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Elimination of fishy taint in eggs from hens fed diets containing canola meal

A report for the Australian Egg Corporation Limited

by Dr Rider Perez-Maldonado and
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Foreword

The demand for eggs and chicken meat is rising with the increasing world population, and the higher disposable income within the developing world. Parallel with this is an increase in the demand for low cost, high nutrition layer and broiler feed.

Canola meal is a relatively low-cost source of highly digestible protein. It has a favourable fatty acid profile compared with other protein meals such as soybean meal and meat and bone meals. Canola grows well in the temperate regions of Australia and an increase in the use of canola meal would likely lead to a decrease in the use of soybean meal, much of which is imported from overseas.

To date, the use of canola meal in layer hen diets has been limited. The primary reason for this is that some brown layer hens, when fed canola meal, lay eggs with a fishy taint which is unacceptable to consumers. This fishy taint is due to a compound called trimethylamine, a metabolite of choline and choline esters. Canola meal is rich in both choline and choline esters, including the ester of choline and sinapic acid, sinapine. While choline is a requirement of layers feeds and is often supplemented, choline esters are poorly metabolised by some layers, and this appears to be the predominate cause of taint. The cause of the problem is a genetic defect which is present in brown layers. This problem is particularly significant in the Australian layer population, where brown layers comprise up to 90% of the total population due to consumer preference for brown eggs.

This phenomenon has been well described in overseas flocks fed canola (or rapeseed) meal which was also grown overseas. The main aim of this project was to identify the levels of sinapine and choline which give rise to unacceptable levels of taint in an Australian population of layer hens. With this knowledge, recommendations may be made as to the highest acceptable levels of canola meal within layer hen diets and avenues to limit taint can be explored.

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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Research Manager
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Abbreviations

AA – Amino Acid
AME-Apparent Metabolisable Energy
CM-Canola Meal
FMO – Flavin-containing Mono-oxygenase
HPLC- High Performance Liquid Chromatography
PCR – Polymerase Chain Reaction
TFA – Trifluroracetic Acid
TMA-trimethylamine
TMAO-trimethylamine oxidase

Executive Summary

Canola meal (CM) is a highly digestible and nutritious source of protein for animal feeds. However, its use in poultry diets has been limited due to the generation of a fishy taint in eggs when fed to brown layer hens. The fishy taint is due to a compound called trimethylamine (TMA), a breakdown product of choline and choline esters. CM is rich in choline (an essential nutrient), and is also rich in choline esters, in particular sinapine (an ester of choline and sinapic acid). Choline esters are poorly metabolised by layers, and will pass through to the gut where bacteria metabolise it, forming TMA. In most layers the TMA is effectively removed. However, some birds have an enzyme deficiency that allows the TMA to pass through to the egg, resulting in fishy taint.

This project investigated the relationship between choline, sinapine and CM and the generation of fishy taint in a population of Isa Brown layers. In feeding trials, choline was included in layer diets up to 3900 mg/kg, three times the recommended daily intake. No increase in taint was observed until the level of choline reached 2600 mg/kg. Some pilot trials of purified sinapine were conducted and the preliminary results suggested that it was more bioavailable than sinapine in CM. Due to the limited amount of sinapine purified we were unable to conduct detailed trials, but an inclusion of 1200 mg/kg (an amount approximately equivalent to an inclusion of 10% CM) led to a significant increase in taint. More detailed trials were conducted with CM which showed that levels up to 12% were well tolerated, while 15% led to an increase in the incidence and severity of taint.

On the basis of these results, we recommend that CM can be safely included at a level of 10-12%. At this inclusion level the amount of sinapine should not exceed 1.5 g/kg. It is further recommended that if CM is included, that no supplemental choline be added as the effects of sinapine and choline appear to be additive. As CM is rich in choline, diets including 10-12% CM should easily supply the recommended daily intake of 1300 mg/kg without supplementation.

The mutation of the gene resulting in fishy taint has recently been described and a PCR-based assay has been devised which can be used to screen for the mutant gene. Screening the breeding rooster population and eliminating those with the mutant gene is the most effective means by which fishy taint can be overcome. The German layer breeding company, Lohmann Tierzucht, who sponsored the research identifying and characterising the mutant gene, expects to market parental stock which is guaranteed free from taint by the end of this year. These new population of layers should be able to be fed maximal levels of CM (up to 20%) without any taint production or adverse egg production performance or quality. The increased use of canola meal at the expense of other protein meals, such as soybean meal and meat and bone meal, should result in significant savings in feed costs and improved nutrition in the eggs.

1. Introduction

The combination of economic growth, elevated disposable income amongst the masses, together with population growth has led to massive developments in the production and global demand for livestock products including eggs and chicken meat. It is predicted that the majority of this growth and food demand will occur in the Eastern Hemisphere; Asia, the Pacific Rim and the Indian sub-continent (Meggisson and Hall, 2005). As a result of this massive development, the demand for highly priced cereal grains and protein-rich ingredients to feed the requested livestock will come particularly from Asia region (Farrell, 1997). Therefore alternative protein sources are being examined.

Canola includes seed from *Brassica napus* and *Brassica rapa* with low levels of glucosinolates in the meal and low concentration of erucic acid as described by the Canola Council of Canada. Canola is one of the Australia's most important oilseed crops which has increased dramatically from around 100,000 tonnes in the early 1990s to a peak of 2.4 million tonnes in 2,000; since then, production has stabilised around 1.6-1.7 million tonnes. (Australian grain, 1998; AOF 2001; Mailer 2004) Canola meal, or flour, is the by-product of the seed after oil has been extracted either physically or by solvent extraction. The major driver of crushing demand is for oil, with meal largely a by-product.

Table 1: Australian annual canola seed and meal production (tonnes)

Year	Seed	Canola meal*
2003/04	1,622,000	274,000
2002/03	790,000	224,000
2001/02	1,608,000	239,000
2000/01	1,681,000	172,000
1999/00	2,402,000	222,000

* Of the canola meal that is produced in Australia, 75% is solvent extracted; 20% is expeller produced, and 5% is cold pressed

About 68% of Australian canola is exported with the remainder (32%) being crushed for oil production in Australia, rendering approximately 300,000 metric tonnes of canola meal (CM) (Mailer *et al*. 1999). Increased canola production and strong growth in the intensive livestock sector has contributed to the increased demand for CM in Australia. CM offers great potential for use in Australia's intensive livestock industry as an economical source of protein, energy, calcium and phosphorus. With increasing animal production and possible banning of meat and bone meal, the demand for vegetable protein meal and grain is likely to increase, leading to higher usage of CM in Australia at the expense of imported soybean meal. CM is a source of highly digestible protein (360-420 g/kg DM; Perez-Maldonado *et al*. 2001) with low anti-nutritive factors making it an ideal feed ingredient for intensive livestock use.

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 (AOF, 2001). In mid 1999 a joint funded research project was launched to investigate the practical inclusion levels for use in broiler and layer diets (RIRDC, project DAQ-264J). These experiments demonstrated that incorporation of CM up to 20% did not adversely affect egg production (Perez-Maldonado RIRDC internal report, 2000; AECL final report 2003). The use of CM also produced eggs with an improved

fatty acid profile (increased monounsaturated and polyunsaturated fats), which is associated with a decreased incidence of coronary artery disease in humans (Perez-Maldonado in press 2001; Farrell and Gibson, 1991). However, these trials also showed that the use of high levels of CM leads to the production of a "fishy" taint in eggs, particularly those from brown layer hens (Centre for Food Technology, 2001). As brown-shelled eggs comprise about 95% of those produced in Australia to satisfy consumer preference, this problem is of considerable importance to the Australian egg industry. Elimination of the "fishy" taint problem is required before the Australian egg industry can take full advantage of CM as an ingredient source in formulated diets for layer hens.

Various researchers have determined that the fish smell in affected eggs is caused by the presence of trimethylamine (TMA). A number of factors are involved in the generation of TMA (for a review, see Butler and Fenwick, 1984). The main factors involved in TMA generation are summarised in Figure 1 (below).

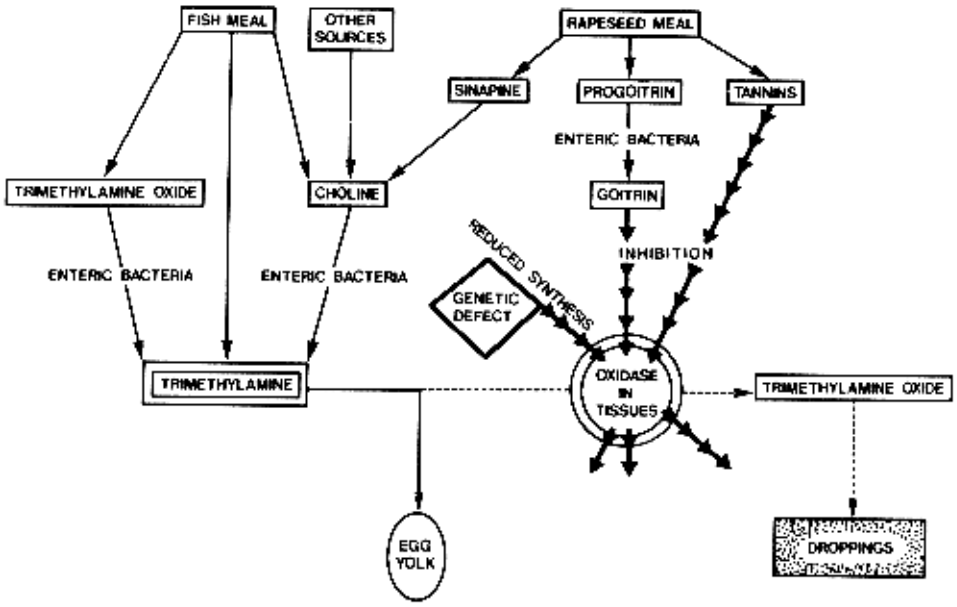

Figure 1: Factors involved in the production of trimethylamine taint in eggs (taken from Butler and Fenwick, 1984).

The major factor resulting in the generation of TMA in layers fed Australian CM is the high level of sinapine. Sinapine, an ester of choline and sinapic acid, is present in CM at about 10-15 g/kg (R Perez-Maldonado and T Treloar, unpublished results). Layer hens do not possess enzymes that effectively metabolise sinapine, which passes to the gastrointestinal tract where it is acted on by enteric bacteria to produce choline. The choline produced in the intestine is then metabolised further by the bacteria to produce TMA. In white layers, the TMA produced is effectively metabolised to odourless trimethylamineoxide, by a microsomal enzyme (TMA oxidase). A significant percentage of brown strain birds have genetically low levels of TMA oxidase. In these birds, when the amount of TMA absorbed from the gastrointestinal tract exceeds the capacity of the hen to metabolise and excrete it, some TMA is diverted to the developing ova, producing a "fishy" taint in the egg. Other research has shown that American and European CM is high in glucosinolates and condensed tannins, which have been shown to inhibit TMA oxidase. These compounds are found at very low levels in Australian CM, suggesting that the high sinapine level is the predominant cause for TMA contamination of eggs from layers fed Australian CM (Perez-Maldonado, 2003). Hence, the removal of sinapine from CM should eliminate the problem of "fishy" taint in eggs.

The effect of dietary choline on the generation of fishy taint has not been thoroughly investigated. Choline is an essential nutrient in layer diets and is a precursor of phosphatidylcholine, a phospholipid which is the primary component of cell membranes, and the neurotransmitter, acetylcholine. Due to the high phospholipid content of eggs, the choline demand of layer hens is relatively high. It has not been established whether high levels of choline itself can exceed the layer hen's capacity to metabolise it and generate taint.

The purpose of this project was to define the effects of sinapine and choline on the production of taint in a population of susceptible hens. This knowledge should allow for the development of strategies allowing for the incorporation of CM in layer hen diets, at the same time minimising taint production.

2. Objectives

1. To investigate the relationship between the amount of choline and sinapine in layer hen diets and the production of fishy taint in eggs.
2. To recommend an optimum layer hen diet (including canola meal) based on the maximum levels of choline and sinapine that can be used without generating excessive fishy taint.
3. To explore methods for the minimisation or eradication of fishy taint in eggs from hens fed canola meal.

3. General Methods

3.1 Measurement of sinapine concentration in canola meal by HPLC

The levels of sinapine in CM were measured by reverse phase HPLC. CM (100-150 mg) was extracted in 5 ml of 70% ethanol in water. Ten microlitre aliquots of this extract were then applied to the HPLC column and the compounds separated using an acetonitrile in water gradient. Chromatographic profiles were acquired at 214 nm and 330 nm and the 330 nm profiles was used for integration and calculation of sinapine concentration.

HPLC conditions

Column: 4.6 mm x 30 mm Phenomenex C18 (Cat. No. #OOA-451-EO)
Mobile phases: Phase A, water containing 0.1% trifluoroacetic acid (TFA); Phase B 60% acetonitrile in water, containing 0.085% TFA
Gradient conditions: 0 minutes – 17 minutes, 5% Phase B to 50% Phase B; 17 minutes-20 minutes, 50% Phase B-100% Phase B; 20 minutes -25 minutes, 100% Phase B wash; 25 minutes-33 minutes re-equilibration in 5% Phase B.

3.2 Purification of sinapine from canola meal

Sinapine was purified from canola meal using a modification of the method described by Clandinin, (1961). Batches of 10 kg of CM were extracted in 50 L of 95% ethanol with constant stirring for two hours at room temperature. The ethanolic extract was then concentrated by distillation at 80°C under pressure to produce a thick syrup. This syrup was diluted five fold with water and then reacted with a one fifth volume of 20% potassium thiocyanate (KSCN). This solution was stored in the cold room for 48 hours to allow the formation of crystals that were collected by filtration in a Buchner flask, and then dissolved in hot 95% ethanol. This was then stored in the cold room for 24 hours for crystals to form. The crystals (sinapine thiocyanate) were collected as above and then stored until needed for conversion to sinapine bisulfate.

Sinapine thiocyanate crystals were converted to sinapine bisulfate by reaction with sulfuric acid. Twenty gram aliquots of sinapine thiocyanate were dissolved in 400 ml of hot ethanol and 5 ml of concentrated sulfuric acid was added dropwise over a period of five minutes. This was then stored in the cold room for the development of crystals. The crystals were then redissolved in water (400ml) at room temperature and the solution was filtered. A further 5 ml of concentrated sulfuric acid was added to the solution as above and the solution was again stored in the cold room until crystals formed. The crystals were collected by filtration and then dissolved in ethanol. This was recrystallised and then the crystals were collected by filtration, dried and used as sinapine bisulfate.

3.3 Selection of Taint-Susceptible Layers

First bird selection*:* Day old pullets (360 Isa Brown) were purchased during August 2002 and reared according to breeder's specifications. At 17 weeks of age, pullets were placed in single-levelled cages housed in a conventional poultry house provided with adjustable shutters and ridge-vent, thermostatically controlled fans and water misters. They were subsequently fed with a Centre commercial mash diet until the commencement of the experiment. 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. At 35 weeks of age these hens were fed a diet containing 16.5% canola

meal in order to identify the layers susceptible to laying eggs with fishy taint. After three weeks in the experimental period, all eggs which had been laid in the previous 24-hour period were collected for testing. Three experienced scientific staff determined the presence of taint in eggs. Eggs were broken into a shallow dish and the presence of taint was assessed by sniffing. An egg was designated as being positive only if two of the three panellists detected fishy taint odour.

Second bird selection: During March 2004, a total of 500 mature Isa Brown layer hens were fed a diet containing 170 g canola meal/kg of diet to ensure the generation of fishy taint eggs in susceptible hens. The house facility and bird management was similar as mentioned previously. Layer hens evaluated during this second selection were used for experiment 2 and 3.

The ingredient composition of the diets used for selecting layer hens able to generate eggs with fishy odour are presented in Table 2.

3.4 Formulation of layer diets and layer experiments

During this study, all layer diets in experiments 1, 2 and 3 were formulated on total AA basis to the breeder recommendations for maximum production using the computer software program Feedmania (ABRI, University of New England). A commercial mineral and vitamin premix with a yolk pigmenter was added to all diets, which were prepared and offered as mash. All dietary treatments for experiment 1A and pilot study 1B were formulated to contain same energy (11.5 MJ/Kg), calcium (Ca, 3.6%) and available phosphorous (AvP, 0.35%). In experiment 2, dietary treatments contained 11.8 MJ/Kg, 3.6% Ca, 0.32-0.35 AvP. In experiment 3 diets contained 11.4 MJ/kg energy, 3.6-4.0% Ca, 0.32-0.35 AvP. Synthetic amino acids were added to all diets which were formulated to obtain commercial production performance.

Each dietary treatment in experiment 1A, was offered to five responder hens whilst three responders and three no responder birds were used in the pilot study 1B (purified sinapine evaluation). In experiment 2, diets were offered to six responder Isa Brown layer hens. In experiment 3 each diet was offered to 10 responder Isa Brown birds. In each trial, diets were offered over a 20 day experimental period after which egg were collected during a week period for sensory evaluation.

Table 2. Ingredient composition (g/kg) of layer diets for the selection of taint-susceptible layers

Ingredient	First selection	Second selection
Sorghum	591.7	534.5
Canola meal	165	170
Meat & bone meal	30	4.64
Soybean meal	0	56.1
Poultry offal meal	30	0
Sunflower meal	0	92.5
Limestone powder	47.3	44.4
Limestone grit	47.3	44.4
Oil	5.37	10
Tallow	65	20
Dicalcium Phosphate	8.6	12.6
Sodium Biocarbonate	0.649	1.72
Salt	0	1.98
Vitamins	1	1
Minerals	1	1
Choline	0.5	0
Pigmenter	2	2
Methionine	1.55	2.08
Lysine	3.12	3.06

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, 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.

Layer Experiment 1

This experiment evaluated the effect of dietary choline on the generation of eggs with fishy taint from selected susceptible birds. The ingredient and calculated choline composition of the diets used in experiment 1 (choline trial) are given in Table 3.

Dietary treatments included a basal diet with no added choline chloride. To this basal diet, graded levels of choline chloride were added to produce a diet with industry recommended levels of choline (1300 mg/kg, normal choline), and three diets with 1.5, 2 and 3 times above the recommended choline levels. These diets were offered to selected responders Isa Brown layers (Experiment 1, choline trail).

Table 3. Ingredient composition (g/kg) of layer diets containing between 1017 mg/kg (no added choline) and 3900 mg/kg choline for choline feeding trial

Ingredients	Without choline	Normal choline	1.5 X choline	2 X choline	3 X choline
Sorghum	300	300	300	300	300
Wheat	400.1	400.1	400.1	400.1	400.1
Poultry offal meal	30	30	30	30	30
Soybean meal	119.6	119.6	118.9	118.3	116.7
Sunflower meal	30	30	30	30	30
Limestone	89.0	89.0	89.0	89.0	89.0
Oil	5.4	5.4	5.4	5.4	5.4
Dicalcium phosphate	12.3	12.3	12.3	12.3	12.3
Salt	2.1	1.9	1.4	0.84	0.48
Sodium Bicarbonate	2.5	2.5	2.4	2.4	2.2
Vitamins	1	1	1	1	1
Minerals	1	1	1	1	1
Choline chloride	0	0.543	1.79	3.04	5.54
Pigmenter	2	2	2	2	2
Methionine	1.81	1.81	1.81	1.81	1.81
Lysine	2.72	2.72	2.72	2.72	2.72
Threonine	0.181	0.181	0.181	0.181	0.181
Calculated Choline (mg/kg)	1017.1	1300	1950	2600	3900

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, 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.

Layer Experiment 2

This experiment was conducted on a second set of layers identified as being susceptible to laying taint-affected eggs to evaluate the effect of choline level on the production of fishy taint. Dietary treatments included a basal diet with no commercial choline added, producing a diet containing 973 mg choline/kg of feed which is lower than normal recommended choline levels of 1300 mg/kg. Commercial choline chloride (60%) was added to this basal diet to produce additional diets containing 1300, 1625, 1950, 2275, and 2600 mg choline/kg of feed. Therefore, dietary treatments contained 1.25, 1.5, 1.75, and 2 times above the recommended daily choline allowance. To evaluate the effect of extracted purified sinapine on the generation of fishy taint, two diets with added purified sinapine (1.2 g/kg) with and without supplemental choline were incorporated into the experimental protocol. To evaluate the effect of dietary sinapine and choline derived from CM, a diet containing 120 g/kg CM, without purified sinapine or choline chloride was added to the experiment. Each of these dietary treatments was offered to six Isa Brown responder hens. To validate the hypothesis that non-responder birds do not generate eggs with fishy taint, a diet equivalent to twice the recommended level of choline was offered to non-responder birds. All dietary treatments were formulated after ingredients were chemically analysed for nitrogen and DM content. The ingredients and calculated energy, protein and choline levels of the diets used in experiment 2 are given in Table 4.

Table 4. Ingredient composition (g/kg) of layer dietary treatments containing choline between 973 mg/kg (no added choline) and 2600 mg/kg choline for choline feeding trial; containing sinapine bisulfate at 1200 mg/kg for a sinapine feeding trial; and a control diet containing 12% canola

Ingredients	Basal, 0 choline	Normal choline	choline 1.25 x	choline 1.5 x	choline 1.75 x	choline 2.0 x	Sinapine	sinapine +choline	canola 120g/kg
Sorghum	693	692	691	691	690	689	693	693	707
Meat & bone meal	67	67	67	67	67	66	67	67	67
Soybean meal	130	130	130	131	131	131	130	130	10
Canola meal	0	0	0	0	0	0	0	0	120
Limestone	96	96	96	96	96	96	96	96	96
Dicalcium Phosphate	3	3	3.1	3.1	3.2	3.2	3.0	3.0	2.6
Salt	1.6	1.4	1.1	0.9	0.6	0.3	1.6	1.3	1.2
Sodium Bicarbonate	0.8	0.5	0.9	1.3	1.6	2.0	0.8	0.4	0.8
Vitamin-Mineral	2	2	2	2	2	2	2	2	2
Choline chloride	0	0.63	1.25	1.88	2.50	3.12	0	0.73	0
Sinapine	0	0	0	0	0	0	0.12	0.12	0
Pigmenter	2	2	2	2	2	2	2	2	2
Methionine	2.9	2.9	2.9	2.9	2.9	2.9	2.9	2.9	2.9
Lysine	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Threonine	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.6
Arginine									1.4
Isoleucine									0.80
Sinapine bisulfate							1.2	1.2	
Calculated analysis									
Energy MJ/kg	11.8	11.8	11.8	11.8	11.8	11.8	11.8	11.8	11.7
Protein	17.5	17.5	17.5	17.4	17.4	17.5	17.5	17.5	16.4
Choline (mg/kg)	973	1300	1625	1950	2275	2600	973	1352	1352

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, 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.

Layer Experiment 3

This experiment was aimed to evaluate the effect of various levels of CM inclusion, of known sinapine content, in the diet of hens known to lay taint affected eggs. A total of six dietary treatments were offered to Isa Brown responder hens. A basal diet with no added choline without CM contained 1082 mg choline/kg. Four dietary treatments were included in which 60, 90, 120 and 150 g CM/kg diet was added to produce a choline level range (mg/kg) 1236, 1355, 1490 and 1595 respectively. To validate the hypothesis that non-responder birds do not generate eggs with fishy taint, a diet with added CM equivalent to 1595 mg/kg level of choline was offered to non-responder birds.

The ingredients and calculated energy, protein and choline levels of the diets used in experiment 3 are given in Table 5.

Table 5. Ingredient composition (g/kg) of layer diets containing 0, 60, 90, 120, and 150 g/kg of canola meal

Ingredients	Basal choline	CM 60 g/kg	CM 90 g/kg	CM120g/kg	CM150 g/kg
Sorghum	516.7	517.8	512.9	476.5	481.3
Maize	150	150	150	150	150
Meat and bone meal		61.3	61.1	70	70
Soybean meal	150	44.0	41.5		
Canola meal		60	90	120	150
Sunflower meal	63.5	69.0	47.2	77.5	45.8
Soyoil				10.7	8.2
Limestone	96.3	86.8	86.6	84.4	84.6
Dical Phosphate	11.6				
Salt	1.75	0.891	1.073	0.854	1.05
Sodium Bicarbonate	1.89	1.71	1.45	1.61	1.31
Vitamins-minerals	2	2	2	2	2
Choline chloride	0	0	0	0	0
Pigmenter	2	2	2	2	2
Methionine	2.00	1.77	1.78	1.67	1.71
Lysine	2.24	2.79	2.42	2.82	2.41
Calculated analysis					
Energy MJ/kg	11.4	11.4	11.4	11.4	11.4
Protein	16.2	16.8	17	17.1	17.2
Choline (mg/kg)	1082	1236	1355	1490	1595

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, 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.

3.5 Sensory Evaluation

Sensory evaluation was conducted by the DPI &F, Sensory Evaluation and Consumer Science Unit at Innovative Food Technology, Hamilton.

Panellists attended a brief round table training session before commencing the assessments. During the training session, the panellists assessed samples of eggs with varying degrees of fishy odour and were familiarised with the scale to be used.

For the actual assessments, each egg was cracked open into a 150 ml 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. All four panellists assessed each egg. Assessments were carried out in individual testing booths under white light. Panellists had filtered water freely available in their booths. Each panellist had a labelled control egg in their booth which they could refer to as required. The four panellists previously checked that the labelled control eggs did not have any fishy odour. The temperature of the eggs on assessment was $22^{o}C – 23^{o}C$.

Panellists indicated the level of fishy odour on a category scale defined as:
None: No fishy odour detectable
Trace: Slight fishy odour detectable
+ : Moderate fishy odour detectable
++ : Strong fishy odour detectable

To aid statistical analysis, the four levels were also given the numerical values 0-3, with 0 indicating the absence of taint and 3 indicating a strong fishy odour.

Panellists were also able to add any other comments regarding each egg. In each trial, panellists were offered either 2 or 3 eggs from each layer hen from different days. Each set of eggs were assessed separately. Within each set, the eggs from all treatments were randomised and presented to the panellists as lots of five samples. Panellists had a short break between each lot of five samples and a 20 minute break between set 1 and set 2. Control eggs, labelled with 3 digit blinding codes, were included where necessary to balance the design.

4. Results

4.1 Selection of birds susceptible to laying taint-affected eggs

Throughout the course of the project, two selection trials were conducted to select a sub-population of birds, susceptible to laying taint-affected eggs, from the wider community. In these selection trials, the layers were fed a diet containing CM expected to generate taint in susceptible birds.

In the first trial, 320 eggs from 360 layers were assessed. Of these, 47 eggs were deemed positive by all three investigators and a further 10 were deemed positive by two of the three. Hence, 57 out of 320 (17.8%) of the birds were selected as being susceptible under the design of the experiment. The results of this selection agrees well with previous reports (Perez-Maldonado, 2003) in which fishy odour in eggs (17 %) was detected when Isa Brown hens were offered a diet containing 150 g canola meal/kg of diet.

In the second selection trial, 500 Isa Brown hens were assessed. Of these, 42 were deemed positive by all three assessors, 17 by two of the three assessors and a further 18 by one of the three assessors. The severity of the taint produced in this trial was small compared with the earlier trial, and thus the confidence in the identification of the susceptible birds was low. For this reason, it was decided to continue feeding the birds for a further week and to reassess eggs from all of the birds which had previously been identified as positive by at least one assessor in the earlier trial. After the retest, 53 birds were determined susceptible to laying taint-affected eggs. This corresponds to an overall percentage of 10.6% of the original population of 500. This is significantly lower than the 17.8% derived from the first selection trial and a previous study in this facility (Perez-Maldonado, 2003).

4.2 Effect of supplemental choline on the production of fishy taint in susceptible chickens

The effect of supplemental choline consumption on the generation of eggs with fishy taint was investigated in two separate feeding trials using two distinct populations of susceptible layer hens (see Section 1.3.4). In both trials, a basal diet was formulated with the primary aim of keeping the choline level to a minimum. Supplemental choline chloride was added to this basal diet to give the specified choline levels in the diet.

The first feeding trials evaluated the effect of dietary choline from the level in the basal diet (1300 mg/kg) up to three times the recommended daily allowance for layer hens (3900 mg/kg). The composition of the basal diet is shown in Table 3. The results for this experiment are shown in Table 6.

Table 6: Effect of supplemental choline on the production of eggs with fish taint in susceptible Isa Brown layer hens

Treatment	Set 1		Set 2		Percentage for set 1 and 2 combined
	No. of eggs with fishy taint	Percentage	No. of eggs with fishy taint	Percentage	
A no added choline (1017 mg/kg)	1/5	20%	1/5	20%	20%
B normal choline (1300 mg/kg)	0/5	0%	0/5	0%	0%
C 1.5x normal choline (1950 mg/kg)	1/5	20%	0/5	0%	10%
D 2x normal choline (2600 mg/kg)	3/5	60%	2/5	40%	50%
E 3x normal choline (3900 mg/kg)	4/5	80%	4/5	80%	80%

These results showed that excessive supplementation levels of choline in the diets produced eggs with fishy taint in susceptible Isa Brown layers. A significant increase in the level of taint was produced when the level of choline supplemented in the diet reached 2600 mg/kg (twice the daily recommended allowance). A further increase in the level of taint was observed at 3900 mg/kg. The severity of the taint also increased as the choline levels in the diet increased (Figure 2).

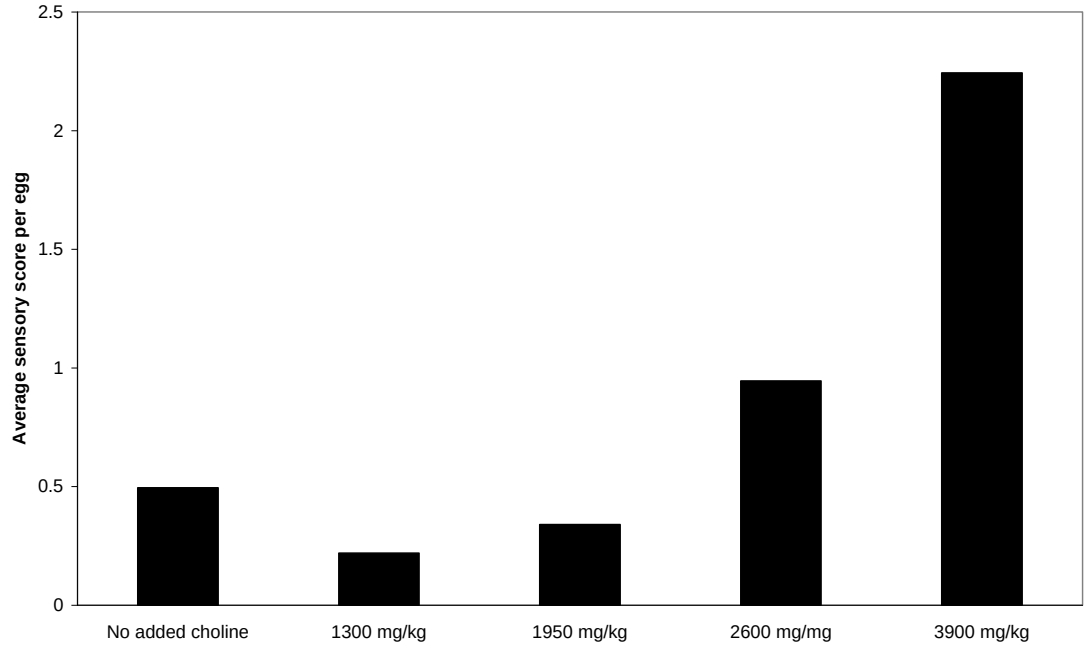

Figure 2: The effect of choline chloride supplementation on the severity of fishy taint in susceptible layers

The second trial assessing the effect of choline level on the production of eggs with fishy taint was performed on a second set of Isa Brown layers, identified as being susceptible to laying taint-affected eggs. This trial investigated levels up to twice the recommended daily choline allowance (2600 mg/kg), looked at six inclusion levels and used six layers per group. The results are shown in Table 7 and Figure 3.

The results from the second choline trial show that there is a significant increase in both the frequency of occurrence and severity of taint at a choline inclusion level of 2600 mg/kg. This agrees well with the results obtained from the first choline feeding trial shown above (Figure 1). Interestingly, the severity of the taint measured in the second trial was less than that seen in the first trial. In both trials the severity of the taint caused, by the feeding of choline (up to twice the recommended daily allowance) rarely rose above trace levels. This level of taint, while detectable by a trained panel, is unlikely to be noticeable to a naïve egg consumer. For this reason, on the basis of these results it is suggested that choline levels up to 2275 mg/kg (1.75 times the current recommended daily intake) can be provided in a layer diet without any significant increase in the level of fishy taint. To our knowledge, this is the first time that dietary choline has been shown to induce taint. Another study in Rhode Island Red layers investigated the effect of 0.05% supplemental choline in a cereal-based diet (Goh *et al*., 1979). This treatment did not lead to any increase in taint. The absolute level of choline (endogenous + supplementary) in this diet is unknown, but would be expected to be below 2600 mg/kg, the threshold level for increases in taint we have shown in our experiment.

Table 7: Effect of dietary choline on the production of eggs with fish taint in a second batch of susceptible Isa Brown layer hens

Treatment	Set 1		Set 2		Percentage for set 1 and 2 combined
	No. of eggs with fishy taint	%age	No. of eggs with fishy taint	%age	
A no added choline (973 mg/kg)	0/6	0%	0/6	0%	0%
B normal choline (1300 mg/kg)	0/6	0%	0/6	0%	0%
C 1.25x normal choline (1625 mg/kg)	0/6	0%	0/6	0%	0%
D 1.5x normal choline (1950 mg/kg)	1/6	17%	0/6	0%	8%
E 1.75x normal choline (2275 mg/kg)	0/6	0%	0/6	0%	0%
F 2x normal choline (2600 mg/kg)	1/6	17%	4/6	67%	42%
G 2x normal choline (2600 mg/kg)*	0/5	0%	0/5	0%	0%

* Diet G was identical to diet F but was fed to a population of non-responders.

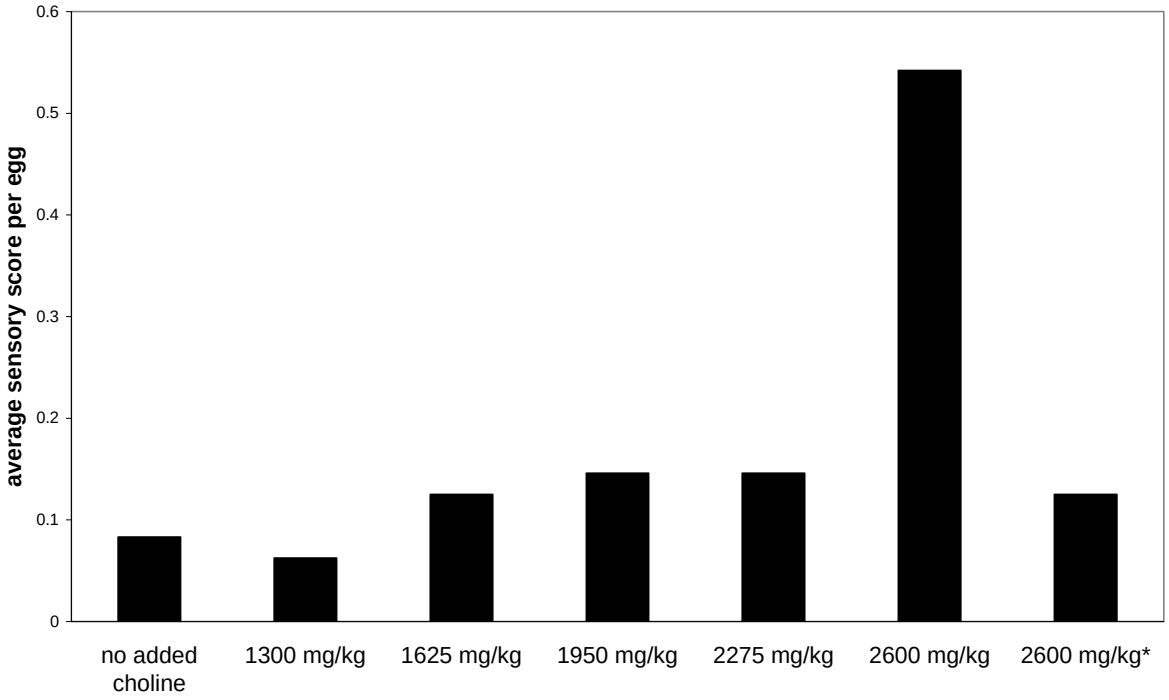

Figure 3: The effect of choline inclusion on the severity of fishy taint in second Isa Brown batch of susceptible layers (* Diet G was identical to diet F but was fed to a population of non-responders)

4.3 Effect of sinapine on the generation of fishy taint in susceptible chickens

4.3.1 Effect of purified chemical sinapine

The effect of choline in the presence of sinapine bisulfate was investigated in a pilot experiment. Two diets were fed to responder layers, one with a minimal choline concentration of approximately 950 mg/kg and 1200 mg/kg sinapine, and the second with the recommended choline intake of 1300 mg/kg in the presence of 1200 mg/kg sinapine. The results are shown in Table 8. The data showing the severity of the taint is shown in Figure 4.

Table 8: Effect of sinapine bisulfate (1200 mg/kg) in a basal (973 mg/kg) and normal (1300 mg/kg) choline diet

Treatment*	Set 1		Set 2		%age for set 1 and 2 combined
	No. with fishy taint	%age	No. with fishy taint	%age	
A Basal choline	0/6	0%	0/6	0%	0%
B Normal choline	0/6	0%	0/6	0%	0%
C Basal choline + sinapine	3/5	60%	2/5	40%	50%
D Normal choline + sinapine	2/5	40%	3/5	60%	50%

*This experiment was done concurrently with the choline feeding trial shown in Figure 2. The data shown for Groups A and B in this experiment is the same date shown for Groups A and B in Table 7.

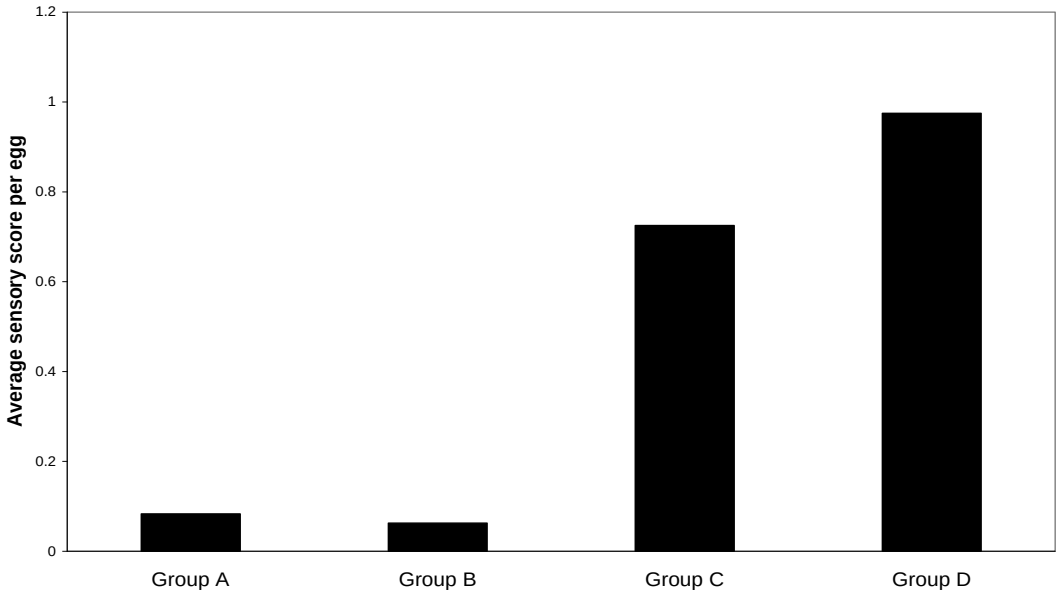

Figure 4: Average sensory scores for eggs from chickens fed a basal choline diet (Group A), a normal choline diet (Group B), a basal choline diet containing 1200 mg/kg sinapine (Group C) and a normal choline diet containing 1200 mg/kg sinapine (Group D).

The supplemental sinapine in the diet caused a significant difference in the incidence of taint compared with their relevant controls. There was a slight increase in the severity of the taint caused by the increase in the amount of choline in the diet in the presence of sinapine. A similar experiment in Rhode Island Red layers showed that 57% of the eggs from layers fed a diet containing 1.4% sinapine bisulfate were affected by taint (Goh *et al*., 1979), which is in reasonable agreement with our results taking into account the different layer populations used.

4.3.2 Effect of the endogenous sinapine in canola meal

After obtaining conflicting results using chemically purified sinapine, a further trial was performed assessing the effect of CM inclusion (of known endogenous sinapine content) in the diet of layer hens known to lay taint affected eggs. Larger numbers of layers hens were able to be fed for longer periods using the endogenous sinapine in CM. In addition, this approach took away the question of differences in bioavailability between endogenous sinapine and added chemically purified sinapine bisulfate. Results from the trial are shown in Table 9 and Figure 5.

These results show that a small amount of taint was present in all of the groups, including the group that was fed zero CM and also the group of non-responders fed the maximum inclusion level of 15%. There was no significant difference in either the occurrence or severity of taint up to an inclusion of 12% CM (containing 1.46 g/kg endogenous sinapine). Increasing the level of CM in the diet to 15% (containing 1.83 g/kg endogenous sinapine) led to a significant increase in the level of taint.

Unfortunately, due to the high level of endogenous choline in CM, it was not possible to control for the amount of total choline in the diets as the inclusion level of CM increased. Hence, the amount of endogenous choline in the 15% CM diets is well in excess of that normally used in layer diets. The total choline level in the diets increased from 1082 mg/kg in the diet with zero added CM, to

1595 mg/kg in the diet containing 15% CM. While our experiments have shown that this level of choline (when using supplemental choline chloride) does not result in an increase in either the incidence or severity of taint by itself (Figures 1 and 2), it is possible that such an increase in the amount of choline (endogenous choline from CM) may influence the observed levels of taint in the presence of sinapine. As CM is high in both choline and sinapine (endogenous source), the combined effect of these two compounds on the generation of taint is an important factor in the usefulness of CM in layer diets and requires more attention.

Table 9: The occurrence of fish taint in layers fed varying levels of CM

Treatment	Sinapine (g/kg)	Choline (mg/kg)	Set 1- Affected Eggs	Set 2- Affected Eggs	Set 3 – Affected Eggs	Overall Percentage of Affected Eggs
A 0% CM	trace	1082	1/10	2/10	1/10	13.3%
B 6% CM	0.73	1236	1/10	1/10	2/10	13.3%
C 9% CM	1.10	1355	1/10	1/9	2/10	13.8%
D 12% CM	1.46	1490	2/10	2/10	1/10	16.7%
E 15% CM	1.83	1595	3/10	4/9	3/9	35.7%
F 15% CM in non-responders	1.83	1595	1/10	0/10	0/9	3.4%

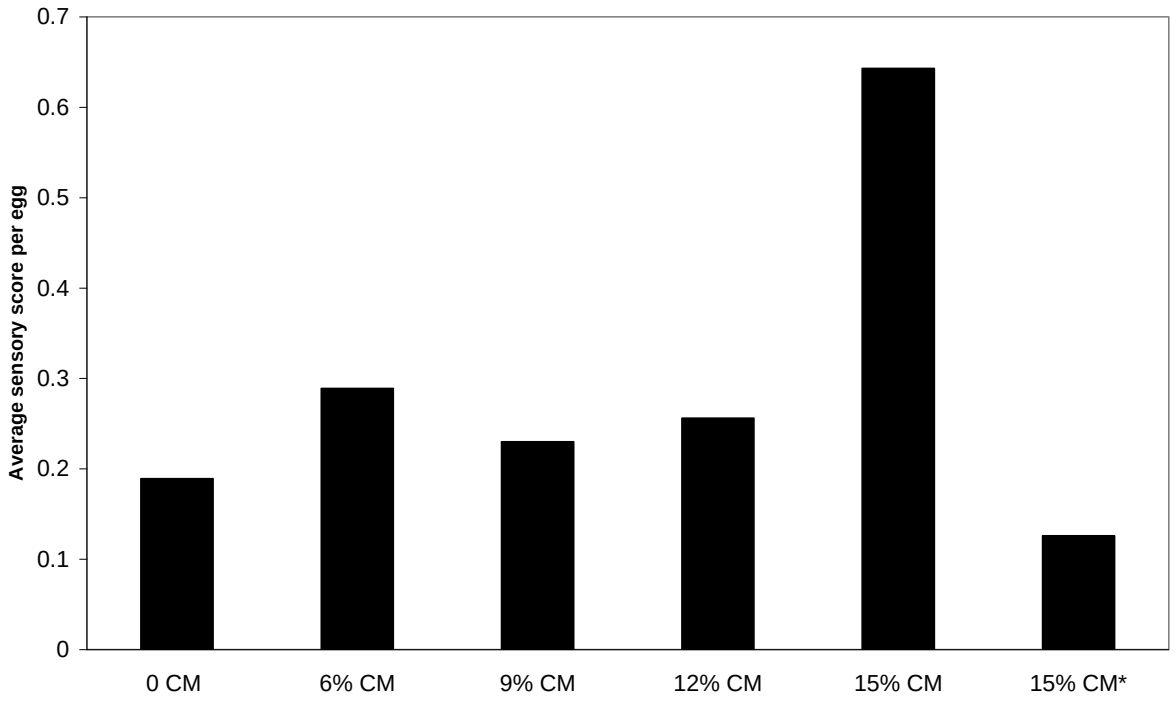

Figure 5: Severity of fish taint in eggs from layers fed varying levels of CM (* 15% CM was fed to a group of layers previously identified as non-responders)

5. Discussion of Results

These results have shown that both sinapine and choline can generate the production of fishy taint in eggs from Isa Brown layer hens independently of the origin (endogenous or supplemental). While taint was occasionally perceived in eggs from layers fed typical layer diets which contained no sinapine, and choline levels in the recommended range (1050-1300 mg/kg), taint levels appeared to increase at sinapine levels above 1.46 g/kg and choline levels of 2275 mg/kg. As Australian CM has sinapine levels between 1 and 1.5% (w/w) or 10-15 g/Kg, an inclusion level of up to 10-12% should not lead to any increase in taint in a similar population of layers. At an inclusion level of 9% and below, we found approximately 13% of eggs to have detectable levels of fishy taint (Table 9). The hens used in this trial were identified as being taint-susceptible from a larger population. Considering only 10-17% of the population were deemed taint susceptible, the incidence of taint in the overall population would be approximately 1.3-2.2% (calculated as the % affected eggs in the restricted population x the % susceptible hens selected for the trial). However, in most cases the levels of taint perceived (especially on diets which contained small amounts of sinapine and/or choline) were only minor and in many cases would not be noticed by naïve consumers.

A number of general observations about the relationship between dietary sinapine and choline, and the appearance of fishy taint in eggs can be made on the basis of these experiments. Our initial experiments attempted to minimise the variation in our treatment groups by removing the layers that were deemed not susceptible to laying taint-affected eggs. Our experiments have established that within the subset of layers that are susceptible, their sensitivity to choline and sinapine varies markedly. This was evidenced by the layers that produced taint-affected eggs in groups which were fed only a basal diet, containing no added choline or sinapine. This suggests that a small percentage of layers of this type will lay taint-affected eggs regardless of their diet.

Contrastingly, we have shown that the layers that do not lay taint-affected eggs will tolerate high levels of sinapine and choline without producing taint-affected eggs. This suggests that there is no restriction to the amount of CM that may be added to diets for these layers on the basis of the production of taint (of course there are other nutritional considerations which will restrict the use of CM). Therefore, if the population of birds that produce taint-affected eggs can be eradicated from the entire population, the level of CM in the typical diets of layers can be increased without the fear of an increase in the levels of fish taint in eggs.

It was also observed that there were considerable differences in the levels of taints perceived in eggs obtained from layers on different days. This suggests that either the levels of TMA in the egg change on different days or that the sensory evaluation used to determine taint was too subjective a technique to determine levels accurately. It would have been better to use a less subjective technique such as headspace gas chromatography. Unfortunately, headspace GC was not practicable for the numbers of eggs used in this project. However, it must be noted that there were only rare instances where the four evaluators had significant discrepancies in the level of taint detected in each egg, suggesting the sensory evaluation was satisfactory. It is possible that the level of TMA, and the amount of taint perceived, varies in eggs layed on different days. Variations in the amount of TMA produced by the gut microflora and absorbed into the bloodstream may account for some of this variability, but this requires further investigation.

On the basis of the feeding trials we have done in the project it would appear that CM can be included into the diets of layer hens up to 10-12% without increasing the incidence of fishy taint in eggs. As CM is relatively rich in choline compared with other protein sources, it would be anticipated that the CM and the other grain products should be sufficient to supply the entire choline requirement without supplementation. Further supplementation with choline chloride is anticipated to increase the levels of taint and should be avoided.

As discussed in the introduction, it has been demonstrated that layers that produce taint-affected eggs have a genetic deficiency in the enzyme TMA oxidase, which converts TMA (the compound responsible for the taint) to the odourless, TMA oxide (Butler *et al*., 1982). Very recently, the genetic defect causing this problem has been described (Honkaturia *et al*., 2005). A single point mutation in the FMO3 gene was shown to cause fish taint in eggs in an experimental population of Lohmann Brown and Rhode Island Red layer hens. This mutation causes a single amino acid change in a highly conserved motif of flavin-containing mono-oxygenase 3 (FMO3), the enzyme responsible for TMA oxidase activity (Honkaturia *et al*., 2005). These authors developed a PCR assay for detecting the variant protein and screened a number of commercial breeds to measure the allelic frequency of the variant. Isa brown layers had the highest frequency of the mutant of all the breeds tested (42% in a population of 71 individuals).

It has recently been reported that the German chicken breeding company, Lohmann Tierzucht (one of only three chicken breeding companies supplying the world market and holding a 25% market share) is currently using this assay to select brown chicken lines which are free from the defect (Food Production Daily.com, 2005; a copy of the report is shown in Appendix 1). This company plans to market the first chicken parent stock free from the defect by the end of this year.

A similar deficiency in this enzyme has been reported in dairy cattle (Lunden *et al*., 2002) and humans (Dolphin *et al*., 1997). In both cases the disorder was found to be an autosomal recessive disorder caused by a mutation in the flavin mono-oxygenase 3 (FMO3) gene which causes a truncation of the FMO3 gene product. This truncated protein is poorly expressed and inactive (Lunden *et al*., 2002). These authors have developed also developed a PCR-based screening assay which is being used to screen the Swedish dairy cattle breeding population in order to eradicate the defect (Leif Andersson, personal communication).

The metabolic defect of fishy taint has been described as an autosomal recessive trait in both chickens and dairy cattle. As an autosomal recessive trait, symptoms are generally seen only in those individuals with two defective gene copies. However, mild symptoms can sometimes be seen in heterozygotes (those with one normal gene and one defective gene) in some circumstances (Lunden *et al*., 2002). This inheritance pattern explains the results we have seen in our feeding trials, whereby a small number of layers will produce taint on control diets (those with two defective genes), a larger number will produce mild taint when exposed to high amounts of sinapine or choline (those with one good gene and one defective gene), whereas the rest do not produce taint regardless of the diet (two good genes). These layers with two normal genes would be what we have described as being non-responders, and comprise over 80% of the total number of layers screened in our initial experiments.

To eradicate the problem of fishy taint, it should only be necessary to screen the breeding rooster population for the mutated gene. If these are eradicated from the breeding population, it is impossible to produce hens that are homozygous for the mutated gene. Furthermore, the mutant gene from the maternal line would be lost through successive generations (the number of mutant genes passed on from the maternal line should halve in each successive generation). It is unclear whether Lohmann Tierzucht is using this approach.

If it proves impractical to remove the taint-susceptible chickens from the population using PCR-screening and selective breeding strategies, the maximum inclusion levels of CM may be increased by treatment of the CM. The most obvious treatment to remove sinapine from CM is enzymatic hydrolysis. As discussed earlier, sinapine is an ester of choline and sinapic acid, and as such would be expected to be a good substrate for a number of esterase enzymes. Recently, a Finnish group assayed a range of commercially-available enzymes for their ability to hydrolyse sinapine and found two enzyme preparations, Ultraflo L and ferulic acid esterase (FAE), which were effective (Vuorela

et al., 2003). Ultraflo L is a crude fungal enzyme mixture which is predominantly a β-glucanase, which is commonly used in the brewing industry. It would not normally be expected to hydrolyse sinapine, the activity seen in this study was ascribed to a contaminant (Faulds *et al*., 2002; Vuorela *et al*., 2003). This enzyme preparation is available in Australia and a sample was made available for assay by Novozymes Australia. This preparation did not contain any sinapine hydrolysing activity (data not shown). Ferulic acid esterase is an experimental product which is not yet available in Australia (S. Sidek, personal communication). Ferulic acid is structurally very similar to sinapic acid, and thus it is not surprising that sinapic acid esters are also good substrates for this enzyme. This shows that enzyme activities capable of catalysing this reaction exist and their use to remove sinapine from CM is feasible. It has also been demonstrated that heat treatment and treatment with strong alkali will also hydrolyse sinapine (Vuorela *et al*., 2003). Although these treatments can be easily applied to CM, they would be expected to decrease the nutrient value of the CM and would not be recommended.

Hydrolysis of sinapine produces choline (and sinapic acid). The maximum amount of sinapine found in Australian CM is approximately 15 g/kg. If all of this is hydrolysed, the choline content of the CM increases by approximately 5 g/kg. Although choline itself can produce taint in susceptible layers, this increase in choline would not be expected to produce taint. Interestingly, it has been shown that while 1.4% sinapine bisulfate gives rise to serious taint, the same quantity of base-catalysed hydrolysed sinapine did not give rise to taint (Goh et al., 1979), showing that the equivalent amount of choline and sinapic acid is acceptable in layer diets. Therefore, the enzymatic hydrolysis of sinapine would be predicted to reduce the incidence and severity of taint in susceptible layers.

6. Implications

1. Currently, the use of CM in commercial layer diets is capped at 7%, but is more commonly used at a lower level of 2-5% (Mailer, 2004) due to concerns over the production of fishy taint in eggs. By eliminating the addition of supplementary choline chloride, the amount of CM that may be included safely in layer diets without producing taint is much higher (up to 10-12%). By increasing the amount of CM, in the diets the amount of other protein meals, particularly soybean meal (SBM) and meat and bone meals, can be markedly reduced. There are a number of benefits to this. CM is currently priced lower than other meals (CM 335AUD/tonne; SBM 480AUD/tonne, L. Jamieson, Riverina Australia Pty. Ltd., personal communication 21/06/2005). Increasing the amount of CM in a layer diet from 5% to 10% at the expense of SBM would represent a saving of approximately $7 per tonne at this price. Use of CM, which is rich in choline compared with other protein meals, obviates the need for supplemental choline in diet formulations, representing a further saving (approximately $1/tonne). Hence, increased use of CM appeals as a means of reducing the cost of layer diets. In addition, it is likely that the use of meat and bone meals in layer diets will be curtailed or banned in coming years due to fears over the spread of disease and contamination issues. **CM represents a nutritious and economical plant protein source and should be more widely utilised in layer diets.**

2. The major limitation in the use of CM in layer diets is the production of fishy taint in eggs from brown layer strains. The genetic defect giving rise to this taint has recently been identified and characterised. The company that identified this genetic defect, expects to market parental stock free of the defect by the end of 2005. Layers without the genetic defect will not produce taint regardless of their diet and thus can be fed CM up to the maximum inclusion limits set by nutritional considerations (previous research has shown that inclusion up to 20% does not have any adverse effect on the egg production performance or egg quality in Isa Brown or Hy-Line Brown layers, Perez-Maldonado 2003). Hence, the use of layers from parent stock, which are genetically free from fishy taint, allows CM to be included up to 20% representing further savings. In addition, if these taint-free layers are used the low levels of taint which are encountered in normal circumstances, will be avoided increasing consumer satisfaction. **If genetically screened layers are used, high levels of CM can be included in layer diets, producing nutritious taint-free eggs at dramatically reduced feed prices.**

7. Recommendations

1. CM can be included up to an inclusion level of 10% (final sinapine level not exceeding 1.5 g/kg) without any increased incidence of fishy taint in Isa brown layers.

2. If CM is being included in layer diets, no supplemental choline chloride should be added.

3. As Australia is a leading producer of CM and Australian consumers strongly prefer brown-shelled eggs, it is recommended that Australian egg producers take advantage of recent advances (*i.e.* the availability of screening assays and defect-free breeding stock) to maximise the use of CM in layer diets.

4. It is apparent that Lohmann Tierzucht will be taking some patent position to protect the technology for this screening assay and/or the genetic composition of their parent stock. Other breeding companies and egg producers should carefully consider this patent position with respect to implementing any strategy to eradicate the problem of taint.

Appendix 1

The following information appeared at the following website www.foodproductiondaily.com on the 15/06/05.

Solved: The fishy brown egg problem

15/06/2005 - **One of the world's largest chicken breeding companies says it will begin selling stock that does not lay fishy smelling brown eggs, *reports Ahmed ElAmin.***

Chicken breeder Lohmann Tierzucht worked on the problem with Finland's MTT Agrifood Research, whose researchers announced yesterday they had identified a genetic defect in brown-coloured chickens that leads them to produce the fishy smelling eggs.

White chickens do not carry the genetic mutation, said Johanna Vilkki, the principal research scientist at MTT and leader of the research team.

"Furthermore, the eggs of brown chickens do not smell unless chickens which have inherited the genetic defect have been given feed containing an ingredient which triggers the taint, for example rapeseed," she said.

About five to 10 per cent of brown chickens that consume such feed produce tainted eggs.

"The fishy taint occurring in brown eggs has caused major problems for chicken breeders, producers and trade in the EU, where most hens' eggs consumed are of the brown variety," MTT said in a statement. *" In Finland, however, brown eggs account for less than 10 per cent of all eggs sold. At worst, the smell of rotting fish can be so strong that it persists even the cooking of eggs. When trade buyers find tainted eggs through spot checks, the producer faces significant loss as the entire batch must be withdrawn from sale. The problem is most severe in central Europe."*

After the discovery the MTT research team developed a procedure to test for the defect. Lohmann Tierzucht is already using the procedure to produce chickens free of the defective gene. The company, which is based in Germany, plans to market the first chicken parent stock free of the defect at the end of the year.

Usually genetic testing is done from taking blood samples, which is not only complicated and expensive, but may also be traumatic for chickens, Vilkki said. MTT's testing procedure only requires taking a sample from one feather to produce an unequivocal result. Cells from the feather shaft indicate whether a chicken has the genetic defect.

The procedure, which is being patented, makes it possible for breeders to select brown chicken lines for crossbreeding. The selection ensures that they produce progeny without the genetic defect.

Lohmann Tierzucht is one of only three chicken breeding companies supplying the international market. The company holds a 25 per cent share of the world market for stock chickens and is the European market leader. It supplies about 70 per cent of the Finnish market.

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