Canola meal and Cottonseed meal in broiler and layer diets

A report for the Australian Egg Corporation Limited

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Foreword

Canola meal (CM) usage in the Australian animal industries has been growing rapidly in recent years, due to increases in the amount of canola grown and processed. There is great potential for increasing the amount of CM used in the poultry industry. It is economical, and has a high protein concentration that is highly digestible. It is also a good source of energy, calcium and phosphorus. Breeding programs in Australia have resulted in the production of varieties of canola having very low levels of antinutritional factors, which are particularly suited to use in chicken feed.

Cottonseed meal (CSM) is Australia's largest oilseed crop but its inclusion at high dietary levels by Australia's poultry industries has been limited. A national survey of feed formulators in the USA indicated that the lack of adequate and accurate information regarding the characteristics, parameters, value and other aspects, including antinutritional factors, of CSM used in animal diets is seriously restricting its use.

Fortunately, Australian CSM contains relatively little of the main antinutritional factor, gossypol. Gossypol concentrations can also be reduced by processing steps.

All this indicates the great potential for higher inclusion levels (> 10%) above normal commercial practice for CM and CSM in poultry diets.

To establish the value of CM in poultry diets, The Rural Industries Research and Development Corporation, the Australian Oilseed Federation, Cargill Australia and the Queensland Government through DPI, commissioned research investigating the use of CM in poultry feed.

This project was funded from industry revenue which is matched by funds provided by the Federal Government.

This report is an addition to AECL's range of research publications and forms part of our R&D program, which aims to support improved efficiency, sustainability, product quality, education and technology transfer in the Australian egg industry.

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Although canola meal (CM) and cottonseed meal (CSM) offer great potential for use in the poultry industry, they are often under-used in poultry diets (4-10%) because of anti-nutritive factors (ANF) and variation in nutritional value due to location, environmental factors, cultivars, and processing.

In Australia, the content of some ANF in CSM and CM ('double zero') have been reduced by genetic selection and by pre-press solvent extraction, which minimises damage to proteins. Addition of soluble iron salts to diets reduces the negative effects of gossypol in CSM.

New strains of laying birds and broiler chickens, improved canola and cotton varieties and better procedures for oil extraction provided a new dimension for poultry research. There is the potential for higher inclusion levels (> 10%) above those normally used in commercial practice for CSM and CM in poultry diets.

The main objective of this project was to provide information on the chemical composition of these meals, their variability with processing, and make recommendations to the poultry industries on the nutritional value of both CM and CSM when included in least-cost poultry diets at levels close to their upper limit.

Broiler Trials

In all broiler experiments, unless otherwise stated, every CM and CSM source was chemically analysed and assayed for apparent metabolisable energy (AME) and ileal digestible amino acid (AA). Iron salts provided a 2:1 iron to gossypol ratio in each CSM diet. Birds were housed in wire cages with feed and water *ad libitum* and 23 h light in an insulated, reversed cycle air-conditioned house. All diets were formulated on a digestible amino acid (AA) basis and fed as crumbled starter and pelleted finisher.

Experiment 1 (Upper limits of inclusion of CM and CSM in broiler diets)

ANF were measured and their impact on chicken's health and performance monitored. Comparisons were made on upper limits of inclusion of Newcastle, Melbourne, Numurkah, and Pinjarra CMs and Narrabri CSM from the 1998-1999 processing. Inclusions of 100, 200, 300 and 400 g/kg were fed to 4 replicate groups of 8 broiler chickens. Sprayed polyethyleneglycol to CSM tested the effect of condensed tannins (CT) on production. Feed intake (FI), liveweight gain (LWG) and feed conversion ratio (FCR) were measured after 25 and 41 days. Organs weights and digesta viscosity were measured at 41 d. Narrabri CSM had an AME of 10.9 MJ/kg DM with low (g/kg): gossypol (0.04), CT (19.5), NDF (181.5). Lysine digestibility was only 45%. Pesticide residue was below the minimum detectable level. Glucosinolates levels in CMs were low (3-7 μmol/g) with varied sinapine (11-15 g/kg), AME, and digestible AA values reflecting differences in processing, environment and soil conditions. Bird performance on starter CSM diets was depressed above 100 g CSM/kg. This was not detected in older birds (25-41 d) giving a satisfactory performance at up to 300 g CSM/kg without signs of anaemia or abnormal organs. Birds fed on starter CM diets, gave satisfactory growth up to 300 g/kg (except Melbourne source) but within CM source and levels, FI and FCR were affected when young chicks were fed high CM levels. During the finisher period, FCR was improved for each CM source at all levels and birds gave a satisfactory growth at up to 300 g CM/kg. Due to unexpected circumstances, all CSM and CM diets were deficient in digestible lysine and this may explain some of the poor chick performance during the starter period. A follow up study in both CSM and CM with adjusted lysine and comparison with diets formulated on total basis was required.

Experiment 2 (High levels of CM in diets formulated on digestible or total AA basis)
This was a repeat of experiment 1 with adjusted lysine coefficients using similar sources of CM from the 1999-2000 processing cycle. CMs were fed at 200, 300 and 400 g/kg. Differences between total and digestible AAs formulations were also studied on Newcastle and Melbourne CMs fed at mentioned levels. Diets were fed to 5 replicate groups of 8 broiler chickens. Crude protein (CP), Ca, P, GSNL, CT, and sinapine content of each CM were similar to previous year. Except for lysine, most essential AA varied slightly from the previous year. The AME obtained with broiler birds were generally lower than in layers but higher than values obtained previously. FCR was not affected by CM inclusion levels but was improved by inclusion of the Newcastle CM. Other CM sources gave satisfactory LWG and FI up to 300 g/kg. In the finisher period, FI was linearly reduced with increasing CM levels for Newcastle and Melbourne sources. Satisfactory LWG was achieved for all sources up to 300 g CM/kg. CM reduced abdominal fat, intestinal viscosity, without affecting liver weight. Pancreas enlargement was observed at 400 g CM/kg. Formulating diets on a digestible AA basis improved growth and FCR only in the starter period. Satisfactory broiler performance can be obtained for both starter and finisher periods when using high levels of CM in broiler diets.

Experiment 3 (High levels of CSM in diets formulated on digestible or total AA basis)
Nutritional value, ANF and variability of CSM using adjusted lysine coefficients were examined. Solvent-extracted Brisbane, and Narrabri CSM, and expeller Gunnedah CSM from the 1999-2000 processing year were fed at 100, 200, 300 and 400 g/kg. Total and digestible AAs formulation comparisons using Narrabri were investigated. Each of the 17 experimental diets was fed to 5 replicate groups of 8 chickens. Narrabri CSM had higher CP, AA, and mineral levels with a low NDF, gossypol, and CT followed by Brisbane and Gunnedah, respectively. AME in both broilers and layers was higher in Narrabri with similar digestible AA values as Brisbane CSM. Gunnedah CSM had the lowest mineral and AA content due to its higher oil (239 g/kg) value. It's levels of NDF, CT, gossypol and CPFA were also highest. Formulating diets on a digestible AA basis improved chick growth and FCR. However, above 200 g CSM /kg, growth is more likely to be impaired. During the finisher period, birds were able to sustain satisfactory growth up to 300 g CSM/kg. Formulating CSM diets on a total AA basis does not account for the low CSM lysine digestibility value depressing FI and LWG in older birds due to low availability of bound lysine. Up to 200 g/kg of solvent extracted CSM can be used during the starter phase, and up to 300 g/kg of either solvent or extruded extracted CSM can be used during the finisher phase in diets formulated on a digestible AA basis.

Experiment 4 (Semi-commercial evaluation of CM and CSM in broiler diets)
Practical levels of CM or CSM were used in a semi-commercial environment. Solvent extracted commercial CSM (Riverina, Australia Pty, Ltd) and CM (Riverland Oilseed Processors Pty, Ltd) from the 2000-2001 processing year were used in crumbled starter (0-21 d) diets at 0, 200 g CSM/kg or 200 g CM/kg, and pelleted finisher (21-43 d) diets at 0, 300 g CSM/kg or 300 g CM/kg. Each of the three experimental diets was fed to 15 replicate pens of 40 birds (20 Males and 20 females) each.
The chemical composition of each meal differed from the previous year's evaluation. Production parameters were satisfactory during the starter and finisher periods and not influenced by the level of CSM or CM in the diet. Results were similar to those in cages. Up to 200 g/kg of either CSM (solvent extracted) or CM (solvent or extruded extracted) can be used during the starter phase, and up to 300 g/kg of either solvent or extruded extracted CSM or CM can be used during the finisher phase in diets formulated on a digestible AA basis.

Layer Trials

Experiment 1 (Evaluation of canola meal and cottonseed meal in layer diets for 1998-1999 harvest)
Three layer experiments evaluated diets containing 0, 100, 150, and 200 g/kg of CM or CSM. Experiment 1a, evaluated Melbourne and Pinjarra CM in Inghams Hisex Brown layers. Trials 1b and 1c evaluated Newcastle CM and Narrabri CSM. Treatments were offered to 26 week old, single

caged IsaBrown and Inghams Supertint White layers reared at QPRDC. The effect of added ferrous sulphate (4:1 iron to gossypol ratio) on egg quality was determined. Ileal digestible AA values from broilers were used to formulate steam pelleted (70-80 °C) diets on each trial. During a 14 weeks experimental period, evaluations on production performance, yolk colour, and egg odour from fresh and cold-stored eggs were performed. Production of the three layer strains was not affected by the source and level of CM or CSM with no mortalities. IsaBrown hens gave higher ($P<0.05$) egg production, lower egg weights, with less FI and better feed efficiency than White Supertint birds which had higher egg weights. CM diets for Hisex Brown and Isa Brown hens produced eggs with a "fishy" taint but with no effect on yolk colour. In eggs from brown birds stored at 10 °C for 2 weeks, "fishy" odour was detected at all CM levels in the Melbourne source but only at 150 and 200 g/kg level in the Pinjarra CM. When eggs were stored at 10 °C for 5 weeks, a "fishy" odour was detected in eggs produced from brown hens on 150 and 200 g CM/kg from the Melbourne source but not from the Pinjarra CM. White Supertint layer did not produce "fishy" eggs at any CM level. Increased yolk colour (12.4) was observed in stored (36 days) eggs derived from the 200 g CSM/kg. Sensory evaluations at the University of Qld found that only eggs from the CSM dietary treatments were different ($P<0.05$) from control eggs.

Experiment 2 (Evaluation of canola meal and cottonseed meal in layer diets 2000 harvest)
There were four layer experiments. Low protein CSM from Brisbane and high protein CSM from Narrabri were included at 120, and 200 g/kg and offered to Hy-line Brown layers (Experiment 2a) and Hy-line White (W-36) layers (Experiment 2b). Newcastle, Melbourne, Numurkah, and Pinjarra CM were included at 120, and 200 g/kg and offered to Hy-line Brown layers (Experiment 2c) and to Hy-line White layers (W-36), in Experiment 2d. Pullet rearing, specifications and measurements were as for Experiment A. During the 15 week experimental period starting at 41 weeks of age, eggs from each bird (brown or white) fed on the CSM treatments were cold-stored for 6 weeks. Eggs were tested for egg odour and sensory characteristics at the Centre for Food Technology (DPI-Queensland).

With high levels of CSM, satisfactory performance was obtained in both layer strains, which consumed more feed from diets based on Brisbane CSM. Gossypol, cyclopropenoid fatty acids levels in the CSM diets, and the addition of ferrous salts (2:1 ratio) did not affect egg production. There was no effect of breed or CM level on bird performance. There was a yolk mottling appearance in stored eggs in both brown and white strains that was significant at 200 g CSM/kg. It is advised to use only solvent extracted CSM of low residual lipid content at 150 g CSM/kg (maximum) in laying hen diets in order to avoid any mottling effect. Ferrous salts (2:1) inhibited the negative effect of gossypol on yolk colour.

A trained sensory panel detected a high incidence of "fishy" odour in raw eggs produced from Hy-line Brown hens fed on CM. Fishy odour was not detected in eggs from hens fed CSM. There was evidence of mottling in the yolks of eggs from hens fed on CSM diets.

Cooked eggs from brown hens fed on Melbourne, and Newcastle CM at 200 g kg, and Numurkah at 120 g/kg, had a higher ($P<0.05$) overall fishy odour intensity than the control eggs from the Hy-line Brown strain. Eggs from brown hens fed on CM diets had a higher ($P<0.05$) level of prawny odour than eggs from brown and white layers fed on the control diet. Eggs derived from CM treatments had more overall egg and yolk flavour and less egg white flavour than eggs from hens fed on a control diet. The levels of seafoody flavour detected in all cooked CM derived eggs were very low, even though a prawny odour was detected. No differences ($P>0.05$) were found in any of the odour attributes between eggs from hens fed the control diet and the CSM treatments.

1. Introduction

1.1 Background to Proposal

It has been forecast that the global demand for eggs and chicken meat will increase in the next five years due to an expected one billion increase in population. The majority of this increase will occur in Asia (Farrell, 1997). The enormous predicted demand for cereal grains and protein-rich ingredients, particularly from Asia, will result in increased demand and higher prices for Australian feedstuffs (Farrell, 1997). Therefore alternative protein sources are being examined.

Canola meal (CM) and cottonseed meal (CSM) offer great potential for usage in the poultry industry as economical protein sources (A$320/tonne and A$350/tonne, respectively), with 32-36% and 37-44% of crude protein in each meal, respectively. Unfortunately, CM and CSM are often limited to low dietary inclusion levels in poultry diets (4-10%) because of the suspected adverse effects of anti-nutritive factors.

In CSM, the major antinutritive factor (ANF) is gossypol, a polyphenolic compound (pigment) found in every part of the cotton plant that binds to iron molecules in the diet, in the bloodstream, and in the yolks of eggs, causing anaemia in the bird and discoloured brown yolks in the eggs. Gossypol may also bind with lysine during processing, thus reducing the nutritional value of the protein.

Fortunately, free gossypol content of CSM can be decreased by pre-press solvent extraction and certain direct solvent processes, rendering meals that can be classified as "low gossypol" containing less than 0.05% free gossypol. Processing methods that generate less heat during oil extraction also reduce the amount of lysine bound by gossypol. Free gossypol can also be inactivated by the use of soluble iron compounds in diets.

In CM, glucosinolates (GSLN) and sinapine are the major ANF and may cause liver haemorrhages, increase in thyroid weight, and egg taint in certain laying flocks; with reduction of feed intake and low weight gain having been reported in broilers. Part of this negative response is due to the presence of excessive levels of sulphur. On the other hand, selected varieties of *Brassica campestris* have resulted in the production of 'double zero' varieties of canola having very low contents of GSNL with less than 20 ug/g and negligible levels of erucic acid in the meal.

It is also well known that in ruminants condensed tannins (CT) from plant material bind to proteins (amino acids) and carbohydrates affecting their metabolism (Perez-Maldonado and Norton, 1996) and this CT effect from both CSM and CM needs to be investigated in poultry. Variation in the nutritional value and ANF of the meals would be expected due to location, environmental factors, cultivars, and industry processing conditions.

The development of new strains of laying birds and broiler chickens in the poultry industry, the improved new canola and cotton varieties in combination with better procedures for industry oil extraction has provided new ground for poultry research.

All this may indicate the potential for higher inclusion levels (> 10%) above normal commercial practice for CSM and CM in poultry diets.

Therefore, a series of experiments were carried out to investigate any possible anti-nutritional effect that these meals could exert on broiler chickens and layer hens when fed upper levels of CM or CSM. Another important objective was to determine the upper limits of inclusion of CM and CSM in diets formulated on a digestible amino acid (AA) basis in order to determine their utilization in broiler chicken and layer hen diets.

1.2 Relevance and Benefits

The proposed research has particular relevance and benefit to the following groups/organisations: Australian crushing and refining plants such as Cargill Australia Ltd., Riverland Oilseed Processors, Australia Country Canola, Pryde's Pty Ltd, Pinjarra Western Australia and Seedex Pty Ltd. These account for more than 90% of Australia's crushing capacity. These oilseeds companies have each expressed a genuine interest, have collaborated with this project and have provided CM and CSM material that was evaluated in this project. The involvement of various crushing plants was important in order to get a wide range of seed meals that were representative of Australia's major environmental conditions. The specific benefits that these companies would obtain are mainly revenues derived from CM and CSM sales to poultry, pig and ruminant livestock producers and some exports to South East Asia. Others revenues are from the sale of the oil to refiners for further processing into a range of end products.

Poultry producers can substantially benefit from the purchase of more cost-effective feed ingredients. The cost of CM and CSM are 40% less than soybean meal, which is at the moment the major protein rich ingredient used in the intensive livestock industry.

If more unconventional oilseed meals such as CM and CSM were introduced to Australia's livestock feed ingredient cycle, substantial savings can be expected in the Australian economy. The actual price of imported soybean meal varies but the price is about $AUS 500-550 per mt. To fill the shortfall in local production Australia imports in the order of 200,000-250,000mt of soybean meal each year. Around $AUS 100-130 million can be saved annually if more CM, CSM and sunflower meals were used by the intensive livestock industry in order to supply their protein requirements. The present research results can also be of particular relevance to plant breeders such as Ag-Seed Research Pty, Ltd which is a major plant breeding company developing new canola varieties. This organisation can develop varieties with improved nutritive value for poultry.

1.3 General Materials and Methods

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In the original methodology it was indicated that the variability of major ANF, bioassays (AME and digestible AAs) and chemical analyses in samples from major processing sites, would be evaluated three times a year. However after consultation with major processing plants it was indicated that the production of CM and CSM is seasonal and therefore to avoid repetition in the evaluation of these meals, a yearly evaluation at the end of each processing cycle would be more effective. Thus, it was decided to carry out sampling of CM or CSM from major processing plants at the end of each oil processing cycle and samples of CM and CSM were obtained during each year (during the course of this project) and the manufacturer ensured that each meal corresponded to the more recent oil crush and not from the year before.

1.3.1 Bioassays and Chemical Analyses

Prior to each broiler and layer experiment, all CM and CSM samples were analysed for proximate analysis according to the methods of the AOAC (1984). Non-starch polysaccharides (NSP) followed the method of Choct and Annison (1992). Condensed tannins (CT) were measured using the method described by Perez-Maldonado and Norton (1996). Total gossypol was determined using the method of Hedin *et al*. (1991). Amino acid (AA) analyses in CSM, CM, feed and digesta samples were undertaken by reverse phase chromatography (Waters HPLC) after hydrolysis with 6 M HCl at 110°C for 18 h under reflux. Cysteine and methionine were determined as cysteic acid and methionine sulphone respectively, following performic acid oxidation. Tryptophan was determined using 4.2 N NAOH at 110°C for 20 h. Amino acids were calculated using a derivatised pre-column by the accq-tag method (Waters). The chromium, concentration (as a marker) in feed, digesta and excreta samples was determined by ICP after acid digestion. Gross energy (GE) was determined

using an AC-350 LECO adiabatic bomb calorimeter. Glucosinolates (GSNL) in all canola meal samples were determined by near infrared spectrometry (NIRS), calibrated as total GSNL based on released glucose following enzymatic hydrolysis of the GSNL using AOF method 4-1.22. All CM and CSM samples were also sent to Singapore-Adisseo to be analysed for digestible amino acid using NIRS. CSM samples in experiment 1 (section 2.1) were analysed for pesticide residues (organophosphates and organochlorines).

Apparent metabolisable energy (AME)

Broiler chickens

In each broiler experiment, the apparent metabolisable energy (AME) of the meals was determined using the classical, total collection of excreta and measurement of food intake (FI) over 4 days (d) made on four replicate cages each of six male broiler chickens (16-21 d old) and accustomed to the diets for 3 d. Inclusion levels of each CM or CSM meal in a basal diet were 300 g/kg and sorghum 667 g/kg. A mineral and vitamin broiler pre-mix plus AA was also added to each diet. Samples of feed and dried excreta were analysed for GE and nitrogen (N). The basal diet contained 967 g/kg sorghum and a mineral and vitamin pre-mix similar to the test diets.

Layer Hens

In all three layer experiments described in Chapter 3 (Experiment 1, section 3.1), AME determination was carried out on a basal diet and six diets containing two samples of cottonseed meal (CSM) from Narrabri (low and high protein) and canola meals (CM) obtained from Newcastle, Melbourne, Numurkah and Pinjarra derived from the 1998-1999 harvests. The AME was measured using chromium III oxide as a marker with six individually caged layer hens (Hi-sex strain) per treatment. The basal diet contained (g/kg): sorghum 350, wheat 374.5, soybean meal 155.7, limestone 94.7, di-calcium phosphate 14.27, sodium bi-carbonate 1.47, salt 2.19, methionine 1.43, lysine 0.8, minerals and vitamins pre-mix 4.5 including chromic oxide (2.0 g/kg) added as indigestible marker. The treatment diets were (g/kg) 700 of the basal diet with 300 of the respective CSM, or CM samples.

The AME determinations for all four layer experiments described in Chapter 3 (Experiment 2, section 3.2) were carried out on a basal diet and six diets containing samples of CSM from two locations, Brisbane and Narrabri and CM from Newcastle, Melbourne, Numurkah and Pinjarra derived from the 1999-2000 harvests. The AME was measured by the total collection method with six individually caged layer hens per dietary treatment. Forty-two individually fed laying hens, Isa Brown, were used for this purpose at 6 hens per treatment. The basal diet contained (g/kg): wheat 762, soybean meal 120, limestone 95.3, di-calcium phosphate 13.2, salt 3.2, methionine 1.9, lysine 1.9 and minerals and vitamins pre-mix 2.5. The treatment diets consisted of (g/kg) 700 of the basal diet with 300 of the respective CSM, or CM sample.

In both AME determinations, an adaptation period of 3 days was followed by a 5 days period in which excreta was quantitatively collected once a day. After each collection, excreta was frozen at – 10 °C and at the end of the trial, this excreta was dried at 70 °C in a forced draught-oven for 2-3 days. Dry excreta was removed from ovens and allowed to equilibrate with ambient temperature and humidity for 3-4 h then weighed and subsequently ground. Gross energy on the feed and excreta samples was determined using an AC-350 LECO adiabatic bomb calorimeter.

Ileal digestible amino acid determination

Broiler chickens

Prior to each experiment, ileal amino acid (AA) digestibility for each CM or CSM, was determined in three replicate groups of four (37-42 d old) broilers as described in Ravindran *et al.,* (1999). The proportions of dextrose and the test meal were varied in each diet to obtain approximately 200 g crude protein/kg. A mineral and vitamin broiler pre-mix and oil were added to each diet. Initially, chromic oxide and acid insoluble ash were used as markers for comparison, but only chromic oxide

was used for evaluating each meal during following experiments. The results obtained using broiler birds were used to formulate diets for both broiler chickens and layer hens.

1.4 Experimental canola meal and cottonseed meal samples and diets

Diet formulations

Prior to the each experiment CM and CSM samples were chemically analysed and bioassayed for apparent metabolisable energy (AME) and ileal digestible AA determination. Ferrous sulphate provided a 2:1 iron to gossypol ratio in each CSM diet. All diets were designed to contain similar calcium, phosphorus, AME with a similar digestible crude protein content, to meet the minimum digestible, or total AA, requirements estimated for maximum growth.

Broiler chicken experiments

All experimental broiler diets were prepared as crumbled starter and pelleted finisher. Diets were formulated on a digestible or a total amino acid (AA) basis using determined AA coefficients. The ideal AA ratios reported in Baker and Han (1994) and by Baker *et al.* (1993) were used for the formulation.

In Experiment 1, section 2.1, commercial CM was obtained from the processing cycle 1998-1999 from four representative Australian processors located in Newcastle (NSW), Melbourne (Vic), Numurkah (Vic) and Pinjarra (WA). All CM samples were processed by solvent extraction except Pinjarra (expeller processed). Solvent-extracted CSM from Narrabri (NSW) was used in this trial.

Diets in the growth experiments, contained graded levels of 100, 200, 300, and 400 g/kg CM or CSM. Additional treatments in which polyethyleneglycol (PEG) was sprayed on to CSM were included to evaluate the effect of condensed tannins (CT) on poultry production parameters. The added CSM+PEG treatments were fed in starter and finisher diets containing 300 and 400 g CSM/kg level.

Determined digestibility coefficients for all AA were used for both meals except for lysine in CSM where a digestibility coefficient of 0.6 was used. Ferrous sulphate provided a 2:1 iron to gossypol ratio in each CSM diet. PEG provided a 1:1 CSM to total CT ratio in each CSM+PEG diet.

In Experiment 2, section 2.2, commercial CM from the processing cycle 1999-2000 was obtained from four representative Australian processors as described in Experiment 1. Diets were prepared as Experiment 1 and were fed at 200, 300, and 400 g/kg CM. Determined AA digestibility coefficient values were used for all CM sources. To investigate differences between total and digestible AA formulations, this study also investigated the effects of adding 200, 300, and 400 g CM/kg from Newcastle and Melbourne sources on diets formulated on digestible or on a total AA basis.

In Experiment 3, section 2.3, commercial CSM was obtained from the processing cycle 1999-2000 from three representative Australian processors located in Brisbane (Qld), Narrabri (NSW), and Gunnedah (NSW). Solvent extraction was used to obtain all CSM, except for the Gunnedah processor, who used expeller extraction.

Diets, in the growth experiment, contained graded levels of 100, 200, 300, and 400 g CSM/kg. Determined AA digestibility values were used for all CSM sources. To investigate differences between total and digestible AA formulations, this study also investigated the effects of adding 100, 200, 300, and 400 g/kg of CSM from the Narrabri processor to crumbled starter and pelleted finisher diets formulated on a total AA basis.

In Experiment 4, section 2.4, commercial CSM (Riverina, Australia, Pty, Ltd) and CM (Riverland Oilseed Processors Pty Ltd) were obtained from the 2000-2001 cycle. Starter diets were formulated to contain 0, 200 g CSM or 200 g CM/kg and finisher diets contained 0, 300 g CSM/kg or 300 g CM/kg. Inghams Enterprises provided the ingredient composition for the control diet. Determined AA digestibility coefficients were used for both CSM and CM sources in diets formulations.

Feeding period

The following table describes the bird's age for each feeding period in each experiment.

Experiment No	Starter period (days)	Finisher period (days)
1	4-25	25-41
2	4-25	25-42
3	4-25	25-42
4	1-21	21-43

Layer hen experiments

All layer diets described in Chapter 3 (sections 3.1 and 3.2) were formulated on digestible AA basis to the breeder recommendations using the computer package Feedmania (ABRI University of New England). A commercial mineral and vitamin pre-mix with a yolk pigment was added to all diets, which were prepared as mash and subsequently steam pelleted (70-80 °C) for all experiments described in (Experiment 1, section 3.1). All diets described in Experiment 2, (section 3.2) were prepared and offered as mash diets.

In Experiment 1a, CM from Melbourne and Pinjarra sources were included at 100, 150 and 200 g/kg and offered to Inghams Hisex Brown layers. In Experiment 1b, graded levels (100, 150 and 200 g/kg) of CM from Newcastle were offered to Isabrown and Inghams White SuperTint layers. In Experiment 1c, similar levels of CSM from Narrabri were included and offered to Isabrown and White Supertint layers. To evaluate the effect of ferrous sulphate on egg quality derived from CSM diets, a 4:1 iron to gossypol ratio was added in each CSM diet.

In Experiment 2a , CSM from Brisbane (of low protein content) and CSM from Narrabri (of high protein content) were included in diets at 120 and 200 g/kg and offered to Hy-line Brown layers. In Experiment 2b, similar CSM sources and levels were offered to Hy-line White (W-36) layers. In Experiment 2c, CM from Newcastle, Melbourne, Numurkah, and Pinjarra were included in diets at 120 and 200 g/kg and offered to Hy-line Brown layers. In Experiment 2d (3.2.4), similar CM sources and levels were offered to Hy-line White (W-36) layers.

All dietary treatments described in Experiment 1, (section 3.1), were formulated to contain the same AME, calcium (Ca), available phosphorus (P) and digestible AA specifications to obtain maximum production. Dietary treatments described in Experiment 2, (section 3.2) were formulated to contain 11.9 MJ/kg, 3.7% Ca, 0.42-0.6% available P and 1.4-2% linoleic acid for Hy-line White birds, while dietary treatments for Hy-line Brown birds contained 11.5 MJ/kg, 3.4-3.6% calcium, 0.4-0.6% phosphorous and 1.1% linoleic acid. Digestible AA specifications were similar for both strains to obtain maximum production. Ferrous sulphate provided a 2:1 iron to gossypol ratio in each CSM diet.

1.5 Birds, housing and measurements

Broiler chickens

Experiments 1-3 used male broiler chicks (Cobb) that were grown in wire cages (66 cm long, 35 cm wide and 40 cm tall) during the starter period and grown in bigger wire cages (95 cm x 70 cm x 40

cm) during the finisher period in an insulated, reverse cycle air-conditioned house. Food and water were offered *ad libitum*; light and temperature followed industry practice. However in Experiment 4, starter and finisher diets were fed from 1 to 21 d of age, and from 21 to 43 d of age respectively, to both male and female broiler chicks (Cobb) grown in an insulated, environmentally-controlled 72 pen, deep-litter broiler experimental shed. Food and water were offered *ad libitum* in each pen from two tube feeders (each 134 cm diameter) and five nipple waterers. These were raised as appropriate throughout the experiment to minimise spillage.

During each broiler experiment, temperature was gradually reduced from day 1 to day 42 according to local commercial conditions. There was a 23 h/d lighting period from 1-42 d. Chickens that died, or were culled during the first 72 h, were replaced by healthy birds. Any bird dying thereafter was not replaced.

The birds in each pen/cage were bulk weighed at the start of the experiment, and on days 21 and 43. Birds that died or were culled and not replaced were individually weighed at the time of removal from the pen/cage and feed residues recorded in affected pens. Feed intake was measured for each pen for the starter (0-21 d) and finisher (21-43 d) by weighing each feeder plus contents at the start and finish of each period and all feed issues during each period. Feed remaining in the feeder at the end of each period was discarded after weighing.

Layer hens

In Chapter 3 (section 3.1), Inghams Hisex Brown was used in Experiment 1a, while Isabrown and Inghams White Supertint pullets were used for experiments 1b and 1c respectively. In section 3.2, Hy-line Brown and Hy-line Whites were used for each CM and CSM evaluation. These birds were purchased as day-old chicks from commercial hatcheries (Inghams Enterprises and Hy-line Australia Pty Ltd) and were vaccinated according to recommended schedules. In the rearing phase chicks were brooded until four weeks of age in electrically heated multi tier wire-floor brooders. They were then transported to a single-tier wire-floor pullet rearing facility following commercial practice.

At 17 weeks of age, the pullets were placed in single-level cages housed in a conventional poultry building provided with adjustable shutters and ridge-vent, and thermostatically controlled fans and water misters. They were subsequently fed with a Centre commercial pelleted layer diet until the commencement of the experiment at 26 weeks of age in Experiment 1 (section 3.1) and at 41 weeks of age in Experiment 2 (section 3.2). Food and water were available *ad libitum* and a photoperiod of 15.5 h was maintained by a combination of natural daylight and tungsten filament lights.

The experimental period in all layer trials described in Experiments 1 and 2, (sections 3.1 and 3.2) were 14 and 15 weeks respectively, during which egg production was recorded for five days each week, egg weight measured weekly, feed intake (FI) measured every 10 days and specific gravity of eggs monthly. All measurements were made on individual birds including the liveweight at the end of the trial, when five hens per treatment at 100, 120 and 200 g/kg levels were euthanased for organ weight measurement.

1.6 Measurements on euthanased birds

Broiler chickens

In Broiler Experiment 1, two birds on diets with 200 and 400 g/kg CM and CSM, were euthanased by cervical dislocation at 41 d, weighed, blood sampled and their liver and pancreas weighed.

In Broiler Experiment 2, two birds on the control diet, 200 and 400 g/kg CM treatments were euthanased and weighed at 42 d. Digesta from the small intestine (SI) was collected; liver, pancreas and fat pad were removed and individually weighed.

In Broiler Experiment 3, two birds from the control, 200 and 400 g CSM/kg treatments were euthanased and weighed at 42 d. Liver, pancreas and fat pad were removed and individually weighed.

Layer Hens

In all experiments described in Section 3.1, five hens per treatment at 100 and 200 g/kg level were euthanased by cervical dislocation. In all experiments described in Section 3.2, five hens per treatment at 120 and 200 g/kg were euthanased by cervical dislocation. These birds were evaluated for organ (liver and pancreas) weight measurements.

Ethical considerations

Before the commencement of each broiler and layers experiments described in this project, animal ethics application forms were submitted to The Animal Research Institute's Animal Ethics Review Committee. All submissions were approved and complied with the "Australian Code of Practice for the Care and Use of the Animals for Scientific Purposes" (The Green Code 6th Ed.), Section 2.2.11.

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2. Broiler Trials

2.1 Experiment 1 - Upper limits of inclusion of canola meal and cottonseed meal in diets formulated on a digestible amino acid basis for broiler chickens

2.1.1 Introduction

The major concern in Australia about the inclusion of canola meal (CM) or cottonseed meal (CSM) in broiler diets is the presence of antinutritive factors (ANF) that are detrimental to broiler production. This negative effect in broiler productivity has been published throughout the years creating a major concern among nutritionists and feed manufacturers in spite of genetic selection programs aimed at reducing these ANF, such as the CM "double zero" varieties and the low gossypol CSM cultivars. Positive changes in the processing of oil extraction have also advanced in the last 10 years and as a result of these protein meals have been improved, containing less of these ANF. Therefore the main objective of these growth experiments was to develop and establish methodologies to evaluate ANF in these meals and their impact on chicken's health and performance. Another objective was to evaluate upper limits of inclusion of CM and CSM in diets formulated on a digestible amino acid (AA) basis in order to determine their utilisation and advantage in broiler starter and finisher diets.

2.1.2 Results and Discussion

Starter and finisher diets

The ingredient and chemical composition of the starter diets for CSM from Narrabri and for CM from Newcastle, Melbourne, Numurkah, and Pinjarra are presented in Tables 2.1.1 and 2.1.2 respectively. The ingredient and chemical composition of the finisher diets for CSM and for CM from Newcastle, Melbourne, Numurkah, and Pinjarra are presented in Tables 2.1.3 and 2.1.4 respectively.

Chemical composition

The nutrient, and chemical composition, and ANF data are presented in Table 2.1.5 The CSM and CMs soluble, insoluble and total NSP are presented in Table 2.1.6 and 2.1.7 respectively. The determined apparent ileal digestibility coefficients of AAs for each meal are presented in Table 2.1.8

Experimental design

There were two experiments using 4 different CM and 1 CSM in starter and finisher diets in the one design layout. In the CM experiment, there were 17 treatments comprising a control diet plus all combinations of four levels x four sources of meal in a factorial design. In the CSM experiment there were seven treatments comprising a control diet + all combination of four levels x one source plus 2 levels x +/- PEG of CSM. In each experiment, a cage of 8 birds was the experimental unit. The layout was randomised blocks, with four replicates in blocks of 22 cages. Data were analysed separately for the CM and CSM experiments. For the CM, the 17 treatments were compared in an initial randomised blocks ANOVA, and then in a follow-up ANOVA in which the full error term (48 degrees of freedom) from the initial ANOVA was used, the main effects and interaction of the embedded four x four factorial design were tested. For the CSM seven treatments were compared in an initial randomised blocks ANOVA, and then a follow-up ANOVA in which the full error term (18 degrees of freedom) from the initial ANOVA was used, the main effects and interactions were tested. For each trial treatment means were compared using a protected LSD procedure ($P<0.05$).

Table 2.1.1 Ingredient composition (g/kg) and levels of cottonseed meal (CSM) and levels of CSM and PEG in starter diets

Ingredients	0	100	200	300	400	300 + PEG*	400 + PEG*
Sorghum	612	590	532	474	405	474	405
Poultry offal meal	50	50	50	50	50	50	50
Meat & bone meal	11.4	50	50	50	50	50	50
Soybean meal	284	182	118	54	8.7	54	8.7
Cottonseed meal	0	100	200	300	400	300	400
Tallow	3	7.6	3	50	50	50	50
Vegetable oil	-	-	-	1.7	18	1.7	18
Limestone	11.7	5.4	6.1	6.8	7.2	6.8	7.2
Dicalcium phosphate	15.6	3.1	2.0	0.8		0.8	
Salt	2.1	1.3	1.2	0.7	0.3	0.7	0.3
Sodium bicarbonate	1.1	0.6	0.2	0.1	0.1	0.1	0.1
Vitamins/minerals	7.1	7.1	7.1	7.1	7.1	7.1	7.1
DL methionine	1.6	1.5	1.4	1.3	1.0	1.3	1.0
Lysine	0.3	1.6	2.5	3.4	3.7	3.4	3.7
Calculated analysis							
Total crude protein	229	245	255	267	286	267	286
Digestible lysine	10.5	10.4	10.3	10.3	10.2	10.3	10.2
Digestible methionine	4.8	4.8	4.7	4.6	4.4	4.6	4.4
Digestible sulphur AA	7.5	7.5	7.5	7.5	7.5	7.5	7.5
Digestible threonine	7	6.9	6.8	6.7	6.9	6.7	6.9
Digestible isoleucine	9.1	8.3	7.6	6.8	6.5	6.8	6.5
Digestible tryptophan	2.6	2.3	2.2	2.1	2.1	2.1	2.1
Calcium	11	10	10	10	10	10	10
Avail. Phosphorous	5	5	5	5	5.1	5	5.1
AME (MJ/kg)	12.5	12.5	12.5	12.5	12.5	12.5	12.5

* PEG= Polyethyleneglycol

Table 2.1.2 Ingredient composition (g/kg) by source and level of canola meal (CM) in starter diets

Ingredients																	
Sorghum	424	387	398	408	392	383	390	451	426	397	418	439	399	381	386	391	379
Wheat	200	200	150	100	50	200	150	50		200	150	100	50	200	150	100	50
Poultry offal meal	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50
Meat & bone meal	50	50	50	50	45	50	50	50	46	50	50	50	45	50	50	50	50
Soybean meal	249	180	112	43		177	106	35		172	96	20		187	126	64	18
CM Newcastle		100	200	300	400												
CM Melbourne						100	200	300	400								
CM Numurkah										100	200	300	400				
CM Pinjarra														100	200	300	400
Tallow	5	15.8	25.1	34.7	49.0	21.2	35.8	46.1	50	11.7	16.9	22.3	37.5	14.3	22.1	30.1	40.5
Vegetable oil									11								
Limestone	6.6	5.3	4.2	2.6	2.4	5.7	5.0	3.9		5.8	5.1	4.0	4.1	5.5	4.5	3.2	1.7
Dicalcium phosphate	4.1	0.6	-	-	-	6.9	0.1	-	-	0.7	0.1	-	-	0.6			
Salt	1.9	1.7	1.5	1.2	0.9	1.8	1.7	1.4	1.7	1.8	1.5	1.3	1.7	1.9	1.8	1.7	1.5
Sodium bicarbonate	0.73	0.6	0.5	0.5	0.5	0.9	1.1	1.4	1.1	1.04	1.4	1.8	1.4	0.6	0.5	0.4	0.5
Vitamins/minerals	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1
DL methionine	1.4	1.2	1.0	0.8	0.4	1.7	1.9	2.3	2.2	1.6	1.9	2.1	1.8	1.3	1.2	1.2	1.0
Lysine	0.3	0.9	1.6	2.2	2.2	0.9	1.51	2.2	1.8	1.1	1.9	2.6	1.81	0.7	1.1	1.6	1.5
Calculated analysis																	
Total crude protein	233	235	236	238	247	233	233	233	245	232	231	230	247	230	227	224	227
Digestible lysine	10.5	10.3	10.1	10.0	9.7	10.4	10.2	10.1	10.0	10.4	10.2	10.1	10.0	10.4	10.3	10.1	10.0
Digestible methionine	4.7	4.6	4.4	4.3	4.0	4.8	4.9	5.0	5.0	4.8	4.8	4.9	4.7	4.6	4.6	4.5	4.4
Digestible sulphur AA	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5
Digestible threonine	7.0	6.8	6.6	6.5	6.6	6.8	6.7	6.5	6.7	6.9	6.7	6.6	7.1	6.9	6.7	6.6	6.7
Digestible isoleucine	9.0	8.3	7.5	6.8	6.4	8.2	7.4	6.7	6.5	8.3	7.4	6.6	6.7	8.3	7.6	6.9	6.3
Digestible tryptophan	2.5	2.3	2.1	2.0	1.8	2.3	2.0	1.8	1.7	2.3	2.1	1.9	2.0	2.4	2.2	2.0	1.9
Calcium	10.7	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
Avail. Phosphorous	5.0	4.5	4.5	4.6	4.5	4.5	4.5	4.6	4.5	4.5	4.5	4.6	4.5	4.5	4.5	4.6	4.8
AME (MJ/kg)	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5

Table 2.1.3 Ingredient composition (g/kg) and levels of cottonseed meal (CSM) and levels of CSM and PEG in finisher diets

Ingredients	Control	100	200	300	400	300 + PEG*	400 + PEG*
Sorghum	692	537	630	548	447	548	447
Wheat		151					
Poultry offal meal	10	50	50	50	21	50	21
Meat & bone meal	10	50	48	44	44	44	44
Soybean meal	250	94	42				
Cottonseed meal	0	100	200	300	400	300	400
Tallow			11.7	38.9	50	38.9	50
Vegetable oil					18.9		18.9
Limestone	10.6	6.1	6.9	8.1	9.2	8.1	9.2
Dicalcium phosphate	15.4	0.59					
Salt	2	1.6	1	0.6	0.4	0.6	0.4
Sodium bicarbonate	1.2	-	-	-	-	-	-
Vitamins/minerals	7.1	7.1	7.1	7.1	7.1	7.1	7.1
DL methionine	1.33	1.0	0.94	0.66	0.37	0.66	0.37
Lysine	0.15	1.26	2.0	2.35	2.44	2.35	2.44
Calculated analysis							
Total crude protein	196	214	229	246	265	246	265
Digestible lysine	8.3	8.3	8.1	7.9	7.8	7.9	7.8
Digestible methionine	3.9	3.8	3.8	3.6	3.4	3.6	3.4
Digestible sulphur AA	6.3	6.3	6.3	6.3	6.3	6.3	6.3
Digestible threonine	5.8	5.9	5.8	5.9	5.9	5.9	5.9
Digestible isoleucine	7.8	6.8	6.3	5.9	5.7	5.9	5.7
Digestible tryptophan	2.2	1.9	1.7	1.8	1.9	1.8	1.9
Calcium	9.6	9.5	9.5	9.5	9.5	9.5	9.5
Avail. Phosphorous	4.5	4.5	4.5	4.5	4.5	4.5	4.5
AME (MJ/kg)	12.5	12.5	12.5	12.5	12.5	12.5	12.5

*PEG= Polyethyleneglycol

Table 2.1.4 Ingredient composition (g/kg) by source and level of canola meal in finisher diets

Ingredients																	
Sorghum	310	434	589	577	472	496	664	556	449	424	581	582	481	410	509	584	478
Wheat	424	271	83	13		211				283	94			288	151		
Meat & bone meal	50	50	50	49	50	50	50	49	50	50	50	49	50	50	50	49	49
Poultry offal meal	50	50	44	50	23	50	50	50	27	45	32	50	21	50	50	50	30
Soybean meal	148	79	19			76	17			80	25			86	24		
Canola Newcastle		100	200	300	400												
Canola Melbourne						100	200	300	400								
Canola Numurkah										100	200	300	400				
Canola Pinjarra														100	200	300	400
Tallow				13.3	46		2.40	30.4	50			4.7	34.4			5.2	33.1
Vegetable oil									11.0								
Dicalcium phosphate	1.6	1.0	0.7				0.6			1.3	1.4		-	1	0.4		
Limestone	5.4	4.2	3.2	1.94	0.97	4.6	3.8	3.03	2.46	4.74	4.2	3.2	2.7	4.4	3.5	2.64	1.91
Salt	2.1	1.8	1.3	1.1	1.12	1.66	1.33	1.86	2.17	1.76	1.31	1.8	2.1	1.9	1.6	1.6	1.69
Sodium bicarbonate	0.3	0.2	0.2	-	-	0.7	0.9	0.3	0.3	0.8	1.4	0.6	0.6	0.3	0.3	-	-
Vitamins/minerals	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1
DL methionine	0.93	0.85	0.81	0.09		1.39	1.7	1.42	1.31	1.26	1.65	1.15	1.05	0.95	0.98	0.66	0.27
Lysine	0.3	1.1	1.9	0.9	0.5	1.2	1.6	0.5		1.2	2.0	0.6	0.1	0.6	1.4	0.8	0.1
Calculated analysis																	
Total crude protein	197	199	202	224	235	198	203	222	235	197	197	224	235	195	192	203	210
Digestible lysine	8.3	8.1	7.9	7.8	7.6	8.2	8.0	7.9	7.5	8.2	8.0	7.9	7.7	8.2	8.1	7.9	7.8
Digest. methionine	3.8	3.7	3.6	3.3	3.3	4	4.1	4	3.9	3.9	4	3.8	3.7	3.8	3.8	3.6	3.3
Digestible sulphurAA	6.3	6.3	6.3	6.3	6.7	6.3	6.3	6.3	6.3	6.3	6.3	6.3	6.3	6.3	6.3	6.3	6.3
Digestible threonine	5.8	5.6	5.4	5.8	5.9	5.6	5.6	6.1	6.3	5.7	5.5	6.7	6.6	5.7	5.6	5.9	6.1
Digestible isoleucine	7.4	6.7	6	6.1	6	6.6	6	6.1	6.1	6.7	5.9	6.4	6.2	6.7	6.1	5.9	5.8
Digestible tryptophan	2	1.8	1.5	1.6	1.7	1.7	1.5	1.6	1.7	1.8	1.6	1.7	1.9	1.8	1.6	1.6	1.7
Calcium	9.5	9.5	9.5	9.5	9.5	9.5	9.5	9.5	9.5	9.5	9.5	9.5	9.5	9.5	9.5	9.5	9.5
Avail. Phosphorous	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.6	4.8
AME (MJ/kg)	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5

Composition of cottonseed meal and canola meal from different processors

Table 2.1.5 Chemical composition (g/kg DM) of the experimental cottonseed (CSM) and canola meals (CM)

Analysis	CSM Brisbane	CSM Narrabri	CM Newcastle	CM Melbourne	CM Numurkah	CM Pinjarra
Dry matter (%)	89.9	89.8	90.5	89.2	89.6	90.2
Crude protein	512	519	414	419	418	335
Phosphorus	15.4	16	12	11.5	11.1	10.9
Calcium	2.1	2.3	8.4	7.0	6.8	7.5
Sulphur	5.2	5.1	7.0	7.2	7.0	7.0
Fat	34	37	49.4	30.7	55.4	129.3
Free-gossypol	0.1	0.04	ND	ND	ND	ND
Free condensed tannis	18.9	12.7	34.2	31.3	38	35.6
Bound condensed tannins	10.2	6.8	10.1	4.8	24	5.2
Total tannins	29.1	19.5	44.3	36.1	62	41.8
Sinapine	ND	ND	11.8	12.7	14.8	14.0
Glucosinolates (µmol/g)	ND	ND	2	4	3	7
Neutral detergent fibre (NDF)	335.9	181.5	327.1	285.9	321.4	248.3
Organochlorine (mg/kg)	< 0.02	< 0.02	ND	ND	ND	ND
Organophosphorous (mg/kg)	< 0.05	< 0.05	ND	ND	ND	ND
Alanine	17.3	17.5	15.8	15.6	15.7	13.3
Arginine	55.8	54.3	25.3	26.1	26.4	21.9
Leucine	24.8	24.9	24.9	23.6	23.9	19.5
Lysine	18.6	19.3	18.8	19.9	19.7	17.7
Methionine	7.6	7.8	8.1	5.2	6.0	6.8
Phenylalanine	23.5	23.0	13.6	13.4	13.7	11.1
Proline	15.7	15.7	21.6	21.2	21.6	17.5
Serine	19.7	19.4	15.4	15.0	15.3	12.9
Aspartic acid	41.6	41.5	24.6	24.3	25.2	20.7
Cystine	7.1	7.7	9.4	6.0	6.6	7.5
Glutamic acid	93.1	91.3	64.8	64.6	66.7	51.8
Glycine	18.6	18.6	17.8	17.7	18.1	15.1
Histidine	11.7	11.8	8.8	8.2	8.6	7.2
Isoleucine	13.1	13.2	13.4	13.7	13.6	11.3
Threonine	15.2	15.1	15.8	15.9	16.4	13.6
Tryptophan	6.4	6.2	4.9	4.4	5.0	4.7
Tyrosine	12.1	12.1	9.5	9.2	9.5	8.1
Valine	18.0	18.0	16.6	16.8	16.6	14.1
Layer hen AME (MJ/kg DM)	10.7	10.9	11.0	10.6	11.2	11.1
Layer hen AMEn	10.0	9.4	10.4	9.7	11.1	10.9
Broilers AME (MJ/kg DM)	10.4	10.9	8.7	9.2	9.7	11.0
Broilers AMEn	9.3	10.0	7.6	8.5	8.6	10.4

ND= not determined.

The chemical analysis (Table 2.1.5) showed that the crude protein (CP) of both CSM was surprisingly similar, but the Narrabri source (use in the feeding trial) was lower in gossypol, condensed tannins (CT) and neutral detergent fibre (NDF). This is to be expected as these ANFs are mostly removed during processing and that CSM was from low gossypol cottonseed varieties. The free CT fraction of all CSM had a mean value of 1.5%, and it is expected that some of this free CT will react and bind with CSM CP making it unavailable for digestion and absorption. The AA profiles of both CSM are in good agreement with expected values. Except for the low levels of lysine, isoleucine, proline, and aspartic acid, most of the AAs were as similar to those in soybean meal which is usually taken as the industry standard. The AME obtained in broilers and layers produced similar results and were marginally higher than those reported in the USA literature (Watkins et al. 1993, 1994; Watkins and Waldroup 1995). Organochlorine and organophosphorus analyses on CSM indicated that these were

not present in concentrations greater than the minimum detectable level and therefore a safe meal for livestock.

Table 2.1.6 Non-starch polysaccharides content of cottonseed meal from two processors (g/kg dry matter)

	CSM Brisbane			CSM Narrabri		
	Soluble	Insoluble	Total	Soluble	Insoluble	Total
Rhamnose	1	2	3	1	2	3
Fucose	0	1	1	0	1	1
Ribose	0	0	0	0	0	0
Arabinose	10	27	37	11	33	44
Xylose	1	32	33	1	30	31
Mannose	1	2	3	1	2	3
Galactose	5	5	10	5	5	10
Glucose	0	46	46	0	40	40
Total	18	115	133	19	113	132

The total NSP values for both CSM were similar (Table 2.1.6). The apparent ileal digestibility values (Table 2.1.8) were obtained using broilers with chromic oxide and bentonite as markers. Both markers produced similar results. The overall AA coefficients of CSM from Brisbane were slightly lower than Narrabri CSM (Table 2.1.8). However both CSM coefficients were in close agreement with those reported for Australian CSM (Ravindran *et al.* 1998, 1999).

Canola meal

The CP for Pinjarra was the lowest of the CM sources (Table 2.1.5). Ca, P, and essential AA content of all CMs were close to expected values. Total AA levels were lower in Pinjarra CM (expeller extracted), most likely due to the dilution effect of higher levels of oil. The GSNL range (3-7 μmols/g) in the CMs was 1/3 of those reported for Canadian "double zero" varieties. The free CT fraction of all CM was similar (mean of 3.5%). Newcastle and Numurkah CM presented a higher CT bound fraction and this is attributed to differences in plant processing, environmental and soil conditions where these crops were harvested. The sinapine content (g/kg) of Newcastle (11.8) and Melbourne (12.7) sources were lower than Numurkah (14.8) and Pinjarra (14.0) indicating differences in processing conditions. As expected, the AME results obtained with broiler birds were much lower than layers with Numurkah and Pinjarra CM processors having higher AME due to their higher residual oil content when compared with Newcastle and Melbourne processors. This is relevant since in many cases, AME values from broilers are commonly used to formulate layer diets. Since all canola varieties currently grown in Australia have low ANF, the AME differences among CM (8.7 MJ/kg Newcastle vs. 9.7 MJ/kg Numurkah) may be attributed to differences in crop location, canola varieties, and in processing conditions.

The analyses of the NSP (Table 2.1.7) and their constituent sugars have not been reported previously in such detail for these CM samples. It is the soluble NSP fraction that causes highly viscous digesta in the small intestine affecting bird performance. Newcastle and Melbourne CM showed more total NSP than Numurkah and Pinjarra due to a higher insoluble fraction. The total soluble fractions for Melbourne, Numurkah and Pinjarra CM were similar, but Pinjarra CM showed the lowest levels for all NSP fractions.

Table 2.1.7 Non-starch polysaccharide content of canola meals (CM) expressed as g/kg dry matter.

	CM Newcastle			CM Melbourne			CM Numurkah			CM Pinjarra		
	S	I	T	S	I	T	S	I	T	S	I	T
Rhamnose	0	3	3	0	4	4	0	2	2	0	3	3
Fucose	0	2	2	0	2	2	0	1	1	0	2	2
Ribose	0	0	0	0	1	1	0	0	0	0	0	0
Arabinose	7	42	49	6	43	49	6	40	46	6	34	40
Xylose	1	16	17	1	16	17	1	16	17	1	13	14
Mannose	1	3	4	1	4	5	1	3	4	0	2	2
Galactose	3	13	16	2	14	16	2	14	16	2	12	14
Glucose	2	59	61	1	57	58	1	54	55	1	10	11
Total	14	138	152	11	141	152	11	130	141	10	76	86

S= soluble. I=insoluble. T= total

Table 2.1.8 Apparent ileal digestibility coefficients of amino acids in cottonseed and canola meals for broilers

Amino acids	CSM Brisbane	CSM Narrabri	CM Newcastle	CM Melbourne	CM Numurkah	CM Pinjarra
Alanine	0.62	0.72	0.71	0.71	0.74	0.77
Arginine	0.82	0.86	0.54	0.67	0.67	0.65
Leucine	0.63	0.67	0.69	0.70	0.73	0.75
Lysine	0.45	0.52	0.63	0.69	0.69	0.75
Methionine	0.66	0.73	0.77	0.79	0.75	0.81
Phenylalanine	0.76	0.78	0.69	0.69	0.72	0.75
Proline	0.66	0.70	0.61	0.65	0.69	0.72
Serine	0.66	0.74	0.62	0.72	0.75	0.75
Aspartic acid	0.68	0.75	0.69	0.72	0.75	0.78
Cystine	0.68	0.76	0.72	0.73	0.76	0.77
Glutamic acid	0.80	0.83	0.82	0.82	0.84	0.85
Glycine	0.67	0.73	0.67	0.71	0.73	0.78
Histidine	0.77	0.80	0.74	0.77	0.79	0.83
Isoleucine	0.61	0.65	0.63	0.64	0.67	0.70
Threonine	0.60	0.68	0.56	0.64	0.69	0.71
Tryptophan	0.65	0.62	0.67	0.73	0.76	0.70
Tyrosine	0.73	0.76	0.64	0.65	0.68	0.71
Valine	0.62	0.65	0.62	0.63	0.67	0.70

The AA digestibility coefficients for CM samples (Table 2.1.8) indicate that except for arginine, the Pinjarra values were higher than the other three sources which may indicate an effect due to differences in meal processing. It is reported that the oil extraction process, and the duration of heat treatment given during this stage, has a significant effect on the apparent ileal digestibility of lysine and perhaps on other AAs (Van Barneveld, 1998).

The Newcastle CM coefficients were generally lowest followed by Melbourne and Numurkah sources. In general, with the exception of methionine, all the CMs coefficients were in close range to those reported on digestible AA by Ravindran *et al*. (1998 and 1999).

The overall AA digestibility coefficients indicative of meal quality for the Newcastle, Melbourne, Numurkah and Pinjarra CM samples were 0.67, 0.70, 0.73, and 0.75 respectively. Pinjarra CM (expeller extracted) had a higher digestible lysine value than other CMs and this should be considered by nutritionists when including expeller CM in diets.

Broiler Diets

A note of clarification

Six months after the conclusion of this trial, it was found that the AA analyses used to determine the AA digestible coefficient values for CSM and CM were wrongly estimated. As a result, lysine was overestimated by 10% and by 20% in CSM and CMs dietary treatments respectively. Therefore, with the exception of the control diet, all CSM and CM diets (starter and finisher periods) were inaccurately formulated for lysine requirements and were just below the minimum digestible AA requirements for maximum growth (see Tables 2.1.1, 2.1.2, 2.1.3, and 2.1.4).

The responses to graded levels of CSM (including PEG treatments) compared with the control diet, for growth performance and other parameters when feeding starter and finisher diets are presented in Tables 2.1.9 and 2.1.10 respectively. The responses to graded levels of CM compared with the control diet, on growth performance and other parameters when feeding starter (4 to 25 d of age) and finisher (25 to 41 d of age) diets are presented in Tables 2.1.11 and 2.1.12 respectively.

Cottonseed meal

Table 2.1.9 Mean feed intake (FI), liveweight gain (LWG) and feed conversion ratio (FCR) for broiler chickens (4-25 d) fed graded levels of cottonseed meal

Diet	FI (g/bird)	LWG (g/bird)	FCR (g FI / g LWG)
Control	1555 ^{a}	1029 ^{a}	1.512 ^{a}
CSM (100 g/kg)	1504 ^{ab}	1008 ^{ab}	1.498 ^{a}
CSM (200 g/kg)	1461 ^{bc}	933 ^{cd}	1.585 ^{b}
CSM (300 g/kg)	1484 ^{bc}	968 ^{bcd}	1.536 ^{ab}
CSM (400 g/kg)	1446 ^{bc}	977 ^{abc}	1.483 ^{a}
CSM (300 g/kg) + PEG	1423 ^{c}	928 ^{cd}	1.554 ^{ab}
CSM (400 g/kg) + PEG	1431 ^{c}	912 ^{d}	1.584 ^{b}
LSD (P=0.05)	61.3	56.3	0.058

Means within a column with no common superscript are significantly different ($P < 0.05$).

During the starter period (Table 2.1.9) chicks fed on CSM diets showed a significant ($P<0.05$) feed intake (FI) reduction at 200, 300 and 400 g/kg. This FI reduction was also observed in birds fed CSM at 300 and 400 g/kg level with added PEG. Since in this trial, values for digestible lysine were lower than recommended values, lysine *per se* became a limiting AA creating a FI depression at all CSM levels. However, it is also possible that this FI reduction was attributed to the increased level of fibre in the diets that went from 2.8 % in the control diet to 7.6 % at 400 g CSM/kg. It is obvious that CT did not affect FI since chicks fed on 300 and 400 g CSM/kg + PEG treatments also gave a reduced FI, and liveweight gain (LWG) responses which negatively affected feed conversion ratio (FCR). On diets without PEG, growth was also affected ($P<0.05$) at 200 and 300 g/kg with no depression at 100 and 400 g/kg. This scatter effect on LWG is difficult to explain. FCR was only affected ($P>0.05$) at 200 g CSM/kg which reflects the lowest LWG obtained at this CSM level. Since the FCR results for the other levels were not significantly different from the control, it looks as if the 200g/kg diet had adverse effects that may have been slightly ameliorated at higher levels due to other factors such as total CP; or possibly the 200g/kg diet was nutritionally deficient.

Measurements made during the finisher period (Table 2.1.10) show that in all CSM treatments without PEG, growth and FCR were not affected ($P>0.05$) by the level of CSM in the diet. In addition, FI was

reduced (P<0.05) only at 400 g CSM/kg level. Similarly, when PEG was supplemented in diets containing 300 and 400 g/kg level LWG and FCR were not negatively affected nor improved, indicating that during the finisher period, dietary CT and PEG did not exert any negative effect on bird's performance. In the current experiment, except for lysine, diets were formulated on a digestible AA basis and although there was an error when supplementing diets with synthetic lysine, the results indicate that older birds were capable of overcoming this deficiency and demonstrated a satisfactory performance. Liver and pancreas weights were not different at any level of CSM in the diets. The blood cell counts (not shown in this report) carried out on these birds were normal and birds did not show any sign of anaemia indicating that the addition of ferrous salts in the diets (ratio 2:1 iron : gossypol) was able to overcome any possible negative effect due of residual gossypol. Therefore satisfactory broiler performance can be obtained during the finisher period with inclusions of up to 300 g/kg of pre-press solvent-extracted CSM. A follow-up experiment was conducted (Broiler Experiment 3, section 2.3) to re-evaluate CSM diets for the starter and finisher periods and formulated with the correct digestible lysine values for CSM.

Table 2.1.10 Mean feed intake (FI), liveweight gain (LWG), feed conversion ratio (FCR) and organ weights for broiler chickens (25-41 d) fed graded levels of cottonseed meal

Diet	FI (g/bird)	LWG (g/bird)	FCR (g FI/g LWG)	Liver (% bodywt)	Pancreas (% bodywt)
Control	2874 [a]	1464	1.962	2.22	0.212
CSM (100 g/kg)	2866 [a]	1500	1.927		
CSM (200 g/kg)	2738 [ab]	1446	1.912	2.31	0.192
CSM (300 g/kg)	2795 [ab]	1450	1.928		
CSM (400 g/kg)	2655 [b]	1418	1.878	2.30	0.197
CSM (300 g/kg) + PEG	2784 [a]	1487	1.931	-	-
CSM (400 g/kg) + PEG	2686 [b]	1441	1.888	-	-
LSD (P=0.05)	139.8	100.4	0.049	0.23	0.049

Means within a column with different superscripts are significantly different (P<0.05).

Canola meal

The results in Table 2.1.11 show that during the starter period, FCR in all inclusion levels was significantly (P<0.05) improved in the Pinjarra CM, due to a reduced (P<0.05) FI and a satisfactory LWG at up to 300 g/kg level that was not different (P>0.05) from the control diet. FCR in the Numurkah source at all inclusions was not different (P>0.05) from the control diet due to a satisfactory chick LWG even at 300 g/kg level, while FI was significantly (P<0.05) reduced at 300 and 400 g/kg level. Melbourne CM on the other hand, had a 8.3, 7.5 and a 10.8 percent LWG reduction at 200, 300 and 400 g/kg level respectively that were significant (P<0.05) when compared with the control diet. This effect on growth may be the result of a linear reduced FI (P<0.05) particularly at 400 g CM/kg. Newcastle FCR was not affected at 100 and 200 g/kg and chicks gave a satisfactory LWG and FI even at 300 g/kg.

Table 2.1.11 Mean feed intake (FI), liveweight gain (LWG) and feed conversion ratio (FCR) of broiler chickens (4-25 d) fed graded levels of canola meal from various sources

Diet	FI (g/bird)	LWG (g/bird)	FCR (g FI / g LWG)
Control	1516 [a]	1024 [a]	1.486 [a]
Newcastle (100 g/kg)	1493 [a]	992 [a]	1.509 [ab]
Newcastle (200 g/kg)	1456 [a]	981 [a]	1.490 [ab]
Newcastle (300 g/kg)	1480 [a]	971 [a]	1.529 [b]
Newcastle (400 g/kg)	1352 [b]	854 [b]	1.595 [c]
Melbourne (100 g/kg)	1498 [a]	1023 [ab]	1.471 [a]
Melbourne (200 g/kg)	1457 [ab]	939 [c]	1.553 [b]
Melbourne (300 g/kg)	1400 [bc]	947 [c]	1.478 [a]
Melbourne (400 g/kg)	1367 [c]	913 [c]	1.503 [a]
Numurkah (100 g/kg)	1510 [a]	1022 [a]	1.478 [a]
Numurkah (200 g/kg)	1500 [ab]	1006 [a]	1.494 [a]
Numurkah (300 g/kg)	1440 [bc]	971 [a]	1.516 [a]
Numurkah (400 g/kg)	1395 [c]	937 [b]	1.499 [a]
Pinjarra (100 g/kg)	1439 [b]	1004 [ab]	1.433 [b]
Pinjarra (200 g/kg)	1423 [b]	989 [ab]	1.442 [b]
Pinjarra (300 g/kg)	1440 [b]	999 [ab]	1.441 [b]
Pinjarra (400 g/kg)	1368 [c]	960 [b]	1.425 [b]
LSD (P=0.05)	69.3	57.1	0.04

Means for each CM source within a column with different superscript are significantly different ($P<0.05$).

During the finisher period, the results in Table 2.1.12 showed that the overall bird FCR was improved ($P<0.05$) for each CM source at all levels compared to control birds. This was largely due to the significantly ($P<0.05$) reduced FI and a satisfactory growth performance, where no adverse effect ($P>0.05$) on LWG was found when birds were fed up to 300 g CM/kg in the Newcastle and Pinjarra source and up to 400 g CM/kg in Melbourne and Numurkah sources. GSNL levels found in all CMs were low, and since CT did not affect bird performance as shown in the CSM experiment, it is possible that differences in the CMs results between the starter and the finisher period were due to younger birds (starter period) being more susceptible to dietary fibre and to lysine deficient diets.

In the present experiment, birds fed on high dietary levels of CM did not show any sign of leg problems although sulphur from CM was increased from 0.16% (control diet) to about 0.31% at 400 g CM/kg. Other sources of sulphur were from supplemental methionine, mineral salts as well as the sulphur content in drinking water. It has been reported that an excess of sulphur in chicken diets results in reduced performance and therefore it is possible that this additional dietary sulphur may have affected FI. The enlargement of the pancreas (Table 2.1.12) on all CM sources at 400 g CM/kg may also indicate that a trypsin inhibitor was present in these meals and this needs to be investigated.

These results demonstrate that higher levels of CM can be used in broiler diets formulated on a digestible AA basis, but more detailed studies, using a corrected lysine value in these meals, are required.

Table 2.1.12 Mean feed intake (FI), liveweight gain (LWG), feed conversion ratio (FCR) and organ weights of broiler chickens (25-41 d) fed graded levels of canola meal from various sources

Diet	FI (g/bird)	LWG (g/bird)	FCR (g FI / g LWG)	Liver (% bodywt)	Pancreas (% bodywt)
Control	3039[a]	1496 [ac]	2.057 [a]	2.026[a]	0.172[a]
Newcastle (100 g/kg)	2919 [a]	1513 [a]	1.952 [b]		
Newcastle (200 g/kg)	2895 [ab]	1484 [a]	1.951 [b]	1.927 [a]	0.179 [a]
Newcastle (300 g/kg)	2750 [bc]	1430 [ab]	1.977 [b]		
Newcastle (400 g/kg)	2634 [c]	1362 [b]	1.951 [b]	2.393 [b]	0.245 [b]
Melbourne (100 g/kg)	2991 [a]	1577 [c]	1.915 [b]		
Melbourne (200 g/kg)	2709 [b]	1451 [a]	1.869 [b]	1.926 [a]	0.185 [a]
Melbourne (300 g/kg)	2631 [b]	1411 [a]	1.872 [b]		
Melbourne (400 g/kg)	2743 [b]	1448 [a]	1.897 [b]	2.074 [a]	0.226 [b]
Numurkah (100 g/kg)	2895 [ab]	1505 [a]	1.924 [b]		
Numurkah (200 g/kg)	2904 [ab]	1495 [a]	1.943 [b]	1.880 [a]	0.170 [a]
Numurkah (300 g/kg)	2816 [b]	1524 [a]	1.897 [b]		
Numurkah (400 g/kg)	2761 [b]	1453 [a]	1.892 [b]	2.341 [b]	0.232 [b]
Pinjarra (100 g/kg)	2765 [b]	1506 [a]	1.861 [b]		
Pinjarra (200 g/kg)	2723 [bc]	1493 [ab]	1.824 [b]	2.269 [a]	0.198 [a]
Pinjarra (300 g/kg)	2827 [b]	1547 [a]	1.825 [b]		
Pinjarra (400 g/kg)	2600 [c]	1414 [b]	1.849 [b]	2.186 [a]	0.235 [b]
LSD (P=0.05)	150.8	86.8	0.0759	0.2693	0.0321

Means for each CM source within a column with different superscripts are significantly different ($P<0.05$) from the control diet.

2.2 Experiment 2 - Maximum inclusion of canola meal in broiler starter and finisher diets formulated on a digestible or total amino acid basis

2.2.1 Introduction

Previous work (Experiment 1) indicated that high levels of canola meal (CM) support satisfactory broiler performance when diets are formulated on a digestible amino acid basis (Perez-Maldonado *et al* 2001). An important objective of this project was to evaluate the nutritional value, anti nutritional factors (ANF) and variability of these meals due to location, environment, cultivars, and industry processing conditions. Because digestible lysine values were wrongly estimated in the previous experiment, the present study re-evaluated this aspect using correct lysine coefficients. Another criticism of the previous experiment was the need for comparison between diets formulated on total amino acid (AA) basis versus digestible AA basis. This study investigated the effect of adding 200, 300 and 400 g CM/kg from four processors (Newcastle, Melbourne, Numurkah and Pinjarra) in diets formulated on a digestible AA basis and compared diets formulated on a total or digestible AA basis from Newcastle and Melbourne processors.

2.2.2 Results and Discussion

Starter and finisher diets

The ingredient and chemical composition of the starter and finisher diets, formulated on total AA basis from Newcastle and Melbourne sources, are presented in Table 2.2.1 and 2.2.2 respectively.

The ingredient and chemical composition of the starter and finisher diets formulated on digestible AA basis from Newcastle, Melbourne, Numurkah, and Pinjarra sources, are presented in Table 2.2.3 and 2.2.4 respectively.

The nutrient, chemical composition and ANF results are presented in Table 2.2.5. The CM soluble, insoluble and total NSP are presented in Table 2.2.6. The determined apparent ileal digestibility coefficients of AAs for each CM are presented in Table 2.2.7.

Experimental design

There were 19 treatments x 5 replicate pens (x 8 birds per pen) in a completely randomised layout of the 95 pens. The structure of the 19 treatments was a control diet plus all factorial combinations of four sources x three inclusion levels formulated on a digestible AA basis, plus all factorial combinations of two of the four sources by the same three inclusion levels formulated in a total AA basis.

Statistical analysis

Analysis of variance (ANOVA) was used to test the effects of treatments using an ANOVA model for a completely randomised design. A cage of eight birds was the experimental unit. The treatment means were compared in an initial randomised ANOVA, and then in a follow-up ANOVA in which the full error term (76 degrees of freedom) from the initial ANOVA was used, the main effects, and interaction, were tested using a protected LSD ($P<0.05$).

Table 2.2.1 Ingredient composition (g/kg) by source and level of canola meal (CM) in starter diets (total amino acid basis)

Ingredients	CM 200	CM 300	CM 400	CM 200	CM 300	CM 400
Sorghum	420	433	400	448	454	412
Wheat	150	80	40	150	100	50
Poultry offal meal	50	50	50	50	50	50
Meat & bone meal	50	50	49	50	50	48
Soybean meal	88	37	-	72	12	-
CM Newcastle	200	300	400	-	-	-
CM Melbourne	-	-	-	200	300	400
Soybean oil	23.7	33.9	47.3	10.7	15.5	25.1
Limestone	3.1	2	1.1	3.9	3.2	2.6
Dicalcium phosphate	1.8	0.8	-	1.9	0.9	-
Salt	1.5	1.5	1.8	1.3	1.3	2
Sodium bicarbonate	0.6	0.6	0.3	1.3	1.7	1
Vitamins/minerals	7.1	7.1	7.1	7.1	7.1	7.1
DL methionine	2.4	2.3	2.1	2.2	2	1.5
Lysine	2.3	2	1.3	2.6	2.6	0.92
Calculated analysis						
Total crude protein	230	233	242	229	233	253
Total lysine	12	12	12	12	12	12
Total methionine	5.2	5.2	5.2	5.2	5.2	5.2
Total sulphur AA	9	9.2	9.6	9.2	9.6	10.3
Total threonine	8.3	8.5	9.1	8.2	8.5	9.5
Total isoleucine	8.5	8.5	8.8	8.5	8.5	9.3
Total tryptophan	3	3	3.1	3	3.1	3.4
Calcium	10	10	10	10	10	10
Avail. Phosphorous	5	5	5	5	5	5
AME (MJ/kg)	12.5	12.5	12.5	12.5	12.5	12.5

Table 2.2.2 Ingredient composition (g/kg) by source and level of canola meal (CM) in finisher diets (total amino acid basis)

Ingredients	CM 200	CM 300	CM 400	CM 200	CM 300	CM 400
Sorghum	472	461	454	499	477	488
Wheat	150	100	-	150	100	-
Poultry offal meal	50	50	50	50	50	50
Meat & bone meal	48	44	39	48	44	11
Soybean meal	47	-	-	31	-	-
CM Newcastle	200	300	400	-	-	-
CM Melbourne	-	-	-	200	300	400
Soybean oil	16.9	29	43.3	3.9	12	23.7
Limestone	3.8	3.1	2.6	4.5	4.3	7.4
Salt	1.6	1.7	1.9	1.4	1.8	2.5
Sodium bicarbonate	0.4	0.3	-	1.1	0.9	0.6
Vitamins/minerals	7.1	7.1	7.1	7.1	7.1	7.1
DL methionine	2.3	2.2	1.9	2.1	1.8	1.4
Lysine	1.8	1.5	-	2.2	1.3	0.3
Calculated analysis						
Total crude protein	214	218	237	214	226	235
Total lysine	10.5	1.05	10.7	10.5	10.5	10.5
Total methionine	4.9	4.9	4.9	4.9	4.9	4.9
Total sulphur AA	8.5	8.7	9.2	8.7	9.3	9.8
Total threonine	7.6	7.9	8.9	7.6	8.2	8.9
Total isoleucine	7.8	7.8	8.7	7.8	8.3	8.9
Total tryptophan	2.8	2.8	3	2.8	3	3.2
Calcium	9.5	9.5	9.5	9.5	9.5	9.5
Avail. Phosphorous	4.5	4.5	4.5	4.5	4.5	4.5
AME (MJ/kg)	12.5	12.5	12.5	12.5	12.5	12.5

Table 2.2.3 Ingredient composition (g/kg) by source and level of canola meal (CM) in starter diets (digestible amino acid basis)

Ingredients													
Sorghum	450	403	379	356	431	421	409	420	405	392	415	393	344
Wheat	250	150	100	50	150	100	50	150	100	50	150	105	94
Poultry offal meal	50	50	50	50	50	50	50	50	50	50	50	50	50
Meat & bone meal	50	50	50	50	50	50	48	50	50	50	50	50	50
Soybean meal	171	59	22	-	42	-	-	49	8	-	93	54	17
Full fat soybean meal	-	50	50	33	50	46.5	-	50	50	11	-	-	-
Sunflower meal	3	-	-	-	-	-	-	-	-	-	22	30	30
CM Newcastle	-	200	300	400	-	-	-	-	-	-	-	-	-
CM Melbourne	-	-	-	-	200	300	400	-	-	-	-	-	-
CM Numurkah	-	-	-	-	-	-	-	200	300	400	-	-	-
CM Pinjarra	-	-	-	-	-	-	-	-	-	-	200	300	400
Soybean oil		19.6	32.6	47.6	6.6	13.4	25.9	10.5	18.8	31.7	-	-	-
Limestone	5.2	3	1.9	0.5	3.7	3	2.6	3.7	3	2.2	3.3	2.4	1.5
Dicalcium phosphate	4.0	1.8	0.6	-	1.9	0.8	-	1.9	0.9	-	2.4	1.6	0.8
Salt	1.1	1.4	1.6	1.7	1.2	1.3	1.4	1.3	1.4	1.5	1.4	1.5	1.8
Sodium bicarbonate	1.1	0.8	0.6	0.4	1.5	1.8	1.8	1.1	1.1	1.1	1.2	1.2	1.2
Vitamins/minerals	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1
DL methionine	2.9	2.2	1.8	1.4	1.9	1.4	0.9	1.9	1.4	1	1.8	1.3	0.9
Lysine	0.3	2.5	2.2	1.9	3	2.9	2.7	2.7	2.4	2.2	2.5	2	1.5
Threonine	1.1	0.7	0.5	0.2	0.8	0.6	0.3	0.8	0.6	0.4	0.8	0.6	0.5
Calculated analysis													
Total crude protein	216	234	243	253	233	242	253	232	241	250	234	242	252
Digestible lysine	10.5	10.5	10.5	10.5	10.5	10.5	10.5	10.5	10.5	10.5	10.5	10.5	10.5
Digestible methionine	4.9	4.6	4.5	4.3	4.5	4.3	4.1	4.4	4.1	3.9	4.3	4	3.8
Digestible sulphur AA	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.7
Digestible threonine	7	7	7	7	7	7	7	7	7	7	7	7	7
Digestible isoleucine	7	7	7	7	7	7	7.1	7	7	7	7	7	7
Digestible tryptophan	2.3	2.4	2.5	2.5	2.4	2.5	2.6	2.4	2.4	2.5	2.4	2.4	2.5
Calcium	10	10	10	10	10	10	10	10	10	10	10	10	10
Avail. Phosphorous	5	5	5	5	5	5	5	5	5	5	5	5	5
AME (MJ/kg)	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5

Table 2.2.4 Ingredient composition (g/kg) by source and level of canola meal (CM) in finisher diets (digestible amino acid basis)

Ingredients													
Sorghum	435	446	463	468	509	479	490	516	474	476	299	176	247
Wheat	304	200	100	-	150	100	-	150	100	-	354	412	264
Poultry offal meal	50	50	50	30	50	50	50	50	50	50	50	50	50
Meat & bone meal	50	48	44	43	22	44	10	32	44	26	50	48	12
Soybean meal	112	20	-	-	34	-	-	-	-	-	-	-	-
Full fat soybean meal	-	3	-	-	-	-	-	-	-	-	-	-	-
Sunflower meal	30	-	-	-	-	-	-	-	-	-	30	-	-
CM Newcastle	-	200	300	400	-	-	-	-	-	-	-	-	-
CM Melbourne	-	-	-	-	200	300	400	-	-	-	-	-	-
CM Numurkah	-	-	-	-	-	-	-	200	300	400	-	-	-
CM Pinjarra	-	-	-	-	-	-	-	-	-	-	200	300	400
Soybean oil	-	16	28.4	45.9	6.2	11.4	23	6.85	16	27.9	-	-	-
Limestone	5.6	3.8	3.1	2.6	7.4	4.3	7.4	6.3	4.2	5.6	3.8	3.2	6.3
Dicalcium phosphate	1.3	-	-	-	7.6	-	8.6	5	-	4.1	0.1	-	9.5
Salt	1.5	1.5	1.8	1.9	1.7	1.8	2.4	1.5	1.8	2.4	1.6	2.4	2.9
Sodium bicarbonate	0.6	0.6	0.3	0.1	1.3	0.9	0.8	1.2	0.54	0.8	1.2	0.4	0.3
Vitamins/minerals	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1
DL methionine	1.9	1.6	1.2	0.9	1.3	0.6	0.1	1.5	0.7	0.4	1	0.6	0.5
Lysine	2.1	2.1	1.5	0.9	2.3	1.4	0.7	2.5	1.3	0.2	2.1	0.7	0.5
Threonine	0.4	0.4	0.8	-	0.4	-	-	0.6	0.3	-	0.6	0.2	-
Calculated analysis													
Total crude protein	201	208	219	229	204	226	235	200	221	235	209	227	230
Digestible lysine	8.3	8.3	8.3	8.3	8.3	8.3	8.3	8.3	8.3	8.3	8.3	8.3	8.3
Digestible methionine	3.8	3.7	3.5	3.4	3.6	3.3	3.1	3.5	3.1	3.1	3.3	3.1	3.1
Digestible sulphur AA	6.3	6.3	6.3	6.3	6.3	6.3	6.4	6.3	6.3	6.6	6.3	6.6	6.9
Digestible threonine	5.8	5.8	5.8	6	5.8	5.9	6.3	5.8	5.8	6.2	5.8	5.8	5.8
Digestible isoleucine	6.3	5.8	5.9	6.2	6	6.4	6.8	5.8	6.2	6.7	5.7	6.1	6.3
Digestible tryptophan	2.1	2.1	2.2	2.4	2.2	2.4	2.6	2.1	2.2	2.4	2.1	2.3	2.4
Calcium	9.5	9.5	9.5	9.5	9.5	9.5	9.5	9.5	9.5	9.5	9.5	9.5	9.5
Avail. Phosphorous	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5
AME (MJ/kg)	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5

Composition of canola meal from different processors

Table 2.2.5 Chemical composition (g/kg DM) of the experimental canola meals (2000-2001)

Analysis	Newcastle	Melbourne	Numurkah	Pinjarra
Dry matter (%)	90.6	88.4	89.9	94.2
Gross energy MJ/kg	19.8	20.0	19.9	21.4
Crude protein	394	428	403	352
Phosphorus	11.8	11.7	11.8	9.3
Calcium	7.8	6.5	6.4	6.2
Sulphur	7.4	7.1	7.5	6.7
Fat	39	39	45	113
Free condensed tannin	36.4	36.4	37.9	31.5
Bound tannins	10.8	6.0	16.1	45.7
Total condensed tannin	47.2	42.4	54.0	77.2
Glucosinolates (μm/g)	3.4	3.5	4.5	10
Sinapine	11.7	ND	13.9	14.4
Neutral detergent fibre (NDF)	284	257	265	239
Alanine	16.5	17.9	17.0	14.8
Arginine	27.7	30.7	27.9	24.6
Leucine	24.5	27.3	25.4	21.7
Lysine	19.6	20.8	20.3	19.1
Methionine	5.5	7.2	6.1	5.0
Phenylalanine	14.7	16.1	15.1	13.1
Proline	23.6	22.4	24.3	18.8
Serine	15.7	16.8	16.1	14.1
Aspartic acid	24.0	27.6	24.8	21.4
Cystine	8.5	10.0	10.0	8.5
Glutamic acid	61.6	67.7	63.8	55.1
Glycine	17.3	19.4	18.1	15.5
Histidine	9.4	10.2	9.7	8.7
Isoleucine	14.7	16.3	15.1	13.0
Threonine	16.0	17.4	16.2	14.1
Tryptophan	5.3	6.1	5.3	4.6
Tyrosine	9.6	10.6	9.9	8.7
Valine	17.6	19.6	18.8	15.7
Layer AME (MJ/kg DM)	11.7	13.1	12.6	12.4
Layer AMEn	10.0	11.6	11.0	11.5
Broiler AME (MJ/kg DM)	9.1	11.2	10.6	12.7

The chemical analyses (Table 2.2.5) showed that the crude protein (CP), and AA levels from Pinjarra (expeller extracted) were the lowest of the CM sources and most likely due to higher levels of residual lipid. The total CP content of each CM was close to the value reported from the previous season (1999). Ca, P, and sulphur contents were also similar to the previous year and except for lysine, most essential AA varied only slightly from the previous year. GSNL levels were low (3.4-10 μmol/g) in all sources indicating that Australian CMs are from truly "double zero" varieties. The mean free condensed tannin (CT) fraction for all CM sources was similar to the previous year (3.5%). Pinjarra CM presented the highest bound CT of 4.6% but this may not cause any detrimental effect to CP since bound CT are not reactive. The sinapine values were similar to those obtained in previous year with Pinjarra (14.4 g/kg) and Numurkah (13.9 g/kg) sources having the highest sinapine content. Except for Pinjarra CM, the AME results obtained with broiler birds were generally lower than in layers, with Newcastle CM processor having the lowest AME due to a higher NDF content when compared with the other CM sources. Interestingly, the overall AME results from this year's CM crop were higher than the values obtained in the previous year (see Experiment 1, Table 2.1.5). Since all the canola

varieties currently grown in Australia have low ANF, the AME differences among CM are attributed to differences in location, seasonal variation, varieties and processing conditions.

The analyses of the NSP (Table 2.2.6) indicate that all CM sources had similar total NSP. However Pinjarra source had the lowest soluble and the highest insoluble NSP fraction when compared with the other three sources. Arabinose was highest NSP in all CM sources and this may have some implication when selecting enzymes for feed improvement.

Table 2.2.6 Non-starch polysaccharide content of canola meals (CM) expressed as g/kg dry matter.

	CM Newcastle			CM Melbourne			CM Numurkah			CM Pinjarra		
	S	I	T	S	I	T	S	I	T	S	I	T
Rhamnose	0	3	3	0	3	3	0	3	3	0	3	3
Fucose	0	2	2	0	2	2	0	2	2	0	2	2
Ribose	0	0	0	0	0	0	0	0	0	0	0	0
Arabinose	11	40	51	10	42	52	11	40	51	10	34	44
Xylose	2	16	18	2	17	19	2	17	19	1	14	15
Mannose	2	3	5	1	3	4	1	3	4	1	3	4
Galactose	5	14	19	5	15	20	5	15	20	4	13	17
Glucose	3	24	27	2	24	26	3	24	27	2	41	43
Total	23	102	125	20	106	126	22	104	126	18	110	128

Table 2.2.7 The apparent ileal digestibility coefficients for amino acids in canola meals

Amino acids	Newcastle	Melbourne	Numurkah	Pinjarra
Alanine	0.74	0.74	0.75	0.73
Arginine	0.85	0.84	0.86	0.85
Leucine	0.75	0.76	0.76	0.76
Lysine	0.73	0.71	0.75	0.76
Methionine	0.86	0.88	0.87	0.91
Phenylalanine	0.75	0.76	0.77	0.76
Proline	0.71	0.72	0.72	0.71
Serine	0.66	0.67	0.65	0.65
Aspartic acid	0.68	0.69	0.69	0.70
Cystine	0.67	0.65	0.69	0.79
Glutamic acid	0.84	0.82	0.83	0.84
Glycine	0.72	0.73	0.73	0.74
Histidine	0.81	0.80	0.82	0.82
Isoleucine	0.71	0.73	0.74	0.73
Threonine	0.66	0.66	0.66	0.64
Tryptophan	0.80	0.79	0.80	0.80
Tyrosine	0.72	0.72	0.74	0.70
Valine	0.70	0.72	0.72	0.72

The AA digestibility values in Table 2.2.7 indicated that, with the exception of Pinjarra CM, the overall digestibility values were generally higher than expected being 0.74, 0.74, 0.77, and 0.76 for the Newcastle, Melbourne, Numurkah and Pinjarra respectively. These higher digestible AA values may suggest that little gain in performance would be obtained when formulating diets on a digestible AA basis. When compared with values obtained in the previous year (Experiment 1), the digestible AA variability among CM in the present study was low. Digestible AA values for Newcastle CM were generally higher when compared with last year's results and this suggests that seasonal variation has an important effect on protein quality in these CMs. In the present study all digestibility coefficients were close to those reported by Ravindran *et al*. (1998 and 1999).

Broiler Diets

Results (digestible amino acid basis)

The responses to graded levels of CM compared with the control diet, on growth performance when feeding starter and finisher diets formulated on digestible AA basis are presented in Tables 2.2.8 and 2.2.9 respectively. Data on liver, pancreas, and fat pad weight and intestinal viscosity, are presented in Table 2.2.10.

Table 2.2.8 Mean feed intake (FI), liveweight gain (LWG), feed conversion ratio (FCR) for broiler chickens (4-25 d) fed graded levels of from various canola meal (CM) sources

Diet	FI (g/bird)	LWG (g/bird)	FCR G FI / g LWG)
Control	1266 [a]	920 [a]	1.382 [a]
Newcastle (200 g/kg)	1235 [a]	921 [a]	1.339 [b]
Newcastle (300 g/kg)	1188 [b]	896 [a]	1.335 [b]
Newcastle (400 g/kg)	1133 [b]	845 [b]	1.348 [b]
Melbourne (200 g/kg)	1218 [a]	889 [a]	1.371 [a]
Melbourne (300 g/kg)	1216 [a]	892 [a]	1.364 [a]
Melbourne (400 g/kg)	1176 [b]	855 [b]	1.375 [a]
Numurkah (200 g/kg)	1282 [a]	930 [a]	1.379 [a]
Numurkah (300 g/kg)	1248 [a]	910 [a]	1.377 [a]
Numurkah (400 g/kg)	1209 [b]	879 [a]	1.375 [a]
Pinjarra (200 g/kg)	1238 [a]	880 [a]	1.410 [a]
Pinjarra (300 g/kg)	1222 [a]	901 [a]	1.357 [a]
Pinjarra (400 g/kg)	1236 [b]	879 [a]	1.406 [a]
LSD (P=0.05)	56.4	45.2	0.0258

Means for each CM source within a column with different superscript are significantly different (P<0.05).

The results in Table 2.2.8 showed that during the starter period, FCR at all inclusion levels was significantly (P<0.05) improved in the Newcastle CM due to a reduced (P<0.05) FI, and a satisfactory LWG that was not different (P>0.05) from the control diet at up to 300 g/kg level. FCR for all other CMs at all inclusion levels was similar to the control diet (P>0.05), and chicks had a satisfactory LWG even at 300 g/kg level in the Melbourne and Newcastle CM and up to 400 g/kg in the Numurkah and Pinjarra sources. Excepting the Newcastle source, FI was only reduced (P<0.05) at 400 g/kg for all others CMs.

In the finisher period (Table 2.2.9), FI was linearly reduced in all CM levels in Newcastle and Melbourne sources but only significant (P<0.05) at 400 g/kg level in the Numurkah and Pinjarra sources. In spite of this reduced FI, a satisfactory LWG was obtained in all CM sources; but a linear declined in LWG was observed with increasing CM inclusion particularly at 400 g/kg level which was not significantly depressed due to an obtained large LSD value. As a result of this FI and LWG combination, FCR for all CM levels and sources was not different (P>0.05) from the control diet. Since all diets were formulated using determined digestible AAs values, the present experiment did not show the problems found during Experiment 1 indicating that satisfactory broiler performance can be obtained in both the starter and finisher periods when using high levels of CM in broiler diets.

Table 2.2.9 Mean feed intake (FI), liveweight gain (LWG), feed conversion ratio (FCR) for broiler chickens (25-41 d) fed graded levels of from various canola meal (CM) sources

CM Treatment	FI (g/bird)	LWG (g/bird)	FCR (g FI / g LWG)
Control	3074 [ac]	1619 [a]	1.901 [a]
Newcastle (200 g/kg)	3016 [a]	1634 [a]	1.855 [a]
Newcastle (300 g/kg)	2923 [b]	1605 [a]	1.848 [a]
Newcastle (400 g/kg)	2831 [b]	1529 [a]	1.851 [a]
Melbourne (200 g/kg)	2927 [b]	1530 [a]	1.939 [a]
Melbourne (300 g/kg)	2870 [b]	1553 [a]	1.865 [a]
Melbourne (400 g/kg)	2797 [b]	1476 [b]	1.887 [a]
Numurkah (200 g/kg)	3158 [c]	1665 [a]	1.902 [a]
Numurkah (300 g/kg)	3010 [a]	1582 [a]	1.905 [a]
Numurkah (400 g/kg)	2923 [b]	1546 [a]	1.889 [a]
Pinjarra (200 g/kg)	3105 [a]	1689 [a]	1.836 [a]
Pinjarra (300 g/kg)	2958 [a]	1664 [a]	1.797 [b]
Pinjarra (400 g/kg)	2931 [b]	1552 [a]	1.934 [a]
LSD (P=0.05)	130.2	106.7	0.0679

Means for each CM source within a column with different superscripts are significantly different ($P<0.05$) from the control diet.

Table 2.2.10 The effect of feeding graded levels of CM from various sources on weights of liver, pancreas, fat pad and viscosity in the small intestine from 25 to 41 days of age

CM Treatment	Liver (% bodywt)	Pancreas (% bodywt)	Fat pad (% bodywt)	Viscosity (centipoise)
Control	2.37 [a]	0.158 [a]	1.453 [a]	4.0 [a]
Newcastle (200 g/kg)	2.76 [b]	0.185 [b]	1.441 [a]	2.48 [b]
Newcastle (400 g/kg)	2.39 [a]	0.198 [b]	0.928 [b]	2.15 [b]
Melbourne (200 g/kg)	2.68 [a]	0.182 [a]	1.046 [b]	2.19 [b]
Melbourne (400 g/kg)	2.68 [a]	0.197 [b]	0.597 [b]	1.64 [b]
Numurkah (200 g/kg)	2.32 [a]	0.182 [a]	1.158 [a]	2.80 [b]
Numurkah (400 g/kg)	2.74 [b]	0.178 [a]	0.670 [b]	1.94 [b]
Pinjarra (200 g/kg)	2.59 [a]	0.186 [b]	1.103 [a]	3.51 [a]
Pinjarra (400 g/kg)	2.64 [a]	0.191 [b]	0.843 [b]	3.28 [a]
LSD (P=0.05)	0.329	0.0248	0.3675	0.821

Means for each CM source within a column with different superscripts are significantly different ($P<0.05$) from the control diet.

In the present experiment, 2% of the birds were culled due to leg problems but this figure is normal for broiler birds raised in wire cages, and a more detailed observation will be performed in a follow up semi-commercial trial using floor pens facilities. Table 2.2.10 indicates that inclusion of CM reduced bird abdominal fat proportion and intestinal viscosity, without affecting liver weight, but relative pancreas weight was increased indicating that a trypsin inhibitor may be present in these meals which needs to be confirmed.

Bird performance on diets formulated on a total versus digestible amino acid basis

The responses to graded levels on growth performance of CM from Newcastle and Melbourne sources compared with the control diet, when feeding starter and finisher diets on digestible and total AA basis are presented in Tables 2.2.11 and 2.2.12 respectively.

Table 2.2.11 Feed intake (FI), liveweight gain (LWG), and feed conversion ratio (FCR) of broiler chickens (4-25 d) fed graded levels of Newcastle and Melbourne sources of canola meal formulated on a total and digestible amino acid basis

CM Treatment	Formulation basis	FI (g/bird)	LWG(g/bird)	FCR (g FI / g LWG)
Control		1266 ^{ab}	920 ^{abc}	1.382 ^{e}
Newcastle 200 g/kg	Total AA	1268 ^{ab}	925 ^{ab}	1.374 ^{cde}
Newcastle 300 g/kg	Total AA	1223 ^{bcd}	896 ^{abc}	1.374 ^{cde}
Newcastle 400 g/kg	Total AA	1185 ^{cde}	864 ^{cd}	1.388 ^{de}
Newcastle 200 g/kg	Digestible AA	1235 ^{abc}	921 ^{abc}	1.339 ^{ab}
Newcastle 300 g/kg	Digestible AA	1188 ^{cde}	896 ^{abc}	1.335 ^{a}
Newcastle 400 g/kg	Digestible AA	1132 ^{e}	845 ^{d}	1.348 ^{abc}
Melbourne 200 g/kg	Total AA	1285 ^{a}	937 ^{a}	1.379 ^{de}
Melbourne 300 g/kg	Total AA	1208 ^{cd}	878 ^{cd}	1.384 ^{de}
Melbourne 400 g/kg	Total AA	1222 ^{bcd}	877 ^{cd}	1.400 ^{e}
Melbourne 200 g/kg	Digestible AA	1218 ^{bcd}	889 ^{bcd}	1.371 ^{cd}
Melbourne 300 g/kg	Digestible AA	1216 ^{bcd}	892 ^{abc}	1.364 ^{bcd}
Melbourne 400 g/kg	Digestible AA	1176 ^{de}	855 ^{cd}	1.375 ^{de}
LSD (P<0.05)		56.4	45.2	0.0257

$^{a-e}$ Means in a column with different superscript differ significantly (P<0.05)

During the starter period, LWG and FCR were influenced by CM source, CM level of inclusion and method of formulation. For both CMs, LWG and FI were depressed at the highest (400 g/kg) level of inclusion. For Newcastle CM at all three levels of inclusion, formulation on a digestible AA basis resulted in a significantly (P<0.05) improved FCR compared to birds given the control diet, or diets formulated on a total AA basis. The relative contribution of FI and LWG to this response, varied with level of inclusion. The results indicate that satisfactory performance can be obtained on broiler starter diets containing up to 300 g CM/kg and that growth and feed efficiency on diets containing CM are likely to be improved by formulating on a digestible AA basis.

During the finisher period, for both CMs, inclusion rate affected both FI and LWG. There was an overall linear negative relationship between both sources of CM and inclusion rate and FI, and a significant (P<0.05) depression in mean LWG on the 400 g/kg CM diets. As a result, mean FCR on the 300 g/kg CM diets was lower than on the control or 200 g/kg CM diets. Thus, very satisfactory growth rate and FCR were obtained on diets containing 300g/kg CM. Contrary to the findings during the starter period, formulating diets on a digestible AA basis did not improve FCR during the finisher period.

Table 2.2.12 Mean feed intake (FI), liveweight gain (LWG), feed conversion ratio (FCR) for broiler chickens (25-42 days) fed graded levels of Newcastle and Melbourne sources of canola meal formulated on a total and digestible amino acid basis

Treatment	Formulation basis	Feed intake (g/bird)	LWG (g/bird)	FCR (g FI / g LWG)
Control		3074 ^{ab}	1619 ^{ab}	1.901 ^{bcd}
Newcastle 200 g/kg	Total AA	2967 ^{bcd}	1605 ^{abc}	1.916 ^{cd}
Newcastle 300 g/kg	Total AA	2893 ^{cde}	1617 ^{abc}	1.808 ^{a}
Newcastle 400 g/kg	Total AA	2866 ^{de}	1504 ^{bc}	1.906 ^{bcd}
Newcastle 200 g/kg	Digestible AA	3016 ^{abc}	1634 ^{ab}	1.855 ^{abc}
Newcastle 300 g/kg	Digestible AA	2923 ^{cde}	1605 ^{abc}	1.848 ^{abc}
Newcastle 400 g/kg	Digestible AA	2831 ^{e}	1529 ^{abc}	1.851 ^{abc}
Melbourne 200 g/kg	Total AA	3103 ^{a}	1661 ^{a}	1.892 ^{bcd}
Melbourne 300 g/kg	Total AA	3010 ^{abc}	1598 ^{abc}	1.911 ^{cd}
Melbourne 400 g/kg	Total AA	2821 ^{e}	1542 ^{abc}	1.843 ^{ab}
Melbourne 200 g/kg	Digestible AA	2927 ^{cde}	1530 ^{abc}	1.939 ^{d}
Melbourne 300 g/kg	Digestible AA	2870 ^{de}	1553 ^{abc}	1.865 ^{abc}
Melbourne 400 g/kg	Digestible AA	2797 ^{e}	1476 ^{c}	1.887 ^{bcd}
LSD (P<0.05)		130.2	141.6	0.0679

Means in a column with different superscript differ significantly (P<0.05)

2.3 Experiment 3 - Maximum inclusion of cottonseed meal in broiler starter and finisher diets formulated on a digestible or total amino acid basis

2.3.1 Introduction

Previous work (Experiment 1) indicated that high levels of cottonseed meal (CSM) supported satisfactory broiler performance when diets are formulated on a digestible amino acid basis (Perez-Maldonado *et al* 2001). An important objective of this experiment is to determine the nutritional value and variation in anti nutritional factors (ANF) of these meals due to location, environment, cultivars, and industry processing conditions. Since digestible lysine values were overestimated for Experiment 1, the present study will re-evaluate broiler production parameters using measured lysine values. Experiment 1 identified the need for a comparison between diets formulated on total amino acid (AA) basis and digestible AA. Thus, this study investigated the effect of including 100, 200, 300 and 400 g CSM/kg from three processors (Brisbane, Narrabri and Gunnedah) in diets formulated on a digestible AA basis. A comparison between diets formulated on a total AA basis and digestible AA was made for CSM from Narrabri only.

2.3.2 Materials and methods

Starter and finisher diets

The ingredient and chemical composition of the starter and finisher diets formulated on a total AA basis from Narrabri CSM are presented in Tables 3.1 and 3.2 respectively.

The ingredient and chemical composition of the starter and finisher diets formulated on a digestible AA basis from Brisbane, Narrabri and Gunnedah CSM are presented in Table 2.3.3 and 2.3.4 respectively.

The nutrient, and chemical composition and ANF results are presented in Table 3.5. The CSM soluble, insoluble and total NSP are presented in Table 2.3.6. The determined apparent ileal digestibility coefficients for AAs for each CM are presented in Table 2.3.7.

Experimental design

There were 17 treatments x 5 replicate cages x 8 birds/cage in a completely randomised block layout of the 85 pens. The structure of the 17 treatments was a control diet plus all factorial combinations of four sources x four inclusion levels formulated on a digestible AA basis, plus all factorial combinations of one of the three sources by the same four inclusion levels formulated on a total AA basis.

Statistical analysis

Analysis of variance (ANOVA) was used to test the effects of treatments using an ANOVA model for a completely randomised block design. A cage of eight birds was the experimental unit. The treatment means were compared in an initial randomised ANOVA, and then in a follow-up ANOVA in which the full error term (64 degrees of freedom) from the initial ANOVA was used. The main effects and interaction were tested using a protected LSD ($P<0.05$).

Table 2.3.1 Ingredient composition (g/kg) of cottonseed meal (CSM) starter diets (total amino acid basis)

Ingredients	CSM 100	CSM 200	CSM 300	CSM 400
Sorghum	582	598	556	472
Meat & bone meal	50	50	47	41
Poultry offal meal	50	50	50	50
Soybean meal	198	81	19	-
CSM Narrabri	100	200	300	400
Soybean oil	-	-	5.8	16.3
Dicalcium phosphate	1.9	0.8	-	-
Limestone	6.0	6.9	8.0	9.5
Salt	1	0.9	0.7	0.4
Vitamins/minerals	7.1	7.1	7.1	7.1
DL methionine	2.71	3.1	2.9	2.3
Lysine	0.9	2.9	3.1	1.9
Calculated analysis				
Total crude protein	252	244	255	280
Total lysine	11.9	11.9	11.9	11.9
Total methionine	5.6	5.8	5.7	5.4
Total sulphur AA	9	9	9	9
Total threonine	8.6	7.9	9.1	8.7
Total isoleucine	8.6	8.6	8	9.1
Total tryptophan	3.4	3.2	3.3	3.6
Calcium	10	10	10	10
Avail. Phosphorous	5	5	5	5
AME (MJ/kg)	12.5	12.5	12.5	12.5

Table 2.3.2 Ingredient composition (g/kg) of cottonseed meal (CSM) finisher diets (total amino acid basis)

Ingredients	CSM 100	CSM 200	CSM 300	CSM 400
Sorghum	500	529	588	501
Poultry offal meal	50	50	50	45
Meat & bone meal	48	44	38	-
Soybean meal	30	-	-	-
Full fat soybean meal	50	33	-	-
Sunflower meal	50	23	-	-
CSM Narrabri	100	200	300	400
Soybean oil	-	-	2	18.8
Limestone	6.5	7.9	9.4	14.7
Dicalcium phosphate	-	-	-	9.6
Salt	1.1	1.1	0.6	0.8
Sodium bicarbonate	0.48	-	-	-
Vitamins/minerals	7.1	7.1	7.1	7.1
DL methionine	2.7	2.7	2.6	2.2
Lysine	2.7	2.7	2.2	1.5
Calculated analysis				
Total crude protein	218	229	245	260
Total lysine	10.5	10.5	10.5	10.5
Total methionine	5.3	5.3	5.2	4.9
Total sulphur AA	8.4	8.4	8.4	8.4
Total threonine	7.1	7.2	7.6	8
Total isoleucine	7.8	7.8	8.1	8.5
Total tryptophan	2.8	3	3.2	3.5
Calcium	9.5	9.5	9.5	9.5
Avail. Phosphorous	4.5	4.5	4.5	4.5
AME (MJ/kg)	12.5	12.5	12.5	12.5

Table 2.3.3 Ingredient composition (g/kg) source and level of cottonseed meal (CSM) in starter diets (digestible amino acid basis)

Ingredients													
Sorghum	653	619	559	489	418	610	523	435	346	577	481	381	275
Poultry offal meal	17	38	50	50	50	50	50	50	50	44	50	50	50
Meat & bone meal	50	50	50	50	50	50	50	50	50	50	50	50	50
Soybean meal	252	167	112	75	38	162	134	108	82	204	187	180	178
CSM Narrabri	-	100	200	300	400	-	-	-	-	-	-	-	-
CSM Brisbane	-	-	-	-	-	100	200	300	400	-	-	-	-
CSM Gunnedah	-	-	-	-	-	-	-	-	-	100	200	300	400
Soybean oil	-	-	4.7	13.6	22.6	3.4	19.0	34.7	50.7	-	7.1	16.1	25.7
Limestone	5.8	6.3	6.8	6.8	6.5	5.8	6.3	6.8	6.6	5.2	5	4.9	4.8
Dicalcium phosphate	5.3	2.8	0.6	-	-	2.8	1.5	0.1	-	4	3.5	3.3	3
Salt	1.0	0.7	0.7	0.4	0.9	0.8	0.9	1	1	1	1	0.8	0.6
Sodium bicarbonate	-	0.7	0.2	-	-	0.8	0.5	0.2	-	0.3	-	-	-
Vitamins/minerals	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1
DL methionine	3.9	3.7	3.4	3.2	2.9	3.4	2.7	2.1	1.5	3.7	3.5	3.3	3.1
Lysine	3.0	3.9	4.1	4.4	4.7	3.6	3.6	3.7	3.7	3	2.9	2.7	2.5
Threonine	1.2	1.3	1.1	0.9	0.8	1.4	1.2	1	0.9	1.3	1.3	1.2	1.1
Calculated analysis													
Total crude protein	221	235	256	277	298	234	253	273	293	227	234	242	251
Digestible lysine	10.5	10.5	10.5	10.5	10.5	10.5	10.5	10.5	10.5	10.5	10.5	10.5	10.5
Digestible methionine	5.7	5.7	5.5	5.4	5.2	5.5	5.2	4.8	4.5	5.7	5.5	5.4	5.2
Digestible sulphur AA	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5
Digestible threonine	7	7	7	7	7	7	7	7	7	7	7	7	7
Digestible isoleucine	7.5	7.1	7	7	7	7	7	7	7	7.2	7.1	7	7
Digestible tryptophan	2.4	2.5	2.6	2.7	2.9	2.4	2.5	2.7	2.8	2.3	2.2	2.1	2.1
Calcium	10	10	10	10	10	10	10	10	10	10	10	10	10
Avail. Phosphorous	5	5	5	5	5	5	5	5	5	5	5	5	5
AME (MJ/kg)	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5

Table 2.3.4 Ingredient composition (g/kg) source and level of cottonseed meal (CSM) in finisher diets (digestible amino acid basis)

Ingredients													
Sorghum	441	462	461	589	485	528	624	537	449	413	337	422	376
Wheat	296	243	199	-	-	158	-	-	-	254	249	60	-
Poultry offal meal	50	50	50	50	50	50	50	50	50	50	50	50	50
Meat & bone meal	50	50	44	9	17	50	48	43	39	50	50	50	50
Sunflower meal	30	30	-	-	-	30	-	-	-	30	-	-	-
Soybean meal	112	25	-	-	-	21	5	-	-	50	42	47	47
Full fat soybean meal	-	19	22	7.8	-	41	50	27	-	30	50	50	-
CSM Narrabri	-	100	200	300	400	-	-	-	-	-	-	-	-
CSM Brisbane	-	-	-	-	-	100	200	300	400	-	-	-	-
CSM Gunnedah	-	-	-	-	-	-	-	-	-	100	200	300	400
Soybean oil	-	-	-	5.8	17.8	-	0.21	19.4	39.3	-	-	-	6.5
Limestone	5.6	6.5	7.9	12.7	12.7	6.1	7	8.1	9.2	5.5	5.4	5.3	5.2
Dicalcium phosphate	1.3	-	-	8.6	4.5	0.4	-	-	-	1.4	1.3	1.2	0.9
Salt	1.4	0.9	1	1	0.5	0.9	0.8	1	1.2	1.2	1.4	1	0.6
Sodium bicarbonate	0.6	0.9	0.3	-	1.3	1.2	0.8	0.4	-	0.8	-	-	-
Vitamins/minerals	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1
DL methionine	2.5	2.6	2.6	2.3	1.3	2.4	2.2	1.7	1.2	2.6	2.5	2.7	2.6
Lysine	2.2	3.6	3.8	3.6	2.3	3.4	3.3	3.4	3.5	3	2.6	2.4	2.1
Threonine	0.8	1.1	1	1	0.3	1.2	1.2	1	0.8	1.3	1.2	1.2	1.2
Calculated analysis													
Total crude protein	202	208	226	235	270	206	221	240	259	197	205	211	219
Digestible lysine	8.3	8.3	8.3	8.3	8.3	8.3	8.3	8.3	8.3	8.3	8.3	8.3	8.3
Digestible methionine	4.4	4.5	4.4	4.7	4.3	4.5	4.4	4.1	3.8	4.5	4.3	4.5	4.4
Digestible sulphur AA	6.3	6.3	6.3	6.3	6.3	6.3	6.3	6.3	6.3	6.3	6.3	6.3	6.3
Digestible threonine	5.8	5.8	5.8	5.8	5.8	5.8	5.8	5.8	5.8	5.8	5.8	5.8	5.8
Digestible isoleucine	6.2	5.6	5.6	5.6	6.2	5.6	5.6	5.6	5.6	5.6	5.6	5.6	5.6
Digestible tryptophan	2.1	2.1	2.3	2.4	2.7	2.1	2.2	2.3	2.5	1.9	1.8	1.8	1.7
Calcium	9.5	9.5	9.5	9.5	9.5	9.5	9.5	9.5	9.5	9.5	9.5	9.5	9.5
Avail. Phosphorous	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5
AME (MJ/kg)	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5

2.3.3 Results and Discussion

Composition of cottonseed meal from different processors

Table 2.3.5 Chemical composition (g/kg DM) of the experimental cottonseed meals (2000-2001)

Analysis	Brisbane	Narrabri	Gunnedah
Dry matter	903	908	950
Gross energy MJ/kg	19.5	19.9	23.1
Crude protein	453	503	221
Phosphorus	13.8	17.1	5.2
Calcium	2.3	2.6	1.6
Sulphur	4.9	5.8	2.3
Fat	18	34	239
Free gossypol	0.06	0.05	1.7
Free condensed tannin	59.0	36.3	72.9
Bound tannins	31.8	19.6	100
Total condensed tannin	90.8	55.9	172.9
Cyclopropanoid fatty acids (μn/g)	54.9	102.2	1342
Neutral detergent fibre (NDF)	228	117	399
Alanine	16.4	18.2	7.5
Arginine	52.4	59.3	21.8
Leucine	22.6	25.5	11.0
Lysine	16.6	18.6	7.8
Methionine	5.8	5.3	2.1
Phenylalanine	21.2	23.9	9.9
Proline	15.5	17.1	6.6
Serine	17.2	19.3	8.8
Aspartic acid	35.5	39.5	17.9
Cystine	7.4	6.7	3.4
Glutamic acid	75.8	84.2	38.5
Glycine	16.2	18.2	7.7
Histidine	10.8	12.5	4.3
Isoleucine	13.6	15.3	6.2
Threonine	13.3	15.0	5.9
Tryptophan	5.5	6.7	2.5
Tyrosine	10.6	12.2	3.9
Valine	16.9	19.0	7.9
Layer AME (MJ/kg DM)	9.3	11.8	ND
Layer AMEn (MJ/Kg DM)	8.1	10.3	ND
Broiler AME (MJ/kg DM)	10.0	11.5	11.9

ND= not determined

The chemical analyses (Table 2.3.5) showed that CP, AA, Ca and P levels where higher in Narrabri followed by Brisbane and Gunnedah respectively. Brisbane and Narrabri CSM had a similar gross energy of about 19 MJ/kg, but Narrabri gave a higher determined AME in both broiler and layers reflecting its lower value in ANF (fibre, gossypol and condensed tannins) and a higher residual lipid content. Gunnedah (expeller extracted) on the other hand, presented the lowest CP, mineral and AA content due to the dilution through higher levels of residual lipid, but Gunnedah also had the highest ANF (NDF, CT, gossypol, and CPFA) and this may be detrimental on AME, digestibility of AA. This may also contribute to mottling in eggs if layers are fed at high levels of this CSM source.

Table 2.3.6 Non-starch polysaccharides content of cottonseed meal (g/kg dry matter)

	CSM Brisbane			CSM Narrabri		
	Soluble	Insoluble	Total	Soluble	Insoluble	Total
Rhamnose	1	3	3	1	2	3
Fucose	0	1	1	0	1	1
Ribose	0	0	0	0	0	0
Arabinose	13	24	37	17	25	44
Xylose	3	76	79	3	28	31
Mannose	1	2	3	2	1	3
Galactose	6	5	11	7	5	10
Glucose	1	86	87	1	35	40
Total	25	197	221	31	97	132

Narrabri gave the lowest values for total NSP (Table 2.3.6) due to a lower insoluble NSP fraction. The NSP composition for both CSM differed slightly for arabinose, xylose and glucose. Fortunately, both CSM sources presented low soluble NSP values and no effect on intestinal viscosity would be expected when using these meals in poultry diets.

The overall AA coefficients (Table 2.3.7) within CSM sources were similar with the exception of lysine, methionine, cysteine and aspartic acid. Brisbane and Narrabri had low lysine values of 0.47 and 0.45 respectively, but Gunnedah, which is an extruded meal, exhibited a better lysine value of 0.56 and this difference may be due to processing conditions in which extrusion uses a lower heat during oil extraction. Surprisingly, Narrabri showed a lower methionine and cystine digestibility coefficient compared with the Brisbane source. This problem can be solved by formulating diets on a digestible AA basis in which synthetic AAs can be added to overcome their low digestibility.

Table 2.3.7 Apparent ileal digestibility coefficients of amino acids in cottonseed meals for broilers

Amino acids	Brisbane	Narrabri	Gunnedah
Alanine	0.64	0.65	0.66
Arginine	0.81	0.82	0.82
Leucine	0.65	0.65	0.67
Lysine	0.47	0.45	0.56
Methionine	0.67	0.57	0.77
Phenylalanine	0.76	0.76	0.74
Proline	0.65	0.68	0.62
Serine	0.67	0.68	0.61
Aspartic acid	0.68	0.69	0.73
Cystine	0.79	0.67	0.62
Glutamic acid	0.81	0.82	0.80
Glycine	0.66	0.68	0.66
Histidine	0.72	0.73	0.70
Isoleucine	0.61	0.61	0.66
Threonine	0.59	0.62	0.57
Tryptophan	0.78	0.73	0.65
Tyrosine	0.68	0.69	0.69
Valine	0.64	0.64	0.64

Broiler Diets

Results (digestible amino acid basis)

The responses to graded levels of CSM compared to the control diet, on growth rate when feeding starter and finisher diets formulated on digestible AAs are presented in Tables 2.3.8 and 2.3.9 respectively. Measurements of liver, pancreas, and fat pad weights are presented in Table 2.3.10.

During the starter period (Table 2.3.8), FI was not depressed at up to 400 and up to 300 g/kg when feeding CSM from Narrabri and Brisbane respectively, but for chicks fed on the Gunnedah source, FI was significantly reduced at 100, 300 and 400 g/kg level. The results also indicated that only up to 200 g/kg supported satisfactory LWG from all CSM sources. Since Narrabri and Brisbane CSM were low in gossypol and in free CT, it is then possible that fibre was the main cause for this poor LWG at above 200 g CSM/kg. It is interesting to observe that chick performance declined linearly as the amount of fibre (NDF) increased from each CSM source. Narrabri, which had a 12%, NDF (see Table 2.3.5), performed better than Brisbane and Gunnedah CSM, which had a NDF of 23% and 40% respectively.

Table 2.3.8 Mean feed intake (FI), liveweight gain (LWG), feed conversion ratio (FCR) for broiler chickens (4-25 d) fed graded levels of from various cottonseed meal sources

CSM treatments	FI (g/bird)	LWG (g/bird)	FCR (g FI / g LWG)
Control	1493 [a]	1004 [a]	1.497 [ac]
Narrabri (100 g/kg)	1438 [a]	962 [a]	1.514 [a]
Narrabri (200 g/kg)	1497 [a]	999 [a]	1.502 [a]
Narrabri (300 g/kg)	1428 [a]	951 [b]	1.503 [a]
Narrabri (400 g/kg)	1458 [a]	927 [b]	1.574 [a]
Brisbane (100 g/kg)	1481 [a]	978 [a]	1.513 [a]
Brisbane (200 g/kg)	1451 [ab]	976 [a]	1.491 [a]
Brisbane (300 g/kg)	1440 [ab]	905 [b]	1.605 [b]
Brisbane (400 g/kg)	1400 [b]	872 [c]	1.606 [b]
Gunnedah (100 g/kg)	1377 [cd]	976 [ab]	1.426 [c]
Gunnedah (200 g/kg)	1466 [ab]	953 [b]	1.538 [a]
Gunnedah (300 g/kg)	1395 [bc]	876 [c]	1.592 [b]
Gunnedah (400 g/kg)	1310 [d]	785 [d]	1.671 [b]
LSD (P=0.05)	77	42	0.084

Means for each CSM within a column with different superscript are significantly different (P<0.05).

During the finisher period (Table 2.3.9) birds fed on Narrabri CSM gave a satisfactory FI, LWG and FCR at all levels. This indicates that during the finisher period, birds are more capable of overcoming any ANF than young chicks fed at 300 and 400 g CSM/kg during the starter period. Brisbane CSM also presented an excellent FI, LWG and improved FCR for all levels. Surprisingly, CSM from Gunnedah also gave a satisfactory FI at up to 200 g CSM/kg with an excellent LWG up to 300 g CSM/kg. As a result of this, FCR was significantly improved at 300 and 400 g/kg level. Since all diets were formulated using the determined digestible AA values, the present experiment did not show the same problems found during Experiment 1 indicating that satisfactory broiler performance can be obtained with CSM at 200 g/kg level during the starter period and up to 300 g/kg during the finisher period.

Table 2.3.9 Mean feed intake (FI), liveweight gain (LWG), feed conversion ratio (FCR) for broiler chickens (25-41 d) fed graded levels of from various cottonseed meal sources

CSM treatments	FI (g/bird)	LWG (g/bird)	FCR (g FI / g LWG)
Control	2861 [a]	1547 [ab]	1.957 [ab]
Narrabri (100 g/kg)	2911 [a]	1582 [a]	1.938 [a]
Narrabri (200 g/kg)	2986 [b]	1594 [a]	1.973 [ab]
Narrabri (300 g/kg)	2860 [a]	1522 [ab]	1.983 [ab]
Narrabri (400 g/kg)	2808 [a]	1468 [b]	2.026 [b]
Brisbane (100 g/kg)	2863 [a]	1602 [a]	1.879 [c]
Brisbane (200 g/kg)	2918 [a]	1608 [a]	1.910 [ac]
Brisbane (300 g/kg)	2884 [a]	1635 [a]	1.873 [c]
Brisbane (400 g/kg)	2841 [a]	1582 [a]	1.899 [ac]
Gunnedah (100 g/kg)	2898 [a]	1556 [a]	1.969 [a]
Gunnedah (200 g/kg)	2796 [a]	1572 [a]	1.872 [d]
Gunnedah (300 g/kg)	2701 [b]	1581 [a]	1.796 [e]
Gunnedah (400 g/kg)	2476 [b]	1428 [c]	1.846 [de]
LSD (P=0.05)	116	89	0.072

Means for each CSM source within a column with different superscripts are significantly different (P<0.05) from the control diet.

Table 2.3.10 The effect of feeding graded levels of CSM from various sources on liver, pancreas, and fat pad weight from 25 to 42 days of age

CSM treatments	Liver (% body wt)	Pancreas (% body wt)	Fat pad (% body wt)
Control	2.26	0.156	1.342 [ab]
Narrabri (200 g/kg)	2.31	0.180	1.245 [a]
Narrabri (400 g/kg)	2.24	0.186	0.866 [b]
Brisbane (200 g/kg)	2.42	0.185	1.454 [a]
Brisbane (400 g/kg)	2.39	0.209	1.187 [a]
Gunnedah (200 g/kg)	2.21	0.182	1.431 [a]
Gunnedah (300 g/kg)	2.28	0.187	1.031 [b]
LSD (P=0.05)	0.210	0.033	0.338

Means for each CSM source within a column with different superscripts are significantly different (P<0.05) from the control diet.

In the present experiment, 2.2% mortality occurred. Only 0.7% of these mortalities were due to leg problems. Upper inclusion of CSM in diets tended to reduce abdominal fat pad, without affecting liver or pancreas weight.

Bird performance comparison on diets formulated on a total versus digestible amino acid basis

A comparison of graded levels of CSM from Narrabri on chicken growth performance, when feeding starter and finisher diets formulated on a total and digestible AA basis, is presented in Tables 2.3.11 and 2.3.12 respectively.

The starter period (Table 2.3.11) results showed that except for total basis at 300 g CSM/kg, FI was not influenced by method of formulation, but a depressed LWG at all CSM levels was observed when formulating on a total basis affecting FCR at 300 and 400 g/kg level. The observed chick LWG depression may be attributed to the amount of fibre and to the amount of total lysine, although present in the meal, that is probably bound as a tannin-protein, or a gossypol-lysine complex and is unavailable for digestion and absorption. When formulating on a digestible AA basis, a satisfactory LWG was obtained up to 200 g CSM/kg but significantly ($P<0.05$) declined at 300 and 400 g CSM/kg without affecting FCR. Since these diets were formulated using digestible AA values, this LWG depression at 300 and 400 g/kg level is more likely to be influenced by the amount of dietary fibre contributed by the CSM. The results during the starter period (0-21 d of age) indicate that, LWG and FCR are likely to be improved by formulation on a digestible AA basis, but above 200 g of CSM/kg, chick LWG performance is most likely to be impaired by dietary fibre.

Table 2.3.11 Feed intake (FI), liveweight gain (LWG), and feed conversion ratio (FCR) for broiler chickens (4-25 d) fed graded levels of Narrabri cottonseed meal formulated on total and digestible amino acid basis

CSM treatment	Formulation basis	FI(g/bird)	LWG (g/bird)	FCR (g FI / g LWG)
Control		1493 [a]	1004 [a]	1.497 [a]
Narrabri 100 g/kg	Total AA	1423 [a]	922 [b]	1.543 [a]
Narrabri 200 g/kg	Total AA	1459 [a]	938 [b]	1.564 [a]
Narrabri 300 g/kg	Total AA	1338 [b]	810 [c]	1.653 [bc]
Narrabri 400 g/kg	Total AA	1428 [a]	851 [c]	1.680 [c]
Narrabri 100 g/kg	Digestible AA	1438 [a]	962 [ab]	1.517 [a]
Narrabri 200 g/kg	Digestible AA	1497 [a]	999 [a]	1.502 [a]
Narrabri 300 g/kg	Digestible AA	1428 [a]	951 [b]	1.503 [a]
Narrabri 400 g/kg	Digestible AA	1458 [a]	927 [b]	1.574 [ab]
LSD ($P<0.05$)		77	42	0.084

Means in a column with different superscript differ significantly ($P<0.05$)

During the finisher period, formulating on a digestible AA basis, FI was significantly ($P<0.05$) improved at 100 and 200 g CSM/kg and was satisfactory at 300 and 400 g/kg without affecting LWG and FCR at all CSM levels. Formulating on a total basis tended to reduce FI which significantly ($P<0.05$) affected LWG at 300 and 400 g/kg level and FCR particularly at 400 g/kg level. Since adult birds (25-42 d), are more able to overcome the negative effects of dietary fibre as demonstrated in previous work, the observed depressed FI and LWG on diets formulated on a total basis are more likely to be explained by the amount of total lysine, although present in the meal, that is probably bound in a tannin-protein or a gossypol-lysine complex, and thus unavailable for digestion and absorption. It is concluded that satisfactory bird performance is possible when feeding high levels of CSM provided diets are formulated on a digestible AA basis.

Table 2.3.12 Feed intake (FI), liveweight gain (LWG), feed conversion ration (FCR) for broiler chickens (25-42 days) fed graded levels of CSM from Narrabri formulated on a total and digestible amino acid basis

CSM treatment	Formulation basis	FI (g/bird)	LWG (g/bird)	FCR (g FI / g LWG)
Control		2861 ^{bc}	1547 ^{abc}	1.957 ^{bcd}
Narrabri 100 g/kg	Total AA	2785 ^{c}	1609 ^{a}	1.825 ^{e}
Narrabri 200 g/kg	Total AA	2774 ^{c}	1511 ^{bc}	1.950 ^{cd}
Narrabri 300 g/kg	Total AA	2656 ^{d}	1398 ^{de}	2.018 ^{bc}
Narrabri 400 g/kg	Total AA	2794 ^{c}	1345 ^{e}	2.207 ^{a}
Narrabri 100 g/kg	Digestible AA	2911 ^{ab}	1582 ^{ab}	1.938 ^{d}
Narrabri 200 g/kg	Digestible AA	2986 ^{a}	1594 ^{ab}	1.973 ^{bcd}
Narrabri 300 g/kg	Digestible AA	2860 ^{bc}	1522 ^{abc}	1.983 ^{bcd}
Narrabri 400 g/kg	Digestible AA	2808 ^{bc}	1468 ^{cd}	2.026 ^{b}
LSD (P<0.05)		116	89	0.072

Means in a column with different superscript differ significantly (P<0.05)

2.4 Experiment 4 - Evaluation of broiler performance in a semi-commercial environment using diets containing upper levels of canola or cottonseed meals

2.4.1 Introduction

Previous experiments indicated that, potentially up to 200 or 300 g/kg of selected cottonseed meals (CSM) and canola meals (CM) could be successfully included in broiler diets during the starter period. Similarly, up to 300 g/kg of selected CM or CSM can be potentially used during the finisher period without affecting broiler production parameters. To obtain this satisfactory performance, bioassays and chemical analyses were undertaken to formulate each broiler diet on a determined digestible amino acid basis and using apparent metabolisable energy values. All broiler experiments were performed in basic environmentally controlled sheds and were adequately replicated in order to obtain information when testing upper levels of these meals in poultry diets. However, these experiments were performed on chickens in cages with raised wire floors and there is a need to evaluate broiler performance using diets containing practical upper levels of canola or cottonseed meals in a semi-commercial environment. This trial was undertaken to provide the poultry industry with practical recommendations for CM and CSM for chicken meat production.

2.4.2 Results and Discussion

Starter and finisher diets

The ingredient and chemical composition of the starter and finisher diets, formulated on a digestible AA basis, using Riverina CSM, Numurkah CM and a control diet are presented in Tables 2.4.1 and 2.4.2 respectively. The ingredients chemical composition and the determined amino acids (AA) and apparent ileal digestibility coefficients for each meal are presented in Tables 2.4.3 and 2.4.4 respectively.

Experimental design

There were three treatments x 15 replicate pens x 40 birds (20 males and 20 females) in a completely randomised block layout of the 45 pens. The 3 treatments were randomly assigned to pens within each block. Dietary treatments during the starter period were a control commercial diet, a CM diet (200 g/kg inclusion) and a CSM diet (200 g/kg inclusion). During the finisher period the inclusion level of each meal was increased to 300 g/kg.

Statistical analysis

Analysis of variance (ANOVA) was used to test the effects of treatments using an ANOVA model for a completely randomised block design. A pen of 40 birds was the experimental unit. The treatment means were compared using an ANOVA. The main effects were tested using a protected LSD ($P<0.05$).

Table 2.4.1 Ingredient composition (g/kg) of control, cottonseed meal and canola meal starter diets

Ingredients	Control	Cottonseed meal	Canola meal
Sorghum	483	442	433
Wheat	193	100	100
Meat & bone meal	70	70	70
Poultry offal meal	21	40	40
Soybean meal	204	114	112
CSM Riverina	-	200	-
CM Numurkah	-	-	200
Soybean oil	5	13.4	27.6
Dicalcium phosphate	2.5	0.46	-
Limestone	6	6	3.8
Salt	-	0.3	0.3
Sodium bicarbonate	2.3	0.63	2.2
Vitamins/minerals	5	5	5
Choline Chloride	0.7	0.7	0.7
Coxistac 12	0.5	0.5	0.5
DL methionine	2.7	2.2	1.9
Lysine	3.4	3.8	2.8
Threonine		1.1	0.9
Calculated analysis			
Total crude protein	230	267	251
Digestible lysine	10.5	10.5	10.5
Digestible methionine	4.9	4.6	4.4
Digestible sulphur AA	7.6	7.6	7.6
Digestible threonine	7	7	7
Digestible isoleucine	7	7	7
Digestible tryptophan	2.2	2.3	2.2
Calcium	10.5	10.5	10.5
Avail. Phosphorous	5.3	5.5	5.2
AME (MJ/kg)	12.5	12.5	12.5

Table 2.4.2 Ingredient composition (g/kg) of control, cottonseed meal and canola meal finisher diets

Ingredients	Control	Cottonseed meal	Canola meal
Sorghum	522	471	450
Wheat	259	100	100
Meat & bone meal	60	48	54
Poultry offal meal	3	50	50
Soybean meal	130	-	-
CSM Riverina	-	300	-
CM Numurkah	-	-	300
Soybean oil	-	9.2	30.7
Dicalcium phosphate	2.5	-	-
Limestone	7.5	8.9	3.5
Salt	-	0.5	1
Sodium bicarbonate	2.8	0.2	1.7
Vitamins/minerals	5	5	5
Choline Chloride	0.6	0.6	0.6
Coxistac 12	0.5	0.5	0.5
DL methionine	2.3	1.4	0.8
Lysine	3.4	3.6	1.2
Threonine	1.5	0.8	0.5
Calculated analysis			
Total crude protein	190	251	231
Digestible lysine	8.3	8.3	8.3
Digestible methionine	4	3.5	3.2
Digestible sulphur AA	6.3	6.3	6.3
Digestible threonine	5.8	5.8	5.8
Digestible isoleucine	5.6	5.8	5.9
Digestible tryptophan	1.8	2.1	2
Calcium	9.6	9.5	9.5
Avail. Phosphorous	4.5	4.5	4.5
AME (MJ/kg)	12.5	12.5	12.5

Composition of cottonseed meal from different processors

The chemical analysis (Table 2.4.3) showed that CSM from Riverina contained less CP, fat and a different AA profile when compared with CSM from Narrabri, evaluated during Experiment 3. CM from Numurkah also differed chemically from a similar CM source evaluated during the previous year. Chemical composition of the meals indicated that the variation depended on seasonal, environmental and plant processing conditions. Hence, it is best to determine the composition of ingredients before formulating poultry diets.

The apparent ileal digestibility values (Table 2.4.4) for CSM from Riverina and CM from Numurkah were obtained using the methods described in previous experiments. The overall AA coefficients of Riverina CSM are higher when compared with previously evaluated cottonseed meals, but the digestibility of lysine and threonine still gave relatively low values of 0.56 and 0.65 respectively, when compared with CMs. Hence, synthetic AA should be added to diets when using upper levels of CSM. CM on the other hand, gave similar digestible AA coefficients to previously evaluated Numurkah CM. Contrary to CSM digestible AA values, CM had satisfactory digestible coefficient values for most AA.

Table 2.4.3 Chemical composition (g/kg DM) of the experimental cottonseed meal and canola meal

Analysis	CSM Riverina	CM Numurkah
Dry matter	902	903
Gross energy MJ/kg	19.9	19.9
Crude protein	476	399
Phosphorus	13.5	10.2
Calcium	2.3	7.4
Sulphur	4.3	6
Fat	29	29
Free gossypol	0.07	37.9
Free condensed tannin	43.5	57.9
Bound tannins	24.6	34.2
Total condensed tannin	68.1	92.1
Glucosinolates (μml/g)	ND	4.4
Sinapine	ND	15.2
Cyclopropanoid fatty acids (μn/g)	102.2	ND
Neutral Determined Fibre	172	288
Alanine	16.5	14.9
Arginine	53.5	23.3
Leucine	24.9	24.8
Lysine	17.5	19
Methionine	4.3	5
Phenylalanine	23.4	14.5
Proline	22.4	27
Serine	19.4	15.7
Aspartic acid	40.8	25.3
Cystine	6.7	9.1
Glutamic acid	93	67.9
Glycine	17.8	17.5
Histidine	10.8	8.4
Isoleucine	14.1	13.8
Threonine	14	15
Tryptophan	5.7	5
Tyrosine	11.2	8.9
Valine	18.2	17.1
AME (MJ/kg DM) in broilers	11.7	9.5

ND= not determined

Table 2.4.4 Apparent ileal digestibility coefficients of amino acids in cottonseed meal (CSM) and canola meal (CM) for broilers

Amino acids	CSM *Riverina*	CM *Numurkah*
Alanine	0.71	0.73
Arginine	0.87	0.81
Leucine	0.72	0.76
Lysine	0.56	0.73
Methionine	0.74	0.86
Phenylalanine	0.81	0.76
Proline	0.73	0.71
Serine	0.72	0.67
Aspartic acid	0.74	0.63
Cystine	0.76	0.74
Glutamic acid	0.84	0.81
Glycine	0.71	0.74
Histidine	0.78	0.80
Isoleucine	0.71	0.72
Threonine	0.65	0.66
Tryptophan	0.75	0.74
Tyrosine	0.76	0.74
Valine	0.72	0.69

Broiler performance

The responses of 200 g/kg level of CSM or CM compared with the control diet, on growth, FI and FCR on starter diets formulated on a digestible AA basis are presented in Table 2.4.5. The responses of 300 g/kg level of CSM or CM compared with the control diet, on production parameters on finisher diets, formulated on a digestible AA basis, are presented in Table 2.4.6.

Table 2.4.5 Feed intake (FI), liveweight gain (LWG), feed conversion ratio (FCR) means for broiler chickens (1-21 d) fed 200 g/kg level of cottonseed meal (CSM) or canola meal (CM).

Dietary treatments	FI (g/bird)	LWG (g/bird)	FCR (G FI / g LWG)
Control	1150	825	1.407
CSM Riverina (200 g/kg)	1134	813	1.408
CM Numurkah (200 g/kg)	1130	829	1.372
LSD (P=0.05)	28	19	0.038
Coefficient of variation %	3	6	4

Means for each CSM within a column with different superscript are significantly different (P<0.05).

The results in the starter period (Table 2.4.5) indicated that FI, LWG and FCR were not influenced by the level of CSM or CM in the diet. During the finisher period (Table 2.4.6) FI of chicks fed on CM was lower (P<0.05) but this did not affect LWG or FCR, which was not different (P>0.05) from the control diet. This semi-commercial broiler experiment indicated that bird production was not affected when fed diets with upper levels of either CSM or CM and confirming our earlier results. In this semi-commercial trial each dietary treatment was replicated in 15 pens using 40 birds each (20 males and 20 females) and diets were formulated on a digestible AA basis. Hence it is concluded that up to 200 g/kg of either CSM (solvent extracted) or CM (solvent extracted or extruded) can be used during the starter phase, and up to 300 g/kg of either CSM (solvent extracted) or CM (solvent extracted or extruded) can

be used during the finisher phase in diets formulated on a digestible AA basis. There were no detrimental effects on chickens during the course of this semi-commercial trial. Mortality and culled birds were not related to leg problems, even though it is well known that CM may influence bird mortality. Litter and environment in the shed were not quantitatively evaluated; the Research Staff at the Centre did not observe any negative effect when using these levels of these meals.

Table 2.4.6 Mean feed intake (FI), liveweight gain (LWG) and feed conversion ratio (FCR) for broiler chickens (21-43 d) fed 300 g/kg level of cottonseed meal (CSM) or canola meal (CM).

Dietary treatments	FI (g/bird)	LWG (g/bird)	FCR (g FI / g LWG)
Control	3383 [a]	1570	2.169 [ab]
CSM Riverina (300 g/kg)	3451 [a]	1579	2.134 [a]
CM Numurkah (300 g/kg)	3263 [b]	1538	2.206 [b]
LSD (P=0.05)	119	68	0.041
Coefficient of variation	5	6	2

Means for each CSM within a column with different superscript are significantly different (P<0.05).

2.5 Broiler General Discussion

Cottonseed meals (CSM) and canola meals (CM) from various processors in Australia were sampled during three years at the end of each processing cycle. Seasonal, environmental and plant processing conditions accounted for most of the variation found in chemical composition and amino acid (AA) profile of CSM and CM from different processors. It is highly recommended that each feed manufacturer determine the chemical composition of these ingredients before formulating poultry diets.

Cottonseed meal

The overall AA digestibility values between CSM sources had only small variation and were consistent during the three year period. Therefore, AA coefficient tables provided in this report could be used for formulating diets on a digestible AA basis. What is important in CSM is its low lysine (range 0.45-0.56) and threonine (0.57-0.68) digestibility values which can be overcome by adding synthetic lysine or threonine to diets when using upper levels of solvent extracted CSM.

When compared with extruded meals, solvent extracted CSM presented an overall low gossypol, condensed tannins and neutral detergent fibre, which are mostly removed during processing. This good quality meal determined the higher AME obtained in both broiler and layers fed high levels of CSM particularly from Narrabri source. The overall levels of non-starch polysaccharides (NSP) was low with arabinose and xylose the highest NSP found in all evaluated meals and this may have some implication when selecting enzymes for feed improvement particularly during the starter period.

The overall results indicated that during the starter period, only up to 200 g CSM/kg supported satisfactory feed intake (FI) and liveweight gain (LWG). However, during the finisher period up to 300 g CSM gave a satisfactory FI, LWG and FCR indicating that older birds were more capable of overcoming any ANF than young chicks. The recommended inclusions of CSM in diets also tended to reduce abdominal fat pad, without affecting liver and pancreas weight. No signs of anaemia were detected in blood samples from birds fed on upper levels of CSM. Addition of iron salts is highly recommended when formulating diets with upper levels of CSM to overcome any residual gossypol effect. Satisfactory bird performance is possible when feeding high levels of CSM provided diets are formulated on a digestible AA basis.

Canola meal

The chemical composition values within various CM sources varied only slightly between year evaluations. However between CM sources, variation in chemical composition was common, particularly between solvent extracted and extruded meals. Therefore, determination of chemical composition is advised before formulating poultry diets. The overall AA digestibility between sources was satisfactory, but variations between solvent and extruded meals were also observed particularly on lysine digestibility where the extruded meals exhibited higher digestibility values due to a lower heat input during processing. The glucosinolates levels found among Australian CM were 1/3 of those reported for Canadian "double zero" varieties and this was consistent. Sinapine content between sources varied in the range of 11-15 g/kg with extruded meals having the highest value suggesting differences in processing conditions.

The overall levels of non-starch polysaccharides (NSP) were low in each CM. Arabinose and xylose were the highest NSP found in all evaluated meals.

Results indicated that during the starter period chicks had a satisfactory performance with inclusion rates up to 200 g CM from Newcastle and Melbourne sources and up to 300 g CM from Numurkah and Pinjarra sources. During the finisher period, satisfactory performance was obtained up to 300 g CM/kg. The recommended inclusion of CM in diets also reduced bird abdominal fat portion and intestinal viscosity, without affecting liver and pancreas weight.

During the starter and finisher period the overall bird performance was improved on diets containing upper levels of CM by formulating on a digestible AA basis.

3. Layer Trials

3.1 Experiment 1. Evaluation of low glucosinolates canola meal and low gossypol cottonseed meal in layer diets. 1998-1999 harvest

3.1.1 Introduction

Canola meal (CM) is often limited to relatively low dietary inclusion levels in layer diets (4-10 %) due to the presence of antinutritive factors (ANF). These ANF contribute to palatability problems, and undesirable anti-nutritional effects such as depression of growth, egg weight and production, thyroid hypertrophy, skeletal abnormalities, liver damage and enlargement, and a 'fishy' or 'crabby' taint in the eggs of brown layers. Fortunately, in the last 10 years Australia has selected canola lines with low ANF in the meal and diets prepared from these new varieties are generally less harmful and are widely used for laying hens with levels as high as 10% causing no apparent adverse effects on performance.

Cottonseed meal (CSM) on the other hand, is also limited in layer diets due to the presence of gossypol, and the cyclopropenoid fatty acids (CPFA), malvalic and sterculic, which produce various unpleasant colour changes in hen's eggs (Phelps, 1966). Gossypol causes chocolate-brown discolouration of yolks and the CPFA are responsible for apricot yolk and pink albumen discolouration and enhancement of the gossypol-produced brown yolk appearance. These compounds are also responsible for negatively affecting production parameters when fed to layer hens. Mottling in yolk eggs is another negative effect on stored eggs which may be caused by feeding high levels of CSM to hens (Panigrahi and Hammonds, 1990). The CPFA-related effects may be avoided by feeding CSM of low residual lipid content, and the gossypol-related effects by treating CSM diets with iron salts.

The development of new strains of laying birds in the poultry industry, and the improved new canola and cotton varieties in combination with better procedures for industry oil extraction, has provided new ground for poultry research.

Section 3.1 provides the results of three layer experiments that evaluated the production performance of the new canola and cotton varieties with low ANF. The influence of oil extraction methods on the nutritional value of CM and CSM were also evaluated.

3.1.2 Materials and methods

Layer Diets

Six hundred kg of commercial CM was obtained from three representative processors located in Newcastle (NSW), Melbourne (Vic), and Pinjarra (WA). Solvent extraction was used to obtain all CM except for the Pinjarra processor who used expeller extraction. Solvent-extracted CSM was obtained from a single supplier located in Narrabri (NSW). These materials were derived from the 1998-1999 harvests.

The ingredient and chemical composition of the diets used in the three layer experiments are given in Tables 3.1.1, 3.1.2 and 3.1.3 respectively. In Layer Experiment 1a, dietary treatments were offered to Inghams Hisex Brown layers. In Layer Experiments 1b and 1c dietary treatments were offered to Isa Brown and Inghams White SuperTint layers.

Chemical analysis and bioassays

Experimental canola and cottonseed meals, chemical analyses and bioassays are described in the general materials and methods section.

Table 3.1.1 Ingredient composition (g/kg) of layer diets with 100, 150 and 200 g/kg of canola meal from Melbourne (M) and Pinjarra (P) sources (Layer Experiment 1a)

Ingredients	Control	M 100	M 150	M 200	P 100	P 150	P 200
Sorghum	481	508	608	582	597	517	540
Wheat	177	143			19	96	44
Meat& bone meal	50	50	50	50	50	50	50
Soybean meal	97	52	48	18	45	34	27
Full fat Soybean meal	41	41	40	36	36	39	39
Sunflower meal	70	22	20	20	70	33	20
CM Melbourne	-	100	150	200	-	-	-
CM Pinjarra	-	-	-	-	100	150	200
Tallow	-	-	1.15	11.5	--	-	-
Dicalcium phosphate	1.16	0.91	0.57	0.24	0.52	3.8	0.07
Limestone	76.4	75.7	72.2	74.8	75.5	75	74.5
Salt	1.19	1.3	1.31	1.32	0.98	1.3	1.42
Sodium bicarbonate	1.06	0.88	0.68	0.72	0.99	0.54	0.18
Vitamins & minerals	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Methionine	1.65	1.98	1.95	2.04	1.6	1.53	1.38
Lysine	0.97	1	0.5	0.28	1.3	0.96	0.54
Tryptosine				0.6			
Calculated analysis							
Total crude protein	170	172	184	185	170	170	175
Digestible lysine	6.9	6.7	6.6	6.5	6.8	6.6	6.6
Digestible methionine	4.0	4.1	4.1	4.1	4.0	3.9	3.8
Digestible sulphur AA	6.1	6.1	6.1	6.1	6.1	6.1	6.1
Digestible threonine	4.9	4.7	5.0	5.0	4.8	4.8	4.9
Digestible isoleucine	6.3	5.6	5.7	5.4	5.8	5.5	5.5
Digestible tryptophan	1.7	1.5	1.5	1.5	1.5	1.5	1.5
Calcium	34	34	34	34	34	34	34
Avail. Phosphorous	4	4	4	4	4	4	4
AME (MJ/kg)	11.5	11.5	11.5	11.5	11.5	11.5	11.5

Vitamin and mineral premixes added per kg of diet: 2.5 mg retinol, 75 ug D_3, 5 mg a-tocopherol acetate, 2 mg menadione sodium bisulfite, 1 mg thiamine, 4 mg riboflavin, 2 mg pyridoxine, 10 ug B_{12}, 1 mg folic acid, 10 mg niacin, 10 mg Ca pantothenate, 30 ug biotin, 225 mg choline, 50 mg Mn, 50 mg Zn, 50 mg Fe, 600 ug Mo, 500 ug Co, 600 ug 1, 4 mg Cu, 70 ug Se, 80 mg Banox.

Table 3.1.2 Ingredient composition (g/kg) of layer diets with 100, 150 and 200 g/kg of canola meal from Newcastle (Layer Experiment 1b)

Ingredients	Control	CM 100	CM 150	CM 200
Sorghum	481	499	516	598
Wheat	177	149	107	
Meat& bone meal	50	50	50	48
Soybean meal	97	43	36	16
Full fat Soybean meal	41	39	40	38
Sunflower meal	70	38	20	20
CM Newcastle		100	150	200
Dicalcium phosphate	1.16	0.8	0.5	0.8
Limestone	76.4	75.3	74.7	74.3
Salt	1.19	1.2	1.3	1.2
Sodium bicarbonate	1.06	0.6	0.2	0.01
Vitamins & minerals	2.5	2.5	2.5	2.5
Methionine	1.65	152	1.29	1.17
Lysine	0.97	1.3	0.9	0.69
Tryptosine				0.48
Calculated analysis				
Total crude protein	170	171	180	185
Digestible lysine	6.9	6.6	6.5	6.4
Digestible methionine	4.0	3.9	3.7	3.6
Digestible sulphur AA	6.1	6.1	6.1	6.1
Digestible threonine	4.9	4.6	4.8	4.8
Digestible isoleucine	6.3	5.6	5.5	5.4
Digestible tryptophan	1.7	1.5	1.5	1.5
Calcium	34	34	34	34
Avail. Phosphorous	4	4	4	4
AME (MJ/kg)	11.5	11.5	11.5	11.5

Vitamin and mineral premixes added per kg of diet: 2.5 mg retinol, 75 ug D_3, 5 mg a-tocopherol acetate, 2 mg menadione sodium bisulfite, 1 mg thiamine, 4 mg riboflavin, 2 mg pyridoxine, 10 ug B_{12}, 1 mg folic acid, 10 mg niacin, 10 mg Ca pantothenate, 30 ug biotin, 225 mg choline, 50 mg Mn, 50 mg Zn, 50 mg Fe, 600 ug Mo, 500 ug Co, 600 ug 1, 4 mg Cu, 70 ug Se, 80 mg Banox.

Table 3.1.3 Ingredient composition (g/kg) of diets with 100, 150 and 200 g/kg of cottonseed meal from Narrabri (Layer Experiment 1c)

Ingredients	Control	CSM 100	CSM 150	CSM 200
Sorghum	481	610	620	573
Wheat	177	31		
Meat& bone meal	50	50	27	2
Soybean meal	97	38	34	10
Full fat Soybean meal	41	40	47	65
Sunflower meal	70	45	20	20
CM Newcastle		100	150	200
Tallow			7.5	25
Dicalcium phosphate	1.16	0.39	6.8	13.5
Limestone	76.4	77.2	80.1	83.2
Salt	1.19	0.80	1.1	1.4
Sodium bicarbonate	1.06	0.7	0.5	0.3
Vitamins & minerals	2.5	2.5	2.5	2.5
Methionine	1.65	1.8	1.8	1.8
Lysine	0.97	2	2.1	2.4
Calculated analysis				
Total crude protein	170	180	183	185
Digestible lysine	6.9	6.7	6.7	6.0
Digestible methionine	4.0	4.0	4.0	3.9
Digestible sulphur AA	6.1	6.1	6.1	6.1
Digestible threonine	4.9	4.7	4.6	4.6
Digestible isoleucine	6.3	5.6	5.3	5.0
Digestible tryptophan	1.7	1.5	1.5	1.5
Calcium	34	34	34	34
Avail. Phosphorous	4	4	4	4
AME (MJ/kg)	11.5	11.5	11.5	11.5

Vitamin and mineral premixes added per kg of diet: 2.5 mg retinol, 75 ug D_3, 5 mg a-tocopherol acetate, 2 mg menadione sodium bisulfite, 1 mg thiamine, 4 mg riboflavin, 2 mg pyridoxine, 10 ug B_{12}, 1 mg folic acid, 10 mg niacin, 10 mg Ca pantothenate, 30 ug biotin, 225 mg choline, 50 mg Mn, 50 mg Zn, 50 mg Fe, 600 ug Mo, 500 ug Co, 600 ug 1, 4 mg Cu, 70 ug Se, 80 mg Banox.

Yolk colour rating and egg odour

On week eight of each layer experimental period, eggs from each bird in each experiment were collected (175, 168, and 168 eggs for Layer Experiments 1a, 1b and 1c respectively) and evaluated as fresh for yolk colour and egg odour. On week nine similar egg collections were performed on each experiment with half of these eggs stored at 10 °C for two weeks while the other half remained cool-stored (10 °C) for five weeks, after which yolk colour and odour were evaluated. Three experienced QPRDC technical personnel carried out the yolk colour and egg odour evaluations. A standard colorimetric system reference (yolk colour fan, Roche 1993) with a scale range 1 to 15 was used for yolk colour evaluation while fresh or stored raw whole egg placed on a white plate was used for odour evaluation. A value coded 0 was recorded if the observer reported no smell or no presence of a different odour to a normal egg smell. A value coded 1 was recorded if an observer reported an unusual odour. The results of this evaluation were sent to the DPI-Animal Research Institute for statistical analyses.

Egg sensory evaluation (Taste evaluation)

On week fourteen, 15 eggs from control, 100 and 200 g/kg level from each layer experiment were collected and sent for a egg sensory evaluation test at the School of Land and Food Sciences, University of Queensland using an untrained group of students from the University. Six (6) eggs from each treatment were broken and albumen and yolk were thoroughly mixed together in a large bowl. The mix was divided into three equal portions then each was placed in a microwave oven and cooked with occasional stirring for 2 minutes until the egg mix had set. The original concept of using hard-boiled eggs had to be abandoned because of the colour variability between individual eggs. Mixing the eggs together as described above overcame this problem as well as providing each taster with a more random sample from the eggs supplied.

The triangle test method was used to determine if a difference existed between the control eggs and the various eggs produced in the various treatments. This test requires panellists to determine which egg sample differ from the other two without identifying the differentiation traits. Approximately 25 untrained panellists were presented with 3 samples, which were coded with 3-digit random numbers. The actual number of panellists varied according to the number of tasters available at any one session. In all, 14 individual tasting sessions were conducted. Twelve of these were the actual samples and two additional sessions were conducted as preliminary trials when the cooking method was being tested.

Experimental Design and Statistical Analysis

There were three layer experiments. In Experiment 1a, there were 7 dietary treatments comprising a control diet plus all combinations of 3 levels (100,150 and 200g/kg) x 2 sources of CM (Melbourne and Pinjarra). Each of the 7 experimental diets was fed to 25 replicates/blocks in a randomised block design.

In Experiment 1b, there were 8 treatments comprising a control diet plus all combinations of 3 levels of CSM (100, 150, and 200 g/kg) fed to 2 strains of bird (Isabrown and Inghams White Supertint) with 24 replicates for each strain in a randomised block design. Data were statistically analysed as 4 levels (cont, 100, 150, 200 g CSM/kg) x 2 strains (brown and white) x 24 blocks/reps.

In Experiment 1c, there were 8 treatments comprising a control diet plus all combinations of 3 levels of CM (100, 150, and 200 g/kg) fed to 2 strains of bird (Isabrown and Inghams White Supertint) with 24 replicates (birds) for each strain in a randomised block design. Data were statistically analysed as 4 levels (cont, 100, 150, 200 g CM/kg) x 2 strains (brown and white) x 24 blocks/reps. Experiments 1b and 1c were conducted together in the one layout, but were statistically analysed separately.

Each experiment was carried out from 26 to 38 weeks of age (14 weeks) and data were analysed by analysis of variance and treatment means compared using the protected LSD at $P = 0.05$.

The treatment means results of odour and yolk colour in the first layer experiments were statistically analysed as percent of relative frequency of odour. This was first calculated as a percent out of 75 odour tests as there were 3 observers x 25 eggs. A frequency percent was then also calculated when either 2/3 or 3/3 operators agreed that the egg had an odour, so the treatment means then become: % eggs (out of 25) for which 2 or 3 operators (out of 3) agree that the egg has an odour. This second method was the better of the two and was chosen as the preferred method for this experiment and Experiments 1b and 1c.

3.1.3 Results and Discussion

Chemical Analysis

Results of the chemical analyses, NSP and digestible AA coefficient results performed on each CM and CSM samples are presented in the broiler section (Chapter 2) in Tables 2.1.5, 2.1.6, 2.1.7, and 2.18 respectively.

Layer Trials
In Experiment 1a, the treatment effect mean values for the production parameters of layer hens (Hisex Brown) fed on Melbourne and Pinjarra CM are presented in Table 3.1.4.

In Experiment 1b, the interaction of two strains of layer hens (Isabrown and Inghams White Supertint) x inclusion level (100, 150 and 200 g/kg) of Newcastle CM is presented in Table 3.1.5 and the bird strain effect is presented in Table 3.1.6.

In Experiment 1c, the interaction of two strains of layer hens (Isabrown and Inghams White Supertint) x inclusion level (100, 150 and 200 g/kg) of CSM from Narrabri is presented in Table 3.1.7 and the bird strain effect is presented in Table 3.1.8.

Yolk colour rating and egg odour

The results for the egg odour and yolk colour evaluations in Experiments 1a, 1b and 1c are presented in Tables 3.1.9, 3.1.10 and 3.1.11 respectively. The results of the individual tasting trials derived from Experiments 1a, 1b, and 1c are presented in Table 3.1.12.

Table 3.1.4 Hisex Brown hens fed on diets with 100, 150, and 200 g/kg of canola meal from Melbourne and Pinjarra (Experiment 1a)

Canola Treatments	Production %	Egg weight (g)	Egg mass (g/d)	FI (g/d)	FCR (g FI/g egg mass)	Hen weight (kg/bird)	Specific gravity	Liver (% bodywt)	Pancreas (% bodywt)
Control	91.8	65.2	59.9	127.9	2.141	2.20	1.081	2.6	0.215
Melbourne 100 g/kg	88.3	64.3	56.9	124.9	2.209	2.21	1.083	2.5	0.201
Melbourne 150 g/kg	91.8	63.2	58.0	127.6	2.206	2.22	1.083		
Melbourne 200 g/kg	93.4	63.8	59.6	131.4	2.214	2.22	1.081	2.4	0.194
Pinjarra 100 g/kg	91.8	62.9	57.8	124.6	2.162	2.18	1.083	2.5	0.205
Pinjarra 150 g/kg	90.9	63.8	58.1	124.2	2.139	2.21	1.084		
Pinjarra 200 g/kg	92.7	63.9	59.1	126.8	2.158	2.22	1.083	2.5	0.193
LSD (P=0.05)	3.82	2.04	4.0	7.34	0.11	0.134	0.0034	0.53	0.036

Table 3.1.5 Isabrown and Inghams White Supertint hens fed on diets with 100, 150 and 200 g/kg of canola meal from Newcastle (Experiment 1b)

Canola treatments	Bird Strain	Production %	Egg wt (g)	Egg mass (g/d)	FI (g/d)	FCR (g FI/g egg mass)	Hen weight (kg/bird)	Specific gravity	Liver (% bodywt)	Pancreas (% bodywt)
Control	Brown	91.5	63.1	57.8	128.3	2.22	2.05	1.082	2.3	0.202
	White	91.3	64.3	58.7	132	2.26	2.13	1.085	2.9	0.178
Newcastle 100 g/kg	Brown	94.2	61.8	58.1	126.7	2.18	2.02	1.084	2.5	0.190
Newcastle 100 g/kg	White	91.3	62.8	57.3	131.6	2.31	2.14	1.085	2.6	0.174
Newcastle 150 g/kg	Brown	91.0	61.6	56.0	124.6	2.24	2.01	1.084	-	-
Newcastle 150 g/kg	White	89.8	63.1	56.7	129.7	2.30	2.00	1.085	-	-
Newcastle 200 g/kg	Brown	94.5	63.0	59.5	130.2	2.20	2.06	1.081	1.9	0.188
Newcastle 200 g/kg	White	90.0	64.1	57.7	131.8	2.30	2.04	1.082	2.3	0.184
LSD (P=0.05)		3.97	2.24	3.12	6.67	0.12	0.12	0.003	0.518	0.0358

Table 3.1.6 The effect of bird strain on production parameters when fed Newcastle canola meal during 14 weeks period (Experiment 1b)

Bird Strain	Production %	Egg wt (g)	Egg mass (g/d)	FI (g/d)	FCR (g FI/g egg mass)	Hen wt (kg/bird)	Specific gravity	Liver (% bodywt)	Pancreas (% bodywt)
Isabrown	92.8 [a]	62.4 [a]	57.9	127.4 [a]	2.21 [a]	2.04	1.083	2.22 [a]	0.193
White Supertint	90.6 [b]	63.6 [b]	57.6	131.3 [b]	2.29 [b]	2.08	1.084	2.60 [b]	0.179
LSD P=0.05	1.99	1.12	1.56	3.3	0.06	0.06	0.002	0.2993	0.0358

Values within a column with different superscripts are significantly different (P<0.05)

Table 3.1.7 Isabrown and White Supertint hens fed on 100, 150 and 200 g/kg of cottonseed meal from Narrabri (Experiment 1c)

Cottonseed meal treatments	Bird Strain	Production %	Egg wt (g)	Egg mass (g/d)	FI (g/d)	FCR (g FI/g egg mass)	Hen wt. (kg/bird)	Specific gravity	Liver (% bodywt)	Pancreas (% bodywt)
Control	Brown	91.5	63.1	57.8	128.3	2.22	2.05	1.082	2.31	0.202
	White	91.3	64.3	58.7	132.0	2.26	2.13	1.085	2.88	0.178
CSM 100 g/kg	Brown	93.6	63.5	59.4	126.9	2.15	2.06	1.082	2.29	0.174
CSM 100 g/kg	White	90.6	64.0	57.9	128.5	2.23	2.10	1.085	2.15	0.178
CSM 150 g/kg	Brown	93.4	62.9	58.7	126.0	2.16	2.09	1.081	-	-
CSM 150 g/kg	White	91.5	64.6	59.1	129.3	2.19	2.15	1.084	-	-
CSM 200 g/kg	Brown	92.7	61.6	57.2	122.3	2.15	2.11	1.083	2.34	0.190
CSM 200 g/kg	White	90.1	63.3	56.9	127.0	2.24	2.15	1.084	2.28	0.196
LSD (P=0.05)		3.67	2.10	3.00	6.27	0.11	0.12	0.003	0.516	0.0288

Table 3.1.8 The effect of bird strain on production parameters when fed on diets containing cottonseed meal during 14 weeks period (Experiment 1c)

Bird Strain	Production %	Egg wt (g)	Egg mass (g/d)	FI (g/d)	FCR (g FI/g egg mass)	Hen weight (kg/bird)	Specific gravity	Liver (% bodywt)	Pancreas (% bodywt)
Isabrown	92.8 [a]	62.8 [a]	58.3	125.9 [a]	2.17 [a]	2.08	1.082 [a]	2.32	0.189
White Supertint	90.9 [b]	64.0 [b]	58.2	129.2 [b]	2.23 [b]	2.13	1.084 [b]	2.44	0.184
LSD (P=0.05)	1.84	1.05	1.50	3.14	0.06	0.06	0.0015	0.298	0.0166

Values within columns with different superscripts are significantly different (P<0.05)

Table 3.1.9 Results of odour (%) and yolk colour evaluation of eggs obtained from Hisex Brown layers fed on graded levels (100, 150, and 200 g/kg) of canola meal from Melbourne and Pinjarra (Experiment 1a)

Canola meal treatments	Fishy odour (fresh eggs)	Yolk colour (fresh eggs)	Odour (after 2 wks stored)	Yolk colour (after 2 wks stored)	Odour (after 5 wks stored)	Yolk colour (after 5 wks stored)
Control	0.0	12.2	0.0	12.1	0.0	12.1
Melbourne 100 g/kg	12	12.2	7.7	12.1	0.0	12.2
Melbourne 150 g/kg	12.0	12.1	1.8	12.1	9.7	12.0
Melbourne 200 g/kg	20.0	12.5	23.1	12.1	8.3	12.4
Pinjarra 100 g/kg	4.0	12.3	0.0	12.2	0.0	12.1
Pinjarra 150 g/kg	20.0	12.2	15.4	11.9	0.0	11.9
Pinjarra 200 g/kg	20.0	12.0	23.1	11.9	0.0	12.1
LSD (P=0.05)	18.4	0.25		0.25		0.38
SEM			8.1		4.7	

Note: eggs stored at 2 and 4 weeks had only 13 reps, therefore only SEM is given

Table 3.1.10.a Results of odour (%) and yolk colour evaluation of eggs obtained from Isabrown layers fed on 100, 150, and 200 g/kg of Newcastle canola meal (Experiment 1b)

Canola meal treatments	Bird Strain	Fishy odour (fresh eggs)	Yolk colour (fresh eggs)	Odour (after 2 wks stored)	Yolk colour (after 2 wks stored)	Odour (after 5 wks stored)	Yolk colour (after 5 wks stored)
Control	Brown	0 [b]	12.2	0	11.9	0 [b]	12.1
Newcastle 100 g/kg	Brown	0 [b]	12.2	8	12.2	8 [ab]	12.2
Newcastle 150 g/kg	Brown	17 [ab]	12.4	8	12.3	0 [b]	12.1
Newcastle 200 g/kg	Brown	42 [a]	12.1	33	12.1	30 [a]	12.1
LSD (P=0.05)		27	0.313	26	0.336	23	0.347

Table 3.1.10.b Results of odour (%) and yolk colour evaluation of eggs obtained from White Supertint layers fed on 100, 150, and 200 g/kg of Newcastle canola meal (Experiment 1b)

Canola meal treatments	Bird Strain	Fishy odour (fresh eggs)	Yolk colour (fresh eggs)	Odour (after 2 wks stored)	Yolk colour (after 2 wks stored)	Odour (after 5 wks stored)	Yolk colour (after 5 wks stored)
Control	White	8	12.3	0	12.1	0	11.8
Newcastle 100 g/kg	White	0	12.4	0	12.2	0	12.2
Newcastle 150 g/kg	White	0	12.4	0	12.3	0	12.1
Newcastle 200 g/kg	White	0	12.4	0	12.3	0	12.1
LSD (P=0.05)		Not analysed	0.313	Not analysed	0.336	Not analysed	0.347

Table 3.1.11 Results of odour (%) and yolk colour evaluation of eggs obtained from Isabrown and White Supertint layers fed on 100, 150, and 200 g/kg of Narrabri cottonseed meal (Experiment 1c)

Canola meal treatments	Bird Strain	Fishy odour (fresh eggs)	Yolk colour (fresh eggs)	Odour (after 2 wks stored)	Yolk colour (after 2 wks stored)	Odour (after 5 wks stored)	Yolk colour (after 5 wks stored)
Control	Brown	0	12.2	0	11.9	0	12.1
Narrabri 100 g/kg	Brown	0	12.1	0	12.0	0	11.9
Narrabri 150 g/kg	Brown	0	12.2	0	12.1	0	12.1
Narrabri 200 g/kg	Brown	0	12.0	0	12.0	0	12.5
Control	White	8	12.3	0	12.1	0	11.8
Narrabri 100 g/kg	White	0	12.0	0	12.1	0	12.1
Narrabri 150 g/kg	White	0	12.3	0	12.3	0	12.3
Narrabri 200 g/kg	White	0	12.4	0	12.3	0	12.4
LSD (P=0.05)		Not analysed	0.388	Not analysed	0.319	Not analysed	0.392

Table 3.1.12 Results of egg's sensory evaluation test (University of Qld Students egg taste panel) Significance of the comparison of CM and CSM sources and levels with untreated controls

Layer consuming	Bird Strain	Probability
CM Melbourne 100 g/kg	Inghams Hisex Brown	0.703 (ns)
CM Melbourne 200 g/kg	Inghams Hisex Brown	0.326 (ns)
CM Pinjarra 100 g/kg	Inghams Hisex Brown	0.846 (ns)
CM Pinjarra 200 g/kg	Inghams Hisex Brown	0.522 (ns)
CSM Narrabri 100 g/kg	Inghams White Supertint	0.719 (ns)
CSM Narrabri 200 g/kg	Inghams White Supertint	0.007*
CSM Narrabri 100 g/kg	Isabrown	0.003*
CSM Narrabri 200 g/kg	Isabrown	0.521 (ns)
CM Newcastle 100 g/kg	Inghams White Supertint	0.956 (ns)
CM Newcastle 200 g/kg	Inghams White Supertint	0.521 (ns)
CM Newcastle 100	Isabrown	0.848 (ns)
CM Newcastle 200	Isabrown	0.848 (ns)

CM= canola meal; CSM= cottonseed meal; ns= not significant * =significant $P<0.05$

The results in Experiment 1a (Table 3.1.4) indicated that the production performance of layer hens (Inghams Hisex Brown) was not affected by the source and level of CM in the diet. Hence, geographical location, processing extraction method and inclusion level did not affect the production performance of layer hens and in particular did not increase mortalities at the higher inclusion levels.

The results in Experiment 1b (Table 3.1.5) also indicate that CM from Newcastle source did not affect the production performance of layer hens ($P>0.05$) Isabrown and Inghams White Supertint at any level of inclusion with no mortalities occurring during the 14 weeks experimental period.

The results in Experiment 1c (Table 3.1.7) indicated that a satisfactory layer performance with inclusions of up to 200 g CSM/kg of diet can be obtained in both brown and white birds.

Tables 3.1.6 and 3.1.8 compared the effect of bird strain on performance when layer hens are fed on CM or CSM respectively. The results indicated that Isa Brown birds (Table 3.1.6) significantly ($P<0.05$) gave higher egg production, lower egg weights with less feed intake and hence a better feed efficiency when compared with White Supertint birds which gave higher egg weights and thus similar egg mass.

Egg quality at QPRDC

The observations made by the QPRDC panel on fresh eggs derived from brown Hisex layers fed on graded levels of CM from Melbourne and Pinjarra sources (Table 3.1.9) indicated that these treatments led to the production of "fishy" tainted eggs. Yolk colour was not affected by storage time or CM level in the diet. When eggs were stored at 10 °C for 2 weeks, an odour was detected at all CM levels in the Melbourne source but only at 150 and 200 g/kg level in the Pinjarra source. When eggs were stored at 10 °C for 5 weeks, a "fishy" odour was detected at 150 and 200 g/kg in the Melbourne source but no abnormal odour was detected at any CM level in the Pinjarra source. It is interesting to observe that a substantial reduction in egg taint occurred after eggs were stored for 5 weeks. Since the staff at QPRDC that carried out this evaluation could be considered untrained the results of this preliminary evaluation are not conclusive. Thus, a more detailed sensory evaluation

was repeated using the Sensory and Consumer Science Unit at the Centre for Food and Technology (Qld-DPI).

The observations on fresh eggs and stored eggs derived from Isa brown layers fed on graded levels of CM from Newcastle source (Experiment 1b; Table 3.1.10.1) indicated that these treatments led to production of "fishy or crabby" odour in fresh eggs at 150 and 200 g CM/kg and at all CM levels in stored eggs with no detrimental effect on yolk colour at any CM level in the diet. However, the QPRDC panellist team did not detect "fishy" odour from eggs when these CM dietary treatments were fed to White Supertint birds (Experiment 1b; Table 3.1.10.2). This problem of "fishy" taint with eggs derived from brown hens feed high levels of CM is due to sinapine which is present in the meal at about 11-15 g/kg. Sinapine is unable to be absorbed and metabolised by hens and passes through the intestine where it is metabolised by enteric bacteria to form choline, and further trimethylamine (TMA). Most brown birds are unable to metabolise TMA which is diverted into the ova, producing a "fishy" taint in eggs. However, eggs derived from White Supertint layer hens (Table 3.1.10.2) did not produce "fishy" eggs indicating that in these birds the TMA produced from sinapine was effectively metabolised to odourless TMA by TMA oxidase (Buttler 1984). This indicates that although high levels of CM support good egg production in brown birds, not more than 100 g/kg in the diet could be added without risking generation of "fishy" taint in eggs. But as shown in this experiment, White Supertint birds were able to support satisfactory performance (similar to brown strains) at high levels of CM without affecting egg quality.

The odour and yolk colour evaluation carried out on eggs derived from birds fed on CSM (Table 3.1.11) indicated that unusual odour was not detected in fresh or stored eggs at all CSM levels. Brown discolouration was also not observed in all evaluated eggs indicating that CSM from Narrabri are low gossypol varieties. Any residual gossypol present in the meal was most likely inactivated by the addition of iron salts in the diets. However, an increased yolk colour value (12.42) that was related to an apricot colour development was observed in stored (36 days) eggs derived from the 200 g CSM/kg. This apricot colour development was suspected to be caused by cyclopropenoid fatty acids (CPFA) indicated by the residual lipid component of 37 g/kg (see Table 3.1.5) in this solvent extracted meal. Thus, this residual lipid component was most probably the cause for this enhancement of yolk colour towards an apricot colour due to the high level of CSM in the diet (200 g CSM/kg). But this change towards an apricot yolk colour was not observed at 150 g CSM/kg, thus indicating the maximum inclusion level of this meal for egg production and acceptable egg quality.

Sensory evaluation

The egg sensory evaluation was carried out at the University of Queensland at the School of Land and Food Sciences. Students from this department were selected to carry out this taste test and eggs from the three layer experiments were sent for the evaluation. The results in Table 3.1.12 showed that of the 12 treatments tested, only the eggs from Isabrown layer fed with CSM at 100 g/kg level and eggs derived from White Supertint strain fed on CSM at 200 g/kg level were found to be significantly different from the untreated control.

This result may indicate that the "fishy" taint in eggs derived from brown layers fed on CM may disappear during cooking and students were not able to detect this problem that is present in fresh eggs. The results also indicate that the untrained panel was not able to detect any difference between eggs from control birds and birds fed CM at either 100 or 200 g/kg levels. However, this does not mean that some consumers would not be able to detect these eggs because only an untrained panel was used and therefore some sensitive individuals may determine a difference.

Only two treatments were detected as being different from the control, and they were both from CSM treatments. Therefore, this would indicate that there is a potential for consumers to detect these samples. However since no questions such as unpleasant odour or taste were asked to the untrained

panel, this detected differences found in CSM may not be necessarily negative or unpleasant to consumers.

This indicates that under the conditions used in our experiments inclusion of up to 200 g CM or CSM/kg allowed excellent egg production. The results from the odour evaluation indicated that consumers would be able to detect "fishy" odour from fresh eggs derived from brown hens fed on CM but not from eggs derived from White Supertint fed on high levels of CM. Consumers would not be able to detect "fishy" odour from eggs from brown or white hens fed on CSM at up to 150 g/kg.

The sensory evaluation (cooked eggs) indicated that consumers would be unlikely to detect the use of CM at the levels used in our experiments. However, the sensory evaluation test on cooked eggs detected differences on eggs derived from CSM diets (Table 3.1.12) and this may indicate that there is a potential for consumers to detect these differences.

Because variations in oil processing and environmental conditions that normally occurred where these CMs and CSMs are produced, plus the constant importation to Australia of new bird strains, these experiments need to be repeated.

To ensure that the sensory evaluation test results are valid, future work should include the use of a trained panel to specifically look for attributes associated with the feed which may affect consumers acceptance of the eggs. This would ensure that the potential benefits of using the meals is not lost due to any negative response by consumers.

3.2 Experiment 2. Evaluation of low glucosinolates canola meal and low gossypol cottonseed meal in layer diets. 2000 harvest

3.2.1 Introduction

During the year 2000, production parameters, egg quality and egg sensory evaluations were undertaken using high levels (100, 150, and 200 g/kg) of canola meal (CM) from Melbourne, Pinjarra, and Newcastle sources and cottonseed meal (CSM) from Narrabri in layer diets formulated on a digestible amino acid (AA) basis. Inghams Hisex Brown, Isa Brown and Inghams White Supertint layer hens were investigated to determine the suitability of these birds to overcome antinutritional factors (ANF) that are present in these meals. The results of these trials indicated that satisfactory production performance was obtained with these bird strains when CM or CSM are included in layer diets at 100, 150 and 200 g/kg. However, subsequent egg evaluations revealed that a "fishy" odour was present in a percentage of eggs derived particularly from Inghams Hisex Brown and Isa Brown layer hens when fed any level of CM. Interestingly, this "fishy" odour in raw eggs seems to disappear when eggs were cooked and submitted to a sensory evaluation. Inghams White Supertint birds did not produce eggs with this taint problem and thus can be suitable to high levels of CM in the diet. CSM which supported good egg production did not produce taint in eggs but there was a yolk colour increase particularly in stored (5 weeks storage) eggs when the levels of CSM were at 200 g/kg in the diet. Yolk colour in CSM derived eggs needs to be re-evaluated as yolk mottling was not evaluated in a previous experiment.

With the introduction of new bird strains into Australia, there is a constant need to re-evaluate the use of high levels of CM and CSM on these birds with emphasis on egg quality as it was shown in previous trial that high levels of these meals might affect consumer's acceptance of the eggs.

The present study was carried out in four experiments aiming to evaluate egg production, egg quality and provide eggs for egg sensory evaluations in Hy-line Brown and Hy-line White (W-36) birds fed on diets containing high levels of CM or CSM. In the present study an independent sensory evaluation was carried out by a qualified panel at the Centre for Food and Technology (Queensland-DPI).

3.2.2 Material and methods

Layer Diets

One tonne of commercial CM was obtained from four representative processors located in Newcastle (NSW), Melbourne (Vic), Numurkah (Vic) and Pinjarra (WA). Solvent extraction was used to obtain all CM except for the Pinjarra processor who used expeller extraction. Solvent-extracted CSM was obtained as high protein from Narrabri (NSW) and a low protein CSM from Brisbane (Qld). These materials were derived from the 1999-2000 harvests.

The ingredient and chemical composition of the diets used in each of the four layer experiments are given in Tables 3.2.1, 3.2.2, 3.2.3, and 3.2.4 respectively. In Experiments 2a and 2b, dietary treatments with CSM were offered to Hy-line Brown and to Hy-line White (W-36) layers. In Experiments 2c and 2d, dietary treatments with CM were offered to Hy-line Brown and to Hy-line White (W-36) layers.

Diets formulation and treatments
Diets formulation procedures and dietary treatments are described in the General Materials and Methods (chapter 1) section for layers.

Chemical analysis and bioassays

Experimental canola and cottonseed meals from the harvests 1999-2000 were chemically analysed and bioassayed (AME and digestible AA determination) as described in the General Materials and Methods section.

Table 3.2.1 Ingredient composition (g/kg) of layer diets with 120 and 200 g/kg of cottonseed meal from Brisbane and Narrabri (Hy-line Brown Layer Experiment 2a)

Ingredients	Control	Narrabri	Narrabri	Brisbane	Brisbane
Sorghum	472	479	406	504	451
Wheat	200	150	232	160	150
Meat& bone meal	50	50	43	50	50
Soybean meal	78	60	-	51	4.2
Full fat Soybean meal	50	27	31	28	50
Sunflower meal	66	30	-	-	-
CSM Brisbane, low CP	-	-	-	120	200
CSM Narrabri, high CP	-	120	200	-	-
Soybean oil	-	-	-	-	8.8
Dicalcium phosphate	1.03	-	-	0.1	-
Limestone	76.5	77	79.7	77	77
Salt	1.2	1	1	1	1
Sodium bicarbonate	0.7	-	-	0.6	0.5
Vitamins & minerals	2.5	2.5	2.5	2.5	2.5
Methionine	1.7	1.7	2	1.7	1.5
Lysine	1.3	1.7	2.9	2.3	2.5
Threonine			0.3	0.4	0.4
Calculated analysis					
Total crude protein	174	195	197	182	194
Digestible lysine	6.6	6.6	6.6	6.6	6.6
Digestible methionine	3.9	3.8	3.9	3.8	3.7
Digestible sulphur AA	5.9	5.9	5.9	5.9	6
Digestible threonine	4.5	4.5	4.5	4.5	4.5
Digestible isoleucine	5.8	5.6	4.9	5.1	5
Digestible tryptophan	1.7	1.8	1.8	1.6	1.7
Calcium	34	34	34	34	34
Avail. Phosphorous	4	4.2	4	4	4.2
AME (MJ/kg)	11.5	11.5	11.5	11.5	11.5

Vitamin and mineral premixes added per kg of diet: 2.5 mg retinol, 75 ug D_3, 5 mg a-tocopherol acetate, 2 mg menadione sodium bisulfite, 1 mg thiamine, 4 mg riboflavin, 2 mg pyridoxine, 10 ug B_{12}, 1 mg folic acid, 10 mg niacin, 10 mg Ca pantothenate, 30 ug biotin, 225 mg choline, 50 mg Mn, 50 mg Zn, 50 mg Fe, 600 ug Mo, 500 ug Co, 600 ug 1, 4 mg Cu, 70 ug Se, 80 mg Banox.

Table 3.2.2 Ingredient composition (g/kg) of layer diets with 120 and 200 g/kg of cottonseed meal from Brisbane and Narrabri (Hy-line White, W-36. Layer Experiment 2b)

Ingredients	Control	Narrabri	Narrabri	Brisbane	Brisbane
Sorghum	452	440	416	466	450
Wheat	200	200	158	150	85
Meat& bone meal	50	50	43	50	50
Soybean meal	100	33	15	47	33
Full fat Soybean meal	50	50	50	50	50
Sunflower meal	37	-	-	-	
CSM Brisbane, low CP	-	-	-	120	200
CSM Narrabri, high CP	-	120	200		
Soybean oil	14.3	13.2	14	14.2	13.8
Tallow	3.7	-	8.2	9.5	25.5
Dicalcium phosphate	2.4	0.6	-	0.2	
Limestone	82.7	83.9	87.6	84.3	84.1
Salt	1.2	1	1.1	1	1
Sodium bicarbonate	1.2	0.9	0.4	1.3	0.9
Vitamins & minerals	2.5	2.5	2.5	2.5	2.5
Methionine	1.9	2.1	1.9	1.8	1.5
Lysine	1.4	2.6	2.6	2.4	2.3
Threonine	0.1	0.3	0.1	0.4	0.3
Calculated analysis					
Total crude protein	174	186	202	183	199
Digestible lysine	7	7	7	7	7
Digestible methionine	4	4	3.9	3.8	3.7
Digestible sulphur AA	6	6	6	6	6
Digestible threonine	4.6	4.6	4.6	4.6	4.6
Digestible isoleucine	5.9	5.2	5.2	5.2	5.2
Digestible tryptophan	1.7	1.7	1.9	1.7	1.8
Calcium	36.5	36.5	36.5	36.5	36.5
Avail. Phosphorous	4.2	4.2	4.2	4.2	4.2
AME (MJ/kg)	11.9	11.9	11.9	11.9	11.9

Vitamin and mineral premixes added per kg of diet: 2.5 mg retinol, 75 ug D_3, 5 mg a-tocopherol acetate, 2 mg menadione sodium bisulfite, 1 mg thiamine, 4 mg riboflavin, 2 mg pyridoxine, 10 ug B_{12}, 1 mg folic acid, 10 mg niacin, 10 mg Ca pantothenate, 30 ug biotin, 225 mg choline, 50 mg Mn, 50 mg Zn, 50 mg Fe, 600 ug Mo, 500 ug Co, 600 ug 1, 4 mg Cu, 70 ug Se, 80 mg Banox.

Table 3.2.3 Ingredients (g/kg) of layer diets with 120 and 200 g/kg of canola meal from Newcastle, Melbourne, Numurkah, and Pinjarra (Hy-line Brown Layer Experiment 2c)

Sorghum	502	464.8	450	450	450	480	450	319
Wheat	150	150	184	142	188	129	169	297
Meat& bone meal	50	48	50	47	50	50	50	50
Soybean meal	53	25	45.2	8	29	-	-	-
Full fat Soybean meal	42	15	35	32	47	17	44.8	3
Sunflower meal	-		30	30	30	30	82	46
CM Newcastle	120	200			-	-	-	-
CM Melbourne	-	-	120	200	-	-	-	-
CM Numurkah	-	-			120	200	-	-
CM Pinjarra	-	-			-	-	120	200
Soybean oil	1	14.7	3.4	8.8	3.5	10.6	-	2.3
Dicalcium phosphate	0.3	-	-	-	0.1	-	0.3	-
Limestone	75.4	74.8	75.8	75.5	75.7	74.8	75.3	74.8
Salt	1.4	1.3	1.4	1.4	1.3	1.1	1	1.4
Sodium bicarbonate	0.3	0.6	0.8	1.1	0.7	1.2	1.4	1.3
Vitamins & minerals	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Methionine	1.6	1.5	1.3	1.2	1.4	1.4	1.4	1.4
Lysine	1.1	1.5	1.2	1.3	1.3	1.8	1.8	1.7
Threonine	-	0.1	-	-	-	0.2	0.2	0.2
Calculated analysis								
Total crude protein	179	180	183	188	179	178	174	178
Digestible lysine	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6
Digestible methionine	3.8	3.7	3.7	3.7	3.7	3.7	3.7	3.7
Digestible sulphur AA	5.9	5.9	5.9	6.1	6	6.1	6	6.1
Digestible threonine	4.5	4.5	4.7	4.7	4.5	4.5	4.5	4.5
Digestible isoleucine	5.6	5.2	5.7	5.6	5.5	5.2	5.2	5.1
Digestible tryptophan	1.7	1.7	1.8	1.8	1.7	1.7	1.7	1.7
Calcium	34	34	34	34	34	34	34	34
Avail. Phosphorous	4	4	4	4	4	4.1	4	4
AME (MJ/kg)	11.5	11.5	11.5	11.5	11.5	11.5	11.5	11.5

Table 3.2.4 Ingredients (g/kg) of layer diets with 120 and 200 g/kg of canola meal from Newcastle, Melbourne, Numurkah, and Pinjarra (Hy-line White, W-36, Layer Experiment 2d)

Sorghum	479	432	450	450	450	480	500	458
Wheat	150	150	160	117	168	92	125	151
Meat& bone meal	50	50	50	50	50	50	50	50
Soybean meal	64	36	51	20	44	-	35	49
Full fat Soybean meal	31	8	32	19	28	32	50	-
Sunflower meal	-	-	30	30	30	30	30	8
CM Newcastle	120	200	-	-	-	-	-	-
CM Melbourne	-	-	120	200	-	-	-	-
CM Numurkah	-	-	-	-	120	200	-	-
CM Pinjarra	-	-	-	-	-	-	120	200
Soybean oil	16.8	19.6	16.1	17.5	16.6	14.9	4.8	0.34
Tallow	5	22.2	6.7	14.1	8.7	17.2	-	-
Dicalcium phosphate	0.3	-	0.1	-	0.2	-	0.7	0.3
Limestone	75.4	74.2	75.7	74.7	75.7	74.8	75.4	74.8
Salt	1.3	1.2	1.2	1.2	1.1	1.1	1.2	1.3
Sodium biocarbonate	1.2	1.4	1.7	2	1.7	1.9	1.7	1.8
Vitamins & minerals	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Methionine	1.7	1.6	1.4	1.2	1.5	1.4	1.5	1.5
Lysine	1.6	1.9	1.6	1.8	1.9	2	1.6	1.5
Threonine	0.1	0.1	-	-	0.1	0.2	0.2	0.2
Calculated analysis								
Total crude protein	178	180	182	186	176	179	176	179
Digestible lysine	6.7	7	7	7	7	7	7	7
Digestible methionine	3.8	3.8	3.7	3.7	3.7	3.7	3.7	3.7
Digestible sulphur	6	6	6	6.1	6	6.2	6	6.2
Digestible threonine	4.6	4.6	4.6	4.7	4.6	4.6	4.6	4.6
Digestible isoleucine	5.5	5.2	5.7	5.5	5.4	5.2	5.4	5.3
Digestible tryptophan	1.7	1.7	1.8	1.8	1.7	1.7	1.7	1.6
Calcium	34	34	34	34	34	34	34	34
Avail. Phosphorous	4	4	4	4.1	4	4.1	4	4
AME (MJ/kg)	11.9	11.9	11.9	11.9	11.9	11.9	11.9	11.9

Yolk colour rating and egg yolk mottling evaluated at QPRDC

Cottonseed meal experiments

On week fifteen of each CSM layer experimental period, eggs from each bird (brown and white strains) in each experiment were collected at 95 eggs/experiment and stored at 10 °C for six weeks. These eggs were evaluated for yolk colour and yolk mottling by four experienced technical and scientific staff from QPRDC and the Centre for Food Technology. A standard colorimetric system reference (yolk colour fan, Roche 1993) with a scale range 1 to 15 was used for yolk colour evaluation. Stored raw whole eggs were placed on a white plate on high light intensity as panellist assessed them. A value coded 0 was recorded if the observer reported no mottling or no presence of a different yolk appearance to a normal yolk egg. A value coded 1 was recorded if an observer reported an unusual yolk colour, spotting or mottling. Data from these evaluations were sent to the DPI-Animal Research Institute for statistical analyses.

Odour and sensory evaluation of eggs from hens fed on canola meal and cottonseed meal diets at Centre for Food Technology (CFT)

Odour assessments and sensory evaluations of eggs were carried out by the Sensory and Consumer Science Unit at the Centre for Food Technology (CFT; Hamilton-Brisbane, Qld). The main aims were (i) to investigate the incidence of fishy taint by odour assessment in raw eggs produced from Hy-line Brown and Hy-line White (W-36) hens fed on various CM and CSM diets; (ii) to record the appearance of the eggs produced from hens fed various CSM diets; (iii) to assess the odour and flavour characteristics of cooked eggs produced from Hy-line Brown and Hy-line White (W-36) hens fed various CM and CSM diets.

Egg samples for assessment at QPRDC and CFT

Canola meal (CM) treatments

A = control diet; white hens (20 hens)	D = Control diet; brown hens (20 hens)
M = Newcastle at 12%; white hens (20 hens)	K = Newcastle 12%; brown hens (20 hens)
N = Newcastle at 20%; white hens (20 hens)	L = Newcastle 20%; brown hens (20 hens)
O = Melbourne at 12%; white hens (20 hens)	Q = Melbourne 12%; brown hens (20 hens)
P = Melbourne at 20%; white hens (20 hens)	R = Melbourne 20%; brown hens (20 hens)
U = Numurkah at 12%; white hens (20 hens)	S = Numurkah 12%; brown hens (20 hens)
V = Numurkah at 20%; white hens (20 hens)	T = Numurkah 20%; brown hens (20 hens)
Y = Pinjarra at 12%; white hens (20 hens)	W = Pinjarra 12%; brown hens (20 hens)
Z = Pinjarra at 20%; white hens (20 hens)	X = Pinjarra 20%; brown hens (20 hens)
Total 180 hens (numbers 1-180)	Total 180 hens (numbers 1-180)

Cottonseed meal (CSM) treatments

A = control diet; white hens (19 hens).	D = Control diet; brown hens (19 hens)
B = HP at 12% in diet; white hens (19 hens).	E = HP at 12% in diet; brown hens (19 hens)
C = HP at 20% in diet; white hens (19 hens).	F = HP at 20% in diet; brown hens (19 hens)
I = LP 12% in diet; white hens (19 hens).	G = LP at 12% in diet; brown hens (19 hens)
J = LP 20% in diet; white hens (19 hens).	H = LP at 20% in diet; brown hens (19 hens)
Total 95 hens (numbers 1-95)	Total 95 hens (numbers 1-95)

HP = high protein CSM LP= low protein CSM

The same diet was used as the control diet for the canola and cottonseed meal trials.

Panellists at CFT

Leaflet drops were made at local shopping centre, libraries, schools and cafes in the North Brisbane area asking for people to apply for positions as casual sensory evaluation panellists. Each applicant

was asked to complete a pre-screening questionnaire to obtain information regarding his or her eating habits, general health, interests and availability. From the 45 people who applied, 35 were selected to complete a series of screening tests based on the results of the pre-screening questionnaires. The screening tests were conducted at the Centre for Food Technology on 15th, 16th and 17th May 2001. All screening tests were completed in individual testing booths.

Raw egg odour assessment methodology at CFT

All eggs for assessment were collected on 22 May 2001 and were stored at 10°C by the client (Rider Perez-Maldonado) at the Poultry Research and Development Centre for three weeks. The eggs were delivered to the Centre for Food Technology (CFT) on Friday 8th June and stored at 10°C until required for assessment. One egg from every hen on each treatment was collected and assessed. The dates of assessment were: Tuesday 12th June 2001: Brown canola eggs from hens number 1 – 45; White canola eggs from hens number 1 – 45. Friday 15th June 2001: Brown canola eggs from hens number 46 – 180; White canola eggs from hens number 46 – 135. Monday 18th June 2001: White canola eggs from hens number 136 – 180 White cottonseed meal eggs from hens number 1 – 95; Brown cottonseed meal eggs from hens number 1 – 95.

One egg from every hen from all treatments (listed under the samples section) was cracked open into a 150ml plastic container and labelled with a three digit blinding code. The egg was lightly beaten with a fork to break the yolk. A tight fitting lid was then placed on the container. The three panellists previously selected on their ability to detect and rate the level of fishy odour in eggs assessed all samples. These panellists received round table training and completed a preliminary booth session prior to completing the assessments. All three panellists assessed each egg. Assessments were carried out in individual testing booths under white light. Panellists had filtered water freely available in their booths. Panellists also had a supply of clean forks to stir the sample with if they wished. Each panellist had a control egg from the trial in the booth which they could refer to as required. The temperature of the eggs on assessment was 18 - 22°C.

Panellists indicated the level of fishy odour on a category scale as shown below.

None Trace + ++

These were defined as:

None: No fishy odour detectable. **Trace:** Slight fishy odour detectable

+ : Moderate fishy odour detectable. **++ :** Strong fishy odour detectable

For the canola meal eggs, the samples were presented in 40 sets (20 brown, 20 white) of 9 eggs (one complete block according to the original experimental design – see Appendix 1) but the panellists had a forced break of 3 minutes between egg samples 5 and 6. Panellists had a five minute break between sets of nine samples.

Prior to lightly beating the cottonseed meal eggs for odour assessment, two staff from the Sensory and Consumer Science Unit made notes regarding the appearance of the eggs. The panellists then assessed the odour of the cottonseed meal eggs in 38 sets (19 brown, 19 white sets) of five treatments from the original experimental design. Panellists had a short break on completing each set of five assessments.

Interpretation of results at CFT

The number of eggs, which were identified as having a trace or fishier odour by at least two out of the three panellists, was used to calculate the percentages of eggs identified as being fishy. No significance testing was done to compare raw odour data.

Experimental Design and Statistical Analysis

Production parameters

There were four layer experiments. In Layer Experiment 2a and 2b, there were five dietary treatments per experiment comprising a control diet in each trial plus all combinations of two levels of CSM (120 and 200g/kg) x two sources of CSM (Narrabri of high protein and Brisbane of low protein) fed to 19 replicates of Hy-line Brown and 19 replicates of Hy-line White (W-36) layer hens allocated in blocks in a randomised block design.

Data in Experiments 2a and 2b were statistically analysed as 3 levels (control, 120or 200 g CSM/kg) x strain x 19 replicates as a completely randomised design.

In Layer Experiment 2c and 2d, there were nine dietary treatments per experiment comprising a control diet in each trial plus all combinations of two levels of CM (120 or 200 g/kg) fed to 20 replicates of Hy-line Brown hens (Experiment 2c) and 20 replicates of Hy-line White hens (Experiment 2d) in a completely randomised design. Data in each trial were statistically analysed as 3 levels (control, 120 and 200 g CM/kg) x strain x 20 reps. Each trial was carried out for 15 weeks from 42 weeks of age. In Experiments 2a and 2b, data were analysed by analysis of variance (ANOVA) and treatment means compared as five distinct treatments using the protected LSD at P=0.05. A second ANOVA was then run in which data was analysed as 2 x 2 factorial using the full error term from run 1. In Experiments 2c and 2d, similar ANOVA were used but as 4 x 2 factorial during the second run.

Mottling rate and colour rating CSM experiments (QPRDC)

The results of mottling and yolk colour in each CSM experiment were statistically analysed as a consensus estimate in which an egg was deemed to have mottling only when either 3/4 or 4/4 operators agreed, so the treatment means become: % eggs for which 3 or 4 operators (out of 4) agreed that the egg has mottling or unusual appearance. Main effects for CSM source and level of inclusion were also analysed.

Sensory evaluation on raw and cooked eggs from CM and CSM experiments (CFT)

The results of these evaluations were statistically analysed by the CFT group and all the results are fully presented as an independent report available from RIRDC. A summary report is attached in the last section of this project.

3.2.3 Results and Discussion

Chemical Analysis

Results of the chemical analyses, NSP and digestible AA results performed on each CM and CSM samples are presented in the broiler section (chapter 2) in Tables 2.2.5, 2.2.6, 2.2.7 (for CM) and Tables 2.3.5, 2.3.6, 2.3.7 (for CSM)

Layer Trials
In Experiments 2a and 2b, the treatment effect mean values for the production parameters, main effects and interactions for CSM sources (Brisbane and Narrabri) and inclusion levels (12% and 20%) in Hy-line Brown and Hy-line White (W-36) layer hens are presented in Tables 3.2.5 and 3.2.6 respectively

In Experiment 2c and 2d, the treatment effect mean values for the production parameters, main effects and interactions for CM sources (Newcastle, Melbourne, Numurkah, and Pinjarra) and inclusion levels (120 and 200 g/kg) in Hy-line Brown and White (W-36) layer hens are presented in Tables 3.2.7 and 3.2.8 respectively.

Mottling rate % and Yolk colour rating at QPRDC

The results for the mottling rate and yolk colour evaluation on six weeks stored eggs derived from Hy-line Brown and Hy-line White (W-36) layer hens in experiment B1 and B2 are presented in Tables 3.2.9 and 3.2.10 respectively.

Sensory evaluation of eggs at the Centre of Food Technology (CFT)

The Sensory and Consumer Science Unit at CFT carried out the sensory evaluation of eggs from hens fed on canola meal and cottonseed meal diets as an independent research. This Centre produced its own final report for which a summary is presented in the final section of this AECL report. The full CFT sensory evaluation report is available from AECL.

Table 3.2.5 Hy-line Brown hens (42-57 weeks) fed on diets with 120, and 200 g/kg of cottonseed meal from Brisbane and Narrabri, Layer Experiment 2a

Cottonseed meal treatments	FI (g/d)	Production %	Egg wt (g)	Egg mass (g/d)	FCR (g FI/g egg mass)	Specific gravity	Hen weight (g/bird)	Liver (% bodywt)	Pancreas (% bodywt)
Control	109.9	90.8	63.1	57.2	1.927	1.085	2009[a]	1.99[c]	0.168
Narrabri 120 g/kg	113.8	88.3	64.4	56.7	2.034	1.085	2230[b]	2.32[ab]	0.194
Narrabri 200 g/kg	110.5	88.8	63.0	55.8	1.982	1.086	2121[ab]	2.57[a]	0.185
Brisbane 120 g/kg	116.2	91.8	64.6	59.3	1.964	1.083	2124[ab]	2.39[a]	0.165
Brisbane 200 g/kg	116.8	92.1	62.9	57.8	2.027	1.085	2214[b]	2.11[bc]	0.179
LSD (P=0.05)	6.2	5.5	2.3	3.5	0.118	0.0025	140	0.28	0.023
Factorial main effects: (1)									
CSM source									
Narrabri (high protein)	112.1	88.5	63.7	56.2	2.008	1.085	2175	2.44	0.190[a]
Brisbane (low protein)	116.5	92.0	63.8	58.6	1.996	1.084	2169	2.25	0.172[b]
LSD (P=0.05)	4.4	3.9	1.6	2.5	0.084	0.0018	99	0.20	0.016
Inclusion levels									
120 g/kg	115.0	90.1	64.5	57.9	1.999	1.084[a]	2177	2.36	0.179
200 g/kg	113.6	90.4	63.0	56.8	2.004	1.085[b]	2167	2.34	0.182
LSD (P=0.05)	4.4	3.9	1.6	2.5	0.084	0.0018	99	0.20	0.016

Where superscripting is used within sections of columns of means, the F-test in the ANOVA was significant and means followed by different letters are significantly different at the 5% level.

Note: (1) The CSM source x inclusion levels interaction was significant for "final body wt and liver wt.

Table 3.2.6 Hy-line White (W-36) hens fed on diets with 120, and 200 g/kg of cottonseed meal from Brisbane and Narrabri, Layer Experiment 2b

Cottonseed meal Treatments	FI (g/d)	Production %	Egg wt (g)	Egg mass (g/d)	FCR (g FI/g egg mass)	Specific gravity	Hen wwight (g/bird)	Liver (% bodywt)	Pancreas (% bodywt)
Control	95.7[a]	85.9	61.5[a]	52.9	1.815ab	1.083	1623	2.19	0.202[ab]
Narrabri 120 g/kg	92.0[ab]	86.4	60.1[ab]	51.8	1.780b	1.081	1596	2.43	0.180[b]
Narrabri 200 g/kg	89.0[b]	87.9	59.2[b]	52.0	1.725b	1.068	1573	2.30	0.186[b]
Brisbane 120 g/kg	92.5[ab]	86.4	60.6[ab]	52.4	1.775b	1.083	1630	2.21	0.203[ab]
Brisbane 200 g/kg	96.4[a]	83.6	62.0[a]	51.7	1.887a	1.081	1709	2.30	0.235[a]
LSD (P=0.05)	5.1	5.3	2.0	3.5	0.107	0.0184	122	0.33	0.037
Factorial main effects: (1)									
CSM source									
Narrabri (high protein)	90.5[a]	87.1	59.6[a]	51.9	1.752[a]	1.075	1.584	2.37	0.183[a]
Brisbane (low protein)	94.5[b]	85.0	61.3[b]	52.0	1.831[b]	1.082	1.670	2.25	0.219[b]
LSD (P=0.05)	3.6	3.7	1.4	2.5	0.076	0.0130	86	0.24	0.026
Inclusion levels									
120 g/kg	92.3	86.4	60.4	52.1	1.777	1.082	1613	2.32	0.192
200 g/kg	92.7	85.7	60.4	51.9	1.806	1.074	1641	2.30	0.211
LSD (P=0.05)	3.6	3.7	1.4	2.5	0.076		89	0.24	0.026

Where superscripting is used within sections of columns of means, the F-test in the ANOVA was significant and means followed by different letters are significantly different at the 5% level.

Note: (1) The CSM source x inclusion levels interaction was significant for FCR.

Table 3.2.7 Hy-line Brown hens fed on diets with 120, and 200 g/kg of canola meal from Newcastle, Melbourne, Numurkah, and Pinjarra. Layer Experiment 2c

Canola meal treatments	FI (g/d)	Production %	Egg wt (g)	Egg mass (g/d)	FCR (g FI/g egg mass)	Specific gravity	Hen weight (g/bird)	Liver (% bodywt)	Pancreas (% bodywt)
Control	113.8 [bc]	89.3	64.6	57.6	1.981	1.085	2160	1.98	0.162
Newcastle 120 g/kg	110.9 [c]	88.3	62.5	55.1	2.017	1.085	2183	1.98	0.162
Newcastle 200 g/kg	113.0 [bc]	87.7	63.3	55.4	2.051	1.086	2089	1.83	0.190
Melbourne 120 g/kg	112.7 [bc]	91.4	62.8	57.3	1.974	1.084	2153	1.89	0.172
Melbourne 200 g/kg	114.8 [abc]	89.2	63.3	56.4	2.040	1.084	2059	1.88	0.172
Numurkah 120 g/kg	115.4 [abc]	84.4	64.4	54.3	2.138	1.084	2086	1.95	0.186
Numurkah 200 g/kg	118.5 [a]	87.4	64.1	56.0	2.128	1.085	2150	1.76	0.168
Pinjarra 120 g/kg	117.1 [ab]	89.3	64.0	57.1	2.064	1.082	2115	1.81	0.182
Pinjarra 200 g/kg	110.3 [c]	86.3	63.2	54.4	2.047	1.083	2026	1.89	0.174
LSD (P=0.05)	4.6	4.3	2.3	2.9	0.113	0.0031	116	0.33	0.033
Factorial main effects: (1)									
CM source									
Newcastle	112.0 [b]	88.0[ab]	62.9	55.3	2.034	1.085	2136	1.91	0.176
Melbourne	113.7 [ab]	90.3[a]	63.1	56.9	2.007	1.084	2106	1.89	0.172
Numurkah	116.9 [a]	85.9[b]	64.2	55.1	2.133	1.084	2118	1.85	0.177
Pinjarra	113.7 [ab]	87.8[ab]	63.6	55.7	2.055	1.082	2071	1.85	0.178
LSD (P=0.05)	3.2	3.0	1.6	2.0	0.080	0.0022	82	0.23	0.023
Inclusion levels									
120 g/kg	114.0	88.4	63.4	56.0	2.048	1.084	2134	1.91	0.176
200 g/kg	114.2	87.6	63.5	55.6	2.066	1.084	2081	1.84	0.176
LSD (P=0.05)	2.3	2.1	1.2	1.4	0.057	0.0016	58	0.16	0.017

Where superscripting is used within sections of columns of means, the F-test in the ANOVA was significant and means followed by different letters are significantly different at the 5% level.

Note: (1) Significant sources x levels interaction due mainly to suspiciously large difference between levels of Pinjarra.

Table 3.2.8 Hy-line White (W-36) hens fed on diets with 120, and 200 g/kg of canola meal from Newcastle, Melbourne, Numurkah, and Pinjarra. Layer Experiment 2d

Canola meal treatments	FI (g/d)	Production %	Egg wt (g)	Egg mass (g/d)	FCR (g FI/g egg mass)	Specific gravity	Hen weight (g/bird)	Liver (% bodywt)	Pancreas (% bodywt)
Control	96.7	85.0	60.9	51.7	1.875	1.082	1658	2.43	0.192
Newcastle 120 g/kg	93.2	84.3	60.7	51.0	1.835	1.081	1628	2.62	0.205
Newcastle 200 g/kg	93.9	83.1	61.0	50.7	1.863	1.081	1710	2.29	0.227
Melbourne 120 g/kg	90.7	79.1	61.5	48.5	1.886	1.081	1617	2.08	0.204
Melbourne 200 g/kg	93.3	83.6	59.6	49.8	1.887	1.081	1582	2.53	0.207
Numurkah 120 g/kg	92.7	80.0	61.7	49.2	1.891	1.080	1612	21.34	0.195
Numurkah 200 g/kg	93.7	80.8	60.7	49.0	1.934	1.080	1674	2.74	0.223
Pinjarra 120 g/kg	95.4	82.4	61.7	50.7	1.886	1.081	1603	2.39	0.190
Pinjarra 200 g/kg	93.4	81.7	59.8	48.8	1.920	1.083	1567	2.57	0.214
LSD (P=0.05)	4.5	5.3	2.1	3.2	0.097	0.0022	106	0.45	0.038
Factorial main effects: (1)									
CM source									
Newcastle	93.5	83.7	60.8	50.9	1.849	1.081	1669	2.46	0.216
Melbourne	92.0	81.3	60.5	49.1	1.886	1.081	1599	2.30	0.205
Numurkah	93.2	80.4	61.2	49.1	1.913	1.080	1643	2.54	0.209
Pinjarra	94.4	82.1	60.8	49.8	1.903	1.082	1585	2.48	0.202
LSD (P=0.05)	3.2	3.7	1.5	2.2	0.068	0.0016	75	0.32	0.027
Inclusion levels									
120 g/kg	93.0	81.4	61.4[a]	49.9	1.874	1.081	1615	2.36	0.198[a]
200 g/kg	93.6	82.3	60.3[b]	49.6	1.901	1.081	1633	2.53	0.218[b]
LSD (P=0.05)	2.2	2.6	1.0	1.6	0.048	0.0011	53	0.22	0.019

Where superscripting is used within sections of columns of means, the F-test in the ANOVA was significant and means followed by different letters are significantly different at the 5% level.

Note: (1) The CSM source x inclusion levels interaction was significant for FCR.

Table 3.2.9 Results of mottling rate (%) and yolk colour evaluation of eggs obtained from Hy-line Brown layers fed on 120 and 200 g/kg of cottonseed meal (Brisbane and Narrabri) and stored for six weeks, Experiment 2a

Cottonseed meal treatments [1]	Mottling rate % [2]	Yolk colour
Control	0.0	11.0 [bc (3)]
Narrabri 120 g/kg	11.8 [abc]	10.9 [c]
Narrabri 200 g/kg	31.6 [a]	11.1 [bc]
Brisbane 120 g/kg	10.5 [bc]	11.6 [ab]
Brisbane 200 g/kg	27.8 [ab]	11.9 [a]
LSD (P=0.05)	23.6	0.56
Factorial main effects: (4)		
Narrabri	21.7	11.0 [a]
Brisbane	19.2	11.8 [b]
LSD (P=0.05)	16.6	0.39
Inclusion levels		
120 g/kg	11.1[a]	11.2
200 g/kg	29.7[b]	11.5
LSD (P=0.05)	16.6	0.39

Note: (1) ANOVA of 0/1 binary data is only approximate due to all-Zero control data. (2) As measured by majority agreement among the 4 operators, that is, 3 or 4 operators agree an egg yolk is mottled then it's classified as "mottled". Similarly for "not mottled". (3) Where superscripting is used within sections of columns of means, the F-test in the ANOVA was significant and means followed by different letters are significantly different at 5% level. (4) The protein level x inclusion level interactions were not significant.

Table 3.2.10 Results of mottling rate (%) and yolk colour evaluation of six weeks stored eggs obtained from Hy-line White layers fed on 120 and 200 g/kg of cottonseed (Brisbane and Narrabri) Experiment 2b

Cottonseed meal treatments [1]	Mottling rate % [2] [3]	Yolk colour (3)
Control	0.0 [b]	8.7 d
Narrabri 120 g/kg	5.6 [b]	11.4 [bc]
Narrabri 200 g/kg	30.6 [a]	11.2 [c]
Brisbane 120 g/kg	5.3 [b]	11.8 [ab]
Brisbane 200 g/kg	26.3 [a]	12.2 [a]
LSD (P=0.05)	20.1	0.53
Factorial main effects: (4)		
Narrabri	18.1	11.3[a]
Brisbane	15.8	12.0[b]
LSD (P=0.05)	14.1	0.37
Inclusion levels		
120 g/kg	5.4 [a]	11.6
200 g/kg	28.4[b]	11.7
LSD (P=0.05)	14.1	0.37

Note: (1) ANOVA of 0/1 binary data is only approximate due to all-Zero control data. (2) As measured by majority agreement among the 4 operators, that is, 3 or 4 operators agree an egg yolk is mottled then it's classified as "mottled". Similarly for "not mottled". (3) Where superscripting is used within sections of columns of means, the F-test in the ANOVA was significant and means followed by different letters are significantly different at 5% level. (4) The protein level x inclusion level interactions were not significant.

The results with CSM fed to both Hy-line Brown and Hy-line White layer hens (Experiments 2a and 2b, Tables 3.2.5 and 3.2.6 respectively) indicated that satisfactory performance was obtained on high levels of CSM in layer diets. The CSM source and level in the diet did not affect any production parameters in brown layer hens but both strains of layers tended to consume more feed when consuming diets based on CSM from Brisbane. This increment in feed consumption was significant ($P<0.05$) in white birds which had higher FCR and final body weight, indicating an inefficient use of this CSM source when compared with Narrabri CSM. It is also possible that the actual AME of diets using the Brisbane source were slightly lower than calculated values and birds had to consume more feed in order to satisfy their need for energy. The results in these experiments showed that neither gossypol or cyclopropenoid fatty acids (CPFA) levels in the CSM diets affected egg production. The addition of ferrous sulphate at the ratio 2:1 did not affect egg production as well in this experiment.

The results when feeding high levels of CM to Hy-line Brown and Hy-line White hens (Experiments 2c and 2d, Tables 3.2.7 and 3.2.8 respectively) indicate that, during the 15 weeks experimental period, the production performance was not different ($P>0.05$) from the control diet for any CM source and level of inclusion. Only brown hens fed on the Numurkah source at 200 g/kg had a significantly ($P<0.05$) higher feed intake that negatively affected FCR when compared to the Newcastle and Melbourne sources. Egg weights for white birds tended to be higher ($P<0.05$) at 120 g CM/kg. All other parameters evaluated in the present experiments indicated that CM supported good egg production. However, the results of these experiments need to be taken with care and in conjunction with the odour, colour and sensory evaluations results described below.

Egg mottling rate

The observations made at QPRDC on eggs derived from brown and white Hy-Line layers fed on graded levels of CSM from Brisbane and Narrabri sources and stored for six weeks (Tables 3.2.9 and 3.2.10) indicated that at least two out of three observers were able to detect mottling in stored eggs in both layer strains. This mottling effect was only significant ($P<0.05$) at 200 g CSM/kg for both bird strains. CSM from Narrabri which had a higher residual lipid level (34 g/kg) and CPFA (102.2 g/kg) in the meal (see Table 2.3.5) tended ($P>0.05$) to impair (by mottling) more eggs than Brisbane CSM, which had a lower lipid (18 g/kg) and CPFA (54.9 g/kg) content. Since CPFA is located within the lipid component, it is advised to use solvent extracted, low residual oil CSM at 150 g/kg maximum in laying hens diets in order to avoid any mottling and other disorders in eggs. Yolk colour in the control diet in white layers was suspiciously low due to the pigment in the diet that was some how absent. CSM from Brisbane gave eggs with significantly ($P<0.05$) higher yolk colour rating suggesting the development of an apricot yolk colour. This effect of Brisbane based diets on yolk colour pigmentation is difficult to explain since Narrabri CSM had a higher CPFA content. Although we did not offer CSM without ferrous salts to layer hens, we recommend to the use of ferrous salts at the ratio of 2:1 (ferrous salt : gossypol) in order to inhibit the potential effect of gossypol in yolk colour discoloration.

Sensory evaluation at Centre of Food Technology (summary)

Raw eggs from Hy-line Brown and Hy-line White layer hens fed on various canola and cottonseed meal sources at QPRDC were assessed at the CFT for the presence of a fishy odour. The highest incidence of fishy odour was found in eggs produced from Hy-line Brown hens and fed on canola meal, although a fishy odour was also detected in eggs produced from Hy-line White (W-36) hens fed on various levels of CM. In general, “fishy” tainted eggs from Hy-line Brown hens fed on CSM diets were not found and only one egg from Hy-line white layer hens fed on CSM diets was identified as having a “fishy” odour.

There was evidence of mottling in the yolks of the eggs from hens fed on cottonseed meal diets.

In the cooked eggs derived from hens fed on CM, significant differences ($P<0.05$) were found in the levels of overall odour intensity and prawny odour. Eggs from brown hens fed on Melbourne, and Newcastle at 200 g CM/kg and Numurkah at 120 g CM/kg, had significantly higher ($P<0.05$) overall odour intensity than the control eggs from Hy-line Brown hens. The overall odour intensity for the control eggs from Hy-line White hens was not significantly different ($P>0.05$) to any of the eggs from Hy-line White layer hens. Eggs from Hy-line Brown hens fed on diets with canola dietary treatments had a significantly higher ($P<0.05$) level of prawny odour than eggs from Hy-line Brown and Hy-line Whites fed on the control diet.

Although no significant differences ($P>0.05$) were found between individual canola treatments for flavour attributes, main effect differences were found. Eggs derived from canola meal dietary treatments had more overall egg flavour and yolk flavour and less egg white flavour than the eggs from hens fed on a control diet. The levels of seafoody flavour detected in all cooked canola meal derived eggs were very low, even though a prawny odour was detected.

No significant differences ($P>0.05$) were found in any of the odour attributes between eggs from hens fed the control diet and any of the eggs from the cottonseed meal dietary treatments. The only egg flavour difference across all eggs from hens fed on CSM dietary treatments and eggs from hens on the control diet was found in the level of yolk flavour. Overall, eggs from hens fed on cottonseed meal had more yolk flavour than the eggs from hens fed on the control diet and eggs from hens fed on diets containing Brisbane CSM (low in protein) treatment had the highest level of yolk flavour and lowest level of egg white flavour.

The panel used for this project was a trained sensory panel able to measure intensities of attributes in eggs. These people are not typical consumers and therefore the question of whether consumers would find any or all of these eggs acceptable/unacceptable cannot be answered within the scope of this project.

3.3 Layer General Discussion

Canola meal (CM) and cottonseed meal (CSM) are often limited in layer diets (4-10%) due to the presence of antinutritional factors (ANF) which negatively contribute to palatability problems, depression of growth, egg weight, unpleasant egg colour changes, and a fishy or 'crabby' taint in the eggs of brown layers.

Production performance of various layers strain

The overall results indicated that production performance of layer hens was not affected by the source and level of CM in the diet. Geographical location, processing extraction method and inclusion level did not affect the production performance and in particular did not increase mortalities at the higher inclusion levels. Therefore, 100, 150 or 200 g CM/kg resulted in a satisfactory layer performance in both brown and white birds. The overall results when feeding high levels of CM to Hy-line Brown and Hy-line White hens indicated that the production performance was not different ($P>0.05$) from the control diet for any CM source and level of inclusion (120 and 200 g CM/kg). Only Hy-line Brown hens fed on the Numurkah source at 200 g/kg had a significantly ($P<0.05$) higher feed intake that negatively affected FCR when compared to the Newcastle and Melbourne sources. Egg weights for white birds tended to be higher ($P<0.05$) at 120 g CM/kg. All other parameters evaluated in the present experiments indicated that CM supported good egg production.

Similar positive responses were obtained when CSM at similar levels were used in the diet. However, Isabrown birds significantly ($P<0.05$) gave higher egg production, lower egg weights with less feed intake and hence a better feed efficiency when compared with White Supertint birds which gave higher egg weights and thus similar egg mass.

The results with two sources of CSM fed at 120 and 200 g CSM/kg to both Hy-line Brown and Hy-line White layer hens indicated that satisfactory performance was obtained on high levels of CSM in layer diets. The CSM source and level in the diet did not affect any production parameters in brown layer hens. However a significant ($P<0.05$) increment in feed consumption, FCR and final body weight was observed in Hy-line White birds consuming CSM from Brisbane, indicating that the actual AME of diets using the Brisbane source were slightly lower than calculated values and birds had to consume more feed in order to satisfy their need for energy.

The effect of gossypol or cyclopropenoid fatty acids (CPFA) levels in the CSM diets did not affect egg production. The addition of ferrous sulphate at the ratio 2:1 also did not affect egg production as in this study.

Egg quality

The observations made on fresh eggs derived from Hisex Brown layers fed on graded levels of CM from Melbourne and Pinjarra sources indicated that these treatments led to the production of "fishy" tainted eggs. However, yolk colour was not affected by storage time or CM level in the diet and 'fishy' egg odour was substantially reduced after eggs were stored for 5 weeks.

When Isabrown layers received diets with graded levels (100, 150, and 200 g/kg) of CM (Newcastle source), these treatments led to production of "fishy or crabby" odour in fresh eggs at 150 and 200 g CM/kg and at all CM levels in stored eggs with no detrimental effect on yolk colour at any CM levels. However, "fishy" odour was not detected from eggs when these CM dietary treatments were fed to White Supertint birds indicating that in white birds the negative effect produced from sinapine was effectively metabolised to odourless compounds and therefore not detected by trained pannelists. White Supertint birds were able to support satisfactory performance (similar to brown strains) at high (100, 150 and 200 g/kg) levels of CM without affecting production performance and egg quality.

Although high levels of CM support good egg production in brown birds, not more than 100 g CM/kg could be added without risking "fishy" taint in eggs.

The odour and yolk colour evaluation carried out on eggs derived from birds fed on CSM indicated that unusual odour was not detected in fresh or stored eggs at all CSM levels. Brown discolouration was also not observed in all evaluated eggs indicating that CSM from Narrabri are low gossypol varieties. Any residual gossypol present in the meal was most likely inactivated by the addition of iron salts in the diets. An increased yolk colour value (12.42) was observed in stored (36 days) eggs derived from the 200 g CSM/kg. This yolk colour increment was not observed at 150 g CSM/kg, indicating the maximum inclusion level of 150g CSM/kg for egg production and acceptable egg quality.

Egg mottling effect

Eggs derived from brown and white Hy-Line layers fed on graded levels of CSM from Brisbane and Narrabri sources and stored for six weeks (Tables 3.2.9 and 3.2.10) presented some degree of mottling that was only significant ($P<0.05$) at 200 g CSM/kg for both bird strains. Because the lipid content of CSM tended ($P>0.05$) to produce mottling, it is advised to use solvent extracted, low residual oil CSM at a maximum of 150 g/kg in laying hens diets in order to avoid any mottling and other disorders in eggs. Although we did not offer CSM without ferrous salts diets to layer hens, we recommend the use of ferrous salts at the ratio of 2:1 (ferrous salt : gossypol) in order to inhibit the potential effect of gossypol in yolk discoloration.

Sensory evaluation

The panel used for this project was a trained sensory panel. These people are not typical consumers and therefore the question of whether consumers would find any or all of these eggs acceptable/unacceptable cannot be answered within the scope of this project.

Raw eggs from Hy-line Brown and Hy-line White layer hens fed on various canola and cottonseed meal sources were assessed for the presence of a fishy odour. The highest incidence of fishy odour was found in eggs produced from Hy-line Brown hens, fed on CM. In general, "fishy" tainted eggs from Hy-line Brown hens fed on CSM diets were not found.

In the cooked eggs derived from hens fed on CM, significant differences ($P<0.05$) were found in the levels of overall prawny odour. Eggs from Hy-line Brown fed on diets with canola treatments had a significantly higher ($P<0.05$) level of prawny odour than eggs from Hy-line Brown and Hy-line Whites fed on the control diet.

No significant differences ($P>0.05$) were found between individual CM treatments for flavour attributes. The levels of seafoody flavour detected in all cooked CM derived eggs were very low, even though a prawny odour was detected.

No significant differences ($P>0.05$) were found in any of the odour attributes between eggs from hens fed the control diet and any of the eggs from the cottonseed meal dietary treatments.