THE INFLUENCE OF PROCESSING OF LUPINS AND CANOLA ON APPARENT METABOLIZABLE ENERGY AND BROILER PERFORMANCE.

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DECLARATION

I, the undersigned, hereby declare that the work contained in this thesis is my own original work and that I have not previously in its entirety or in part submitted it at any university for a degree, and to the best of my knowledge, does not include material previously published or written by another person, except where due reference is made in the text.

SIGNATURE DATE

Abstract

1. The influence of processing of *Lupinus angustifolius* and full-fat Canola seed on Apparent Metabolizable Energy and broiler performance.

The extrusion and dehulling of sweet blue lupins (*Lupinus angustifolius*, cultivar Wonga) and the expansion of full-fat canola seed were evaluated in terms of their effect on the nitrogen corrected apparent metabolizable energy (AMEn) value and broiler performance. Two separate trials were conducted for the lupin and canola test materials respectively. Four lupin products were tested and consisted of: lupin meal (LM), dehulled lupin meal (DLM), extruded lupin meal (ELM) and extruded dehulled lupin meal (EDLM). The two canola products were: canola full-fat (CFF) and expanded canola full-fat (ECFF). In each trial two summit diets were formulated with similar energy and crude protein values. The one did not contain any test material (control) and the other contained either lupin meal (170 g/kg for the starter test diet and 174 g/kg for the finisher test diet) or full-fat canola seed (169 g/kg for both the starter and finisher tetst diets). The other test diets were then prepared by replacing the LM with equal quantities of DLM, ELM and EDLM respectively and the CFF were replaced by ECFF. Each of these test diets were then blended with the control diet at six inclusion levels (0, 20, 40, 60, 80 and 100%) to produce experimental diets with increasing levels of the test material. These diets were fed to three groups of 80 as hatched Ross 308 broilers in the lupin trial and 40 in the case of the canola trial. Each trial lasted 42 days. Firstly, the influence of processing was measured in terms of its effect on the AME value of the test material and secondly on broiler performance. Body weight, feed intake and feed conversion ratio (g feed / g weight) were the parameters measured. The extrusion of lupin meal decreased AMEn from 8.61 MJ/kg to 7.52 MJ/kg. The ELM also resulted in inferior broiler performance in comparison with LM and DLM for all parameters measured. The combination of dehulling and extrusion (EDLM) did not result in significant improvements above that observed for ELM. The dehulling of lupin meal (DLM), however, increased the absolute value of lupin AMEn from 8.61 MJ/kg to 8.81 MJ/kg and this effect was also seen when ELM was compared to EDLM (7.52 MJ/kg to 8.04 MJ/kg). The expansion of full-fat canola (ECFF) increased the AMEn from 15.51 MJ/kg to 19.20 MJ/kg, but it did not significantly improve the body weight, feed intake or feed conversion ratio of broilers. Dietary levels above 10% CFF resulted in lower body weights and feed intakes were reduced in comparison to birds on the control diet from 6.8% CFF. The utilization of canola diets (FCR) were superior to those of the control from the 6.8% inclusion level of CFF.

Thus, the dehulling of lupins rendered a more nutrient dense product and this was reflected in the superior broiler performances observed with DLM in comparison with the other lupin products. If body weight is the main criteria, ground, raw canola seeds can be included in broiler diets up to 10%, but dietary levels of up to 16.9% will perform well in terms of feed conversion ratios.

Uittreksel

1. Die invloed van prosessering van Lupiene (*Lupinus angustifolius)* en Volvet Kanola op die Skynbare Metaboliseerbare Energie en braaikuiken prestasie.

Die invloed van ekstrusie en ontdopping van soet blou lupiene (*Lupinus angustifolius*, kultivar Wonga) en die ekspandering van volvet kanola saad is ge-evalueer in terme van hul effek op die stikstof gekorregeerde skynbare metaboliseerbare energie (SMEn) waarde asook braaikuikenprestasie. Twee aparte proewe is uitgevoer vir die lupiene en kanola toetsmateriale afsonderlik. Vier lupien produkte is getoets naamlik: lupienmeel (LM), ontdopte lupienmeel (DLM), geëkstrueerde lupienmeel (ELM) en geëkstrueerde ontdopte lupienmeel (EDLM). Die twee kanola produkte is: volvet kanola saad (CFF) en geëkspandeerde volvet kanolasaad (ECFF). Vir elke proef is twee rantsoene met dieselfde energie en proteïen waardes geformuleer. Die een bevat geen toetsmateriaal nie (kontrole) en die ander een bevat óf lupiene (170 g/kg in die aanvangsrantsoen en 174 g/kg in die afrondingsfase) óf kanola saad (169g/kg vir beide die aanvangs- en afrondingsrantsoene). Die ander toetsrantsoene is voorberei deur die lupienmeel met gelyke hoeveelhede DLM, ELM of EDLM te vervang, terwyl die CFF met ECFF vervang is. Elk van hierdie toetsrantsoene is dan met die kontrole rantsoen vermeng in verskillende verhoudings (0, 20, 40, 60, 80 en 100%) om sodoende eksperimentele rantsoene te skep met toenemende vlakke van die toetsmateriaal. Die rantsoene is aan drie groepe van 80 Ross 308 braaikuikens gevoer tydens die lupien-proef en 40 in die geval van die kanola-proef. Die afsonderlike proewe is in ‘n 42-dag siklus uitgevoer. Die invloed van prosessering van die toetsmateriaal is gemeet in terme van die veranderng in SME, asook in braaikuikenprestasie. Liggaamsmassa, voerinname en voeromsettingsverhouding (g voer / g massa) is gebruik as maatstawwe vir braaikuikenprestasie.

Die ekstrusie van lupienmeel het die SMEn van 8.61 MJ/kg tot 7.52 MJ/kg verlaag. In vergelyking met LM en DLM het die ELM ook ‘n swakker liggaamsmassa en voeromset tot gevolg gehad. Die invloed van beide ontdopping en ekstrusie van lupiene (EDLM) het nie tot enige betekenisvolle verbetering gelei in vergelyking met dié van ELM nie. Die ontdopping van lupienmeel (DLM) aan die ander kant, het die absolute waarde van SMEn van lupiene van 8.61 MJ/kg tot 8.81 MJ/kg verhoog. Hierdie effek is ook waargeneem tydens die vergelyking van ELM met EDLM (7.52 MJ/kg tot 8.04 MJ/kg).

Die ekspandering van volvet kanola het die SMEn van 15.51 MJ/kg tot 19.20 MJ/kg verhoog, maar dit het nie die liggaamsmassa, voerinname of voeromsettings verhouding (VOV) van braaikuikens betekenisvol verbeter nie. Insluitingsvlakke bo 10% CFF het ‘n laer liggaamsmassa tot gevolg gehad, terwyl voerinnames noemenswaardig verlaag het in vergelyking met die braaikuikens op die kontrole dieët vanaf 6.8% CFF. Die benutting van kanola rantsoene (VOV) was egter beter as dié van die kontrole vanaf die 6.8% insluitingspeil van CFF.

Die ontdopping van lupiene het dus ‘n meer nutriënt-digte produk daargestel en is weerspieël in die beter braaikuikenprestasie wat waargeneem is met DLM in vergelyking met die ander lupien produkte. Indien liggaamsmassa as die hoof kriteria beskou word, kan rou, gemaalde kanolasaad by braaikuikenrantsoene ingesluit word tot en met 10%, maar insluitingspeile van tot 16.9% sal goed presteer in terme van voeromsettings verhoudings

This Thesis is dedicated

to my soul partner, my husband and friend André

for his patience, trust and understanding

&

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CONTENTS

General Introduction

Increased global poultry production and the pressure it exerts on conventional protein sources has driven the need to investigate locally produced protein sources as an economic alternative. In this instance, lupins and canola have been identified as being well adapted to the Western Cape. This study aims to contribute information towards the suitablility of these feedstuffs as protein sources, by means of assessing the nutritive value thereof for broilers.

The most important lupin species cultivated today are *Lupinus albus*, *Lupinus luteus* and *Lupinus angustifolius*. Recent cultivars of lupin has been genetically engineered towards a far superior composition and reduced alkaloid content (less than 0.01%) and are referred to as sweet lupins. Large variation exists between lupin species and even cultivars from different areas or parts of the world. In order to make the best prediction of its nutritional value, the feed compounder should be aware of the specific type and composition of lupins that are being used. *Lupinus angustifolius* have been selected for this study, seeing as little work has been done on estimating its nutrient availability for broilers.

The use of canola meal in poultry diets have far exceeded that of the canola seed. Moreover, a general lack of information therefore exists regarding the inclusion of this oilseed in its full-fat form in poultry diets. Currently, the market capacity for protein concentrates for animal feed exceeds that of the oil-pressing capacity in the Western Cape and provided further grounds for selecting canola seed for this study. It also provides the added advantage of utilizing a commodity that is not only a good source of protein, but one that is high in energy. This could prove especially beneficial for poultry nutrition in the hot South African climate.

Firstly, the goal is to provide information regarding the nutritive value and inclusion of *Lupinus angustifolius* and full-fat canola seed in broiler diets. Secondly, it investigates ways of improving its nutritive value. This study was done on a semi-commercial scale and consisted of two trials. In both trials the available energy were evaluated by means of an AME-bioassay with a total faecal collection procedure. The improvement in nutritional value of lupins and canola due to processing was measured by means of broiler performance. Body weight, feed intake and feed conversion ratio (g feed / g weight) were recorded weekly. In the first trial, the effect of dehulling and extrusion of *Lupinus angustifolius* was evaluated. It was expected that the dehulling of lupins will greatly improve the energy-diluting effect of the high fiber contents of these legumes. The thermo-mechanical extrusion treatment was expected to improve the nutritive value of lupins for broilers through the increased accessibility of nutrients to digestive enzymes. In the second trial, the expansion of full-fat canola was tested. Unprocessed whole canola seed is not warranted for use in poultry diets due to the presence of myrosinase that hydrolyses the glucosinolates into toxic and goitrogenic components upon crushing or grinding of the seed. The heat treatment was therefore expected to inactivate this enzyme as well as modifying the protein structure, thus rendering it more readily assimilable by poultry.

The following chapter reviews the literature pertaining to oilseeds and legumes in a global context, as well as summarising the relevant literature on the nutritional values of *Lupinus angustifolius* and Canola.

CHAPTER 1

Literature Review

1.1 Introduction to Legumes and Oilseeds

Global poultry production makes substantial use of plant protein sources to satisfy their dietary protein requirements. Legumes and oilseeds are especially important in providing valuable protein of vegetable origin (Liener, 1990; Larbier & Leclercq, 1994). According to FAO forecasts (Gillin, 2003), the total world poultry meat production will reach 100 million tonnes by 2015 and increase even further to approximately 143 million tonnes by 2030. The global *per capita* consumption of poultry meat (kg / person / year) is also expected to continue its upward trend and was reported to average around 10.9 kg in 2000 (Gillin, 2003). This growth in demand for livestock products has been largely fuelled by the growth that occurred in the developing world, most notably in China and South-East Asia (Dean, 2002). The developing world will be contributing more than 60% of the world poultry meat production by 2030 (Gillin, 2003). Population growth, income growth, cultural factors as well as urbanization, all play a role in determining the demand for livestock products.

To be able to sustain the high rates of increase in livestock production, the manufacturing feed industry must match the increasing demand by increased levels of feed production. Since almost 70% of the total world industrial feed tonnage is intended for the pig and poultry industry (Gill, 2003), it places additional pressure on the feedstuff resources for monogastric nutrition. The high costs of animal protein sources such as fish meal (McDonald *et al*., 1995), and the BSE-imposed ban on the feeding of meat-and-bone-meal by the European Union in 2001, dramatically altered the feed protein market in Europe, increasing demand for vegetable protein sources (Jurgens, 2001; Partridge & Hruby, 2002). Table 1.1 indicates some of the potential legume and oilseed crops of the world.

Legume seeds represent an extremely important source of protein for poultry (Wiseman & Cole, 1988) and contain approximately 20 to 25 % crude protein (Elkin, 2002). Some leguminous seeds, such as beans and peas are low in oil, but rich in starch and can be directly incorporated into poultry diets (Larbier & Leclercq, 1994). Other oil- and legume seeds with higher oil content, such as canola and soya, are employed as raw materials in the edible oil industry, but may also be used as full-fat seeds. These by-products of the oil food industry, also known as oilseed meals, have varying levels of oil content, depending on whether mechanical force (expeller-extraction) or solvent (hexane) extraction methods were used to extract the oil from the seeds (McDonald *et al*., 1995). The latter being the method most widely implemented, but produces the least amount of residual oil in the oilseed meal. This usually averages around 1 to 2 %, compared with the 4 % or more of residual oil in expeller-extracted oilseed meals (Salunkhe *et al*., 1992). Oilseed meals are, however, high in protein and provide the feed compounder with a valuable source of protein. Oilseeds are thus all subjected to manufacturing processes (Dale, 1996) and even though the quality of protein in a particular oilseed is relatively constant, that of the meal derived from it may vary depending on the conditions of processing (McDonald *et al*., 1995). This also holds true for the amino acid composition of oilseed meals (McDonald *et al*., 1995). It has generally been accepted to consider soyabean meal as the universal standard for comparison with other protein

supplements (Ravindran & Blair, 1992; Kohlmeier, 1997 and Leeson & Summers, 2001). The successful incorporation of soyabean meal in monogastric diets can be attributed to various factors. It has a high protein content, usually standardized at 44% or 48% crude protein, depending on the fraction of hulls included, and an amino acid profile that is especially complementary to that of low-lysine cereal-based diets (Elkin, 2002). The amino acid availabilities in soyabean meal are higher than those for other oilseed meals (Aherne & Kennelly, 1982) and the anti-nutritional factors are eliminated in properly processed soyabean meal (Ravindran & Blair, 1992). These valuable characteristics are reflected in the fact that it accounts for 75% of all protein used in industrial feeds (Gill, 2003). The whole group, legumes and oilseeds, constitute an important source of protein, which may or may not be associated with oil production.

Table 1.1 Potential Legume and Oilseed crops of the world

	Scientific Name
Oilseeds[1]	
Castor	*Ricinus communis*
Coconut	*Cocos nucifera*
Cottonseed	*Gossypium spp.*
Linseed / Flax seed	*Linum usitatissimum*
Palm kernel	*Elaeis guineensis*
Peanut/ Groundnut	*Arachis hypogaea*
Rapeseed / Canola	*Brassica campestris / napus*
Safflower	*Carthmus tinctorius*
Sesame	*Sesamum indicum*
Soyabean	*Glycine max*
Sunflower	*Helianthus annus*
Legume seeds[2]	
Chick pea	*Cicer arietinum*
Common dry bean	*Phaseolus vulgaris*
Cowpeas	*Vigna unguiculata*
Faba bean	*Vicia faba*
Field pea	*Pisum sativum*
Green gram	*Vigna radiata*
Lupin	*Lupinus spp.*
Lentil	*Lens culinaris*

1. Salunkhe et al. (1992); 2. Ravindran & Blair (1992).

As livestock production becomes more commercialized in response to increased consumer demand, a major consideration will be the extent to which a particular country can satisfy increased demand for feedstuffs on its own account and the extent to which it will have to rely on imports. Europe has been relying substantially on soyabean meal imports, but the strong drive towards non-GMO feedstuff sources and the high cost involved in soyabean meal imports, prompted them to investigate the production and use of alternative protein-rich

ingredients (Gatel, 1994). This phenomenon is also found in other countries, such as South Africa where expensive soyabean meal imports are required to satisfy local demand (Jurgens, 2001), and in other areas of the world where soyabean meal is primarily intended for the human food chain and thus not readily available for animal feed use, such as Asia and the Pacific (Ravindran & Blair, 1992). The partial or complete replacement of soyabean meal by indigenous protein sources could be an economically attractive alternative and may lead to savings in valuable foreign exchange.

Grain legumes are potential substitutes for soybean meal because of their similar amino acid profiles (Gatel, 1994). Although the use of grain legumes in poultry production is mostly directed towards supplying a source of protein (Brandt, 1998), due to the carbohydrate (mainly starch) and oil content of some of the legume seeds, they can also be viewed as potential sources of energy (Reddy *et al*., 1984; Ravindran & Blair, 1992). Table 1.2 compares the nutritional value of selected protein concentrates in terms of protein, fat (ether extract) and fiber content. It is evident from these values that the various methods of oil-extraction had an influence on the protein and fat content of these sources, the solvent extraction method yielding a concentrate source with higher protein and lower fat values than the expeller-extraction method.

Table 1.2 Average protein, fat and fiber contents (% dry matter) of selected protein concentrates utilized in poultry nutrition

Protein Concentrate Sources	Protein (%)	Ether extract (%)	Crude Fiber (%)	Source*
Soyabean meal, expeller-extracted	42.0	6.0	8.0	1
Soyabean meal, pre-press, solvent	49.4	0.9	8.2	2
Soyabean meal, dehulled, solvent	53.9	1.1	4.3	2
Rapeseed/ Canola meal, expeller-extracted	36.0	7.0	12.0	1
Rapeseed/ Canola meal, pre-press, solvent	37.2	1.9	13.2	2
Cottonseed meal, expeller-extracted	38.0	6.0	14.0	1
Cottonseed meal, pre-press, solvent	45.0	1.6	12.3	2
Peanut/ Groundnut meal, dehulled, expeller-extracted	44.0	7.0	13.0	1
Peanut/ Groundnut meal, pre-press, solvent	52.2	1.3	14.6	2
Sunflower meal, dehulled, expeller-extracted.	36.0	8.0	13.0	1
Sunflower meal, dehulled, pre-press, solvent	45.2	3.1	13.1	2
Common dry bean	24.0	2.0	4.0	1
Chick pea	22.0	4.0	9.0	1
Faba bean	23.0	2.0	7.0	1
Field pea	25.0	1.5	6.0	1
Lupin meal, whole seed	33.7	5.0	18.7	3
Lupin meal, dehulled	43.7	8.2	5.5	3

* Source: 1. Ravindran & Blair (1992); 2. Aherne & Kennelly (1982); 3. Fernández & Batterham (1995).

Despite their favourable amino acid profile and relatively high energy content, the use of grain legumes in commercial poultry production is still limited because of uncertainty about their effective nutritional quality (Wiryawan & Dingle, 1995). A major constraint in the use of grain legumes (Wiseman & Cole, 1988) and

oilseeds (Aherne & Kennelly, 1982) in poultry diets is that they contain anti-nutritional factors (ANFs) that depress poultry performance. Some of the ANFs of these plant protein sources are displayed in Table 1.3.

Table 1.3 Anti-nutritional Factors in Seeds (Huisman & Tolman, 1992)

Seeds	Anti-nutritional Factors			
	Trypsin Inhibitors	Lectins	Polyphenolic compounds	Other ANFs
Legume seeds				
Soya	++ / +++	++	-	++ / +++ [A, C]
Vicia faba bean	+	+	+ / ++ / +++	+ / ++ / +++ [B]
Ph. Vulgaris bean	- / + / ++	+ / ++ / +++	+ / ++	+ / ++ / +++ [A]
Pisum sativum	+ / ++	+ / ++	+ / ++	-
Lentils, cowpeas, chick peas	+ / ++	+ / ++	- / + / ++	-
Lupins	-	-	-	+ / ++ / +++ [C]
Other seeds				
Rapeseed/ Canola	-	-	+ / ++	+ / ++ / +++ [D]
Sunflower seed	- / +	-	+ / ++ [E]	-
Cotton seed	- / +	-	-	+ / ++ / +++ [F]
Peanut	-	-	+ / ++ [G]	-

below detection level; + low level; ++ medium level; +++ high level. Different varieties of the same material may have different characteristics. A, antigenic proteins; B, vicine/convicine; C, alkaloids; D, glucosinolates and sinapins; E, 3-3.5 % phenolic compounds; F, gossypol; G, 16-18 % in the shell around the nut.

Various treatments have been tested to improve the nutritive value of grain legumes to their full potential. The nutritional value depends not only on the chemical composition of the plant protein source, but also on the extent to which nutrients are digested, absorbed and utilized. ANFs interfere with these digestion, absorption and utilization processes (Huisman & Tolman, 1992), but could be reduced or eliminated by means of suitable processing techniques, enzyme supplementation and plant breeding (Wiseman & Cole, 1988; Liener, 1990; D'Mello, 1995). The resulting improvements in nutritive value are related to increases in metabolizable energy values and in the digestibilities of the legume proteins (Wiryawan & Dingle, 1999).

In the context of the global trading economy, it is appropriate to look at oilseed and legume production and utilization on a worldwide basis.

1.2 Global production and trade

World trade in oilseeds and oilseed products rose steadily in the years following the Second World War. The rate of growth showed a continuous increase in the following decades with rape and soya contributing the greatest proportional volume increases. This is clearly visible from the data of world oilseed production in Table 1.4. It is noteworthy that not all seeds are processed to obtain oil. A part of the produce is used as seed; some are fed unprocessed to animals, or used directly for human consumption.

Table 1.4 Calculated world production (million tonnes) of selected oilseeds (Weiss, 2000)

	1960	1970	1980	1990	2000*
Castor	0.7	0.9	1.0	1.3	1.5
Copra	4.0	4.0	5.0	5.0	5.0
Cottonseed	20.0	21.0	24.0	34.0	35.0
Groundnut	12.0	12.0	14.0	17.0	20.0
Linseed	4.0	4.0	3.0	2.5	2.5
Rapeseed	4.0	7.0	12.0	25.0	40.0
Safflower	0.5	0.6	1.0	1.0	0.5
Sesame	2.0	2.0	3.0	3.0	3.0
Soya	27.0	45.0	93.0	104.0	180.0
Sunflower	7.0	10.0	15.5	23.0	28.0
Total	81.2	106.5	171.5	215.8	315.5

* Estimate

The great increase in rape and soya production is a major factor affecting international trade in oilseeds. Traditionally, the availability of soya or its products, especially from the USA and Argentina, dominated the world oilseed trade and had a direct impact on the price levels of competitive crops (Willemse, 2004). This dominance was put on hold by the growth in Malaysian palm oil production as well as the introduction of canola oil from Canada. Meal and oil from double zero rapeseed cultivars can now directly substitute for soya bean products (Weiss, 2000). The worlds major producers of selected oil crop meals are listed in Table 1.5.

Table 1.5 Major producers of selected oilcrop meals around the world

Oilseed meals	Major Producers[1]
Soyabean meal	USA, Brazil, Argentina
Rape/Canola seed meal	Canada, EU*, China, India, Japan
Cottonseed meal	China, India, Russia, USA
Groundnut meal	India, China
Sunflower meal	Argentina, EU, Russia
Copra meal	Philippines, Indonesia
Linseed meal	Argentina, China, EU, Asia
Lupinseed meal	Australia[2]

1. Weiss (2000); 2. Cox (1998); * EU – European Union

Lupins have shown to be an excellent substrate for both bacterial and fungal fermentations, used in making foods such as Indonesian tempe, miso and traditional soy sauces (Petterson, 1998). Apart from its use in foods, lupins are generally utilized as a source of protein and energy in livestock feeds (Edwards & Barneveld, 1998). The utilization of oilseeds (Salunkhe *et al*., 1992) can take the form of either the whole seeds or the products that are derived by a partial removal of one of the major seed components. The seeds can be directly processed into various edible products, including roasted, fermented and cooked products. Although the oils are mostly utilized as edible oils for the human food chain, many oils are also used for industrial purposes (Sonntag, 1995) and include coconut, soybean, linseed and castor oil. Coconut oil is mostly utilized in

cosmetics, soaps, detergents, pharmaceuticals and as base material in paints, whereas soybean and linseed oils are used as plasticizers or stabilizers for vinyl plastics (Weiss, 2000). Castor oil is used in lubricants, plasticizers, coatings, surfactants and pharmaceuticals (Weiss, 2000). The demand for oilseed meals have exceeded that for vegetable oils and can be contributed to the rise in demand by the intensive livestock production sector especially pigs, poultry and aquaculture.

1.3 Production and trade in South Africa

South Africa relies strongly on imports of oilseeds and oilseed products to satisfy local demand for protein sources. As with global trends, the demand for these protein sources has also increased in South Africa. The oilcake inclusion rate for animal feed in South Africa increased by 0.48% during the 2002/2003 period (Briedenhann, 2003) and resulted in increased oilcake imports. According to Table 1.6 the total available oilcakes increased by 5.3% during the period 2001/2002 and 2002/2003 and pushed the available oilcakes to over 1,2 million tonnes for the first time.

Table 1.6 Summary of total available oilcake (tonnes) in South Africa from 1999/2000 to 2003/2004 (Briedenhann, 2003)

Year	Local Production	Imports	Total
1999 / 2000	554 903	508 435	1,063,338
2000 / 2001	514 020	635 134	1,149,154
2001 / 2002	482 448	666 776	1,149,224
2002 / 2003	472 311	738 085	1,210,396
2003 / 2004*	417 359	782 641	1,200,000

*estimate

The largest contributor towards total available oilcake in South Africa is soya, followed by sunflower, cotton, canola, lupins and groundnuts. These are the major plant protein sources and their respective contributions during 2002/2003 are visually represented in Figure 1.1. The other oilcakes include copra, linseed, palm and rapeseed.

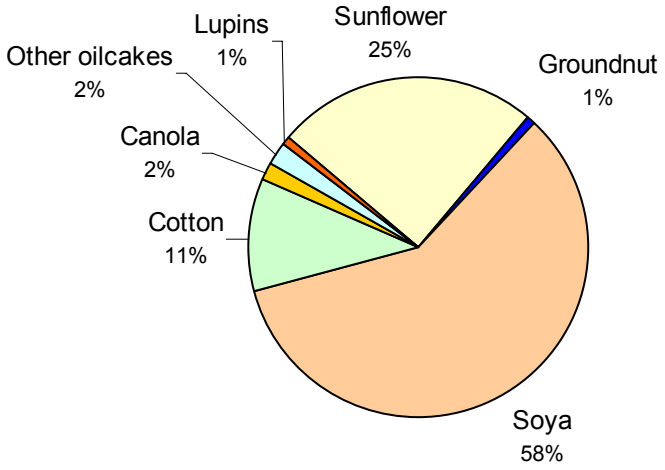

Figure 1.1 Breakdown of total available oilcakes in SA for 2002/2003.

The estimated local production of oilseeds and oilcakes for the marketing season 2003/2004 is shown in Table 1.7. From this data it is clear that even though soya is the largest contributor towards total oilcake availability, it is sunflower that is produced on the largest scale in South Africa. The four most important oilcrops for poultry production in particular, are sunflower, soya, canola and lupins.

Table 1.7 Estimated availability of oilcakes (Tonnes) for the period: 1 April 2003 – 31 March 2004 (Briedenhann, 2003)

OILSEEDS	2002/03 CROP ESTIMATES [1]	AVAILABLE FOR CRUSHING	CONVERSION RATE (SEED)	EQUIVALENT OILCAKE
LOCAL PRODUCTION				
Sunflower	706,700	706,700	42.0%	296,814
Soya [3]	139,420	90,320	80.0%	72,256
Groundnut	66,205	15,000	53.5%	8,025
Cotton seed	26,081	26,081	50.0%	13,041
Canola [2]	40,770	40,770	55.0%	22,424
Lupins [2]	4,800	4,800	100%	4,800
ESTIMATED LOCAL PRODUCTION				417,359
Total Estimated Requirements				1,200,000
IMPORT REQUIREMENT				782,641

Sources:
1. Crop Estimates Committee (20 August 2003).
2. Crop Estimates Committee - Preliminary area estimate (20 August 2003 - calculation).
3. 44 000 tonnes for human consumption. 5100 tonnes for seed.

This thesis aims to investigate some of the most promising locally produced protein sources for utilization in broiler diets and will specifically focus on the winter oilseed crops lupins and canola. It could be especially advantageous to the South African conditions to provide a concentrated source of protein and energy for the feeding of poultry in relatively hot climates. In the Western Cape in particular, it is often more economical to import energy and protein sources from countries such as Argentina, than it is to transport similar products from the Highveld. Locally produced conventional protein sources are also becoming less available and more expensive (Wiryawan & Dingle, 1995), and thus driving the importance of investigating all possible alternatives that could provide an adequate source of protein and energy in broiler diets. Lupins (Brand & Brundyn, 2001) and canola (Grobbelaar, 1999) have been identified as being well adapted to the winter rainfall area of the Western Cape and if successfully cultivated, have the possibility of providing a much needed local supply of protein. With this study it is intended to determine the nutritional value of *Lupinus angustifolius* and Canola for broilers and to investigate whether any additional processing of the full-fat seeds will lead to improvements in the nutritional value and thus a further savings effect on the expensive imports. The following sections summarise the relevant literature on lupins and canola respectively, and provide grounds for the research conducted for this thesis.

1.4 Nutritional value of Lupins (*Lupinus angustifolius*) for broilers

Lupins (*Lupinus spp*.) belong to the family Leguminosae and have been cultivated by ancient cultures of the Mediterranean basin and the Andean highlands. Several hundred different species of lupins exist, but only a few are actually cultivated (Todorov *et al*., 1996). Some, such as *Lupinus albus* and *Lupinus mutabilis,* have been cultivated for human consumption since the earliest of times (Petterson, 1998), but others have only come into cultivation more recently (Olver, 1994). The type of lupin being produced today bears little resemblance to its predecessors and has been genetically engineered towards a far superior composition and reduced alkaloid content. These new cultivars have been reported to contain less than 0.01% alkaloids and are referred to as sweet lupins (Leeson & Summers, 1997). The high alkaloid content of the more traditional bitter lupin cultivars suppress both food intake and growth, but a significant decrease in food conversion efficiency for broilers fed increasing levels (25, 50, 75 & 100%) of bitter lupins was also found (Guillaume *et al*., 1979). Olver (1994) defined alkaloids as nitrogen-containing, water soluble compounds that are produced in the chloroplasts of certain plants and serve to repel or kill insect parasites. Cultivars of sweet varieties must be strictly controlled to assure that cross-pollination does not occur and result in bitter progeny (King *et al*., 1985). Sweet lupins can either be of the white (*Lupinus albus*), yellow (*Lupinus luteus)* or blue seeded (*Lupinus angustifolius)* varieties. These are currently the most important lupin species cultivated and are shown in Figures 1.2, 1.3 and 1.4 respectively. The colours refer to the colour of the lupin plant flower and together with the shape of the plant leaves (narrow-leaved or broad-leaved) can be used to distinguish between the species. Lupins are widely used as a source of protein and energy in livestock feeds (Edwards & Van Barneveld, 1998), but it is especially the low alkaloid lupins that are increasingly being investigated as an alternative protein feedstuff for poultry.

Figure 1.2. *Lupinus albus* (white lupins)

Figure 1.3. *Lupinus luteus* (yellow lupins)

Figure 1.4. *Lupinus angustifolius* (narrow-leaved or blue lupins)

Wide variation exists in the compositional characteristics of lupin seeds between species as well as between cultivars of the same species and may primarily be due to genetic differences between them (Sathe *et al*., 1982). Variation also exists within cultivars from different areas and are mostly influenced by environmental and location effects (Jimenez *et al*., 1991). This makes animal feeding trial-comparisons

between lupin species and even cultivars from different areas or parts of the world extremely difficult and the feed compounder should therefore be aware of the specific type and composition of lupins that are being used, in order to make the best prediction of its nutritional value. The chemical and amino acid composition of the three best known lupin species: *Lupinus angustifolius, Lupinus albus* and *Lupinus luteus* have been summarized from literature and are presented in Table 1.8 as minimum and maximum reported values for the individual lupin species respectively. In the following section the chemical composition of lupins will be discussed in more detail.

Table 1.8 The chemical (% dry matter) and amino acid composition of three major lupin species

Components	L.angustifolius [1,2,3,4,5,6,7,8,9,10]		L. *Albus* [2,5,6,8,9,11,12,13,14,15]		L. *Luteus* [1,5,9,16,17]	
	Min.	Max.	Min.	Max.	Min.	Max.
DM	91.5	94.29	90.20	94.43	90.30	93.00
Protein (N x 6.25)	28.33	34.70	31.45	41.3	35.74	45.41
Fat	4.71	6.50	8.13	10.89	4.50	5.89
Crude Fiber	16.26	18.66	12.80	16.50	15.53	17.70
ADF	21.65	25.02	16.06	18.01	20.06	20.06
NDF	23.65	27.70	17.31	21.22	22.80	22.80
Ash	2.90	4.10	3.00	4.23	3.91	4.60
Ca	0.19	0.33	0.16	0.22	0.15	0.27
P	0.33	0.42	0.33	0.45	0.48	0.51
Mn (ppm)	17.00	70.8	404.97	3750	59.13	115
Amino acids (% Protein)						
Arginine	9.77	11.30	8.14	11.60	9.10	13.63
Cystine	0.80	2.10	1.08	2.29	1.49	2.47
Histidine	2.30	3.00	1.74	2.35	3.30	5.58
Isoleucine	3.40	4.51	3.10	5.01	3.50	4.99
Leucine	5.82	8.11	5.67	7.30	7.14	7.61
Lysine	4.11	5.49	3.73	5.70	4.10	6.41
Methionine	0.39	0.90	0.45	0.90	0.51	1.43
Phenylalanine	3.11	4.18	3.29	4.01	3.51	6.07
Threonine	3.11	3.85	3.39	4.29	2.71	5.05
Valine	3.40	4.23	3.07	4.30	3.65	4.25

[1] Hove, 1974; [2] Batterham, 1979; [3] Barnett & Batterham, 1981; [4] Batterham *et al*., 1986a; [5] Múzquiz *et al*., 1989a; [6] Prinsloo, 1993; [7] Fernández & Batterham, 1995; [8] Brand, 1996; [9] Brand & Brundyn, 2001; [10] Steenfeldt *et al*., 2003; [11] Aguilera *et al*., 1985; [12] Kemm *et al*., 1987; [13] Brand *et al*., 1995; [14] Brenes *et al*., 1993; [15] Olver & Jonker, 1997; [16] Petterson, 1998; [17] Seabra *et al*., 2001; ADF - Acid detergent fiber; NDF - Neutral detergent fiber

1.4.1 Chemical evaluation

The crude protein content of lupin seeds generally fall in the range from 28 to 45 % (dry matter) with both *L.albus* and *L.luteus* averaging higher crude protein contents than *L.angustifolius* (Table 1.8). Mossé *et al*. (1987) analyzed 20 samples of *Lupinus albus* seeds from 10 different cultivars and found an even wider variation in protein content (23.8 – 48.4%) for that particular species. Petterson (1998) reported the typical

average protein values (air-dry basis) for the major lupin species to be 32.16% for *L.angustifolius,* 36.10% for *L.albus* and 41.3% for *L.luteus*. The protein content of lupins compare well to the 37% protein of full fat soyabeans (NRC, 1994), and can thus also be seen as a valuable source of protein for broiler diets. The major proteins of lupin seeds can be divided into two main classes: albumin and globulin (conglutin), with the latter comprising about 85% of the total protein and the remaining 15% consists of albumins. The globulin fraction can be separated into three major proteins: conglutin α, conglutin β and a lupin-specific protein conglutin δ. These are similar to the storage proteins of field peas, soyabeans and other legumes in terms of size and physical properties (Petterson, 1998). There is, however, variation in the proportion of conglutin fractions among the different lupin species (Van Kempen & Jansman, 1994), which could contribute to the differences in functional properties of the various lupin proteins.

It is well known that pigs and poultry do not have a requirement for crude protein *per se*, but rather for specific levels of amino acids. Despite this, crude protein level is often used as a guide to the amino acid content of lupins. This could, however, affect the efficiency of use of lupins in pig and poultry diets, since large variation exists in the crude protein content. Typical amino acid profiles for the domesticated lupin species (Table 1.8) show that they resemble that of many other legume proteins in being low in methionine (0.39-1.43 g /100g protein) as well as lysine (3.73-6.41 g /100g protein), but are viewed as a good source of arginine (8.14-13.63 g /100g protein). Comparable average amino acid values (% protein) for methionine, lysine and arginine from soyabeans are 1.38, 6.49, and 7.57 % respectively (Waldroup, 1982). Mossé *et al*. (1987) reported that the proportion of lysine and alanine in each lupin species falls as the total nitrogen content increases, whereas that of some of the non-essential amino acids rises. It should be noted that genetic engineering of some of the more recently domesticated species of lupins have generated amino acid levels that are significantly higher than the more traditional varieties (Atkins *et al*., 1998).

Energy storage within the lupin seed cotyledons comes from variable proportions of oil, oligosaccharides and non-starch polysaccharides. The oligosaccharides provide available energy to the bird through the successful absorption of its fermentation products (Carré *et al*., 1995). Lupins contain a significant amount of oil (Table 1.8) in comparison with other grain legumes such as peas (1.37 – 2.80%) and field beans (1.20 – 1.90%) (Welch & Griffiths, 1984). The extraction thereof is, however, not economically justifiable, but it makes a valuable contribution to the metabolisable energy value of the seed (Múzquiz *et al*., 1989a). Within lupin species the oil content of *L.albus* (8-11%) is almost double that of *L.angustifolius* and *L. luteus* (4-7%). According to Hansen & Czochanska (1974), the fatty acid composition of lupin oil is similar to that of soyabean seed, but the absolute quantity and profile of lupin fatty acids was found by Jimenez *et al*. (1991) to vary substantially between different species and cultivars according to environmental influences. Jimenez *et al*. (1991) reported a total unsaturated fatty acid content for *Lupinus albus* to be between 70 and 80 %, with oleic acid (C18:1) comprising 53 % of the total oil content. The total fat and oleic acid contents were also found to be positively correlated. The high level of oleic acid found in *Lupinus albus* oil is typical for this species, whereas *Lupinus angustifolius* and *L.luteus* usually contain more linoleic acid (C18:2), which is also characteristic of plant oils such as corn and soyabean (Watkins *et al*., 1988). The linoleic acid content of *L.angustifolius* ranges

from 33.7 to 48.3 % of total oil content (Hansen & Czochanska, 1974; Jimenez *et al*., 1991) and provides a valuable source of the precursor needed for the biosynthesis of the essential fatty acid arachidonic acid.

It can be seen in Table 1.8 that lupins have a high crude fiber content (12 to 18%). This is a consequence of the thick seed coats of lupin seeds, which comprise about 30% of the seed weight for *L.luteus*, 25% for *L.angustifolius* and 15% for *L.albus* (Petterson, 1998). According to classical fiber analysis for monogastric diets, feed ingredients are analyzed for crude fiber (CF) by means of extractions with alkali and acid. Within these CF fractions, variable portions of insoluble non-starch polysaccharides (NSPs) are also incorporated, but it does not allow for the inclusion of soluble NSPs (Smits & Annison, 1996). Pectic substances like galactans are abundant in lupin cell walls, but are solubilized and lost during fibre measurements, suggesting that it could underestimate the 'unavailable' carbohydrate content of lupins. NSPs generally include all the polysaccharide molecules except for starch and are classified according to Choct (2002) into an insoluble cellulosic component, and components that are partially soluble, consisting of non-cellulosic and pectic polymers (Figure 1.5). The total NSP content of whole lupin seed (*Lupinus angustifolius*) is about 38% with the insoluble portion comprising almost 89% thereof (Smits & Annison, 1996). These insoluble components have a minimal effect on nutrient utilization by monogastrics and contribute to the maintenance of normal gut motility, due to their ability to hold large quantities of water (Petterson, 1998). Many authors, however, have reported on the adverse effects of soluble NSPs on nutrient availability for poultry, and this will be discussed in more detail at a later stage. From the three major lupin species in Table 1.8, *Lupinus angustifolius* generally contains the highest CF levels (16.3-18.7%), followed by *Lupinus luteus* (15.3-17.7%) and *Lupinus albus* (12.8-16.5%). Bitter lupin varieties are also known to contain more CF than their sweet counterparts (Olver & Jonker, 1997).

Lupin seed hulls and cotyledons contain different types of carbohydrates. Lupin hulls are low in lignin and consist predominantly of structural polysaccharides such as cellulose, hemi-cellulose and pectins, whereas the cotyledons consist mainly of the non-structural polysaccharides of cell walls, including galactose, arabinose and uronic acid residues (Brillouet & Riochet, 1983). They account for approximately 67%, 13% and 10% respectively of total cotyledon NSP (Evans *et al*., 1993). These constituent sugars of lupin NSPs are similar to those found in cereal grains, but are not necessarily linked in the same way. Most legumes contain pectic polysaccharides as their main NSP, but these polymers also differ widely in terms of their molecular structures (Choct, 2002). It is noteworthy that the cell wall material content of lupin seed cotyledons varies greatly among species and cultivars, ranging from 7.5% to 32.1% dry matter (Brillouet & Riochet, 1983). In lupins, the major polysaccharide is β-(1-4)-galactan, consisting of a mixture of D-galactose, L-arabinose, L-rhamnose and galacturonic acid (Van Kempen & Jansman, 1994). Only trace amounts (0.4%) of starch are found in lupin species (Cerning-Beroard & Filiatre, 1976; Steenfeldt *et al*., 2003).

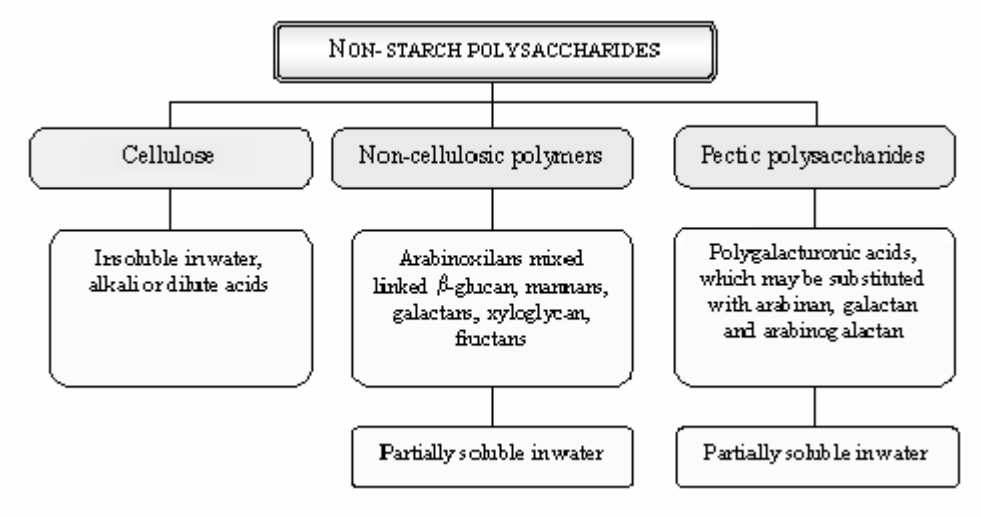

Figure 1.5 Classification of non-starch polysaccharides (Choct, 2002).

The calcium content of lupin seeds (1.5 – 3.3 g kg $^{-1}$) is higher than that for peas but lower than that of soyabean meal, and the total phosphorous content (3.3 – 5.1 g kg $^{-1}$) is similar to that of peas but much lower than for soybean meal (Petterson, 1998). The lupin seed coats are unique in having no detectable phosphorous (Hove, 1974). The percentage of phytate phosphorous in lupin species is comparable to the other grain legumes. The trace mineral content of lupins is sufficiently high to make them a valuable contributor of these essential nutrients, but is influenced by genotype and also tends to reflect the soil types on which they are grown (Petterson, 1998). The accumulation of manganese by *L.albus* is well known and levels of up to 6900 ppm have been reported (Van Kempen & Jansman, 1994). That is a rather extreme value, however, and the average manganese content for this lupin species grown in Australia (Victoria) was closer to 2287 ppm, with a range including values from 900 to 3920 ppm (Karunajeewa & Bartlett, 1985). Very high manganese values (4000 ppm) will reduce feed intake and growth (NRC, 1994) and may cause toxicity and lead to the oxidation of oils and vitamins in feeds (Van Kempen & Jansman, 1994).

The availability of a raw material in a particular area plays an important role and influences the feed compounders choice of raw materials, and thus the cost-effectiveness of feed formulation in terms of utilizing local resources. For this thesis, one of the predominant sweet lupin species of the Western Cape namely *Lupinus angustifolius* was used. The nutritional value for this particular species have been summarized from the literature and are presented in Table 1.9. It includes the highest and lowest reported values for each nutrient from various cultivars.

In this section, the chemical composition of lupin seeds have been described. However, quantifying these components is only the first step in evaluating the nutritional value of lupins, and are usually well documented in literature as indicated in Table 1.9. When dealing with poultry, however, the nutritional value of a feed ingredient is more adequately described by the available nutrient content and should reflect closely on broiler performance parameters such as body weight gain, feed intake and feed conversion efficiency. Unfortunately, little work has been done on estimating the nutrient availability of *Lupinus angustifolius* for broilers in particular, as illustrated by the few authors reporting on apparent or true metabolizable energy in Table 1.9. The

following section reviews the pertaining literature on biological analysis for poultry in terms of assessing the available energy content of lupins.

Table 1.9 Summary of reported nutritional values for *Lupinus angustifolius*

Components (10 % moisture basis)		Lowest reported value	Highest reported value	Average	Source
Ash	%	2.14	3.69	2.96	1,2,3,5,8,9,12,14,15,16,17,18,19, 20
Crude Protein	%	24.03	32.03	29.33	1,2,3,4,5,6,8,9,10,11,12,13,14,15,16,17,18,19, 20
Ether Extract	%	3.84	7.52	5.02	1,4,5,8,9,10,11,12,13,14,15,16,17,18,19,20
Crude Fiber	%	13.00	19.22	15.89	3,4,6,9,10,11,12,13,16,17,19,20
ADF	%	16.29	24.64	21.04	1,3,6,8,9,11,13,14,16,17,19
NDF	%	19.57	30.55	24.52	1,3,6,8,9,11,12,13,16,17
GE	MJ/kg	17.47	18.53	18.02	6,9,12,14,18
AME	MJ/kg	6.71	10.46	8.73	4,13,14
TME	MJ/kg	11.07	11.07	11.07	10
TMEn	MJ/kg	9.41	12.47	10.46	1,5,8
Tot Alkaloid	%	0.002	0.008	0.005	7,10,16
Ca	%	0.17	0.30	0.22	1,4,5,9,10,11,13,20
Phosphorous	%	0.29	0.41	0.36	1,4,5,9,10,11,13,20
avail. P	%	0.20	0.20	0.20	4,5,9,10,11,20
Na	%	0.04	0.27	0.16	5,8,15,18,20
Mg	%	0.14	0.18	0.16	1,5,8,15,18,20
Cu	mg/kg	3.07	5.94	4.21	1,5,8,15,18,20
Zn	mg/kg	31.18	39.78	34.08	1,5,8,15,18,20
Mn	mg/kg	24.24	63.72	38.14	1,5,8,15,18,20
Fe	mg/kg	39.06	51.63	46.04	1,5,8,15,18,20

[1] Brand *et al*., 2004; [2] Steenfeldt *et al*., 2003; [3] Mariscal-Landín *et al*., 2002; [4] Swick, 2001; [5] Brand & Brundyn, 2001; [6] Edwards & Tucek, 2000; [7] Brand & Brandt, 2000; [8] Brandt, 1998; [9] Petterson, 1998; [10] Olver & Jonker, 1997; [11] Brand, 1996; [12] Fernández & Batterham, 1995; [13] Prinsloo, 1993; [14] Johnson & Eason, 1991; [15] Múzquiz *et al*., 1989a; [16] Batterham *et al*., 1986a; [17] Barnett & Batterham, 1981; [18] Batterham, 1979; [19] Pearson & Carr, 1976; [20] Hove, 1974; ADF-Acid detergent fiber; NDF-Neutral detergent fiber; GE-Gross Energy; AME-Apparent metabolizable energy; TME-True metabolizable energy; TMEn-nitrogen corrected true metabolizable energy.

1.4.2 Bioassays - Energy

Apart from species and age differences, there are a large number of factors that can influence the bioavailable energy content of a feedstuff. The assaying method applied, the expression of results as true or apparent metabolizable energy, correction for the nitrogen status of the bird, as well as the interaction of the dietary components of the test ingredient with those of the assay diet are but to name a few. Consequently, detailed information regarding the analytical methods and procedure applied should be included at all times in order to facilitate reasonable comparisons between results.

A species difference in the bioavailable energy value of foodstuffs were investigated by Sibbald *et al*. (1983) and a very close relationship between the apparent digestible energy (ADE) value for pigs and the true metabolizable energy value (TME) for adult cockerels were found. Similar results were obtained by Sibbald *et al*. (1990) when they analyzed 84 feedstuffs for available energy content and compared the results obtained from adult cockerels (TMEn) with those from pigs (AMEn). A linear relationship was found between these parameters in cereal-based and mixed diets.

Literature findings regarding the influence of age on the metabolizable energy (ME) value of poultry diets are inconclusive, but the influence thereof was found to be negligible. Carré *et al*. (1995), however, studied the differences in various nutrient digestibilities between adult cockerels and 3-week old broilers, and found the greater apparent digestion of carbohydrates and lipids in adults to have the biggest contribution towards the difference experienced in the AMEn value of the diet. Zelenka (1968) found a rapid decrease in the ME of practical diets during the first days after hatching and it lasted until the 7th or 9th day, whereafter it progressively increased until the 14th day. Thus, differences between ME values observed are dependent on the age that is chosen for the balance periods under comparison.

Johnson & Eason (1991) reported the apparent metabolizable energy (AME) of two cultivars of Australian sweet lupins (*L.angustifolius*) to be 7.2 and 9.6 MJ kg $^{-1}$ DM (Table 1.9), using a rapid broiler assay technique. Other authors also reported AME values for *L.angustifolius* that fall within this range, but it was either determined with layers (Perez-Maldonado *et al.,* 1999) or a calculated estimate using regression equations with nutrient values of European origin (Olkowski *et al*., 2001). Brand & Brundyn (2001) determined the average nitrogen corrected true metabolizable energy content (TMEn) of eight samples of *L.angustifolius* for roosters (10.46 MJ kg $^{-1}$ DM), and indicated a significantly higher (P< 0.05) TMEn content for *L.albus* (12.49 MJ kg $^{-1}$ DM). A number of authors also found *L.albus* to be superior to *L.angustifolius* in terms of AME content for adult roosters (Guillaume *et al*., 1979; Karunajeewa & Bartlett, 1985; Perez-Escamilla *et al*., 1988) and this could partly be contributed to the higher fat content and lower hull:kernel ratio of *L.albus* in comparison with *L.angustifolius*. Generally, the AME of *L.angustifolius* for poultry is inferior to that of other grain legumes such as field peas (11.70 MJ kg $^{-1}$) and faba beans (11.04 MJ kg $^{-1}$), and could be contributed to the absence of any appreciable hindgut recovery of energy (Petterson, 1998). The high fiber contents of lupins are also responsible for a decrease in the available energy content, but this energy-diluting effect could be greatly improved by the dehulling of lupins. The nutritional significance of dehulling and other processing methods that can enhance the available energy content of lupins will be discussed at a later stage in this chapter. Similar to this section where the available energy content of lupins were discussed, the following section will review literature on biological analysis for poultry in terms of assessing the available amino acid content of lupins.

1.4.3 Bioassays – Amino acids

Available amino acids represent the proportion of amino acids that are in a form suitable for utilization and are certainly a much more effective basis than 'total amino acid content' to formulate poultry diets with. Unfortunately, this approach has not been widely adopted due to the lack of uniform and accurate methods of determining amino acid availability (Papadopoulos, 1985) as well as a general lack of need and confidence on the part of the nutritionist to switch over to formulating with digestible amino acids rather than total amino acids (Creswell & Swick, 2001a). These authors have published a series of articles with regards to formulating with digestible amino acids and provides important information for the nutritionist (see Creswell & Swick, 2001a; 2001b; 2001c). Methods of determining available amino acids include *in vivo* and *in vitro* approaches,

and must generally conform to three main criteria as described by Johnson (1992): precision, sensitivity and ease of execution. The most common and accurate method for determining available amino acids are by means of *in vivo* digestibility studies and are divided into either ileal or faecal digestibility. The advantages and disadvantages of using these digestibility techniques, together with that of intact or caecectomized cockerels have been discussed in detail by Johnson (1992). The combining and averaging of digestibility values as determined by different techniques and laboratories are also strongly discouraged by Johnson (1992). The author recommends that amino acid digestibility measurements be specifically adapted for a particular feedstuff, since the large variation in protein contents between feedstuffs utilized in poultry nutrition, as well as the fiber content and its influence on endogenous secretions, may lead to considerable inaccuracies.

The total amino acid composition (% protein) of *Lupinus angustifolius* is summarized in Table 1.10, but due to the lack of a complete set of digestibility values for this lupin species, the reported values in Table 1.10 have been pooled to include values determined by true excreta digestibility (Prinsloo, 1993; Brand *et al*., 2004) as well as apparent ileal digestibility (Creswell & Swick, 2001b). The apparent ileal digestibility method tends to give lower estimates than those based on true digestibility measurements, and in particular lower digestibility coefficients for threonine due to the high threonine content of intestinal secretions (Creswell & Swick, 2001b).

A high degree of variability in total amino acid values between laboratories may also be expected for identical samples (Engster *et al*., 1985). There are a number of factors that contribute to the increased variability of results. They include the specific bioassay- and analytical methods applied, expression of results as apparent or true digestibility, the age or physiological stage of experimental animals used, as well as between- or within-species variability (Gatel, 1994). Literature findings tend to reveal only selective information regarding the methodology used, thereby masking some of the effects of the factors that contribute to the increased variability of results. A need therefore exists to establish a standard, practical method for determining and reporting the amino acid digestibility of feedstuffs, particularly for lupins and other grain legumes.

Table 1.10 Summary of reported values for amino acid composition (% protein) and digestibility of *Lupinus angustifolius*

Amino acid composition [1 - 15]	Minimum reported value	Maximum reported value	Average
Arginine	9.88	12.00	11.17
Histidine	2.39	2.75	2.52
Isoleucine	3.29	4.52	3.86
Leucine	6.25	7.24	6.60
Lysine	4.32	5.47	4.83
Methionine	0.41	0.72	0.58
Phenylalanine	3.43	4.25	3.74
Threonine	2.98	3.39	3.28
Tyrosine	3.11	3.51	3.31
Valine	3.04	4.25	3.68
Amino acid digestibility [1,5, 9] (%)			
Arginine	87.99	95.36	92.48
Histidine	87.90	92.11	89.63
Isoleucine	80.00	92.74	84.30
Leucine	86.50	93.91	89.60
Lysine	75.30	84.40	83.04
Methionine	75.30	82.35	78.96
Phenylalanine	82.90	93.16	87.60
Threonine	77.01	92.31	84.16
Tyrosine	94.87	95.79	95.28
Valine	79.04	90.08	83.57

[1] Brand *et al*., 2004; [2] Steenfeldt *et al*., 2003; [3] Mariscal-Landín *et al*., 2002; [4] Brand & Brundyn, 2001; [5]Creswell & Swick, 2001b; [6] Petterson, 1998; [7] Olver & Jonker, 1997; [8] Fernández & Batterham, 1995; [9]Prinsloo, 1993; [10] Múzquiz *et al*., 1989a; [11] Batterham *et al*., 1986; [12] Barnett & Batterham, 1981; [13] Batterham, 1979; [14] Pearson & Carr, 1976; [15] Hove, 1974

What separates the inclusion of these feedstuffs from other raw material sources, is the influence of their anti-nutritional factors on digestive functions, which results in altering the release of endogenous secretions (Gatel, 1994). This has specific reference when using true digestibility values. These values generally takes into account the endogenous contribution of animals fed an experimental protein-free diet, but this contribution could differ substantially from animals fed a natural diet, especially those containing grain legumes such as lupins, and may lead to inaccuracies in the estimation of the endogenous secretion component (Gatel, 1994). However, one could argue that this characteristic should be taken into account when establishing a nutritional value for lupins, and therefore it has been suggested that apparent digestibility values may be more reliable. When using apparent digestibility values, the level of food (protein) intake becomes critical, with too low intakes resulting in the endogenous secretion amounting to a proportionately larger part of the total digesta, thus leading to severe underestimation of digestibility for certain amino acids. The effect of a species difference was indicated by Batterham *et al*. (1986a), who found a higher digestibility coefficient for chickens (0.81-0.95) than for pigs (0.53). Ten Doeschate *et al.* (1993) evaluated the influence of age on nutrient digestibility values with

broilers. They concluded that even though an age effect was observed, it was rather inconsistent regarding its effect on both nitrogen and amino acid digestibilities and indicated that nitrogen does not describe true protein digestion accurately. The authors also indicated that the method of determining faecal nitrogen (i.e. with or without uric acid) influenced the results. If urinary nitrogen excretion is not constant (as in the case of older chickens that tend to experience increased excretion), differences between amino acid and nitrogen digestibilities could be observed, resulting in an underestimation of N digestibility with older chickens. This could possibly explain the significantly lower apparent protein digestibility (N x 6.25) values for adult birds that were observed by Carré *et al*. (1991), when they fed peas (*Pisum sativum*) to young and adult birds.

Factors that influence the availability of nutrients from lupins are mostly associated with the energy diluting effect of CF as well as the anti-nutritive properties of oligosaccharides and NSPs. These NSPs can influence the digestion of fat and protein in broilers (Edwards & Van Barneveld, 1998), thereby influencing the available energy and amino acid contents. Semino *et al*. (1989), for instance, have characterized carbohydrates in lupins that were bound to proteins, and resulted in interference with proteolysis. It is thus important to not only include CF as a nutritional constraint when formulating diets for monogastrics, but also to make provision for other dietary fiber components. Lupins have a carbohydrate content of more than 40% (Bach Knudsen, 1997), which consists of a wide range of components, including oligosaccharides and soluble NSPs. These components do not affect digestion in the same way and to the same extent, and not even the same category of NSP always exerts the same effect. The α-galactosides of lupins were found to produce flatulence for instance, but the α-galactosides of peas did not (Gatel, 1994). The large variation in the physicochemical properties of NSPs could account for their variable effect on nutrient digestibilities. Unfortunately, the relationship between the structure and physiological activity of these polysaccharides are still poorly understood (Åman & Graham, 1990). Hence, when lupins are used in monogastric diets, the physiological effects of other dietary fiber components must be accounted for.

It is therefore, not surprizing to find similar variation amongst literature findings regarding the maximum inclusion level of lupins in monogastric diets. Brenes *et al*. (1993) found that broiler chickens can tolerate up to 25% of low-alkaloid lupin seed meal without adversely affecting growth, provided that adequate supplements of lysine and methionine are given. The importance of supplementing lupin diets with methionine and lysine were also indicated in several reports. Zaviezo & McGinnis (1980) fed unsupplemented diets containing sweet *L.albus* seeds to day-old chicks, and found it resulted in poor growth performance. Subsequent supplementation of methionine resulted in a significant improvement in growth and feed conversion efficiency, however, the birds showed no response to the addition of lysine. Karunajeewa & Bartlett (1985) concluded that broiler starter diets could contain 22.4% *L.albus* cv. Hamburg with no adverse effect on growth performance when adequately supplemented to meet the chick's requirements for methionine, lysine and metabolizable energy. These synthetic amino acids are now competitively priced and could be added to improve the protein value of lupins. Some authors reported a maximum inclusion level of sweet *L. albus* seeds in broiler diets of up to 30% (Watkins *et al*., 1988; Perez-Escamilla *et al*., 1988) and even as high as 40% (Olver, 1987; Olver &

Jonker, 1997) and found no significant differences in growth and feed efficiency when compared with a lupin-free control diet.

The level of inclusion for *L.angustifolius* for broiler diets has been reported to be slightly different to that of *L. albus*, which could be due to the small number of authors reporting on the nutritive value and maximum inclusion level for broiler diets of this particular species of lupin. Yule & McBride (1976) observed that broilers fed diets containing up to 24% ground lupin seed (*L.angustifolius* cv. Uniwhite) grew as rapidly as those fed wheat-based diets when these were balanced for amino acids and energy. Johnson & Eason (1990) also found similar results with *L.angustifolius* cv. Yandee. The inclusion of 180 g/kg resulted in growth and performance of broilers equal to those of the soyabean-control. A year later, the same authors observed a 2% reduction in liveweight of broilers at 42 days when they were fed diets containing only 150 g/kg *L.angustifolius* from Victoria, Australia (Johnson & Eason, 1991). Although this difference was not significant, the authors suggested that such inclusion levels could result in an economic loss. When interpreting these results, however, it should be noted that the diets were formulated on a total amino acid basis, and no allowance were made for differences in nutrient digestibilities between that of lupins and soybean meal. The use of different *L.angustifolius* cultivars could also have influenced the results and highlights the need to establish a complete, reliable database of nutritional information regarding the local lupin species and cultivar. The highest inclusion level of *L.angustifolius* for broilers was at 40%, when Olkowski *et al*. (2001) fed diets containing the cultivar Troll for 21 days and experienced significant decreases in feed intake and growth rate in all birds fed lupin-based diets. Acute signs of toxicity were also observed in some individuals. This was, however, the only study reporting on adverse effects of such magnitude due to the feeding of sweet *L.angustifolius* to broilers. The feeding of bitter varieties of lupins should be avoided, since the anti-nutritive properties of the alkaloids suppress both feed intake and growth (Guillaume *et al*., 1979). Results reported by Olver & Jonker (1997), support these findings, as the 6-week old broilers fed on the 40% bitter lupin diet weighed only 72% of and consumed only 88% of the food eaten by those broilers on the soyabean control diet. These effects were also more marked during the initial 3-week feeding phase. Halvorson *et al*. (1983) found that the inclusion of 20% *L.albus* cv. Ultra had no adverse effects on the growth performance of young turkeys, but 30% or more white lupins depressed weight gains significantly. These findings were also supported by Perez-Escamilla *et al*. (1988), who showed that diets containing 40% or 60 % lupins significantly reduced food intake and weight gain for turkey poults.

Farrell *et al*. (1999) recommended an optimum inclusion of sweet lupins (*L.angustifolius* cv. Gungurru) for broiler starter diets to be less than 10% but slightly higher (12-15%) for finisher feeds. The reason being that older birds appear to be better adapted to withstand the incidence of increased gut viscosity and wet droppings that are usually associated with the feeding of high levels of lupins. Constraints on the maximum inclusion level of lupins in broiler diets are not necessarily due to drops in production above this level, but due to the incidence of wet-sticky droppings that may be promoted by high levels of lupin NSPs. These wet-sticky droppings pose a health risk to broilers through adverse affects on litter moisture levels and respiratory stress from high ammonia levels.

The high incidence of wet-sticky droppings is not of great concern for caged laying birds. Edwards & Van Barneveld (1998) indicated that a maximum inclusion level of 25-35% of raw *L.angustifolius* or *L.albus* will not affect laying performance. The results are in agreement with those of Prinsloo *et al*. (1992), where the inclusion of 30% raw, sweet *L.albus* seeds had no deleterious effect on performance and egg quality of laying hens. Perez-Maldonado *et al*. (1999) concluded that sweet lupins (*L.angustifolius* cv. Gungurru) also support excellent production when included in layer diets at 25%, but warns that the incidence of increased digesta viscosity could warrant even lower inclusions. Watkins & Mirosh (1987), however, found that the inclusion of 25% raw lupins (*L.albus* cv. Ultra) resulted in lower egg weights and egg production also dropped when 30% raw lupins were fed for 32 weeks. Similar to broiler diets, it is recommended that raw lupins should not be included in excess of 10-15% in layer diets, since the higher inclusion levels may increase the incidence of dirty eggs as a result of the wet droppings, even though laying performances are not jeopardized. Lupins appear to be an excellent source of egg yolk pigment. El-Difrawi & Hudson (1979) determined the carotenoid content of several lupin species with *L.angustifolius* containing 133 mg/100g β-carotene and 500 mg/100g zeaxanthin, which suggest that they are good sources of provitamin A for animal feeds. The mean egg yolk colour score was significantly ($P<0.001$) affected by lupins as well as the level of lupins, with the highest yolk colour score of 8.86 (Roche yolk colour fan) observed when hens were fed 25% lupins (Watkins & Mirosh, 1987).

Other applications of lupins in monogastric nutrition include that of a protein source for pig diets. The Standing Committee on Agriculture in Australia recommends an inclusion level of *L.angustifolius* for pig starter or weaner diets at 10-15%, for grower diets at 20-25%, for finisher diets at 30-35% and dry and lactating sow diets can include 20% lupins. This recommendation have been supported by various authors who have successfully incorporated seeds of *L.angustifolius* in diets for grower-finisher pigs (Taverner, 1975; Pearson & Carr, 1976; Batterham, 1979; Barnett & Batterham, 1981) when these diets were adequately supplemented with the limiting amino acids lysine and methionine. Pearson & Carr (1977) evaluated the inclusion of *L.angustifolius* and *L.albus* in diets for growing-finishing pigs and found considerable differences between the species regarding their suitability as protein sources. They concluded that either of the two *L.angustifolius* cultivars (Uniwhite or Uniharvest) could be included at levels of up to 43%, supporting similar growth rates and feed efficiency of the barley-based control, but that the inclusion of *L.albus* cv. Neuland resulted in severe feed refusal. The high manganese content (1303 ppm) as well as the alkaloid content (0.09%) and composition (Ruiz *et al*., 1977) of this particular lupin cultivar has contributed to the undesirable effects. Other authors experienced similar results of depressed feed intake and growth with the inclusion of various cultivars of *L.albus* seeds in pig diets (Kemm *et al*., 1987; Donovan *et al*., 1993; Ferguson *et al*., 2003). Together with the more variable results obtained with *L.albus* than with *L.angustifolius* (Farrell *et al.,* 1999) it has lead to the conclusion that pigs do not perform as well on *L.albus* when compared to *L.angustifolius,* and this specific lupin species is currently not recommended for use in pig diets.

1.4.4 Anti-nutritional factors in Lupins

All legumes contain a number of ANFs which interfere with nutrient availability to various extents, and whose presence is responsible for reports of suboptimal animal performance and sometimes even toxicity. It should be kept in mind that the sensitivity to these ANFs appears to be influenced by species and age of animals, and varies according to the different ANFs under consideration. For instance, chickens were found to be more sensitive to saponins than pigs, but in the case of tannins they were less sensitive than piglets (Huisman & Tolman, 1992). On the other hand, older birds appear to be more tolerant towards the ANFs of chick peas than younger birds (Farrell *et al*., 1999), but this is again dependent on the source of ANFs, since the opposite has been found for beans (Davidson, 1973), where chicks seem to be the least effected between the two age groups.

The main ANF that limits the use of lupins in monogastric diets is their alkaloid content (Edwards & Van Barneveld, 1998). Alkaloids reduce the palatability of lupin-containing diets, and for monogastric animals even relatively low alkaloid contents can suppress feed intake and growth (Olver, 1994). Poultry appear less sensitive to alkaloids than pigs (Huisman & Tolman, 1992). Guillaume *et al.* (1979) observed a significant decrease in food intake, growth and food efficiency of broiler chickens, when the amount of sweet lupins in their diet were replaced by increasing levels of lupin alkaloids, mainly lupanine.

Lupins contain variable levels of quinolizidine alkaloids with the total content varying from 0.1-4% in bitter varieties to < 0.1% in sweet varieties. The most common alkaloids are lupanine, sparteine, lupinine and angustifoline (Petterson, 1998). The various proportions of these alkaloids in three species of lupins are presented in Table 1.11. In *L.albus* and *L.angustifolius* the major alkaloid present is lupanine, while in *L.luteus* it is lupinine.

Table 1.11 Total alkaloid contents (g/kg) and proportions of alkaloid components (%) in three species of *Lupinus* (Múzquiz *et al*., 1989b)

	L.angustifolius	*L. albus*	*L.luteus*
Total alkaloid content (g/kg)	0.0 – 15.0	0.0 – 38.0	0.4 – 19.0
Lupanine (%)	21.8 – 66.6	80.7 – 98.7	_
Angustifoline (%)	20.2 – 49.5	0.0 – 0.8	_
Sparteine (%)	_	_	0.0 – 2.5
Lupinine (%)	_	_	35.2 – 100.0
Gramine (%)	_	_	0.0 – 11.0

Plant breeders have successfully cultivated sweet lupin varieties with low (< 0.1%) alkaloid contents and even with prolonged intake of these sweet lupin seeds, there should be no concern regarding toxicity. Contamination with bitter seeds, however, could result in lupinosis (Olver, 1994). Lupinosis is a mycotoxicosis that is primarily recognized as a disease of sheep, but natural outbreaks have also been reported in cattle, goats, donkeys, horses and pigs (Allen, 1998). It is mainly characterized by severe liver damage, which results in inappetence, loss of condition, lethargy and often death. Neural inhibition have been reported as the primary metabolic effect of alkaloids (Huisman & Tolman, 1992).

The alkaloid contents of South African lupin cultivars, from all three major lupin species, have been analyzed by Brand & Brandt (2000) and the results are presented in Table 1.12. It is clear from the data that the variability in alkaloid content of *L. albus* is much greater than for the other two species and special attention is needed in this regard when *L. albus* is included in monogastric diets.

Table 1.12 Alkaloid content of South African lupin cultivars (Brand & Brandt, 2000)

Lupin species and cultivar	Number of samples (n)	Average alkaloid content (%)
Lupinus luteus		
Juno	2	0.004
Borsaja	2	0.002
Lupinus albus		
Astra	2	0.092
Hamburg	3	0.312
Kiev	5	0.087
Buttercup	3	0.159
Lupinus angustifolius		
Eureka	2	0.006
Helderberg	2	0.003
Merrit	2	0.005
Moredou (bitter)	2	1.521

Low-alkaloid lupins are generally regarded as very safe with respect to ANFs. The seed does, however, contain several anti-nutrients (Table 1.13) of which the oligosaccharides and soluble NSPs probably exert the biggest effect on nutrient availability (Steenfeldt *et al*., 2003). It is also likely to be responsible for a large part of the variation observed in nutrient digestibilities. The influence of oligosaccharides and soluble NSPs on nutrient availability of lupins will therefore be discussed hereafter. Other ANFs such as phytic acid, tannins, saponins, protease inhibitors and lectins are also found in variable quantities in lupins (Table 1.13), but their concentrations are generally lower than found in most other legumes and oilseeds (Hove & King, 1979; Múzquiz *et al*., 1989b; Ruiz *et al*., 1995; Allen, 1998; Petterson, 1998). These anti-nutrients may also be classified according to their ability to withstand thermal processing, the most commonly employed treatment for destroying them (D'Mello, 1995). Heat-labile factors include lectins and protease inhibitors, whereas saponins and NSPs are regarded as heat-stable factors. Oligosaccharides become somewhat more digestible after heat treatment, whereas the effect on tannins remains uncertain (Francis *et al*., 2001).

As previously shown by Carré *et al*. (1985), lupin cotyledons have a high percentage of cell wall material (20%) that is rich in pectic substances. These α-galacto-oligosaccharides are extremely susceptible to fermentative breakdown, and was found to act mainly as diluents in poultry feeds (Carré & Leclercq, 1985). Because of the lack of intestinal enzyme α-galactosidase in mammalian tissue, these oligosaccharides escape hydrolysis and absorption in the small intestine, but are readily fermented by bacterial α-galactosidase in the lower gut. The bacterial digestion produces carbon dioxide, hydrogen and methane, and may result in flatulence, diarrhoea, nausea, cramps, and abdominal discomfort with a resultant decrease in animal performance (Van Kempen & Jansman, 1994). It has even been reported that the high concentration of stachyose in lupin seeds (more than twice that of other legumes) have been responsible for the death of one pig, which suffered from stomach distention with gas, followed by rupture (Mariscal-Landín *et al*., 2002). The

energetic efficiency of the volatile fatty acids that are generated by hindgut fermentation of cell wall polysaccharides, is considerably lower than the sugar release in the small intestine, and in the case of pigs, this could create a wider disparity between the apparent digestible energy and the net energy values for lupins relative to other feedstuffs. The oligosaccharides, however, also play a role in the osmotic regulation of the gastrointestinal tract, which may be beneficial (Petterson, 1998).

Table 1.13 Comparing anti-nutritional factors in lupins to those of other legumes (Allen, 1998)

	LUPINS		SOYABEAN	FIELD PEA
ANTI-NUTRITIONAL FACTORS	*L.angustifolius*	*L.albus*	*Glycine max*	*Pisum sativum*
Oligosaccharides (%)	5.16	6.69	5.75	3.69
Phytic acid (%)	0.58	0.79	1.59	0.48
Tannins total (%)	0.32	0.37	0.57	0.25
Tannins condensed (%)	< 0.01	0.01	-	0.06
Saponins (%)	573	10 – 23	3500	1800
Trypsin inhibitors (mg/g)	0.14	0.13	17.9	1.01
Chymotrypsin inhibitors (mg/g)	0.08	-	-	1.60
Lectins (dilutions)	ND	ND	> 4	4

ND = not detected

The anti-nutritive properties of some NSPs for poultry have been clearly demonstrated with rye (Bedford *et al*., 1991), wheat (Choct & Annison, 1992) and barley (Campbell *et al*., 1989). It is especially the water-soluble portion, about 4-7% of *L.angustifolius* (Evans *et al*., 1993) that is considered to have an anti-nutritional effect. The mechanisms by which soluble NSPs exert their anti-nutritive effect are complex and have been studied by various authors (Smits & Annison, 1996; Chesson, 1990). In addition to the direct impairment of nutrient absorption by high gut viscosity (Mohanna *et al*., 1999), the detrimental effect of soluble NSPs on bird performance appears to be indirectly related to a proliferation of fermentative microflora in the small intestine (Carré *et al*., 1995; Choct *et al*., 1996). These microflora can compete directly with the host for potentially absorbable substrates if the absorption rates are reduced by the presence of viscous polysaccharides (Chesson, 1990). As a result of these microbial effects, an overestimation of nutrient uptake by the bird can occur when faecal digestibility values are estimated for diets that are high in soluble NSPs, and have special reference when determining the AME value of a diet (Choct *et al*., 1996). The soluble portion of total NSP content in sweet lupins (6.7%) is almost twice that of other grain legumes such as field peas (3.25%) and faba beans (3.1%) and Perez-Maldonado *et al.* (1999) found these high NSP contents to be in accordance with the higher gut viscosity observed in laying hens. The increased digesta viscosity will decrease the rate of diffusion of substrates and digestive enzymes, as well as interfering with their effective interaction at the mucosal surface. Viscous NSPs may also be able to entrap bile salts and thereby reducing their effectiveness in solubilizing the fat components and subsequently lipid absorption (Smits & Annison, 1996). These soluble polysaccharides are also related to the incidence of wet-sticky droppings, which as discussed in this section is one of the major factors limiting the inclusion of lupins in broiler diets (Chesson, 1990).

Theoretically, the adverse effects of soluble NSPs on growth could be diminished with the addition of enzymes that depolymerize the NSPs, leading to reduced viscosity of the gut contents and improved nutrient absorption. The addition of exogenous enzymes to lupin diets has, however, been met with variable results. Reported improvements ranged from no effect to a significant increase in digestibility, AME content and performance (Brenes *et al*., 1993; Annison *et al*., 1996 and Kocher *et al*., 2000). The use of exogenous enzymes is beyond the scope of this study, but in the following section dealing with processing methods of lupins, some literature findings regarding the use of exogenous enzymes will briefly be mentioned.

1.4.5 Processing of Lupins

Even though sweet lupins do contain a few ANFs, they are mostly at low concentrations. Unlike whole soybeans and many other grain legumes, lupins do not require specific heat treatments to destroy the heat labile ANFs that may interfere with nutrient availability. It is, however, evident from the literature that the nutritive value of lupins is not as high as would be predicted from knowledge of its chemical composition. Several methods of processing have been tested to improve the nutritional value of lupins, particularly through the destruction of anti-nutritional factors and possibly through increased accessibility of protein to enzymatic hydrolysis.

One way to counteract possible anti-nutritive effects caused by both the NSPs and α-galactose-containing oligosaccharides in lupins is to supplement the diets with enzymes to degrade these carbohydrates. The response to mixed enzyme supplementation is extremely variable, but tends to be greater for diets with a high cell wall content (Wiryawan *et al*., 1997), as well as high NDF and low ME values (Wiryawan *et al*., 1995). Brenes *et al*. (1993) supplemented lupin-based diets with crude enzyme preparations (combinations of carbohydrases, proteases and galactosidases) and found that it resulted in substantial improvement in terms of weight gains and feed efficiency for chickens. The same improvement in nutritive value, however, was not observed when α-galactosidase was added to dehulled lupins (Brenes *et al*., 2003). It has been suggested that the α-galacto-oligosaccharides (raffinose and stachyose) do not greatly affect chick performance, and this may contribute to the lack of significant response in chick performance when these enzymes are added (Brenes *et al*., 2003). Protease, xylanase and cellulase enzymes were added to broiler mash diets, containing 40% *L.albus* seeds, to investigate the possible improvement in the growth depression and reduced feed efficiency that was induced by the lupin-based diet. Supplementation of the diet with either xylanase or cellulase improved performance, by reducing the deleterious effects of cellulose and NSP, but no benefit was observed with the addition of protease or a mixture of the enzymes (Naveed *et al*., 1998). Kocher *et al*. (2000) also showed that the addition of a commercial enzyme preparation to a lupin-based diet for broilers resulted in a significant increase in the ileal digestibility of NSP. This was mainly related to the digestibility of glucose, xylose, arabinose and polymers that were found predominantly in the hull fraction. The effect of enzymes on the NSPs of lupins could have resulted in an improvement in their fermentability in the hindgut (increased solubility), leading to an improvement in AME of the diet (Annison *et al*., 1996). The inclusion of pectinases, however, maintained high digesta viscosity, but this in turn did not result in a depression of growth performance or

protein digestion (Annison *et al.,* 1996; Kocher *et al*., 2000). The mechanism of enzymatic hydrolysis is substrate specific, and because of the highly variable content and composition of non-starch polysaccharides in lupins, it is not surprizing to find just as many inconclusive results regarding the effect of enzyme supplementation. The benefits, in terms of improved bird performance, that have occasionally been observed with the appropriate enzyme supplementation, provides a challenge for further research into this field, but the difficulty in determination of individual NSP components that serve as substrates, will first have to be addressed.

The lupin seed coat has a negligible protein value and more than 50% CF, which is of limited nutritional value to monogastrics. At 20 to 25% of the seed, the hull substantially dilutes the protein nutritional value (Hove, 1974). In pigs there are partial recovery of the energy in lupin hulls via hindgut fermentation, but in poultry they act mainly as diluents. Removing the hull, will therefore lead to an increase in the CP and fat content with a decrease in the CF content. The chemical and amino acid composition of whole lupin seed, -kernels and -hulls of *L.angustifolius* cv. Gungurro are presented in Table 1.14.

Table 1.14 The chemical and amino acid composition (%, air-dry basis) of whole lupin seed, dehulled lupin seed and lupin hulls (Fernández & Batterham, 1995)

Composition (%)	Whole lupin seed meal	Dehulled lupin seed (kernel)	Lupin hulls
Approximate yield	100.00	75.00	25.00
Dry matter	92.20	92.60	94.00
Crude protein (N x 6.25)	31.10	40.50	5.60
Crude Fat	4.60	7.60	1.50
Crude Fiber	17.20	5.10	61.90
NDF	31.30	11.00	7.47
S-NSP	15.00	19.70	7.60
I-NSP	31.50	13.00	70.90
Ash	3.00	2.86	6.67
Alkaloids	0.07	0.13	<0.01
Gross energy (MJ/kg)	17.90	19.30	15.80
Essential amino acids			
Lysine	1.60	2.00	0.39
Methionine	0.16	0.21	<0.4
Cystine	0.25	0.31	<0.3
Valine	1.30	1.70	0.29
Threonine	1.20	1.50	0.24
Histidine	0.80	1.10	0.13
Isoleucine	1.40	1.90	0.25
Leucine	2.20	2.90	0.40
Tyrosine	1.20	1.60	0.18
Phenylalanine	1.30	1.70	0.25

NDF, Neutral detergent fiber; S-NSP, Soluble non-starch polysaccharides; I-NSP, Insoluble non-starch polysaccharides.

It should be noted that the actual kernel:hull ratio in lupin seed is approximately 80:20 but in the commercial dehulling process the yields are closer to 75:25 due to some kernel chips and flour being removed with the hulls during the dehulling process (Edwards & Van Barneveld, 1998). For the same reason the protein content of the lupin seed components (kernels and hulls) as reported in Table 1.14, are not always achieved with commercial dehulling, where values closer to 35% for the kernel and 7-10 % for the hulls are more common (Petterson, 1998). The dehulling of *L.angustifolius* increased the protein (40.5 vs. 31.1%), fat (7.6 vs.

4.6%) and lysine content (2.0 vs. 1.6%) in relation to the whole lupin seed. The removal of the fibrous hull of lupin seed also resulted in a kernel component with a substantially reduced CF (5.1 vs. 17.2%) and insoluble NSP content (13.0 vs. 31.5%). Fernández & Batterham (1995) also reported that dehulled lupins increased the digestible energy content and the ileal digestibility of amino acids (lysine, 0.88 vs. 0.82) for pigs.

Steenfeldt *et al*. (2003) reported a 30% increase in the protein value (445 g/kg DM) as well as the fat content (94 g/kg DM) of *L.angustifolius* cv. Emir with dehulling. The total NSP content of the seed was also reduced from 45% to 32.3%, seeing as though the major portion of NSPs are located in the hull (824 g/kg DM). This could explain the higher digestibility of energy (+18%) and that of protein (+7%) observed when Brenes *et al*. (1993) compared dehulled to whole lupins. Improved broiler weight gains (151g vs. 135g) and feed to gain ratios (1.66 vs.1.78) were also observed for the dehulled lupin diet after an 8-day feeding trial (from 10 to 17 days of age), but these differences were not significant when compared to the whole lupin diet. Lupin kernels tend to be of greater economic value to poultry than the whole lupin seed, but the economics of dehulling are dependent on the opportunity to be able to utilize the hull component effectively. Lupin hulls could be incorporated into ruminant or sow diets, and even as diluent in pig finisher diets (Edwards & Van Barneveld, 1998). Brand (1996) concluded that the dehulling of lupins could increase its value by 30%.

The successful processing of lupins also depends on the physical properties of the seed and to what extent it complements the processing techniques. The spherical character of the seed provides good flowing attributes and ease of handling at the processing plant and can easily be crushed or ground. The fine grinding of lupins is especially important when included in pig diets, since it has been found that coarse crushing reduces the digestible energy value of lupins (Edwards & Tucek, 2000). Consequently, the authors recommended a hammer mill screen size of 3.0 to 3.25 mm in diameter. The high fiber content of the lupin hull and the rubbery nature of the kernel fraction provides some resistance to grinding, and usually results in a lower throughput rate for lupins than for grain. The production rate through the pellet press on the other hand, depends largely on the conditioning parameters such as temperature, time and steam, with high steam and fine grinding accentuating the "stickiness" associated with the high NSP content of lupins (Edwards & Tucek, 2000). This property is extremely useful as a pellet binder and improves pellet quality. Edwards & Tucek (2000) suggested that these advantages will even offset the reduced throughput rate of lupins at the pellet press. Another positive attribute of lupins is the deep golden colour of the lupin seed endosperm, which enhances the physical presentation of the feed, especially in mash diets.

The absence of significant levels of heat-labile ANFs in lupins, such as protease inhibitors and lectins that are found in other legume species, could limit the improvement in the nutritive value of lupins when subjected to heat treatments. The benefits of thermo-mechanical treatments such as pelleting or extrusion are therefore more likely to be contributed to the breakdown of the cell walls, which allows accessibility of nutrients to digestive enzymes, rather than to the inactivation of ANFs (Gatel, 1994). This was illustrated by Farrell *et al*. (1999) who steam-pelleted (70 – 80°C) legume diets and observed improved broiler growth rates and feed conversion ratios for all diets fed from 0 to 42 days. While the beneficial influence of steam pelleting on the productive energy value of some poultry diets have been shown (Reddy *et al*., 1962), it appears as if the

effects are more pronounced when the feedstuff contains high levels of fiber or heat labile growth depressants. The differences in energy availability attributed to the steam pelleting process may reflect differences in energy expenditure during the act of eating (birds spend more time and energy consuming a mash diet than pellets), as well as differences in feed consumption, since broilers are known to consume more food in pelleted form than in a mash form (Reddy *et al*., 1962). This effect on voluntary feed intake could have influenced the increased AME values observed for pelleted *vs*. mash diets, but due to the fact that TME values are independent of feed intake levels, it is not surprizing to find that steam pelleting will not influence the TME value of poultry diets (Sibbald, 1977b).

To use heat treatments effectively, the temperature and duration of processing have to be carefully controlled. Excessive heating can result in reduction of protein solubility and may even destroy or reduce the availability of certain amino acids, especially lysine. Van Barneveld *et al*. (1993) reported that the availability of lysine from heat-treated peas for pigs was decreased by 21% when the heating temperature was increased from 110 to 150 °C for 15 min. With prolonged or elevated heating, basic amino acids such as lysine, undergo a Maillard reaction that reduces their digestibility and biological availability. This process is characterized by the nonenzymatic browning reactions that occur between the ε-amino group of lysine and the carbonyl groups of reducing sugars such as the oligosaccharides raffinose and stachyose (Parsons *et al*., 1992). Consequently, the heat treatment of feedstuffs such as lupins with a high content of these sugars will have to be monitored carefully. However, the damage to proteins that are heated in the absence of carbohydrates have also been studied (Bjarnason & Carpenter, 1969; Varnish & Carpenter, 1975), and it has been found that cross-linkages in proteins can also occur with the amide groups of asparagine and glutamine during severe heat treatment. These cross-linkages generally reduce the rate of protein digestion by hindering enzyme attack *in vivo* (Hurrel *et al*., 1976). The influence of temperature and duration of heat treatment on the nutritional value of oilseeds such as soyabeans (Herkelman *et al*., 1993; Perilla *et al*., 1997) and sunflowers (Zhang & Parsons, 1994) have been well documented and guidelines exist regarding the optimum processing conditions. However, when it comes to legumes and other feedstuffs that are not readily heat treated, very little information exists. The biological response to heat treatment will depend on the composition of the feedstuff as well as the presence of heat-labile ANFs. The extended steam heating of *Phaseolus vulgaris* beans from 40 to 80 min. resulted in lower weight gains and higher feed conversion ratios in piglets (Van der Poel, 1990). It is therefore important to find the exact conditions of heating for a specific feedstuff that will maximize the improvement of the nutritive value, but without implicating the economics of processing.

Heat treatment of *L.albus* seed has been shown to produce variable effects on nutrient utilization (Boldaji *et al*., 1986) and hen performance (Watkins & Mirosh, 1987). The treatment procedure used by Boldaji *et al*. (1986) included cooking the lupin seeds in boiling water for 30 or 60 minutes, as well as autoclaving at 120°C for 15, 30 and 60 min. respectively. The GE, TME and TMEn content for White Leghorn roosters were determined and the results are presented in Table 1.15.

It is noteworthy that these procedures were conducted at different time periods (Experiment 1 & 2 in Table 1.15). The authors reported a significant improvement in the TME and TMEn values when the

autoclaving time was increased from 15 to 30 min. During experiment 2, they found that the TME and TMEn values for lupin seed could be improved with 30 minutes cooking and 60 minutes autoclaving, but that prolonged cooking (60 min.) resulted in a small numerical decrease in the available energy content. In aggreement with these results Ravindran & Blair (1992), showed that a relatively short period of cooking could improve the biological value of protein, whereas extended cooking, on the other hand, could adversely affect protein quality and should be avoided. Other researchers have also examined the effect of autoclaving on the nutritive value of lupins for poultry (Watkins & Mirosh, 1987; Perez-Escamilla *et al*., 1988; Brenes *et al*., 1993) and pigs (Batterham *et al*., 1986a,b), but have either showed no beneficial effects, or the results were inconsistent. The potential damage of protein with autoclaving, however, is high because of the long treatment times. It is also an impractical process for large-scale commercial production and are only used on an experimental basis.

Table 1.15 Effect of autoclaving and cooking on gross energy (GE), true metabolizable energy (TME) and nitrogen corrected TME (TMEn) content of *Lupinus albus* seed (adapted from Boldaji *et al*., 1986)

Treatment	GE	TME[1]	TMEn[1]
		MJ/kg	MJ/kg
Experiment 1			
Raw	20.21	12.05 [ab]	9.46[ab]
Autoclaved, 15min.	20.50	10.50 [a]	8.45 [a]
Autoclaved, 30 min.	21.63	13.26 [b]	10.84 [b]
Experiment 2			
Raw	20.20	10.96 [a]	9.58 [a]
Cooked, 30 min.	20.05	11.80 [a]	10.25 [a]
Cooked, 60 min.	20.38	10.59 [a]	9.50 [a]
Autoclaved, 60 min.	20.59	11.92 [a]	10.75 [a]

[1]Each value is the mean of 3 determinations.
[ab]Mean values within a column with different superscripts are significantly different (P<0.05).

Other alternative processes have been developed where feed or feed components are treated by extrusion. The extrusion method utilizes friction as the sole source of heat, accompanied by pressure. Heat and pressure are developed by passing the product through an extruder barrel by means of a screw with increasing restrictions. Then, the sudden decrease in pressure when the product is discharged through the die into the atmosphere results in expansion of the product. By vaporizing moisture, cell structures are ruptured. The amount of expansion depends on several factors, such as the composition of the product, temperature, pressure and the amount of moisture (Woodroofe, 1999). Traditionally the dry extruder was used solely for the processing of whole soybeans, since the high internal oil content of the beans acted as lubricant during the extrusion process and the addition of moisture was thus not necessary (Woodroofe, 1999). However, more recently it has been shown that preconditioning the soyabeans with steam prior to extrusion, has lead to more efficient processing of the oilseed (Woodroofe, 1999). Extruders are simple screw machines, but can be extremely versatile in their application, with or without steam preconditioning. During extrusion it is the cooking process (as the product moves through the barrel with increasing heat) as well as the expansion process (as the product explodes from the die opening to the atmosphere) that are responsible for the changes occurring

in the major constituents of feed materials. These changes that occur with extrusion have been researched by Woodroofe (1999) and will briefly be discussed in terms of protein, starch, fat and fiber.

The cooking action of extrusion creates sufficient heat to break down the secondary bonds of protein, but does not adversely affect the primary bonds between amino acids. It is this effect of denaturization that is useful, since the mild breakdown of protein structure could improve digestibility. Also many proteinaceous constituents inhibit normal digestion (trypsin inhibitors in soyabeans) or reduce shelf life of products (lipase in rice bran), and could therefore be inactivated by extrusion.

Raw starch has little water holding capacity, but when heated in the presence of water, the starch granules swell and then break up to form a more homogenous, loosely packed structure that resembles a gel. This process of conversion of starch from the raw state to the gel state is known as gelatinization. Gelatinized starches have a strong binding ability and tend to be more digestible than raw starch. The relevance of gelatinization in monogastric nutrition has been reviewed by De Wet (2000), but this is not considered to be of special interest to our study with lupins, since the legume contains too little starch to influence the nutritive value thereof through extrusion.

The main effect of extrusion processing on fats are the release of the fat or oil that is encapsulated within the cells by either the shear force or expansion of the product once it makes contact with the atmosphere. The fat quality in terms of off odors and oxidation, will also not be adversely affected by the heat generated from extrusion.

The effect of extrusion on fiber is related primarily to its bulk density. The mechanical action of the extruder breaks up and compresses the fibrous material, thereby changing their bulk density. High fiber ingredients such as lupins are normally low in moisture and also take on moisture slowly. These materials usually require some type of pre-conditioning with extrusion.The beneficial nutritional effects of extrusion will vary with species, age and type of ration being fed, but in general these benefits can be summarized in terms of the change that occurs in the protein, energy and fiber content of the ration.

Plavnik & Sklan (1995) examined the effect of short time extrusion processing on the digestibility of energy and nutrients of feeds and grains in 18-21 day old broiler chicks (Table 1.16). A complete maize-based feed or wheat or barley were exposed to extrusion (125°C) and compared with the same untreated material. All diets were subsequently milled to equal size. The results are presented in Table 1.16.

The extrusion of complete diets enhanced the GE digestion and increased the AME and AMEn values by 1.5 and 3.5% respectively. The extrusion of wheat or barley and their addition to basal diets also resulted in similar increases in their AME and AMEn values. The main contributor towards the increases observed for AME and AMEn values of maize-based diets was due to enhanced fatty acid digestion. It is the mechanical shear force during extrusion processing that ruptures oil cells and thereby influencing the fatty acid digestion. Since this was not the case for the wheat- and barley diets, it has been suggested that a change in the digestion of other non-starch carbohydrate fractions could have resulted from the heat treatment, as was previously demonstrated by Choct & Annison (1992). This could have important implications for legumes such as lupins that are high in NSPs.

Table 1.16 Effect of extrusion on apparent digestibilities of nitrogen (N), fatty acids, starch, gross energy (GE), apparent (AME) and nitrogen-corrected (AMEn) metabolizable energy of maize or wheat/barley based diets (Plavnik & Sklan, 1995)

	N %	Fatty acids %	Starch %	GE MJ/kg	AME MJ/kg	AMEn MJ/kg
Maize-based diets						
Mash	68.0	75.8[b]	96.9	79.4[b]	14.19[b]	13.04[b]
Extruded	69.8	81.3[a]	97.7	80.2[a]	14.40[a]	13.55[a]
Wheat-/barley based diets						
Mash	67.3	77.8	97.6	76.8[b]	13.57[b]	12.58[b]
Extruded	66.7	77.7	98.0	77.5[ab]	13.84[a]	12.81[a]

[ab] Means within the same column (per diet) with different superscripts differ significantly (P<0.05)

Mariscal-Landín *et al.* (2002) examined the effect of extrusion on the apparent ileal digestibility (AID) of protein and amino acids of winter peas for pigs and found a significant increase in the dry matter, protein and amino acid ileal digestibilities. A large part of the improvement in amino acid digestibility, however, was related to the inactivation of trypsin inhibitors found in winter peas. Some of the improvement that was observed for the AID of protein from extruded peas, were attributed to the gelatinization effect of extrusion on starch as well as the denaturing of the protein structure due to the heat treatment, which enhances the action of digestive enzymes on seed protein. It is especially the effect of extrusion on the protein structure of peas that could prove beneficial to similar treatments for lupins, since lupins do not contain any significant levels of trypsin inhibitors or starch. Unfortunately no details were given regarding the time, temperature or pre-conditioning that were used in the extrusion processing for this trial.

There are very few studies that have reported on the influence of extrusion of lupins on broiler performance. Watkins *et al*. (1988), extruded lupin meal (*L.al*bus cv. Ultra) by heating the ground seed at 80°C for 20 seconds under pressure and formulated broiler diets to contain either raw or extruded lupins at 10, 20 and 30% of the basal diet by partial substitution of corn and soyabean meal. These diets were supplemented with DL-Methionine and L-lysine. Similar inclusion levels were used for autoclaved lupin meal (121°C for 30 min.). The diets were fed for 18 days and the body weight gains, feed efficiencies and mortalities of broilers were determined for the experimental period. The results are shown in Table 1.17. The gain and feed efficiency of chicks fed diets containing raw, extruded or autoclaved lupins were not inferior to those of the control diet. Extruding lupins also improved the feeding value of the seed over autoclaved and raw lupins, resulting in a significant improvement (P<0.05) in chick growth. The 10% extruded lupin diet yielded growth performance values superior to the control diet.

Watkins & Mirosh (1987) used the same method of processing (extruding lupins at 80°C for 20 min.) and included it at levels of 10 and 20% of a corn-soya diet, but did not observe any significant (P<0.05) improvements in egg production, egg weight and feed consumption of laying hens.

Table 1.17 Average gains, feed efficiencies (total gain/total feed) and mortality of chicks fed raw, extruded and autoclaved lupin diets (Watkins *et al*., 1988)

% Inclusion	Average gain (g)	Total gain / total feed	Mortality (No./total)
Lupin %			
0	428[bc]	0.671[ab]	0/32
Average	428	0.671	
Raw			
10	418[c]	0.675[ab]	4/32
20	431[abc]	0.680[ab]	0/32
30	428[abc]	0.671[ab]	1/32
Average	428	0.675	
Extruded			
10	462[a]	0.689[ab]	0/32
20	445[abc]	0.680[ab]	0/32
30	460[ab]	0.704[a]	2/32
Average	456*	0.689*	
Autoclaved			
10	418[c]	0.662[b]	1/32
20	450[abc]	0.680[ab]	0/32
30	441[abc]	0.675[ab]	1/32
Average	436	0.675	
Pooled SEM	10	0.02	

[a-c]Values within columns with different superscripts are significantly different ($P<0.05$).
*Average values significantly different from other average values within column ($P<0.05$).

Very little information is published regarding the effect of heat treatment on the nutritive value of lupins for broilers. There are, however, indications that extrusion processing could enhance the AME value through its influence on the digestibility of non-starch carbohydrates as well as increasing the protein and amino acid availability through the denaturing of the protein structure. The following section will summarise the relevant literature on canola seed and provide grounds for the research conducted for this thesis.

1.5 Nutritional value of Canola seed (*Brassica* spp.) for broilers.

Over the past couple of decades *Brassica* oilseed production has increased to become the world's third-most important source of oil for human consumption and second-most important source of protein meal for animals (Arkcoll *et al*., 1998). *Brassica rapa* (formerly known as *Brassica campestris*) was introduced to Canada in 1936 from Poland and a few years later *Brassica napus* was introduced from Argentina (Bell, 1984). Even though the origin of the different *Brassica* species is not entirely clear, it is believed that *Brassica rapa* is the oldest species with the widest distribution, and that *Brassica napus* was derived from a cross between *B. rapa* and *B. oleracea* (Kimber & McGregor, 1995). *B.rapa* and *B.napus* subsequently became the source of seeds in Canada on which extensive research was done in order to improve on the unacceptably high levels of erucic acid and glucosinolates that was common to rapeseed. The first low erucic acid cultivar was produced in Canada in 1968, followed by another improvement in 1974, with a cultivar that was low in both erucic acid and glucosinolates (Bell, 1984). The Canola Council of Canada decided to distinguish the nutritionally superior seed and oil derived from these double low rapeseed cultivars from those of the original high erucic acid and high glucosinolate rapeseed cultivars by referring to them as Canola (Clandinin *et al*., 1989). The remarkable history of rapeseed in Canada has been reviewed by Bell (1982) and special recognition has been given to the breakthroughs achieved in plant breeding. Canola generally refers to rapeseed containing less than 2% of the total fatty acids in the oil as erucic acid and less than 30 μmoles of glucosinolates per gram of oil-free dry matter (DM) of the seed (Bell, 1993).

Canola seed contains about 40% oil (Fenwick & Curtis, 1980), which are usually removed by crushing, solvent extraction or a combination of both techniques. As described previously in this chapter, the quality and composition of the residual meal is affected by the method of processing and could vary considerably. The prepress solvent extraction method is widely used in Canada and the various processing stages of this oilseed into its oil and meal components are described by Pickard *et al.* (1989) and Carr (1995). The meal represents about 60% of the original weight of the seed (Bell, 1995) and is mainly used as a protein supplement for animal feeding. It contains between 36 and 39% protein (Aherne & Kennelly, 1982) and a good balance of essential amino acids. However, its potential use for broiler feeds may be decreased due to the relatively high CF content (see Table 1.18) which results in a relatively low level of ME (Dale, 1996). The introduction of yellow seeded cultivars with a thinner, less fibrous seed coat, resulted in a 4% reduction in fiber content (Fenwick & Curtis, 1980) and could prove highly beneficial in poultry nutrition. These cultivars are sometimes referred to as the "000" cultivars (Van Kempen & Jansman, 1994).

Figure 3.1 Canola flower.

In addition to the meal, certain other by-products of canola crushing and oil refining are useful in animal feeds and are sometimes blended with the meal at the crushing plant. These include gums (containing glycolipids, phospholipids, sterols, etc.) and acidulated fatty acids, also termed soapstocks (Pickard *et al.*, 1989; Bell, 1995). In Canada, gums are frequently added back to canola meal at levels of 1.5%, but even levels of 6% have been shown to have no detrimental effects on the feeding value of canola for broilers and layers (Robblee *et al*., 1989). Such additions serve to reduce the dustiness of the meal and increase its ME value.

The incorporation of full-fat canola seed in poultry diets have also been investigated due to its increased contribution towards the ME content of the diet when compared to that of oil-extracted canola meal (Olomu *et al*., 1975b; Summers *et al*., 1982). Full-fat canola seed contains approximately 40% ether extract (fat) and 21 to 23% crude protein (Robblee *et al*., 1989) and could thus have potential as a high energy and protein feed ingredient for poultry diets.

It is evident from literature that the use of canola meal in poultry diets has been researched far more extensively than full-fat canola in terms of composition, inclusion levels, as well as the influence of processing conditions on the nutritional value thereof. Some form of heat treatment is necessary to inactivate the enzyme myrosinase that is present within the seed and that hydrolyses the glucosinolates into their goitrogenic and toxic components when the seeds are crushed (Bell, 1984). Heat treatment in the form of cooking/conditioning is usually applied prior to the expeller or solvent extraction phase in the processing of canola meal for the purpose of inactivating myrosinase (Pickard *et al*., 1989). The role of glucosinolates and their undesirable breakdown products will be discussed in more detail in further sections.

Canola meal is classified as a protein supplement in the feed trade and usually competes directly with soyabean meal. For this reason a comparison of the chemical and amino acid composition of canola, in its full-fat and meal form with that of soyabean meal is presented in Table 1.18 (with both meals obtained from solvent extraction). Variation in chemical composition of canola meal and full-fat seed, as with many other feed ingredients, are the result of many factors and include the cultivar of *Brassica* used, the soil type on which they are grown, the weather and other environmental effects, as well as the processing conditions applied in the crushing plant in the case of canola meal (Bell & Keith, 1991; Bell, 1995). In the following section the chemical composition of canola will be evaluated in more detail.

1.5.1 Chemical Evaluation

Canola protein is generally of high nutritional value as indicated by the levels of essential amino acids (Table 1.18) and compares well with that of soyabean meal. Uppström (1995) reported that the protein content of *Brassica* seed shows an inverse relationship to its oil content, with the protein content being higher and oil content lower when the seeds are grown under warm dry conditions, and vice versa. The absolute quantity of amino acids in canola vary in proportion to the CP content, which in turn also varies considerably according to the factors mentioned previously, especially environmental factors during seed development (Bell, 1995). This variation in amino acid content is more pronounced for specific amino acids in canola meal than for full-fat canola because of the additional influence of processing conditions (Bell & Keith, 1991). Apart from the

variation in amino acid content, Bell & Keith (1991) also observed differences among amino acid profiles (g 16 g N^{-1}) of canola protein from different geographical regions, suggesting varying proportions of globulins (storage proteins) and albumins (metabolically active proteins) among regions.

Table 1.18 Chemical (10% moisture basis) and amino acid (% crude protein) composition of full-fat canola and canola meal in comparison with soyabean meal (44%)

Components	Unit	**Full-fat Canola** [a] (*Brassica* spp.)	**Canola meal** [b] (*Brassica* spp.) Solvent extracted	**Soyabean meal** [c] (*Glycine max*) Solvent extracted
Crude Protein	%	20.27	36.34	44.50
Ether extract	%	40.69	3.28	0.81
AMEn	MJ/kg	18.67[d]	8.29[d]	9.44
TMEn	MJ/kg	19.08[d]	8.75[d]	-
Crude Fiber	%	12.17	11.16	7.08
Ca	%	0.34	0.67	0.29
Total P	%	0.71	1.14	0.66
Avail. P	%	0.17	0.29	0.27
K	%	0.49	1.26	2.02
Mg	%	0.29	0.63	0.27
Fe	mg/kg	194.05	155.54	121.35
Mn	mg/kg	32.02	52.73	29.33
Zn	mg/kg	24.26	69.85	40.45
Amino acids				
Arginine	% CP	7.35[d]	6.08	7.14
Histidine	% CP	3.23[d]	2.80	2.66
Isoleucine	% CP	5.63[d]	3.45	4.45
Leucine	% CP	9.55[d]	6.92	7.70
Lysine	% CP	8.22[d]	5.95	6.11
Methionine	% CP	1.96[d]	2.05	1.41
Phenylalanine	% CP	5.83[d]	3.90	4.91
Tyrosine	% CP	3.53[d]	2.61	4.34
Threonine	% CP	6.56[d]	4.52	3.91
Valine	% CP	7.00[d]	4.41	4.70

[a]Nwokolo & Sim, 1989; [b] Clandinin *et al*., 1989; [C] NRC, 1994; [d] Lee *et al*., 1995; AMEn – nitrogen corrected apparent metabolizable energy; TMEn - nitrogen corrected true metabolizable energy

Another factor that should be considered when comparing protein contents of feedstuffs is the conversion factor used to convert Kjeldahl nitrogen content to protein. Although the conversion factor of 6.25 is widely used, it has been recognized that a factor of 5.53 for *Brassica* oilseeds and 5.69 for soyabean would be more appropriate (Tkachuk, 1969). These factors take into account the differences in amino acid profiles and amounts of non-protein nitrogen obtained from nucleic acids, purine and pyrimidine bases, nitrogen-containing lipids and glucosinolates (Uppström, 1995). Finlayson (1974) also found that because of the insolubility of the nitrogen-containing hull component of rapeseed, at least 7% of the seed protein should not be considered as protein nitrogen.

The amino acid content of canola meal could be considered similar to that of soyabean meal (Table 1.18), however, canola contains more sulphur-containing amino acids; methionine and cystine (data not shown) than soyabean meal, but less lysine (Clandinin *et al*., 1989). Lysine also represents a higher percentage of full-fat canola seed protein than of the canola meal protein and this may be due to Maillard reactions occurring from

processing conditions, especially during the desolventizer toaster stage of processing of canola meal (Bell & Keith, 1991). The complementary amino acid profile of canola and soyabean makes the combination of these protein-rich feedstuffs well suited in poultry nutrition.

The ether extract of full-fat canola is generally around 40% and are the biggest contributor towards the available energy content of canola. Other benefits include a reduction in the dustiness of the feed as well as modification of the fatty acids in eggs, meat and milk products (Bell, 1995). In recent times, with consumer health at the forefront, the increased n-3 fatty acid content of poultry products, due to the inclusion of canola in the diet, was found to be especially beneficial. Full-fat canola seed is a good source of α-linolenic acid (C18:3n3) and Ajuyah *et al*. (1991) found that the inclusion of this oilseed in poultry diets significantly modified tissue fatty acid composition of broiler carcasses. The ether extract of canola meal (Table 1.18) tends to be higher than that of soyabean meal, mostly because of the addition of gums to canola meal (Clandinin *et al*., 1989).

The CF content of canola meal is higher (11.16%) than that of soyabean meal (7.08%) and is partly responsible for the relatively low ME value of the meal. Most of the fiber is present in the hulls with lesser amounts present in the embryo (Bell & Shires, 1982). The hulls comprise about 16% of the seed weight and 30% of the weight of the oil-free seed meal (Bell, 1984). The carbohydrates of canola meal comprise almost 50% of the dry matter (Bell, 1995) and therefore have an important influence on the nutritional value. The main components of the available carbohydrate fraction include sugars such as sucrose, stachyose, D-fructose and D-glucose. Sucrose and starch are readily digested by monogastric animals in their free form and contributes to the ME value. The starch content of the seed is relatively high during early development, but as the seed matures, it declines and Blair & Reichert (1984) found only trace amounts in the seed cotyledons. The carbohydrate constituents of rapeseed have been reviewed by Siddiqui & Wood (1977). The cellulose content of canola meal is about 5%, the NDF 26%, total dietary fiber 33% and over 90% of the NSPs are insoluble (Bell, 1995). The seed or meal from brown canola cultivars are known to have higher ratios of insoluble to soluble fiber (Bell, 1995) and this has provided further encouragement for the conversion to yellow-seeded cultivars. These yellow-seeded cultivars have a thinner seed coat with resultant lower CF content in the meal derived from it and it is viewed that the future use of these cultivars will have improved nutritional value (Slominski *et al*., 1994; Simbaya *et al*., 1995). Another approach to reducing the CF content of canola is by means of dehulling. Bell (1993) found a significant reduction in the CF and ADF content of dehulled canola meal, as well as improved digestibility of energy (79 vs. 66%) and protein (86 vs. 76%) for pigs when compared to oil-free canola meal. The removal of the hull fraction of canola meal, however, also alters the amino acid profile of the meal since the protein of the hull fraction contains more lysine than the embryo fraction (Bell, 1995). An important consideration for the improvement of canola meal quality is also to keep the processing costs low enough to remain competitive with soyabean meal and since the offset for canola hulls is rather limited, this method is probably only second best to the use of yellow-seeded canola cultivars. There may also be less polyphenols, lignin and tannins in the hulls of these light-coloured canola seeds, which would be desirable for improving digestibility (Bell, 1995).

The mineral content of canola meal (Table 1.18) generally exceeds that of soyabean meal, especially with regard to phosphorous, calcium, magnesium, selenium and manganese that are about double the amount. The sulphur content is also higher in low glucosinolate canola meal than in soyabean meal, but this varies with the glucosinolate level (Bell, 1995). While 70% of the phosphorous in canola meal is present in the inorganic form, it has been shown that the phytic acid and fiber in canola meal reduce the availability of P, Ca, Mg, Zn, Cu and Mn (Clandinin *et al*., 1989). However, in spite of the lower availability of minerals in canola meal versus those of soyabean meal, canola meal is still a better source of available Ca, Fe, Mn, P, Mg and Se.

ME has been widely used as an index of the available energy in feedstuffs for poultry and it is knowledge of this availability of nutrients (energy) that is essential when formulating diets for poultry. The following section reviews the pertaining literature on biological analysis for poultry in terms of assessing the available energy content of canola.

1.5.2 Bioassays - Energy

Unfortunately, estimates of ME for canola seed vary widely and even more so for canola meal due to the influence of the different oil extraction methods used during processing (Nwokolo & Bragg, 1978; Salmon, 1984 and Bell, 1995). A large number of other variables should also be kept in mind when comparing ME values of canola and include the following: the method used to assess the energy content (chick growth assay or rooster force feeding), whether provision is made for endogenous energy losses (TME) or not (AME), and whether a correction is made for protein stored or lost during the trial (Fenwick & Curtis, 1980). Differences between samples in their energy components and CF content usually account for most of the differences observed in ME values (Nwokolo & Bragg, 1978). It has been found that the age of the chicken also influences the results (Lodhi *et al*., 1968; March *et al*., 1973), where about 10% greater ME values have been found for adult hens compared to chicks (Bell, 1993). Bayley *et al*. (1974), however, concluded that the wide variations observed in the ME values of rapeseed could be attributed to factors other than the ones they have investigated, such as the level of rapeseed meal in the diet, its variety and subsequent processing, the duration of feeding and the age of the birds. It has also been postulated that variations in the response to rapeseed meal might be due to differences in the microflora present in the digestive tract of the birds (March *et al*., 1975). It is thus very difficult to assign a single energy value to canola, since so many variables could influence it.

There is considerable variation among values reported in the literature and this is demonstrated in Table 1.19. Even though these authors all used a rooster force-feeding assay to determine TME and a total faecal collection assay to determine AME, there are considerable differences between the different *Brassica* species, between the seeds fed as ground or whole seeds, as well as whether the seeds were fed alone or in combination with an assay diet. Muztar *et al*. (1978) found that the major discrepancy in ME values observed for whole seeds apparently resulted form the differing responses of individual birds to the palatability and texture of the seeds. Grinding the seed has also been shown to reduce the variability in TME and AME between birds for both the Tower and Candle cultivars of canola seed and this was recommended for future energy evaluations (Muztar *et al*., 1978). In addition, low glucosinolate levels in modern cultivars have apparently resulted in an

improved ME value, as well as the reduction in fiber contents by means of breeding (yellow seeded cultivars) or dehulling (Bell, 1993).

Table 1.19 Reported values of Metabolizable energy of full-fat Canola seed (dry matter basis)

Species, Cultivar & Form	Units	Range (MJ / kg)	Reference
(*B.napus*), Tower, Ground	TME [1]	20.38 – 21.30	Sibbald, 1977a;Muztar et al.,1978
(*B.napus*), Tower, Ground	TME [2]	21.05	Sibbald & Price, 1977
(*B.napus*), LELG, Ground	TME [2]	20.30 – 21.39	Sibbald & Price, 1977
(*B.napus*), Tower, Whole	TME [1]	20.38 – 22.77	Muztar et al., 1978; Muztar *et al.,* 1980
(*B.napus*), Tower, Whole	TME [2]	19.46 – 23.18	Muztar et al., 1978; Muztar *et al*., 1980
(*B.rapa*), Candle, Ground	TME [1]	22.43	Muztar et al., 1978
(*B.rapa*), LELG, Ground	TME [2]	16.36 – 19.96	Sibbald & Price, 1977
(*B.rapa*), Candle, Whole	TME [1]	18.33 – 22.81	Muztar et al., 1978; Muztar *et al.,* 1980
(*B.rapa*), Candle, Whole	TME [2]	17.79 – 21.09	Muztar et al., 1978; Muztar *et al.,* 1980
(*B.rapa*), Candle, Ground	AME [1]	18.33	Muztar et al., 1978
(*B.rapa*), Candle, Ground	AMEn[1]	18.04	Muztar et al., 1978
(*B.rapa*), Candle, Whole	AME [1]	11.51 – 12.64	Muztar et al., 1978
(*B.rapa*), Candle, Whole	AMEn[1]	11.59 – 12.43	Muztar et al., 1978
(*B.napus*), Tower, Ground	AME [1]	18.87	Muztar et al., 1978
(*B.napus*), Tower, Ground	AMEn[1]	18.54	Muztar et al., 1978
(*B.napus*), Tower, Whole	AME [1]	15.19	Muztar et al., 1978
(*B.napus*), Tower, Whole	AMEn[1]	15.02	Muztar et al., 1978

TME – True metabolizable energy; AME – Apparent metabolizable energy; AMEn – nitrogen corrected AME;
[1] Test ingredient assayed with basal diet; [2] Test ingredient fed alone; LELG – low erucic acid low glucosinolate

1.5.3 Bioassays – Amino acids

The digestibility of protein (and amino acids) are generally more difficult to evaluate than energy because of the differences in digestive systems and requirements for essential amino acids among various species and ages of animals (Bell, 1995). It is common practice to use the same TME force-feeding procedure of Sibbald (1979) to determine true protein (and amino acid) availability and therefore the factors influencing the variability of TME assays are also applicable to the evaluation of protein and amino acid availabilities. A second method that is also used to determine apparent or true amino acid availability is the total excreta collection assay (Nwokolo & Sim, 1989). The true amino acid availability (TAAA) values are usually higher than the apparent amino acid availability (AAAA) values, since compensation is made for the metabolic faecal and endogenous urinary amino acid excretions in the TAAA assay (Lee *et al*., 1995). Again, as with the ME values, a lot more research has been done on the availability of amino acids from canola meal when compared to that of full-fat canola seed (FFCS). A few authors have evaluated amino acid availabilities of both canola products and found the amino acids in the seeds to be more available than those in the meal form (Muztar *et al*., 1980; Lee *et al*., 1995), conversely Barbour & Sim (1991) found these values to be similar for both the seed and

meal products. A summary of true amino acid availability values for FFCS is presented in Table 1.20. These values were all determined with a rooster force feeding assay where the test ingredient (FFCS) was fed alone (Muztar *et al*., 1980; Barbour & Sim, 1991 and Lee *et al*., 1995). Nwokolo & Sim (1989) used a total excreta collection assay on broiler chicks, in which the test ingredient (FFCS) was blended 50:50 with a nitrogen-free diet. In order to determine the metabolic faecal and endogenous urinary losses, the nitrogen-free diet was used as a control.

Table 1.20 Summary of true amino acid availabilities (% dry matter) of full-fat canola seed.

	Muztar *et al.* (1980)	Nwokolo & Sim (1989)	Barbour & Sim (1991)	Lee *et al*. (1995)	Average
Arginine	94.0	84.8	100.0	90.5	92.33
Histidine	92.0	83.6	90.0	93.7	89.83
Isoleucine	91.0	85.3	93.0	84.6	88.48
Leucine	93.0	87.8	94.0	86.8	90.38
Lysine	89.0	81.9	95.0	87.9	88.45
Methionine	94.0	n.a.	89.0	90.4	91.13
Phenylalanine	92.0	87.4	96.0	87.6	90.75
Serine	90.0	83.5	94.0	84.3	87.95
Threonine	86.0	72.9	n.a.	82.2	80.37
Tyrosine	90.0	84.4	n.a.	84.1	86.17
Valine	90.0	85.6	93.0	83.6	88.05

n.a. – not assayed

From Table 1.20 it can be seen that the availability of amino acids from full-fat canola seed generally falls within the range of 80.4 to 92.3%. Muztar *et al*. (1980) and Barbour & Sim (1991) also found that the availability of some of the amino acids in canola seeds and -meal were similar to those in soyabean meal. This could prove highly beneficial for the incorporation of canola in poultry diets. Muztar *et al.* (1980) observed that the standard errors of the TAAA values were higher when the test ingredient were mixed with a basal diet than when fed alone and this was confirmed by Lee *et al.* (1995). Muztar *et al.* (1980) also suggested that the use of ground canola seed will reduce the variability between birds, and an excreta collection period longer than 24 hours, as well as an increased number of replicates will improve the reproducibility of TAAA assay results.

Robblee *et al*. (1989) conducted broiler trials to evaluate the use of canola seed in broiler rations. Raw and flaked canola seed was included in the rations at levels of 10, 15 and 20%. Body weights and feed:gain ratios were evaluated against that of chickens fed a wheat-soyabean control diet. The broiler performance results are presented in Table 1.21.

Table 1.21 Effect of different levels of flaked canola seed on the performance of broiler chickens (Robblee *et al*., 1989)

Rations	Body weight (g)	Feed/gain
Control, Wheat-soyabean meal	1740	1.99
+ 10% flaked canola seed	1733	1.95
+ 15% flaked canola seed	1666	1.97
+ 20% flaked canola seed	1563	2.02

These authors recommended that 10% raw flaked canola seed may be used in broiler rations without negatively affecting performance. These results are in agreement with Leeson *et al*. (1987) who also found that

a 10% inclusion level was suitable to sustain similar broiler growth rates as those fed a soyabean control diet. In another study, however, broiler body weight gain was reduced by the inclusion of 10% FFCS when compared to a barley-based diet, but no significant differences were found for feed consumption or feed:gain ratio (Nwokolo & Sim, 1989). In a study by Ajuyah *et al*. (1991) the inclusion of 20% FFCS did not result in any adverse effects on growth, carcass weight or cut-up yields, but affected the fatty acid composition of the carcass by enriching it with linolenic acid (C18:3n3), docosapentaenoic acid (C22:5n3) and docosahexaenoic acid (C22:6n3). To present, no consensus has been reached regarding the maximum inclusion level of FFCS in broiler diets, since some studies supported the finding that the feeding of a diet with 20% raw, canola seed did not result in growth depression (Leeson *et al*., 1978; Lee *et al.,* 1991), whereas others showed that more than 17.5% FFCS resulted in reduced feed intake and weight gain (Summers *et al*., 1982). The latter authors concluded that the major problem associated with the reduced performance of broilers consuming full-fat canola-based diets, was the reduced feed intake and that it could be connected to a palatability problem. Some of the conflicting reports concerning the value of full-fat rapeseed for poultry are most likely due to the level of erucic acid and glucosinolates, since these vary from cultivar to cultivar. Olomu *et al*. (1975a) observed a decreased weight gain, increased feed:gain ratios and leaner carcasses for broilers when they were fed diets containing 20% of a low erucic acid cultivar (Span), but these effects were reduced when the seed was autoclaved for 10 minutes at 120°C. In a companion paper the importance of these ANFs was highlighted when they fed broilers on a diet with 20% raw Span (a low erucic acid variety of *B.rapa*) or raw Bronowski (a low glucosinolate variety of *B.napus*) rapeseed and observed depressed weight gains and feed conversion ratios with both cultivars when compared to that obtained from the control (Olomu *et al*., 1975b). A superior weight gain and feed conversion ratio for Bronowski was observed in comparison with Span rapeseed. Clement & Renner (1977) showed that high erucic acid rapeseed oils resulted in significant depressions in growth, while low erucic acid rapeseed oil did not result in any depression in growth performance. This should theoretically not be a problem with canola cultivars, since they are bred for low erucic acid as well as low glucosinolate levels. However, as seen from the previous studies, conflicting results of broiler performance with the inclusion of FFCS in broiler diets still exists and further research is needed in order to clarify this issue. Promising results have been obtained with ways to improve the nutritive value and inclusion levels of canola seed and are mostly directed at the effect of various heat treatments and grinding of the seeds. These studies will be discussed in section 1.5.5.

1.2.1 Anti-nutritional factors in Canola

One of the remarkable events that distinguish modern canola from its more traditional rapeseed varieties is the breakthrough that has been achieved in the 1970's when plant breeders succeeded in breeding a variety of rapeseed that has a low erucic acid content in the oil as well as low glucosinolate content in the meal. In the animal feeding industry it is especially the low glucosinolate canola meal that has guaranteed its future use in animal feeds, since glucosinolates are most toxic and has been known to reduce feed intake and growth performance in animals, as well as interfering with thyroid activity (Van Kempen & Jansman, 1994).

1.5.4.1 Glucosinolates

More than 100 glucosinolates have been identified in the plant kingdom, of which six occur in *Brassica napus* and *Brassica rapa* seeds. They can generally be divided into aliphatic and indole glucosinolates with the former being more predominant in *Brassica* seeds. The aliphatic glucosinolates consists of butenyl, pentenyl and their 2-hydroxy analogues, whereas the indole glucosinolates consists of 3-indolylmethyl and 4-hydroxy-3-indolylmethyl (Uppström, 1995).

The problems concerning glucosinolates are related to both the total amount of glucosinolates present as well as the type of glucosinolates and their breakdown products (Olsen & Sørensen, 1980). Earlier varieties of rapeseed contained 110-180 μmoles of aliphatic glucosinolates per gram of oil-free dry matter and with the advent of the low glucosinolate cultivars, these glucosinolates have been reduced to 10-20% of their former levels (Bell, 1995). New canola varieties today contain less than 30 μmol of glucosinolates per gram of oil-free dry matter and generally refer to the aliphatic glucosinolates, since the indole glucosinolates are not primarily goitrogenic and are thus of less concern (Bell, 1995). It is not the intact glucosinolates that are toxic *per se*. The presence of the enzyme myrosinase (β-thioglucosidase, EC 3.2.3.1) in separate compartments within the seed, hydrolyses the glucosinolates into their goitrogenic and toxic compounds when the seeds are crushed during processing. This enzyme may also be present in weed seeds contaminating rapeseed or may be produced by microflora in the intestinal tract of animals (Van Kempen & Jansman, 1994). The hydrolysis of one of the hydroxy analogues of aliphatic glucosinolates (2-hydroxy-3-butenyl) produces goitrin, which is the most active compound of the goitrogenic factors. It results in a net reduction in circulating tri-iodothyronine and thyroxine, consequently leading to the stimulation of the hypophysis to produce more thyroid-stimulating hormones and finally results in the enlargement of the thyroid gland (Fenwick & Curtis, 1980). Another goitrogenic factor is isothiocyanate that is converted to thiocyanate and tends to prevent active uptake of iodine. Thus, the effects of thiocyanate are most noticed when iodine is limiting (Aherne & Kennelly, 1982). Depending on the nature of the glucosinolates and the reaction conditions, such as low pH, nitriles and thiocyanate ions are also formed (Van Kempen & Jansman, 1994). Aside from the goitrogenic and other anti-nutritional properties of glucosinolates (or rather their breakdown products), they are regarded as generally unpalatable and responsible for reduced feed intake with diets containing rapeseed meal (Bell, 1995). Animal species also appear to differ in response to ingestion of glucosinolates and this may in part be explained by differences in intestinal microflora activity (Bell, 1993). It has also been suggested that the myrosinase originating from the bacteria in the gastro-intestinal tract of laying hens may contribute to the incidence of liver haemorrhage when glucosinolates are ingested (Slominski *et al*., 1988).

Numerous attempts have been made to remove, reduce or counteract the effects of the goitrogenic products by inactivating myrosinase before glucosinolate hydrolysis can occur. This usually takes place during the cooking phase prior to expeller or solvent extraction in the processing of canola meal. A reduction of 15 to 77% in glucosinolates compared with original seed levels have been obtained at some crushing plants (Table 1.22).

Table 1.22 Effect of commercial processing on glucosinolate content of canola meal (Bell, 1995).

Glucosinolate	Glucosinolate content (μmol g^{-1} of oil extracted meal)	
	Seed	Meal
Aliphatic glucosinolates		
3-Butenyl	7.44	4.97
4-Pentenyl	2.55	1.67
2-Hydroxy-3-butenyl	13.44	8.82
2-Hydroxy-4-pentenyl	0.99	0.74
Total	24.42	16.20
Indole glucosinolates		
3-Indolylmethyl	0.63	0.38
4-Hydroxy-3-indolylmethyl	13.37	4.48
Total	14.00	4.86
Overall total, canola origin	38.42	21.06
Contaminant glucosinolates		
2-Propenyl (allyl)	1.41	1.05
4-Hydroxybenzyl	2.31	2.25

The development of low glucosinolate canola cultivars has been a major improvement on the high glucosinolate rapeseed cultivars of the past and the prospect of reducing the glucosinolate content even further is very promising. A recommended maximum dietary level of 2.5 μmol g^{-1} has been established and levels as low as 1.43 5 μmol g^{-1} diet are advised to minimize the liver haemorrhage problem in laying hens (Bell, 1995).

Three other types of anti-nutritional factors may also be found in canola and include sinapine, tannins and phytic acid (Table 1.23).

Table 1.23 Sinapine, tannin and phytic acid contents of canola meal and their main effects on animals (Bell, 1993).

Compound	Amount in canola meal (%)	Effects on animals
Sinapine	0.6 – 1.8	Bitter flavor "Fishy eggs" (susceptible layers)
Tannins	1.5 – 3.0	Impairs digestion, especially protein
Phytic acid	3 - 6	Binds minerals

1.5.4.2 Sinapine

Sinapine is the major compound among several phenolic choline esters present in the embryo of rapeseed and has a bitter flavour. This may affect feed intake, even though it has been found that glucosinolates have a greater adverse effect on palatability or feed intake than either sinapine or tannins (Bell,1995). Probably the most important effect of sinapine is the production of off-flavour or "fishy eggs" by susceptible hens, which lack the liver enzyme trimethylamine (TMA) oxidase. These hens cannot effectively handle the high yield of choline following hydrolysis of sinapine in the gut, and results in a build-up of trimethylamine that is transferred to the developing egg (Goh *et al*., 1979). "Fishy eggs" are usually observed if the diet contains more than 1g of sinapine per kilogram of diet. This problem appears to relate mainly to hens that lay brown-shelled eggs and the inclusion of canola should thus be restricted for these layers, since the sinapine content of canola meal exceeds the minimum acceptable level (Blair & Reichert, 1984). The value of canola meal could

be considerably enhanced with the development of new cultivars with reduced sinapine contents (Fenwick & Curtis, 1980).

1.5.4.3 Tannins

These compounds can be subdivided into hydrolysable and condensed tannins and exist mainly in the seed coat. They are more abundant in dark-hulled canola cultivars than in the yellow-seeded cultivars and are known to interfere with digestive enzymes, especially those affecting protein hydrolysis (Bell, 1993). This effect is, however, disputed by other authors who failed to show any effect of rapeseed meal tannins on protein digestibility and growth of chickens (Van Kempen & Jansman, 1994). Bell & Shires (1982) found that the protein in hulls of the yellow-seeded canola cultivars were more digestible for pigs than those of the dark varieties and contributed it to the lower tannin and lignin contents of the hulls. There are also indications that tannins may have other anti-nutritional effects and that it could be involved with sinapine in the "fishy egg" syndrome by inhibiting TMA oxidase, thereby preventing the enzyme from converting trimethylamine to a water-soluble odourless oxide (Bell, 1995). Tannins may also form complexes with carbohydrates such as starch and may impair absorption of certain minerals and vitamins in the digestive tract of poultry (Bell, 1995).

1.5.4.4 Phytic acid

Phytic acid is found mainly in the embryo of cereal grains and oilseeds. This compound is strongly negatively charged at the usual pH of feeds, and can therefore react with positively charged groups such as cations and proteins. Depending on the pH, phytic acid may form complexes (phytates) with certain mineral ions of varying solubility and thereby reducing the availability of certain minerals (Nwokolo & Bragg, 1977). In rapeseed the presence of phytates have caused Zn, Ca and Mg deficiency syndromes in chickens (Nwokolo & Bragg, 1977). They may also form complexes with proteins in the food, with digestive enzymes or with proteins closely associated with starch (Bell, 1995). Phytic acid may account for 60-90% of the total phosphorous and exists mainly as salts of calcium, magnesium and potassium (Bell, 1993). Canola meal contains about 1.22% total P of which 0.53 is phytate-bound (Bell, 1993). The corresponding values for soyabean meal (SBM) are 0.66% and 0.38, indicating that even though canola meal contains more phytate than SBM, it provides about twice as much non-phytate P due to its higher levels of minerals. Dietary supplementation with phytase could significantly improve phosphorous availability for broiler chicks and have been described by Żyła & Korelski (1993). The hydrolysis of phytate in canola meal with phytase is, however, not a simple matter and continuous research is being done to improve the process. Newkirk & Classen (1998) have reported on additional meal pre-treatment that is necessary for the effective hydrolysis of phytate in canola meal.

Canola does contain a few other anti-nutritional factors such as trypsin inhibitors and saponins, but the levels are too low to be of any nutritional significance. The only other component that could have an anti-nutritional effect are pectins. They comprise approximately 14.5% of dehulled rapeseed and because of its effect on the viscosity of the digesta, may retard digestion and absorption of nutrients (Bell, 1984). The

addition of pectinases may therefore be useful in increasing the nutritive value of rapeseed, but for the purpose of this study we have concentrated on the use of heat treatment to try and improve the nutritive value and these methods will be discussed in the following section.

1.2.2 Processing of Canola

Previous research regarding the processing of canola has almost been exclusively devoted to its oil and meal components, as well as the influence of every stage of the prepress solvent extraction method on the various nutritional and anti-nutritional components of this feed (Fenwick *et al*., 1986; Pickard *et al*., 1989; Campbell & Slominski, 1990; Carr, 1995 and Mustafa *et al*., 2000). Even though full-fat canola has the potential of being a high-energy high-protein ingredient for poultry, the use of unprocessed whole canola seed is not warranted due to the presence of myrosinase that hydrolyses the glucosinolates into their toxic and goitrogenic components upon crushing or grinding of the seed. It has therefore been considered essential that the seed be processed with some form of heat treatment prior to inclusion in poultry diets in order to inactivate this enzyme.

Pickard *et al.* (1989) have outlined a few basic principles that should be followed when processing canola to keep the hydrolysis of glucosinolates to a minimum:

Moisture content should be kept between 6 and 10%, since levels above that will enhance hydrolysis and levels. Temperature should be raised as quickly as possible to the optimum temperature, since the rate of enzymatic hydrolysis increases with increasing temperature until inactivation occurs. A slow rate of heating will thus favour hydrolysis. Temperatures should be monitored and excessive heating must be avoided to control thermal decomposition of the glucosinolates.

Fenwick *et al*. (1986) have reported that dry extrusion of rapeseed at 150°C effectively inactivated myrosinase but had little effect on glucosinolate content. However, it was emphasized that successful detoxification should maintain the biological and nutritional value of the product and that this was unlikely to be the case with such high temperatures. A decrease in protein quality and digestibility due to Maillard-product formation are more likely to occur. The authors have also found that the effectiveness of extrusion as a means of removing glucosinolates can be increased by the addition of chemicals such as formaldehyde, alkali or ferrous sulphate prior to the extrusion process. There are, however, certain risks that the products formed as a result of the chemical treatments may differ from those produced upon enzymatic hydrolysis and that these may in fact be even more toxic.

Indole glucosinolates are more susceptible to heat treatment than aliphatic glucosinolates (Slominski & Campbell, 1989) and upon hydrolysis yields products such as thiocyanate ion (SCN) and indoleacetonitriles that are also more toxic than the breakdown products of aliphatic glucosinolates (Campbell & Slominski, 1990). These authors have determined the thermal degradation products of indole glucosinolates during the processing of canola and have established that there are as of yet unidentified breakdown products present. The presence in canola meal of some products, which releases SCN upon hydrolysis in the gastro-intestinal tract of poultry, has also been demonstrated by Campbell & Slominski (1989).

Pigs seem to be somewhat more sensitive than poultry to low levels of glucosinolates and Keith & Bell (1983) found that steam treatment reduced the total glucosinolate content of canola from 40 to 15 μmol/g and that it had a positive response on the growth performance of the pigs. Heat treatment such as extrusion also had no significant influence on other anti-nutritional factors of canola such as the sinapine or tannin content (Van Kempen & Jansman, 1994).

Heat treatment may also cause a certain modification of the protein structure and this is generally considered as desirable since it renders the protein more readily assimilable by poultry (Pickard *et al*., 1989). Excessive heat treatment may, however, result in the loss of some of the essential amino acids of which lysine is the most susceptible (Gray *et al*., 1957). Pickard *et al*.(1989) have described two types of damage that can occur. Firstly, the amino acids may become bound in such a form that they are not liberated by digestion *in vivo* or by enzyme hydrolysis *in vitro,* but are liberated by acid hydrolysis. Secondly, the amino acids are irreversibly altered and are not recovered by acid hydrolysis.

Gray *et al*. (1957) determined the effect of heat treatment, in the form of autoclaving and dry heating (115-120°C), on the lysine content of rapeseed and concluded that both heat treatments resulted in the reduction of lysine with the latter resulting in a lesser degree of destruction. They have also reported that autoclaving improved chick growth despite the lowered level of lysine in the raw seed. As for other oilseeds such as soyabeans (Kohlmeier, 1997), it has been suggested that a protein solubility test might be applicable to determine the effectiveness of canola processing. Goh *et al*. (1980), however, have established that the protein solubility of rapeseed meal in 0.2% KOH is not a reliable test for indicating the protein quality, since it showed poor correlation with total protein efficiency (TPE – calculated as: weight gain of chicks divided by the total weight of protein consumed by the chicks) as determined by a chick growth assay. These results support the relevance of assessing the effectiveness of canola processing in terms of the bioavailability of nutrients by means of a chick growth assay.

Woodly *et al*. (1972) indicated that heat treatment was indeed necessary to improve the nutritive value of full-fat canola and that it resulted in a marked improvement in the average weight gain and feed:gain ratios from those obtained by feeding raw canola. These findings were supported by Olomu *et al.* (1974) who found that autoclaving raw canola at 120°C for 10 minutes gave the best results and significantly improved broiler weight gain and feed conversion efficiency over that of raw canola.

Bayley & Summers (1975) extruded rapeseed at 120°C and formulated broiler diets containing graded levels (10, 20, 30, 40 and 50%) of either raw or extruded rapeseed. Broiler performance was measured in terms of feed intake, body weight and feed:gain ratio and compared with an isocaloric and isonitrogenous corn-soyabean control diet. The results (Table 1.24) showed that the extrusion of rapeseeds did improve the value of the oilseed in diets for growing chicks, but that the inclusion of more than 10% extruded rapeseed have reduced the growth performance in comparison to the chicks that were receiving the corn-soyabean meal control diets.

Table 1.24 Effect of adding raw or extruded rapeseeds to the diet of growing light hybrid chicks on performance from 7 to 21 days of age (Bayley & Summers, 1975)

Control			Rapeseed (%)						
Corn-soy	Corn-soy-rapeseed		10	20	30	40	50	Sx*	HSD**
			Feed consumed (g/bird/day)						
19.0	19.9	Raw rapeseed	16.5	13.8	11.6	9.4	8.2	0.44	2.2
		Extruded rapeseed	19.2	18.1	16.9	15.2	14.9		
			Final weight (g/bird)						
191	189	Raw rapeseed	162	133	119	96	88	2.99	14.7
		Extruded rapeseed	188	177	167	153	149		
			Feed:gain						
2.14	2.26	Raw rapeseed	2.44	2.92	3.20	4.52	5.75	0.13	0.64
		Extruded rapeseed	2.21	2.30	2.37	2.53	2.57		

*Based upon error mean square from analysis of variance 33 df.
**Honestly significant difference, Tukey (12 means, 33 df P = 0.05).

Heat treatment of canola diets in the form of steam-pelleting has also proven to be beneficial (Summers *et al*., 1982; Shen *et al*., 1983). However, when diets are given in a meal form, it is advisable that the canola seeds are well ground. Shen *et al.* (1983) have examined their ground canola material and found approximately 4% whole seeds in the "once ground" batch, 0% in the "twice ground" batch, 26% in the 50:50 maize:canola mix "once ground" and 3.5% in the same mixture "twice ground". There were also a number of seeds that were in various stages or degrees of pulverization for all ground samples. The level of grinding had a marked influence on the fat digestibility of canola seed (mashed diets), with twice ground canola resulting in the highest fat digestibility of the canola seed diets. The performance of broilers fed these canola seed diets in mashed form was, however, consistently lower that those obtained from either the maize-soya control or the steam-crumbled canola seed diets, highlighting the need for heat treatment when full-fat canola seeds are used.

Apart from heat treatments, there are also other processing methods that are being investigated regarding the improvement of the nutritional value of canola. One such method is the reduction of the CF content by means of dehulling. Leslie *et al*. (1973) showed that the dehulled fraction of canola seed had a higher protein and fat content with a lower fiber content than the whole seed, consequently leading to an increased digestible nitrogen and digestible energy content of dehulled canola seed. Zuprizal *et al*. (1991) showed that in adult roosters the true digestibilities of protein and total amino acids were increased when dehulled meal were compared to whole canola meal (82.3 vs. 72.7% and 85.7 vs. 80.2%, respectively). Bell (1993) also found that the digestible energy content for pigs of dehulled canola meal was superior to that of whole canola meal (13.57 MJ/kg). The results look promising, but the difficulty in dehulling such a small seed effectively as well as the high processing cost involved, will more than likely offset these nutritional benefits. Another method of improving the nutritional value of canola involves the use of exogenous enzymes such as phytases and carbohydrases. This is mainly due to the anti-nutritional impact of phytic acid and non-starch polysaccharides such as pectin. Promising results have been obtained in this field (Bell, 1993; Newkirk & Classen, 1998), but will not be discussed as part of this study.

The potential effect of expansion on the nutritional quality of canola will be discussed hereafter. Very little information exists for this particular heat treatment on full-fat canola, but relevant results of the expansion of other oilseed products and whole diets will be given in the hope of possibly predicting the outcome of the expansion of full-fat canola.

During expander processing steam- or liquid-conditioned meal is fed into a mixing and conveying screw. A cone-shaped valve is mounted near the end of the mixing tube and can be hydraulically or electrically controlled to ensure that the expansion process can be adapted to appropriate requirements, with regard to the nutritional, hygienic and physical quality of the end product (Van Zuilichem *et al.,* 1997). The feedstuff is subjected to increasing pressure and shear action caused by the rotating single screw and when it leaves the expander, a sudden drop in pressure occurs and some of the added water evaporates spontaneously. This, along with the sudden increase in volume of the material as it leaves the expander, is known as "expansion".

Expander processing has been used in the poultry industry for some time (Kwakkel & Van der Poel, 1997) and is generally considered as being a cheaper form of heat treatment than both single- and twin-screw extrusion processes (Van Zuilichem *et al.,* 1997). There are in fact many variables during or after the expansion process that can influence the nature of the final product and this makes it very difficult to evaluate the efficiency of the expansion treatment (Bos *et al.,* 1997). Some of the advantages of expander processing for the feed manufacturer will include improved pellet durability, the inactivation of ANFs, sterilization of the feed, increased fat addition, gelatinization of starch and reduced dust levels (van Zuilichem *et al.,* 1997).

The effects of processing – both desired and undesired – on the nutritional properties of feed are highly dependent on the conditions during thermo-mechanical processes such as pelleting or expander treatments. The time/temperature combination is in most instances the major factor influencing the extent of reactivity of the various chemical components of the feed ingredients (Bos *et al.*, 1997). It is thus essential to optimize processing conditions in order to limit the occurrence of undesirable reactions such as denaturation, caramelization, oxidation and maillard reactions. Some of the more desirable effects of expansion on protein, amino acids, fat and cell wall structures will be discussed hereafter

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1.5.5.1 Effect on proteins and amino acids

Expander processing has no significant influence on the feed protein content as well as on the stability and availability of certain amino acids (Peisker, 1992). Up to a temperature of 120 ^{0}C neither the total lysine nor the reactive lysine changed when a pig diet was exposed to expander treatment, but at 130 ^{0}C there was a slight reduction in the content and availability of the amino acids even though it was not significant. Considering the widespread use of synthetic amino acids such as lysine and methionine in poultry rations today, the fact that they have proven to be "expander stable" during high processing conditions are of extreme importance (Peisker, 1992).

Expander processing may also be employed to reduce the levels of proteinaceous ANFs, thereby increasing plant protein digestibility (Bos *et al.,* 1997). Many basic treatment procedures exist and are dependent on various parameters. The effectiveness of such treatment is therefore related to a complete

knowledge of both the ANFs and composition of a specific feed material as well as the process technologies involved.

1.5.5.2 Effects on fat and fatty acids

Expander processing can influence the quality of fatty acids in feed by inactivating the naturally occurring lipases, thereby limiting the amount of free fatty acids that are formed as well as the extent of hydrolysis taking place (Peisker, 1992). This is especially important in high-oil feedstuffs such as canola where the amount of free fatty acids increases with the duration of storage (Table 1.25).

Table 1.25 Free fatty acid content (g/kg) in different stored products with and without expander treatment [a] (Peisker, 1992).

		Storage time (weeks)		
Treatment		0	4	8
Untreated	Beans	12.9	14.7	18.9
	Rapeseed	1.9	8.6	11.1
Expanded	Mixture 50:50	2.7	2.5	2.8

[a] No additional processing conditions were described.

As can be seen from Table 1.25 the free fatty acid content of the expanded feedstuffs did not exceed the initial value of the untreated feedstuffs even after 8 weeks of storage. The oxidation of fat in feeds and feedstuffs may also occur due to the enzyme, lipoxidase that causes the fats to turn rancid (Bos *et al.,* 1997). The products formed during this oxidation give the feed a characteristic taste and smell, and usually result in reduced feed intakes. According to the work of Peisker (1992), expander processing reduces the incidence of fat oxidation by inactivation of the relevant enzymes during treatment.

1.5.5.3 Effects on cell wall structures

Cell walls of older plants contain pectins, hemicellulose, cellulose and some lignin and have a very low digestibility for poultry (Bos *et al.,* 1992). Just like milling and pelleting could result in the disruption of cell walls due to the high shear forces of these treatments, the same could be applied to expansion with a resultant increase in the digestibility of cell walls.

1.5.5.4 Effect on micro-organisms

The high temperatures reached during the expansion of feed ingredients or complete diets will lead to a reduction in the amount of micro-organisms present (Beumer & Van der Poel, 1997). Temperature, treatment time, moisture content or a combination of these is certainly the most important parameters determining the extent of the reduction in microbial numbers (Beumer & Van der Poel, 1997). It is important to control and understand these variables in order to successfully reduce the amount of micro-organisms such as *E.coli* and moulds. Expander treatment also appears to be an effective means of eliminating *Salmonella* spp. from animal feeds, but this needs to be supported by a quality control system such as HACCP or the "farm-to-fork"concept throughout the production chain.

The influence of expansion on other nutrients such as starch has been well researched (Bos *et al*., 1997; Thomas & Van der Poel, 1997), but since it is not completely relevant to our study of the expansion of canola, it will not be discussed here. Unfortunately, information on the expansion of single feed ingredients, especially canola seed, is not available in the literature.

1.5.5.5 Effect on nutritional value and broiler performance

It is well known that young broiler chicks have a restricted feed intake capacity and needs to consume highly digestible and concentrated feeds in order to satisfy their rapid growth potential (Kwakkel & van der Poel, 1997). High CF fractions, such as those in canola, will have a negative effect on the apparent digestibility of protein and amino acids and can severely impair their growth. Exposure of the feed to expander treatment may reduce these negative effects (Kwakkel & van der Poel, 1997).

The effects of pelleting, expanding and expanding-pelleting on broiler performance have been investigated and the results are shown in Table 1.26.

Table 1.26 Effects of dietary treatments on feed characteristics [1] and broiler performance at 36 days of age (Kwakkel & van der Poel, 1997)

	Pelleting	Expander Treatment	Expander-Pelleting
Body weight at 36 d (g)	1504[a]	1432[b]	1523[a]
Cumulative feed intake (g)	2551[b]	2433[c]	2592[a]
Feed conversion ratio	1.74	1.75	1.75

[a,b,c] Values with different superscripts in the same row are significantly different ($P<0.05$)
[1] No additional processing conditions were described.

The best growth performance was observed for the group fed the expanded-pelleted feed, with the single expanded feed resulting in inferior growth. The expansion of the feed increased its bulkiness and due to the restricted feed intake capacity of broilers, it resulted in a reduction in feed intake. This problem can be avoided with the pelleting of expanded feeds. The feed conversion ratios were not affected by treatment.

In another trial, the influence of pelleting, expansion-pelleting and first expanding the maize portion and then pelleting the whole diet was determined on nutrient digestibilities. The results are shown in Table 1.27.

Table 1.27 Digestibility coefficients of crude nutrients in differently treated broiler mixed feeds[1] (Peisker, 1992)

	Processing		
Digestibility coefficients	Standard pelleted	Expanded-pelleted	Maize expanded Mix pelleted
Organic matter	68.6 ± 3.4	70.2 ± 1.2	67.7 ± 2.3
Crude protein	78.5 ± 1.9	77.2 ± 0.8	76.9 ± 0.8
Crude fat	70.6 ± 4.5[b]	82.3 ± 6.1[a]	67.7 ± 10.0[b]
Starch	97.8 ± 0.7[b]	98.9 ± 0.1[a]	98.1 ± 0.7[ab]
NDF	8.4 ± 2.1[b]	13.8 ± 3.5[a]	13.8 ± 4.6[a]
ADF	19.8 ± 4.5	22.3 ± 6.0	20.0 ± 3.2

[1] Mixture consisted mainly of maize (40%), soyabean meal (14.4%) and maize gluten feed (10%); counter pressure in expander was 80 bar; mixture was conditioned at 70°C; no further processing conditions were described
[a,b] Values with different superscripts in the same row differ significantly (P<0.05)

No significant differences were observed in either organic matter or CP digestibilities. Expander-pelleting did, however, increase starch, fat and NDF digestibility and could be contributed to the rupture of fat containing cells and possibly starch gelatinization.

Plavnik & Sklan (1995) examined the effect of expansion processing on the digestibility of energy and nutrients of feeds and grains in 18-21 day old broiler chicks. A complete maize-based feed or wheat or barley were exposed to expansion (90°C) and compared with the same untreated material. All diets were subsequently milled to equal size. The results are presented in Table 1.28.

Table 1.28 Effect of expansion on apparent digestibilities of nitrogen (N), fatty acids, starch, gross energy (GE), apparent (AME) and nitrogen-corrected (AMEn) metabolizable energy of maize or wheat/barley based diets (Plavnik & Sklan, 1995).

	N %	Fatty acids %	Starch %	GE MJ/kg	AME MJ/kg	AMEn MJ/kg
Maize-based diets						
Mash	68.0	75.8[b]	96.9	79.4[a]	14.19[b]	13.04[b]
Expanded	66.1	79.8[a]	97.2	79.6[a]	14.33[a]	13.26[a]
Wheat-/barley based diets						
Mash	67.3	77.8	97.6	76.8[b]	13.57[b]	12.58[b]
Expanded	67.6	78.1	97.9	77.9[a]	13.96[a]	12.90[a]

[ab] Means within the same column (per diet) with different superscripts are different (P<0.05)

The expansion of complete maize-based diets as well as that of wheat or barley and their addition to basal diets increased the AME and AMEn values of the diets by 1.5 - 2.5% (P<0.05). From the research findings presented here it seems that expander processing can be successfully employed to destroy anti-nutritional factors and enhance nutrient and energy utilization. Its main effect is through the rupturing of fat containing cells as well as the breakdown of cell walls. From a physical point of feed quality, it also has a positive effect on pellet quality and durability.

1.6 Discussion

From the literature it is evident that a large variation exists in the chemical composition (especially protein and fibre content) of different species and cultivars of lupins. This could lead to considerable inaccuracies when establishing the nutritional value of a particular lupin species for broilers. It is therefore necessary to specifiy between the different species and cultivars of lupins that were used in a particular trial.

When dealing with poultry, however, the nutritional value of a feed ingredient is more adequately described by the available nutrient content and should reflect closely on broiler performance parameters such as body weight gain, feed intake and feed conversion efficiency. From the previous literature review it is evident that very few reports have been made on the apparent or true metabolizable energy value of *Lupinus angustifolius* for broilers. This study will aim to provide that information.

Detailed information regarding analytical methods and procedures applied during animal-feeding trials are necessary in order to facilitate accurate comparisons between results. This was seldom found in the literature. In the following two chapters, all details regarding the analytical methods used and the procedures applied in the trials with *Lupinus angustifolius* and Canola are included.

It is also evident from the literature that the nutritive value of lupins is not as high as would be predicted from knowledge of its chemical composition. Several methods of processing have been tested to improve the nutritional value of lupins. Dehulling typically resulted in a product with lower fibre and higher protein and fat values. Thermo-mechanical treatments such as pelleting or extrusion are likely to contribute to the breakdown of the cell walls, thereby allowing greater accessibility of nutrients to digestive enzymes. Very few studies in the literature have, however, reported on the influence of extrusion of *Lupinus angustifolius* on broiler performance. In this study the possible improvements in chick growth and feed conversion efficiency with additional heat treatment of dehulled lupin seeds were investigated.

As seen from the literature studies, conflicting results of broiler performance with the inclusion of full-fat canola seed in broiler diets exist and further research is needed in order to clarify this issue. Various heat treatments and the grinding of the seeds showed promising results in improving the nutritive value of canola. In the work conducted for this study full-fat canola seed were subjected to heat treatment and the influence thereof assessed by broiler performance. Initially extrusion was tried, but due to the high oil content of full-fat canola, it was found that expansion resulted in a better product and less oil-clogging of the equipment. The expander treatment in this study was without the traditional pre-conditioning phase since the addition of moisture before heat has been found to accelerate the hydrolysis of glucosinolates.

These shortcomings from the literature review will be addressed in the following two chapters dealing with the influence of processing of lupins and canola respectively on the apparent metabolizable energy and broiler performance.

CHAPTER 2

The influence of extrusion and dehulling of *Lupinus angustifolius* on apparent metabolizable energy (AME) and broiler performance.

2.1 Introduction

South Africa relies strongly on imports of oilseeds and oilseed products to satisfy local demand for protein in animal feed. As with global trends, the demand for these protein sources has also increased in South Africa (Briedenhann, 2003). Local conventional protein sources are becoming less available and more expensive (Brand & Brundyn, 2001), thus driving the need to investigate possible alternatives in providing an adequate source of protein and energy for broiler diets. *Lupinus angustifolius* have been identified as being well adapted to the winter rainfall area of the Western Cape and have a high concentration of protein that could possibly provide a much needed local supply of this nutrient for inclusion in broiler diets. Like most legume proteins, however, the methionine content of lupins is low, and diets must be supplemented with the sulphur-containing amino acid to optimize broiler performance (Zaviezo & McGinnis, 1980).

The inclusion of lupin seeds in poultry diets have been limited in the past due to high levels of toxic and bitter alkaloids that suppresses both feed intake and growth (Guillaume *et al*., 1979). Since most modern varieties of *L.angustifolius* contains low levels of alkaloids (<0.01%), the main constraint seems to be the high level of CF and possibly NSPs that influence the availability of nutrients for broilers (Brenes *et al*., 1993). At 20 to 25% of the seed, the hull substantially dilutes the protein nutritional value (Hove, 1974). Dehulling have increased both the fat and protein content, as well as reduced the total NSP levels of *L.angustifolius* cv. Emir (Steenfeldt *et al*., 2003). The beneficial effect of dehulling on apparent metabolizable energy (AME) and broiler performance has, however, only been determined with *Lupinus albus* (Brenes *et al*., 1993).

The nutritive value of sweet *L.angustifolius* is not as high as would be predicted from knowledge of its chemical composition. Even though this legume species does not contain sufficient levels of heat-labile ANFs, the benefit of thermo-mechanical treatments such as pelleting or extrusion are more likely to be contributed to the breakdown of the cell walls, which allows accessibility of nutrients to digestive enzymes, rather than to the inactivation of ANFs (Gatel, 1994). Very little information exists regarding the influence of heat treatment on *L.angustifolius*, but positive results in terms of chick growth have been obtained with extrusion (Watkins *et al* 1988).

The suitability of various levels of *L.angustifolius* in broiler diets have been evaluated in several studies (Yule & McBride, 1976; Johnson & Eason, 1990 and Olkowski *et al*., 2001), but no consensus have been reached with respect to the maximum level of this lupin species that could sustain broiler growth rates similar to those of a control diet. Constraints on the maximum inclusion level are not necessarily due to drops in production above this level, but due to the incidence of wet-sticky droppings that may be promoted by high levels of lupin NSPs (Chesson, 1990; Farrell *et al*., 1999).

Given their potential future importance to the poultry industry, there is a surprising lack of information on *L.angustifolius* in broiler diets under local conditions and the aim of this study is to evaluate the effect of processing methods such as dehulling and extrusion on improving the AME value, broiler growth rates and feed conversion efficiency.

2.2 Materials and Methods

Sweet blue lupins (*Lupinus angustifolius*) cultivar Wonga, used in these experiments were grown in the Western Cape, South Africa and screened for alkaloids at Irene (Agricultural Research Center, Pretoria) by the method of Ruiz (1976). The concentration of total alkaloids was found to be less than 0.01%. Some of the lupins were then dehulled at Nola (Randfontein, Johannesburg) with the percentage hulls reported as 13.7%. Due to technical difficulties with the equipment, the lupins had to be sent through the rollers twice, resulting in a rather large powder component of 27.8% and the kernels thus making up only 58.5% of the total weight. All the lupin seeds were first milled in a hammer mill to pass a 3 mm screen, whereafter a part of the whole and dehulled seeds were extruded at Equifeeds (Fisantekraal, Cape Town) using a single screw extruder at a barrel jacket temperature of 120°C. This resulted in four test products: lupin meal (LM), extruded lupin meal (ELM), dehulled lupin meal (DLM) and extruded dehulled lupin meal (EDLM).

2.2.1 AME Bioassay

Ground test samples of the lupin products were analyzed for dry matter, ash, CP, ether extract and CF by methods detailed by the Association of Official Analytical Chemists (AOAC, 2000). NDF and ADF fractions of the products were analyzed according to the procedures described by Goering & Van Soest (1970) and Robertson & Van Soest (1981). All analyses were performed in triplicate. AME values were determined according to the European reference method described by Bourdillon *et al.* (1990). Three hundred and fifteen 21-day-old broilers (Ross 308) were used in the AME bioassay. Five birds were allocated per cage and housed in an environmentally controlled facility. The test products (LM, ELM, DLM & EDLM) were assayed by means of a 50:50 blend with yellow maize and each test diet were fed at four levels of intake (45, 60, 75 & 90% of *ad libitum*). A treatment consisted of a test diet fed at a specific intake level. Each treatment was replicated three times. A summary of the treatments used in the AME bioassay is shown in Table 2.1. Energy retention was calculated according to the method of Bourdillon *et al.* (1990) and AMEn was obtained by applying the factor 34.4 kJ/g nitrogen retained or excreted (Hill & Anderson, 1958).

Table 2.1 Summary of treatments used in the AME bioassay.

Treatment	Description	Level of intake (% of ad libitum)
1	Control (Maize)	40
2	Control (Maize)	50
3	Control (Maize)	60
4	Control (Maize)	75
5	Control (Maize)	90
6	50% LM : 50% Maize	45
7	50% LM : 50% Maize	60
8	50% LM : 50% Maize	75
9	50% LM : 50% Maize	90
10	50% ELM : 50% Maize	45
11	50% ELM : 50% Maize	60
12	50% ELM : 50% Maize	75
13	50% ELM : 50% Maize	90
14	50% DLM : 50% Maize	45
15	50% DLM : 50% Maize	60
16	50% DLM : 50% Maize	75
17	50% DLM : 50% Maize	90
18	50% EDLM : 50% Maize	45
19	50% EDLM : 50% Maize	60
20	50% EDLM : 50% Maize	75
21	50% EDLM : 50% Maize	90

LM-Lupin meal; ELM-Extruded Lupin meal; DLM-Dehulled Lupin meal; EDLM-Extruded Dehulled Lupin meal

The test diets were fed in a mash form during the energy determination. Gross energy content of the test diets and excreta samples was measured using an adiabatic bomb calorimeter (Calorimeter CP500, Digital Data Systems, Randburg, South Africa). Complete amino acid analysis was done on the test products by Degussa Africa (Pty) Ltd. The amino acids were analyzed with a Biochrom 20 Amino Acid Analyzer (Amersham Biosciences) according to the official method 994.12 of the Association of Official Analytical Chemists (AOAC, 2000).

2.2.2 Broiler Performance Trial

The broiler performance trial was conducted in a starter phase (from day 1 to 20) and a finisher phase (from day 21 to 42). For each phase two basal diets were formulated to contain all nutrients to fulfill or exceed the nutritional requirements of broiler chickens for that specific developmental period (NRC, 1998). The one diet was without any lupin product (control) and the other contained 170g/kg lupin meal (test diet) in the starter phase and 174 g/kg lupin meal in the finisher phase. These diets were formulated with Winfeed 2 Feed Formulation to contain similar energy and nitrogen values, using the analyzed values for AMEn, chemical and amino acid content of the test products and main ingredients. Three more test diets were formed by replacing the lupin meal with equal quantities of either extruded lupin meal (ELM), dehulled lupin meal (DLM) or extruded dehulled lupin meal (EDLM). Each of the four test diets were then blended with the control diet at

five levels (20%, 40%, 60%, 80%, 100%), resulting in a total of 20 treatments plus a control diet. The summary of all experimental diets is shown in Table 2.2.

Table 2.2 Summary of treatments used in the broiler performance trial for both the starter and finisher phase.

Treatment	% of Control diet	% of Test diet
1	80	20% of LM Test Diet
2	60	40% of LM Test Diet
3	40	60% of LM Test Diet
4	20	80% of LM Test Diet
5	0	100% of LM Test Diet
6	80	20% of ELM Test Diet
7	60	40% of ELM Test Diet
8	40	60% of ELM Test Diet
9	20	80% of ELM Test Diet
10	0	100% of ELM Test Diet
11	80	20% of DLM Test Diet
12	60	40% of DLM Test Diet
13	40	60% of DLM Test Diet
14	20	80% of DLM Test Diet
15	0	100% of DLM Test Diet
16	80	20% of EDLM Test Diet
17	60	40% of EDLM Test Diet
18	40	60% of EDLM Test Diet
19	20	80% of EDLM Test Diet
20	0	100% of EDLM Test Diet
21	100	0% of Test Diet

LM-Lupin meal; ELM-Extruded Lupin meal; DLM-Dehulled Lupin meal; EDLM-Extruded Dehulled Lupin meal

Each of the dietary treatments was fed to three replicate groups of 80 as hatched Ross 308 broilers. Five thousand and forty day-old broiler chicks were housed in 63 litter pens at a stocking density of 21 birds/ m^2. Wood shavings at a depth of 60mm was used as floor material and checked regularly by means of visual inspection for wetness. The birds were housed in a closed, environmentally controlled unit with localized brooding. Initial starting temperature was 33°C, decreasing gradually to 21°C at 21 days of age and maintained at that level. A 23-hour light: 1 hour dark lighting programme was followed. The diets were fed *ad libitum* in the starter (Table 2.3) and finisher phase (Table 2.4) and were crumbed for the starter and cold pelleted for the finisher diets. The birds were starved for 3 to 5 hours prior to weighing to reduce the error associated with the digesta contents of the individual birds.

Body weight gain and feed consumption were recorded weekly, with mortalities being recorded daily to correct the calculated feed conversion ratio (FCR) values. The experiment had a factorial design with main effects being the processing procedure and blend. The results obtained from the experiment were subjected to analysis of variance using the General Linear Models (GLM) procedure of SAS ® software (SAS Institute, 2000). The interaction between blends and treatments for both the starter and finisher diets were found to be

significant (P< 0.001) and will be discussed accordingly. Differences between treatment means were identified using a Student's T test.

Table 2.3 Composition and calculated nutrient content of starter diets (g/kg on a 10% moisture basis)

Ingredients	Control	Test
Maize	503.0	428.0
Maize gluten 60	42.0	22.1
Wheat bran	20.0	0.5
Test Ingredient*	-	170.0
Soyabean Oilcake 46	152.6	113.2
Sunflower Oilcake 37	87.5	87.5
Fish meal 65	110.0	88.5
Oil - sunflower	56.3	59.2
L-lysine HCL	0.5	-
DL-methionine	5.0	4.8
Limestone	9.4	10.0
Monocalcium-P	9.1	11.5
Salt	0.4	1.0
Sodium bicarbonate	2.7	2.2
Vit+min premix[1]	2.5	2.5
Coccidiostat (Salinomycin, 12%)[2]	0.5	0.5

Calculated nutrient content[3]	Control	LM*	ELM*	DLM*	EDLM*
AMEn (MJ/kg)	12.68	12.32	12.13	12.35	12.22
Crude protein	244.8	239.6	245.0	255.7	259.4
Crude fiber	38.3	61.6	59.6	41.3	39.5
Ether extract	94.9	100.3	100.4	101.4	102.1
Lysine	10.7	10.3	10.5	10.9	11.1
Methionine	10.0	8.9	8.9	9.0	9.0
Threonine	11.7	10.8	10.9	11.2	11.4
Tryptophan	2.6	2.4	2.5	2.6	2.6
Arginine	13.1	15.1	15.9	17.1	17.9
Calcium	9.9	10.1	10.1	9.8	9.8

*Test ingredient includes either of the following lupin products: LM – lupin meal; ELM – extruded lupin meal; DLM – dehulled lupin meal; EDLM – extruded dehulled lupin meal. [1] Vitamin and mineral premix: Commercial broiler premix supplied by Roche Ltd. (Pty). [2] Salinomycin (12%) supplied by Profile Feeds, Cape Town. [3] Nutrient content calculated with Winfeed 2 Feed Formulation program

2.3 Results and Discussion

3.3.1 AME Bioassay

The chemical and amino acid composition of the four test products are shown in Table 2.5. The most noticeable changes occurred with the dehulling of lupins. It increased the protein (27.9 vs. 37.4%), fat (5.9 vs. 6.6%) and lysine contents (1.2 vs. 1.6%) in relation to lupin meal (LM) and the removal of the fibrous hull also resulted in a kernel component (DLM) with a substantially reduced CF (5.4 vs. 17.3%) content.

These results are similar to those reported by previous studies (Fernández & Batterham, 1995; Steenfeldt *et al*., 2003). This effect of dehulling was also portrayed by the extruded dehulled lupin meal (EDLM) diet with similar increases than those observed in DLM for protein, fat and lysine contents as well as a decrease in the CF content in relation to extruded lupin meal (ELM). The AMEn value of DLM is superior to that of LM (8.81 vs. 8.61 MJ/kg) just as the AMEn value of EDLM is higher than that of ELM (8.04 vs. 7.52 MJ/kg). This is most probably due to the removal of the fibrous pericarp that resulted in a more nutrient dense product. Brenes *et al*.

(1993) also observed a higher digestibility of energy when comparing dehulled to whole lupins. Steenfeldt *et al*. (2003) postulated that another possible beneficial effect of dehulling on the AME value of lupins could be related to the reduction of NSPs that occurs with dehulling, thereby leading to the alleviation of their anti-nutritional effect on the availability of ME (Choct *et al*., 1996). It is also interesting to note that the dehulling of lupin meal resulted in a substantial reduction in the Ca content of DLM, but that the P content remained similar (Table 2.5). This could be due to the fact that the lupin seed coats are unique in having no detectable phosphorous (Hove, 1974).

Table 2.4 Composition and calculated nutrient content of finisher diets (g/kg on a 10% moisture basis)

Ingredients	Control	Test
Maize	574.6	499.5
Maize gluten 60	10.0	15.0
Wheat bran	10.6	9.0
Test Ingredient*	-	174.0
Soyabean Oilcake 46	151.0	128.7
Sunflower Oilcake 37	71.0	21.5
Fish meal 65	103.3	59.8
Oil - sunflower	53.8	59.7
L-lysine HCL	-	0.3
DL-methionine	5.0	4.7
Limestone	8.2	10.5
Monocalcium-P	7.6	12.1
Salt	0.6	2.4
Sodium bicarbonate	2.7	1.3
Vit+min premix[1]	3.0	3.0

Calculated nutrient content[2]	Control	LM*	ELM*	DLM*	EDLM*
AMEn (MJ/kg)	12.84	12.48	12.29	12.51	12.38
Crude protein	218.8	207.4	212.9	223.8	227.6
Crude fiber	35.6	53.7	51.7	32.9	31.1
Ether extract	93.4	100.8	100.9	101.9	102.6
Lysine	9.7	8.8	8.9	9.4	9.6
Methionine	9.4	8.0	8.0	8.1	8.1
Threonine	10.6	9.1	9.2	9.5	9.7
Tryptophan	2.4	2.1	2.1	2.2	2.3
Arginine	12.1	13.0	13.8	15.1	15.9
Calcium	8.9	9.1	9.1	8.9	8.8

*Test ingredient includes either of the following lupin products: LM – lupin meal; ELM – extruded lupin meal; DLM – dehulled lupin meal; EDLM – extruded dehulled lupin meal
[1] Vitamin and mineral premix: Commercial broiler premix supplied by Roche Ltd. (Pty)
[2] Nutrient content calculated with Winfeed 2 Feed Formulation program

From Table 2.5 it can be seen that the extrusion of lupin products (ELM and EDLM) resulted in an increased protein and fat content when compared to their raw counterparts (LM and DLM). Woodroofe (1999) indicated that the cooking action of extrusion is responsible for the mild breakdown of the protein structure and that either the shear force during extrusion processing or the expansion of the product once it makes contact with the atmosphere, ruptures the oil cells and releases the additional fat. As can be expected, the amino acid content of the extruded lupin products showed similar increases to that of the CP contents when compared with LM and DLM. The increase in protein content (31.05% vs. 27.88%) of the ELM compares well to that observed by Prinsloo (1993), where the extrusion of *L.angustifolius* under similar conditions resulted in an

increase in protein content from 27.81% to 30.91% (10% moisture basis). It was expected that the extrusion of LM would result in an increased AMEn value, especially in the light of the increased fat contents. Plavnik & Sklan (1995) have observed that such an increase are one of the main contributors towards an increased ME value. This was, however, not found with either the ELM or the EDLM products. Prinsloo (1993) evaluated the effect of extrusion of *L.angustifolius* on the AME value with adult cockerels and reported an increase from 6.71 MJ/kg to 7.58 MJ/kg. This AME value for LM is lower than the value observed for AMEn in this study (8.61 MJ/kg). The expansion that occurs during extrusion could have resulted in a product with increased bulkiness. Due to the mash form in which the products were presented during the energy assay, it may have led to a decreased energy intake of the extruded products (with a more constant energy excretion) and could thus have contributed to the decreased AMEn value observed. For future energy assays, extruded lupin products should be pelleted in order to minimize differences in feed intake.

Table 2.5 Chemical and amino acid composition of different lupin products (10% moisture basis)

Composition[1]	Lupin Meal (LM)	Extruded Lupin Meal (ELM)	Dehulled Lupin Meal (DLM)	Extruded Dehulled Lupin Meal (EDLM)
Crude Protein	27.88	31.05	37.35	39.53
AME_n MJ/kg	8.61	7.52	8.81	8.04
Ether extract	5.89	5.95	6.55	6.94
Crude Fiber	17.25	16.10	5.35	4.30
ADF [2]	24.93	22.88	11.07	9.84
NDF [3]	27.09	24.54	10.59	7.38
Ash	3.31	3.35	3.05	3.05
Ca	0.31	0.28	0.15	0.12
P	0.38	0.44	0.44	0.53
Mn (mg/kg)	37.39	39.99	32.13	38.50
Amino acids				
Arginine	2.67	3.15	3.88	4.36
Aspartic acid	2.58	2.90	3.52	3.85
Glutamic acid	5.16	6.04	7.25	8.20
Glycine	1.09	1.21	1.45	1.58
Histidine	0.76	0.83	1.04	1.11
Isoleucine	1.04	1.15	1.45	1.58
Leucine	1.75	1.94	2.37	2.58
Lysine	1.22	1.32	1.58	1.68
Methionine	0.15	0.16	0.19	0.19
Phenylalanine	1.01	1.13	1.39	1.51
Threonine	0.92	1.00	1.18	1.26
Tryptophan	0.23	0.25	0.31	0.33
Valine	1.05	1.13	1.46	1.52

[1] % DM unless stated otherwise; [2] Acid detergent fiber; [3] Neutral detergent fiber

2.3.2 Broiler Performance Trial

The broiler performance results have been divided between the effects observed with the starter diets at the end of 3 weeks of age (Table 2.6) and those with the finisher diets at the end of 6 weeks of age (Table 2.7). The trends evident at 3 weeks of age were generally very similar to those observed for 6 weeks of age, especially for feed intake and broiler body weight.

2.3.2.1 0 to 3 Weeks

There was a significant ($P<0.001$) interaction between treatments (LM, DLM, ELM, EDLM) and blends (0, 20, 40, 60, 80 & 100%). Body weight at 21 days of age (Table 2.6), for birds that were fed diets containing up to 80% of the LM test diet were heavier ($P<0.05$) than the control birds, but the 100% inclusion of the LM test diet resulted in broiler body weights that were lower ($P<0.05$) than the control birds. Most of the increased body weights that were observed can be explained by the higher feed intakes of broilers fed various levels of the LM test diet, when compared to those of the control birds, even though only the 60, 80 and 100% blends resulted in significant increases ($P<0.05$). It is also apparent from the data that LM does not contain any ANFs that could negatively influence the feed intake of broilers, since the increasing levels of LM test diet did not result in equally significant ($P<0.05$) reductions in feed intake. These findings are in agreement with those reported by Olver & Jonker (1997), indicating that when the alkaloid level of sweet lupin seed is less than 0.1 g/kg, there are no deleterious effects on broilers in terms of feed intake. It is, however, also possible that the anti-nutritional effect of the lupin NSPs on the availability of nutrients such as lysine may have resulted in the increased feed intake that was observed for broilers fed diets containing LM. FCR was positively affected ($P<0.05$) up to 60% inclusion of LM in the test diet. The decreased body weight ($P<0.05$), despite the increased feed intake of the 100% inclusion of LM test diet, resulted in a higher ($P<0.05$) FCR. This demonstrates that the inclusion of 17% lupin meal in a broiler starter diet does have a significant ($P<0.05$) influence on the efficiency with which broilers are able to convert the feed ingested into body weight. Perez-Maldonado *et al.* (1999) found that the high NSP contents of lupins are in accordance with the higher gut viscosity ($P<0.05$) they have observed in laying hens. This increased digesta viscosity could result in a decreased rate of diffusion of substrates and digestive enzymes as well as interfering with their effective interaction at the mucosal surface (Mohanna *et al*., 1999), thereby influencing the efficiency with which nutrients are absorbed and utilized by the bird. This anti-nutritional effect of the high NSP content of lupins might thus be responsible for a lower lysine availability than was anticipated. Since lupins already contain a rather limiting amount of lysine (Larbier & Leclercq, 1994) and the starter test diets were only supplemented with DL-methionine, it could have contributed to the increased feed intakes that was observed with increasing levels of LM. It is to be noted that the incidence of wet-sticky droppings, which are normally associated with high levels of soluble polysaccharides (Chesson, 1990), were observed in all the pens fed a 100% blend of the LM test diet.

Table 2.6 Effect of increasing levels of lupin meal, dehulled lupin meal, extruded lupin meal and extruded dehulled lupin meal on broiler performance to 3 weeks of age

Lupin Test Ingredient	Level of inclusion of test diet (g/kg)	Body weight (g)	Feed Intake (g)	Feed conversion ratio (g feed /g weight)
Lupin Meal (LM)	0	709.79	1011.34	1.423
	20	738.49	1028.61	1.390
	40	727.11	1020.50	1.404
	60	741.88	1036.34	1.396
	80	728.10	1029.28	1.416
	100	691.82	1037.25	1.501
Dehulled Lupin Meal (DLM)	0	709.79	1011.34	1.423
	20	744.19	1025.21	1.377
	40	777.76	1069.99	1.373
	60	788.01	1077.15	1.364
	80	804.87	1085.96	1.348
	100	793.98	1066.38	1.345
Extruded Lupin Meal (ELM)	0	709.79	1011.34	1.423
	20	711.41	1006.76	1.416
	40	713.68	1012.14	1.417
	60	709.62	1010.77	1.425
	80	699.55	1003.73	1.437
	100	687.50	994.04	1.445
Extruded Dehulled Lupin Meal (EDLM)	0	709.79	1011.34	1.423
	20	715.59	1015.23	1.419
	40	719.57	1013.23	1.411
	60	718.36	1012.18	1.408
	80	716.57	1009.91	1.409
	100	710.50	1000.21	1.407
LSD		9.51	14.34	0.015

Broilers fed DLM performed better ($P<0.05$) than the control group in terms of body weight for all levels of inclusion of the test diet. Body weight of broilers fed DLM was also higher ($P<0.05$) than those fed LM from the 40% inclusion level. This effect could be explained by the higher feed intakes ($P<0.05$) that were observed from the same inclusion level when DLM were compared to the LM test diets. The reduction in CF content with the dehulling of lupin meal resulted in a more nutrient dense product with an increased protein, energy and fat content (Table 2.5). The broilers fed the DLM diets thus performed better ($P<0.05$) at each level from the 40% inclusion level in terms of body weight, feed intake and FCR when compared with the LM diets. Similar improvements in broiler performance was also observed by Brenes *et al*. (1993) when they compared dehulled to whole lupins in an 8-day feeding trial (from 10 to 17 days of age), but the differences were not found to be significant.

The use of ELM up to 80% inclusion did not affect body weight. However, 100% inclusion resulted in a significant decrease in body weight ($P<0.05$). These differences in body weight can be explained by the similar pattern in feed intake observed in birds fed LM. There were no differences ($P<0.05$) in feed intake when birds

were fed the test diets up to the 80% level. Birds fed the diet containing 100% ELM consumed less ($P<0.05$) feed than those on the control diet. Since FCR is dependent on both these variables, it is not surprizing to find that the FCR was also similar to that of the control up until the 80% inclusion of ELM test diets, whereafter the ratio increased ($P<0.05$). In comparison with the LM diets, it is clear that the ELM diets did not perform as well. For body weight, the broilers fed ELM diets performed worse ($P<0.05$) at all levels of inclusion of the test diet and for the other performance parameters, feed intake and FCR, the birds also performed worse at all levels, but only significantly so ($P<0.05$) for the 20, 60, 80 and 100% inclusion of ELM test diets. Prinsloo (1993) also investigated the effect of extrusion of *L.angustifolius* on broiler performance and found significant reductions ($P<0.05$) in body weight, feed intake and feed conversion efficiency at 25 days of age. Conversely, Watkins & Mirosh (1987) observed an improvement ($P<0.05$) in broiler growth rate and feed efficiency when they compared extruded *vs*. raw lupins. The decreased feed intake that was observed in this study for ELM in comparison with LM, may have resulted from the increased bulkiness of the feed due to the extrusion process. In addition, the high oil content of the diets (± 10%) resulted in a relatively high breakage of the starter crumbs and may have negatively influenced the feed intake of the birds.

The combination of dehulling and extrusion of lupin meal (EDLM) performed similar to that of the ELM diets for feed intake, body weight and FCR. Even though the EDLM diets resulted in numerically superior results for all inclusion levels in all the performance parameters, the only significant differences ($P<0.05$) between the two diets were found to be at levels 80 and 100% for body weight and 60, 80 and 100% for FCR. The reduction in CF level with the additional dehulling of ELM may have been responsible for the slightly better response observed in comparison with ELM diets, but as mentioned before the results are not significant at all levels of inclusion. When comparing the results from the inclusion of DLM with those of the EDLM, it is clear that the additional extrusion process resulted in significantly ($P<0.05$) lower broiler performances for all parameters at all inclusion levels, except the 20% level for feed intake. Reasons for the decreased performance of broilers with the EDLM are similar to those given for comparison between LM and ELM diets.

The interaction between blends and treatments for the starter diets is highly significant ($P<0.001$). Dehulling increased broiler performance at all levels, while extrusion decreased the nutritional value of lupins for broilers.

2.3.2.2 3 to 6 Weeks

The finisher diets were fed from day 21 until 42 days of age and broiler performance was measured in terms of body weight, food intake and feed conversion ratio (g feed / g weight). The results at the end of 6 weeks are shown in Table 2.7. A significant ($P<0.001$) interaction was also observed between all treatments (LM, DLM, ELM, EDLM) and blends (0, 20, 40, 60, 80 & 100%). The 6-week body weight of broilers being fed the LM test diets were higher ($P<0.05$) than those of the control group for all levels of inclusion up to the 80% level, whereafter it decreased to less ($P<0.05$) than those of the control group. These results are similar to those recorded during the starter period. With increasing levels of LM test diet, the feed intake of broilers also increased, but was only significantly higher than the control group from the 60% inclusion level of LM test

diet. It is possible that the anti-nutritional effect of the lupin NSPs on the availability of nutrients such as lysine may have influenced the feed intake of broilers with diets containing LM. The FCR at 42 days of age progressively increased with increasing levels of LM, but was only significantly higher ($P<0.05$) than those of the control group from the 60% inclusion level. These results are somewhat different from those observed at the end of 3 weeks of age, suggesting that despite the increased feed intakes of the birds during the finisher phase, they still could not compensate for their increased requirements. This is especially significant ($P<0.05$) for the 60%, 80% and 100% inclusion levels of LM test diet. The 100% inclusion of LM test diet also showed similar results at the end of 6 weeks than was observed at the end of 3 weeks and again demonstrates that the inclusion of 17.4% LM in a broiler finisher diet does have a significant ($P<0.05$) influence on the efficiency with which broilers are able to convert the feed ingested into body weight.

Table 2.7 Effect of increasing levels of lupin meal, dehulled lupin meal, extruded lupin meal and extruded dehulled lupin meal on broiler performance to 42 days of age

Lupin Test Ingredient	Level of inclusion of test diet (g/kg)	Body weight (g)	Food Intake (g)	Feed conversion ratio (g feed /g weight)
Lupin Meal (LM)	0	2278.51	4020.17	1.77
	20	2295.88	4071.36	1.77
	40	2301.25	4080.12	1.78
	60	2284.24	4098.92	1.80
	80	2272.04	4113.76	1.82
	100	2214.22	4138.26	1.87
Dehulled Lupin Meal (DLM)	0	2278.51	4020.17	1.77
	20	2303.72	4069.36	1.77
	40	2372.47	4197.95	1.77
	60	2359.40	4206.47	1.77
	80	2359.58	4209.12	1.78
	100	2341.73	4183.22	1.79
Extruded Lupin Meal (ELM)	0	2278.51	4020.17	1.77
	20	2264.37	4027.96	1.78
	40	2271.91	4044.79	1.78
	60	2259.25	4035.99	1.79
	80	2239.92	4047.88	1.81
	100	2194.20	4046.35	1.84
Extruded Dehulled Lupin Meal (EDLM)	0	2278.51	4020.17	1.77
	20	2282.83	4025.30	1.77
	40	2276.31	4038.54	1.77
	60	2263.09	4020.18	1.78
	80	2243.15	4010.23	1.79
	100	2227.05	3989.75	1.79
LSD		23.70	40.69	0.01

The body weight of broilers fed DLM test diets were higher ($P<0.05$) than the control group from the 40% inclusion level upwards and can be explained by the higher feed intakes ($P<0.05$) that were observed at all inclusion levels when DLM were compared to the control diets. In comparison with the control group, the broilers that were fed the DLM test diets had similar FCRs up until the 60% inclusion level, whereafter it

increased significantly (P<0.05). Similar to the 3-week results, the broilers fed the DLM test diets performed better (P<0.05) than those on the LM test diets in terms of body weight and feed intake from the 40% inclusion level. For FCR, however, the trend as seen at 3 weeks of age was reversed in that the ratio increased significantly (P<0.05) with increasing levels of DLM from the 60% inclusion level. The improved body weight and feed intake of broilers fed DLM in comparison with LM could be explained by the effect of dehulling on the nutrient density of the lupin product, but the reason for the change from 3 to 6 weeks in the efficiency with which broilers can convert high levels of DLM into body weight is not exactly clear. A possible explanation might be that at these high intake levels (almost four times as much as the starter feeds), the anti-nutritional influence of NSPs might exert a bigger effect on the FCR. Unfortunately, no other broiler performance studies with similar comparisons between increasing levels of dehulled and whole lupins have been reported that could support these findings.

When ELM was fed to broilers up until the 60% inclusion level, it resulted in body weights that did not differ significantly from those of the control group, but the 80 and 100% levels did result in lower body weights (P<0.05). Feed intake of broilers fed the ELM test diets was similar to that of the control birds for all levels of inclusion, with the FCR being increased (P<0.05) in comparison to the control group for all levels of inclusion of ELM. This effect might indicate that the intake of ELM not only have a negative effect on the FCR from the 80% inclusion level as seen at 3 weeks of age, but that the higher feed intakes needed at 6 weeks of age could have resulted in the overall negative effect on FCR observed for all levels of inclusion of the ELM test diets. The bulkiness of the feed and the possible negative effect of extrusion processing (Maillard reaction) on the availability of lysine might also have reduced the potential growth of broilers at these high intake levels. The ELM diets did not perform as well when compared with the LM diets. Similar results were observed at 3 weeks. At each inclusion level, the broilers fed the ELM diets weighed less than those on the LM diets and significantly (P<0.05) so up to the 80% inclusion level. The broilers also consumed less of the ELM feed than the LM at all inclusion levels, but only the 20, 60, 80 and 100% levels proved to be significant (P<0.05). The FCR of broilers fed the ELM test diets were, however, similar to those of the LM test diets at all levels of inclusion, except for the 100% blend where the LM test diet resulted in a higher (P<0.05) average value.

Broilers fed EDLM performed similar to that of the ELM diets. There were no differences (P<0.05) in body weight for these birds compared to those of the control group up to the 60% inclusion level, but the 80 and 100% inclusion of EDLM test diets resulted in lower (P<0.05) body weights. Similar feed intakes to the control group were observed for all levels of inclusion of the EDLM test diets and just as with the ELM diets, the FCR also increased progressively with increased inclusion levels of EDLM, but this was only significant from the 80% level when compared with the control group. In comparison with the ELM diets, broilers fed the EDLM diets weighed more and ate less at each level of inclusion, but these were only significant (P<0.05) at the 100% inclusion level for both these parameters. It is thus not surprizing that the FCR for EDLM diets were generally lower than those obtained from the ELM diets for all inclusion levels, but again these were only found to be significantly (P<0.05) lower for the 20, 80 and 100% levels. The reduced CF level of EDLM vs. ELM (Table 2.5) also resulted in test diets with a lower CF content (Table 2.4) and thus a more nutrient dense

diet that will reduce the amount of feed intake and increase broiler weight per unit of feed intake (i.e. reducing FCR). Since the advantage of the additional processing procedure (EDLM) were not significant ($P<0.05$) throughout the inclusion levels, it is unlikely that the additional cost will benefit broiler production above that of using only ELM. When comparing the results from the inclusion of DLM with those of EDLM, it is clear that the additional extrusion process resulted in lower ($P<0.05$) broiler body weights at all inclusion levels as well as lower ($P<0.05$) feed intakes from the 40% inclusion level of EDLM. The difference in FCR between EDLM and DLM at all levels of inclusion was, however, not significant ($P<0.05$) and might indicate that the beneficial effect of dehulling could override the negative response seen with the extruded lupin products.

2.4 Conclusion

It can be concluded from the results presented that the dehulling of lupins have resulted in superior broiler performance in terms of body weight, feed intake and feed conversion ratio (g feed / g weight) when compared with LM, ELM or EDLM. The dehulling of lupin meal produced a more nutrient dense product and was also responsible for the highest AME measured for the lupin products. The extrusion of lupins on the other hand did not perform as well when compared to LM and DLM and this method of processing, under the specific conditions as indicated for this trial, is not recommended for lupins at this stage. The combination of extrusion and dehulling of lupins did not produce any further benefits in terms of a better broiler response.

CHAPTER 3

The influence of expansion of Full-fat Canola seed on apparent metabolizable energy (AME) and broiler performance.

3.1 Introduction

Full-fat canola seeds contain approximately 40% oil and 21 to 23% CP (Robblee *et al*., 1989) and its inclusion in poultry diets as a high energy-high protein feed ingredient could prove especially beneficial in the hot South African climate. It is, however, evident from literature that research on the use of canola meal in poultry diets have far exceeded that of the canola seed and a general lack of information therefore exists regarding the inclusion of this oilseed in its full-fat form in poultry diets.

The amino acid content of canola compares favourably with that of soyabean meal, with canola consisting of more of the sulphur-containing amino acids methionine and cystine (Clandinin *et al*., 1989). The lysine content of the canola seed protein is also superior to that of soyabean meal (Lee *et al*., 1995). The complementary amino acid profile of canola and soyabean therefore makes the combination of these protein-rich feedstuffs well suited in poultry nutrition. Appreciable variation exist amongst reported values for ME of canola seed (Sibbald, 1977a; Sibbald & Price, 1977; Muztar *et al*., 1978 and Muztar *et al.,* 1980) and have been contributed to differing responses of individual birds to the palatability and texture of the seeds as well as the influence of grinding of the seed (Muztar *et al*., 1978).

Even though it has been recommended that canola seeds are grinded to reduce the variability in ME values (Muztar *et al*., 1978), it also results in the activation of myrosinase that hydrolyses the glucosinolates into their toxic and goitrogenic components with resultant detrimental effects for the birds ingesting these components (Pickard *et al*., 1989). It has, however, been found that some form of heat treatment of the canola seed prior to inclusion in poultry diets will inactivate myrosinase and lead to an improvement in broiler performance (Woodly *et al*., 1972; Leeson *et al*., 1978). Bayley & Summers (1975) have also reported that extrusion resulted in an improvement in the nutritional value of this oilseed in diets for growing chicks above that of a maize-soyabean control diet, but only to a maximum 10% inclusion level. The literature provides conflicting results in broiler performance trials with the inclusion of full-fat canola (Leeson *et al*., 1978; Summers *et al*., 1982 and Lee *et al.,* 1991) and further research is needed to establish a maximum inclusion level of canola seed that will optimize broiler performance. The aim of this study is to contribute information regarding the influence that a heat treatment, such as expansion, has on the AME value of full-fat canola and to establish whether an improvement in broiler performance is evident with such treatment.

3.2 Material and Methods

Full-fat canola seeds (*Brassica rapa*) cultivar Outback, were obtained from a producer in the Western Cape, South Africa. The canola seeds were mixed in a 50:50 ratio with maize and milled in a hammermill to pass a 3 mm screen. The batch had to be sent through the mill twice in order to reduce the amount of whole canola seeds in the mixture. The addition of maize to the canola seeds assisted with the processing of the seeds,

since the high oil content of the canola could hamper the efficiency of the machines during processing (Summers *et al*., 1982). The canola-maize mixture was then expanded at Equifeeds (Fisantekraal, Cape Town). Initially extrusion was tried, but due to the high oil content of full-fat canola, it was found that expansion resulted in a better product and less oil-clogging of the equipment. The expander treatment (120°C) was without a pre-conditioning phase, since the addition of moisture before heat has been found to accelerate the hydrolysis of glucosinolates (Pickard *et al*., 1989). This resulted in two test products: canola full-fat (CFF) and expanded canola full-fat (ECFF).

3.2.1 AME Bioassay

The AME determination for the test ingredients was performed as described in Chapter 2. A total of 195 21-day-old broilers (Ross 308) were used in this trial. A summary of the treatments used in the AME bioassay is shown in Table 3.1.

Table 3.1 Summary of treatments used in the AME bioassay

Treatment	Description	Level of intake (% of ad libitum)
1	Control (Maize)	40
2	Control (Maize)	50
3	Control (Maize)	60
4	Control (Maize)	75
5	Control (Maize)	90
6	50% CFF : 50% Maize	45
7	50% CFF : 50% Maize	60
8	50% CFF : 50% Maize	75
9	50% CFF : 50% Maize	90
10	50% ECFF : 50% Maize	45
11	50% ECFF : 50% Maize	60
12	50% ECFF : 50% Maize	75
13	50% ECFF : 50% Maize	90

CFF-Canola full-fat; ECFF-Expanded Canola full-fat

3.2.2 Broiler Performance Trial

Two basal diets were formulated to contain all nutrients to fulfill or exceed the nutritional requirements of broiler chickens (NRC, 1998) during a starter phase (from day 1 to 20) and a finisher phase (from day 21 to 42). The one diet was without any canola product and served as the control, while the other contained 169g/kg CFF (test diet) in both the starter and finisher phase. Another test diet was formed by replacing the CFF with an equal quantity of ECFF. The diets were formulated and blended as described in Chapter 2. A summary of all experimental diets used in this broiler performance trial is shown in Table 3.2.

Each of the dietary treatments was fed to three replicate groups of 40 as hatched Ross 308 broilers. One thousand three hundred and twenty day-old broiler chicks were housed in 33 litter pens at a stocking density of 21 birds/ m^2. Wood shavings at a depth of 50mm was used as floor material. The birds were housed in a closed, environmentally controlled unit with localized brooding. Initial starting temperature was 33°C, decreasing

gradually to 21°C at 21 days of age and maintained at that level. A 23-hour light: 1 hour dark lighting programme was followed. The diets were fed *ad libitum* in the starter (Table 3.3) and finisher phase (Table 3.4) and were crumbed for the starter and cold pelleted for the finisher diets. The birds were starved for 3 to 5 hours prior to weighing to reduce the error associated with the digesta contents of the individual birds.

Table 3.2 Summary of treatments used in the broiler performance trial for both the starter and finisher phase

Treatment	% of Control diet	% of Test diet
1	80	20% of CFF Test Diet
2	60	40% of CFF Test Diet
3	40	60% of CFF Test Diet
4	20	80% of CFF Test Diet
5	0	100% of CFF Test Diet
6	80	20% of ECFF Test Diet
7	60	40% of ECFF Test Diet
8	40	60% of ECFF Test Diet
9	20	80% of ECFF Test Diet
10	0	100% of ECFF Test Diet
11	100	0% of Test Diet

CFF-Canola full-fat; ECFF-Expanded Canola full-fat

Table 3.3 Composition and calculated nutrient content of starter diets (g/kg on a 10% moisture basis)

Ingredients		Control diet	Test diet
Maize		503.0	473.0
Maize gluten 60		42.0	38.1
Wheat bran		20.0	0.5
Test Ingredient*		-	169.0
Soyabean Oilcake 46		152.6	138.4
Sunflower Oilcake 37		87.5	62.8
Fish meal 65		110.0	88.4
Oil - sunflower		56.3	0.5
L-lysine HCL		0.5	-
DL-methionine		5.0	4.6
Limestone		9.4	9.2
Monocalcium-P		9.1	10.9
Salt		0.4	1.1
Sodium bicarbonate		2.7	2.3
Vit+min premix[1]		2.5	2.5
Coccidiostat (Salinomycin, 12%)[2]		0.5	0.5
Calculated nutrient content[3]	Control	CFF*	ECFF*
AMEn (MJ/kg)	12.68	12.17	12.79
Crude protein	244.8	241.2	241.5
Crude fibre	38.3	53.8	53.1
Ether extract	94.9	100.7	102.2
Lysine	10.7	10.9	10.8
Methionine	10.0	9.6	9.5
Threonine	11.7	11.4	11.4
Arginine	13.1	13.2	13.1
Calcium	9.9	10.1	10.0

*Test ingredient includes either of the following canola products: CFF – canola full-fat; ECFF – expanded canola full-fat.
[1] Vitamin and mineral premix: Commercial broiler premix supplied by Roche Ltd. (Pty). [2] Salinomycin (12%) supplied by Profile Feeds, Cape Town. [3] Nutrient content calculated with Winfeed 2 Feed Formulation program

Body weight gain and feed consumption were recorded weekly, with mortalities being recorded daily to correct the calculated feed conversion ratio (FCR) values. The results obtained from the experiment were subjected to analysis of variance using the General Linear Models (GLM) procedure of SAS ® software (SAS Institute, 2000). Differences between treatment means were identified using the appropriate SED (LSD) and a Student's T test.

Table 3.4 Composition and calculated nutrient content of finisher diets (g/kg on a 10% moisture basis)

Ingredients		Control diet	Test diet
Maize		574.6	539.3
Maize gluten 60		10.0	21.7
Wheat bran		10.6	-
Test Ingredient*		-	169.0
Soyabean Oilcake 46		151.0	165.0
Sunflower Oilcake 37		71.0	6.5
Fish meal 65		103.3	61.2
Oil - sunflower		53.8	1.7
DL-methionine		5.0	4.6
Limestone		8.2	9.3
Monocalcium-P		7.6	11.5
Salt		0.6	1.7
Sodium bicarbonate		2.7	2.5
Vit+min premix[1]		3.0	3.0
Calculated nutrient content[2]	Control	CFF*	ECFF*
AMEn (MJ/kg)	12.84	12.27	12.90
Crude protein	218.8	213.6	213.9
Crude fiber	35.6	46.4	45.7
Ether extract	93.4	101.3	102.7
Lysine	9.7	9.5	9.4
Methionine	9.4	8.6	8.6
Threonine	10.6	9.9	9.9
Arginine	12.1	11.4	11.3
Calcium	8.9	9.1	9.1

*Test ingredient includes either of the following canola products: CFF – canola full-fat; ECFF – expanded canola full-fat; [1] Vitamin and mineral premix: Commercial broiler premix supplied by Roche Ltd. (Pty). [2] Nutrient content calculated with Winfeed 2 Feed Formulation program

3.3 Results and Discussion

3.3.1 AME Bioassay

The chemical and amino acid composition of full-fat and expanded canola seed are presented in Table 3.5. The heat treatment increased the AMEn value of full-fat canola from 15.51 MJ/kg to 19.20 MJ/kg. From the results it is apparent that the increase in available energy content stems mainly from the increase in the fat content (39.07 % to 39.94%). Even though no other studies were found reporting similar effects of heat treatment on the chemical composition of full-fat canola, Plavnik & Sklan (1995) reported that the expansion of maize based diets resulted in a significant increase in the AMEn value and contributed it to the significant increase in the fat digestibility with the expansion of the diets. The shear force action that takes place when the feed is projected forward inside the expander barrel as well as the expansion of the product once it makes contact with the atmosphere, ruptures the oil cells and releases the additional fat (Woodroofe, 1999). A similar

effect of extrusion on cell walls have also been found by Bos *et al*. (1992) and could explain the decrease in CF, ADF and NDF contents observed in Table 3.5.

Table 3.5 Chemical and amino acid composition of full-fat and expanded canola seed (10% moisture basis)

Composition[1]	Full-fat Canola (CFF)	Expanded Canola Full-fat (ECFF)
Crude Protein	20.30	20.50
AME_n MJ/kg	15.51	19.20
Ether extract	39.07	39.94
Crude Fibre	13.88	13.47
ADF [2]	25.50	21.96
NDF [3]	25.76	22.85
Ash	2.48	2.44
Ca	0.52	0.51
P	0.71	0.70
Amino acids		
Arginine	1.35	1.30
Isoleucine	0.86	0.79
Leucine	1.47	1.45
Lysine	1.33	1.26
Methionine	0.43	0.41
Threonine	0.91	0.91
Valine	1.12	1.03

[1] % DM unless stated otherwise; [2] Acid detergent fiber; [3] Neutral detergent fiber

The CP content of CFF showed a slight numerical increase with the expansion treatment, but most of the individual amino acid contents measured, decreased with the heat treatment. Expander processing up to a temperature of 120^0C is not known to have a significant influence on feed protein content nor on the total lysine content, but Peisker (1992) reported a slight numerical decrease in the content and availability of amino acids at 130^0C. The modification of the protein structure that occurs with heat treatment is generally considered as desirable since it renders the protein more readily available for poultry. Excessive heat treatment, however, may cause some damage in that the amino acids, especially lysine, could become irreversibly altered and may not be recovered by acid hydrolysis (Pickard *et al*., 1989). The results in Table 3.5 might indicate that even though our heat treatment was measured at an outer barrel temperature of 120°C, our expansion treatment did in fact result in a slight reduction of total lysine (and other amino acid) contents as measured by acid hydrolysis.

3.3.2 Broiler Performance Trial

The broiler performance results have been divided between the effects observed with the starter diets at the end of 3 weeks of age (Table 3.6) and those with the finisher diets at the end of 6 weeks of age (Table 3.7).

3.3.2.1 0 to 3 Weeks

Increasing levels of both CFF and ECFF reduced broiler body weights, but this was only found to be significant ($P<0.001$) from the 60% inclusion level of either of the test diets (i.e. 10% of the test ingredients) when compared with a maize-soyabean control. The goitrogenic products produced by the action of myrosinase during the milling of canola seeds, have a negative influence on feed intake (Pickard *et al*., 1989) and might

therefore be responsible for the reduction in broiler body weights at levels higher than 10% CFF. These results are similar to those reported by Bayley & Summers (1975) for the inclusion of more than 10% extruded rapeseed and that of Leeson *et al*. (1987) for the inclusion of more than 10% CFF. The expansion treatment of CFF seeds did not result in any significant improvement ($P<0.05$) in broiler body weight at any inclusion level during this period. Leeson *et al*. (1978) found that the body weight gain of broilers (4 weeks of age) was unaffected ($P<0.05$) when they were fed heat-treated whole rapeseed at 10 and 20% of the dietary level. These results are in agreement with those reported by Olomu *et al.* (1974). These authors found that neither oven-heating nor autoclaving (120°C for 10 minutes) resulted in a significant ($P<0.05$) improvement in broiler body weight gains when fed at 10 and 20% of the diet. Other reports from the literature, however, show the opposite effect. Bayley & Summers (1975) fed increasing levels of raw and extruded rapeseed (10, 20, 30, 40 and 50%) to broilers and found that extrusion did in fact increase the final weight of the bird in relation to the raw rapeseed. However, the inclusion of more than 10% extruded rapeseed reduced the growth performance in comparison to the chicks that were receiving the maize-soyabean meal control diets.

Table 3.6 Effect of increasing levels of canola full-fat and expanded canola full-fat on broiler performance to 3 weeks of age

Test Ingredient	Level of inclusion of test diet (%)	Body weight (g)	Food Intake (g)	Food conversion ratio (g food /g gain)
Canola Full-fat (CFF)	0	725.59	1000.94	1.39
	20	712.59	980.24	1.37
	40	709.43	962.26	1.36
	60	705.57	954.93	1.36
	80	702.19	942.34	1.36
	100	695.77	939.93	1.35
Extruded Canola FF (ECFF)	0	725.59	1000.94	1.39
	20	713.15	986.99	1.37
	40	701.17	962.00	1.37
	60	697.67	959.00	1.37
	80	690.19	952.81	1.38
	100	684.17	951.17	1.39
LSD		10.78	19.43	0.03

The feed intake of broilers showed a marked response ($P\leq0.001$) with increasing levels of CFF, and for all inclusion levels from the 40% level a decrease ($P<0.05$) in feed intake was noticed in comparison with the maize-soyabean control diet. Studies with laying hens reported reductions in feed intake with full-fat rapeseed (Leslie & Summers, 1972), and Summers *et al*. (1982) reported significantly lower feed intakes for broilers when canola seed diets were fed from 7-24 days of age. Similarly, Bayley & Summers (1975) observed reductions in feed intake in comparison with a maize-soyabean control when broilers were fed increasing levels of CFF seeds. The expansion of CFF did not influence the feed intake of broilers at any level of inclusion and could explain the similar trend that was observed for the body weights during the same starter period. This form

of heat treatment as described in this study seemed to be unsuccessful in improving the nutritive value of CFF and did not influence the negative effect that the ANFs present in canola seed had on feed intake.

Despite the negative effect on body weight, the FCR was improved with the diets containing CFF in comparison with the control diet when the level of inclusion was above 40% for both weeks 3 and 6. It seems as if feed intake *per se* are affected by canola seeds, but that the utilization of the diets are not. Similar results have been reported by Summers *et al*. (1982). From the data it also appears as if the reduced feed intakes with increasing levels of CFF had a more pronounced effect on broiler body weight during the starter phase than during the finisher phase. These results suggest that as the bird ages it is more capable of utilizing the nutrients provided by CFF, or that they become less susceptible at the potential ANFs present in the seeds.

3.3.2.2 3 to 6 Weeks

The influence of increasing levels of CFF on broiler body weight was found not to be significant and even though the body weights were lower than that of the control, none of the inclusion levels resulted in significant reductions in body weight. For ECFF, the influence of increasing levels on body weight was found to be significant ($P<0.05$). When compared with the control diet, the 100% inclusion level resulted in a significant ($P<0.05$) reduction in broiler body weight. Similar to the starter period, the expansion treatment of CFF did not result in significant improvement ($P<0.05$) in broiler body weights. The 60 and 100% inclusion levels of ECFF test diet showed a reduction ($P<0.05$) in broiler body weights with expansion.

Table 3.7 Effect of increasing levels of canola full-fat and extruded canola full-fat on broiler performance to 6 weeks of age.

Test Ingredient	Level of inclusion of test diet (%)	Body weight (g)	Food Intake (g)	Food conversion ratio (g food /g gain)
Control	0	2263.35	3927.77	1.74
Canola Full-fat (CFF)	20	2245.91	3901.10	1.73
	40	2233.60	3864.04	1.73
	60	2224.77	3831.64	1.72
	80	2229.95	3833.18	1.72
	100	2230.67	3819.51	1.71
Control	0	2263.35	3927.77	1.74
Extruded Canola FF	20	2225.97	3844.16	1.73
(ECFF)	40	2211.08	3811.50	1.72
	60	2203.75	3798.27	1.72
	80	2190.09	3780.23	1.72
	100	2167.67	3739.56	1.73
LSD		36.75	53.20	0.01

The trend observed for feed intake during the starter period was maintained to week 6 and the increasing levels of CFF had a significant influence ($P<0.01$) on the feed intake of broilers during the finishing period. The 60, 80 and 100% inclusion of the CFF test diet resulted in significant ($P<0.05$) reductions in feed intake in comparison with the control diet. Similar results were reported by Leeson *et al.* (1986) and it is suggested that

the reduced feed intakes observed with increasing levels of CFF could be associated with the general undesirability of these diets. The influence of expansion treatment on the feed intake of broilers was found not to be significant ($P<0.05$) and increasing levels of ECFF resulted in reduced feed intakes.

The main difference between the treatments (ECFF and CFF) was found for FCR, where the influence of increasing levels of ECFF did not result in significant ($P<0.05$) differences between birds fed the control diets and those fed ECFF for week 3 and week 6. For CFF the influence of increasing levels was found to be significant for both the starter and finisher diets ($P<0.001$). The effect of expansion may not only have influenced the amino acid content as such (Table 3.5), but also have lead to the decreased availability of some of the amino acids and thereby negatively influencing the utilization of diets containing ECFF. This effect was, however, not found to be significant ($P<0.05$) in relation to the utilization of CFF diets. The increased AMEn content that was observed for the ECFF test diets in comparison with the CFF test diets (Table 3.3 and Table 3.4) did not lead to any significant differences in broiler performance.

3.4 Conclusion

It can be concluded from the results that the expansion treatment of full-fat canola did not significantly improve the body weight, feed intake or feed conversion ratio of broilers. Dietary levels above 10% of full-fat canola resulted in lower body weights and from a 6.8% level the feed intakes were reduced in comparison to birds on the control diet. The feed intake *per se* of canola full-fat has been identified as the biggest concern, since the utilization of the canola diets were found to be better than the control. Thus, the use of ground, low glucosinolate, low erucic acid Canola seeds can be included in broiler diets up to 10% if body weight is the main criteria, but dietary levels of 16.9% will perform well in terms of feed conversion ratios.

CHAPTER 4

General Conclusion

With this study it was intended to determine the nutritional value of Lupins and Canola for broilers and to investigate whether any additional processing of the full-fat seeds will lead to improvements in the nutritional value, and thus a further savings effect on the expensive imports of protein sources. The effects of extrusion and dehulling of lupins and the expansion of canola were investigated. The influence of these processing methods on the AME value as well as on broiler response in terms of body weight, feed intake and feed conversion ratio (g feed / g weight) was measured.

From the results obtained it is clear that the process of dehulling resulted in the most pronounced effect on the AMEn value of lupins. Due to the reduction in CF content with dehulling, it increased the AMEn value of *L.angustifolius* from 8.61 MJ/kg to 8.81 MJ/kg. The CP (27.9 vs. 37.4%), fat (5.9 vs. 6.6%) and lysine contents (1.2 vs. 1.6%) were also increased in relation to LM. This resulted in a more nutrient dense product that, with inclusion in broiler diets at the same concentration as LM, produced superior broiler body weights, feed intakes and a better FCR.

It was expected that the shear force and cooking action of the extrusion process would have resulted in higher AMEn values, especially due to the greater availability of fat from the rupturing of oil cells, but this was not found to be the case. The opposite happened and the AMEn value of *L.angustifolius* was reduced from 8.61 MJ/kg to 7.52 MJ/kg. The CP (27.9 vs.31.1%) and amino acid (Lysine: 1.22 vs. 1.32%) contents did increase, but it is suspected that the formation of Maillard reaction products during heat treatment might have indeed reduced the availability of certain amino acids, especially that of lysine. Since lupins are typically low in lysine contents and the diets were not further supplemented by L-lysine HCL, it might have caused a lysine deficiency. Broilers would try to compensate by increasing their feed intake up until a certain point, but at the end of week 6 this only resulted in an increased FCR with increasing levels of ELM. The fact that they did not increase their feed intake throughout the increasing levels of ELM could be because of the viscosity effect of the NSPs. Lupins are well known to contain a large amount of NSPs and their anti-nutritional effect stems from an increased digesta viscosity that reduces the rate of diffusion of substrates and digestive enzymes. This will influence the efficiency with which nutrients are absorbed and utilized by the bird. Even though the NSP content was not measured during this trial, the incidence of wet-sticky droppings, which are normally associated with high levels of soluble polysaccharides, were most pronounced in the pens fed a 100% blend of lupins. The effect of extrusion on lupins generally resulted in inferior broiler response in comparison with LM and DLM. It is suggested that this method of processing, as indicated for this study, is not an economically viable option of improving the nutritive value of *L.angustifolius*.

Future studies with lupins should concentrate on the influence of soluble polysaccharides and possibly focus on the application of various carbohydrases in an attempt to improve the nutritive value of lupins. Various successes have been achieved in this field, but the variability of birds in their ability to adapt to diets containing lupins or lupin products may be responsible for large variation in the results. This could be related

to the extent of proliferation of fermentative microflora in the small intestine of each bird. These microflora compete directly with the host for potentially absorbable substrates, especially if the absorption rates are reduced by the presence of viscous polysaccharides. It is thus suggested that the number of repetitions for nutritional studies with lupins be greatly increased. The inclusion of lupins or dehulled lupins in broiler diets are usually limited by safety margins in feed formulation and should be guided by the incidence of wet-sticky droppings at inclusion levels of above 10% (starter) and 13% (finisher) inclusion. Diets should also be supplemented with lysine and methionine.

The expansion treatment of full-fat canola seed did not significantly improve the body weight, feed intake or feed conversion ratio of broilers. From the chemical and amino acid evaluation of the test ingredients it is evident that the heat treatment increased the AMEn value of canola seed. This was expected since the shear force and expansion process during the heat treatment is known to rupture oil cells, thereby releasing the oil and increasing the fat content of the product. Since the fat content can be seen as the major contributor towards the available energy content, this effect of expansion treatment on the AMEn value was not surprising. A similar mechanism during the expansion treatment is responsible for the decrease in cell wall content as indicated by the decreased CF, ADF and NDF contents. The CP content of expanded CFF was also increased, but the individual amino acid contents did not follow the same pattern. Possible overprocessing could be responsible for the decreased amino acid contents that was observed. Care should be taken when canola seed is subjected to thermo-mechanical treatments such as expansion or extrusion. Apart from the difference in available energy content, the canola and expanded canola test diets did not differ much in their calculated nutrient content. The resulting similarities in broiler performances indicated that the expansion process did not eliminate any anti-nutritional or other factor in canola seed that might have reduced broiler performance. The inclusion of increasing levels of CFF resulted in significantly lower body weights than those of the control birds from the 10% level. The feed intake of broilers was also decreased from the 6.8% level of inclusion of full-fat canola. Since it was found that the utilization of canola diets was better than those of the control from the 6.8% inclusion of full-fat canola, it appeared as if the feed intake *per se* can be regarded as the major problem with canola seed diets for broilers. These results indicate that well grounded canola seeds can be incorporated into broiler feeds and that heat treatment will not necessarily improve the nutritive value of this oilseed for broilers. Levels of up to 10% full-fat canola seeds are recommended if body weight is the main criteria, but for the efficiency of diet utilization, levels of up to 16.9% in broiler starter and finisher diets are acceptable.

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