{a}	81.4^{a}	79.7^{b}	***
N	5	5	5	5	5	1	

[abc]Means within a row without common superscript differ significantly $P<0.05$.

* $P < 0.05$, *** $P < 0.001$.

[1]Plant 7 did not contribute non-toasted canola meal samples.

Table 4.8 Apparent ileal amino acid digestibility of toasted canola meal (TCM) samples and the control TCM (Experiment 2)

Amino Acid	TCM samples Mean	TCM samples Min	TCM samples Max	TCM control Mean	SEM
	(%)				
Lys	78.0	65.5	85.7	73.8	0.5
Arg	86.2	76.5	90.5	83.4	0.4
His	83.5	73.9	88.8	80.4	0.4
Cys	73.5	57.2	83.8	72.4	0.6
Met	79.1	38.0	87.9	84.2	0.9
Asn[1]	75.1[a]	58.4	84.9	68.6[b]	0.6
Gln[2]	85.3	74.8	90.1	82.4	0.4
Gly	85.2	60.3	83.6	72.6	0.6
Ser	70.5	53.1	81.4	70.6	0.7
Thr	68.9	48.6	79.7	68.6	0.7
Ala	79.1	64.5	87.0	77.6	0.5
Val	75.7	62.0	82.9	71.1	0.6
Leu	78.8	62.7	87.8	76.2	0.6
Ile	73.3	56.2	82.2	71.6	0.7
Pro	75.3	55.5	84.3	75.6	0.6
Phe	81.1[a]	66.3	88.4	71.6[b]	0.5
CP	71.0	55.1	78.5	68.0	0.5
N	31			1	

[ab]Means within a row without a common superscript differ significantly $P<0.05$.

[1]Includes Asp and Asn.

[2]Includes Gln and Glu.

Table 4.9 The digestibility of amino acids in toasted canola meal samples from seven crushing plants (Experiment 2)

Amino Acid	Plant 1	Plant 2	Plant 3	Plant 4	Plant 5	Plant 6	Plant 7	Probability
	(%)							
Lys	81.6^{ab}	79.0^{abc}	77.4^{c}	70.4^{d}	82.1^{a}	77.3^{c}	77.9^{bc}	***
Arg	86.5^{abc}	87.8^{ab}	85.9^{abc}	83.5^{c}	88.5^{a}	84.8^{bc}	86.0^{abc}	**
His	84.8^{ab}	85.4^{a}	82.4^{ab}	81.4^{b}	84.5^{ab}	81.7^{ab}	85.1^{ab}	*
Cys	76.7^{ab}	74.4^{ab}	72.0^{bc}	68.2^{c}	77.8^{a}	72.3^{abc}	74.2^{ab}	***
Met	83.3^{a}	81.8^{a}	79.1^{a}	66.9^{b}	82.2^{a}	79.3^{a}	86.0^{a}	***
Asn[1]	76.2^{ab}	75.4^{ab}	73.2^{bc}	70.5^{c}	80.1^{a}	75.5^{ab}	72.5^{bc}	***
Gln[2]	85.9^{ab}	86.3^{ab}	84.2^{bc}	81.9^{c}	88.0^{a}	84.7^{bc}	85.9^{ab}	***
Gly	76.1^{ab}	75.3^{ab}	73.7^{bc}	69.9^{c}	80.0^{a}	75.3^{ab}	75.3^{ab}	***
Ser	70.8^{b}	70.2^{b}	68.5^{b}	65.3^{b}	76.7^{a}	71.1^{b}	70.5^{b}	***
Thr	70.6^{a}	70.1^{a}	68.5^{a}	61.3^{b}	73.4^{a}	68.5^{a}	71.1^{a}	***
Ala	79.5^{ab}	79.3^{ab}	77.4^{bc}	74.4^{c}	83.4^{a}	79.3^{ab}	80.1^{ab}	***
Val	76.5^{ab}	76.6^{ab}	75.4^{abc}	71.0^{c}	79.3^{a}	73.5^{bc}	78.4^{ab}	***
Leu	79.3^{ab}	79.6^{ab}	77.5^{ab}	74.8^{b}	82.4^{a}	78.1^{ab}	80.2^{a}	**
Ile	75.9^{ab}	74.0^{ab}	72.1^{b}	66.3^{c}	78.6^{a}	71.5^{bc}	73.4^{ab}	***
Pro	77.9^{ab}	73.1^{bc}	71.8^{c}	72.2^{bc}	79.7^{a}	77.2^{abc}	74.1^{bc}	***
Phe	82.4^{ab}	82.4^{ab}	79.8^{bc}	77.4^{c}	85.0^{a}	80.4^{abc}	83.2^{ab}	***
CP	72.1^{ab}	72.0^{ab}	69.6^{bc}	65.9^{c}	76.3^{a}	69.6^{bc}	68.9^{bc}	***
N	5	5	5	5	5	5	1	

[abc]Means within a row without a common superscript differ significantly $P<0.05$.

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

[1]Includes Asp and Asn.

[2]Includes Gln and Glu.

Table 4.10 The glucosinolate content of non-toasted (NTCM) and toasted (TCM) canola meal samples and the statistical probability of meal type or plant effect, or a meal type by plant interaction

Glucosinolate	NTCM			TCM			SEM	Probability		
	Mean	Min	Max	Mean	Min	Max		Meal	Plant	Interaction
	(μmol/g)									
3-Butenyl	3.40[a]	2.70	5.30	1.94[b]	0.50	4.30	0.14	***	***	***
4-Pentenyl	0.67[a]	0.30	2.00	0.38[b]	0.00	1.60	0.05	***	***	NS
2-OH-3-Butenyl	6.28[a]	4.70	9.00	3.64[b]	0.70	6.20	0.25	***	***	***
2-OH-Pentenyl	0.20	0.10	0.50	0.20	0.00	0.60	0.02	NS	***	**
Total aliphatic	10.55[a]	7.8	15.10	6.16[b]	1.20	12.50	0.43	***	***	***
3-Methylindoyl	0.58[a]	0.30	1.00	0.22[b]	0.00	0.50	0.03	***	***	***
4-OH-3-Methylindolyl	4.20[a]	2.50	5.90	0.78[b]	0.10	1.70	0.24	***	***	***
Total indolyls	4.78[a]	2.80	6.90	1.00[b]	0.10	2.10	0.27	***	***	***
Allyl	0.52[a]	0.00	2.40	0.37[b]	0.00	1.10	0.06	***	***	***
N	26			31						

[ab]Means within a row without common superscript differ significantly $P<0.05$.

** $P < 0.01$, *** $P < 0.001$, NS = not significant ($P > 0.05$).

Table 4.11 The glucosinolate content (μmol/ g) of canola meal samples (includes both toasted and non-toasted meals) from seven crushing plants

Glucosinolate	Plant 1	Plant 2	Plant 3	Plant 4	Plant 5	Plant 6[1]	Plant 7[1]
	(μmol/g)						
3-Butenyl	3.50^{a}	2.64^{bc}	2.23^{cd}	2.01^{d}	2.84^{b}	2.58	0.60
4-Pentenyl	1.16^{a}	0.52^{b}	0.26^{c}	0.30^{c}	0.32^{c}	0.62	0.10
2-OH-3-Butenyl	5.54^{b}	4.96^{b}	4.07^{c}	3.57^{c}	6.34^{a}	5.00	1.40
2-OH-Pentenyl	0.33^{a}	0.15^{b}	0.07^{c}	0.07^{c}	0.27^{a}	0.45	0.0
Total aliphatic	10.53^{a}	4.98^{b}	4.99^{c}	4.88^{c}	9.77^{a}	8.65	2.10
3-Methylindoyl	0.39^{b}	0.29^{c}	0.40^{b}	0.36^{b}	0.61^{a}	0.25	0.00
4-OH-3-Methylindolyl	2.50^{b}	2.12^{c}	2.29^{bc}	2.41^{bc}	3.10^{a}	1.55	0.10
Total indolyls	2.89^{b}	2.41^{c}	2.69^{bc}	2.77^{bc}	3.71^{a}	1.80	0.10
Allyl	0.75^{a}	0.72^{a}	0.14^{b}	0.13^{b}	0.09^{b}	1.07	0.20
n	10	10	10	10	10	6	1

^{abcd}Means within a row without a common superscript differ significantly $P<0.05$.

[1]Means were excluded from the Duncan's analysis due to an imbalance between toasted canola meal (TCM) and non-toasted canola meal (NTCM) samples in the mean. Plant 6 contributed 5 TCM and 1 NTCM. Plant 7 contributed 1 TCM and no NTCM samples.

Table 4.12 The interaction between toasting and plant on glucosinolate content (μmol/g)

Amino Acid	Meal	Plant 1	Plant 2	Plant 3	Plant 4	Plant 5	Plant 6	Plant 7
3-Butenyl	NTCM[1]	3.86^{ab}	3.32^{bc}	2.80^{cd}	3.36^{bc}	3.46^{bc}	4.30^{a}	NA[3]
	TCM[2]	3.14^{bc}	1.96^{e}	1.66^{e}	0.66^{f}	2.22^{de}	2.24^{de}	0.60^{f}
2-OH-3-Butenyl	NTCM	5.92^{bc}	6.18^{b}	5.14^{bcd}	6.14^{bc}	7.78^{a}	7.50^{a}	NA
	TCM	5.16^{bcd}	3.74^{ef}	3.00^{f}	1.00^{g}	4.90^{cde}	4.50^{de}	1.40^{g}
2-OH-Pentenyl	NTCM	0.36^{ab}	0.20^{cd}	0.10^{de}	0.14^{de}	0.18^{cd}	0.40^{ab}	NA
	TCM	0.30^{bc}	0.10^{de}	0.04^{de}	0.00^{e}	0.36^{ab}	0.46^{a}	0.30^{bc}
Total aliphatic	NTCM	11.42^{ab}	10.36^{bc}	8.36^{cde}	10.16^{bc}	11.88^{ab}	13.50^{a}	NA
	TCM	9.64^{bcd}	6.18^{ef}	4.90^{f}	1.74^{g}	7.66^{de}	7.68^{de}	2.10^{g}
3-Methylindoyl	NTCM	0.48^{cd}	0.38^{de}	0.58^{bc}	0.64^{b}	0.84^{a}	0.50^{c}	NA
	TCM	0.30^{ef}	0.20^{f}	0.22^{f}	0.08^{g}	0.38^{de}	0.20^{f}	0.00^{g}
4-OH-3-Methylindolyl	NTCM	3.60^{b}	3.50^{b}	3.96^{b}	4.70^{a}	5.10^{a}	5.00^{a}	NA
	TCM	1.40^{c}	0.74^{de}	0.62^{de}	0.12^{e}	1.10^{cd}	0.86^{cd}	0.10^{e}
Total indolyls	NTCM	4.08^{b}	3.88^{b}	4.54^{b}	5.34^{a}	5.94^{a}	5.50^{a}	NA
	TCM	1.70^{c}	0.94^{d}	0.84^{de}	0.20^{ef}	1.48^{cd}	1.06^{cd}	0.10^{f}
Allyl	NTCM	0.88^{b}	0.84^{b}	0.20^{de}	0.26^{cde}	0.04^{e}	2.4^{a}	NA
	TCM	0.62^{bc}	0.60^{bcd}	0.08^{e}	0.00^{e}	0.04^{e}	0.62^{bc}	0.60^{bcd}

^{abcdef}Means within a glucosinolate category without a common superscript differ significantly $P<0.05$.

[1]NTCM = non-toasted canola meal.

[2]TCM = toasted canola meal.

[3]NA= not available.

4.6 DISCUSSION

Toasted canola meal samples contained substantially lower levels of amino acids on a proportional basis than did non-toasted canola meal. The level of Lys was, on average, 7% lower and in the extreme case 11.2% lower. The level of all the other amino acids except Met and Gln, were also lower in toasted canola meal samples. These data support the hypothesis that the final stages of meal processing, and in particular desolventizing/toasting, reduce meal amino acid content. This is in agreement with previous work (Chapter 3) where desolventizing/toasting caused a similar effect in samples obtained from one processing plant. The exact reason for the loss in amino acid content cannot be determined from this research, but the major effect on Lys, the brown colour and knowledge of the conditions of the desolventizing/toasting step indicate that Maillard reactions are likely responsible.

The change in amino acid composition of the meal with desolventization/toasting may be due to the addition of screenings or gums to the meal in the DT. The screenings cleaned from the seed prior to processing are often added back to the meal in the DT as a means of disposal. Depending on the composition of these screenings, this could affect the amino acid and protein composition of the meal. However, the screenings are believed to consist primarily of fragments of straw or seed pods. These materials contain low levels of protein so should have very little impact on the level of amino acids in the meal when expressed as a percentage of the protein. Gums recovered from the oil are also commonly added to the meal but the protein content of this lipid/wax material would be very low and would not have a major impact on the amino acid composition as a proportion of the protein. Therefore, the changes in amino acid composition are likely due directly to the desolventization/toasting process.

Not only is the desolventization/toasting process reducing amino acid content but it appears to be introducing a significant amount of variation. The interaction of plant and meal type on the contents of Lys, Gln, Ser and Thr revealed that some crushing plants were reducing the amino acid content during desolventization/toasting more than others. However, there was a significant amount of variation in the amino acid contents of the toasted meals within each plant, which indicates that the desolventization/toasting process increases the potential for introduction of variation, even within a plant. This supports the

hypothesis that the desolventization toasting process, not the seed, is responsible for much of the variability in amino acid content in conventional canola meal.

Although amino acid digestibility was done separately for the NTCM and TCM samples because of large numbers, the data still provide useful information on their comparative digestibility. In addition, the inclusion of a commercial TCM control provided evidence that the experimental digestibility procedure was relatively repeatable in both experiments. The digestibilities of all amino acids from the NTCM were higher than for the TCM samples, whereas the variability of the NTCM digestibility values was less. For example, Lys digestibility ranged from 92.3 to 87.1% for NTCM samples, which is less than the range of 85.7 to 65.5% for TCM samples and the 86 to 74% range shown for canola meal in the most recent NRC publication (NRC, 1994). These results are also supported by the lower amino acid contents of the TCM samples shown above. Although processing plant effects on amino acid digestibility were significant for both NTCM and TCM samples, the differences were much smaller for the former meals. The differences in Lys digestibility as affected by toasting and processing plant are shown in Figure 4.1. This visual presentation supports the hypothesis that desolventizing/toasting reduces amino acid digestibility and that variability within and between plant is high for TCM samples but relatively uniform for NTCM. It is of interest that some samples of TCM had Lys digestibilities that were only marginally lower than those for NTCM. It is probable that these samples were not as severely heat processed, which suggests that a high quality but lightly toasted meal can be produced if the process is carefully controlled. However, the elimination of the toasting process would likely reduce the risk of reducing protein quality during solvent extraction.

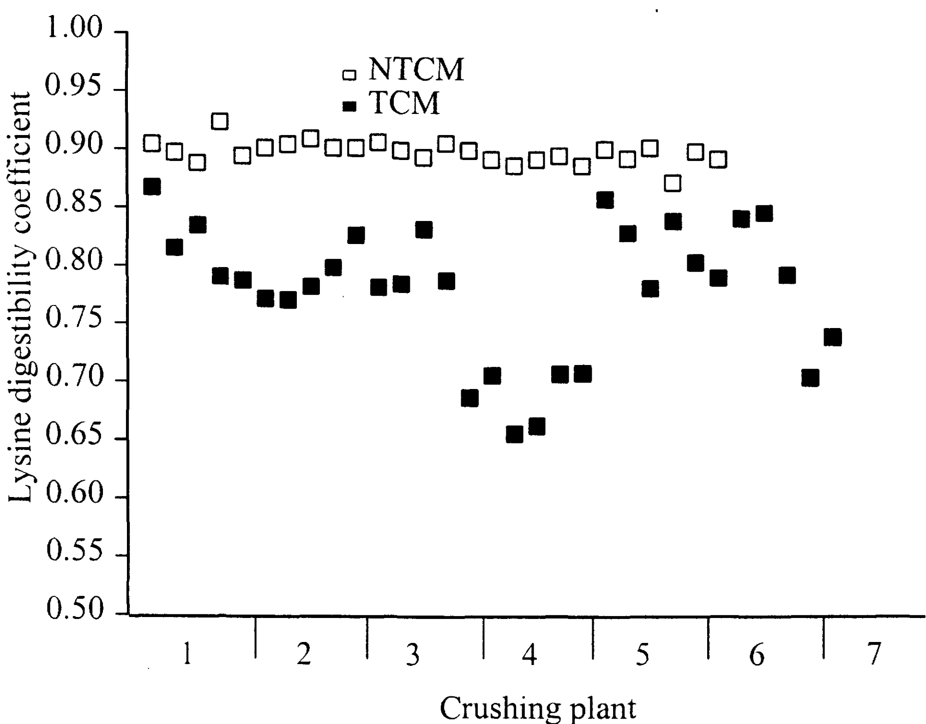

Figure 4.1 Apparent Lys digestibility (%) of toasted (TCM, n=31) and non-toasted (NTCM, n=26) canola meal samples

There were some significant effects of the crushing plant on amino acid availability in NTCM, indicating that stages of processing prior to desolventization/toasting or genotypic variation can affect protein quality. However, the ranges of values were generally small as indicated by differences between extremes of 1.3, 2.1, 8.4, 1.9, 5.1, 4.4 and 2.5% for Lys, His, Met, Gly, Thr, Ile, and Phe, respectively. Overall, the apparent digestibilities of the non-toasted meals were high and relatively consistent, indicating production of a non-toasted canola meal would result in a highly digestible product. This is consistent with earlier findings (Anderson-Haferman et al., 1993; Section 3) and supports the hypothesis that the variation and reduced amino acid digestibility, especially Lys, is due to the desolventization/toasting stage of oil extraction.

The reduced digestibility values in toasted canola meal were likely due to formation of early products of Maillard reaction that are not available to digestion and absorption, but may still be measured as Lys after acid hydrolysis for HPLC analysis. This sugar bound or 'non-reactive' Lys can be corrected for (Hurrell and Carpenter, 1981), but this would mask the effect of processing on digestibility, and the potential of the meal if processing methods were to be changed. The effects of Maillard reactions on amino acid levels and digestibility have been reviewed extensively by Mauron (1981). Lys is most susceptible to Maillard reactions due to the presence of the ε-amino group that can react directly with reducing sugars under moist, hot conditions. The loss of digestibility of the other amino acids indicated that advanced Maillard reactions such as Strecker degradations were also occurring. Desolventization/toasting changed the colour of the meal from a light yellow colour to the characteristic brown of canola meal, and this too supported the hypothesis that advanced Maillard reactions were occurring during this process.

The desolventization/toaster (DT) was designed, as the name implies, to both remove the solvent used to extract the oil and to toast the meal in order to reduce the content of anti-nutritional factors. This is achieved in a relatively simple piece of equipment that applies both direct and indirect heat to the meal. The indirect heat is encountered as the hexane-laden material enters the unit and falls onto heated plates. Since hexane boils at approximately 69°C, most of the hexane evaporates off very quickly. This material is agitated for a time on one plate before falling to the next and

this continues for a number of plates. Since the meal entering the DT contains a low level of moisture and evaporation of hexane cools the product, there is little opportunity for Maillard reactions to proceed. However, in the final tray(s), the meal encounters direct heat by means of steam injection into the meal. Some of the steam injected into the meal increases the moisture content and likely increases the rate of Maillard reactions. This direct heat is generally considered to cause the toasting process and reduce anti-nutritional factors such as trypsin inhibitors and lectins in soybean meal, but the requirement for canola meal is not well defined (Anderson-Hafermann et al., 1992). To date, glucosinolates are the only anti-nutritional factors in canola known to be reduced by this process, but again, the time, temperature and moisture required have not been defined.

When solvent extraction was first used to extract oil, rapeseed contained high levels of glucosinolates and reducing them by toasting improved animal performance (Nasi and Siljander-Rasi, 1991). However, the glucosinolate level of the seed has decreased dramatically since then, particularly with the introduction of low glucosinolate varieties. Prior to the early 1970's, the seed contained approximately 150 μmol of aliphatic glucosinolates/g oil free seed and this had a dramatic negative effect on animal performance, especially if the meal was under heated. Low glucosinolate varieties of canola, by definition, contain less than 30 μmol aliphatic glucosinolates per gram of oil free meal (Anon, 1992). This reduces the negative effects associated with rapeseed and, in turn, should reduce the need for toasting the meal. However, there is no indication that the desolventization/toasting process was changed with the introduction of canola varieties. The meals tested in this experiment contained only 10.55 μmol of aliphatic glucosinolates/g of meal prior to desolventization/toasting, which is much lower than that of the toasted meals from the original varieties of canola. Desolventization/toasting did reduce the aliphatic glucosinolate level to 6.16 μmol/g of meal, but it is not known if this provides any added benefit. Desolventization/toasting also reduced the indolyl and allyl glucosinolates but the levels prior to toasting were so low it would seem likely that they were of little consequence.

Reducing glucosinolates in canola meal by toasting can potentially be more detrimental than beneficial to the nutritional value of canola meal. For example, plant 4

had the lowest glucosinolate levels (1.2 μmol aliphatic glucosinolates/g of meal in one instance) but also had the lowest amino acid content and digestibility values. This would suggest that even though glucosinolates were reduced, the additional toasting was detrimental to meal quality. It is unclear if the level of glucosinolates in non-toasted meal is a problem, but it would seem unlikely. Further studies are needed to assess whether the levels of glucosinolates in non-toasted meal are an issue in terms of the nutritional value of meal. Even if further reductions in the level of glucosinolates in the diet are beneficial it may be more advisable to simply reduce the maximum inclusion level of canola meal in the diet rather than reduce the content and utilization of amino acids in the meal by overheating.

There are some indications that toasting in certain circumstances can be beneficial to animal performance. Shires et al. (1983) reported that heating air-desolventized, oil-free meal to 100°C increased chick performance, but this was not further improved with direct steam injection. The improvement observed with heating was determined to be due to the elimination of myrosinase activity which had not been eliminated by the cooking stage of the pilot crush. Commercial crushing conditions are designed to completely deactivate myrosinase prior to solvent extraction to prevent myrosinase from breaking down glucosinolates to oil soluble sulphur compounds that interfere with the catalyst used in the hydrogenation of the oil (Anon., 1992). Therefore, the level of residual myrosinase activity remaining prior to desolventization/toasting is very low in commercial samples (Newkirk and Classen, unpublished data), which would not need to be toasted to reduce levels further. It is not known if performance would still have been improved by heating in the study by Shires et al. (1983) if the myrosinase had been deactivated prior to desolventization, but dry heat should have little effect on amino acid quality as no moisture would be available for Maillard reactions to proceed. Maillard reactions require both high temperatures and moisture to proceed at a rapid pace. The steam injected directly into the meal in the final tray(s) of the DT provides this type of environment. Therefore, even if the meal requires heating to optimize performance, avoiding direct steam injection should ensure meal quality is not compromised.

In addition to reducing anti-nutritional factors, direct steam injection, which is likely responsible for the toasting process, is used to assist the desolventization process. Steam

penetration increases the rate of migration of hexane out of the meal, which reduces the amount of time required in the desolventizer, especially in the final stages when most of the surface hexane has already evaporated. The steam also dilutes the solvent vapours, enhancing the rate of evaporation of the last remnants of hexane. Therefore, if the DT is to be modified and the sparge steam is eliminated, a longer residence time may be required or a slight vacuum may have to be applied to the final tray(s) of the DT to minimize the residual hexane content of the meal. However, elimination of sparge steam may reduce processing costs, as steam is expensive to generate and it adds moisture to the meal that has to be removed prior to shipping to prevent spoilage.

Modification of the desolventization process should result in a meal with both higher levels and better digestibilities of amino acids, but almost as importantly should result in a more consistent meal quality. One of the factors preventing the use of high levels of canola meal in the diet is the need to increase safety margins for amino acids due to variability among canola meal samples. A non-toasted meal would, therefore, be of higher value and easier to incorporate into diets on a routine basis. Another potential benefit of modifying the desolventization process is that non-toasted meals are a lighter colour than in the toasted counterparts. All of the non-toasted meals used in this study were a light yellow colour, whereas all of the toasted meals were a brownish colour. This would be an advantage in meal markets that prefer light-coloured diets. The yellow-coloured meals would also provide visual assurance of meal quality. In toasted meals, dark colour was associated with reduced Lys digestibility but the difference is highly subjective and is not a reliable indicator of quality.

It is not known if there are other reasons to continue to process the meal in the current manner, since the meal does not contain significant quantities of the commonly known heat labile anti-nutritional factors. It is possible that this heat treatment may be necessary to reduce levels of an as yet unidentified factor(s).

If it is found that toasting cannot be eliminated for either nutritional or engineering reasons, it is recommended that the level of toasting be carefully controlled so that a high quality product is produced. There are currently no definitive guidelines for the degree of toasting of canola meal (Anderson-Hafermann et al., 1993). Therefore, further research is

required to develop chemical assays and guidelines for meal quality that can be used by both canola processors and nutritionists to assess the quality of the meal.

4.7 CONCLUSIONS AND RECOMMENDATIONS

The desolventization/toasting procedure used in commercial processing plants reduces the level and digestibility of many amino acids in canola meal. Elimination of the sparge steam from this stage of the process may result in a meal with higher levels of more digestible amino acids, particularly Lys. The non-toasted product would also be of more consistent quality. The non-toasted meals are a light yellow colour, which is more desirable than the current brown colour in certain markets and provides a means of identity preservation. Toasting reduces the glucosinolate content of the meal but the levels prior to toasting are already low and may not cause any anti-nutritional effects. Even if the levels of glucosinolates in the meal are shown to still be an issue, it may be more desirable to reduce inclusion levels of canola meal rather than toasting to reduce overall levels in the diet. Modification of the current desolventization/toasting process may require some engineering design and the retrofitting of current systems to ensure effective desolventization without toasting the meal, but the potential for reduced processing costs, increased meal value and better acceptance of canola meal should offset any additional expenses. If toasting is still required or cannot be eliminated, then toasting conditions should be carefully controlled, which requires the development of quality control assays and processing guidelines.

5 THE EFFECTS OF TOASTING CANOLA MEAL ON BODY WEIGHT, FEED CONVERSION EFFICIENCY AND MORTALITY IN BROILER CHICKENS

5.1 ABSTRACT

It is hypothesized that the moisture incorporated into canola meal during desolventization as sparge steam promotes toasting. Elimination of toasting of canola meal (CM) would result in higher digestible amino acid content, but it is not known if heat processing is required to reduce anti-nutritional factors. Therefore, the objectives of this study were to determine if suspending the use of sparge steam will prevent toasting and to study the effects of toasting on broiler chicken performance. Conventional toasted canola meal (TCM) and a hexane laden, non-toasted canola meal (NTCM) were collected from a commercial crushing plant. Desolventizing NTCM in a research desolventizer/toaster (DT) without the use of sparge steam produced a yellow coloured meal. This meal and TCM were fed to broiler chickens from 0-39 d and replaced 0, 20, 40, 60, 80 and 100% of the soybean meal (SBM) in wheat based diets. Non-toasted canola meal increased ($P<0.05$) broiler weight from 0.606 and 2.148 to 0.618 and 2.181 kg at 19 and 39 d of age, respectively, and gain to feed ratio (0-19 d) from 0.637 to 0.642 but did not affect mortality. There was a quadratic effect of soybean meal replacement level on average body weights. Maximum body weight occurred between 20 and 60% replacement of soybean meal by canola meal and lowest body weights were observed with 100% replacement. Total mortality and chronic heart failure (CHF) between 19 and 39 d increased ($P<0.05$) with level of CM addition from 5.2 to 13.9% and 1.9 to 9.6%, respectively for NTCM and TCM. Chronic heart failure in males, but not females, was increased ($P<0.05$) from 3.3 to 17.4% with increasing CM level. In conclusion, desolventization without sparge steam produced a non-toasted meal and improved broiler growth and feed efficiency in comparison to TCM. Therefore, NTCM can be fed to broiler chickens.

5.2 INTRODUCTION

Canola meal (CM) has an excellent balance of amino acids, but has lower amino acid digestibility than soybean meal (Zuprizal et al., 1992). Previous work has shown that the desolventization/toasting stage of prepress-solvent extraction of canola reduces both the content and digestibility of amino acids, Lys in particular (Sections 3 and 4). The desolventization/toasting stage removes the hexane used to extract the oil in a prepress-solvent extraction system, and also imparts a toasting process to reduce the level of heat labile anti-nutritional factors in the meal.

The Schumacher DT[19] is the most prevalent design in North America and consists of a series of heated trays stacked vertically. The solvent laden meal is conveyed onto the top tray and falls from tray to tray until exiting to a drier/cooler with low residual hexane levels remaining. The first trays are internally heated with steam thereby heating the meal indirectly and causing the majority of the solvent to evaporate. The final trays apply direct heat in the form of sparge steam injected directly into the meal. This steam condenses and increases the moisture content of the meal exiting the DT to approximately 14.5 to 18.5%.

Entering the DT, the solvent extracted CM is a yellow colour, but on exiting it is a brown colour, indicating that Maillard reactions are occurring in the process (Section 3). The extent of Maillard reactions is seen by the lower Lys content and digestibility in toasted meal (Section 3).

The optimal conditions for Maillard reactions have been defined previously in other proteinaceous products as being between 15-18% moisture in combination with elevated (>100°C) temperatures (Mauron, 1981). Since sparge steam is increasing the moisture content, it may contribute to losses in amino acid content and digestibility during desolventization. Therefore, the elimination of additional moisture in the form of sparge steam during desolventization/toasting may result in a yellow-coloured meal with an elevated concentration of amino acids and enhanced digestibility. However, it is not known if the toasting process imparted by the sparge steam is necessary to reduce the levels of heat labile anti-nutritional factors in the meal and is therefore, required to optimize the nutritional value of the meal.

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[19] Crown Iron Works Company, Minneapolis, MN, USA, 55440-1364

The objectives of the current experiment were to determine if elimination of sparge steam during desolventization would reduce the level of browning and toasting of the meal, and to determine if the toasting process imparted by the sparge steam is required to reduce anti-nutritional factors and, therefore, optimize broiler performance.

5.3 MATERIALS AND METHODS

5.3.1 Meal Collection and Desolventization

Conventional desolventized/toasted (TCM) and non-desolventized canola meal samples were collected on the same day from a commercial canola crushing plant located in Western Canada. The TCM has small amounts of gums and screenings added at the processing plant whereas the NTCM did not. The oil extracted, solvent laden meal (Marc) was collected in steel drums (160 L capacity) with sealed lids and transported to POS[20] for desolventization. The Marc was desolventized in a DT (77.5 cm inside diameter) built by Crown Iron Works[21] equipped with two steam heated trays (53.3 cm between trays). The line for feeding sparge steam into the meal was disconnected such that the meal was desolventized solely by indirect heat in the form of steam heated trays. The rate of flow through the unit was adjusted such that the exit temperature of the meal was approximately 100°C and no residual hexane could be detected by smell. Samples of meal exiting the DT were analyzed for residual hexane according to the method of the American Oil Chemists Society (1997), erucic acid according to the method of the American Oil Chemists Society (1991, 1992) and glucosinolates according to the method of Daun et al. (1989).

5.3.2 Broiler Study

Day old, Peterson x Hubbard broiler chickens (3240) were randomly assigned to one of 72 straw litter pens (45 birds per 2.3 x 2 m pen) located in six rooms. Each room contained 12 pens and the 12 dietary treatments were randomly assigned and replicated once per room. Each sex was randomly assigned to three of the six rooms. Feed and

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[20] POS Pilot Plant Corp., 118 Veterinary Road, Saskatoon, SK, Canada, S7N 2R4
[21] Crown Iron Works Company, Minneapolis, MN, USA, 55440-1364

water were provided *ad libitum* for the duration of the trial. The initial room temperature was 35° C and declined in a step wise fashion to 22°C by day 35 where it was maintained to trial end (39 d). The lighting program consisted of 24L:0D, 18L:6D, 8L:7.5D:1L:7.5D, 10L:6.5D:1L:6.5D, 14L:4.5D:1L:4.5D, and 18L:6D for the periods of 0-4, 5-7, 8-14, 15-21, 22-28 and 29-39 d, respectively. Light intensity was 20 lux for 0 to 4 d of age and 5 lux thereafter.

TCM and non-toasted canola meal (NTCM) replaced 0, 20, 40, 60, 80 and 100% of dietary soybean meal (SBM). Diets were formulated to be isoenergenic and to meet bird requirements according to the recommendations of Leeson and Summers (1997). All diets were formulated to have at least the same level of digestible Lys, Met+Cys, and Arg as the control treatment using the digestibility values reported by NRC (1994) for wheat and soybean meal and Section 4 for CM. Prior to formulation, total amino acid content of feed ingredients was analyzed by the performic acid method (Llames and Fontane, 1994) using a Beckman 7300 High performance Amino Acid Analyzer[22]. Neutral detergent insoluble nitrogen (NDIN) content of the NTCM and TCM was determined using an Ankom 200 Fibre Analyzer[23]. The procedure supplied by the manufacturer was used except sodium sulphite was not added to the NDF solution (Mustafa et al., 2000). AAFCO[24] check samples were also analysed for amino acid, crude protein, NDIN and moisture and the values obtained were comparable to those reported by other laboratories participating in the program. Tables 5.1 to 5.4 show the composition of the starter and grower diets used in this study. Starter diets were allocated on the basis of 1.1 kg per starting chick (~ 23 d) and then birds were switched to the grower ration until the end of the experiment. Body weight (on a pen basis) was determined at 0, 19 and 39 d and feed consumption was measured throughout the trial. Gain to feed was calculated by dividing weight gain, including weight gain of dead birds, by feed intake. Serum samples were collected from four and two birds per pen at 19 and 39 d of age, respectively, for the determination of total triiodothyronine (T_3) and thyroxine (T_4) levels using a DPC Coat a

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[22] Beckman Instruments, Inc., Fullerton, CA, USA 92834-3100

[23] Ankom Technology Coporation, 140 Turk Hill Park, Fairport, NY, 14450

[24] Association of American Feed Control Officials, Inc., College Station, TX, USA, 77841-3160

Count® kit[25]. At 39 d of age, two birds per pen were individually weighed and sacrificed by cervical dislocation and weights of the heart, liver, bursa, pancreas, empty proventriculus, gizzard, and the full and empty weights of the duodenum, jejunum, ileum, and cecum determined. The animal care protocol used in this experiment was approved by the Universtity of Saskatchewan Animal Care Committee. All experiments were conducted according to the guidelines of the Canadian Council on Animal Care (1993).

5.3.3 Statistical Analysis

The data were analyzed as 2 x 2 x 6 (two sexes x two meal types x six inclusion levels) factorial with sex nested within room (12 dietary treatments within each of three rooms of males and three rooms of females) using the GLM procedure of the SAS program (SAS Institute, 1989) using the model $Y_{ijkl} = \mu + \alpha_i + \beta_j + \gamma_{k(ij)} + \varepsilon_{ijkl}$ where Y_{ijkl} is the dependent variable, μ is the overall mean, α_i is the main effect of meal source, β_j is the main effect of level, $\gamma_{k(ij)}$ is the main effect of sex nested within room and ε_{ijkl} is the normally distributed residual. Differences were considered significant when $P < 0.05$. When a significant effect of dietary level was found, regression analysis was conducted using the REG procedure of the SAS program (SAS Institute, 1989).

[25] Diagnostic Products Corporation, Los Angeles, CA, USA, 90045-5597

Table 5.1 Starter diet composition

	Replacement Level[1]					
Ingredients %	0	20	40	60	80	100
Wheat	73.52	70.98	67.93	63.24	58.55	53.85
Soybean meal	20.84	16.64	12.79	8.32	4.16	0.00
Canola meal	0.00	6.27	12.48	20.83	28.87	36.91
Canola oil	1.0	1.63	2.33	3.3	4.28	5.25
Dicalcium phosphate	1.45	1.42	1.39	1.34	1.30	1.25
Limestone	1.74	1.68	1.61	1.52	1.44	1.35
Sodium chloride	0.37	0.38	0.38	0.39	0.40	0.40
Vit/Min premix [2]	0.50	0.50	0.50	0.50	0.50	0.50
Choline chloride	0.10	0.10	0.10	0.10	0.10	0.10
DL-Met	0.10	0.09	0.07	0.05	0.03	0.01
L-Lys HCl	0.18	0.20	0.21	0.20	0.18	0.17
Endoxylanase[3]	0.05	0.05	0.05	0.05	0.05	0.05
Coccidiostat[4]	0.10	0.10	0.10	0.10	0.10	0.10
Growth promotant[5]	0.05	0.05	0.05	0.05	0.05	0.05

[1]Soybean meal was replaced with non-toasted or toasted canola meal.

[2]Supplied per kilogram of diet: 11,000 IU vitamin A (retinyl acetate + retinyl palmitate), 2,200 IU vitamin D_3, 30 IU vitamin E (dl-α-tocopheryl acetate), 2.0 mg menadione, 1.5 mg thiamine, 6.0 mg riboflavin, 60 mg niacin, 4 mg pyridoxine, 0.02 mg vitamin B_{12}, 10.0 mg pantothenic acid, 6.0 mg folic acid, 0.15 mg biotin, 0.625 mg ethoxyquin, 500 mg calcium carbonate, 80 mg Fe, 80 mg Zn, 80 mg Mn, 10 mg Cu, 0.8 mg I, and 0.3 mg Se.

[3]Avizyme 1302, Finnfeeds International Ltd., Marlborough, Wiltshire, UK, SN8 1AA, contains 5,000 units xylanase/g and 1,600 units protease/g.

[4]Coxistac, Pfizer Canada, London, ON, Canada, N6A 1K7, contains 60 g salinomycin sodium /kg.

[5]Stafac 22, SmithKline Beecham, Mississauga, ON, Canada, L5N 6L4, contains 22g Virginiamycin /kg.

Table 5.2 Calculated nutrient composition of the starter diets (%)

	Replacement Level[1]					
Ingredients %	0	20	40	60	80	100
AME_n (kcal/kg)	2926	2925	2925	2925	2925	2925
Crude protein[2]	21.14	21.43	21.41	21.80	21.99	22.85
Calcium	1.00	1.00	1.00	1.00	1.00	1.00
Non-phytate P	0.42	0.42	0.42	0.42	0.42	0.42
Lys	1.06	1.07	1.08	1.09	1.10	1.12
Available Lys	0.95	0.95	0.95	0.95	0.95	0.95
Met + Cys	0.83	0.84	0.86	0.88	0.91	0.93
Available Met + Cys	0.68	0.68	0.69	0.71	0.72	0.73

[1]Soybean meal was replaced by toasted or non-toasted canola meal.

[2]Analyzed content.

Table 5.3 Grower diet composition

Ingredients %	Replacement Level[1]					
	0	20	40	60	80	100
Wheat	77.12	74.71	72.31	69.91	67.51	64.61
Soybean meal	15.02	12.01	9.01	6.01	3.01	0.00
Canola meal	0.00	4.81	9.60	14.39	19.18	24.40
Canola oil	2.00	2.00	2.00	2.00	2.00	2.00
Tallow	1.41	2.09	2.77	3.46	4.14	4.92
Dicalcium phosphate	1.39	1.37	1.34	1.32	1.30	1.27
Limestone	1.60	1.55	1.50	1.45	1.39	1.34
Sodium chloride	0.37	0.37	0.38	0.38	0.39	0.39
Vit/Min premix[2]	0.50	0.50	0.50	0.50	0.50	0.50
Choline chloride	0.10	0.10	0.10	0.10	0.10	0.10
DL-Meth	0.10	0.08	0.07	0.06	0.05	0.04
L-Lys HCl	0.20	0.21	0.21	0.22	0.23	0.23
Endoxylanase[3]	0.05	0.05	0.05	0.05	0.05	0.05
Coccidiostat[4]	0.10	0.10	0.10	0.10	0.10	0.10
Growth promotant[5]	0.05	0.05	0.05	0.05	0.05	0.05

[1]Soybean meal was replaced with non-toasted or toasted canola meal.

[2]Supplied per kilogram of diet: 11,000 IU vitamin A (retinyl acetate + retinyl palmitate), 2,200 IU vitamin D_3, 30 IU vitamin E (dl-α-tocopheryl acetate), 2.0 mg menadione, 1.5 mg thiamine, 6.0 mg riboflavin, 60 mg niacin, 4 mg pyridoxine, 0.02 mg vitamin B_{12}, 10.0 mg pantothenic acid, 6.0 mg folic acid, 0.15 mg biotin, 0.625 mg ethoxyquin, 500 mg calcium carbonate, 80 mg Fe, 80 mg Zn, 80 mg Mn 10 mg Cu, 0.8 mg I, and 0.3 mg Se.

[3]Avizyme 1302, Finnfeeds International Ltd., Marlborough, Wiltshire, UK, SN8 1AA, contains 5,000 units xylanase/g and 1,600 units protease/g.

[4]Coxistac, Pfizer Canada, London, ON, Canada, N6A 1K7, contains 60 g salinomycin sodium /kg.

[5]Stafac 22, SmithKline Beecham, Mississauga, ON, Canada, L5N 6L4, contains 22g Virginiamycin /kg.

Table 5.4 Calculated nutrient composition of the grower diets

	Replacement Level[1]					
Ingredients %	0	20	40	60	80	100
AME (kcal/kg)	3100	3100	3100	3100	3100	3100
Crude protein[2]	18.76	19.38	19.24	19.13	19.44	19.37
Calcium	0.92	0.92	0.92	0.92	0.92	0.92
Non- phytate P	0.40	0.40	0.40	0.40	0.40	0.40
Potassium	0.65	0.64	0.63	0.62	0.61	0.61
Sodium	0.18	0.18	0.18	0.18	0.18	0.18
Lys	0.91	0.92	0.92	0.93	0.94	0.94
Avail. Lys	0.81	0.81	0.81	0.81	0.81	0.81
Met + Cys	0.77	0.78	0.78	0.79	0.80	0.82
Avail. Met + Cys	0.62	0.62	0.62	0.63	0.64	0.64

[1]Soybean meal was replaced with non-toasted or toasted canola meal.

[2]Analyzed content

5.4 RESULTS

The solvent-laden white flake was desolventized, without sparge steam, at a rate of 128 kg per hour producing 87 kg per hour of meal with a residual hexane content of 256 μg/g exiting the DT. Hexane analysis was not conducted on the conventional TCM as it was collected after the drier cooler and, therefore, would not be representative of meal exiting the DT directly. Non-toasted canola meal and TCM had similar levels of protein, Lys, Met, cystine and erucic acid (Table 5.5). The NDIN content of the TCM was 1.7 times higher than that of the NTCM. The NTCM contained approximately twice the level of glucosinolates as the TCM, with the largest difference being the level of 4-OH-3-methylindoyl glucosinolate (Table 5.5). The NTCM contained similar levels of total glucosinolates as reported for earlier verities of canola meal after toasting (Table 5.5) Both the TCM and NTCM contained markedly higher levels of glucosinolates than a strain of canola which has been reported to have very low levels of glucosinolates (Table 5.5) indicating that genetic selection will continue to reduce the levels in the meal. Prior to desolventization, NTCM meal had a bitter smell that was no longer apparent once the meal had been heated to 100°C. The TCM did not have this smell. Desolventization in the absence of sparge steam did not change the colour of the meal which was therefore, a light yellow colour. In contrast, the meal desolventized in the conventional manner with sparge steam was a light brown colour.

Feeding NTCM resulted in higher body weight at 19 and 39 d than did feeding TCM (Table 5.6). Replacing soybean meal with canola meal had a quadratic effect on 19 d body weight. Replacing 20 to 60% of the soybean meal resulted in the highest 19d body weights, while complete replacement of the soybean meal with canola meal produced the lowest. There was also a similar quadratic effect of replacement level on 39d body weight ($P<0.06$) where replacing 80 or 100% of dietary soybean meal with CM had a negative effect. Males had higher body weights at 19 and 39 d than did females. There was an interaction between soybean replacement level and sex on 19d body weight. Increasing the level of replacement from 60 to 80% resulted in a significant reduction in 19d body weight in females, but in males a depression was not observed until the level was increased from 80 to 100% (Table 5.7). There was also a significant interaction

between meal type, soybean meal replacement level and sex on 19 d body weight. The heaviest female 19d body weights were achieved at 40 and 60 % replacement with NTCM and TCM (Table 5.8), respectively. However, maximum 19 d male body weights were achieved at 60 and 40% replacement with NTCM and TCM, respectively.

Table 5.5 Chemical composition (% of dry matter) of toasted canola meal (TCM), non-toasted canola meal (NTCM), Tobin TCM from 1988 and a very low glucosinolate variety of TCM from 1988

	NTCM	TCM	1988 Tobin Meal[1]	1988 Very Low Glucosinolate meal[1]
Crude protein	42.51	41.13	39.73	39.66
Lys (g/16 g N)	5.29	5.47	5.51	5.62
Met (g/16 g N)	1.98	2.14	2.09	1.99
NDIN (% of protein)[2]	11.31	19.73	ND[3]	ND
Erucic acid (% of meal)	0.003	0.008	ND	ND
Erucic acid (% of oil)	0.130	0.190	ND	ND
Glucosinolates (umoles/g)				
Allyl	0.15	0.27	0.00	0.00
3-Butenyl	3.21	1.94	4.21	0.00
4-Pentenyl	0.55	0.34	2.12	0.00
2-OH-3-Butenyl	5.81	3.88	7.78	0.00
2-OH-4-Pentenyl	0.12	0.04	0.87	0.00
Methylthiobutenyl	0.14	0.12	ND	ND
Methylthiopentenyl	0.09	0.16	ND	ND
4-OH-Benzyl	0.73	0.78	0.00	0.00
3-Methylindolyl	0.72	0.31	0.17	0.04
4-OH-3-Methylindolyl	10.37	1.88	2.21	0.49
Total glucosinolates	21.89	9.72	17.36	0.58

[1]Adapted from Bell et al., 1995.

[2]NDIN = Neutral Detergent Insoluble Nitrogen.

[3]ND = not determined.

Table 5.6 Body weight and average feed consumption in broiler chickens fed toasted (TCM) and non-toasted (NTCM) canola meal

	Average body weight		Average feed consumption	
	19 d	39 d	0-19 d	19-39 d
	(kg)		(kg/bird)	
NTCM	0.618	2.181	0.905	3.193
TCM	0.606	2.148	0.891	3.140
Level of Replacement				
0	0.614	2.184	0.905	3.140
20	0.623	2.207	0.911	3.179
40	0.621	2.184	0.913	3.150
60	0.623	2.200	0.909	3.190
80	0.609	2.137	0.893	3.157
100	0.582	2.072	0.856	3.176
Regression	Quadratic	Linear	Quadratic	NS[1]
Male	0.629	2.270	0.919	3.373
Female	0.594	2.058	0.877	2.957
SEM	0.003	0.015	0.005	0.030
Type	***	**	**	0.09
Level	***	***	***	NS[1]
Sex	***	***	***	***
Type x level	NS	NS	*	NS
Type x sex	NS	NS	NS	NS
Level x sex	*	0.09	NS	NS
Type x level x sex	*	NS	NS	*

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$.

[1]NS = not significant ($P > 0.05$).

Table 5.7 The interaction between level of replacement of soybean meal with canola meal and sex on body weight (19d), gain to feed ratio (0-19 d) and chronic heart failure (20-39 d)

	Body weight (19 d)		Gain : feed (0-19 d)[1]		Chronic heart failure (19-39 d)	
Replacement	Males	Females	Males	Females	Males	Females
	(Kg)		(Kg/Kg)		(%)	
0	0.630	0.597	0.642	0.628	3.3	0.4
20	0.645	0.601	0.654	0.632	5.6	1.9
40	0.640	0.602	0.647	0.632	5.2	1.5
60	0.642	0.603	0.648	0.643	11.1	2.2
80	0.629	0.589	0.649	0.634	7.8	5.9
100	0.589	0.574	0.633	0.636	17.4	1.9
Regression	Quadratic	Quadratic	Quadratic	NS[2]	NS	NS
SEM	0.003		0.004		0.7	

[1]Gain to Feed ratio includes the weight gain of birds that died during the experimental period.

[2]NS= not significant (P>0.05).

Table 5.8 The interaction between sex, level of soybean meal replacement, and toasted (TCM) and non-toasted canola meal (NTCM) on body weight (19d), feed consumption (19-39 d) and mortality (20-39 d)

		Body weight (0-19 d)		Feed Consumption (19-39 d)		Mortality (19-39 d)	
Sex	Level	NTCM	TCM	NTCM	TCM	NTCM	TCM
		(kg)				(%)	
Female	0	0.597	0.597	0.892	0.892	2.6	2.6
	20	0.608	0.594	0.896	0.885	2.2	6.7
	40	0.611	0.593	0.908	0.872	2.2	1.5
	60	0.602	0.605	0.886	0.882	3.0	8.2
	80	0.594	0.585	0.863	0.869	11.1	8.2
	100	0.589	0.561	0.852	0.829	6.7	3.0
Regression		Quadratic	Quadratic	NS[1]	Quadratic	Linear	NS
Male	0	0.630	0.630	0.919	0.919	7.8	7.8
	20	0.657	0.634	0.952	0.910	14.1	8.2
	40	0.637	0.643	0.947	0.926	15.6	8.2
	60	0.661	0.623	0.966	0.904	17.8	11.9
	80	0.638	0.621	0.908	0.930	11.1	16.3
	100	0.600	0.577	0.884	0.860	18.5	27.4
Regression		Quadratic	Quadratic	NS	NS	Linear	Linear
SEM		0.003		0.030		0.9	

[1]NS = not significant ($P > 0.05$).

Birds fed the NTCM consumed more feed between 0-19 d than those fed TCM but toasting had no effect on feed consumption between 19-39 d (Table 5.9). Replacement level affected feed consumption between 0-19 d, but not between19-39 d. Replacing 40% of the soybean meal resulted in the highest feed consumption between 0-19 d but complete replacement of soybean meal resulted in the lowest feed consumption. Males consumed more feed between 0-19 and 19-39 d than did females. There was a significant interaction between meal type and replacement level on 0-19 d feed consumption. Replacement of dietary soybean meal with NTCM had a quadratic affect on feed consumption, with 40% resulting in the highest intake and 100% the lowest (Table 5.10). There was also a quadratic effect of replacement with TCM on 0-19 d feed consumption ($P<0.07$; Table 5.10) where feed consumption was similar between 0 and 80% replacement, but 100% had a negative effect. There was a significant interaction of meal type, level and sex on 19-39 d feed consumption (Table 5.8), but the cause of the interaction is not clear.

Feeding NTCM resulted in a higher gain to feed ratio between 0-19 d than did TCM (Table 5.9). Males had a higher gain to feed ratio between 0-39 d than did females. There was a significant interaction of meal type and soybean meal replacement level on gain to feed ratio between 0-19 d. Increasing the level of TCM, but not of NTCM, had a quadratic effect on gain to feed ratio between 0-19 d (Table 5.10). There was an interaction between soybean meal replacement level and sex on gain to feed ratio where males responded in a quadratic manner to inclusion level, but there was no effect in females (Table 5.7).

Table 5.9 Gain to feed ratio, mortality and chronic heart failure (CHF) in broiler chickens fed toasted (TCM) and non-toasted (NTCM) canola meal

	Gain to feed[1]		Mortality 19-39 d	
	0-19 d	19-39 d	Total	CHF
	(kg/kg)		(%)	
NTCM	0.642	0.545	9.6	5.2
TCM	0.637	0.546	8.9	5.5
Level of Replacement				
0	0.635	0.543	5.2	1.9
20	0.643	0.550	7.8	3.7
40	0.640	0.544	6.9	3.3
60	0.645	0.551	10.2	6.7
80	0.642	0.546	11.7	6.9
100	0.634	0.542	13.9	9.6
Regression	NS[2]	NS	Linear	Linear
Male	0.646	0.554	13.7	8.4
Female	0.634	0.538	4.8	2.3
SEM	0.004	0.005	0.9	0.7
Type	*	NS	NS	NS
Level	NS	NS	***	***
Sex	***	***	***	***
Type x level	**	NS	NS	NS
Type x sex	NS	NS	NS	*
Level x sex	*	NS	**	**
Type x level x sex	NS	NS	**	NS

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$.

[1]Gain to feed ratio includes the weight gain of birds that died during the experimental period.

[2]NS = not significant ($P > 0.05$).

Table 5.10 The interaction between soybean meal replacement and toasted (TCM) and non-toasted canola meal (NTCM) on feed consumption and gain to feed from 0-19 d , serum triidothyronine (T_3) levels at 19 d and heart weight (% of body weight) at 39 d of broiler chickens

	Feed consumption (0-19d)		Gain : feed (0-19d)[1]		Serum T_3 (19 d)		Heart weight (39 d)	
Replacement	NTCM	TCM	NTCM	TCM	NTCM	TCM	NTCM	TCM
	—— (kg) ——		—— (kg/kg) ——		—— (ηg/dL) ——		(% body weight)	
0	0.905	0.905	0.635	0.635	239	239	0.46	0.46
20	0.924	0.897	0.645	0.641	232	237	0.48	0.43
40	0.927	0.899	0.634	0.646	261	233	0.43	0.50
60	0.926	0.893	0.645	0.645	231	267	0.50	0.47
80	0.886	0.900	0.650	0.633	242	270	0.46	0.50
100	0.868	0.845	0.642	0.627	246	257	0.48	0.50
Regression[2]	Q	Q *P*<0.07	NS[3]	Q	NS	L	NS	L
SEM	0.005		0.004		4.0		0.01	

[1]Gain to Feed ratio includes the weight gain of birds that died during the experimental period.

[2]Q= quadratic relationship, L= linear relationship

[3]NS = not significant ($P > 0.05$).

There was no effect of treatment on mortality up to 19 d. Toasted canola meal and NTCM resulted in similar levels of mortality and chronic heart failure (Table 5.9). Mortality increased with the level of CM inclusion between 19 and 39 d. The increased mortality was primarily due to chronic heart failure. Males had a higher level of mortality and chronic heart failure between 20-39 d. There was an interaction between meal type and sex on chronic heart failure. Males fed NTCM tended to have a higher level of chronic heart failure than those fed TCM (Table 5.11). However, females fed TCM tended to have a higher level of chronic heart failure than those fed NTCM. There was an interaction between soybean meal replacement level and sex on mortality and chronic heart failure between 19 and 39 d. Chronic heart failure in males increased with replacement of soybean meal by CM but the same effect was not observed with females (Table 5.7). The same effect was observed for mortality but the data is not shown since the primary effect on mortality is reflected by the chronic heart failure values. There was an interaction between meal type, replacement level and sex on mortality between 19 and 39 d. Mortality in females increased linearly with increasing NTCM, but not with TCM level (Table 5.8). Mortality in males increased linearly with increasing inclusion of both types of meals; TCM resulted in the highest level of mortality with complete replacement of soybean meal.

Meal type or level had no effect on organ weights other than liver, full jejunal and empty cecal weights at 39 d of age (Table 5.12). Conventional TCM increased liver weight and full jejunal weight at 39 d of age. Empty cecal weight as a percentage of body weight at 39 d was reduced linearly with replacement of soybean by CM. Females had higher proportional liver weights than did males at 39 d. There was a significant interaction between meal type and replacement level on 39 d heart weight. Replacement of soybean meal with TCM but not NTCM resulted in a linear increase in heart weight at 39 d (Figure 5.1).

Table 5.11 The interaction between sex and toasted (TCM) and non-toasted canola meal (NTCM) on the incidence of chronic heart failure between 19 and 39 d and serum triiodothyronine (T_3) in broiler chickens at 39 d

	Chronic heart failure (%)[1]	
Canola meal type	Males	Females
NTCM	9.8	2.4
TCM	9.0	3.0
SEM	0.7	
	Serum T_3 (ηg/dL)[1]	
Canola meal type	Males	Females
NTCM	235	241
TCM	271	257
SEM	5	

[1]Excludes data from treatments 1 and 7 since they did not contain either type of canola meal.

Table 5.12 The effect of feeding toasted (TCM) and non-toasted canola meal (NTCM) on liver, jejunal (full) and cecal weight (empty) at 39 d of age

	Organ weight			
	Liver	Full jejunal	Empty cecal	Heart
	(% body weight)			
NTCM	2.79	2.14	0.29	0.48
TCM	2.92	2.30	0.28	0.47
Level of Replacement				
0	2.78	1.98	0.37	0.46
20	2.82	2.20	0.31	0.46
40	2.76	2.22	0.29	0.46
60	2.94	2.32	0.27	0.48
80	2.89	2.29	0.27	0.48
100	2.94	2.16	0.26	0.49
Regression	NS	NS	Linear	NS
Male	2.77	2.08	0.28	0.48
Female	2.92	2.35	0.29	0.47
SEM	0.03	0.04	0.01	0.01
	Probability			
Type	*	*	NS[1]	NS
Level	NS	NS	*	NS
Sex	*	***	NS	NS
Type x level	NS	NS	NS	***
Type x sex	NS	*	NS	NS
Level x sex	NS	NS	NS	NS
Type x level x sex	NS	*	NS	NS

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$.

[1]NS, not significant ($P > 0.05$).

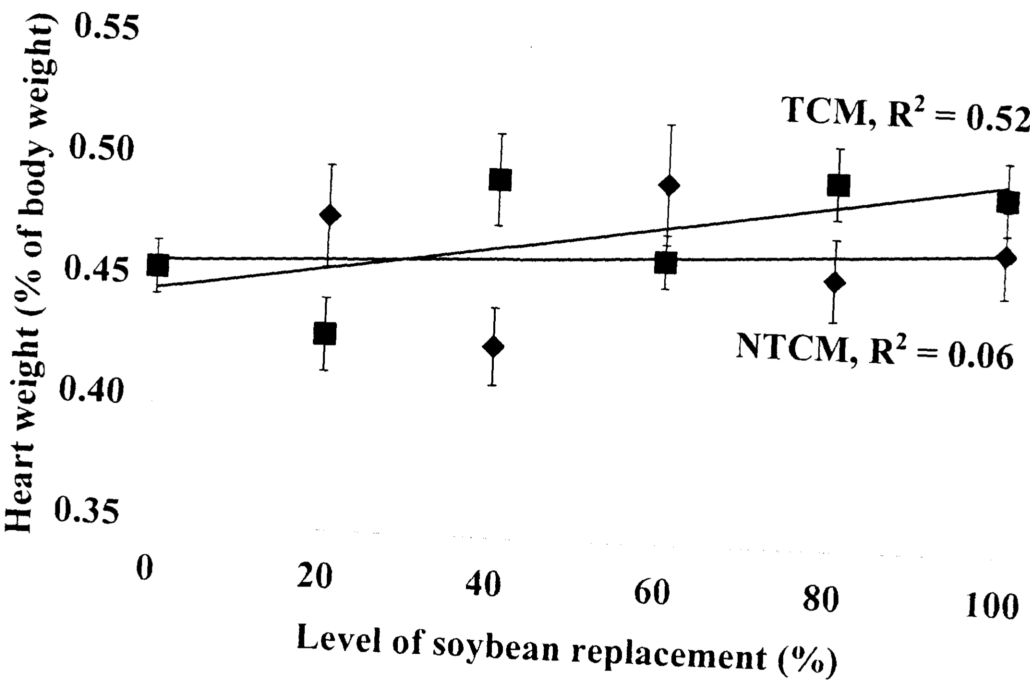

Figure 5.1 The effect of soybean replacement level with toasted (TCM, ■) and non-toasted (NTCM, ♦) canola meal on heart weight in broiler chickens at 39 d. The effect of level of TCM on heart weight is linear ($P < 0.05$). The effect of level of NTCM on heart weight was not significant ($P < 0.05$).

TCM resulted in higher serum T_3 at 39 d of age than did NTCM (Table 5.13). Serum T_3 at 39 d was increased by replacement level. Males had higher serum T_3 levels than females at 19 d, but not at 39 d (Table 5.13). There was a significant interaction between meal type and soybean replacement level on serum T_3 at 19 d. There was no effect of inclusion level on serum T_3 when NTCM was fed, but serum T_3 increased with TCM inclusion level (Table 5.14).

Males had lower serum T_4 levels at 19 d and 39 d (Table 5.13). There was a significant effect of soybean meal replacement level on serum T_4 level at 19 d but regression analysis failed to show either a linear or quadratic effect. There was a significant interaction of level and sex on serum T_4 at 19 and 39 d. There was a quadratic effect of replacement level on serum T_4 in females at 19 d, but no effect in males at the same age (Table 5.14). At 39 d, males fed the soybean control had the highest level of serum T_4, but 20% replacement with CM yielded the lowest value. There was no apparent effect of inclusion level on T_4 in females at 39 d.

5.5 DISCUSSION

Within the crushing industry, it is a commonly held belief that direct heating through sparge steam injection is required to produce a meal with low residual hexane levels in a conventional DT. As a result, when the desolventization process was planned for this research, it was assumed that three times as much residence time in the desolventizer would be required to effectively desolventize the meal. Typically, POS is able to desolventize at a rate of 100 to 150 kg per hour with sparge steam in the DT used in this study. In this experiment desolventization (128 kg/hour) without sparge steam was achieved. Therefore elimination of the sparge steam appeared to have little or no impact on the rate of desolventization in this instance.

Table 5.13 The effect of feeding toasted (TCM) and non-toasted canola meal (NTCM) on serum triiodothyronine (T_3) and thyroxine (T_4) levels at 19 and 39 d of age

	19 d		39 d	
	T_3	T_4	T_3	T_4
	(ηg/dL)			
NTCM	241	558	235	711
TCM	253	565	263	653
Level of Replacement				
0	239	565	224	759
20	234	524	230	683
40	247	529	236	673
60	249	589	256	706
80	256	495	262	633
100	252	669	267	695
Regression	NS	NS	Linear	NS
Male	258	458	250	601
Female	235	661	246	763
SEM	4	21	5	24
	Probability			
Type	NS	NS	**	NS
Level	NS	*	*	NS
Sex	***	***	NS	***
Type x level	*	NS	NS	NS
Type x sex	*	NS	NS	NS
Level x sex	NS	*	NS	*
Type x level x sex	*	NS	NS	NS

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$.

[1]NS, non-significant ($P > 0.05$).

Table 5.14 The interaction between level of replacement of soybean meal with canola meal and sex on serum thyroxine (T_4, 19 and 39 d) and the interaction between soybean meal replacement and toasted (TCM) and non-toasted canola meal (NTCM) on serum triiodothyronine (T_3) levels at 19 d

	Serum T_4 (19 d)		Serum T_4 (39 d)		Serum T_3 (19 d)	
Replacement	Males	Females	Males	Females	NTCM	TCM
			(ηg/dl)			
0	389	756	750	768	239	239
20	445	603	428	853	232	237
40	466	589	671	673	261	233
60	557	618	693	718	231	267
80	361	623	509	757	242	270
100	507	824	566	813	246	257
Regression	NS	Quadratic	NS	NS	NS	Linear
SEM	21		24		4	

[1]NS = not significant ($P > 0.05$).

The meal contained an acceptably low level of residual hexane (256 μg/g) even though sparge steam was not utilized. The residual hexane levels in the meal were higher than that normally achieved in this unit (~100 μg/g) but were lower than previously reported (452 μg/g) for a sample of 30 commercial meals (American Oil Chemists Society, 1997). The level of residual hexane in the meal exiting the DT in commercial crushing plants is not regulated directly, but overall solvent losses during processing are. The United States Environmental Protection Agency has recently enacted a new rule (40 CFR Part 63) which limits the loss of hexane during processing to 2.65 L/tonne of canola seed processed for existing crushing plants and 1.136 L/tonne for new crushing plants. Losses of hexane in the meal and in the drier/cooler exhaust air are believed to account for 70-80% of the losses of hexane in a commercial solvent extraction plant (Myers, 1983). Therefore, assuming 80% of the loss is due to residual hexane in meal exiting the DT, and the seed is 60% oil, the maximum allowable hexane in the residual hexane exiting the DT is 2332 μg/g for existing crushing plants and 999 μg/g for new crushing plants. The level of hexane in the final meal would have to be, and is, considerably lower, however, as hexane is lost from the meal during the drying/cooling process after exiting the DT. Residual hexane levels in finished soybean meal have been reported to range from 100 to 3000 μg/g with average values being approximately 500 μg/g (Myers, 1983). The rate of desolventization and residual hexane content were not determined with sparge steam in this experiment, so our results can only be compared to those normally obtained in this plant. It is premature to recommend elimination of sparge steam during desolventization in a commercial process, but the current data suggest that it might be possible.

One of the hypotheses of this research was that the elimination of sparge steam during desolventization should reduce or eliminate browning due to a reduction in moisture content during desolventization. If water, in the form of condensed sparge steam, is not added to the meal during desolventization, browning should be prevented because moisture is required to promote Maillard reactions (Lea and Hannan, 1949a) at an appreciable rate. Desolventization without sparge steam did result in a light yellow-coloured product and did not appear to brown the product to any extent, which supports the hypothesis. The NDIN content of NTCM, which has been shown to be positively

correlated with protein damage during desolventization processing (Section 6), was lower than that of TCM, which also indicates the elimination of sparge steam prevented damage during desolventization.

Prior to desolventization, the meal had a bitter smell, this smell was no longer detectable once the meal had been heated to 100°C. These volatile bitter compound(s) might be degradation product(s) of glucosinolates such as thiocyanate, 5-vinyl oxazolididine-2-thione, 1-cyanyo-2-hydroxy-3 butene, isothiocyanate or nitriles (Subuh et al., 1995) produced during the crushing process prior to desolventization. Previous research found that heating CM to 100°C, with or without steam, improved feed intake and growth in broiler chicks (Shires et al., 1983). Part of the improvement in growth with heating was related to the denaturation of myrosinase activity in the meal, but it may be possible that this (these) volatile compound(s) is also a factor. Effective commercial desolventization, with or without sparge steam, requires heating the meal to ensure the residual hexane content is minimized. In the current experiment, effective desolventization did not occur until the meal reached approximately 100°C so it is unlikely that this bitter compound will become an issue if canola crushers reduce the use of sparge steam during desolventization.

The NTCM contained approximately twice the level of glucosinolates as the TCM, which also indicates the degree of toasting was reduced. Glucosinolates are heat labile and reduction during desolventization/toasting is well known (Jensen et al., 1995a); it appears that sparge steam enhances the rate of their degradation. Gums and screenings were added back to the TCM but not the NTCM and might account for some of the differences in glucosinolate content. However, the levels added to the meal were very low (<2%) and are not thought to contain significant quantities of glucosinolates.

The NTCM in the current experiment contained less 3-butenyl, 4-pentenyl, 2-OH-3-butenyl and 2-OH-4-pentenyl glucosinolates than reported for a conventional TCM from 1988 (Table 3) and a survey of meals published in 1991 (Bell and Keith, 1991), indicating plant breeding has further reduced the aliphatic glucosinolates since that time. The TCM contained similar levels of 4-OH-3-methylindolyl glucosinolates as the 1988 conventional meal, indicating genetic selection has not reduced this compound. The 4-OH-3-methylindolyl glucosinolate (neoglucobrassicin) was most affected by the

conventional toasting process, which is consistent with the findings of Jensen et al. (1995a), but the toxic effects of glucosinolates are generally thought to be attributed to the aliphatic not the indolyl glucosinolates (Darroch and Bell, 1991). Therefore, it is unlikely that the reduction in glucosinolates in this case would have any major impact on toxicity of the meal. Conventional toasting reduced neoglucobrassicin by 82% and total glucosinolates by 55%; however, it did not improve bird performance. The birds fed the non-toasted meal were significantly heavier at 19 and 39 d, indicating the levels of glucosinolates prior to toasting were not impairing performance. Instead, it appears that the glucosinolate breakdown products produced during toasting may have reduced performance.

Complete replacement of soybean meal with CM in the starter, and 80% replacement in the finisher, did impair body weight as compared to the wheat/soybean meal control. However, it does not appear that the effect is due to glucosinolates as the non-toasted meals exceeded performance of the toasted meals, even at the highest levels of inclusion. Serum thyroid hormones, T_3 and T_4, were not reduced by the CM as would be expected if the glucosinolates were impairing thyroid function (Bell et al., 1991), further suggesting the depression at high inclusion levels is not due to the glucosinolate concentration. The current findings are in general agreement with those of Swierczewska et al. (1995), who demonstrated that feeding low glucosinolate varieties as whole seed (0.9 μmol/g seed) to broiler chickens did not depress serum T_3 levels. Previous studies have also demonstrated similar reductions in body weight of broiler chickens with high levels of CM inclusion (Kocher et al., 2000), but the cause was not known. However, replacement of more than 60% of the soybean meal in broiler diets is impractical due to the low nutrient density of CM.

The toasted meal resulted in lower body weights at 19 and 39 d than did the non-toasted meal, possibly due to a reduction in nutritional value as a result of toasting (see Sections 3 and 4). The increase in NDIN content supports this hypothesis, but the fact that Lys content was similar between the two meals does not. The improvement in gain to feed ratio with 20, 60 and 80% soybean meal replacement by NTCM, but not with conventional meal, also suggests the nutrient composition of the non-toasted meal may have been superior to that of the toasted meal. Replacement of up to 60% of the soybean

meal with either TCM or NTCM resulted in final body weights as high as the control, indicating that both TCM and NTCM can be used to replace a significant portion of the soybean in the diet with no adverse affects on performance.

Toasting increased the full jejunal weight but the cause is not known, but may be related to a reduced rate of digestion and, therefore, more materials remaining in this section of the gut. The reduction in empty cecal weight with CM is likely due to decreased fermentation in the cecum as soybean meal contains more indigestible but fermentable sugars, such as stachyose and raffinose, than does CM (Leske and Coon, 1999).

CM inclusion increased mortality levels. A previous study also showed that feeding high levels of CM increased mortality levels (Classen et al., 1991). The primary cause of mortality in the study by Classen et al. (1991) was due to leg abnormalities, in contrast to chronic heart failure in this experiment. Mortality was increased by both toasted and non-toasted meals, indicating that the cause was not thermal breakdown products of glucosinolates or due to heat labile toxic factors that are removed by the DT. The effect of replacing soybean meal with CM on chronic heart failure was more evident and consistent in males, which are more prone to chronic heart failure. This is generally thought to be to due to the faster rate of gain and higher rate of metabolism in males (Olkowski and Classen, 1998). The earliest varieties of rapeseed were associated with heart disease, which was attributed to the high level of erucic acid in the oil (Abdellatif, 1979). However, the levels of erucic acid in the meals in the present study were low so erucic acid is unlikely to have contributed to the incidence of heart failure. Chronic heart failure (ascites) has also been associated with increased metabolic rate in broiler chickens as measured by increased serum T_3 levels (Scheele et al., 1992). The increase in chronic heart failure with higher T_3 levels is thought to be a result of the increase in metabolic rate caused by the elevated serum T_3, which increases the demand for oxygen and places additional demands on the heart. Therefore, the increased chronic heart failure with CM inclusion may be related to the elevation of serum T_3 levels. The reason the broilers in this study were more prone to chronic heart failure than in previous studies is not known. However, increased growth rate of modern broilers and environmental factors may have interacted with feeding CM to predispose birds to chronic heart failure. The reason T_3

levels increased with canola meal inclusion is not known. Schone et al. (1993) also observed an increase in plasma T_3 levels when canola meal was fed to broiler chicks. They suggested that anti-thyroid compounds in the meal bound or destroyed cellular T_3 receptors, and therefore, plasma T_3 levels increased as a result of impaired transfer from the blood to the tissues.

Feeding TCM did result in a linear increase in heart weight as a proportion of the body weight, which may be a sign of chronic heart failure due to an increased rate of metabolism. However, feeding the NTCM did not impact heart weight in the same manner.

CM is thought to have a bitter flavour due to the presence of sinapine, which may reduce feed intake (Clandinin, 1961). Between 0-19 d, the highest level of CM inclusion did reduce feed intake, which would support this hypothesis. However, there was no effect on feed intake between 20 to 39 d, suggesting CM is palatable to older birds. Toasting appears to reduce palatability, as feed intake was reduced in comparison to NTCM (0-19 d). It is not known why toasting would reduce feed intake, but numerous flavoured compounds are produced as a result of Maillard reactions. The products of toasting are normally associated with enhanced flavour (Mauron, 1981). Toasting does cause glucosinolate degradation and the resulting products may be reducing feed intake in young birds. Toasting did not appear to affect palatability in older birds, as feed intake was not affected.

The increase in gain to feed ratio in males (0-19 d) with 20% replacement of soybean meal may be due to an underestimation of the metabolizable energy content of the canola oil used in the diet. However, gain to feed ratio of the males did not continue to increase with CM inclusion, nor was there any effect in females at the same age. Overall (0-39 d) there was no effect of inclusion level on gain to feed ratio, indicating the diets were likely iso-energenic and iso-nitrogenous.

Birds fed conventional toasted meal had larger livers than those fed non-toasted meal, which may indicate the toasting process is producing toxic compounds that have to be detoxified in the liver. Since toasting not only increased liver weight but also heart weights and serum T_3, and reduced body weights and feed consumption, it appears that toasting may be producing toxic compounds in the meal. Toasting degraded some of the

glucosinolates in the meal and, therefore, the breakdown products may have been responsible for the toxic effects observed in this study (Subuh et al., 1995). The primary objective of this study was to determine if toasting is required to reduce toxic or anti-nutritional factors in canola meal. However, the results suggest that the toasting process may be producing toxic components instead of destroying them. Therefore, it may be advisable to eliminate toasting, not only to increase amino acid digestibility and to reduce processing costs, but to prevent the production of toxic compounds in the meal.

5.6 CONCLUSIONS AND IMPLICATIONS

In conclusion, desolventization without sparge steam results in a light-coloured meal and appears to reduce, if not eliminate, toasting. Desolventization without sparge steam also resulted in the highest body weights, which suggests that there are no anti-nutritional factors in CM that are reduced by the toasting process. Toasting appears to produce toxic compounds, likely degradation products of glucosinolates, that negatively affect bird performance, so it not only appears that toasting is not necessary but that it may be detrimental. If the use of sparge steam can be suspended, the cost of producing the steam will be avoided and the potential to destroy essential amino acids almost eliminated. However, prior to a general recommendation being made to eliminate CM toasting, the impact of this process must be studied on other species, such as swine or cattle. CM can be used to replace a portion of the soybean meal in broiler diets, but complete replacement of soybean meal by CM impairs performance. High levels of CM in the diet also increased metabolic rate and the incidence of chronic heart failure in males, but the cause is not known.

6 ASSESSMENT OF PROTEIN SOLUBILITY, NEUTRAL DETERGENT INSOLUBLE NITROGEN AND NEAR INFRARED REFLECTANCE AS PREDICTORS OF THE DIGESTIBLE AMINO ACID CONTENT OF PROCESSED CANOLA MEALS

6.1 ABSTRACT

Protein solubility in 0.5% KOH, neutral detergent insoluble nitrogen (NDIN) and near infrared reflectance spectroscopy (NIRS) were used to predict the digestibility and digestible content of amino acids in canola meal. Commercial canola meal samples (31) and non-toasted canola meal samples (26) were analysed for amino acid content and digestibility using broiler chickens, and these values were correlated with protein solubility, NDIN and NIRS values. Digestibility and digestible content of Lys increased linearly with protein solubility in 0.5% KOH (R^2=0.65 and 0.74, respectively) when both toasted and non-toasted canola meals were included in the analysis. However, regression analysis without the non-toasted canola meals reduced the coefficient of determination, resulting in a poorly fitting regression line (R^2=0.17). Therefore, protein solubility would not be a good *in vitro* indicator of amino acid quality in toasted canola meal samples. There was a negative linear relationship between NDIN content and the digestibility and digestible content of Lys (R^2=0.78) and all of the other essential amino acids when both toasted and non-toasted meals were included in the analysis. Elimination of non-toasted meals reduced the coefficients of determination, but reasonable fitting regression lines were still obtained for Lys (R^2=0.54 and 0.58 for digestibility and digestible content, respectively), indicating that NDIN could be used as an indicator of protein quality in toasted canola meal. NIRS accurately predicted Lys digestibility (R^2=0.92) as well as the digestible content of Lys (R^2=0.84) and cystine (R^2=0.77), indicating it has the potential to be used to monitor canola meal quality. Due to limited access to the NIRS, calibration sets were only determined with both toasted and non-toasted meals. Although NIRS shows promise, effective development of a

NIRS calibration requires a larger validation set than is currently available and, therefore, NDIN is recommended for prediction of digestible amino acid content for broiler chickens until additional samples are available for calibration and validation.

6.2 INTRODUCTION

Canola meal is used as a source of protein for agricultural animals as it possesses an excellent balance of amino acids. However, during desolventization/toasting of the meal, both the content and availability of the amino acids can be reduced (sections 3.51 and 3.52). Currently, if there is any question about meal quality after processing, only amino acid content and protein solubility in 0.5% KOH are used to assess meal damage during the desolventization/toasting process.

The amino acid content of canola meal, particularly Lys content, is reduced by excessive toasting (Section 3.51). The amino acid analysis procedure requires expensive HPLC equipment and dedicated technicians to conduct the analysis. As a result, Canadian canola crushing plants are not currently equipped to conduct the analysis and are not likely to adopt this technology for routine quality control. Since the analysis is expensive and time consuming, it is more probable that only suspect samples will be sent to commercial laboratories for amino acid analysis.

Protein solubility in canola meal is reduced during heat processing as the native structure of the protein is disrupted (Pastuszewska et al., 1998). Therefore, protein solubility should provide a measure of how much heat has been applied to the meal during the desolventization/toasting process (Parsons et al., 1991) . The procedure is relatively simple, requires only basic laboratory equipment, and can be completed in a matter of hours. Although low protein solubility has been demonstrated with excess heating of canola meal in the laboratory, it is not known if low protein solubility correlates with amino acid content and digestibility in commercial canola meal samples (Pastuszewska et al., 1998).

Neutral detergent insoluble nitrogen (NDIN) is that portion of the protein that is not solubilized during the analysis of neutral detergent fibre and, therefore, remains in the residue. The NDIN content of canola meal has been shown to increase from 7.1% to 19.1% of the crude protein during desolventization/toasting (Mustafa et al., 2000) and,

therefore, could indicate if meal has been overheated. Due to the insoluble nature of the NDIN fraction, one might expect the digestibility of this protein to be low. Therefore, NDIN might also be an indicator of amino acid digestibility after heat processing.

Near infrared reflectance spectroscopy (NIRS) is a commonly used procedure to estimate protein and moisture content in feed ingredients. The analysis of the sample is simple and rapid, requiring only a few minutes. It is based on the principle that C-H, O-H, N-H, C-C and C=O bonds absorb thermal radiation at specific wavelengths. By applying thermal radiation to the sample and measuring the amount of reflected light at specific wavelengths between 1100 and 2500 nm, one can determine the chemical composition of feed ingredients. Near infrared reflectance has been shown to be able to predict the metabolisable energy content of wheats (Swift et al., 1998) suggesting it can predict digestibility of starch. However, it is not known if NIRS can be used to predict the digestible amino acid content of canola meal.

Therefore, the objective of this study was to determine if protein solubility in 0.5% KOH, NDIN and/or NIRS can be used to predict the available amino acid content of processed canola meals.

6.3 MATERIALS AND METHODS

As previously described in section 4, 31 toasted and 26 non-toasted canola meal samples were collected from crushing plants located across western Canada and analysed for amino acid content and apparent digestibility in broiler chicks. The meal samples were analysed in duplicate for protein solubility in 0.5% KOH as described by Pastuszewska et al. (1998).

NDIN content of the canola meal samples was determined in duplicate using an Ankom 200 Fibre Analyzer[26]. Canola meal (0.5 g) ground to pass through a 0.5 mm screen was weighed in to filter bags ({Ankom Technology} F57) and heat sealed. The filter bags (20), neutral detergent solution (2 L {Ankom Technology} premixed chemical solution- FND20) and 1.36 million modified Wohlgemuth Units of heat stable amylase ({Ankom Technology} FAA) were added to the analyzer and allowed to agitate for 60 minutes at 100°C. The solution was then drained from the analyzer and washed

[26] Ankom Technology Corporation, 140 Turk Hill Park, Fairport, NY, 14450

with an additional 2 L of hot (90-100°C) distilled water with agitation for 5 minutes; this wash procedure was repeated three times. The water was gently squeezed out of the filter bags by hand prior to soaking in acetone for 3 minutes. The acetone was allowed to evaporate prior to drying the bags in an oven at 105°C for 4 hours. The protein content of the residue in the filter bags was then analysed according to the methods of the Association of Official Analytical Chemists (1990) using the Kjeldahl procedure. NDIN was expressed as the proportion of the protein in the meal remaining in the NDF residue. AAFCO[27] check samples were also analysed for crude protein, NDIN and moisture and the values obtained were comparable to those reported by other laboratories participating in the program.

Samples were scanned from 400 to 2500 nm at 2 nm intervals with a NIRSystems 6500[28] equipped with a standard sample cup and using NSAS v3.53 software. The calibrations were developed with WinISI v1.04. Calibrations were developed using modified PLS under WinISI, first derivative math treatment was used for cystine, Lys and Lys/CP availability. Second derivative math treatment was used for Met availability. The spectral region used in developing the calibration was 650 to 2500 nm. Due to limited access to the NIRS system used in this study, calibration sets were only produced for the apparent Lys digestibility and apparent digestible Lys, Met and cystine content of canola meals.

Regression analysis of the data was conducted using the Proc RSREG and REG procedures of the SAS program (SAS Institute, 1989).

6.4 RESULTS

The regression equations and coefficients of determination for protein solubility in 0.5% KOH versus amino acid digestibility and digestible amino acid content are shown in Tables 6.1 and 6.2, respectively. Inclusion of both toasted and non-toasted canola meals in the regression analysis resulted in a significant relationship between protein solubility and apparent digestibility and the digestible content of all of the essential amino acids tested. However, eliminating the non-toasted meals from the

[27] Association of American Feed Control Officials, Inc., College Station, TX, USA, 77841-3160

regression analysis reduced the coefficients of determination for all amino acids (Tables 6.1 and 6.2 and Figures 6.1and 6.2). Exclusion of the non-toasted meals from the analysis also reduced the significant effects of protein solubility to only Lys and Met for digestibility and to Lys and His for digestible content.

Similarly, regression analysis of the NDIN contents of toasted and non-toasted meals revealed significant effects on both amino acid digestibility and digestible amino acid content (Tables 6.3 and 6.4). Eliminating the non-toasted meals from the regression analysis reduced the coefficients of determination for amino acid digestibility and digestible amino acid content (Tables 6.3 and 6.4 and Figure 6.3). With the exclusion of non-toasted meals from the regression analysis, NDIN still revealed significant effects for all the essential amino acids for digestibility and all essential amino acids for digestible content except for His.

Due to limited access to the NIRS system used in this study, calibration curves were only developed for Lys digestibility and digestible Lys, cystine and Met content. Similarly, the calibration was only developed with the full set of samples which included the non-toasted canola meals. Therefore, coefficients of determination determined without the non-toasted meals are not available. The scanned reflectance spectra (450 to 2500 nm) of three samples with varying amounts of digestible Lys are shown in Figure 6.4. The scan spectra used in the calibration of the system were limited to 1100 to 2500 nm as many of the NIRS systems available commercially do not operate below 1100 nm. Using this range of spectra, effective prediction equations ($P<0.001$) were produced by NIRS for both apparent Lys digestibility ($R^2=0.92$, Figure 6.5) and digestible content ($R^2=0.85$, Figure 6.6). Near infrared spectroscopy also predicted available cystine content ($R^2=0.77$, Figure 6.7), but was less effective in predicting the digestible Met content ($R^2=0.53$, Figure 6.8).

[28] FOSS-NIRSystems, Inc., Silver Spring, MD

Table 6.1 Regression analysis of KOH protein solubility versus amino acid digestibility coefficient in toasted and non-toasted (n=57) or toasted canola meal (n=31) samples only

	Toasted + Non-toasted meals			Toasted meals only		
Amino Acid	Slope	Intercept	R^2	Slope	Intercept	R^2
Arg	0.002***	0.80	0.59	0.001	0.83	0.07
Cys	0.003***	0.62	0.58	0.001	0.69	0.04
His	0.002***	0.77	0.59	0.001	0.79	0.10
Ile	0.003***	0.63	0.48	0.002	0.66	0.07
Leu	0.002***	0.72	0.40	0.001	0.76	0.02
Lys	0.003***	0.67	0.65	0.002*	0.70	0.15
Met	0.003***	0.65	0.50	0.003*	0.65	0.16
Phe	0.002***	0.75	0.45	0.001	0.77	0.06
Thr	0.003***	0.57	0.57	0.002	0.63	0.06
Val	0.003***	0.67	0.53	0.001	0.70	0.07

* $P < 0.05$.

*** $P < 0.001$.

Table 6.2 Regression analysis of KOH protein solubility versus digestible amino acid content (% as is) in toasted and non-toasted (n=57) or toasted canola meal (n=31) samples only

	Toasted + Non-toasted meals			Toasted meals		
Amino Acid	Slope	Intercept	R^2	Slope	Intercept	R^2
Arg	0.006***	1.67	0.56	0.003	1.81	0.06
Cys	0.003***	0.59	0.26	-0.001	0.70	0.07
His	0.003***	0.88	0.57	0.002*	0.92	0.14
Ile	0.005***	1.022	0.54	0.003	1.10	0.08
Leu	0.007***	1.89	0.44	0.002	2.06	0.02
Lys	0.011***	1.26	0.74	0.005*	1.45	0.18
Met	0.002***	0.54	0.25	0.002	0.55	0.08
Phe	0.004***	1.06	0.49	0.002	1.13	0.05
Thr	0.006***	0.93	0.60	0.002	1.08	0.04
Val	0.006***	1.36	0.57	0.003	1.48	0.07

* $P < 0.05$.

*** $P < 0.001$.

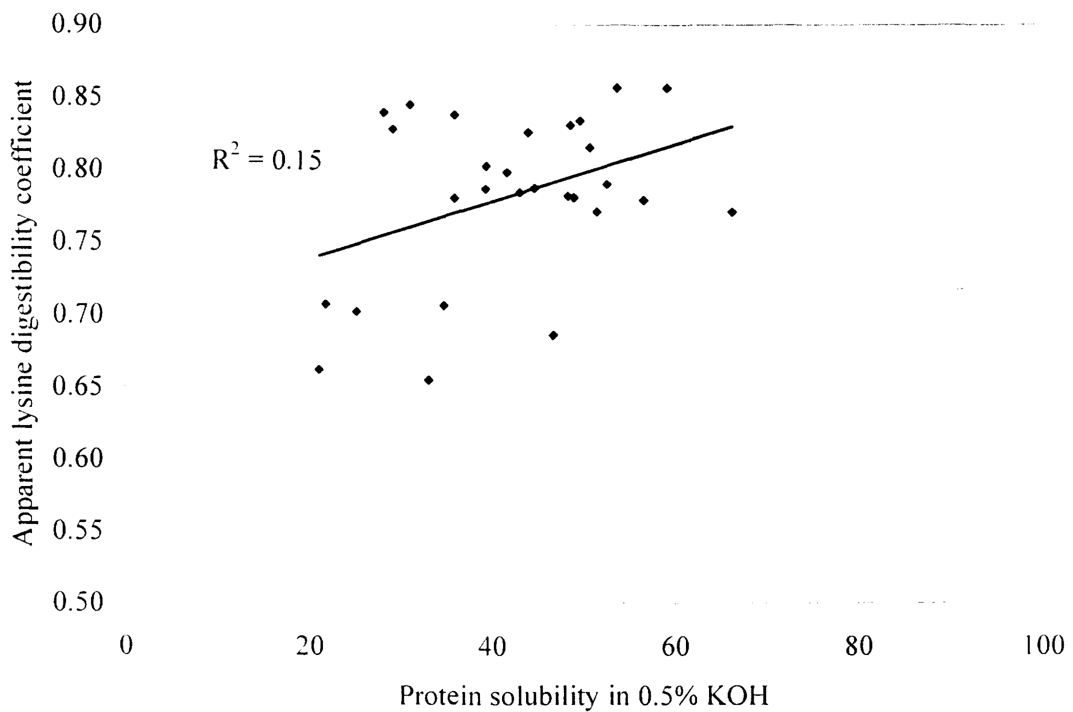

Figure 6.1 Protein solubility in 0.5% KOH of toasted canola meal samples (n=31) versus apparent Lys digestibility in broiler chickens ($P<0.05$).

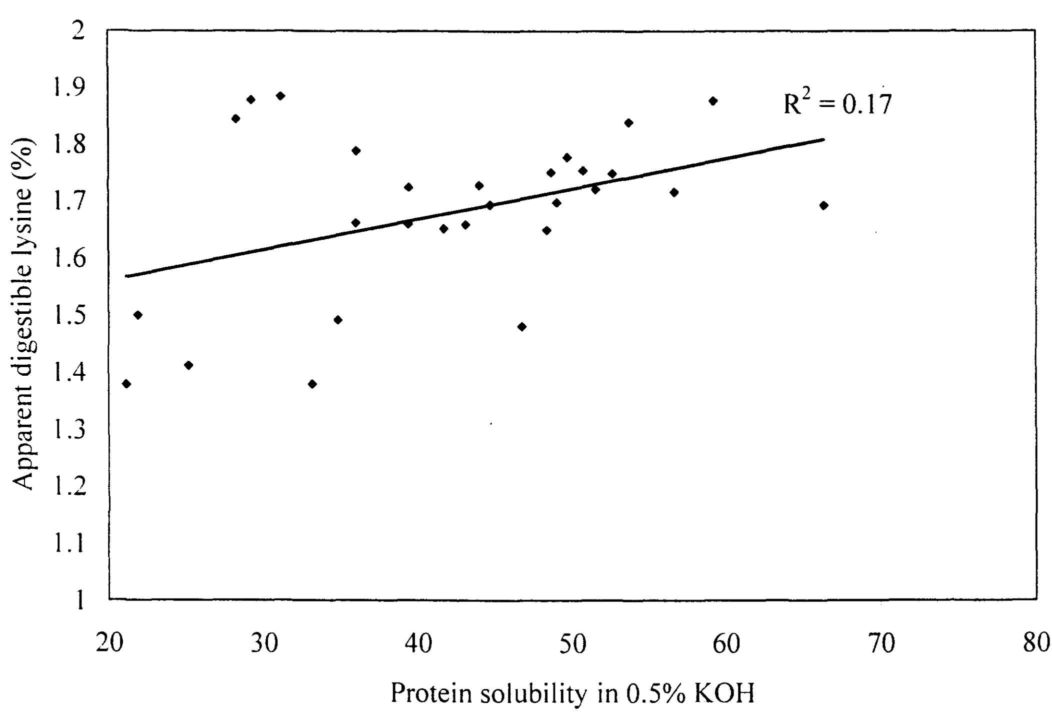

Figure 6.2 Protein solubility in 0.5% KOH versus apparent digestible Lys content of toasted canola meal (n=31) for broiler chickens ($P<0.05$).

Table 6.3 Regression analysis of neutral detergent insoluble nitrogen (NDIN) versus amino acid digestibility coefficient in toasted and non-toasted (n=57) or toasted canola meal (n=31) samples only

	Toasted + Non-toasted meals			Toasted meals only		
Amino Acid	Slope	Intercept	R^2	Slope	Intercept	R^2
Arg	-0.009***	1.00	0.65	-0.006**	0.94	0.26
Cys	-0.017***	0.98	0.69	-0.011***	0.885	0.33
His	-0.009***	0.96	0.56	-0.005*	0.90	0.18
Ile	-0.015***	0.95	0.64	-0.013***	0.91	0.36
Leu	-0.011***	0.95	0.56	-0.009**	0.91	0.25
Lys	-0.016***	1.01	0.78	-0.013***	0.96	0.54
Met	-0.017***	1.04	0.47	-0.013**	0.97	0.22
Phe	-0.010***	0.95	0.56	-0.008**	0.92	0.25
Thr	-0.018***	0.95	0.67	-0.012***	0.86	0.36
Val	-0.013***	0.94	0.63	-0.009**	0.88	0.29

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$.

Table 6.4 Regression analysis of neutral detergent insoluble nitrogen (NDIN) versus digestible amino acid content (% as is) in toasted and non-toasted (n=57) or toasted canola meal (n=31) samples only

	Toasted + Non-toasted meals			Toasted meals only		
Amino Acid	Slope	Intercept	R^2	Slope	Intercept	R^2
Arg	-0.034***	2.43	0.58	-0.018*	2.18	0.21
Cys	-0.016***	0.92	0.40	-0.008*	0.80	0.18
His	-0.014***	1.22	0.43	-0.005	1.07	0.06
Ile	-0.030***	1.67	0.63	-0.020*	1.51	0.31
Leu	-0.037***	2.67	0.49	-0.021*	2.45	0.15
Lys	-0.056***	2.51	0.78	-0.033***	2.14	0.58
Met	-0.012***	0.81	0.28	-0.009*	0.76	0.15
Phe	-0.020***	1.50	0.53	-0.012*	1.37	0.19
Thr	-0.033***	1.67	0.62	-0.019**	1.44	0.27
Val	-0.030***	2.08	0.58	-0.017*	1.83	0.19

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$.

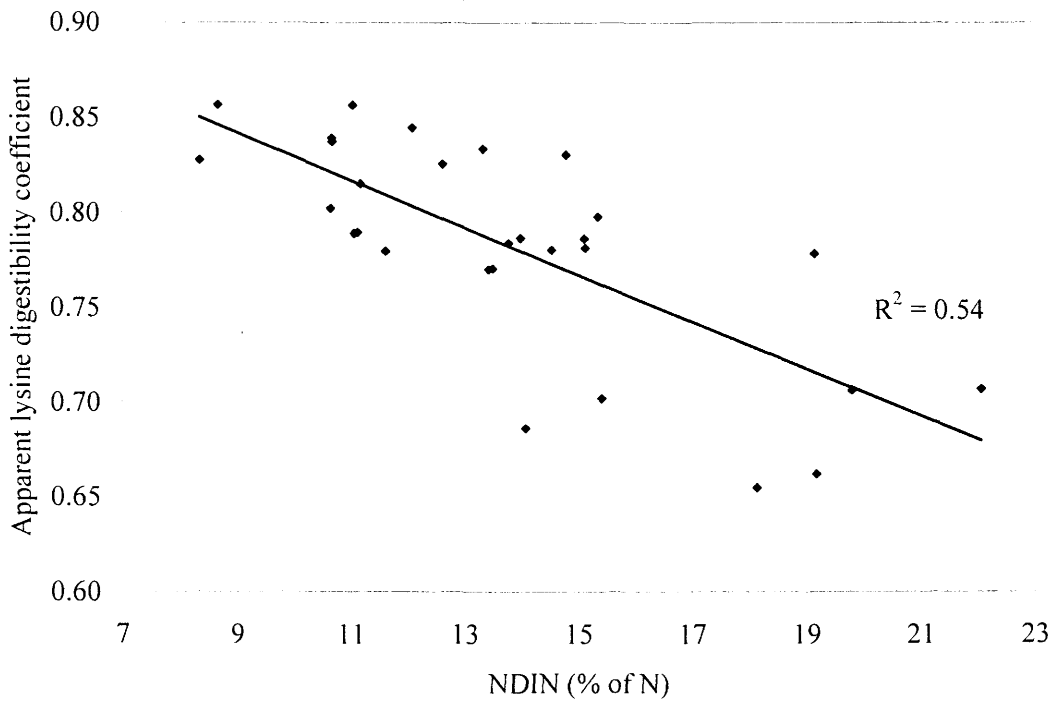

Figure 6.3 Neutral detergent insoluble nitrogen content (NDIN, as % of N) of toasted canola meal samples (n=31) versus Lys digestibility in broiler chickens (P<0.001).

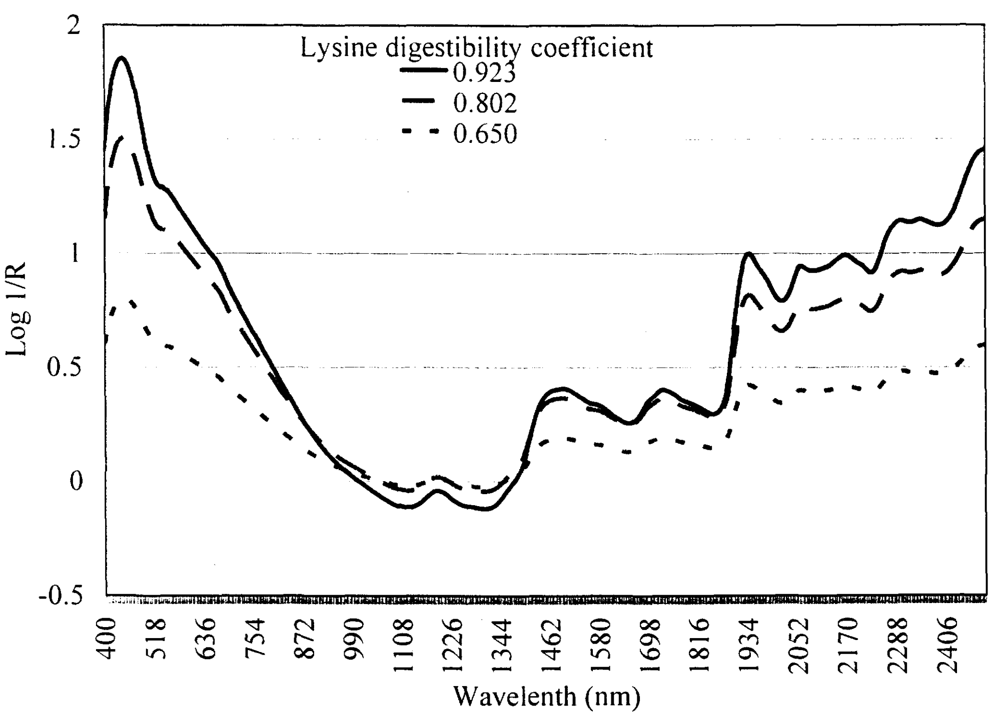

Figure 6.4 NIRS scan spectra (log of 1/reflectance versus wavelength) of three canola meal samples differing in apparent Lys digestibility.

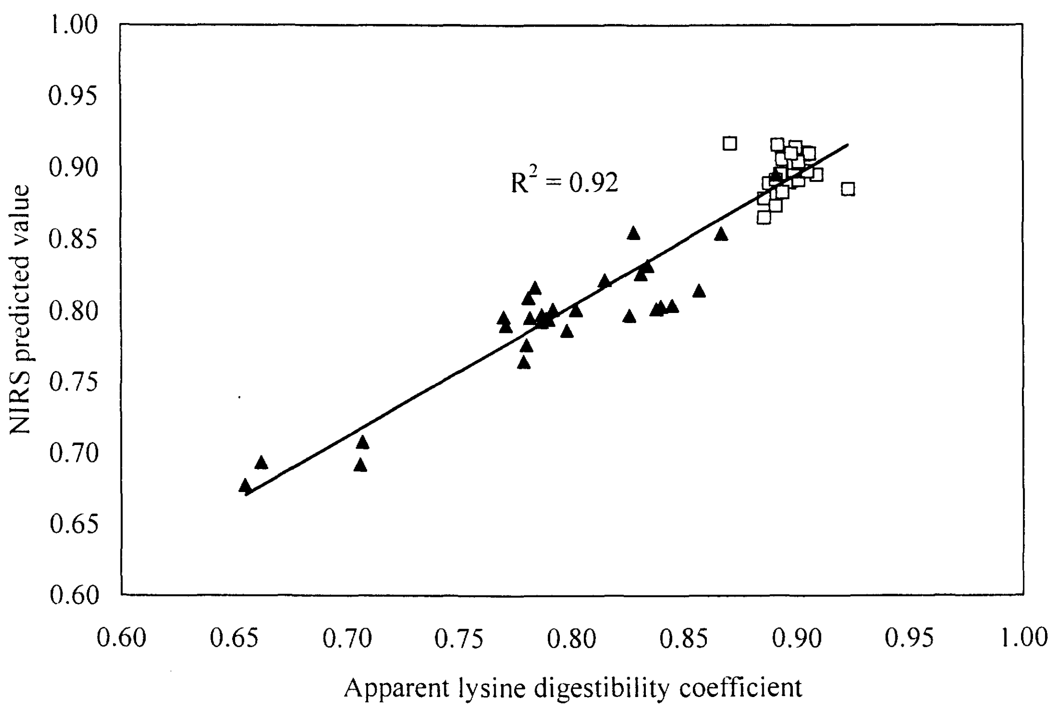

Figure 6.5 Apparent Lys digestibility coefficient in non-toasted (□) and toasted (▲) canola meal samples (n=57) versus the NIRS predicted values ($P<0.001$).

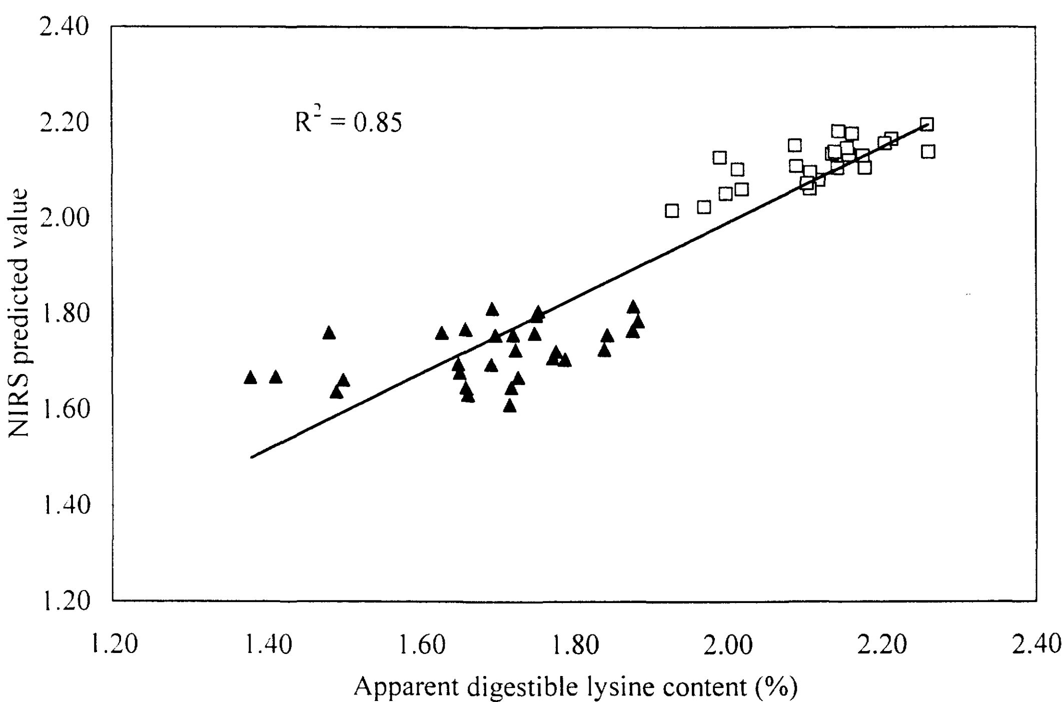

Figure 6.6 Apparent digestible Lys content in non-toasted (□) and toasted (▲) canola meal (n=57) versus values predicted by NIRS (P<0.001).

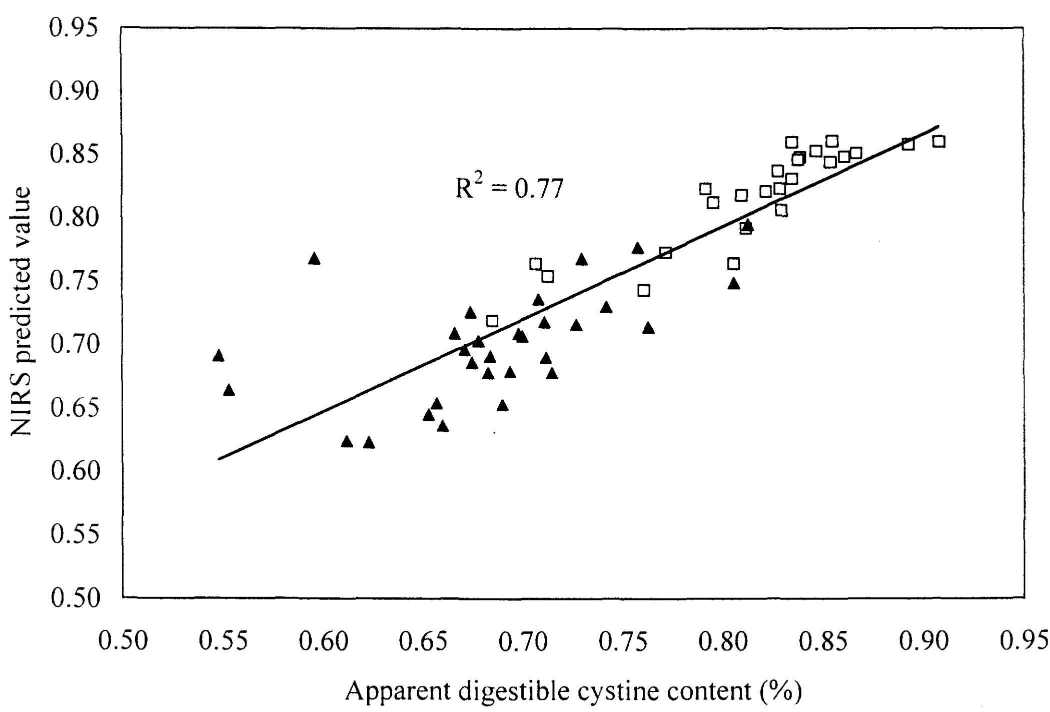

Figure 6.7 Apparent digestible cystine content in non-toasted (□) and toasted (▲) canola meal (n=57) versus the values predicted by NIRS ($P<0.001$).

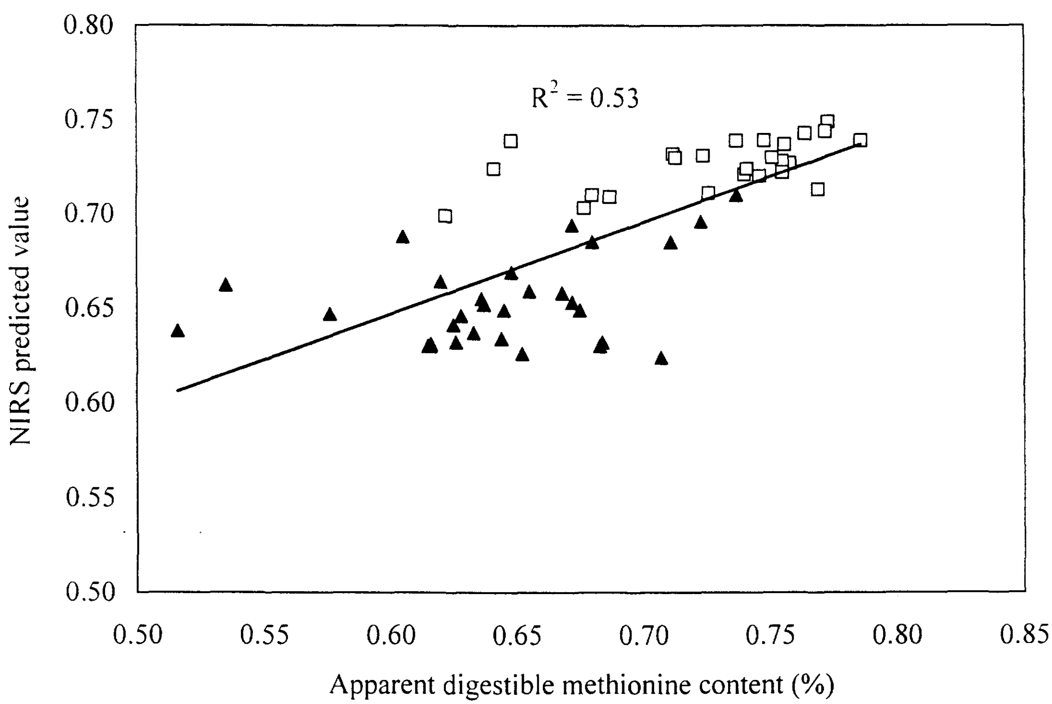

Figure 6.8 Apparent digestible Met content in non-toasted (□) and toasted (▲) canola meal (n=57) versus the values predicted by NIRS ($P<0.001$).

6.5 DISCUSSION

Both commercially produced toasted meals and commercially extracted but air desolventized meals (non-toasted) were included in the initial regression analysis and provided a broad range of products with a wide range of protein solubility and digestibility values. However, one of the objectives of this experiment was to determine if protein solubility could be used to determine the amino acid digestibility of conventionally toasted meals. Although the regression analysis showed promise when both toasted and non-toasted meals were included in the analysis, there appears to be little relationship between protein solubility and amino acid digestibility and digestible content in toasted meals. Anderson-Hafermann et al. (1993) reported that protein solubility is reduced during heat processing and would indicate over processing and suspect protein quality. However, there is little information on correlations between protein solubility in commercially toasted canola meals and *in vivo* amino acid digestibility. The data from the current study suggest that protein solubility is not a good indicator of either amino acid digestibility (Figure 6.1) or digestible amino acid content (Figure 6.2)

The negative relationship between NDIN and apparent digestibility of amino acids, especially Lys (Figure 6.3), suggests the NDIN fraction is poorly utilized by broiler chickens and could be used to estimate the apparent digestibility of Lys in canola meal. Neutral detergent insoluble nitrogen is routinely used to estimate the amount of protein that is only slowly degraded by the rumen (Mass et al., 2001). However, in contrast to the findings of this study, this fraction of the protein is assumed to be partially or completely digestible depending on the acid detergent insoluble nitrogen content. This would suggest that poor quality canola meal, as judged by a high NDIN content, may still be good quality feed for ruminant animals.

Similar to protein solubility, coefficients of determination using NDIN were reduced by eliminating the non-toasted meals from the analysis. However, NDIN still afforded reasonable prediction equations when only toasted meals were used, indicating it could be used as a screening tool to identify poor quality meals. NDIN values appear to deviate from the regression line above 15% NDIN (Figure 6.3), indicating this assay would not accurately predict Lys digestibility in this range. Neutral detergent insoluble

nitrogen has the advantage of being available in most feed testing laboratories. It is low in cost and has a fast turnaround time, so it would be a practical assay for quality control during production. In addition, feed manufacturers could have each new batch of canola meal tested to ensure the meal meets the desired specifications. Based on this study, it would appear that meals with less than 12% of the protein as NDIN are of very good nutritional value.

Near infrared spectroscopy, can be, and is, used to estimate the protein and moisture content of feed ingredients (Williams et al., 1983). There is also recent evidence that NIRS can be used to predict the amino acid content of canola and other feed ingredients with a relatively high degree of precision (Fontaine et al., 2001). Digestion is the result of a series of complex events; therefore, one might expect that predicting digestibility of amino acids may be more difficult. Since digestibility is often a function of chemical and physical characteristics, NIRS may be able to predict digestibility (Swift et al, 1998). In the current study, there were marked differences in NIR spectral scans of meals differing in amino acid digestibility (Figure 6.4), suggesting it should be possible to determine digestible amino acid levels in canola meal by NIRS. The high coefficients of determination obtained in this study suggest that NIRS has the potential to be used to predict the available content of amino acids in canola meal and agrees with the findings of van Kempen (1998) for other common feed ingredients. However, effective calibration of NIRS requires a larger set of samples with known available amino acid contents than is currently available. In addition, year to year variation in canola meal spectra would likely require the NIRS systems to be recalibrated and validated at least on an annual basis. Therefore, additional work is required before NIRS can be used commercially to predict amino acid digestibility and digestible content of amino acids. However, the current data suggests that NIRS may be more accurate, cost effective and rapid than the protein solubility or NDIN assays.

6.6 CONCLUSIONS

The availability and content of amino acids in canola meal can be adversely affected during processing, making it necessary to monitor meal quality on a routine basis. Protein solubility (0.5% KOH) is reduced by heating and correlates with the digestible Lys content of canola meals. However, the relationship between protein solubility and amino acid digestibility or digestible amino acid content is poor when only toasted canola meal samples are tested, indicating it would not be an effective assay for conventional canola meal samples. The NDIN fraction of the protein in canola meal is increased by heating during processing, and is correlated with amino acid digestibility (especially Lys) and apparent digestible amino acid content. Although R^2 values for NDIN were lower when only non-toasted meals were tested, they were sufficiently related that this test could be used to monitor meal quality after processing. Near infrared spectroscopy appears to accurately predict Lys digestibility, digestible Lys and cystine content. Near infrared spectroscopy is the fastest and simplest of procedures but requires expensive equipment. In addition, effective NIRS calibration requires a larger number of samples than is currently available, as well as constant validation. Therefore, until standard calibration sets become available for NIRS, it is recommended that NDIN be used to monitor canola meal quality.

7 THE EFFECT OF CANOLA PROCESSING CONDITIONS ON THE APPEARANCE AND NUTRITIONAL VALUE OF CANOLA MEAL

7.1 ABSTRACT

During desolventization/toasting, canola meal is heated to cause the hexane to evaporate and produce a meal low in residual solvent. However, the protein in the meal is susceptible to heat damage. Experiments were conducted to determine the conditions during desolventization that can adversely affect protein quality in the meal, as determined by the level of neutral detergent insoluble nitrogen (NDIN). In Experiment 1, two samples of seed were collected, solvent extracted and air-desolventized prior to heat treatment in an isothermal autoclave at 85 and 120°C for 0-60 min. Below 100°C, there was little change in NDIN content. However, above 100°C, NDIN content increased in a quadratic manner with time, suggesting that temperatures below 100°C should be used during desolventization. The *Brassica rapa* sample had a lower level of NDIN before heating but higher after heating than the *B. napus* sample, suggesting that there may be species differences in susceptibility to heat damage during processing.

In Experiment 1 it was not possible to control the level of moisture during heat processing, and the rate of protein damage may be dependent on moisture content. Therefore, a second experiment was conducted to examine the effects of moisture, time and temperature on meal quality as measured by NDIN, amino acid content and changes in meal colour. In the absence of added moisture (7%), heating had very little effect on NDIN, amino acid content or colour even at temperatures up to 115°C. However, at higher moisture contents (10-18%), the level of protein damage was affected in a quadratic manner with temperature, indicating that maximal protein damage occurs at approximately 14% moisture content. Lys content was most adversely affected by heat treatment with added water, and the meal became a darker brown colour as well, indicating that the reductions in nutritional value during heat processing were due in part

to Maillard reactions. Therefore, canola meal should be desolventized without the addition of moisture to the meal. This would result in a light coloured meal with maximal amino acid content and digestibility. However, if moisture must still be incorporated in the form of sparge steam to effectively desolventize the meal, the level of moisture and heat should be minimized to produce the highest quality meals.

7.2 INTRODUCTION

Canola meal is used as a source of protein in the diets of most commercial animal species. It has an excellent balance of amino acids, but during desolventization the availability and content of amino acids is reduced (Section 4.0). In addition, desolventization changes the colour of the meal from light yellow with black flecks of hull to dark brown (Section 4.5). The change in colour and Lys content is likely the result of Maillard reactions which produce brown pigments. It appears that the direct injection of sparge steam during desolventization may be responsible for promoting Maillard reactions, as suspending its use produces a light yellow meal (see Section 5). Desolventization of the meal after solvent extraction requires the application of heat, but at low moisture content, the Maillard reactions proceed slowly (Lea and Hannan, 1949a). Direct injection of sparge steam increases the moisture content of the meal as the steam condenses, and it is hypothesized that the increase in moisture content during heating is promoting Maillard reactions and is, therefore, responsible for the losses in lysine content and digestibility during processing. The effects of time, temperature and moisture during heat processing on the rate of Maillard reactions are known for food products, but have not been defined for canola meal. Production of the highest quality canola meal requires an understanding of which conditions preserve the quality of the product and which ones should be avoided.

Amino acid content, especially Lys, is reduced during advanced Maillard reactions and, therefore, can be used to assess the effects of processing conditions on the nutritional value of meal. However, the products of early Maillard reactions have reduced amino acid availability, but not content. Therefore, simply just monitoring meal

amino acid content can underestimate the impact of processing on meal quality. Apparent digestible Lys content decreases with increased neutral detergent insoluble nitrogen (NDIN) content as a result of processing (see section 6.4). Therefore, NDIN is a good indicator of the effects of processing on the nutritional value of canola meal. Advanced Maillard reactions result in browning of products and, therefore, the degree to which these reactions are occurring during processing can also be assessed by the colour of the meal.

The objective of the first experiment was to determine the effect of temperature and exposure time to direct steam heat on the NDIN content of meal from two samples of canola. The objective of the second experiment was to assess the effects of time, temperature and moisture on the protein quality and colour of canola meal.

7.3 MATERIALS AND METHODS

7.3.1 Experiment 1 – Effect of Time and Temperature with Steam Injection

Two samples of seed grade canola, *Brassica napus* variety "Bounty" and *Brassica rapa* variety "Klondike", were expelled in a lab screw press[29] and extracted with hexane over a period of 4 h. The meal was desolventized in a fume hood for 2 to 3 d. Samples (12 g) of the meal were then placed in folded 18.5 cm Fisherbrand® P8 course filter paper[30]; the filter paper edges were folded and stapled to form an envelope that was approximately 1 cm thick, 8 cm wide and 17 cm long. The envelopes were then placed vertically in steel racks such that the steam entering the autoclave could penetrate into the meal. A 2x8x4 factorial design was used to study meal treatment conditions. Two samples of meal were exposed to eight temperatures (85, 90, 95, 100, 105, 110, 115,

[29] Komet Oil Expeller, IBG Monforts Gmbh and Co., Mönchengladback, Post fach 20 08 53, D-41, Germany
[30] Fisher Scientific, Pittsburgh, PA, 15275

120°C) for 15, 30, 45 or 60 minutes in an Amsco Eagle Series 2322 Isothermal Autoclave[31]. The samples were then dried at 50°C in a forced air oven.

NDIN content of the canola meal samples was determined in duplicate using an Ankom 200 Fibre Analyzer[32]. Canola meal (0.5 g) ground to pass through a 0.5 mm screen was weighed in to filter bags ({Ankom Technology}, F57) and heat sealed. The filter bags (20), neutral detergent solution (2 L, {Ankom Technology}, premixed chemical solution- FND20) and 1.36 million modified Wohlgemuth Units of heat stable amylase ({Ankom Technology}, FAA) were added to the analyzer and allowed to agitate for 60 minutes at 100°C. The solution was then drained from the analyzer and washed with an additional 2 L of hot (90-100°C) distilled water with agitation for 5 minutes; this wash procedure was repeated three times. The water was gently squeezed out of the filter bags by hand prior to soaking in acetone for 3 minutes. The acetone was allowed to evaporate prior to drying the bags in an oven at 105°C for 4 hours. The protein content of the meal and the residue in the filter bags were then analysed according to the methods of the Association of Official Analytical Chemists (1990) using the Kjeldahl method. NDIN was then expressed as the proportion of the protein in the meal remaining in the NDF residue. AAFCO[33] check samples were also analysed for amino acids, crude protein, NDIN and moisture and the values obtained were comparable to those reported by other laboratories participating in the program.

7.3.2 Experiment 2 - Effect of Moisture Level During Toasting

In Experiment 1, moisture content of the meal during heating was not directly controlled but moisture content may directly influence the level of protein damage during heating. Therefore a second experiment was conducted to examine the effects of heating at controlled moisture levels on the NDIN, amino acid content and colour of the resulting meals.

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[31] 51 International Blvd., Etobicoke, ON, M9W 6H3
[32] Ankom Technology Coporation, 140 Turk Hill Park, Fairport, NY, 14450
[33] Association of American Feed Control Officials, Inc., College Station, TX, USA, 77841-3160

Prepress/solvent extracted canola meal was collected prior to entering the desolventizer/toaster in a commercial crushing plant. The meal was placed in burlap sacks and allowed to air desolventize at 20°C over a 72 hour period. The meal was then ground through a 6.0 mm screen with a Retsch SM 2000 Forage grinder[34]. The moisture content was adjusted by adding the appropriate amount of water to 1.5 kg of meal and mixing with a stainless steel wire whisk prior to placing 50 g in 600 ml glass jars and sealing with a lid. The moisture content of the extracted, air desolventized, ground meal was 7%. The experiment was a 4x4 factorial with four moisture levels (7, 10, 14 and 18%) and four temperatures (100, 105, 110 and 115°C). Temperature of the meal was monitored by a thermometer inserted into the meal samples through a tight fitting hole in the lid of the jars. The oven temperature was initially set 20°C above the desired meal temperature in order to achieve the desired temperature more quickly. Once the meal achieved the desired temperature, the meal was maintained at that temperature for 10 minutes. All treatments were replicated three times. Sample analyses were conducted in duplicate except amino acids which were analysed once.

The samples were analysed for crude protein and NDIN as described in section 7.3.1. The meals were analyzed for amino acids using a Beckman[35] amino acid analyzer equipped with a Spheroge 1 IEX anion exchange column, System Gold 126AA solvent module, 508 auto-sampler, 166 detector, ninhydrin 232 post column reactor, Mistral[36] column following hydrolysis using the performic acid oxidation with acid hydrolysis and sodium metabisulfite method as described by Llames and Fontaine (1994, Alternative I). The colour of the meals was measured using a Hunter Lab – Miniscan XE[37] which was set at L*m a* and b*, observer 10 and illuminant A.

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[34] Retsch GmbH & Co. KG, Rheinische Straße 36, 42781, Haan, Germany
[35] Beckman Instruments, Inc., Fullerton, CA, 92834-3100
[36] Holland Spark, P.O. Box 388, 7800 AJ Emmen, The Netherlands
[37] 11491 Sunset Hills Road, Reston, VA, 22090-9902

7.3.3 Statistical Analysis

For Experiment 1, the data were analysed as a 2x4x8 (2 species x 4 times x 8 temperatures) factorial using the GLM procedure of the SAS program (SAS Institute, 1989). For Experiment 2, the data were analysed as a 4x4 (4 moisture levels x 4 temperatures) factorial. Regression analysis was conducted using the RSREG procedure of the SAS program (SAS Institute, 1989). Differences were considered significant when $P<0.05$. Means separation was conducted using the method of Duncan (1955) when significant main effects were observed.

7.4 RESULTS

7.4.1 Experiment 1 – Effect of Time and Temperature of Steam Heating

There was no effect of processing conditions on NDIN content at temperatures below 100°C (Figure 7.1). At higher temperatures, however, NDIN levels in meals from both samples were increased ($P<0.05$) by heat treatment. The *Brassica rapa* sample had higher NDIN levels than the *Brassica napus* sample when heated between 105 and 120°C (Figure 7.1), in spite of having marginally lower levels prior to heating (6.6 vs 8.0). There results indicate that the *Brassica rapa* sample was more susceptible to heat damage than the *Brassica napus* sample. After 30, 45 and 60 minutes of heat treatment, the *Brassica rapa* sample had higher levels of NDIN than did the *Brassica napus* sample (Figure 7.2). There was a significant interaction between processing temperature and time on NDIN content in meals (Figures 7.3 and 7.4). Below 100°C, the rate of NDIN formation was slow, but at higher temperatures the rate increased in a quadratic manner.

7.4.2 Experiment 2 – Effect of Moisture, Time and Temperature During Processing

Since the moisture level was not controlled in Experiment 1, but may have an impact on the rate of protein damage, Experiment 2 was conducted to determine the

effects of moisture and temperature on protein quality. The NDIN content of canola meal increased linearly with moisture content during heating (average values across all temperatures, Figure 7.5). There was a significant interaction between moisture content and temperature on the NDIN content of meal. In the absence of added moisture (7% level), there was very little change in NDIN content with increased processing temperature (Figure 7.6). However, above 7%, the higher the moisture content, the greater the amount of NDIN formed with increasing temperature.

The final products of the Maillard reactions, melanoidins, absorb light at 420 ηm and as a result, absorbance or, inversely, reflectance at 420 ηm is often used as a measure of browning (Martins et al., 2001). Therefore, the extent of advanced Maillard reactions occurring during processing was estimated by measuring reductions in reflectance at 420 ηm. Reflectance at 420ηm was affected in a quadratic manner by moisture content during heating (Figure 7.7). The lightest coloured meal was produced at 7% moisture and reflectance decreased as moisture was increased to 14%. The meals produced at 14 and 18% moisture were similar in colour. There was an interaction between moisture and temperature on the colour of the meal. Reflectance decreased linearly with temperature at all moisture levels, but the 18% moisture treatment had the steepest slope (Figure 7.8).

There was a significant linear or quadratic effect of moisture level on the content of all amino acids measured, except valine. Amino acid content was reduced as moisture content increased (Table 7.1). Lys content was most adversely affected by moisture content, with a 13.3% decrease as the moisture content was increased from 7 to 18%. There was also a significant effect of temperature on the content of Ile, Leu and Lys. The content of Ile, Leu and Val increased whereas Lys content was linearly reduced, with increasing temperature (Table 7.2).

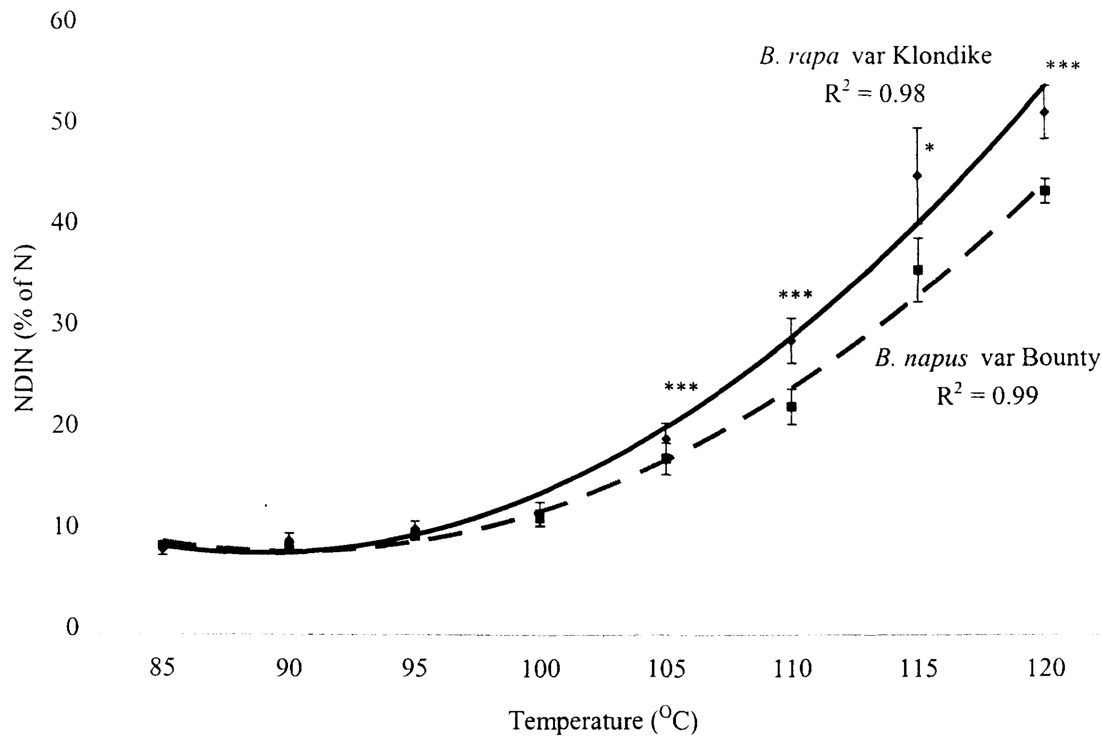

Figure 7.1 The effect of processing temperature on the neutral detergent insoluble nitrogen content (NDIN, as a proportion of N) of a *Brassica rapa* and a *Brassica napus* sample (data point is average of 15, 30, 45 and 60 minutes heating). Superscripts above data points within temperature indicate significant differences between canola samples (*, *** denote $P<0.05$ and $P<0.001$, respectively).

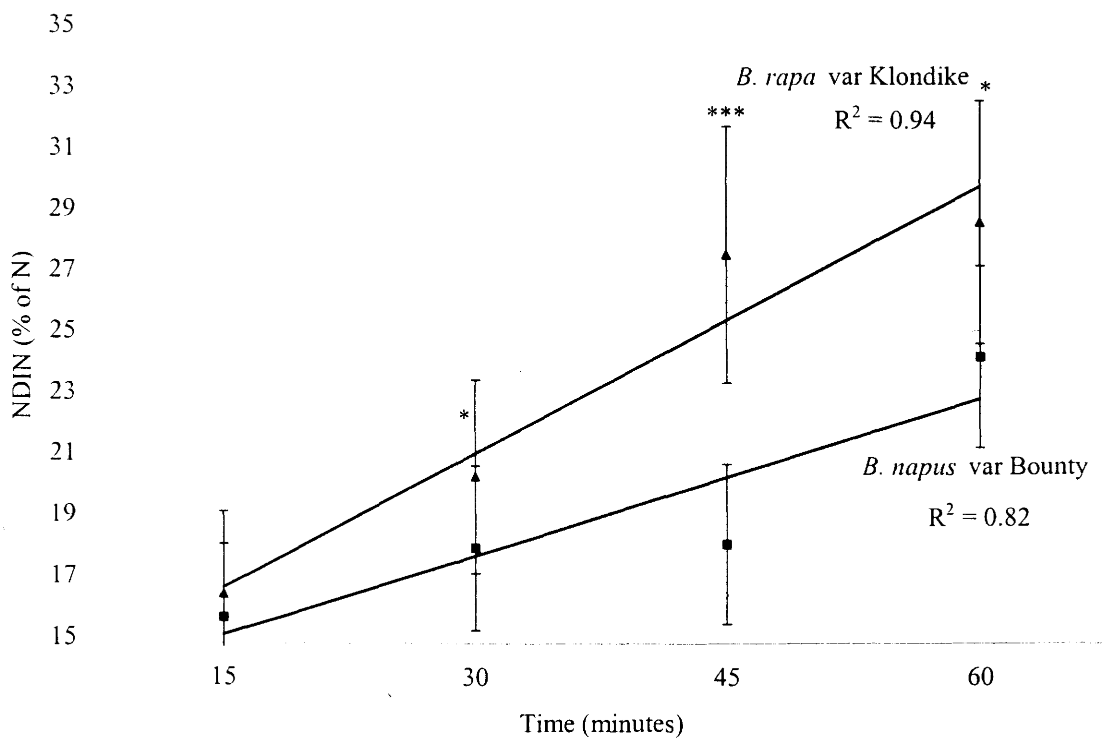

Figure 7.2 Neutral detergent insoluble nitrogen content (NDIN, as a proportion of N) of canola meals (± standard error) heated for 15, 30, 45 or 60 minutes (average of all temperatures). Statistically significant effect of species within time is noted by * (*, *** denotes $P<0.05$ and 0.001, respectively).

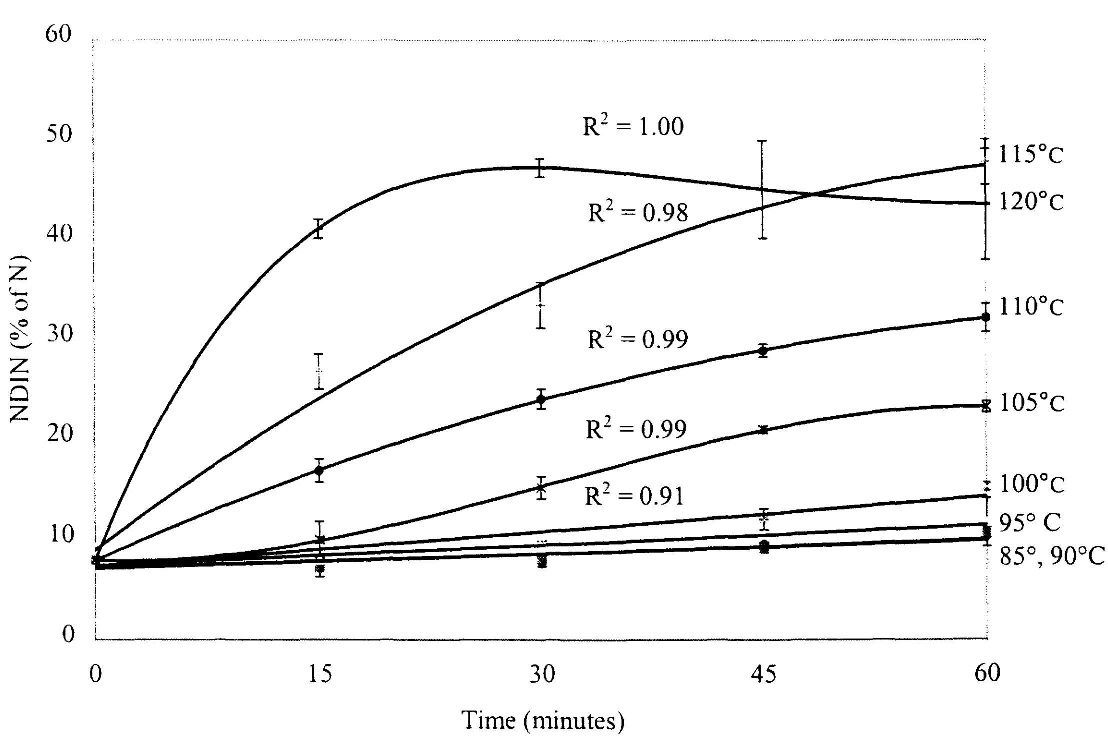

Figure 7.3 Neutral detergent insoluble nitrogen content (NDIN, as a proportion of N) of *Brassica napus* meal (± standard error) heated in an isothermal autoclave for 0 - 60 minutes at 85 - 120°C. Coefficients of determination for 85, 90 and 95°C are 0.71, 0.94 and 0.74, respectively.

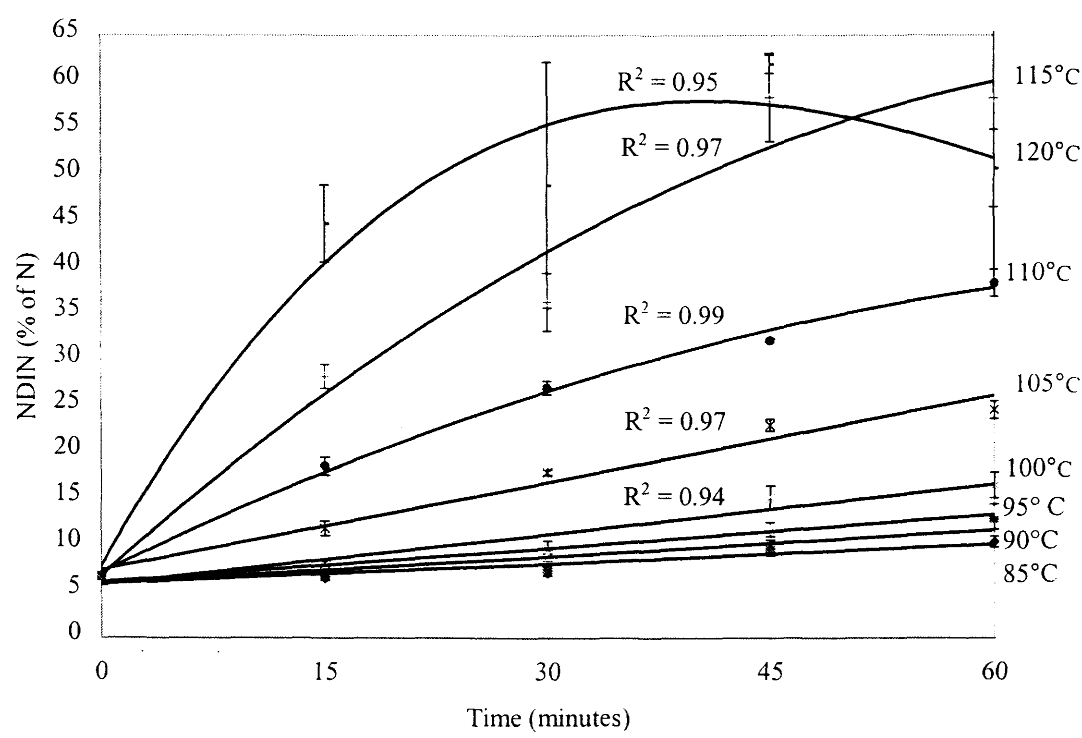

Figure 7.4 Neutral detergent insoluble nitrogen content (NDIN, as a proportion of N) in *Brassica rapa* canola meal (± standard error) after isothermal autoclaving for 0 - 60 minutes at 85 - 120°C. Coefficients of determination for 85, 90 and 95°C are 0.84, 0.96 and 0.82, respectively.

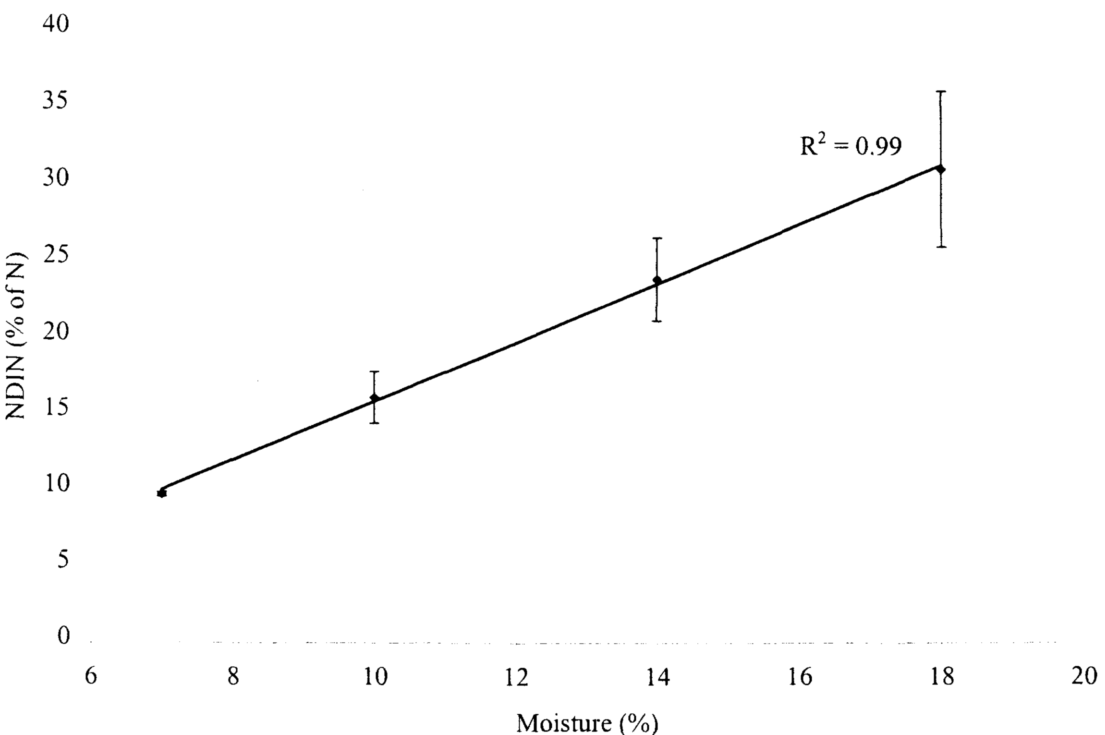

Figure 7.5 The effects of moisture content during heat treatment (average value for all temperatures shown) on the neutral detergent insoluble nitrogen content (NDIN, as a proportion of nitrogen) of canola meal (± standard error).

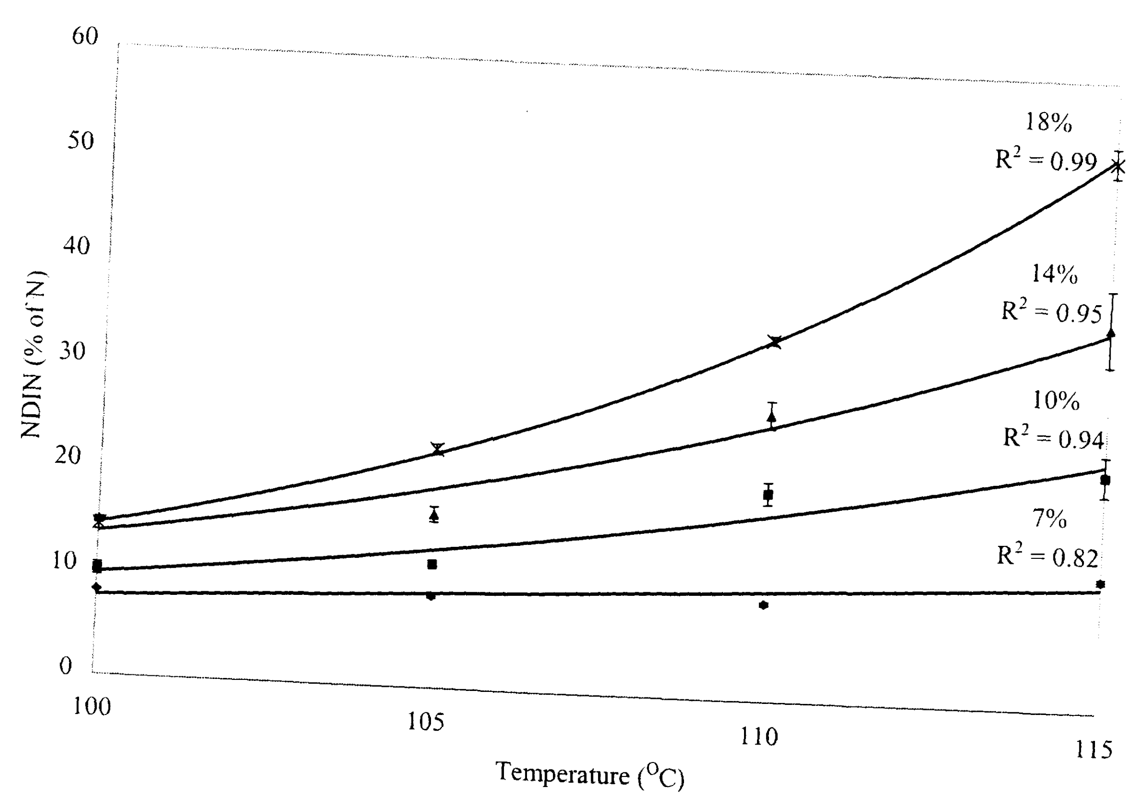

Figure 7.6 The effect of moisture and temperature on the neutral detergent insoluble nitrogen content (NDIN, as a proportion of N) of canola meal (± standard error) after 10 minutes of heating.

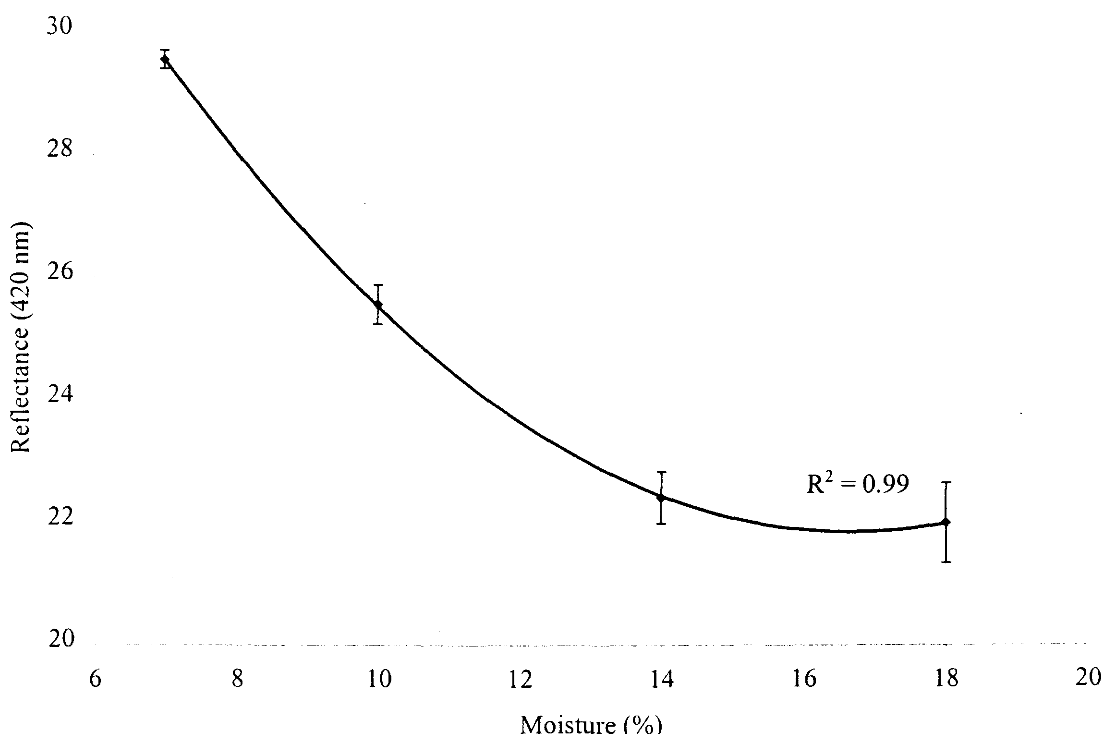

Figure 7.7 The effect of moisture content during heating (average value for all temperatures shown) for 10 minutes on the reflectance at 420 ηm (± standard error) of canola meal.

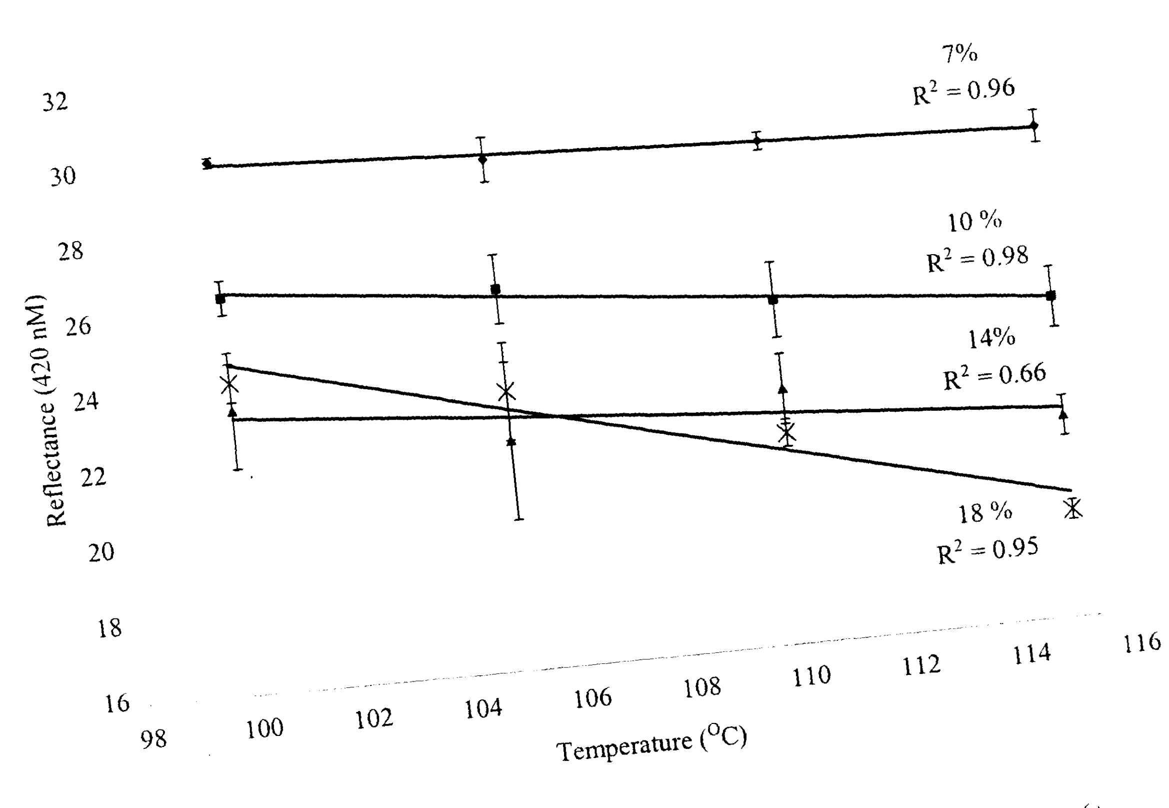

Figure 7.8 The effect of moisture and temperature on the reflectance at 420ηm (± standard error) of canola meal after heating for 10 minutes.

Table 7.1 The effect of moisture content of canola meal heated 10 minutes on the amino acid content (% dry matter basis) of canola meal and the probability of the effects of moisture, temperature and their interaction

	Moisture Level (%)					Main Effect		
	7	10	14	18	SEM	Moisture	Temperature	Interaction
	(%)					Probability		
Arg	2.71[a]	2.56[b]	2.51[b]	2.48[b]	0.02	***, L[1]	NS	NS
Cys	0.94[a]	0.89[b]	0.86[c]	0.88[b]	0.01	***, Q[2]	NS	NS
His	1.13[a]	1.10[b]	1.07[c]	1.04[d]	0.01	***, L	NS	*
Ile	1.66[a]	1.67[a]	1.62[b]	1.67[a]	0.01	*	*, L	**
Leu	2.98[a]	2.90[b]	2.86[b]	2.89[b]	0.01	***, Q	*, L	*
Lys	2.40[a]	2.25[b]	2.15[c]	2.08[c]	0.03	***, L	***	*
Met	0.79[a]	0.76[b]	0.76[b]	0.75[b]	0.00	***, Q	NS	NS
Phe	1.69[a]	1.66[b]	1.63[c]	1.64[bc]	0.01	***, Q	NS	*
Thr	1.77[a]	1.70[b]	1.73[b]	1.72[b]	0.01	***, Q	NS	*
Val	2.05	2.06	2.02	2.07	0.01	NS	*, L	***

[a,b,c] Means within a row without a common superscript differ significantly ($P<0.05$).

*,**,*** Significant at $P<0.05$, $P<0.01$, and $P<0.001$ respectively; NS, non-significant ($P>0.05$).

[1]L, linear

[2]Q, Quadratic

Table 7.2 The effect of temperature on the amino acid content (% dry matter) of canola meal after heat treating for 10 minutes

Temperature (°C)	100	105	110	115
		(%)		
Ile	1.63^{b}	1.66^{ab}	1.65^{ab}	1.69^{a}
Leu	2.88^{b}	2.90^{ab}	2.93^{a}	2.92^{a}
Lys	2.26^{a}	2.29^{a}	2.22^{a}	2.10^{b}
Val	2.02^{b}	2.04^{b}	2.04^{b}	2.09^{a}

^{a,b} Means within a row without a common superscript differ significantly (P<0.05).

There was a significant interaction between the effects of moisture and temperature on the content of His, Ile, Leu, Lys, Phe, Thr and Val. At 7% moisture, the content of Lys was not affected by temperature; however, at 10 and 14% moisture, Lys content decreased linearly with increasing temperature (Figure 7.9). At 18% moisture, Lys content increased with temperature over the range of 100 to 110°C, then declined rapidly up to 115°C. The effects on His, Ile, Leu and Phe, Thr and Val were relatively small and inconsistent (Tables 7.3 and 7.4).

7.5 DISCUSSION

The objectives of this study were to examine the effects of desolventization/toasting conditions on the protein quality and colour of canola meal. Neutral detergent insoluble nitrogen was used in this study as an indicator of the quality of protein in canola meal, as it is negatively correlated with amino acid availability in broiler chickens (section 6.4). The increase in NDIN content with processing temperature indicates that the protein in canola meal is susceptible to damage at temperatures above 100°C, and that higher temperatures should be avoided during desolventization. The *Brassica rapa* sample had marginally lower initial levels of NDIN (6.6 versus 8.0%) than the *Brassica napus* sample, and this may be due to the thinner hull of the yellow seed and the resultant lower levels of insoluble fibre (Slominski et al., 1999). However, the *Brassica rapa* sample was more susceptible to damage by heat, as indicated by the higher NDIN levels in the meals treated at 105°C or higher. The reason that the *Brassica rapa* sample was more susceptible to heat damage than the *Brassica napus* sample is not known, but one could speculate that it may be related to differences in hull structure, such as thickness or degree of lignification. Since only one sample of each species was tested, it is not known if the differential response is due to species or sample. The formation of NDIN was slow in both samples when the temperature was below 100°C, which might be explained by the fact that Maillard reactions are not as rapid below 100°C (Hurrell, 1984).

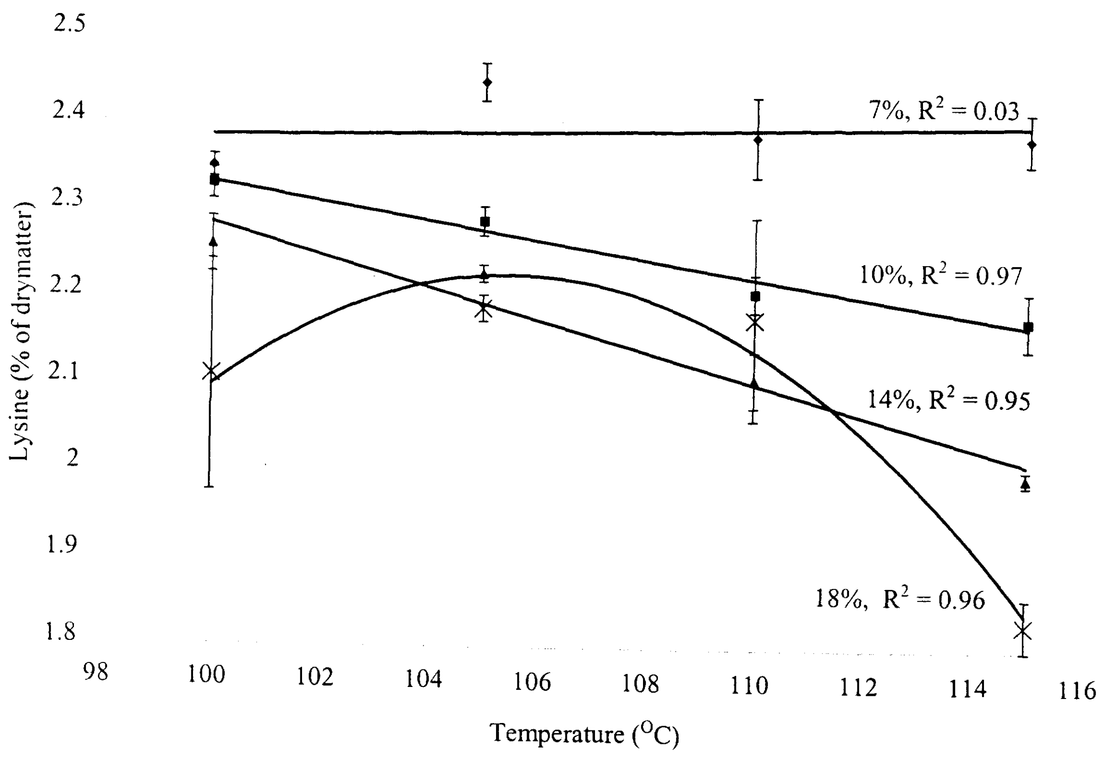

Figure 7.9 The effect of moisture and temperature during heat processing of canola meal on Lys content (% of dry matter ± standard error).

Table 7.3 The effects of moisture and temperature during processing on the content of histidine, isoleucine and leucine in canola meal heated for 10 minutes

	Temperature	Moisture (%) 7	10	14	18
	°C	(%)			
His	100	1.11	1.13	1.09	1.03
	105	1.13	1.11	1.08	1.04
	110	1.14	1.09	1.04	1.06
	115	1.15	1.08	1.06	1.04
Ile	100	1.60	1.66	1.66	1.59
	105	1.68	1.66	1.63	1.65
	110	1.66	1.71	1.54	1.69
	115	1.71	1.66	1.63	1.74
Leu	100	2.95	2.89	2.86	2.81
	105	3.00	2.88	2.83	2.88
	110	3.00	2.93	2.86	2.91
	115	2.96	2.87	2.97	2.95

Table 7.4 The effects of moisture and temperature during processing on the content of phenylalanine, threonine and valine in canola meal heated for 10 minutes

		Moisture (%)			
	Temperature	7	10	14	18
	°C	(%)			
Phe	100	1.66	1.66	1.64	1.60
	105	1.71	1.66	1.61	1.63
	110	1.71	1.66	1.62	1.65
	115	1.70	1.65	1.65	1.67
Thr	100	1.77	1.72	1.69	1.71
	105	1.80	1.70	1.71	1.71
	110	1.80	1.68	1.75	1.72
	115	1.71	1.71	1.74	1.72
Val	100	1.97	2.04	2.10	1.99
	105	2.05	2.06	2.05	2.02
	110	2.05	2.10	1.91	2.09
	115	2.13	2.06	2.03	2.16

The most common design of desolventizer/toaster used in western Canada is the Schumacher design by Crown Iron Works[38]. The general design of the Schumacher is shown in Figure 7.10, and consists of a series of heated plates or decks in a vertical tube. Meal enters the top of the unit, falls on a preheat tray where some of the hexane is evaporated prior to the meal being swept from tray to tray. The last tray(s) inject steam directly into the meal (sparge steam). The steam is forced through the meal and through the plates above it through hollow stay bolts, producing a counter current flow of steam and meal. The vapours exit only from the top, so the sparge steam is required to force the hexane up through the trays and meal prior to exiting the unit. Commercially, meal resides in the desolventizer/toaster for up to one h, but the temperature is not constant during that period. When the meal enters the desolventizer/toaster, it contains approximately 30% hexane and is heated indirectly. The temperature of the meal rises, but this is counteracted by evaporative cooling as hexane boils at approximately 69°C. The meal is constantly being agitated, so is only in contact with the plate for short periods of time, which allows the meal to cool as the hexane evaporates. By the time the meal reaches the last tray(s), most of the hexane has evaporated and, therefore, evaporative cooling is less of a factor. In addition, steam is injected directly into the meal in the last two trays, thereby applying a constant source of heat to all the meal as well as increasing its moisture content. The meal is heated to approximately 105 to 110°C, and resides for approximately 10-15 min on the final tray. Autoclaving at 105 to 110°C for approximately 10 min produced a meal from *Brassica napus* that was similar to commercially produced meals (Section 6.4), suggesting the conditions in the isothermal autoclave approximated the conditions in the final stages of the desolventizer/toaster.

[38] Minneapolis, MN, USA, 55440-1364

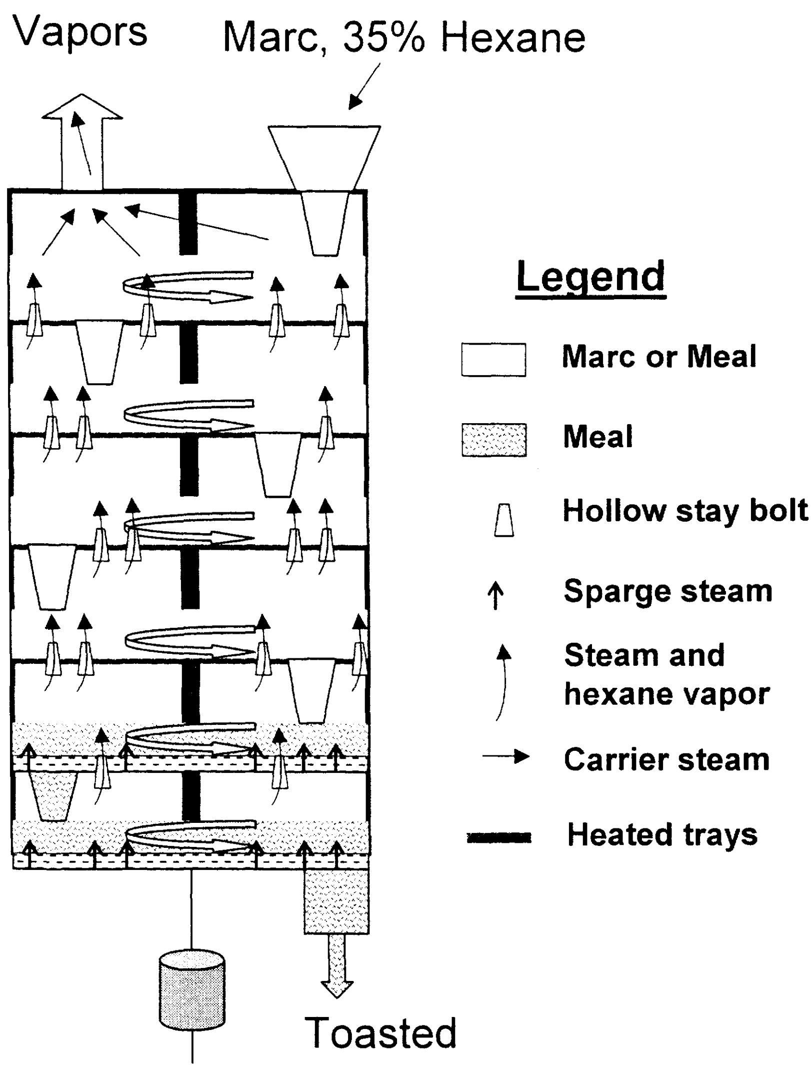

Figure 7.10 General design of Schumacher desolventizer toasters used in western Canada. Not drawn to scale.

Although the isothermal autoclave method approximates the conditions in the desolventizer/toaster, there is no control on the level of moisture incorporated, which may have a direct and significant impact on the degree of protein damage as moisture is required for Maillard reactions (Mauron et al., 1981). In this experiment, it was not possible to control the level of moisture in the meal during heating, but it would be expected to increase with temperature as more steam is injected into the autoclave. The moisture content of the meal exiting the autoclave could not be accurately determined as some of the moisture flashed off as soon as the steam pressure was released and the samples were exposed to the atmosphere. Therefore, it was not possible to isolate the effects of moisture and temperature on meal quality in this experiment. Commercially, it is necessary to heat the meal to desolventize it, but there are two ways of providing this heat. It can be transferred indirectly through steam-heated trays; this does not increase the moisture content of the meal. Alternatively, heat can be applied directly in the form of steam that is injected directly into the meal (sparge steam), but this increases the moisture content of the meal. The moisture content of the meal exiting the desolventizer/toaster is typically between 15 and 18% (Unger, 1990). However, it is not known if this is the ideal combination of direct and indirect heat to optimize protein quality. Therefore, Experiment 2 was conducted to assess the effects of moisture and temperature on protein quality after processing. The moisture content of the air-desolventized meal used in this study was 7%, indicating that if no sparge steam was used during desolventization, one would expect the meal to be at 7% moisture or less when exiting the desolventizer/toaster.

Four moisture conditions were used in this study; no added water (7%), and 10, 14 and 18%. Since meal is exposed to high moisture and temperature conditions in the desolventizer/toaster for approximately 10 min this was the processing time used in Experiment 2. Meal exiting the DT can range in temperature from 100 to 115°C, so four temperatures in this range were used in this experiment.

The linear increase in NDIN content with moisture content suggests that addition of moisture during desolventization/toasting is detrimental to amino acid availability in canola meal. In the absence of added moisture (7% treatment), the NDIN content of the

meal was not affected by heating, indicating that direct steam injection, (i.e., moisture incorporation) should be avoided. This is consistent with the conditions that promote Maillard reactions. In a mixture of casein and glucose, no browning was observed at low moisture content, but maximal loss of Lys was observed between 15 and 18% moisture (Lea and Hannan, 1949b).

Higher temperatures, in the presence of added moisture, also promote additional protein damage, which is likely the result of the increased rate of Maillard reactions (Martins et al., 2001). Therefore, if direct heat must be used during desolventization of the meal, it would be advisable to use the least amount of heat possible. If no additional moisture is added to the meal during desolventization, temperatures of up to 115°C for 10 min can be tolerated with little effect on meal quality as indicated by NDIN content.

Meal colour reflects the amount of browning that has occurred during processing (Mauron, 1981). Meals heated with added moisture were a noticeably darker brown colour (lower reflectance at 420ηm). Similar to the situation with NDIN values, heating the product at 7% moisture had little effect on the colour of the meal, indicating that Maillard reactions were likely repressed. Heating in the presence of added moisture resulted in a darker colour, which would be consistent with the formation of brown pigments as a result of Maillard reactions. This also suggests that the losses in amino acid content and availability are at least partially attributable to Maillard reactions.

Below 110°C, meal heated at 18% was a lighter colour than meal heated at 14% moisture, indicating that the additional moisture might be preventing Maillard reactions at lower temperatures. At higher temperatures, the highest moisture contents resulted in the darkest meals, indicating that the reaction was no longer repressed. Maillard reactions require water, but too much moisture impedes the dehydration steps involved in the advanced reactions that lead to the formation of melanoidins (Lea and Hannan, 1949b). The early and intermediate stage Maillard products do not absorb visible light, but are indigestible (Mauron, 1981) and, therefore, colour is not an accurate method of assessing amino acid availability. For example, increasing the moisture content to 18% during processing at 105°C will lead to a lighter-coloured meal giving the appearance of

a better quality meal, but the NDIN content will be higher and the digestibility will likely be lower than that of a darker coloured meal produced at 14% moisture.

As with NDIN and colour, temperature did not affect amino acid content in the absence of added moisture, further suggesting that the addition of moisture through direct sparge steam injection should be avoided, if possible. Lys content was most adversely affected by heat treatment at elevated moisture contents, which is consistent with the hypothesis that Maillard reactions are responsible for the loss in protein quality as the ε-amino group in Lys is most susceptible to reactions with aldose sugars (Martins et al., 2001). However, other amino acids are also susceptible when they are the terminal α-amino acid or when they react with intermediary Maillard products such as hydroxymethyfurfural or furfural to form aldimines or ketimes. It is unlikely that the amino acids undergo Strecker degradation as this Maillard reaction usually occurs at alkaline pH and the canola meal used in this study had a pH of 5.9. Similarly, it is unlikely that amino acids reacted with fission products such as acetol or diacetyl to form aldimines or ketimes, as this is also promoted by alkaline pH (Martins et al., 2001).

At the highest moisture content, amino acid content was reduced by heating, but the effect of increasing temperature was not as consistent as at lower moisture contents. This might suggest that moisture level, rather than temperature, is the rate limiting factor at that point.

7.6 CONCLUSIONS AND IMPLICATIONS

In conclusion, heating canola meal in a moist environment reduced the quality of its protein. The level of damage increased with both temperature and time. Heating under low moisture conditions had little effect on protein quality, even at elevated temperatures. Desolventization of canola without added moisture should, therefore, result in a non-toasted, higher quality meal. Therefore, if possible, the use of direct sparge steam during desolventization of canola meal should be avoided. If it must be used to effectively desolventize the meal, the amount of direct steam injection and the temperature and duration should be minimized to produce the highest quality canola meal possible.

8 THE EFFECT OF PLANT GENOTYPE ON THE NDIN CONTENT OF CANOLA MEAL BEFORE AND AFTER HEAT TREATMENT

8.1 ABSTRACT

During prepress-solvent extraction of canola, the meal is exposed to heat and moisture, resulting in a toasted product. This toasting reduces the digestibility of protein by poultry and increases the neutral detergent insoluble nitrogen content (NDIN) of the meal. Commercial canola meal is derived from a number of varieties from within two species (*B. napus* and *rapa*), but it is not known if there are species or varietal differences in susceptibility to heat damage during toasting. The NDIN content of canola meal is negatively correlated with amino acid digestibility and can, therefore, be used to determine the amount of protein damage incurred during processing. An experiment was conducted to determine genotype or varietal differences in susceptibility to heat damage. Canola meal produced from twenty varieties of *B. napus* and ten varieties of *B. rapa* were analysed for NDIN content before and after toasting at 95°C and 110°C for 10 min in an autoclave. On average, the *B. rapa* samples had lower NDIN contents than the *B. napus* samples before (8.6 versus 11.7%, respectively) and after toasting at 110°C (19.2 versus 22.0%, respectively), suggesting that *B. rapa* meal may be more digestible. The change in NDIN content after light toasting (95°C for 10 min) was not influenced by variety within either species. However, after heavy toasting (110°C for 10 min), there were varietal effects within species on the NDIN content, suggesting that genetic variation in susceptibility to heat damage during toasting exists.

8.2 INTRODUCTION

Canola meal is defined as the oil-free residue of seed from low glucosinolate, low erucic acid varieties of the *Brassica* genus. The chemical composition of various commercial canola meal samples has been determined and related to nutritional value (Bell and Keith, 1991). However, the effect of species and variety on the nutritional

value of canola meal has only been studied to a limited degree. It has been shown that yellow-seeded varieties contain less crude fibre and lignin, but that total dietary fibre content was similar to that of brown-seeded varieties (Slominski et al., 1999). The desolventization/toasting process can reduce the content and availability of amino acids in canola meal (Section 4.5), but it is not known if species or varietal differences contribute to the relative susceptibility to heat damage. The protein remaining in the neutral detergent fibre residue (NDIN) of canola meal provides a measure of how much of the protein is indigestible especially as a result of heat damage (Section 6.4). Two Brassica species, *B. napus* and *B. rapa* are commonly used to produce canola meal and oil. In a previous study comparing a *Brassica napus* and a *Brassica rapa* variety of canola meal, the *Brassica rapa* sample contained less NDIN before desolventization/toasting, but appeared to be more susceptible to damage as it had a higher NDIN level after heat processing (section 7.4.1). However, only a single variety within each species was examined, and it is not known if variation between or within species exists. Therefore, the objectives of this study were to determine the effect of species and variety on the NDIN content of canola meal and the relative change in NDIN content during conditions that approximate those of commercial desolventization/toasting.

8.3 MATERIALS AND METHODS

Samples of canola seed from the 1997 Regional Spring Grain Variety Trial conducted by the Crop Development Centre at the University of Saskatchewan were collected. Three replicates of each of ten varieties of *Brassica rapa* and 20 varieties of *Brassica napus* were grown in Canora, Saskatchewan and samples of the seed collected for this study. The *Brassica napus* varieties tested were AC Excel, 46A65, 44A89, AC-H102, Beacon, Clavet, Dakini, PERF9124, Jewel, LG3220, LG3430, OAC Dynamite, Optimum 500, Sentry, Sprint, Battleford, Synbrid 220, 45A71 (HT), Innovator (HT) and Quest. The *Brassica rapa* varieties tested were AC Parkland, 41P04, 41P55, AC Boreal, AC Sunbeam, Hysyn 110, Hysyn 111, Hysyn 120 CS, Norwester, Foothills. The seed was ground to pass through a 1 mm screen and placed in a folded 18.5 cm Fisherbrand®

P8 Course filter paper[39] the edges were folded and stapled forming an envelope approximately 1 cm thick, 8 cm high and 17 cm long. The samples were then placed in a soxhlet extractor and extracted with n-hexane for 8 hours. Over the 8 hour period of extraction, the meal was flushed with distilled hexane approximately 16 times, as the unit cycled approximately every 30 minutes. After solvent extraction, the filter paper pouches containing the meal were placed in a fume hood and allowed to evaporate prior to drying at 50°C for 4 hours.

Each of the dried canola meal samples (30 varieties x 3 replications = 90) were then weighed (0.5 g) into three Filter bags (F57[40]) and heat sealed. One filter bag of sample per replication was analyzed directly to determine the NDIN content of the raw meal. The other two filter bags per replication were then heat treated in an isothermal autoclave[41] at 95°C or 110°C for 10 minutes to expose the meal to conditions similar to that of light or heavy toasting during commercial processing. The NDIN content of all three types of samples (raw unheated, toasted at 95°C and toasted at 110°C) were analysed for NDIN content as follows. The filter bags (20 per batch), neutral detergent solution (2 L premixed chemical solution- FND20[33]) and 1.36 million modified Wohlgemuth Units of heat stable amylase (FAA[33]) were added to the analyzer and allowed to agitate for 60 minutes at 100°C. The solution was then drained from the analyzer and washed with an additional 2 L of hot (90-100°C) distilled water with agitation for 5 minutes; this wash procedure was repeated three times. The water was gently squeezed out of the filter bags by hand prior to soaking in acetone for 3 minutes. The acetone was allowed to evaporate prior to drying the bags in an oven at 105°C for 4 hours. The protein content of the meal and the residue in the filter bags was then analysed according to the methods of the Association of Official Analytical Chemists (1990) using the Kjeldahl method. NDIN was then expressed as the proportion of the protein in the meal remaining in the neutral detergent fibre residue. AAFCO[42] check

[39] Fisher Scientific, Pittsburgh, PA, 15275
[40] Ankom Technology Corporation, 140 Turk Hill Park, Fairport, NY, 14450
[41] Amsco Eagle Series 2322 Isothermal Autoclave, 51 International Blvd., Etobicoke, ON, M9W 6H3
[42] Association of American Feed Control Officials, Inc., College Station, TX, USA, 77841-3160

samples were also analysed for crude protein, NDIN and moisture and the values obtained were comparable to those reported by other laboratories participating in the program.

8.4 STATISTICAL ANALYSIS

The data were analyzed by analysis of variance with variety nested in species using the GLM procedure of the SAS program (SAS Institute, 1989) using the model $Y_{ijk} = \mu + \alpha_i + \beta_{j(i)} + \varepsilon_{ijk}$ where Y_{ijk} is the dependent variable, μ is the overall mean, α_i is the main effect of species, $\beta_{j(i)}$ is the main effect of variety nested within species and ε_{ijk} is the normally distributed residual. Differences were considered significant when $P < 0.05$. Means separation of variety within species was conducted using the method of Duncan (1955) using separate analysis of variance analyses for each species. Differences were considered significant when $P<0.05$ unless indicated otherwise.

8.5 RESULTS

Meal from *Brassica rapa* had lower levels of NDIN than meal from *Brassica napus* before and after heat treatment (Table 8.1). There was a significant effect of variety on the NDIN content of *Brassica napus* meal prior to heat treatment, ranging from a low of 9.37 % of N for LG 3220 and a high of 14.6% of N for OAC Dynamite (Table 8.2). There was no significant effect of variety within *Brassica rapa* on the NDIN content prior to heat treatment, but it ranged from 7.7 to 10.40% of N (Table 8.3). There was no effect of variety on the change in NDIN content of the meal after heating the meal to 95°C for 10 minutes for either the *B. napus* or *B.rapa* species. The increase in NDIN content after heating ranged from 2.80 to 6.85% of N for *Brassica napus* varieties and from 1.80 to 8.84% of N for *Brassica rapa* varieties. There was a significant effect of variety on the change in NDIN content after heating from 95 to 110°C within *B. napus*. The change in NDIN content ranged from 3.05% of N for OAC Dynamite to 9.50% of N for PERF 9124. There was a significant effect ($P<0.08$) of variety on the increase in NDIN content with increasing temperature from 95 to 110°C within *Brassica rapa*. The NDIN content increased the least within Hysyn110 (4.73%

of N) and the most within Foothills (8.65% NDIN). The NDIN content (as % of nitrogen) of the final toasted (110°C) meals ranged from 19.57 to 28.00% for *B. napus* and from 16.76 to 23.31% for *B. rapa* (Table 8.3).

8.6 DISCUSSION

NDIN (Neutral Detergent Insoluble Nitrogen expressed as a percentage of total nitrogen) has been shown previously to be a good indicator of the level of indigestible protein in canola meal (Section 6.4). Therefore, the NDIN content of canola meals was used as an *in vitro* indicator of amino acid digestibility in this experiment. The reduced level of NDIN content of the *B. rapa* varieties prior to heating relative to the *B. napus* varieties is likely a function of the colour and size of the seeds. *Brassica rapa* varieties are often yellow-seeded as a result of having thinner hulls with lower lignin content (Slominski et al., 1999) than *B. napus* varieties. In addition to the thinner hull fraction, the seeds are generally larger and, therefore, have lower proportional contents of hull (Jensen et al., 1995b) and dietary fibre. The hull accounts for between 21 and 30% of the meal, and has a protein content which ranges from 15 to 18% (Slominski et al., 1999). The protein in the hull fraction is likely the source of the NDIN content of the meal prior to heat treatment, and since *B. rapa* varieties have less hull, they also contain less NDIN. This is in agreement with an earlier study which compared the NDIN content of a variety of *B. napus* and a variety of *B. rapa* (Section 7.4.1).

The lower level of NDIN content in the *Brassica rapa* samples prior to heating suggests that meal from this species may have a higher level of digestible amino acids. Slominski et al. (1999) compared meals from *B. rapa* and *B. napus*, but no significant differences in amino acid digestibility were observed. The meals in that study were heat treated to mimic commercial desolventization, so it is not known if they had different levels of digestible amino acids prior to heat treatment. The NDIN content of the meals was not reported, so it is not known if they varied in this experiment. Similarly, Newkirk et al. (1997) compared the nutritional value of heat-treated canola meal from *B. napus* and *B. rapa*, and there was no significant difference in ileal protein digestibility.

Table 8.1 Effects of heat processing and *Brassica* species on the neutral detergent insoluble nitrogen (NDIN) content of solvent extracted meal

	Processing Conditions		
Species	Prior to heating	After 95°C for 10 min	After 110°C for 10 min
Rapa (n=10)	8.6^{b}	13.2^{b}	19.2^{b}
Napus (n=20)	11.7^{a}	16.4^{a}	22.0^{a}
SEM	0.3	0.3	0.4

^{a,b} Means within a column without a common superscript differ significantly ($P<0.05$).

Table 8.2 The effect of heat processing on the neutral detergent insoluble nitrogen (NDIN) content of *Brassica napus* variety

Variety	Raw sample	Change in NDIN level		110°C for 10 min
		95°C – raw value	110°C- 95°C	
	NDIN (% of N)			
AC Excel	11.27[cde]	5.40	4.75[bcd]	21.42[b]
AC H102	11.60[bcde]	4.15	4.50[cd]	20.25[b]
Battleford	10.00[de]	5.50	5.60[bcd]	21.10[b]
Beacon	12.45[abcd]	3.00	5.40[bcd]	20.85[b]
Clavet	10.43[cde]	5.53	5.93[bc]	21.89[b]
Dakini	14.50[ab]	3.50	4.63[bcd]	22.63[b]
OAC Dynamite	14.60[a]	3.20	3.05[d]	20.85[b]
Innovator	13.33[abc]	3.60	7.30[ab]	24.23[ab]
Jewel	10.63[cde]	4.17	5.93[bc]	20.73[b]
LG 3220	9.37[e]	5.07	5.13[bcd]	19.57[b]
LG 3430	11.70[abcde]	5.00	5.03[bcd]	21.73[b]
Optimum 500	11.37[cde]	5.87	6.89[bc]	24.13[ab]
PERF 9124	12.15[abcde]	6.35	9.50[a]	28.00[a]
Quest	12.85[abcd]	5.40	6.30[bc]	24.55[ab]
Sentry	11.63[bcde]	3.97	5.33[bcd]	20.93[b]
Sprint	12.17[abcde]	3.37	4.65[bcd]	20.19[b]
Synbrid 220	10.75[cde]	6.85	5.13[bcd]	22.73[b]
45A71	11.85[abcde]	3.80	5.15[bcd]	20.80[b]
46A65	11.95[abcde]	2.80	5.60[bcd]	20.35[b]
44A89	11.77[abcde]	4.67	5.17[bcd]	21.61[b]
Mean	11.70	4.61	5.58	21.97
SEM	0.22	0.24	0.22	0.37

[a,b,c,d,,e] Means within a column without a common superscript differ significantly

Table 8.3 The effect of heat processing on NDIN content of a *Brassica rapa* variety

Variety	Raw sample	Change in NDIN level		110°C
		95°C – raw	110°C- 95°C	
	NDIN (% of N)			
AC Parkland	7.57	8.84	6.90^{ab}	23.31^{ab}
41P04	7.93	3.93	4.90^{b}	16.76^{b}
41P55	8.40	4.15	8.00^{ab}	20.65^{ab}
AC Boreal	9.30	7.25	5.75^{ab}	22.30^{ab}
Foothills	9.55	5.25	8.65^{a}	23.45^{a}
Hysyn110	8.90	3.05	4.73^{b}	16.68^{ab}
Hysyn111	10.40	1.80	8.50^{a}	20.70^{ab}
Hysysn120CS	7.70	5.30	5.30^{ab}	18.30^{ab}
Norwester	8.27	4.13	5.87^{ab}	18.27^{ab}
AC Sunbeam	9.05	3.45	4.80^{b}	17.30^{ab}
Mean	8.60	4.79	6.00^{ab}	19.39
SEM	0.27	0.57	0.37	0.67

^{a,b} Means within a column without a common superscript differ significantly ($P<0.08$).

Previously, it was shown that there was an apparent difference between species in susceptibility to heat damage, with a *B. rapa* sample being most adversely affected (Section 7.4.1). However, in the current experiment, both species were equally susceptible to heat damage as measured by the level of NDIN.

The change in NDIN content with light toasting was not influenced by variety within either species, suggesting there exists little genetic variation in susceptibility to protein damage at this temperature. However, the varietal effects on the susceptibility to heat damage at higher temperatures within both species indicate that there is genetic variation in this trait.

Within each species, the level of NDIN in the meal after toasting at 110°C was similar for most varieties. However, a few varieties such as PERF 9124 within *B. napus* and AC Parkland within *B. rapa* had relatively high levels of NDIN after toasting (28.00 and 23.31%, respectively), suggesting that they would have reduced amino acid digestibilities after processing. Although it may not be practical to use NDIN after heat processing as a selection criterion during genetic selection, it may be desirable to screen potential new varieties for NDIN content after toasting to ensure they do not have markedly lower amino acid digestibilities after processing.

Based on the NDIN content of raw seed, it appears that some varieties have lower amino acid digestibilities. However, research is required to confirm a relationship between NDIN content in raw seed and amino acid digestibility so that it might be used as a genetic selection tool.

8.7 CONCLUSIONS AND IMPLICATIONS

B. rapa varieties contained less NDIN than *B. napus* and may contain higher levels of digestible amino acids prior to toasting. Therefore, it may be beneficial to produce more *Brassica rapa* varieties, or *Brassica napus* with lower levels of NDIN. However, it is not known if the NDIN content in raw seed is correlated with amino acid digestibility, so this requires further clarification. It may be desirable in the future to select varieties that are less susceptible to damage during moderate to heavy heat

treatment. If it is not practical to use NDIN content after heat processing as a general selection criterion, it may be desirable to analyse potential new varieties prior to registration to ensure amino acid digestibility is not compromised in those lines. If the toasting process is changed such that the meal is not toasted significantly, however, there would be no need to select for heat resistance.

9 GENERAL CONCLUSIONS AND DISCUSSION

Canola meal is commonly used as a source of protein in poultry diets, but the digestibility of the amino acids is lower than that in soybean meal making it necessary to formulate diets on an available rather than total amino acid basis. The lower relative Lys content, amino acid digestibility and meal consistency reduce the value of the meal and represent a significant loss to both the canola industry and the feed industries. The research in this thesis demonstrated that processing is the main reason for the differences in these characteristics between canola and soybean meals. Prior to the desolventization/toasting process, the digestibility of canola meal was comparable to that of soybean meal, and therefore, is a more valuable feed ingredient than the toasted meal for non-ruminant species. Therefore, one has to ask why meals are toasted in the current manner.

The rationale for current desolventization/toasting procedures for canola meal is not completely clear. The industry consists of a small number of large crushing companies (ADM, Cargill, CanAmera, Canbra and Central Soya) and, as a result, very little information is available on the nature of processing conditions. In addition, published research on canola meal processing is virtually non-existent. It would appear that industry practice is based on past experience and anecdotal evidence. In addition, the process and equipment were originally designed for soybean crushing and then applied to other oilseeds such as canola and cottonseed. There is a significant body of published work on the effects of processing on the nutritional value of soybean, and there are also laboratory tests available to monitor processing conditions. Heat processing in soybean meal is necessary to reduce or eliminate heat labile anti-nutritional factors, trypsin inhibitors in particular. Since canola meal no longer contains significant levels of heat labile anti-nutritional factors, there may be no need for heat processing of canola meal during desolventization/toasting. At the very least, the requirements for heat processing canola meal are not well defined.

Early studies with rapeseed indicated that feeding under-heated meal reduced the growth rate of chickens due to the level of residual glucosinolates. However, the conditions required to minimize glucosinolates but still maximize meal quality were never clearly established. There is also no evidence that toasting conditions were changed in the 1970's, when production shifted from rapeseed to new low-glucosinolate varieties of canola, to reflect the new type of meal. Since most of the data was not published, some modifications may have been made but not reported. It would appear that the level of toasting was not lowered to reflect the decreased need to reduce glucosinolate levels.

Slominski (1997) reported *in vitro* evidence that suggested canola meal should be heated to approximately 107°C in order to maximize protein utilization, but this result was never confirmed *in vivo*. It is possible that this work validated the procedures used by canola crushing companies and, therefore, they have remained relatively unchanged to the present day. The finding that flaked canola, which has not been exposed to high temperature, had excellent amino acid digestibility (Section 3) suggested that, unlike meat products, canola does not have to be heat treated to maximize protein digestibility. These findings, and other research in this thesis, support the concept that the meal should be exposed to as little heat as possible.

Another issue that may be preventing the canola industry from reducing toasting level is the evidence that high temperature exposure produces a meal with higher levels of rumen-undegraded protein (McKinnon et al., 1991). With the exception of Canbra Foods, crushing plants are not specifically toasting meal to increase ruminal by-pass value, but there is a perception that high temperature processing is not necessarily an issue and may be beneficial. This issue requires further clarification.

Based on the findings presented in this thesis there does not appear to be any requirement for toasting of canola meal for broiler chickens. Therefore, it should be possible to minimize toasting and produce a more consistent meal with higher amino acid digestibilities. Two options for producing a higher quality meal are to monitor meal

temperature and moisture more closely during desolventization/toasting to minimize protein damage, or to eliminate the toasting process altogether.

At first glance, it would seem that monitoring the desolventization/toasting process more carefully would be most appealing, as it would not require significant changes to the equipment used in the process. However, controlling the process may prove more challenging than it first appears. One of the challenges is quickly assessing canola meal quality in a quality control program to optimize the process. Since Lys digestibility and content is the most susceptible to over processing, the assay of choice should accurately predict Lys digestibility.

The current method of assessing meal quality is to measure protein solubility in 0.5% KOH. Protein solubility is reduced in protein meals after denaturation by heating, but this does not necessarily impact nutritional value. The results of this study clearly show that protein solubility is a poor indicator of Lys digestibility. Therefore, this assay has little or no value as a quality control measure for either the processor or meal buyer.

Both NDIN and NIRS appear to predict Lys digestibility and could be included in a quality control program. Near infrared spectrascopy was the most accurate and rapid method but would require constant recalibration. It would be advisable for the canola industry to establish a calibration program so that NIRS could be used in quality control programs. An ongoing program should be implemented where meal samples are collected and tested *in vivo* for digestible amino acid content, so that the assay does not lose its predictive value for broiler chickens. Since some canola crushing plants are not currently equipped with NIRS systems, but do have the ability to conduct wet laboratory analysis, NDIN could readily be incorporated into quality control programs. Based on findings reported in this thesis, it would be advisable to produce meals with less than 12% of its nitrogen being found in the NDIN fraction. Additional research may be useful in further refining this recommendation using samples derived from a wider variety of canola genotypes and growing conditions.

By setting targets for digestible amino acid content and monitoring meal in a quality control program, it should be possible to produce a higher quality, higher valued

canola meal. However, the nature of the current processing system can still introduce variation in meal quality and produce over-toasted meal. Factors of this type include unplanned processing stoppage and the nature of the desolventizer/toaster. One of the practical issues facing the canola crushing industry is maintaining a constant level of toasting while production levels routinely fluctuate. The crushing plants in western Canada are relatively large (1,000-4,000 tonnes per day of seed crushing capacity), but their storage space for meal and oil is relatively small (2-3 days on average). Therefore, product sales dictate the level of production. It is normally impractical to shut down and start up production on a routine basis, so the crush level is changed instead. When sales for either oil or meal are low, plants reduce crush levels to match sales. For example, a plant could shift from running at 100% of capacity to 40% of capacity. For many of the stages of the process, this is not an issue because either the residence time is very short (for example – the flaker) or there is redundancy so some of the equipment can be shut down temporarily (for example – expellers). However, as shown in this report, time and temperature in the desolventizer/toaster can impact meal quality but neither residence time nor steam input per unit of meal is controlled on a real time basis. As described previously, the desolventizer/toaster consists of a series of heated plates or trays stacked in a vertical tube. Meal is mixed on each tray by a sweep arm and eventually falls from one tray to another through a chute until it exits the system. The residence time in the DT is not controlled directly but is determined by the level of meal on the plate. This tray level can only be changed manually when the system is shut down. Therefore, the lower the level of production, the longer the residence time, and consequently, the greater the potential for producing over-toasted meals. This could be rectified by installing variable speed rotary valves between trays, similar to the one that already exists at the exit from the DT. When the production level drops, the valve speed could be increased so that the residence time in the desolventizer is maintained. However, this would require capital expenditure and training of the staff involved.

Low plant production not only increases residence time but also increases the temperature of the meal. Increased temperature is the result of a combination of extended residence time and a reduced evaporative load on the DT. Normally, the level of steam input into the desolventizer toasted is manually controlled and, therefore, is not

directly controlled by the evaporative requirements of the unit. Tray and dome temperature (temperature of the hexane vapours leaving the DT) are monitored at most plants, but adjustments are seldom made to the level of steam input. The level of steam input into the DT could be controlled thermostatically by installing electrically-controlled steam pressure regulators that are linked to temperature sensors in the DT. Again, this would require capital expenditure, but savings in energy usage and the increased value of the meal could offset the additional costs.

Since there is no known benefit of toasting of canola meal, and it will likely be difficult to produce a toasted meal with consistent levels of digestible amino acids, the option of producing a non-toasted canola meal is appealing. Clearly, the main objective of desolventization/toasting is to recover as much of the hexane from the meal as possible. Therefore, regardless of toasting practice, hexane must be effectively removed from the meal. Not only does hexane loss increase cost, but it can be both a safety and an environmental issue as well. Although the canola crushing industry has decades of experience in the desolventization of canola meal, most of that experience has been under a specific range of conditions. The study described in Section 7 explored methods of applying heat to the meal without toasting it. In this way, the meal could be desolventized while producing a non-toasted meal. Heating in the absence of added moisture (~7% moisture content) resulted in an essentially non-toasted meal even at high temperatures and extended processing times. Therefore, if the meal were to be desolventized without added moisture, not only would the meal not be toasted, but the process would not be so sensitive to changes in production level.

It appears that most of the additional moisture incorporated during desolventization comes from steam that is directly injected into the meal and condenses as hexane is evaporated. Therefore, one option might be to reduce or eliminate the use of sparge steam during desolventization. As shown in Figure 7.10, the most popular design of desolventizer used in western Canada, the Schumacher, uses the direct steam as both a source of heat input and a method of driving the hexane counter current through the trays and out a single vent at the top. Therefore, if direct steam usage was to be suspended, two changes would have to be made. Since direct steam injection is a

source of heat, additional heat would have to be available in the form of indirect heat. In addition, since the steam would not be available to dilute the vapours around the meal and to drive them through the meal in higher trays, vents would have to be added to each deck. In this way the hexane vapours could be vented directly to the condenser. Small amounts of steam injection into the airspace above the meals may be desirable in the lower trays to ensure that the hexane level in the head space is low enough to promote maximal evaporation. An example of such a DT is depicted in Figure 9.1.

In general, the canola crushing industry is unwilling to attempt desolventization without added sparge steam, as it is felt that direct steam injection is required to "strip" the residual hexane from canola meal. In the study described in Section 5, the meal produced in a DT similar to the one depicted in Figure 9.1 had low residual hexane content, suggesting that this is not necessarily the case. However, further studies are required with commercial scale equipment to assess the requirement for direct steam injection. If direct steam is still required in the final tray, steps should be taken to ensure that a minimum amount of steam condenses into the meal. One method of achieving this would be to use superheated steam for direct steam injection. Superheated steam contains more energy per tonne of steam than conventional steam and, therefore, would impart less moisture to the meal when the hexane evaporates. Super heated steam involves high pressures and it would likely be expensive to incorporate the necessary modifications to the DT and boiler. Another option would be to inject the steam directly into the meal in the final tray but then provide a vent so the steam does not go through the meal in the next trays and condense. However, this is not the most efficient use of steam as the steam would have to be dealt with in the condensers.

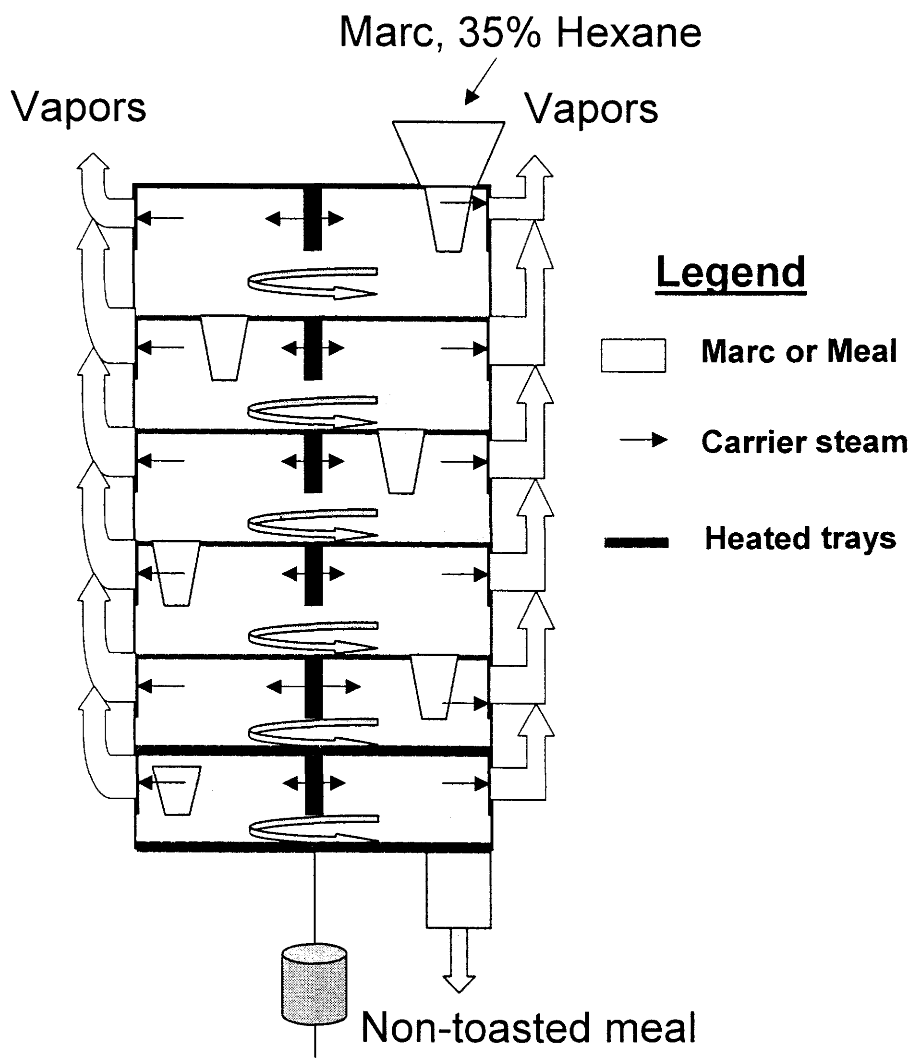

Figure 9.1 Potential design of a non-toasting desolventizer for canola meal.

One of the other reasons that sparge steam is thought to be needed for effective desolventization is that it provides additional surface area for heat transfer. If direct steam injection is eliminated, heat transfer would occur when the meal was in contact with the steam-heated plates. Direct steam injection is a very efficient method of transferring energy to the meal, as the heat transfer area is effectively the entire surface of the particles. Therefore, eliminating sparge steam from an existing desolventizer/toaster would likely increase the requirement for heated surface area. This means that additional trays would need to be added to the DT and the level of the meal on the trays reduced. The other option would be to design a new system with increased surface area using technologies found in the drying industry. In any case, it would appear that the elimination of direct steam injection would be the most effective method of producing a higher quality, lighter coloured, non-toasted canola meal. However, producing this meal commercially will require some equipment retrofitting or replacement and therefore will take some time to be implemented.

The feed industry has no experience with non-toasted canola meal and the only production information available is provided in Section 5. Additional studies are required on the effects of feeding non-toasted canola meal on animal performance. The data in this thesis indicates that eliminating toasting has positive effects on broiler chicken performance, but since the protein will be more soluble, it is not known whether this will affect the performance of ruminant animals. Since the toasting of canola meal reduced digestibility in broiler chickens, a similar effect might be expected in pigs. However, pigs are considered to be more sensitive to diet palatability, which might be affected by processing, so studies should be conducted with this species as well.

Until now, it has always been assumed that a reduction in the glucosinolate content of canola meal by toasting is beneficial to meal quality. In contrast to this assumption, research presented in this thesis suggests that toasted canola meal is more toxic than the non-toasted product. The responsible factor(s) is (are) not known, but it can be speculated that the toasting process degrades the glucosinolates to toxic compounds that are not measured during routine glucosinolate analysis. This highlights the danger of assuming that a compound has no effect simply because it is not detected

by traditional methods. The confirmation that toasting promotes production of toxic compounds and the identification of these compounds requires additional study. The possibility that toasting promotes production of toxic compounds adds support to the suggestion that toasting be eliminated in canola meal processing.

The conversion of rapeseed to canola by traditional breeding has been a great success story and demonstrates the potential to select for unique traits. There does appear to be some variation in the level of NDIN between varieties in both the raw seed and after toasting. It is not known if the differences in NDIN content in the seed indicate higher nutritional value of the meal, but it would seem likely. The relationship between NDIN content in the seed and amino acid content and digestibility should be clarified before it is considered as a selection tool. Neutral detergent insoluble nitrogen content after heat processing has been shown to be correlated with amino acid availability in canola meal, and there appears to be variation among genotypes in their susceptibility to heat treatment. Therefore, it may be useful to select for varieties that are less susceptible to heat damage during desolventization. However, if the industry were to move towards the production of a non-toasted meal, genetic selection for this trait would not be necessary.

In conclusion, the main factor affecting digestible amino acid content in canola meal appears to be the level of toasting applied during desolventization. The inability to accurately measure meal quality, combined with issues of maintaining a constant level of heat application during swings in production, can result in inconsistent meal quality. Neutral detergent insoluble nitrogen can be used to monitor meal quality, but it appears that NIRS offers the greatest potential to assess the digestible amino acid content of processed canola meal. Only enough heat should be applied to canola meal to desolventize the meal, as additional toasting appears to have no beneficial effects on broiler chicken performance. Desolventization without the incorporation of added moisture in the form of direct steam injection should result in a light coloured, non-toasted canola meal. Therefore, it is recommended that the level of toasting of canola meal be minimized by reducing steam input. If possible, desolventizer/toasters should be modified so that hexane is evaporated without incorporating additional moisture into

the meal during desolventization such that a non-toasted canola meal is produced. Prior to producing non-toasted canola meals commercially, studies should be conducted with other animal species to ensure that the new product is well suited to them.

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