RURAL INDUSTRIES RESEARCH
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Developing a Slope Ratio Chick Assay for Amino Acid Availability

In this report three canola meals and one cottonseed meal were assayed for lysine availability

A report for the Rural Industries Research and Development Corporation

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Foreword

There is an increasing trend in the international poultry industry to adopt digestible amino acid values rather than total amino acid for diet formulation. A relatively well known, simple technique to determine undigested amino acids (AA) appearing at the terminal ileum or in excreta has been used to determine dietary amino acid digestibility.

However, dietary AA digestibility in the ileum section is the preferred measure (ileal digestibility), and the assumption is made that, if an AA is not recovered at the terminal ileum, then it has been absorbed in a form that can be utilised for maintenance and production by the bird. This is not always true, for reasons only partially understood, a fact which has led to the concept of "availability". Availability differs from ileal digestibility in that it involves a measure (liveweight gain or feed conversion ratio) of utilisation or potency of the absorbed AA. Available AAs are those which are actually supplied at the sites for protein synthesis for incorporation into body protein or metabolites for other body uses.

The proposed research will establish lysine availability values in canola meal (CM) and cottonseed meal (CSM) samples form major processors in Australia. Lysine has been chosen for this initial investigation because of its known susceptibility to heat damage during processing of CM and CSM. Therefore, this study will identify the extent to which the utilisation of the critical amino acid lysine is impaired in current CM and CSM from major Australian processors, some of which use more heat than others and also different oil extraction methods are used.

This project was funded by DPI-Queensland and assisted with support from industry revenues matched by funds provided by the Australian Federal Government.

This report is a new addition to RIRDC's diverse range of over 600 research publications. It forms part the Chicken Meat R&D program, which aims to support increased sustainability and profitability in the chicken meat industry by focusing research and development on those areas that will enable the industry to become more efficient and globally competitive and to assist in the development of a good industry and product image.

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Abbreviations

TAA	Total amino acid
DAA	Digestible amino acid
AA	Amino acid
AAs	Amino acids
CM	Canola meal
CSM	Cottonseed meal
CP	Crude Protein
CT	Condensed tannins
CPFA	Cyclopropanoid fatty acid
QPRDC	DPI, Queensland Poultry Research and Development Centre
DPI-Q	Department of Primary Industries Queensland

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Executive Summary

The slope ratio chick bioassay is the most appropriate model used for determining the availability of amino acids in feedstuffs. The aim of this project was developed to perfect the slope ratio chick bioassay methodology for the determination of available lysine in three canola meals (CM) and one cottonseed meal (CSM) obtained from various Australian oilseed processors.

There were three main stages during the course of this study; a pilot assay followed by two full-scaled replicated slope ratio assay trials to evaluate the availability of CM from Boree, Riverland and Melbourne and one CSM from Narrabri. The pilot study established the lower, middle and upper dose levels to ensure a linear response to standard lysine and the test proteins. The values of lysine levels selected in the pilot work to be used for the slope ratio bioassays were (g lysine/kg): 4.7, 5.4, 6.1, and 6.8. However, the first slope ratio assay failed to be statistically validated, with low bird feed intake, inappropriate feed texture and low dietary amino acid concentration in the diet being the major causes for failure. Thus, a modified slope ratio assay (third activity) was carried out which included steam-crumbled diets and a higher supply of amino acid, which improved by nearly 40% the overall bird feed intake and live-weight gain (LWG).

Central to the project was to prove and validate that the observed chickens responses were due to the amino acid under test. Therefore, the test of validity of the bioassay as described by Finney (1971) were statistically analysed for LWG and for feed conversion ratio (FCR). For LWG the slope ratio assay was conducted within the linear portion of the bird's LWG response to lysine, and that the intersecting lines regression model was statistically valid. But, the validity for FCR failed due to curvature present particularly in the basal diet points. However, bioavailability figures obtained with FCR are also reported as well as for LWG and explanations are given within the report.

The lysine bioavailability estimates for the four test proteins meals as assessed by chick slope ratio assay using either LWG or FCR as the criteria for availability were (LWG, FCR): CSM 0.555, 0.609, CM Boree 1.114, 0.919, CM Riverland 0.905, 0.878, CM Melbourne 0.828, 0.876. In general the estimates of availability when using FCR were in close agreement with the LWG figures.

Our CSM lysine availability was lower by 24 and 30% for LWG and FCR, respectively, than values reported in Major and Batterham (1981). This discrepancy may be attributed to differences in plant cultivars, processing methods for oil extraction, and the type of condensed tannins present in the meal.

Among the three CM, Boree exhibited the highest lysine availability compared with the other CM sources. Since Boree CM is an extruded extracted meal, this may suggest processing conditions as the main factor affecting lysine availability in these meals.

In our study, the availability of lysine from various CM was 49-100% higher than that of CSM. Plant processing conditions for oil extraction (particularly heating conditions), and differences in the type of condensed tannins which are present in both protein meals are responsible for the difference in lysine availability. Further experiments are needed to confirm this.

One of the main objectives of this research was to compare the slope ratio assay for availability determined in this work with the apparent ileal digestibility method undertaken during a previous study at QPRDC (see Perez-Maldonado, 2003). For CSM, both methods agreed well (slope ratio assay 0.555 c.f. ileal digestibility method 0.515), thus ileal digestibility of lysine appears to provide a reasonable estimate of lysine availability in CSM from Narrabri.

The lysine availability of CM from Boree, Riverland and Melbourne were 1.114, 0.905, and 0.828, respectively which were 51, 25, and 20% higher than the lysine ileal digestibilities obtained on similar CM samples using the ileal digestibility method. These results suggests that the two methods

compare poorly for CM. The explanation for this outcome is difficult and further investigation is needed. However, a recent review, (Sales and Janssens, 2003) indicated that use of markers such as chromium oxide may be the cause for low digestibility determinations. Therefore, CM digestibility can not be relied on as a measure of the lysine availability, but more evaluations of the lysine availability in other protein meals may give insight into the relative merits of the two methods.

In conclusion:

- A major aspect of this work was to establish the chick slope ratio assay methodology.

- Three major assays trials were conducted including a pilot assay and two full-scale replicated bioassays to establish the bioassay and to determine lysine bioavailability in four protein meals.

- Of the protein meals tested, cottonseed meal has much lower available lysine than canola meals. There are biological significant differences in available lysine among the three canola meals tested which are most likely due to primarily to the processing conditions used to extract oil to produce the CM.

- It was found that in CM ileal digestibility method underestimate lysine availability compared with the slope ratio bioassay and more research is needed in this area.

- Condensed tannins, a poliphenolic compound found in CSM and CM, may play an important role when assaying the digestibility and availability of lysine. Further research including other protein meals is needed to confirm this.

1. Introduction

1.1 Background to Proposal

Growth assays, such as the slope ratio assay for an available amino acid (AA) involve the addition of graded levels of a protein concentrate to a basal diet deficient in the AA under study, in order to establish a relationship between the growth response and the level of the AA in the diet (Carpenter *et al.* 1972). This ability is normally compared with the response obtained with a crystalline synthetic AA which is assumed to be 100% available. Chick growth assays have been developed to determine the availability of several amino acids (AAs) such as lysine (Major and Batterham 1981, Nordheim and Coon 1984, Parsons 1986 and Parsons *et al.* 1997), digestible lysine (Fernandez and Parsons 1996), methionine (Carpenter *et al.* 1972), digestible valine (Fernandez and Parsons 1996) and tryptophan (Harwood and Shrimpton 1969). Although such bioassays are not without their limitations they are the ultimate measure of an amino acid's availability and the only direct means whereby the validity of other less time-consuming methods can be tested (McNab 1994).

Numerous studies with pigs (Batterham 1994) have shown that for cereals and some good quality protein meals such as soybean meal, ileal digestible and available AA values are similar while for heat damaged meals ileal digestibility seriously overestimates availability. However, heat damage is not always implicated as ileal digestible lysine in lupinseed meal may also be poorly utilised.

After several literature searches, there appears to be no comparable data for poultry on the availability of digestible AAs in canola mela (CM), cottonseed meal (CSM) or other protein feedstuffs where digestibility and availability have both been determined in broiler chickens. Fernandez and Parsons (1996) used a slope ratio chick assay and a caecectomised cockerel digestibility assay to determine the availability of digestible lysine and valine in CSM and soybean meal. Their results indicate that in these feed ingredients, digestible lysine and valine are almost totally available. This finding confirmed earlier results from a similar study of digestible lysine, methionine and cystine in meat and bone meal (Parsons 1986) but contrasts with another report (Parsons *et al.* 1992) in which lysine digestibility overestimated its availability by 17% and 11% in autoclaved and non-autoclaved soybean meal, respectively. In Australia, Major and Batterham (1981) used a slope ratio chick assay to determine the availability of lysine in several protein meals using chicks. They found quite high values (>93%) for sunflower, soybean and fish meals but lesser values for meat and bone meal (86%) and CSM (83%). High lysine availability values imply high digestibility and therefore, most probably, close agreement between the two measures for those feed ingredients. These authors also reported higher lysine availabilities when measured in chicks than in pigs. While Batterham's (1994) conclusion that ileal digestibility may seriously overestimate amino acid availability in pigs cannot be extended to poultry, it nevertheless remains a possibility until clear evidence to the contrary is available.

At the DPI-Queensland's Poultry Research and Development Centre (QPRDC) in a 3½ year study (Perez-Maldonado, 2003) determined lysine digestibility coefficients for various CM and CSM samples ranged 0.69-0.76 and 0.45-0.56 respectively which are in agreement with data published earlier by Ravindran *et al*, 1998. It was interesting to observe that these digestibility range values for CM and CSM are much lower than the values reported for these meals on lysine availability these conflicting results need to be addressed. Thus, this proposed study had the follow objectives:

- Establish and validate a slope ratio chick assay, determine the availability of lysine in selected samples of Australian CM and CSM derived from different processors.
- To evaluate and compare the availability of lysine in similar samples used in previous studies evaluated at QPRDC.
- To compare lysine availability versus ileal apparent digestibility on similar samples of CM and CSM derived from various Australian processors.

2. Materials and Methods

There were three main stages during the course of this work; a preliminary study called a pilot study and two slope ratio assays used to evaluate lysine availability of three CM and one CSM.

For all stages of the work, birds were kept in a controlled environment (air-conditioned) building in metabolisable cages designed to house birds from 0-21 days of age. This new fully insulated building provides complete environmental control heating and cooling by an integrated, reversed cycle air-conditioning system with artificial lighting. Cages for brooding chicks from 0-21 days old measure 66 cm (long) x 35 cm (wide) x 40 cm (tall). Feed and water were available at all times in each cage from portable feeders and two nipple waterers, respectively. During the first activity (pilot study), male broilers (from 1 to 20 days of age) were caged at 5 birds/cage providing a floor space of 462 cm^2/bird. During the slope ratio assays, male broilers were caged at 6 birds per cage providing a floor space of 385 cm^2/bird. The facilities in which these trials were performed comply in all respects with SCARM welfare codes. The stocking density in the cages reached a maximum of approximately 17.1kg/sq m, which is well under the recommended maximum density of 30kg/sq m as stipulated by the Model Code of Practice for the Welfare of Animals – Domestic Poultry (4th Edition).

2.1 Background - pilot study and two slope ratio assays

Pilot study

Initially a pilot study was conducted since we had not previously performed this type of activity (slope ratio chick assay) at QPRDC. Thus, an un-replicated pilot slope ratio response to increasing dose level of standard lysine was performed to establish the lower, middle and upper dose levels to ensure a linear part of the response to standard lysine and test proteins. This pilot study also provided an insight into the availability of lysine from test proteins. The obtained values were then used in two replicated slope ratio assays to fully evaluate the responses to test proteins (three canola meals and one cottonseed meal) using a valid experimental design. By doing the pilot experiment first, we ensured the slope ratio assay worked without risking the use of a high number of birds, and that the availability of lysine was evaluated using a valid standard lysine linear regression.

Two slope ratio assays

After completion and analysis of the first slope ratio assay in late 2002, major errors were observed in the results which did not allow for valid statistical analysis of the obtained data. All four test ingredients (three canola meals and one cottonseed meal) produced or suggested a flatter response which did not "line up" well with the lysine response in the control diet (basal). Also, technical staff at QPRDC expressed concern over the low level of bird feed intake. After some discussion and advice it was decided to repeat this assay with some modifications on amino acid requirements and feed texture aspects of dietary treatment preparation. Therefore, during April-May 2003 a new slope ratio assay was carried out including similar test ingredients as in the previous assay performed during 2002, but this time steam pelleted-crumble diets were prepared instead of mash diets. The specification requirements for the formulation of diets were also lifted; all of these changes were aimed at improving the overall bird intake, feed physical structure and higher supply of dietary amino acid. The results of this new slope ratio assay were satisfactory and in combination with the pilot work are presented in this report.

2.2 Pilot study

Diets

During this preliminary work only one canola and one cottonseed meal were assayed. A total of fourteen diets were formulated which included the basal diet without added lysine (blanks), plus seven diets to determine the chick's response to standard lysine, and six diets which included either CM or CSM. The basal diet in the pilot study (Table 2.1) was formulated using wheat in combination with wheat gluten and wheat starch in order to produce a lysine deficient diet, chosen to be 4.0 g lysine/kg, but was actually 3.7 g lysine/kg (Table 2.2). The design was unreplicated and the experimental unit was a cage of five birds.

Table 2.1. Composition (g/kg) of the basal diet used for the pilot study

Ingredients	
Wheat	408.5
Wheat starch	265.2
Wheat gluten	238.4
Soybean oil	35
Dicalcium phosphate	24.4
Limestone	12.4
Salt	2.25
Sodium bicarbonate	3.1
Vitamin and mineral pre-mix	5.0
Choline	2.0
Coccidiostat	0.5
Arginine	1.51
Methionine	1.2
Threonine	0.62

Table 2.2. Composition (g/kg dry matter) of the wheat and wheat gluten used for the pilot study

Composition	Wheat	Wheat gluten
Crude protein	16.6	84.4
Dry matter	898	933
Fat	15	10
Neutral detergent fibre	90	44
Essential amino acids		
Threonine	4.0	18.7
Glycine	6.7	26.7
Valine	6.1	28.6
Cysteine + Methionine	3.6	28.5
Isoleucine	4.9	28.0
Leucine	9.1	51.9
Phenylalanine + tyrosine	10.9	67.7
Histidine	3.3	14.9
Lysine	3.5	10.9
Arginine	8.5	32.6

To each diet, amino acids such as arginine, methionine and threonine were added to ensure near adequacy according to the estimates of the National Research Council 1994. For each treatment containing a protein meal, three chosen levels of protein were used. Each of the protein concentrates (CM and CSM) was incorporated into the basal diet to provide three levels of lysine: low, medium and a high. The eight basal dietary treatments were formulated so as to be adequate in all amino acids except lysine. Basal diets were supplemented with seven levels of pure lysine (L-lysine

monohydrochloride anhydrous, 99% pure; P.T. Cheil Samsung Indonesia) (Table 2.3). In the case of CSM, ferrous sulphate was added to inactivate any effects of residual free gossypol. The diets were equalised in respect of calcium, available phosphorus, sodium, chloride, AME and crude fibre content. Dietary energy was maintained at 12.5 MJ metabolisable energy/kg diet using wheat starch and soybean oil as non-protein energy sources.

Table 2.3. Dietary treatments used in the pilot study

Diet	Treatment	Standard Lysine (g)	Meal (g)	Dietary lysine (g/kg)
1	Basal diet (Blanks)	0	0	3.7
2	Basal diet	1.674	0	5.0
3	Basal diet	2.702	0	5.8
4	Basal diet	3.731	0	6.6
5	Basal diet	4.76	0	7.4
6	Basal diet	5.79	0	8.2
7	Basal diet	6.82	0	9.0
8	Basal diet	7.834	0	9.8
9	Basal diet	8.765	0	10.6
10	Basal diet + canola meal	1.674	46.7	5.8
11	Basal diet + canola meal	1.674	93.5	6.6
12	Basal diet + canola meal	1.674	140.2	7.4
13	Basal diet + cottonseed meal	1.674	46	5.8
14	Basal diet + cottonseed meal	1.674	91.9	6.6
15	Basal diet + cottonseed meal	1.674	138	7.4

Broiler management and experimental design (pilot study)

For the pilot study, chicks (Cobbs) were purchased from a local commercial hatchery as day old. They were allocated to cages and offered a starter commercial diet *ad libitum*. During the course of the experiment the birds' health was closely monitored and any abnormality was recorded. At day 6 birds that were fasted overnight (day 5), were individually weighed and then assigned by stratified randomisation to their treatment cages to achieve five birds per cage. On day 15 after fasting three hours, remaining food and chicks from each pen were weighed. The experiment continued until day 19 when remaining feed and chicks were weighed (after three hours fast) and the experiment terminated. All birds were transferred to a floor pen shed.

Chick response to treatments was assessed in terms of live-weight gain per day (LWG) and feed conversion ratio (FCR) plotted against dietary lysine concentration, to check for linearity of the response.

2.3 Chick slope ratio assay

Protein concentrates, ingredients and chemical analysis

In this the main assay, lysine availability was estimated in four protein meals produced in Australia. The four meals were: two solvent extracted CMs from Melbourne (Cargill Aust.) and Numurkah (Riverland), and an expeller extracted CM from Boree (Borenore, NSW), together with a solvent extracted CSM (Cargil Aust, Narrabri). These protein meals were surplus from previous work on digestible amino acid performed at QPRDC (Perez-Maldonado, 2003) and were used to allow comparison between lysine digestibility versus availability of lysine. The composition of the wheat, wheat gluten and the four protein concentrates used during the assay are presented in Table 2.4 All feed ingredients were analysed for dry matter, fat, nitrogen and amino acids. The apparent

metabolisable energy (AME) used for wheat, sorghum, and protein meals were determined using the total collection method as described in Perez-Maldonado, (2003) on six birds per cage, replicated three times in male broiler chickens (14 to 21 days old). The AME for wheat starch, wheat gluten (Penford Australia Limited, 176-182 Marius St. Tamworth NSW 2340) and rice hulls (Coprice Feeds, PO Box 561 Leeton NSW 2705) were obtained from their manufacturers.

Nitrogen was measured using a CNS-2000 LECO combustor analyser (St. Joseph, MA, USA) whilst the analyses of amino acids were performed by ion-exchange chromatography (Waters HPLC) after hydrolysis with 6 M HCL at 110 °C for 18 h under reflux conditions (Spackman et al., 1958; Finalyson, 1964). Cysteine and methionine were determined as cysteic acid and methionine sulphone, respectively, by performic acid oxidation (Moore, 1963)

Table 2.4. Composition (g/kg dry matter) of the wheat, wheat gluten, sorghum, three canola meals from Melbourne, Riverland and Boree and a cottonseed meal from Narrabri used in the slope ratio assay

	Wheat	Wheat gluten	Sorghum	CM Melbourne	CM Riverland	CM Boree	CSM Narrabri
Crude protein	12.9	78.1	13.2	41.4	40.0	36.4	50.9
Dry matter	897	933	879	893	903	937	909
Fat	14	10	34	1.23	2.9	14.8	3.2
Phosphorous	3.3	0.19	ND	1.1	1.0	0.9	1.3
Calcium	ND	0.05	ND	0.71	0.74	0.75	0.23
Fibre	ND	4.4	ND	13.4	13.2	12.8	10.6
Essential amino acids							
Threonine	3.2	19.1	4.3	1.6	1.5	1.4	1.4
Glycine	5.2	26.5	3.9	1.9	1.7	1.6	1.5
Valine	5.2	29.0	6.2	1.9	1.7	1.6	1.7
Sulphur amino acid	3.6	22.5	4	1.5	1.4	1.3	1.2
Isoleucine	4.1	27.4	4.9	1.5	1.4	1.3	1.6
Leucine	8.0	51.0	16.5	2.71	2.5	2.3	2.7
Phenylalanine + tyrosine	8.5	64.8	11.1	2.6	2.3	2.2	3.8
Histidine	2.4	15.0	2.7	0.74	0.84	0.81	1.2
Lysine	2.7	11.0	2.4	2.0	1.9	2.0	1.9
Arginine	5.2	26.6	5.5	2.6	2.3	2.2	5.9
AME (MJ/Kg)				9.1	9.5	16.8	11.7
Antinutritional factors							
Free gossypol							0.07
CPFA (mg/kg)*							102.2
Glucosinolates umole/g				3.4	4.4	11.7	
Sinapine				13.2	13.7	14.5	
Free condensed tannins				53.1	57.9	50.4	43.5
Bound condensed tannis				42.8	34.2	38.6	24.6
Total condensed tannins				95.9	92.1	89.0	68.1

CM= Canola meal; CSM= Cottonseed meal; ND= not determined. *= cyclopropenoid fatty acids

Diets

The basal diet was formulated (Table 2.5) using wheat and sorghum, which in combination with gluten produced a lysine deficient diet (4.0 g lysine/kg). Rice hulls and sucrose were added to improve fibre content and texture in all pelleted-crumbled diets. The four protein meals were evaluated in a single assay, which involved the formulation of twenty-one diets: the basal diet without added lysine (blanks), four diets to determine the chick's response to pure standard lysine, and sixteen diets for the four protein meals at four levels each (Table 2.2). The four levels of lysine used to determine the chick's response to standard lysine were in increments of 700 mg lysine/kg (as determined in pilot study) and were obtained by the addition to the basal diet of L-lysine (monohydrochloride anhydrous,

99% pure; P.T. Cheil Samsung Indonesia). The protein meals (test ingredients) were incorporated into the diets to provide (g/kg diet): 4.7, 5.4, 6.1, and 6.8 of total lysine, with standard lysine at the expense of rice hulls, starch, and dextrose. Nutrient requirements used in the slope ratio assay were obtained from Baker *et al.,* 2002. The mineral and vitamin premix contributed (per kg diet): 3.75 mg retinol, 112 ug D_3, 30 mg α-tocopherol acetate, 3 mg menadione sodium bisulfite, 1.5 mg thiamine, 6 mg riboflavin, 3 mg pyridoxine, 15 ug B_{12}, 1.5 mg folic acid, 55 mg niacin, 15 mg Ca pantothenate, 180 ug biotin, 600 mg choline, 75 mg Mn, 75 mg Zn, 75 mg Fe, 900 ug Mo, 750 ug Co, 900 ug I, 6 mg Cu, 105 ug Se, 120 mg Banox.

All diets were isoenergetic (12 MJ/Kg diet) and were formulated using the Feedmania® software package (ABRI University of New England Armidale, NSW 2351).

Table 2.5. Composition (g/kg as is basis) of the basal diet used for the slope ratio assay

Ingredient	
Wheat	200
Sorghum	200
Wheat starch	60
Dextrose	57.8
Rice hulls	119.9
Wheat gluten	300
Soybean oil	10.96
Dicalcium phosphate	22.5
Limestone	12.3
Salt	1.67
Sodium bicarbonate	3.48
Vitamin and mineral pre-mix	5.0
Choline	1.0
Coxidiostat	0.65
Arginine	3.48
Methionine	1.2
Threonine	0.5
Estimated composition	
AME MJ/kg	12.5
Lysine	4.0
Protein (N X 6.25)	272

Broiler management and experimental design

Day old chicks from a hatchery were allocated to cages and offered a commercial starter diet *ad libitum*. On day seven, birds were fasted overnight, and on day eight individually weighed and assigned to cages by stratified randomisation so that cage mean bodyweights were nearly similar. On day nineteen (12 day experimental period) after a three hour fast, the chickens and any residual feed from each cage were weighed and the experiment terminated. Chick response (dependant variable in the multiple regressions) was assessed in terms of daily live-weight gain (LWG) and feed conversion ratio (FCR), whilst the independent variable in the regressions was g lysine/kg diet.

The experimental design for the lysine assay was a completely randomised layout of 92 cages (6 birds/cage) to which 21 diet treatments were randomly allocated so that there were 12 replicate cages for diet 1 (blanks) and 4 replicates of each of the other 20 diets (Table 2.6). Lysine availability was determined by "the slope ratio assay for relative potency" described by Finney (1971). In the chick slope ratio assay for availability used here, linear regression coefficients of response to increasing dose levels of test protein meals (CM or CSM) and standard amino acid (lysine) are calculated simultaneously in a multiple regression model. The ratio of the test protein meal's linear regression

coefficient to the standard amino acid's linear regression coefficient provides a measure of the availability of lysine in the test protein meal.

Table 2.6. Dietary treatments showing the sources of lysine added to the basal diet, as used in the slope ratio assay

Diet	Treatment	Pure standard Lysine (g)	Protein meal (g)	Dietary lysine (g/kg)
1	Basal diet (Blanks)	0	0	4.0
2	Basal diet 1	0.89	0	4.7
3	Basal diet 2	1.78	0	5.4
4	Basal diet 3	2.67	0	6.1
5	Basal diet 4	3.55	0	6.8
6	CM Melbourne	0	38.9	4.7
7	CM Melbourne	0	77.8	5.4
8	CM Melbourne	0	116.7	6.1
9	CM Melbourne	0	155.6	6.8
10	CM Riverland	0	41.2	4.7
11	CM Riverland	0	82.4	5.4
12	CM Riverland	0	123.5	6.1
13	CM Riverland	0	164.7	6.8
14	CM Boree	0	38.9	4.7
15	CM Boree	0	77.8	5.4
16	CM Boree	0	116.7	6.1
17	CM Boree	0	155.6	6.8
18	CSM Narrabri	0	41.2	4.7
19	CSM Narrabri	0	82.4	5.4
20	CSM Narrabri	0	123.5	6.1
21	CSM Narrabri	0	164.7	6.8

2.4 Statistical analyses

Overview of the slope ratio assay for lysine availability

An intersecting lines multiple linear regression model for a slope ratio assay was fitted, following methodology as described in Finney (1971). Whereas Finney's example was of an assay with a standard and only one test substance, leading to a regression model with just 2 independent variables, our assay involved a standard (basal diet BAS) and four test protein meals: CSM, BOR (CM Boree), RIV (CM Riverland), and MEL (CM Melbourne), leading to a regression model with five independent variables. Also, our lysine assay involved a "blanks" level, which was non-zero whereas the slope ratio assay methodology is usually presented with the blanks X value at zero.

The bioavailability of lysine in each of the test protein meals relative to lysine in the basal diet was estimated as the ratio of the linear regression slope coefficients of test over basal. Approximate 95% confidence limits were calculated for the bioavailability estimates (Finney, 1971).

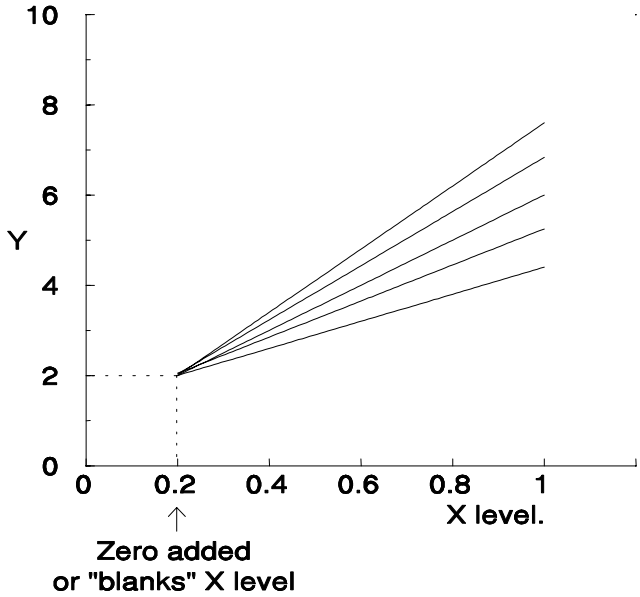

Figure 1

Intersecting lines multiple regression model when lines intersect at non-zero "blanks"

A family of intersecting lines (also called convergent lines, Figure 1) have a common point of intersection. If the low value, called the zero added level or "blanks" level, is zero then the family of lines can be said to have a common intercept. Otherwise, when the blanks X level is non-zero as shown, a simple re-scaling of the X-axis to X' = X - 0.2 where the X'-axis is now called "added X level", is equivalent to the common intercept case. This distinction seems trivial but is important when setting up the X matrix for the regression.

The multiple regression shown in Figure 1 can be represented either as a single multiple regression equation involving five X variables:

$Y = a + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_4 + b_5X_5$

with "suitably defined" values for the X variables, or as a set of five individual single-variable (simple linear regression) equations:

$Y = a_1 + b_1X_1$, $Y = a_2 + b_2X_2$, $Y = a_3 + b_3X_3$, $Y = a_4 + b_4X_4$, $Y = a_5 + b_5X_5$

(Note that although the latter representation is not the same as fitting separate regressions for each of the five X variables, it is still an intersecting lines model fitted simultaneously to all of the data).

These five individual equations were derived from the single multiple equation by setting the X values for the other four X variables not in the equation equal to the "blanks" value. And if the "blanks" value is non-zero as shown in Figure 1, the five resulting equations have different intercepts, as shown, even though the set of lines they describe have a common point of intersection. But, as mentioned previously, if X is simply re-scaled to X'= (X – blank value) then the two representations of the intersecting lines equations both simplify to a common-intercept form:

$Y = a + b_1X'_1 + b_2X'_2 + b_3X'_3 + b_4X'_4 + b_5X'_5$

and

$Y = a + b_1X'_1$, $Y = a + b_2X'_2$, $Y = a + b_3X'_3$, $Y = a + b_4X'_4$, $Y = a + b_5X'_5$

Tests of validity

Fundamental requirements for validity of the slope ratio assay are:

- That the regression lines for standard and test (the 5 lines in our case, one for each of the 5 diets) have a common point of intersection at an X value within the range of the X values of the experimental design, usually at X = zero (or x= blanks level if non-zero). Finney (1971) describes a statistical test of significance for this.

- That if there is a common point of intersection, then the Y value for the common point of intersection and the Y value of the mean response measured at X= blanks level, are not significantly different - this is also testable as part of the regression modelling.

These tests labelled as the "intersection" and "blanks" terms in the regression ANOVA table, given elsewhere, are sequential with the test for a common intercept preceding the test for its coincidence with the blanks mean.

So, in a hypothetically ideal slope ratio assay without error, the regression lines when fitted to the non-blanks data would intersect at the point: (X= blanks level, Y= blanks mean) as shown in Fig. 1.

Accommodating the second validity requirement
There are 3 ways in which the second validity requirement (that the lines pass through the blanks mean) might be accommodated through choice of the type of regression model:

i) All lines are forced to pass through the blanks mean point, or

ii) A significance test is done to compare the blanks mean with the Y value of the common point of intersection when the regression lines are fitted to only the non-blanks data points. If there is a significant difference, then the blanks mean is not included in the data used for fitting the regression – this is the approach recommended by Finney (1971). or

iii) The blanks mean point is not given the special importance as described in i) above, and it is regarded as just another data point in the fitting of the regression model; ie. the blanks mean is included in the fit without the testing described in ii) above.

Our approach to dealing with the validity requirements differs from Finney. We accept the first requirement, that there is a common point of intersection, as fundamental. And, as in Finney, we test whether the blanks mean differs significantly from the common point of intersection of the non-blanks regression lines, but after an informal graphical assessment to check that the lack-of-fit at the blanks point is no worse than at the other data points, we include the blanks mean in the final fitted regression. This seems to be the most reasonable compromise between the 3 choices of regression model listed above.

Setting up for fitting the regression model
The experimental design for the lysine assay was a completely randomised layout of 92 cages to which 21 diet treatments were randomly allocated so that there were 12 replicate cages for diet 1 (blanks) and 4 replicates of each of the other 20 diet treatments. This design provides 92 data points in total for the regression but it's the variation between the 21 design points (diets) that is of interest – in ANOVA terms, the broad split-up of variation is as follows:

Replicates within design points = ERROR	71 df	Intersections	4 df
		Lack of fit	10 df
Total	91 df	——	

The equation for estimating the intersecting lines multiple linear regression model, relating responses y to values X_{ik1} , … X_{ik5} of the five X variables, is:

$y_{ik} = a + \{ b_0X_{ik0} \} + b_1X_{ik1} + b_2X_{ik2} + b_3X_{ik3} + b_4X_{ik4} + b_5X_{ik5} + e_i + e_{ik}$,
where :
y_{ik} are values of either LWG or FCR;
j= 1 to 21 and either k= 1 to 12 (for j= 1, the blanks) or k= 1 to 4 (for j= 2 to 21, the design points);
a is the common intercept;
X_0 is a (0/1) dummy variable for "blanks" which may be in or out of the model depending on a significance test;
X_{ik1} , … X_{ik5} are values of the X variables for the 5 diets (% lysine in the basal diet BAS and the 4 test diets CSM, BOR, RIV, MEL);
e_i , e_{ik} are lack-of-fit and experimental error terms, respectively.

The X values (g lysine/kg) written in matrix form, where rows of the matrix are design points and columns are X variables, is as follows:

Table 2.7. X matrix for fitting the intersecting lines multiple regression model (Replicates, which just correspond to copies of rows of the matrix, are indicated but not shown)

Diet	j	k	blanks	BAS	CSM	BOR	RIV	MEL
(Treat)	(Point)	(rep)	X_0	X_1	X_2	X_3	X_4	X_5
Blanks	1	1-12	1	0.40	0.40	0.40	0.40	0.40
BAS	2	1-4	0	**0.47**	0.40	0.40	0.40	0.40
BAS	3	1-4	0	**0.54**	0.40	0.40	0.40	0.40
BAS	4	1-4	0	**0.61**	0.40	0.40	0.40	0.40
BAS	5	1-4	0	**0.68**	0.40	0.40	0.40	0.40
CSM	6	1-4	0	0.40	**0.47**	0.40	0.40	0.40
CSM	7	1-4	0	0.40	**0.54**	0.40	0.40	0.40
CSM	8	1-4	0	0.40	**0.61**	0.40	0.40	0.40
CSM	9	1-4	0	0.40	**0.68**	0.40	0.40	0.40
BOR	10	1-4	0	0.40	0.40	**0.47**	0.40	0.40
BOR	11	1-4	0	0.40	0.40	**0.54**	0.40	0.40
BOR	12	1-4	0	0.40	0.40	**0.61**	0.40	0.40
BOR	13	1-4	0	0.40	0.40	**0.68**	0.40	0.40
RIV	14	1-4	0	0.40	0.40	0.40	**0.47**	0.40
RIV	15	1-4	0	0.40	0.40	0.40	**0.54**	0.40
RIV	16	1-4	0	0.40	0.40	0.40	**0.61**	0.40
RIV	17	1-4	0	0.40	0.40	0.40	**0.68**	0.40
MEL	18	1-4	0	0.40	0.40	0.40	0.40	**0.47**
MEL	19	1-4	0	0.40	0.40	0.40	0.40	**0.54**
MEL	20	1-4	0	0.40	0.40	0.40	0.40	**0.61**
MEL	21	1-4	0	0.40	0.40	0.40	0.40	**0.68**

3. Results and Discussions

As mentioned in the materials and methods section, three activities were conducted during the course of this project; a pilot study, and two slope chick ratio assays. During the pilot study it was possible to establish the lower, and upper doses necessary to ensure that the main assay was conducted in the linear part of the response to standard lysine and test proteins. The values of lysine levels selected in the pilot work to be used for the slope ratio bioassays were (in g lysine/kg diet) 4.7, 5.4, 6.1, and 6.8. However, the first slope ratio assay did not work as expected and reasons were stated previously. Therefore with the experience collected from the pilot study and the failed bioassay activity, a modified slope ratio bioassay was carried out and the results from this third assay are presented in this section.

3.1 Bird performance and diets

The chick's performance mean values for live-weight gain (LWG) and feed conversion ratio (FCR) when birds were offered the basal diet and each of the protein meals are presented in Table 3.1
All chicks throughout the 12 days experimental period remained healthy, with only 0.9% mortalities. (Five chicks out of 552 birds were culled and euthanised; there were two in the basal diets at 0.54 and 0.61% lysine, one in the CSM diets at 0.68% lysine, and two in the CM diets, Boree and Riverland at 0.68 and 0.54% lysine respectively). This low number of mortalities is a positive indicator of the overall results.

Table 3.1 Live-weight gain (g/bird; LWG) and feed conversion ratio (feed intake/weight gain; FCR) of chicks fed on three canola meals (CM) and one cottonseed meal (CSM) diets for the chick slope ratio assay for lysine.

Form of lysine addition					
Lysine dose level (g/kg)	Basal diets (Pure lysine)	CM			CSM
		Boree (extruded)	Riverland	Melbourne	Narrabri
Live-wt gain (g/bird)					
0 (blanks)	98.6	-------	-------	-------	-------
4.7	116.5	127.5	117.7	126.7	117.1
5.4	175.2	167.5	144.8	146.8	135.1
6.1	188.9	198.1	191.3	171.1	140.6
6.8	239.3	264.1	220.0	218.1	176.1
LSD (P=0.05) Blanks vs any of the others = 17.4 LSD (P=0.05) between the others = 21.3					
Feed conversion ratio					
0 (blanks)	2.742	-------	-------	-------	-------
4.7	2.336	2.415	2.524	2.383	2.541
5.4	2.096	2.093	2.244	2.162	2.334
6.1	1.982	2.128	2.025	2.021	2.258
6.8	1.812	1.822	1.866	1.950	2.102
LSD (P=0.05) Blanks vs any of the others = 0.099 LSD (P=0.05) Between the others = 0.122					

The mean LWG obtained over the 12 days experimental period of chicks offered the basal diets ranged 98.6 to 239.3 g/bird (8.3 to 19.9 g/bird/day) with a mean feed intake (FI, not shown in table) of 22.5g/bird/day. These figures for LWG and FI on the basal diet were 35 and 37%, respectively, better than the corresponding values obtained in the previously failed chick ratio assay. That previous assay had treatment diets with the same lysine content but were prepared and offered as mash, whereas in this third assay diets were crumbled rather than mash.

Slope ratio bioassays are complex in terms of experimental design and diet formulation, and dietary treatment preparation and bird management. However, the improved results for FI, LWG and FCR due to pelleted-crumbled diets, higher fibre inclusion level and higher amino acid supply to chicks demonstrated that it's possible to achieve substantial gains in the quality of the data with seemingly small changes to methodology.

3.2 Slope ratio bioassay statistical analysis

Results of the regression analysis of variance (ANOVA) for LWG and FCR are presented in Tables 3.2, and 3.3 respectively.

The tests of validity of the bioassay as described by Finney, (1971) are shown in the ANOVA tables (3.2 and 3.3). For LWG (Table 3.2), the results showed that the overall regression was significant and the tests for "intersections and "blanks" were each non-significant (P>0.01) indicating that the validity requirements were met. However, the ANOVA for FCR (Table 3.3) shows that the validity tests failed.

Table 3.2. Regression ANOVA table for live-weight gain

	DF	SSQ	MSQ	F	
Regression	5	191,247	38,249	167.0	**
Blanks	1	1,079	1,079	4.7	NS
Intersection	4	729	182	0.8	NS
Pooled lack of fit	10	4,199	420	1.8	NS
Between points	20	197,254			
Pure error (rep cages)	71	16,273	229		
Total	91	213,257			

** and NS denote significance and non-significance, respectively, at the 1% level.

Table 3.3. Regression ANOVA table for feed conversion ratio

	DF	SSQ	MSQ	F	
Regression	5	6.8338	1.3668	183.5	**
Blanks	1	0.1717	0.1717	23.1	**
Intersection	4	0.1157	0.0289	3.9	**
Pooled lack of fit	10	0.1572	0.0157	2.1	NS
Between points	20	7.2784			
Pure error (rep cages)	71	0.5287	0.0074		
Total	91	7.8071			

** and NS denote significance and non-significance, respectively, at the 1% level.

The fitted multiple regression models for LWG and FCR are presented graphically in Figures 2 and 3 respectively.

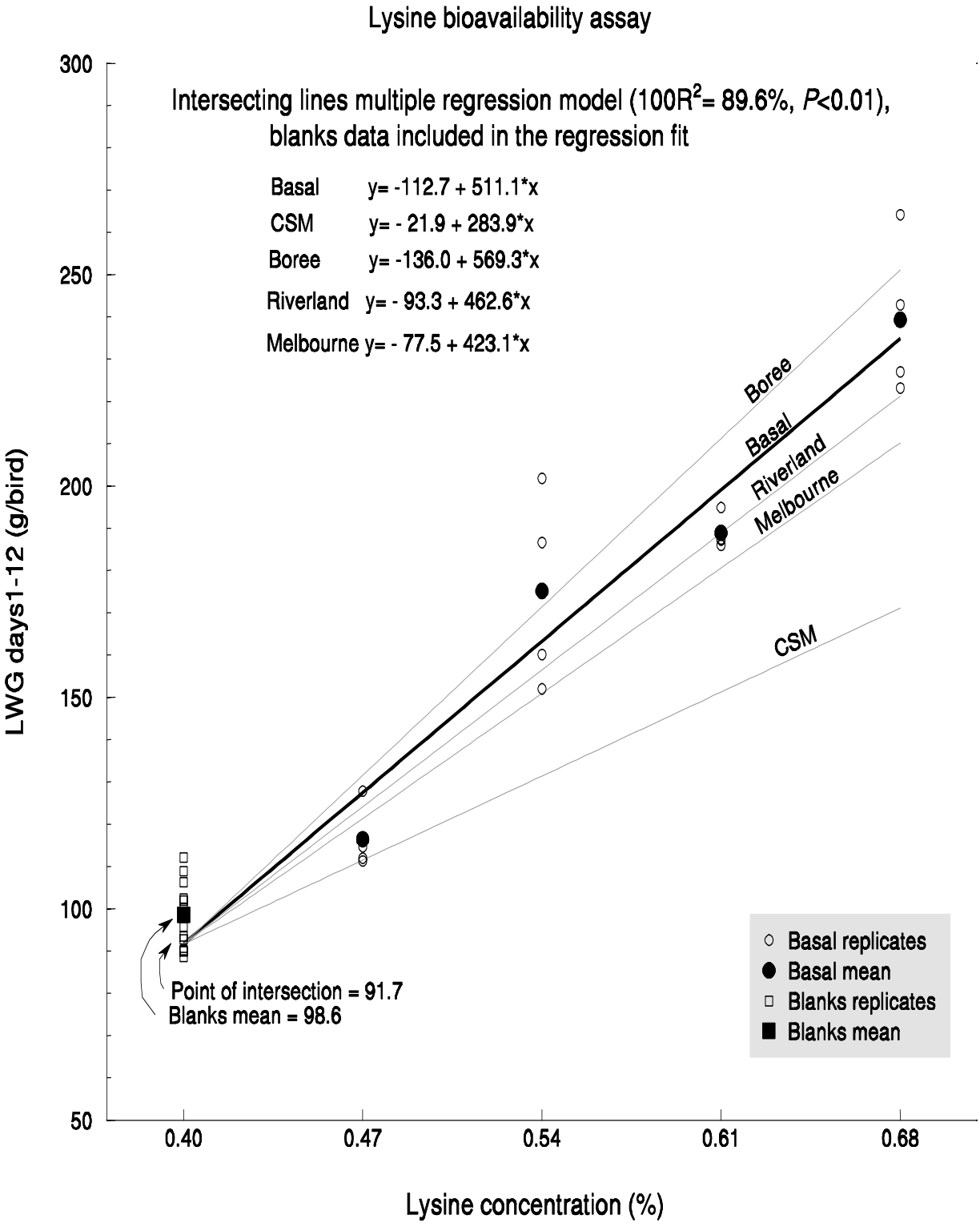

Figure 2. Graph showing the multiple regression model (with blanks data included in the regression fit) for live-weight gains of chicks in response to supplemental lysine (see also Tables 3.1 (means) and Table 3.2 (ANOVA)).

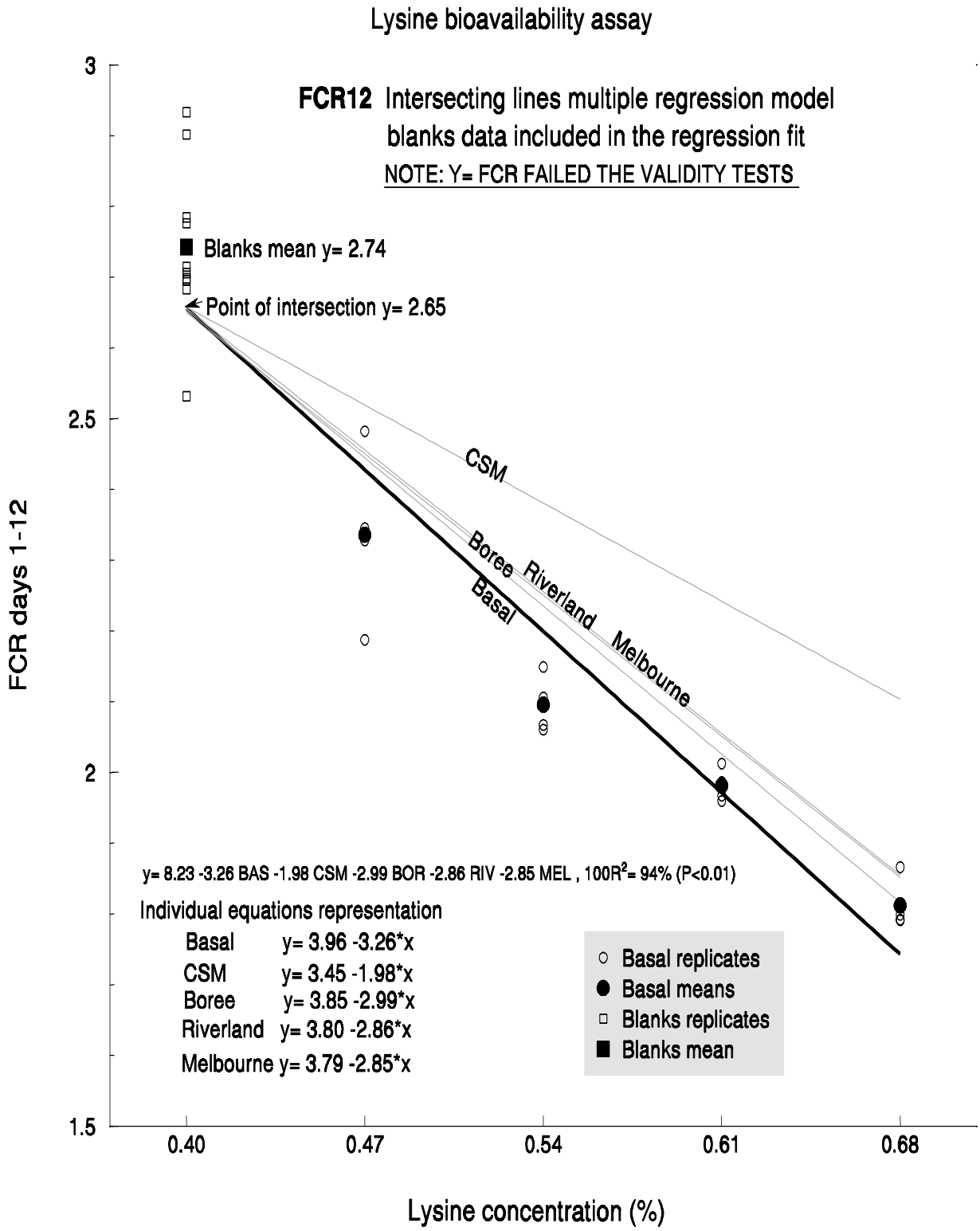

Figure 3. Graph showing the multiple regression model (with blanks data included in the regression fit) for feed conversion ratio of chicks in response to supplemental lysine (see also Table 3.1 (means and Table 3.3 (ANOVA))

The illustration in Figure 2 and the ANOVA shown in Table 3.2 for LWG clearly indicated that slope ratio assays were conducted within the linear portion of the chick's LWG response to lysine, and that the intersecting lines regression model was statistically valid. The point of intersection of the straight

lines was 91.7 g/bird which was not significantly different (P>0.01) from the point representing the blanks mean (98.6 g/bird).

The illustration in Figure 3 shows that for FCR the point of intersection of the straight lines representing the basal diet and all the test proteins intersected at 2.65 which was significantly different (P<0.01) from the blanks mean (2.74). In this situation, Finney (1971) suggested dropping the blanks data from the regression. However in our study, by excluding the blanks, the re-fitted regression lines still did not intersect at a common point close to the blanks mean, and therefore, the validity test using FCR is not valid. Separate regressions for the basal diet and for each of the test proteins (graphs and equations not shown) indicated that curvature was present, particularly in the basal points, and this was the main reason for the failure of the validity test when using FCR. However, in this report bioavailability figures obtained with the FCR will be reported as well as for LWG, since FCR is one of the main parameters suggested in the literature for estimating availability of amino acid as it takes into account any differences in food intake (Major and Batterham 1981).

3.3 Calculating availabilities from the multiple regression slope coefficients

The lysine bioavailability estimates for the four test protein meals along with the regression slope coefficients from which the availabilities are calculated, are shown in Table 3.4 for both LWG and FCR. The approximate 95% confidence limits for the availability estimates are given in Table 3.5.

Table 3.4. Lysine bioavailability estimates of the four test protein meals (cottonseed meal and canola meals) as assessed by chick slope ratio assay using live-weight gain (LWG) and feed conversion ratio (FCR) as the criteria for availability (standard error given in parenthesis).

Test protein meal	LWG Regression equation	LWG Availability	FCR Regression equation	FCR Availability
Basal	Y= - 112.7 + 511.1 X	----------	Y= 3.96 – 3.26 X	---------
CSM Narrabri	Y= - 21.9 + 283.9 X	0.555 (0.046)	Y= 3.45 – 1.98 X	0.609 (0.042)
CM Boree	Y= - 136 + 569.3 X	1.114 (0.058)	Y= 3.85 – 2.99 X	0.919 (0.047)
CM Riverland	Y= - 93.3 + 462.6 X	0.905 (0.052)	Y= 3.80 – 2.86 X	0.878 (0.046)
CM Melbourne	Y= - 77.5 + 423.1 X	0.828 (0.050)	Y= 3.79 – 2.85 X	0.876 (0.046)

Table 3.5. Slope ratio estimates of lysine bioavailability using Live-weight gain (LWG) and Feed Conversion Ratio (FCR) with approximate 95% confidence limits given in parenthesis (lower, upper limits).

Test protein meal	LWG Availability	FCR Availability
Basal	-------	-------
CSM Narrabri	0.555 (0.464, 0.647)	0.609 (0.526, 0.692)
CM Boree	1.114 (0.999, 1.230)	0.919 (0.825, 1.013)
CM Riverland	0.905 (0.801, 1.009)	0.878 (0.786, 0.970)
CM Melbourne	0.828 (0.728, 0.928)	0.876 (0.784, 0.968)

3.4 Possible reasons for unexpected availability estimates

The major unexpected result is the high lysine availability estimate for CM Boree, particularly when using LWG (Table 3.5).

In the present study, the lysine levels were selected to be in the linear response range, with the upper lysine levels at 60% bellow the estimated requirement of lysine by chicks (Baker *et al,* 2002). All diets were iso-caloric and were maintained at 12.5 MJ/kg diet by varying starch, sucrose, and soybean oil as non-protein energy sources. The dietary crude protein (CP) in the basal diets for various lysine levels varied little (272 - 275 g/kg). But the CP content for the treatment diets containing the test protein meals varied from 288 to 343 and 285 to 324 (g/kg) for CSM and CM, respectively. This CP variation was due to an increment in the concentration of lysine and other amino acids present in each protein meal and this is inevitable when the graded supplementation technique is used. There is another technique called the 'diet dilution technique' (Gous, 1980) which is claimed to be superior, but D'Mello (1982) concluded that there is little difference between graded supplementation or diet dilution.

Nevertheless, it can be argued that the CP variation shown in the present study may produce imbalanced diets which can depress amino acid availability estimates due to the addition of imbalanced test proteins that normally reduce bird FI (Batterham *et al*, 1979). Thus, Major and Batterham (1981) have suggested the use of FCR for availability estimates as FI is taken into account. However, in our study LWG is used for estimating lysine bioavailabilities as chick's FI was not affected and consumed all their diets at acceptable levels indicating that the CP variation among treatments did not exert any confounding effect in the present work. Nevertheless, in the present study it's clear (Table 3.5) that the FCR estimate of lysine bioavailability for CM Boree (0.919, 95% confidence limits 0.825, 1.013) is more credible than that for LWG (1.114, 95% confidence limits 0.999, 1.230).

3.5 Cottonseed meal lysine availability

In our study, LWG figures are preferred for lysine availability estimates as this bioassay was statistically valid (as discussed by Finney, 1971). Also, FI was not affected by the CP variation among dietary treatments. However, bioavailability estimates using FCR are also given due to the scarcity of reports available in this area, and because in the present work the slope ratio estimates of bioavailability when using FCR and LWG did not differ greatly, although the FCR validity test failed as mentioned earlier.

The CSM lysine availability (expressed as a proportion of the total in the present work) was 0.555 when using LWG and 0.609 for FCR. Our values are much lower when compared with 0.89 (LWG) and 1.1 (FCR) by Fernandez and Parson (1996). This difference is most probably due to differences in methodology as dietary treatments (in the Fernandez and Parson report) were formulated on a digestible amino acid basis and therefore the utilisation of digestible lysine and not proportion of the total lysine in CSM was measured by those authors.

Our CSM lysine availability results are also lower (by 24 and 30 % for LWG and FCR, respectively) than the values of 0.69 (LWG) and 0.79 (FCR) reported in Major and Batterham (1981) who measured availability of lysine as a proportion of the total, as we did. This discrepancy may be attributed to differences in plant cultivars, processing methods for cottonseed oil extraction and the type and level of any antinutritional factors present in the meal. In another report using pigs, Batterham *et al* (1990) reported an even lower CSM availability (proportion of total) of 0.31 (LWG) and 0.29 (FCR) indicating that chicks are somehow more able to efficiently incorporate lysine from CSM than pigs. Therefore, further research is warranted to determine the causes of species differences in lysine availability in CSM, and to determine whether it occurs with other protein meals.

The low CSM lysine availability in pigs was attributed by Batterham *et al* (1990) to severe heat and moisture conditions necessary for the extraction of the oil, but which also have the effect of binding free gossypol to an inactive form. Antinutritional factors (ANF) such as gossypol and cyclopropanoid fatty acids (CPFA) were dismissed as responsible for this low CSM lysine availability in pigs since

ferrous sulphate added to diets counteracted any residual gossypol present in CSM, and that these meals were low in CPFA due to the low fat content (about 3.0%) found in solvent extracted meals.

However, condensed tannins (CT) are an important ANF found in CSM which has the potential to interfere with pig performance, but this was not mentioned in those reports. CT are powerful polyphenolic compounds able to complex to dietary proteins thereby interfering with absorption and metabolism, in both ruminants and monogastric animals (Perez-Maldonado, 1994). CT in monogastric animals appear to have a deleterious effect due to strong interactions with proteins, resulting in inhibition of intestinal enzymes and depressing the availability of amino acids such as methionine (Ford and Hewitt, 1979; Burns, 1971; Reichert *et al* 1980).

Recent research carried out at QPRDC evaluating various Australian sources of CM and CSM in poultry diets, indicated the presence of CT in these meals (Perez-Maldonado 2003). The current CSM produced in Australia exhibits little free gossypol (0.05-0.07 g/kg) and low CPFA (102.2 mg/kg) but shows a relatively high total CT content (56-70 g/kg), of which 36-44 g/kg are in a free form and about 20-25 g/kg are bound to either fibre or proteins fractions (Perez-Maldonado 2003).

The level and duration of heat treatment imparted during the oil extraction process in the production of protein meals, is said to have a significant effect on the ileal digestible lysine of protein meals such as CM (Van Barneveld, 1998). It is expected that this heat input will also affect the availability of lysine in CSM, since heat during processing, will cause the free CT fraction to form complexes mainly with plant proteins rendering it unavailable for utilisation by the host animal. During digestion, further complexes can be formed between the free CT fraction and the host digestive enzymes reducing their activity and efficiency even further. This effect on enzymes will further reduce the availability of lysine and other amino acids present in diets containing CSM. The results obtained by Batterham *et al* (1990); Major and Batterham (1981) in which lysine was less available in pigs than in fowls can be explained by CT having more time for exerting its powerful binding effect in pigs than in fowls which have a shorter digestive transit time.

3.6 Canola meal lysine availability

The lysine availability estimates when using LWG for CM protein meals were 1.114, 0.905, and 0.828 for Boree, Riverland and Melbourne, respectively. When FCR was used, the estimates were 0.919, 0.878 and 0.876, respectively. As for CSM, the estimates of availability when using FCR were in close agreement to the LWG figures.

Among the three CM, Boree consistently exhibited the highest lysine availability than CM from Riverland and Melbourne sources. Since Boree CM is an extruded extracted meal, this may suggest processing conditions as the main factor affecting lysine availability in these meals. Manufactures of the extruded Boree CM (Mac Smith Milling, personal communication) indicated that heat is normally applied at 90-100 °C during seed cooking and conditioning during oil extraction. But this heat input is for only 5 to 10 minutes which is approximately 83% less than the time used by other CM processing plants in Australia. A report on the use of CM in growing pigs by Van Barneveld, 1998, described the manufacturing steps used for the various CM produced in Australia during oil extraction. Van Barneveld, 1998 found that excessive heating during processing can result in reduced animal digestibility of some amino acids, particularly lysine.

3.7 Cottonseed meal versus canola meals lysine availability

In our study, the availability of lysine from various CM was 49-100% higher than that of CSM. This difference in lysine availability could be related to differences in plant processing conditions for oil extraction from canola and cottonseed (particularly heating conditions), and also to differences in the types of ANFs found in CM and CSM. Since both protein meals contained substantial amounts of

total CT, it is possible that differences in CT structure, molecular weight or units of anthocyanidins (CT building molecules) in CM versus CSM, are responsible for differences in the lysine availability.

CT measured in CSM and various CM samples during 2000-2001 (Perez-Maldonado, 2003) indicated substantial differences in the type of anthocyanidin molecules. Previous work (Perez-Maldonado, 1994) also indicated substantial differences in purified CT from the pasture legumes *Desmodium intortum, Lotus pedunculatus and Leucaena leucocephala*. These CT differences were responsible for the variation in CT protein binding capacity among these different forage legumes, and therefore a similar argument applies to the CT differences between the protein meals investigated here. It is quite possible that in CSM the CT protein binding capacity is higher than CT from CM and this is reflected in the difference found in lysine availability values, but this need to be investigated.

3.8 Digestibility versus availability

When reviewing the literature, a general misunderstanding arises on the use of "availability" and "digestibility" terminology. There are many reports which claim to have evaluated the availability of nutrients from various feed ingredients, when they are in reality only digestibility figures (true or apparent) since the methodology used in those reports involves only disappearance of nutrients from any point of the gastro intestinal tract of the host animal. Another assumption usually claimed is that if a nutrient is not recovered at the terminal ileum, or in excreta (from caecectomised birds), then the nutrient has been absorbed in a form that can be fully utilised for maintenance and production by the bird. This assumption is not always the case, but the difficulty can be circumvented by going over to the measurement of availability rather than digestibility. Availability differs from ileal digestibility in that involves a measure (live-weight gain or feed conversion ratio) of utilisation or potency of the absorbed nutrient. Available nutrients such as amino acids are those which are actually supplied at the sites for protein synthesis for incorporation into body protein or metabolites for other body uses.

One of the main objectives of the research reported here was to compare the slope ratio assay for availability versus the apparent ileal digestibility to assess nutrient quality of diets, in this case lysine availability versus lysine digestibility. To make this comparison, the availability of lysine on one CSM and three CM from various sources was determined in chickens during the present study, whilst digestibility bioassays on similar protein meals samples were undertaken during a previous study at QPRDC (see Perez-Maldonado, 2003).

A summary result for the availability of lysine as assessed with the slope ratio assay as a proportion of the total for CSM from Narrabri and CM from Boree, Riverland and Melbourne is presented in Table 3.6 along with ileal digestibilities on similar samples values obtained in previous work during the period 1999-2002.

The results in Table 3.6 show that for CSM, the bioavailability of lysine of 0.556 agrees well with the CSM lysine ileal digestibility value (mean 0.515). Therefore, for CSM from Narrabri, the low lysine digestibility at the terminal ileum of 0.515 appears to be the major cause of the poor availability of CSM (0.555). Thus for CSM, the ileal digestibility of lysine appears to provide a reasonable estimate of lysine availability. Unfortunately, due to the time frame for this project, it was not possible to compare broiler performance on diets containing CSM formulated on available lysine basis versus digestible basis - this research needs to be done.

Table 3.6. Lysine bioavailability estimates by slope ratio assay using Live-weight gain (LWG) and Feed Conversion Ratio (FCR), and ileal digestibilities of lysine determined in chickens 35-42 days of age on cottonseed meal (CSM) from Narrabri and canola meal (CM) from various Australian oilseed processing plants during 1999 to 2002. Figures given in parenthesis following the mean digestibilities are values for the years shown.

Protein meal	LWG	FCR	Mean Digestibility (1999, 2000, 2001, 2002)	
CSM Narrabri	0.555	0.609	0.515	(0.52, 0.45, 0.53, 056)
CM Boree	1.114	0.919	0.740	(ND, ND, 0.74, ND)
CM Riverland	0.905	0.878	0.727	(0.70, 0.75, 0.73, 0.73)
CM Melbourne	0.828	0.876	0.690	(0.69, 0.71, 0.67, ND)

ND= not determined.

In a previous broiler growth trial at QPRDC (Perez-Maldonado, 2003) on broilers fed on CSM diets with inclusion levels of up to 400 g CSM/kg comparing diets formulated on a total or on a digestible amino acid basis, clearly indicated a superior bird performance from CSM diets formulated on a digestible amino acid basis. Up to 200 g CSM/kg during the starter period and up to 300 g CSM/kg during the finisher period, broilers achieved a satisfactory performance. However, during the starter period, diets formulated on digestible AA basis containing 300 and 400 g CSM/kg a depressed chick performance was observed without a satisfactory explanation, and this needs to be further investigated. It appears that other factors apart from AA digestibility are responsible for this problem with chick performance in the starter period. Since values of lysine availability and digestibility in CSM are similar, it is possible that the availability of other AA may be responsible for this mentioned low performance and more research is needed if substantial amounts (> 200 g CSM/kg) are to be included during the starter chick growth phase.

The lysine availability on the three CM of 1.114, 0.905, and 0.828 for Boree, Riverland and Melbourne, respectively were 51, 25 and 20 % higher than the lysine digestibilities obtained on similar CM (Tables 3.6) suggesting lack of agreement of lysine values between the two techniques. The explanation for this outcome is difficult and further investigation is needed. In recent broiler trials (Perez-Maldonado, 2003) in which CM was included at up to 400 g/kg in diets formulated on a total or on a digestible AA basis, to compare the formulation effect on broiler performance, the results indicated a similar good bird performance when using either formulation system. Contrary to the results shown with CSM diets (as explained above), little gain was obtained in CM diets when total AA values were substituted for digestible figures. Therefore, this growth trail showed that the total AA present in CM was mostly utilised by birds. This suggests that the digestibility of AA in CM may be near as possible to the availability figures obtained in the present work, in which lysine from various CM sources were at least 83% available. Therefore not much difference in performance would be expected when broiler diets are formulated on either a digestible AA or on lysine availability basis.

3.9 Use of markers and the effect on digestible amino acid determination

The problems associated on the estimation of digestibilities in avian species using various digestive markers such as chromium oxide or acid insoluble ash was recently reviewed by Sales and Janssens, 2003). From 13 studies using chromic oxide as marker, seven found lower values than when using the total excreta method indicating that the use of markers can underestimate feed utilisation, particularly when it is well accepted that the recovery rate of chromium oxide varied from 85 to 101%.

When chromium oxide was used in diets offered to 21-28 days old broilers, the lysine digestibility of soybean meal (SBM) was 0.82 (Perez-Maldonado *et al*., (on press, 2003) which is only five percent lower than previously reported values in SBM fed to 35-42 days old broilers (Ravindran and Bryden,

1998), indicating that the difference was due to bird's age. In regards to availability, Major and Batterham, (1981) using the slope ratio chick found that the lysine availability in SBM was 0.93 which is 13% higher than our SBM digestibility value (0.82) and determined using chromium oxide. This discrepancy between the two methods, suggests that the use of markers may underestimate ileal digestibility and more research is needed to investigate all this area.

4. Conclusions

- A major aspect of this work was to establish the chick slope ratio assay methodology.
- Three major assays trials were conducted including a pilot assay and two full-scale replicated bioassays to establish the bioassay and to determine lysine bioavailability in four protein meals.
- Of the protein meals tested, cottonseed meal has much lower available lysine than canola meals.
- There are biological significant differences in available lysine among the three canola meals tested which are most likely due to primarily to the processing conditions used to extract oil to produce the CM.
- It was found that in CM ileal digestibility method underestimate lysine availability compared with the slope ratio bioassay and more research is needed in this area.
- Condensed tannins, a poliphenolic compound found in CSM and CM, may play an important role when assaying the digestibility and availability of lysine. Further research including other protein meals is needed to confirm this.

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