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& DEVELOPMENT CORPORATION

Amino Acid and Energy Requirements of

Imported Brown Layer Strains

A report for the Rural Industries Research and Development Corporation

by D. Balnave & D. Robinson

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Foreword

This project resulted from a workshop organised by RIRDC to develop a program of research on the nutrition of the high-producing brown egg layers recently introduced from overseas, now generally known as “imported” strains.

The task assigned to the present three year project was to investigate the protein, amino acid and energy requirements of an imported strain at different cage densities. The work related to protein and amino acid requirements was conducted at the University of Sydney, supervised by Associate Professor D. Balnave, and that relating to energy requirements was carried out at the Queensland Poultry Research and Development Centre, supervised by Mr D. Robinson.

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

This report, a new addition to RIRDC’s diverse range of over 600 research publications, forms part of our Egg R & D program, which aims to initiate, support and manage R & D to meet the requirements of a profitable and responsible Australian egg industry.

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Contents

Abbreviations

AME	Apparent metabolisable energy
B’wt	Body weight
CP	Crude protein
Ca	Calcium
LSD	Least significant difference (P<0.05)
ME	Metabolisable energy
QDPI	Queensland Department of Primary Industries
QPRDC	Queensland Poultry Research & Development Centre

Executive Summary

During the past decade egg producers in Australia have to a large extent discarded established layer strains in favour of new coloured overseas genotypes. These imported brown-egg strains produce considerably more egg mass and generally convert feed to egg mass more efficiently than local strains, so it might be expected that their nutritional requirements are more exacting than those of local strains.

However, no estimates of the nutritional requirements of these "imported" strains have been made in Australia using Australian diets. Previous attempts to evaluate the performance and to determine the dietary nutrient specifications of these overseas strains in the Australian environment have been impeded by a high mortality problem related to Marek's disease and cannibalism. The recent reduction in the incidence of Marek's disease provided an opportunity to evaluate the nutrient requirements of these imported stocks under Australian conditions.

The aim of this project was to improve the economic value of imported commercial brown layer strains by defining their protein, amino acid and energy requirements under Australian conditions. IsaBrown laying hens were used as representative of the new imported coloured strains. The aim of the energy study was to determine how variation in dietary energy concentration influenced the performance of IsaBrown laying hens housed in single-bird and two-bird cages in an open-sided, flat-deck, cage shed in southeast Queensland. The shed was provided with adjustable shutters and ridge-vent, and thermostatically controlled fans and foggers.

Three dietary ME levels were fed to IsaBrown hens housed in single-bird and two-bird cages. The nominal ME values of the diets were 10.6 (L), 11.4 (M) and 12.2 (H) MJ/kg, while the ME values obtained by metabolism studies, using cockerels, were 9.78±0.29 (L), 11.41±0.25 (M) and 12.52±0.37 (H) MJ/kg. Amino acids, total protein, calcium and phosphorus were maintained in approximate proportion to the nominal ME levels.

The results indicated that the IsaBrown bird is inefficient at adjusting feed intake to meet energy requirements. The ME intake on diet H appeared to be excessive while intake of ME and/or other nutrients on diet L may have been too low to support maximum egg mass output. Feed intake of diet L, which was a very bulky (low density) diet, was substantially lower than predicted on the basis of energy requirement of the birds and energy content of the diet, suggesting that dietary bulk was a limiting factor. Nevertheless profit was maximised with this diet under current Queensland cost/price conditions.

Thus, it appears that for imported brown egg layers, diets with a low to medium energy content and proportionately lower protein content are likely to be more economical than higher density diets in most circumstances. The results suggest that a minimum dietary AME of 11.4 MJ/kg appeared to be required for optimal biological efficiency. This ME concentration is similar to the current breeder recommendation.

The diets used to examine the protein, lysine and methionine requirements during lay contained calculated ME concentrations of 11-11.25 MJ/kg. Diets containing 160 or 180 g crude protein/kg in one experiment, lysine concentrations ranging from 7.35 to 8.95 g/kg in a second experiment and methionine concentrations ranging from 2.83 to 3.83 g/kg in a third experiment were fed to IsaBrown hens in single- and 5-bird cages. In the first study the effect of feeding a pre-layer diet containing 2 g Ca/kg from 15 to 18 weeks of age was also examined to determine whether this procedure influenced subsequent feed intake.

These three studies were conducted in a newly built, high-rise, windowless layer house built from insulated panels in which computerised control of fans and evaporative cooling pads allowed the temperature to be maintained below heat-stress levels. The results clearly showed no advantage from increasing the calcium concentration of a grower diet for the three weeks prior to sexual maturity.

Likewise, the production advantages of increasing the layer diet from 160 to 180 g/kg were limited to a small, significant increase in egg mass output due mainly to a significant increase in egg weight. The lysine and methionine requirements for hens in single cages were lower than for hens in multiple 5-bird cages. The requirements of the latter hens are more applicable to the commercial situation and were shown to approximate 970 mg lysine/day and 430 mg methionine/day. At the calculated dietary ME concentration of 11.25 MJ/kg used in these studies these intakes were attained with dietary concentrations of 7.75 g lysine/kg and 3.33 g methionine/kg.

Important observations from the lysine study were that increasing the dietary lysine concentration to 8.15 g/kg improved albumen quality and consistent, if non-significant, increases in serum total immunoglobulin titres were observed with increases in dietary lysine. The latter response contrasted with that observed in the methionine study where increases in dietary methionine concentration reduced albumen quality and gave non-significant reductions in serum total immunoglobulin titres.

The main difference between hens in single- and multiple- bird cages was the higher mortality, mostly resulting from cannibalism, observed in the multiple cages. This had a significant effect on hen-housed egg production in the lysine and methionine studies. In both studies this difference due to cage density was ameliorated by increasing the dietary concentration of lysine and methionine, respectively.

Other effects of cage density were in the lysine study where hens in the multiple-bird cages produced eggs with significantly lower % shell but significantly improved albumen height and in the methionine study where hens in multiple-bird cages again produced eggs with significantly lower % shell. There was a tendency (P=0.081) in the methionine study for hens housed in multiple-bird cages to have increased serum total immunoglobulin titres compared to hens in single cages.

Current breeder recommendations for the IsaBrown hen consuming a diet containing approximately 11.4 MJ of ME/kg are 19.5 g crude protein, 880 mg lysine and 430 mg methionine/day. Estimates based on hen-housed egg production in 5-bird multiple cages in the present work confirm these recommendations for crude protein and methionine but are considerably higher than the suggested requirement for lysine (970 vs 880 mg/day).

Introduction

During the past decade egg producers in Australia have largely discarded established layer strains in favour of new coloured overseas genotypes. These imported brown-egg strains produce considerably more egg mass and generally convert feed to egg mass more efficiently than local strains, so it might be expected that their nutritional requirements are more exacting than those of local strains. However, no estimates of the nutritional requirements of these "imported" strains have been made in Australia using Australian diets. Previous attempts to evaluate the performance and to determine the dietary nutrient specifications of these overseas strains in the Australian environment have been impeded by a high mortality problem related to Marek's disease and cannibalism. The recent reduction in the incidence of Marek's disease provided an opportunity to evaluate the nutrient requirements of these imported stock under Australian conditions.

Objectives

The present studies were carried out to determine the protein, lysine and methionine requirements, and the optimal dietary ME concentration, for IsaBrown laying hens (as representative of the new imported coloured genotypes) housed in single-bird or multiple-bird cages. In addition, the effect of feeding a pre-layer diet containing 20 g Ca/kg from 15 to 18 weeks of age was also examined in one study to determine whether this procedure influenced subsequent feed intake.

PART 1
Protein, lysine and methionine requirements (Experiments 1-3)

Materials and Methods

Beak-trimmed IsaBrown pullets (RIR x RIW) were purchased at 15 (Experiment 1) and 16 (Experiments 2 and 3) weeks of age from a commercial supplier (Baiada Poultry Pty Ltd, Pendle Hill NSW 2145). The pullets were housed in cages at the University of Sydney, Camden, in a high-rise windowless house with computerised control of fans and evaporative cooling pads to maintain house temperatures below 30^{o}C. The hens in Experiment 1 were the first to be housed in this newly built layer house.

In Experiment 1 the birds were randomly allocated to 20 replicates of 5 pullets on each of 4 treatments. These consisted of a grower diet (Table 1) or this grower diet containing an additional 10 g Ca/kg, fed to pullets housed in either single or 5-bird multiple cages. The single-bird cages measured 25 cm wide by 54.5 cm deep and the multiple 5-bird cages measured 50 cm wide by 45 cm deep. At 18 weeks of age 10 replicates from each of these 4 treatments were fed one of 2 layer diets containing either 160 or 180 g CP/kg (Table 1).

In Experiments 2 and 3, pullets were fed the grower diet without the additional Ca supplement to 19 weeks of age and then one of five layer diets similar in all ingredients (Table 1) except that the lysine (Experiment 2) and methionine (Experiment 3) concentrations were varied by substituting L-lysine HCl and DL-methionine, respectively, for solkafloc, an inert cellulose filler, to provide dietary lysine concentrations from 7.35 to 8.95 g/kg in increments of 0.4 g/kg and dietary methionine concentrations from 2.83 to 3.83 g/kg in increments of 0.25 g/kg. Ten replicates of 5 pullets in either single or 5-bird multiple cages were fed each of the layer diets in Experiments 2 and 3.

Birds commenced laying in August 1997 (Experiment 1), July 1998 (Experiment 2) and August 1999 (Experiment 3). Production records were kept from 20 to 56 weeks of age (Experiment 1), 19-61 weeks of age (Experiment 2) and 20-52 weeks of age (Experiment 3).

Table 1. Experiment 1-3. Composition of experimental diets

(Values are g/kg unless stated otherwise)

	Grower	Pre-layer	Layer			
			Experiment 1		Experiment 2	Experiment 3
Crude protein (g/kg):	**160**	**160**	**160**	**180**	**160**	**160**
Wheat	200.0	200.0	200.0	200.0	200.0	200.0
Sorghum	457.4	457.4	457.4	413.4	460.45	461.95
Soybean meal	64.0	64.0	64.0	108.0	64.0	64.0
Meat meal	95.0	95.0	95.0	95.0	95.0	95.0
Millrun	100.0	100.0	100.0	100.0	100.0	100.0
Rice hulls	65.5	40.0	-	-	-	-
Tallow	9.0	9.0	9.0	9.0	9.0	9.0
Limestone	-	25.5	65.5	65.5	65.5	65.5
Sodium chloride	1.4	1.4	1.4	1.4	1.4	1.4
L-lysine	1.05	1.05	1.05	1.05	-	0.5
DL-methionine	1.65	1.65	1.65	1.65	1.65	0.40
Vitamins/minerals	5.0[A]	5.0[A]	5.0[A]	5.0[A]	1.0[B]	1.0[B]
Solkafloc	-	-	-	-	2.0	1.25
Determined amino acid concentrations						
Lysine	7.87	8.60	7.79±0.53[C]	10.32±0.00[C]	7.35±0.67[D]	6.83±0.29[E]
Arginine	9.96	9.33	9.65±0.01	10.48±0.16	9.30±0.48	9.23±0.37
Histidine	4.06	3.82	3.87±0.03	4.47±0.00	4.18±0.25	3.67±0.09
Threonine	5.24	4.97	5.11±0.06	5.75±0.11	5.38±0.21	5.33±0.12
Phenylalanine	7.01	6.83	6.88±0.06	7.63±0.08	8.17±0.15	7.43±0.17
Tyrosine	4.70	4.41	4.63±0.08	5.30±0.07	5.39±0.13	4.97±0.09
Leucine	13.32	12.99	13.04±0.03	13.78±0.22	14.68±0.30	14.93±0.27
Isoleucine	5.96	5.75	5.81±0.08	6.60±0.00	6.52±0.14	6.03±0.17
Valine	7.87	7.52	7.64±0.05	8.52±0.03	8.97±0.28	7.67±0.19
Serine	6.27	6.06	6.09±0.04	6.81±0.02	6.10±0.17	6.80±0.21
Glycine	11.55	10.60	10.79±0.15	12.28±0.28	9.60±0.71	10.63±0.63
Methionine						2.83±0.03
Cystine						2.80±0.06
Calculated concentrations						
Methionine	4.0	3.8	4.0	4.2	4.0	2.83
Cystine	2.7	2.6	2.7	2.9	2.6	2.62
ME (MJ/kg)	11.20	11.20	11.20	11.0	11.25	11.25

[A] Supplied (per kg diet): vitamin A, 6000 IU; vitamin D_3, 1200 IU; vitamin E adsorbate, 4 mg; vitamin K_3, 2 mg; riboflavin, 5 mg; calcium pantothenate, 6 mg; niacin, 15 mg; pyridoxine, 2 mg; folic acid, 0.5 mg; vitamin B_{12}, 5 µg; Mn, 50 mg; Zn, 50 mg; Fe, 30 mg; Cu, 2 mg; I, 2 mg; Co, 0.2 mg and ethoxyquin, 125 mg.

[B] Supplied (per kg diet): vitamin A, 11000 IU; vitamin D_3, 2200 IU; vitamin E, 5 mg; vitamin K (hetrazeen), 6 mg; calcium pantothenate, 5 mg; niacin, 10 mg; folic acid, 0.5 mg; vitamin B_{12}, 7 µg; Mn, 60 mg; Zn, 50 mg; Fe, 35 mg; Cu, 2.5 mg; I, 0.5 mg; Se, 0.1 mg and ethoxyquin, 0.33 mg.

[C] Mean ± s.e.m. of 2 feed mixes fed prior to peak-lay.

[D] Mean ± s.e.m. of 4 randomly selected feed mixes.

[E] Mean ± s.e.m. of 3 randomly selected feed mixes.

A constant daily photoperiod of 16 h was used and hens were allowed free access to feed and water at all times. Each replicate was treated as an experimental unit, feed intake and production being recorded for the complete group. Egg production was recorded daily and all eggs were weighed on one day every two weeks. Egg quality measurements were carried out on 30 eggs randomly selected from each treatment at 50 weeks of age in Experiment 2 and at 48 weeks of age in Experiment 3. Egg shell breaking strength (quasi-static compression) and albumen height were measured using equipment supplied by Technical Services and Supplies, Chessington Park, Dunnington, York, UK (J.R. Roberts, personal communication).

The immunocompetence of the hens in the three studies was measured by determining the serum total immunoglobulin levels as described by Hornbeck (1994). Blood samples (5 ml) were taken from all hens in three randomly selected replicates on each treatment at 54 weeks of age (Experiment 1), from all hens in two randomly selected replicates from each treatment at 50 weeks of age (Experiment 2) and from 10 randomly selected hens from all replicates in each treatment at 50 weeks of age (Experiment 3). In Experiment 1, comparisons were made with 15 hens of a local layer strain (Inghams SuperBrown), of similar age to the IsaBrown hens, which had been transferred four weeks previously from another poultry house on the University farm.

The data from Experiment 1 were analysed as a 2^3 factorial ANOVA with the main effects being the diets fed prior to lay, the CP concentration of the diets fed during lay and the cage density. The data from Experiments 2 and 3 were analysed as a 5 x 2 ANOVA with the main effects being the dietary lysine (or methionine) concentrations and the cage density (Steel and Torrie, 1982). The experiment was conducted under a protocol authorised by the Animal Care and Ethics Committee of the University of Sydney.

Results

For most of Experiment 1 the daily house temperature ranged from 15^{o}C to 30^{o}C. From December to March a minimum night temperature of 20^{o}C was recorded with the maximum day temperature only occasionally rising above 30^{o}C for short periods of time. No humidity data were recorded in Experiment 1. In Experiment 2, mean minimum and maximum house temperatures varied between approximately 16^{o}C and 23^{o}C during the first 20 weeks of the study and between 22^{o}C and 28^{o}C during the remainder. Corresponding ranges in mean relative humidity in Experiment 2 were 50-80% and 55-80%, respectively. Equipment malfunction prevented the recording of temperature and humidity in Experiment 3 but the control settings for the house fans and evaporative cooling pads were similar to those used in Experiment 2. Mortality was low in all three experiments. A total of 9 birds died in Experiment 1(2.25%), 24 in Experiment 2 (4.8%) and 6 in Experiment 3 (1.2%). The majority of these occurred in the multiple-bird cages (1.25% in Experiment 1, 4.2% in Experiment 2 and 0.75% in Experiment 3) and were diagnosed as vent pecking.

In Experiment 1, the egg production on all treatments peaked between 95 and 98% and the mean rate of lay was above 88% at the end of the study at 56 weeks of age. The production responses of the hens on the various treatments to 56 weeks of age are shown in Table 2. The diet fed prior to lay from 15 to 18 weeks of age had no significant effect on production during lay. Increasing the CP content of the layer diet from 160 to 180 g/kg had no effect on feed intake or egg production but significantly improved egg mass output through a significant increase in egg weight. Multiple-caged hens ate significantly less feed than hens housed in single-bird cages. This resulted in a significant reduction in

daily egg mass output and a significant improvement in feed conversion. The only significant interactions were pre-lay x lay diet interactions with hen-day and hen-housed egg production (Table 3). Feeding the pre- layer diet significantly reduced both hen-day and hen-housed egg production of hens subsequently fed the lower-protein layer diet and significantly increased the hen-housed egg production of hens fed the 180 g CP/kg layer diet compared with those fed the 160 g CP/kg layer diet.

Table 2. Experiment 1. Production measures for main effects between 20 and 56 weeks of age

	Feed intake (g/day)	Hen-day production (%)	Hen-housed production (%)	Egg weight (g)	Egg mass (g/day)	Feed conversion (g feed:g egg)	L'wt gain (g)
Grower diet	124.9	89.4	88.0	60.5	54.0	2.32	482
Pre-layer diet	124.7	88.9	87.4	60.0	53.3	2.34	521
Significance	NS	NS	NS	NS	NS	NS	NS
16% layer diet	124.8	89.3	87.4	59.4	53.0	2.36	494
18% layer diet	124.9	89.0	88.0	61.1	54.3	2.30	509
Significance	NS	NS	NS	***	*	NS	NS
Single cages	128.2	89.7	88.8	60.4	54.2	2.37	536
Multiple cages	121.6	88.6	86.5	60.1	53.2	2.29	467
Significance	***	n.s.	n.s.	n.s.	*	*	n.s.
s.e.m.	1.14	0.83	0.95	0.40	0.43	0.022	25.4

** P<0.05; *** P<0.001; n.s., non significant.*

Table 3. Experiment 1. Interaction between diet fed prior to lay and the protein concentration of the layer diet on hen-day and hen-housed egg production

Diet fed prior to lay	Protein concentration of layer diet (g/kg)	
	160	180
	Hen-day	
Grower	90.5a	88.2ab
Pre-layer	88.0b	89.8ab
s.e.m.	0.83	
	Hen-housed	
Grower	89.5a	86.5ab
Pre-layer	85.3b	89.4a
s.e.m.	1.34	

Values followed by the same letter are not significantly different (P>0.05)

Although there were no significant treatment effects on serum total immunoglobulin titres in Experiment 1, hens fed the pre-layer diet had numerically lower titres than hens fed the grower diets prior to lay. Similar reductions (11-12%) were observed with hens fed both layer diets. Cage density had no effect and there was no difference between the mean value for the IsaBrown hens (46.0%) and the SuperBrown hens (47.6%) which had been transferred from another poultry house to the experimental house four weeks prior to measurement (Table 4).

Table 4. Experiment 1. Serum total immunoglobulin titres (% of hyperimmunised birds)

Diet fed prior to lay	Layer diet crude protein (g/kg)	Cage density 1	Cage density 5	Mean
Grower	160	52.9	50.6	51.8
	180	53.3	38.9	46.0
Pre-layer	160	47.7	44.6	46.2
	180	33.0	47.3	40.2
Mean		46.7	45.4	
s.e.m. (interaction)		5.0		

In Experiment 2, peak rates of lay on the dietary treatments varied between 94 and 99% and hen-day egg production remained above 90% to 43 weeks of age. Mean hen-day egg production at the end of the study was 82%. The production responses to 61 weeks of age of the hens on the various treatments are shown in Table 5. Feed intake declined significantly while lysine intake increased significantly with increases in dietary lysine concentration. The increases in lysine intake at the higher dietary lysine concentrations were not reflected in significant responses in production suggesting that the lowest daily lysine intake of 938 mg/day was sufficient to satisfy the requirement of hens producing 53 g of egg mass daily.

Table 5. Experiment 2. Production measures for main effects between 19 and 61 weeks of age

	Feed intake (g/day)	Lysine (mg/day)	Hen-day production (%)	Hen-housed production (%)	Egg weight (g)	Egg mass (g/day)	Feed conversion (g feed:g egg)	B'wt gain (g)
Dietary lysine (g/kg)								
7.35	127.6a	938d	88.8	85.1	59.6	52.9	2.41	393
7.75	125.9a	976c	88.9	88.0	59.9	53.3	2.37	333
8.15	126.2a	1029b	89.0	86.2	60.2	53.5	2.36	331
8.55	122.4b	1047b	87.6	85.4	59.2	51.9	2.37	361
8.95	122.4b	1095a	88.4	87.0	60.3	53.3	2.41	392
s.e.m.	1.13	8.0	1.05	1.6	0.38	0.72	0.036	27.7
Significance	**	***	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Cage density (birds/cage)								
1	124.3	970	88.4	87.8b	59.9	52.9	2.36	349
5	125.4	980	88.7	84.9a	59.8	53.0	2.37	375
s.e.m.	0.71	4.0	0.66	1.01	0.24	0.46	0.023	17.5
Significance	n.s.	n.s.	n.s.	*	n.s.	n.s.	n.s.	n.s.

Values followed by the same letter are not significantly different (P>0.05)
** P<0.05; ** P<0.01; *** P<0.001; n.s., non significant.*

In Experiment 2, the feed and lysine intakes were similar at both cage densities. However, cage density had a significant effect on hen-housed egg production due to the significantly (P<0.001) higher mortality observed with multiple-caged hens (8.4% *vs* 1.2%). In fact, a significant dietary lysine x cage density interaction was observed with hen-housed egg production in Experiment 2 (Table 6). The increased mortality (Table 6) observed with the multiple-caged birds fed the diet with the lowest lysine concentration (7.35 g/kg) resulted in the hen-housed egg production of this treatment group being significantly inferior to that of hens fed the same diet and housed in single cages. In all treatments other than the diet with the maximum lysine concentration the mortality was numerically greater and the hen-housed egg production was numerically inferior for hens housed in multiple-bird cages.

Table 6. Experiment 2. Lysine x cage density interaction for hen-housed egg production (s.em. = 2.27) and mortality

(Percentage mortality in parentheses. s.e.m. of transformed √(% + 0.5) data = 0.50)

Cage density	Dietary lysine (g/kg)				
(birds/cage)	7.35	7.75	8.15	8.55	8.95
1	90.0a	89.0a	88.5a	87.0a	84.7ab
	(0%)	(0%)	(2%)	(2%)	(2%)
5	80.2b	87.1a	84.0ab	83.7ab	89.3a
	(14%)	(4%)	(16%)	(6%)	(2%)

Values followed by the same letter are not significantly different (P>0.05)

Increasing the dietary lysine concentration had a significant influence on albumen height and egg shell breaking strength with improvements being observed up to a dietary lysine concentration of 8.55 g/kg (Table 7).

Hens in the multiple-bird cages produced eggs with significantly lower % shell but significantly improved albumen height, the latter response being consistent at each dietary lysine concentration. There were significant lysine x cage density interactions for % shell and shell thickness due to the fact that the greater values observed with hens fed the lowest dietary lysine concentration in the single cages compared to the multiple-bird cages were gradually reversed with increasing lysine supplementation.

Table 7. Experiment 2. Egg quality measurements on eggs collected at 50 weeks of age

Dietary lysine (g/kg)	Cage density (birds/cage)	Egg weight (g)	Albumen height (mm)	Shell breaking strength (N)	Shell (%)	Shell thickness (μm)
7.35	1	65.3	5.07	36.8	9.62	406
	5	63.8	5.83	34.7	9.21	396
7.75	1	65.1	5.85	35.8	9.64	406
	5	66.0	6.00	34.8	9.31	399
8.15	1	65.6	6.99	38.1	9.66	412
	5	62.6	7.06	34.7	9.38	398
8.55	1	63.8	6.71	37.7	9.57	402
	5	64.1	7.52	36.4	9.60	405
8.95	1	65.3	6.85	32.4	9.25	389
	5	63.3	7.04	34.1	9.47	403
Probability (s.e.m.)						
Lysine		n.s.	0.0001 (0.23)	0.041 (0.9)	n.s.	n.s.
Cage density		0.041 (0.4)	0.054 (0.15)	n.s.	0.041 (0.05)	n.s.
Interaction		n.s.	n.s.	n.s.	0.036 (0.12)	0.028 (4.7)

n.s., not significant.

Consistent, but non-significant, increases in serum total immunoglobulin titres were observed with increases in dietary lysine concentration in Experiment 2. Similar mean titres were obtained for the single- and multiple-caged hens but, as with % shell and shell thickness, there was a reversal in the relative values observed with the different cage densities as the dietary lysine concentration increased (Table 8).

Table 8. Experiment 2. Serum total immunoglobulin titres (% of hyperimmunised birds)

Dietary lysine (g/kg)	Cage density 1	Cage density 5	Mean
7.35	80.2	75.1	77.6
7.75	82.9	72.8	77.9
8.15	84.4	81.8	83.1
8.55	80.1	86.5	83.3
8.95	82.5	86.4	84.5
Mean	82.0	80.5	
s.e.m. (interaction)	6.0		

In Experiment 3, peak rates of lay on the dietary treatments varied between 91 and 99% and the mean rate of lay was 88% at the end of the study. The production responses to 52 weeks of age are shown in Table 9. Methionine intake increased significantly with increases in dietary methionine concentration. However, this response was not reflected in significant increases in production suggesting that the lowest methionine intake of 370 mg/day was sufficient to satisfy the requirements of hens producing 54 g of egg mass daily. Total feed and methionine intakes were significantly greater in the single cages.

The significantly higher mortality in hens housed in multiple-bird cages resulted in a significant reduction in hen-housed egg production. At a dietary methionine concentration of 3.08 g/kg the hen-housed egg production in multiple-5 bird cages was significantly lower than in the single-bird cages suggesting that the dietary requirement in the single-bird cages was 2.83 g/kg and 3.33 g/kg in the multiple-bird cages (Table 10).

Increasing the dietary methionine concentration had a significant influence on albumen height, shell breaking strength and % shell (Table 11). However, the only trend evident was a reduction in albumen height with increasing dietary methionine. Hens housed in single-bird cages showed significant and consistent increases in % shell and shell thickness compared to hens in multiple-bird cages. A significant methionine x cage density interaction was also observed with egg shell breaking strength due mainly to an abnormally low value for eggs from hens fed the 3.58 g/kg diet in single cages.

Table 9. Experiment 3. Production measures for main effects between 20 and 52 weeks of age
(Met. = Methionine)

Dietary Met.	Feed intake	Met.	Hen-day production	Hen-housed production	Egg weight	Egg mass	Feed Conversion	B'wt gain	Mortality
(g/kg)	(g/d)	(mg/d)	(%)	(%)	(g)	(g/d)	(g food:g egg)	(g)	(%)
2.83	130.6	370	89.8	88.5	59.9	53.9	2.43	315	1.4
3.08	128.0	394	87.5	84.8	60.3	52.9	2.43	287	1.4
3.33	130.0	433	90.8	88.9	60.6	55.2	2.36	299	1.2
3.58	127.5	456	88.3	86.9	59.7	52.7	2.43	317	1.3
3.83	127.6	489	90.0	90.0	60.3	54.3	2.37	325	0.7
s.e.m.	1.13	3.8	1.16	1.78	0.34	0.70	0.039	22.4	0.3[1]
Significance	n.s.	***	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Cage density (birds/cage)									
1	132.3	440	70.1	89.7	60.2	54.3	2.45	327	0.9
5	125.1	418	88.5	86.0	60.1	53.3	2.36	289	1.5
s.e.m.	0.71	2.4	0.73	1.13	0.22	0.44	0.025	14.2	0.2[1]
Significance	***	***	n.s.	*	n.s.	n.s.	*	n.s.	*

[1] s.e.m. of transformed √(%+0.5) data

** P<0.05; *** P<0.001; n.s., not significant*

Table 10. Experiment 3. Hen-housed egg production (%) of single- and multiple-caged hens

Cage density (birds/cage)	Dietary Methionine (g/kg) 2.83	3.08	3.33	3.58	3.83
1	91.5	88.8	91.1	87.8	89.3
5	85.5	80.9	86.8	86.0	90.7
Significance	n.s.	*	n.s.	n.s.	n.s.
s.e.m.			2.5		

Table 11. Experiment 3. Egg quality measurements on eggs collected at 48 weeks of age

Dietary Methionine (g/kg)	Cage density (birds/cage)	Egg weight (g)	Albumen height (mm)	Shell breaking strength (N)	Shell (%)	Shell thickness (µm)
2.83	1	62.7	7.16	34.7	9.35	399
	5	66.1	7.20	32.9	9.01	392
3.08	1	64.1	6.80	31.3	9.69	400
	5	64.4	6.80	29.7	9.43	398
3.33	1	65.8	6.42	36.8	9.42	405
	5	66.5	6.23	35.0	9.33	405
3.58	1	64.3	6.32	26.6	9.61	405
	5	63.6	6.69	34.1	9.17	391
3.83	1	64.8	6.71	35.0	9.46	406
	5	64.7	6.34	33.0	9.19	393
Probability (s.e.m.)						
Methionine		n.s.	0.001 (0.16)	0.0004 (1.04)	0.026 (0.08)	n.s.
Cage density		n.s.	n.s.	n.s.	0.001 (0.05)	0.015 (2.04)
Interaction		n.s.	n.s.	0.003 (1.47)	n.s.	n.s.

n.s., not significant

Although treatment effects were not significant in Experiment 3, the serum total immunoglobulin titre values declined with increasing dietary methionine concentration and there was a 12% reduction in the mean titre value for hens in single cages compared with hens in multiple-bird cages (P=0.081) (Table 12).

Table 12. Experiment 3. Serum total immunoglobulin titres (% of hyperimmunised birds)

Dietary Methionine (g/kg)	Cage density 1	5	Mean
2.83	51.4	75.8	63.6
3.08	57.3	56.2	56.8
3.33	49.5	68.8	59.2
3.58	57.8	54.3	56.1
3.83	53.0	49.4	51.2
Mean	53.8	60.9	
s.e.m. (interaction)	6.4		

Discussion

These experiments were notable for the low mortalities observed. No mortality from Marek's disease occurred and, in agreement with previous reports (Cumming *et al.* 1995, 1998; Nolan *et al.* 1998), cannibalism, or more specifically vent pecking, was the major cause of mortality in the multiple-bird cages.

There was no indication of low feed consumption and poor egg production at peak-lay as reported in some Australian layer flocks by Parkinson and Almond (1995). Egg production was exceptionally good, with hens on all treatments in all three experiments peaking between 91 and 99% lay. The data obtained in Experiment 1 provided no evidence to indicate any advantage from feeding a pre-layer diet containing additional Ca from 15 to 18 weeks of age. Feeding the pre-layer diet prior to the onset of lay gave no significant improvement in performance during the subsequent laying period compared with birds fed the conventional grower diet to 18 weeks. Also, in this experiment the main response to feeding the higher dietary CP concentration during lay was a significant increase in egg weight. Feed intake and egg production were unaffected but the increase in egg weight resulted in a significant improvement in egg mass output without any significant change in feed utilisation. Increasing the dietary lysine in Experiment 2 or dietary methionine in Experiment 3 had no significant effect on egg weight, egg mass or feed utilisation.

The influence of cage density on feed intake and performance differed between experiments. In Experiments 1 and 3 the hens in multiple cages ate significantly less feed. Those in Experiment 1 produced significantly, and those in Experiment 3 produced numerically, less daily egg mass than hens in single cages. Also, in Experiment 1, significant interactions were observed between the diet fed prior to lay and the protein concentration of the layer diet with respect to both hen-day and hen-housed egg production. Hens fed the conventional grower diet prior to lay produced significantly more eggs than hens fed the pre-layer diet when given the layer diet containing 160 g CP/kg during lay. Hens fed the pre-layer diet prior to lay tended to produce more eggs than hens fed the grower diet when given the layer diet containing 180 g CP/kg during lay.

In Experiments 2 and 3 the hen-housed egg production was significantly reduced in the multiple-bird cages due to higher mortality, mainly from cannibalism. There was also a significant dietary lysine x cage density interaction with hen-housed egg production in Experiment 2. At the lowest dietary lysine

concentration the maximum production was achieved with hens in the single cages whereas the reverse was observed at the highest dietary lysine concentration. A similar, non-significant, trend in hen-housed egg production was observed with increasing dietary methionine concentration in Experiment 3. Similar reverses in the % shell, shell thickness, and serum total immunoglobulin titre measures were also observed with increasing dietary lysine supplementation in Experiment 2.

Finally, it is worth noting that the significant increase in albumen height resulting from dietary lysine supplementation and the consistently greater albumen height observed in eggs from hens in the multiple cages compared with those from single-caged hens in Experiment 2 were not a reflection of reduced egg weight, in contrast to the data of Scott *et al.* (1999) which indicated an inverse relationship between egg weight and albumen height. Similarly, the significant reduction in albumen height with dietary methionine supplementation in Experiment 3 did not appear to be related to changes in egg weight.

A lysine concentration of 7.79 g/kg in the diet containing 160 g CP/kg, corresponding to a daily lysine intake of 972 mg daily, satisfied the lysine requirements of the IsaBrown hens used in Experiment 1. In Experiment 2, at the lowest dietary lysine concentration of 7.35 g/kg, a mean daily lysine intake of 938 mg between 19 and 61 weeks of age satisfied the lysine requirement of IsaBrown hens housed in single, but not in multiple-bird, cages. In terms of hen-housed egg production, the requirement of the latter hens approximated 7.75 g/kg or 976 mg lysine daily, a value similar to that obtained in Experiment 1. However, if egg quality was used as the criterion of assessment the lysine requirement approximated 8.15 g/kg or 1029 mg daily. These values compare with determined daily lysine intakes of between 899 and 1023 mg for maximum egg output of 51-52 g daily obtained by Al Bustany and Elwinger (1987a,b) for a Rhode Island Red hybrid (Hisex Brown).

In Experiment 3, at the lowest methionine concentration of 2.83 g/kg, a mean daily methionine intake of 370 mg between 20 and 52 weeks of age satisfied the methionine requirements of IsaBrown hens housed in single cages. However, when comparisons were made between the hen-housed egg production of the single- and multiple-caged hens the data suggested that the methionine requirement for the multiple caged hens was 3.33 g/kg or 433 mg/day.

The measures of serum total immunoglobulin carried out at 50-54 weeks of age in Experiments 1-3 were not significantly affected by treatment. Nevertheless, definite trends were noted. These were reduced titres from hens previously receiving the pre-layer diet rather than the grower diet for 3 weeks prior to the onset of lay, increased titres with increasing dietary lysine concentrations and decreased titres with increasing dietary methionine concentrations.

PART 2
Energy requirements (Experiment 4)

Materials and Methods

Beak-trimmed IsaBrown pullets were obtained from a commercial supplier (Baiada Poultry Pty Ltd) and were part of the same flock as used in Experiment 2. Five hundred and seventy six pullets were housed at eighteen weeks of age in an open-sided flat-deck cage shed at the Queensland Poultry Research & Development Centre. The shed was provided with adjustable shutters and ridge-vent, and thermostatically controlled fans and foggers. The pullets were distributed at random into 72 experimental groups in a random split plot arrangement. There were 24 replicates of each of two cage-types (single-bird and two-bird cages), each of which was subdivided into three diet types (low, medium and high energy). Each of the resultant 72 replicate-groups contained eight birds, either in eight single-bird cages or in four two-bird cages. The single-bird cages measured 23 cm wide by 46 cm deep and the two-bird cages measured 30.5 cm wide by 46 cm deep.

The composition of the three diets are shown in Table 13. The diets were designed to contain nominal ME levels of 10.6, 11.4 and 12.2 MJ/kg (values calculated by Feedmania® using average values for ingredients). Actual ME levels as determined by the rapid method for apparent metabolisable energy (AME) are given in the Results section. The diets were formulated so that the concentrations of limiting amino acids, total protein, calcium and phosphorus were maintained in approximate proportion to the nominal ME levels. Protein, fat and amino acid concentrations of ingredients were determined prior to diet preparation and these values were used in the feed formulation program (Feedmania®). Feed and water were available continuously and a constant daily light period of 15.5 hours was provided.

The metabolisable energy content of the experimental diets was determined by the rapid AME method using cockerels (Farrell *et al*. 1991). Individually caged cockerels that had been trained to consume most of their daily feed requirement within one hour were used. Each diet was fed to six cockerels. Prior to the day on which the test diets were presented the cockerels were given only small fixed amounts of food. The test diets were then given during two 30-minute periods separated by a 20-minute rest period. All excreta voided over the following 42 hours were collected, oven dried at 70^{o}C, finely ground, mixed and subsampled. Gross energy of the feed and excreta were measured by combustion in an AC-350 Leco adiabatic bomb calorimeter. AME values were then calculated using the following formula after converting all data to an as fed basis:

AME = { (Feed intake x feed GE) – (Excreta output x excreta GE) } / Feed intake

Data collection commenced at 19 weeks of age. All performance records were maintained on a group basis; mortalities were recorded as they occurred, eggs were recorded on five consecutive days each week and the percentage production was calculated as 100 x number of eggs / number of hen-days in the five-day period. Feed intake and egg weights were recorded weekly for the first 16 weeks and at four-weekly intervals thereafter. At four-week intervals all eggs laid on one day were individually weighed in air and in water at 21^{o}C to obtain estimates of specific gravity. Birds were group weighed on arrival from the grower at 16 weeks of age and individually weighed at 19, 22 and 35 weeks and at termination of the trial (83 weeks of age or 64 weeks of production). Maximum and minimum shed temperatures were recorded five days per week. At termination of the trial sixteen birds from each of the six treatments were slaughtered and autopsied and the weight of the abdominal fat pad was measured. Statistical analyses of cumulative data were done using Statistix® analysis of variance programs. The economic evaluations were based on current Queensland average price information.

Table 13. Experiment 4.
Average composition and nutrient analysis of the experimental diets.

Ingredient composition g/kg	Low energy	Medium energy	High energy
Sorghum	432.8	480.7	496.2
Wheat	200.0	200.0	200.0
Millrun	60.0	80.0	-
Rice husk	83.0	-	-
Soybean meal (49%)	90.9	100.4	124.4
Meat & bone meal (51%)	40.0	40.0	57.5
Tallow	1.13	-	22.9
Sunflower oil	1.54	1.42	3.35
Limestone	82.0	89.2	87.2
Salt	1.50	1.50	1.30
Sodium bicarbonate	1.50	1.00	1.20
Lysine mono HCl	2.06	2.19	2.03
DL-methionine	1.13	1.16	1.48
Vitamin & mineral premixes[1]	2.44	2.44	2.44
	1000.00	1000.00	1000.00
Nutrient analysis/kg			
ME (nominal, MJ)	10.6	11.4	12.2
ME (determined, MJ)	9.78	11.41	12.52
Density (kg/litre)	0.57	0.77	0.79
Protein (g)	155.0	166.7	175.1
Fat (g)	24.2	25.0	47.4
Lysine (g)	7.2	7.80	8.30
Methionine (g)	3.74	4.01	4.43
Met + Cys (g)	6.20	6.70	7.10
Iso-leucine (g)	5.40	5.90	6.30
Threonine (g)	4.82	5.12	5.46
Tryptophan (g)	1.77	1.91	2.06
Linoleic acid (g)	9.2	10.0	10.6
Calcium (g)	34.5	37.0	38.2
Total Phosphorus (g)	5.40	5.70	6.15
Available Phosphorus (g)	3.10	3.21	4.00
Sodium (g)	1.80	1.73	1.70
Chloride (g)	1.81	1.86	1.86

[1] Premixes supplied (mg/kg diet): 2.5 retinol, 0.075 cholecalciferol, 5 α-tocopherol acetate, 2 menadione sodium bisulphite, 1 thiamine, 4 riboflavin, 2 pyridoxine, 0.01 cyanocobalamin, 1 folic acid, 10 niacin, 10 calcium pantothenate, 0.03 biotin, 150 choline, 50 Mn, 50 Zn, 50 Fe, 0.6 Mo, 0.5 Co, 0.6 I, 4 Cu, 0.07 Se, 80 Banox (BHA + BHT), yolk pigment.

Results

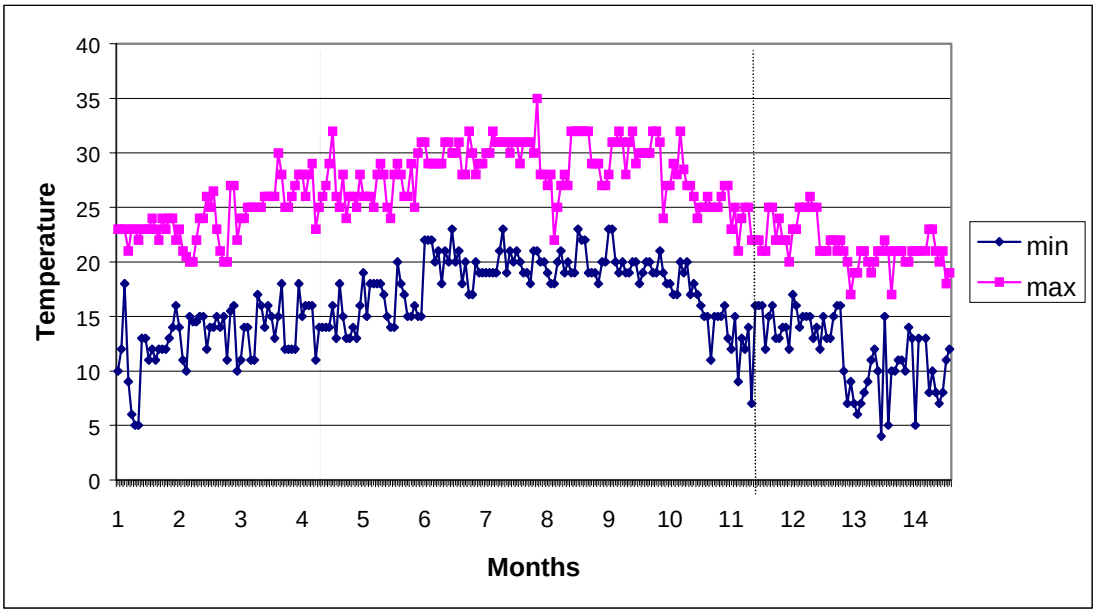
The results of the AME assays were as follows:

Low energy diet	9.78 ± 0.29 MJ/kg
Medium energy diet	11.41 ± 0.25 MJ/kg
High energy diet	12.52 ± 0.37 MJ/kg

The determined AME values for the medium and high energy diets were consistent with the calculated values but the determined value for the low energy diet was unexpectedly low. The experimental flock commenced lay at approximately 19 weeks of age and reached a peak rate of lay at approximately 29 weeks of age. The birds maintained excellent health throughout the trial and the general mortality rate was low.

Air temperature in the shed varied considerably during the trial. Figure 1 shows a graph of maximum and minimum temperatures over a fourteen-month period. The daily average temperature ranges in the shed were approximately 13-24^{o}, 19-29^{o} and 10-21^{o}C during the early, middle and late phases of the trial respectively.

Figure 1. Maximum and minimum shed temperatures throughout the trial



Mean performance results for the main treatments over the 64-week trial period are presented in Tables 14 and 15, and the principal results for all treatment combinations over the trial period are in Table 16. Principal results for the first 16 weeks (cool season), the next 28 weeks (warm to hot season) and the last 20 weeks (mainly cold season) are shown in Tables 17-19 respectively.

Birds on the high ME diet reached peak production five to seven days earlier than birds on the other diets but their peak rate of lay was lower (93.3% compared with medium ME 94.4% and low ME 95.4%). Over the 64-week laying period average egg weight, ME intake, body weight gain and mortality increased and feed efficiency improved with increasing ME level in the diet, while feed intake, efficiency of conversion of energy to egg mass and egg specific gravity declined with increasing ME level (differences between low and high ME diets $P<0.001$ for feed and energy intake and conversion, $P<0.05$ for other parameters) (Table 14). Final bodyweight also increased with increasing dietary ME level, and abdominal fat pad weight (absolute or as a proportion of body weight) at termination of the trial was lower ($P<0.01$) for the low ME diet than for the other diets.

Table 14. Experiment 4. Performance of energy treatments from 19 to 83 weeks of age[1]

ME level	Eggs/ 100 bird-days	Egg Weight (g)	Egg mass (g/d)	Egg specific gravity	Feed intake (g/d)	Feed conversion (g feed:g egg)
Low	81.35	64.05[a]	52.10	1.0837[b]	125.0[c]	2.399[c]
Medium	82.21	64.34[ab]	52.90	1.0826[ab]	123.1[b]	2.327[b]
High	82.14	65.07[b]	53.45	1.0811[a]	117.6[a]	2.200[a]
LSD (P<0.05)	*2.50*	*0.77*	*1.51*	*0.0016*	*1.9*	*0.072*

ME level	Mortality (%)	Final b'wt (kg)	B'wt gain (g/d)	Fat pad (g/kg b'wt)	ME intake (MJ/d) Nominal	ME intake (MJ/d) Determ'd	ME/g egg (kJ)
Low	1.04[a]	2.142[a]	0.958[a]	44.2[a]	1.325[a]	1.223[a]	23.47[a]
Medium	4.69[ab]	2.189[ab]	1.019[a]	55.1[b]	1.403[b]	1.404[b]	26.54[b]
High	7.29[b]	2.226[b]	1.136[b]	57.9[b]	1.435[c]	1.472[c]	27.54[c]
LSD	*4.25*	*0.058*	*0.115*	*8.3*	*0.020*	*0.021*	*0.77*

[1] *Means in a column with a similar superscript in are not significantly different (P<0.05)*

Table 15. Experiment 4. Performance of cage treatments from 19 to 83 weeks of age[1]

Birds /cage	Eggs/ 100 bird-days	Egg weight (g)	Egg mass (g/d)	Egg specific gravity	Feed intake (g/d)	Feed conversion (g feed:g egg)	Fat pad (g/kg b'wt)
1	80.61[a]	64.69	52.15[a]	1.0823	121.2	2.323	50.4
2	83.17[b]	64.29	53.48[b]	1.0827	122.6	2.294	54.3
LSD	*1.80*	*0.67*	*1.14*	*0.0013*	*1.6*	*0.053*	*7.3*

[1] *Means in a column with a similar superscript are not significantly different (P<0.05)*

Table 16. Experiment 4. Performance results from 19 to 83 weeks of age

ME level	Birds /cage	Eggs/100 bird-days	Egg weight (g)	Egg mass (g/d)	Feed intake (g/d)	Feed conversion (g feed:g egg)	B'wt gain (g/d)	Fat pad (g/kg b'wt)
Low	1	80.07	64.66	51.77	125.3	2.421	0.983	45.9
	2	82.63	63.46	52.44	124.7	2.378	0.933	42.4
Medium	1	81.35	64.44	52.42	122.2	2.330	1.004	51.3
	2	83.07	64.25	53.37	124.0	2.323	1.034	58.8
High	1	80.42	64.97	52.27	116.0	2.219	1.081	53.9
	2	83.82	65.17	54.62	119.1	2.181	1.192	61.8
LSD (P<0.05)		*3.54*	*1.08*	*2.14*	*2.68*	*0.101*	*0.163*	*11.7*
Low		81.35	64.05	52.10	125.0	2.399	0.958	44.2
Medium		82.21	64.34	52.90	123.1	2.327	1.019	55.1
High		82.14	65.07	53.45	117.6	2.200	1.136	57.9
LSD (P<0.05)		*2.50*	*0.77*	*1.51*	*1.9*	*0.072*	*0.115*	*8.3*
	1	80.61	64.69	52.15	121.2	2.323	1.023	50.4
	2	83.17	64.29	53.48	122.6	2.294	1.053	54.3
LSD (P<0.05)		*1.80*	*0.67*	*1.14*	*1.6*	*0.053*	*0.110*	*7.3*

In the first sixteen weeks of lay (Table 17) average egg weight was lower on the low ME diet than on the other diets. In the next 28 weeks of lay (Table 18) feed efficiency improved with increasing ME level. Body weight at 35 weeks and egg mass output were lower on the low ME diet than on the other diets, while egg weight was higher and feed intake was lower on the high ME diet than on other diets. In the last 20 weeks (Table 19) egg weight was higher, feed intake lower and feed efficiency better on the high ME diet than on diets of lower energy content.

Individually caged birds came into lay sooner than birds caged in pairs, but achieved a lower peak rate of lay (93.5% compared with 95.2%). Cage type had little influence on most performance factors averaged over the entire 64-week laying period (Tables 16 and 17). However, birds caged in pairs had a slightly higher rate of lay and produced more egg mass than individually caged birds ($P<0.05$).
In the first sixteen weeks of lay (Table 17) individually caged birds ate more feed and converted feed to eggs less efficiently than birds caged in pairs. In the following 28 weeks (Table 18) individually caged birds consumed less feed and tended to produce fewer eggs than birds caged in pairs. In the final 20 weeks (Table 19) individually caged birds laid fewer but larger eggs and produced less egg mass than birds caged in pairs.

Over the 64-week laying period as a whole, feed intake of individually caged birds was more responsive than that of birds caged in pairs to changes in dietary ME level, while egg mass output and ME intake of birds caged in pairs were more responsive than for individually caged birds to changing dietary ME level. As indicated above, the abdominal fat pad weight at termination of the trial was lower for the low ME diet than for the other diets: this difference was greater in birds that had been caged in pairs than in individually caged birds.

In the first sixteen weeks of lay individually caged birds on the low ME diet ate substantially more feed than birds caged in pairs on the same diet. In the following 28-week period individually caged birds on the high ME treatment laid fewer eggs and produced less egg mass than those caged in pairs on the same diet. In the last 20 weeks individually caged birds on the low ME diet produced substantially fewer but heavier eggs and had a lower egg mass output and poorer feed efficiency than birds caged in pairs on the same diet.

Table 17. Experiment 4. Performance results from 19 to 35 weeks of age

ME level	Birds /cage	19-week b'wt (kg)	22-week b'wt (kg)	Rate of lay (%)	Egg weight (g)	Egg mass (g/d)	Feed intake (g/d)	Feed conversion (g feed:g egg)
Low	1	1.711	1.756	85.90	59.01	50.68	130.14	2.568
	2	1.714	1.790	84.64	58.10	49.17	120.67	2.454
Medium	1	1.706	1.787	83.80	59.67	50.00	126.76	2.535
	2	1.758	1.844	85.90	59.53	51.14	125.69	2.458
High	1	1.716	1.793	83.79	59.36	49.74	126.55	2.544
	2	1.718	1.809	86.09	59.80	51.49	123.35	2.396
LSD (P<0.05)		*0.053*	*0.057*	*3.13*	*0.99*	*1.98*	*4.41*	*0.175*
Low		1.713	1.773	85.27	58.55	49.93	125.41	2.511
Medium		1.732	1.816	84.85	59.60	50.57	126.22	2.496
High		1.717	1.801	84.94	59.58	50.61	124.95	2.470
LSD (P<0.05)		*0.038*	*0.040*	*2.21*	*0.70*	*1.40*	*3.12*	*0.124*
	1	1.711	1.779	84.50	59.34	50.14	127.82	2.549
	2	1.730	1.814	85.54	59.15	50.60	123.24	2.436
LSD (P<0.05)		*0.031*	*0.033*	*1.80*	*0.57*	*1.14*	*2.55*	*0.101*

Table 18. Experiment 4. Performance results from 35 to 63 weeks of age

ME level	Birds /cage	35-week b'wt (kg)	Rate of lay (%)	Egg weight (g)	Egg mass (g/d)	Feed intake (g/d)	Feed conversion (g feed:g egg)
Low	1	1.896	83.74	65.83	55.13	125.65	2.279
	2	1.928	85.21	64.36	54.84	127.49	2.325
Medium	1	1.924	86.53	65.16	56.38	121.97	2.163
	2	1.987	87.82	65.25	57.30	126.03	2.200
High	1	1.947	84.94	65.86	55.94	113.60	2.031
	2	1.963	88.71	66.19	58.72	119.83	2.041
LSD (P<0.05)		*0.058*	*3.76*	*1.13*	*2.62*	*3.86*	*0.153*
Low		1.912	84.47	65.10	54.98	126.57	2.302
Medium		1.956	87.17	65.20	56.84	124.00	2.181
High		1.955	86.82	66.02	57.33	116.71	2.036
LSD (P<0.05)		*0.041*	*2.67*	*0.80*	*1.85*	*2.73*	*0.108*
	1	1.922	85.07	65.62	55.82	120.41	2.158
	2	1.959	87.25	65.26	56.95	124.45	2.188
LSD (P<0.05)		*0.033*	*2.18*	*0.65*	*1.51*	*2.23*	*0.088*

Table 19. Experiment 4. Performance results from 63 to 83 weeks of age

ME level	Birds /cage	Rate of lay (%)	Egg weight (g)	Egg mass (g/d)	Feed intake (g/d)	Feed conversion (g feed:g egg)	Egg specific gravity
Low	1	70.26	68.18	47.93	121.0	2.558	1.0805
	2	77.41	66.80	51.70	124.0	2.407	1.0795
Medium	1	72.14	67.70	48.84	118.7	2.453	1.0777
	2	74.16	67.06	49.66	119.7	2.428	1.0778
High	1	71.50	68.84	49.16	110.9	2.285	1.0789
	2	75.15	68.42	51.40	114.7	2.265	1.0763
LSD (P<0.05)		*5.24*	*1.20*	*2.89*	*4.8*	*0.128*	*0.0027*
Low		73.84	67.49	49.81	122.5	2.483	1.0800
Medium		73.15	67.38	49.24	119.2	2.441	1.0778
High		73.33	68.63	50.28	112.8	2.275	1.0776
LSD (P<0.05)		*3.71*	*0.85*	*2.04*	*3.4*	*0.091*	*0.0019*
	1	71.30	68.24	48.64	116.9	2.432	1.0790
	2	75.57	67.43	50.92	119.5	2.367	1.0779
LSD (P<0.05)		*3.03*	*0.69*	*1.67*	*2.8*	*0.074*	*0.0016*

Discussion

The determined AME value of the low energy diet was considerably lower than the calculated value and appears to be incompatible with the determined values of the other diets, unless it is assumed that rice husks not only have a near-zero ME content but also contain factors that interfere with the utilisation of energy from the other ingredients.

The small but significant increase in egg numbers and egg mass from birds caged in pairs over individually caged birds was most pronounced in the high energy feeding treatment and appeared to be associated with higher feed intake and increased fat deposition. However, in the last five months the rate of lay of birds on the low energy diet in two-bird cages was also significantly higher than in individual cages. The reasons for these differences are not clear, although the worsening performance of the individually caged birds towards the end of lay may have been due to the colder weather combined with behavioural factors.

Previous trials at the QPRDC have indicated that the characteristic ME intake of IsaBrown hens in Queensland is within the range 1.35-1.4 MJ/day. At each stage of the trial this intake was generally met by the medium energy diet, exceeded by the high energy diet but never attained by the low energy diet. These differences in energy intake may account for the small but sometimes significant differences in egg weight, egg mass output, body weight gain and abdominal fat body weight between the three treatments. It is perhaps surprising that the reduced ME intake on the low energy diet did not have a more adverse effect on performance. Despite their somewhat lower egg mass output, birds on the low ME diet converted energy and protein to egg mass more efficiently than those on the high ME diet.

The warm period from 35 to 63 weeks of age was also the period of maximum egg mass output. It is difficult to assess the part played by temperature in improving the efficiency of feed conversion in this period compared with the preceding and following periods. An analysis of weekly production and temperature changes from 19 to 35 weeks of age may provide some information on this point. There was little evidence of an interaction between diet and temperature in respect of any performance parameter.

As the nominal ME values are more compatible with one another and with the performance results, in the following discussion these values will be used rather than the determined ME values. If the birds on the low and high energy diets had adjusted their feed intake so that their ME intake was the same as those on the medium energy diet (1.403MJ/kg), the feed intakes on the low and high energy diets would have been approximately 7% higher and 7% lower, respectively. However, the birds on the high energy diet consumed only 4.47% less feed than those on the medium energy diet, while those on the low energy diet consumed only 1.54% more feed. The "efficiency of adjustment" was therefore 64% for the high energy diet and only 22% for the low energy diet.

This result is compromised by the fact that the decline in egg mass output with declining energy level may have been caused, at least partly, by the concomitant reduction in essential amino acid intake. Thus, part of the decline in energy intake may have been due to a reduced requirement for productive energy. If efficiency of feed conversion rather than feed intake is taken as the effective criterion, the proportional changes of -5.4% and +3.1% observed for the high and low energy diets compared with the medium energy diet represent efficiencies of adjustment of 77% and 44% respectively. (The true adjustment presumably lies between the "feed intake" estimate and the "feed efficiency" estimate). In the first 16 weeks of the trial, however, there was virtually no adjustment of feed intake or feed efficiency to compensate for variation in dietary energy content.

The results, therefore, suggest both that the IsaBrown bird is rather poor at adjusting feed intake to meet energy requirement and that birds on the diet with the lowest energy content may have been unable to consume sufficient feed to meet energy and/or other nutrient requirements. In particular, this strain seems unable to compensate for low dietary ME content by increasing its feed intake sufficiently. The results suggest that for part of the laying period the birds on the diet with the lowest energy content may have been marginally unable to consume sufficient feed to meet energy and/or other nutrient intakes required to maximise egg mass yield. There is some indication that a dietary ME level in excess of the median level used in this trial (11.4 MJ/kg) may be required for optimum biological efficiency. However, feedstuff prices will have a strong bearing on which energy level is most economic (see economic assessment below).

The density of the feed appears to have been a constraint on intake of the low energy diet. The density of this diet was only 0.57 compared with 0.77 for the medium energy diet and 0.79 kg/litre for the high energy diet. The inverse values ("dietary bulk") are 1.754, 1.299 and 1.266 litres/kg respectively. Table 20 shows the large volume of feed consumed by the birds on the low energy diet (column E), and suggests that daily ME intake (column F) is inversely related to dietary bulk (column B) and positively related to dietary ME content expressed on a volume basis (column C). None of these relationships appear to be linear, however. Nevertheless, the relative effect of dietary ME content and dietary bulk on feed intake appears to be fairly constant over the whole range of ME and bulk levels studied. In terms of percentage change of each factor, the data are consistent with the assumption that 70-80% of the change in feed intake can be ascribed to dietary ME content (per unit weight) and 20-30% to dietary bulk. The results of this trial therefore agree with the findings of Gleaves *et al.* (1968) and Cherry *et al.* (1983) that dietary energy content alone cannot be used to predict feed intake and that dietary bulk must also be taken into account.

Table 20. Experiment 4. Relationships between energy level, dietary bulk and feed intake

Diet (ME level)	A Nominal ME content of diet (MJ/kg)	B Dietary bulk (litres/kg)	C Nominal ME content of diet (MJ/L)	D Feed intake (g/day)	E Feed intake (ml/d)	F ME intake (kJ/d)
Low	10.6	1.754	6.05	125.0	219	1325
Medium	11.4	1.299	8.78	123.1	160	1403
High	12.2	1.266	9.64	117.6	149	1435

Table 21 illustrates the economics of feeding IsaBrown hens on diets ranging in ME content from 10 to 12.5 MJ/kg, assuming that the relationship between dietary energy and performance follows the same pattern as in the trial. The cost of the diet per kg is relatively flat at energy levels below 11 MJ/kg (but this depends very much on the price paid for low-energy ingredients such as millrun). However, price/kg rises steeply as ME content increases from 11.5 MJ/kg upwards.

This results in a minimum energy cost at a dietary energy level around 11 MJ/kg. The feeding cost per bird per day is lowest at the lowest energy level and so is the cost per 50g of egg, by a small margin, despite the lower egg output of these birds. With eggs priced at $1.00/dozen the difference in return between the high and low ME diets used in the trial is approximately 0.18 cents/bird/day but this is more than offset by an increased feeding cost of approximately 0.34 cents/bird/day. Thus, the egg price/feed cost ratio would need to double for the high energy diet to become the more profitable.

This calculation does not take into account the lower mortality rate to be expected in the birds on the lower energy diets. If the effect of mortality is included the high ME diet is strongly disadvantaged within normal price structures. However, although the mortality in the high energy treatment was significantly higher than in the low energy treatment, this result is not entirely convincing as the figures represent quite small numbers of birds (42 and 6).

Table 21. Experiment 4. Current economics of feeding diets with different ME levels, extrapolated from results to 56 weeks of age

Diet ME[1] (MJ/kg)	Diet cost (c/kg)	Energy cost (c/MJ)	Feed intake (g/d)	Feeding cost (c/d)	Feeding cost (c/50g egg)
10	22.3	2.23	125.3	2.79	2.68
10.5	22.8	2.17	125.0	2.85	2.72
11	23.4	2.13	124.4	2.91	2.76
11.5	25.4	2.21	123.5	3.14	2.97
12	27.0	2.25	121.8	3.29	3.09
12.5	32.0	2.56	118.4	3.79	3.55

[1] *It is assumed that major nutrients are included at concentrations proportional to ME level.*

General Discussion

The present studies were carried out to determine the protein, lysine and methionine requirements, and the optimal dietary ME concentration, for IsaBrown laying hens housed in single-bird or multiple-bird cages. IsaBrown hens were used as the representative of the newly imported coloured genotypes which produce considerably more egg mass and generally convert feed to egg mass more efficiently than local genotypes. Although it has been assumed that the nutritional requirements of the new genotypes are more exacting than those of local layer strains no estimates of these requirements have been made previously in Australia using Australian diets.

The results of the energy study, where peak rates of lay at the three ME concentrations ranged between 93 and 95%, indicated that the IsaBrown hens were poor at adjusting feed intake to meet energy requirements. Furthermore, hens fed a low-density diet with a determined AME concentration of 9.8 MJ/kg did not appear to consume sufficient feed to meet energy and other nutrient needs. A minimum dietary AME of 11.4 MJ/kg appeared to be required for optimal biological efficiency. This ME concentration is similar to the current breeder recommendation.

The diets used to examine the protein, lysine and methionine requirements of the IsaBrown hens in Experiments 1-3 contained calculated ME concentrations of 11-11.25 MJ/kg. Mortality was low and peak rates of lay varied between 91 and 99% on the various treatments in the three experiments. The results of Experiment 1 clearly showed no advantage from increasing the calcium concentration of a grower diet for the three weeks prior to sexual maturity.

Likewise, the production advantages of increasing the layer diet from 160 to 180 g/kg were limited to a small, significant, increase in egg mass output due mainly to a significant increase in egg weight. The lysine and methionine requirements for hens in single cages, obtained in Experiments 2 and 3, respectively, were lower than for hens in multiple 5-bird cages. From a commercial point of view the requirements of the latter hens are more important and were found to approximate 970 mg lysine/day and 430 mg methionine/day. At the calculated dietary ME concentration of 11.25 MJ/kg used in these studies these intakes were attained with dietary concentrations of 7.75 g lysine/kg and 3.33 g methionine/kg.

Important observations from Experiment 2 were that increasing the dietary lysine concentration to 8.15 g/kg improved albumen quality and consistent, if non-significant, increases in serum total immunoglobulin titres were observed with increases in dietary lysine. The latter response contrasted with that observed in Experiment 3 where increases in dietary methionine concentration reduced albumen quality and gave non-significant reductions in serum total immunoglobulin titres.

The main difference between hens in single and multiple cages was the higher mortality, mostly resulting from cannibalism, observed in the multiple cages. This had a significant effect on hen-housed egg production in Experiments 2 and 3. In both studies the difference due to cage density was ameliorated by increasing the dietary concentration of lysine (Experiment 2) and methionine (Experiment 3).

Other effects of cage density were in Experiment 2 where hens in the multiple-bird cages produced eggs with significantly lower % shell but significantly improved albumen height and in Experiment 3 where hens in multiple-bird cages again produced eggs with significantly lower % shell. There was a tendency (P=0.081) in Experiment 3 for hens housed in multiple-bird cages to have increased serum total immunoglobulin titres compared to hens in single cages.

Current breeder recommendations for the IsaBrown hen eating 125 g/day of a diet containing approximately 11.4 MJ of ME/kg are 19.5 g crude protein, 880 mg lysine and 430 mg methionine/day. Estimates based on hen-housed egg production in 5-bird multiple cages in the present work confirm these recommendations for crude protein and methionine but are considerably higher than the suggested requirement for lysine (970 vs 880 mg/day).

Implications

The current breeder recommendations for dietary energy, crude protein and methionine appear to be satisfactory for IsaBrown hens under Australian conditions. The determined lysine requirement was higher than breeder recommendations and further examination of this requirement should be carried out.

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