Chapter 4

Effects of shellgrit shape on the performance of choice-fed laying hens

4.1 Introduction

The individual constituents of the shellgrit had not been considered prior to the commencement of the previous experiments reported in this thesis. It was noted in the experiments reported in the previous chapter that the hens chose flat pieces of shellgrit and rejected cone-shaped shellgrit. Shape may have been the reason for rejection of these shells but it was also noted that these cones contained the dead, dry carcases of the occupants so palatability of the different shells may have influenced intake.

If a great proportion of the shellgrit is cone-shaped it may reduce the calcium intake by the birds and this may lead to a calcium shortfall in hens provided with the shellgrit intermittently. This would be so particularly if the birds were to be presented with fixed amounts of calcium as has been the practice in numerous experiments with substitution of part of the ground calcium with particulate sources (Watkins et al., 1977; Rao and Roland, 1989; Cheng and Coon, 1990c and Keshavarz *et al.*, 1993).

The experiment presented in this chapter was designed to examine the effects of shellgrit shape on shellgrit preferences, feed and calcium intake and egg production..

4.2 Materials and methods

Layer stock, feed, housing and management were as described in Section 3.2, Chapter 3.

At 58 weeks of age, two weeks after the completion of Experiment 3, 56 hens in cages (treatment n=7) in the north side bank of cages (Section 3.2.3 Cages, Chapter 3) were allocated, within each group of four adjacent cages in a randomised block design, to one of four *ad libitum* shell-grit treatments. The treatments were;

Tr 1. Flat shellgrit held by a sieve 4 mm in diameter.

Tr 2. Conical shells held by a sieve 4 mm in diameter.

Tr 3. Flat shellgrit, broken then sieved to between 0.5 and 2 mm in diameter.

Tr 4. Conical shells, broken and then sieved as per Treatment 3.

The experiment ran for 10 d.

All shellgrit was sorted by hand to ensure only the required shape was present in each treatment.

Feed was covered by a grid (Experiment 2, Chapter 3) to prevent wastage and 400 g was presented each day. Wheat and protein concentrate were provided in the 80:20 ratio operating during Experiment 2 (Chapter 3). Shellgrit (100 g per cage) was provided daily. Total feed and shellgrit consumption were determined by weigh-back daily at lights on. Total daily calcium intakes were determined from shellgrit and estimated protein concentrate intakes.

Eggs were collected at 0900 h daily and production was recorded on a hen day percentage (HD%) basis.

Hens in cages where "wet feeders" had been identified (see Chapter 3) were rejected for use in this experiment.

Statistical analysis was as described in Section 3.2, Chapter 3. Where a significant effect of treatment, but no significant interaction of treatment and time, was found by repeated measures analysis (Section 3.2.8, Chapter 3), daily results were pooled across treatments and analysed by the General Linear Models procedure of SAS. Treatment LS Means were then separated using paired-sample t-tests and are presented with the appropriate SE.

Regression analysis was performed on daily egg production versus daily total feed, shellgrit and total calcium intake using the appropriate procedures of the statistical package Minitab.

4.3 Results

Egg production (HD%) was not significantly different (P>0.05) across the treatments (Tr 1=82.5, Tr 2=85.7, Tr 3=73.8 and Tr 4=77.0 SE 5.4).

Daily feed consumption (Table 4.1) by the hens was not significantly different across the 4 treatments (Tr 1=125.6 Tr 2=114.3, Tr 3=124.5 and Tr 4=121.2 SE 4.04) although the hens on Treatment 2 ate approximately 8 % less than those fed the other 3 treatments. There was a highly significant time effect (P<0.01) which may be related to both daily temperature variation and egg production status which was highly variable within treatments from day to day.

Table 4.1. Experiment 4: Total feed intake (g/d per bird) of hens offered flat shellgrit (Tr1); cones (Tr2); fine, flat shellgrit (Tr3); or fine cones (Tr4) (LS Means).

Tr	Day									
	1	2	3	4	5	6	7	8	9	10
1	121	116	126	123	128	126	123	144	121	128
2	114	113	119	114	107	115	115	121	117	109
3	115	122	129	127	121	128	122	132	123	125
4	118	119	122	125	120	125	113	128	122	119
SE	6.1	5.2	5.3	4.7	5.8	5.0	4.5	5.4	3.5	6.2

Shellgrit consumption (Table 4.2) differed across treatments (P<0.05) and over time (P<0.001). Hens on Treatment 2 (whole cones) consumed less shellgrit on the first and sixth days of the experiment than hens on the flat shellgrit (Treatment 1) with a general trend to eat less shellgrit than the hens on the other treatments. Hens on Treatments 3 and 4 displayed a lower shellgrit consumption on the first day of the experiment than those on Treatment 1 but rapidly adjusted their intake to match that of hens on Treatment 1.

Table 4.2. Experiment 4: Shellgrit intake (g/d per bird) of hens offered flat shellgrit (Tr1); cones (Tr2); fine, flat shellgrit (Tr3); or fine cones (Tr4) (LS Means).

Tr	Day									
	1	2	3	4	5	6	7	8	9	10
1	6.1 $_{a}$	4.6	3.7	4.7	7.4	7.9 $_{a}$	5.7	6.3	7.4	6.8
2	0.8 $_{c}$	3.6	3.8	4.7	5.7	3.7 $_{b}$	6.8	5.9	4.9	5.2
3	2.4 $_{bc}$	6.1	3.9	5.4	7.4	6.4 $_{ab}$	7.1	7.0	8.2	6.1
4	2.9 $_{b}$	4.9	4.7	5.4	6.9	6.2 $_{ab}$	4.9	6.6	7.7	6.8
SE	0.64	0.91	1.03	0.68	0.97	0.93	1.01	0.95	1.18	1.04

Values within columns with different subscripts are significantly different ($P<0.05$).

Total calcium intake (Table 4.3) was, as may be expected, highly correlated ($r = 0.99$) with shellgrit intake and also showed a significant ($P<0.05$) effect across treatments and a highly significant ($P<0.001$) time effect. Feed residues were not separated each day to determine the proportion of protein concentrate consumed. The amount of calcium eaten in the protein concentrate was estimated to be 0.9 g/d (calculated from the mean intake of protein concentrate in Experiment 2). No differences in the proportions of wheat or protein concentrate eaten were apparent between treatments. This was determined by visual observation of feed remnants each day. Total daily calcium intake of the hens was derived from the daily shellgrit intake plus 0.9 g.

Table 4.3. Experiment 4: Total calcium intake (g/d per bird) of hens offered flat shellgrit (Tr1); cones (Tr2); fine, flat shellgrit (Tr3); or fine cones (Tr4) (LS Means).

Tr	Day									
	1	2	3	4	5	6	7	8	9	10
1	3.2 $_{a}$	2.6 $_{ab}$	2.3	2.7	3.7	3.9 $_{a}$	3.1	3.4	3.8	3.5
2	1.1 $_{c}$	2.2 $_{b}$	2.3	2.6	3.0	2.2 $_{b}$	3.4	3.1	4.0	2.8
3	1.8 $_{bc}$	3.2 $_{a}$	2.4	3.0	3.7	3.3 $_{ab}$	3.6	3.6	2.7	3.2
4	1.9 $_{b}$	2.7 $_{ab}$	2.7	3.0	3.5	3.3 $_{ab}$	2.7	3.5	3.7	3.4
SE	0.25	0.35	0.39	0.26	0.39	0.37	0.39	0.37	0.46	0.38

Values within columns with different subscripts are significantly different ($P<0.05$).

The hens on Treatment 2 (whole cones) had a lower ($P<0.05$) total calcium intake than those on Treatment 1 on the first and sixth days of the experiment with a general, though not statistically significant, trend for intake to be less for these hens than those on the other treatments Hens given the crushed calcium sources (Treatments 3 and 4) displayed a lower calcium intake on the first day of the experiment than those given large, flat shellgrit (Treatment 1) but rapidly adjusted their intake upwards to match that of Treatment 1 hens.

No significant relationships were found by regression analysis between daily egg production and daily total feed, shellgrit or total calcium intake.

4.4 Discussion

It appears that there are no published studies of the effects of shape on the intake of particulate calcium sources. Perhaps Roland (1986b) may have been alluding to shape when he noted that the great variation in the physical characteristics of different sources of granular calcium used in comparative trials contributed to the inconsistency in experimental reports. The effect of shape on the preferences for various whole grains by birds has been studied (Wood-Gush, 1971) but not, apparently, particulate calcium sources. This appears to be a deficiency, given the plethora of research on the relative merits of shellgrits versus limestones, either ground or in various sizes, on their effects on eggshell quality.

A change in the form and size of the shellgrit offered to the hen affected the amount eaten but for only one day when both flat and conical shellgrits were crushed and used in Treatments 3 and 4. As the hens on Treatment 4 (crushed cones) ate a similar amount to those on Treatment 1 after the first day, it would appear that neither taste nor palatability was the reason of rejection of the conical shells in Experiments 1 and 2 (reported in the previous chapter).

Although the hens fed Treatment 2 (whole cones) ate more shellgrit after the very low amount eaten on day 1, they generally ate less shellgrit than the hens on the other treatments. The reason for the rejection of the cones is therefore apparently shape, but the hens, having targeted the shellgrit as their calcium source, will eat the cones if nothing else is available.

The reason for the unacceptability of the cones is unclear. The cones may be difficult for the bird to manipulate with its beak. An indirect effect of pain may be incurred from consumption of the cones which have sharp-pointed tips, but many of the apparently acceptable flat pieces of shellgrit actually have sharp edges. Pain or discomfort due to the intake of coned shells may also explain the trend toward lower feed intake in the hens on Treatment 2

Shape *per se* appears to be the most important factor in determining selection of the shellgrit. Gentle (1979) found preferences for the shape of objects that chicks pecked at with flat disks preferred by day-old chicks and spherical objects preferred at three days of age. Rogers (1995) confirmed the shape preferences of birds for round or "insect shaped" objects. These studies may not be relevant to mature layers but do indicate that shape preferences exist in the bird.

The trend to a slightly lower intake of calcium due to the provision of coned shellgrit did not have a significant effect on egg production in this experiment. Protein concentrate consumption was not measured in this experiment but it appeared, by close observation, that the hens had not increased their intake of the concentrate to obtain more calcium.

Chapter 5

Effects of the ratio of protein concentrate to wheat on the performance and calcium intake of choice-fed laying hens

5.1 Introduction

In the initial experiment (reported in Chapter 3) the original formulation ratio (70:30) of wheat to protein concentrate was changed after one week to 75:25 and then altered again to 80:20 for the final three weeks. This was due to protein concentrate accumulating in the feed troughs because the birds were eating wheat at a rate greater than the formulation determined. As temperatures were dropping consistently during the first four weeks of this experiment, the birds were increasing their wheat intake to meet the higher energy requirement for the maintenance of body temperature.

Towards the completion of Experiment 2 however, the change in the appearance of the diet presented to the hen was of concern. The lower level of concentrate offered may have altered the feeding pattern of the birds, as wheat predominated in the feed trough. As temperatures increased during the course of Experiment 2, total feed consumption declined but no alteration in the proportion of wheat to concentrate intake occurred as in Experiment 1. Whilst the actual amount of protein concentrate eaten decreased as temperatures rose, the calcium contributed by the concentrate also decreased. The effect of this decrease may have been relatively minor but may have led to a change in the overall calcium economy of the bird.

The experiment presented in this chapter was designed to examine the effects on calcium intake of alteration to the level of provision of the protein concentrate in response to the abovementioned factors observed in the first two experiments (see Chapter 3).

5.2 Materials and methods

Layer stock, feed, housing and management were as described in Section 3.2, Chapter3.

Simultaneously with Experiment 4, at 58 weeks of age, 42 hens in cages (treatment n=8) in the south side bank of cages (Section 3.2.3 Cages, Chapter 3) were allocated, within each group of three adjacent cages in a randomised block design, to one of three levels of protein concentrate inclusion. The treatments were protein concentrate at 20%, 25% or 30% of total daily feed provision (Tr 1, Tr 2 and Tr 3 respectively). The experiment ran for 10 d.

Feed was covered by a grid (Experiment 2, Chapter 3) to prevent wastage and 400 g was presented each day. Shellgrit (100 g per cage) was provided daily. Total feed, the proportion of protein concentrate, total crude protein and shellgrit consumption were determined daily at lights on. Total daily calcium intakes were determined from shellgrit and protein concentrate intakes. Total daily crude protein intakes were determined by addition of the protein intake from the protein concentrate and wheat sources.

Eggs were collected at 0900 h daily and production was recorded on a hen day percentage (HD%) basis. Egg weights were not recorded but daily egg collection involved egg pick-up by a very experienced egg collector to monitor variation in egg weights and the method was as described in Chapter 3 (Section 3.2.7).

Hens in cages where "wet feeders" had been identified (see Chapter 3) were rejected for use in this experiment.

Statistical analyses performed were as described for Experiment 4 (Section 4.2, Chapter 4) with the addition of the proportion of protein concentrate and crude protein intakes to the regression analyses.

5.3 Results

Egg production (HD%) was not different (P>0.05) across the treatments (Tr 1=83.3, Tr 2=89.6 and Tr 3=85.4 SE 3.29). Egg size was appeared to be similar across the treatments at each day's collection.

Feed consumption by the hens (Table 5.1) was not different (P>0.05) across the 3 treatments (Tr 1=120, Tr 2=126 and Tr 3=122 g/d per bird SE 3.80) although there was a highly significant time effect (P<0.001) with feed intake increasing from the lower quantity eaten on the first day. This may be related to both daily temperature variation and egg production status which was highly variable.

Table 5.1. Experiment 5: Total feed consumption (g/d per bird) of hens offered protein concentrate at 20% (Tr1), 25% (Tr2) or 30% (Tr3) of total feed provision (LS Means).

Tr	Day									
	1	2	3	4	5	6	7	8	9	10
1	106	110	116	122	122	117	123	127	127	127
2	113	127	129	127	127	125	128	132	129	124
3	108	123	124	120	126	122	131	124	121	125
SE	3.8	5.5	4.7	6.3	4.3	4.1	5.7	4.6	3.8	3.9

Intakes of protein concentrate (Table 5.2) as a percentage of total feed intake were significantly different (P<0.05) across treatments (Tr 1=12.4, Tr 2=17.9 and Tr 3=20.5 % SE 0.81). There was a highly significant (P<0.01) time effect with lower proportions chosen on the first day and then subsequent increases. This was more pronounced for hens on Treatment 1, less so on Treatment 2 and negligible on Treatment 3. When the data were statistically analysed, variation within treatments indicated that an overall SE was not appropriate and, hence, separate SEs for Treatment 1 and for Treatments 2 and 3 were produced.

Total crude protein intake (Table 5.3) differed across treatments (Tr 1=16.5, Tr 2=19.4 and Tr 3=19.8 g/bird d SE 0.69) as would be expected (above) with hens on Treatment 1 eating less (P<0.05) than those on Treatments 2 and 3. As with total feed and the proportion of concentrate intakes, there was, as may be expected, a highly significant ($P<0.001$) time effect with crude protein intakes increasing from the lower amounts recorded on day 1.

Table 5.2. Experiment 5: Intake of protein concentrate as a percentage of total feed intake of hens offered protein concentrate at 20% (Tr1), 25% (Tr2) or 30% (Tr3) of total feed provision (LS Means).

Tr	Day									
	1	2	3	4	5	6	7	8	9	10
1	11.0_{a}	11.7_{a}	12.1_{a}	13.1_{a}	12.4_{a}	11.4_{a}	13.1_{a}	13.0_{a}	13.0_{a}	12.7_{a}
SE^{1}	1.21	1.20	1.47	1.25	0.83	0.67	0.90	1.02	0.94	1.09
2	17.9_{b}	16.4_{b}	19.4_{b}	18.0_{b}	18.1_{b}	17.6_{b}	17.6_{b}	17.9_{b}	18.1_{b}	17.5_{b}
3	20.9_{b}	18.1_{b}	21.4_{b}	21.9_{c}	21.0_{c}	19.8_{c}	20.6_{c}	20.1_{b}	21.4_{c}	20.0_{b}
$SE^{2.3}$	1.13	1.12	1.37	1.17	0.77	0.63	0.85	0.95	0.88	1.02

Values within columns with different subscripts are significantly different ($P<0.05$).

SE^{1} Standard Error Treatment 1

$SE^{2,3}$ Standard Error, Treatments 2 and 3 pooled

Table 5.3. Experiment 5: Crude protein intake (g/d per bird) by hens offered protein concentrate at 20% (Tr1), 25% (Tr2) or 30% (Tr3) of total feed provision (LS Means).

Tr	Day									
	1	2	3	4	5	6	7	8	9	10
1	14.5_{a}	15.1_{a}	15.9_{a}	17.0	16.8_{a}	15.9_{a}	17.3_{a}	17.7_{a}	17.7	17.7_{a}
SE^{1}	0.74	1.04	1.04	1.20	0.83	0.76	0.98	0.87	0.82	0.72
2	17.3_{b}	18.9_{b}	20.4_{b}	19.5	19.7_{b}	19.1_{b}	19.5_{ab}	20.3_{b}	19.9	18.9_{ab}
3	17.5_{b}	19.1_{b}	20.4_{b}	19.9	20.6_{b}	19.4_{b}	21.2_{b}	19.8_{ab}	19.9	20.0_{b}
$SE^{2,3}$	0.69	0.97	0.98	1.22	0.78	0.71	0.92	0.81	0.77	0.67

Values within columns with different subscripts are significantly different ($P<0.05$).

SE^{1} Standard Error Treatment 1

$SE^{2,3}$ Standard Error, Treatments 2 and 3 pooled

Shellgrit consumption (Table 5.4) did not differ significantly across treatments (Tr 1=5.8, Tr 2=6.1 and Tr 3=6.3 g/d per bird SE 0.28).

Table 5.4. Experiment 5: Shellgrit intake (g/d per bird) of hens offered protein concentrate at 20% (Tr1), 25% (Tr2) or 30% (Tr3) of total feed provision (LS Means).

Tr	Day									
	1	2	3	4	5	6	7	8	9	10
1	4.4	6.4	5.5	6.3	6.6	5.1	6.9	6.0	4.7	6.0
2	3.1	6.4	6.1	7.8	6.7	6.3	8.1	5.4	5.5	5.9
3	4.3	5.6	7.1	7.2	6.2	5.6	7.4	7.5	5.7	6.4
SE	0.90	0.87	0.88	1.00	0.80	0.67	0.99	1.00	0.95	0.67

Total calcium intake (Table 5.5) was highly significantly different ($P<0.01$) across treatments with hens on Treatment 1 consuming less than those on Treatments 2 and 3 (2.8, 3.2 and 3.4 g/d per bird respectively, SE 0.13). As with the intake of all feed ingredients, there was a highly significant ($P<0.001$) time effect on both shellgrit and calcium intake. Day 1 of the experiment was hot and this may have caused the low intakes of all feed items.

Table 5.5. Experiment 5: Total calcium intake (g/d per bird) of hens offered protein concentrate at 20% (Tr1), 25% (Tr2) or 30% (Tr3) of total feed provision (LS Means).

Tr	Day									
	1	2	3	4	5	6	7	8	9	10
1	2.1	2.9	2.7_{b}	3.0	3.1	2.5_{b}	3.3	2.9	2.5	2.9
2	2.0	3.2	3.3_{ab}	3.9	3.5	3.3_{a}	4.0	3.0	3.1	3.1
3	2.6	3.0	3.8_{a}	3.8	3.4	3.1_{ab}	3.9	3.9	3.2	3.4
SE	0.33	0.37	0.34	0.41	0.31	0.26	0.37	0.38	0.38	0.27

Values within columns with different subscripts are significantly different ($P<0.05$).

No significant effect on daily egg production of daily total feed, proportion of protein intake, total crude protein intake, shellgrit or total calcium intake was found by regression analysis. Calcium intake was positively related ($P<0.05$) to the proportion of protein concentrate eaten by the hens.

5.4 Discussion

This short experiment showed that it is imperative to maintain good management standards when attempting to manipulate proportions of feedstuffs presented to choice-fed hens. The effect of what may have been essentially protein restriction highlights the problem raised by Taylor and Kirkley (1967) and Morris and Taylor (1967) of attempting, in balance experiments, to keep variations in feed intake as small as possible by limiting the amount of feed presented to layers. By restricting total feed intake the true effect of calcium levels in the diet may be confounded such as may have occurred in the experiment of Rao and Roland (1990) who presented old layers with fixed amounts of particulate calcium in a small quantity of feed and found that calcium intake parallelled the level of presentation in calcium-deprived hens.

The lower protein concentrate intake by hens on Treatment 1 caused a lower total intake of calcium and, again, the short-term nature of Experiment 5 precluded a determination of how long the hens might take to increase shellgrit intake to make up this shortfall, if ever. Although no effects on egg production were apparent, the longer term effects of lower calcium intake on bone metabolism must be considered. Riddell (1992) found that egg production and eggshell quality could be maintained in hens with osteoporosis up to the point of acute death or posterior paralysis. Whitehead and Wilson (1992) suggested that short-term sub-optimal nutrition may contribute to structural loss of bone which is apparently not replaced for the period that a hen continues to lay.

Variation in protein concentrate intake by the hens in Experiment 5 confirmed the indications of a lower protein concentrate intake at the completion of Experiment 2. Hens on Treatments 2 and 3 consumed similar amounts of crude protein but more than those on Treatment 1. Even though no differences in egg production or egg weight were apparent, significant differences in egg production or size, due to the level of protein concentrate intake, may have emerged in a longer term trial as the hens on Treatment 1 were eating less crude protein than that recommended by the NRC (1994) for brown egg layers.

An explanation for the lower protein concentrate consumption is that the birds had limited access to the smaller amounts of protein concentrate presented. The feed trough was constantly agitated by bird movement throughout the day and the finer concentrate was shaken to the bottom of the trough. (Gently shaking the feed trough by hand showed that this occurred within a few minutes). The grid further limited the capacity of the hens to search for the concentrate by raking over the feed mix with their beaks. For many cages of hens it was noted, during the course of Experiments 2 and 5, that as feed levels dropped in the feed trough, small cone-shaped holes in the remaining feed were formed within the grid pattern. Observation of the hens showed that these formed as the hens pecked through the covering layer of wheat to the bottom of the trough. It is possible that some hens had learned to find the remaining protein concentrate in this way.

The hens on Treatment 2 possibly compensated for the lack of protein concentrate intake by consuming more wheat, as indicated by their non-significantly higher feed intake. Even though the hens offered 80:20 wheat:protein concentrate increased their feed intake over the 10 d of the trial, they did not make up the crude protein shortfall and amino-acid and/or mineral imbalances which may affect metabolism in the longer term must be considered. This may explain why some hens were affected by Fatty Liver and Haemorrhagic Syndrome (Section 3.6.2, Chapter 3) in Experiment 2. This may not have been manifested in the majority of the hens in Experiment 2 as the overall level of feed provision and, hence, the quantity of protein concentrate provided was higher due to the cold weather energy requirement. The hens could have eaten more protein concentrate early in the day whilst more was available prior to being agitated to the bottom of the feed trough.

Chapter 6

The intermittent feeding of shellgrit calcium to individual choice-fed laying hens

6.1 Introduction

The experiments reported earlier in this thesis were conducted in cold conditions and the effect of feeding shellgrit on an intermittent basis in hot weather, particularly to aged hens is unknown. Eggshell quality, and to a lesser extent egg production, are adversely affected by both hot conditions and bird age. Hot weather, exacerbated by humidity, is a problem in most of the major Australian egg production areas.

A reduction in (values of standard measures of) egg quality reported in Experiment 1 (Chapter 3) occurred between days 2 and 4 of denial of shellgrit to the hens. The rate of removal of shellgrit from the gizzards of hens in Experiment 3, and the poorer quality of eggshells after 4 days of shellgrit denial, indicated the need to monitor calcium and phosphorus excretion to gain some measure of bone mineral turnover.

The intermittent provision of shellgrit had an effect on coning of excreta under the cages. Hens on Treatment 4 produced lower, apparently wetter, cones during the warmer weather towards the end of Experiment 2 (Chapter 3). The high calcium content of the gut after re-feeding of shellgrit may have caused higher water consumption and, thus, wetter excreta as the hens may have had a need to remove excess calcium. Leeson and Summers (1991) and Leeson (1993) mentioned the higher water intake and subsequently increased moisture content of excreta from layers on high calcium diets which may be the cause of this reduced coning. Wetter excreta may indicate that the digesta was moving rapidly through the gut with a consequent reduction in the apparent metabolizable energy (AME) of the diet.

In both Experiments 1 and 2 particular hens were noticed to be dribbling thick fluid, presumably crop contents, into the feed troughs. This habit was also noted by

Cumming (1984) but no reasons were offered as to its cause. More birds did this when the weather was warmer. In three cages this was particularly troublesome, due to the volumes of fluid involved, as it made feed weigh-back difficult. The habit was also a problem due to the rapidity, generally within 2-3 hr, with which decomposition of the feed proceeded. It was noticed that some birds appeared to reject the feed after decomposition had reached the point where humans found the odour offensive.

Experiment 6 was undertaken to examine, with individual hens, the effects on production and eggshell quality of intermittent feeding of shellgrit to choice-fed layers under warm conditions. The issues introduced above, calcium and phosphorus excretion, excreta coning and fluid dribbling and dietary AME levels, resulting from the earlier experiments, were also examined.

6.2 Materials and methods

Layer stock, management, feed and methods used for egg measurements, statistical analysis and behavioural and other observations were as described in Section 3.2, Chapter 3. A new batch of the protein concentrate mash (see Chapter 3, section 3.2.4) was prepared to the same specifications for this experiment.

At 59 weeks of age and at the conclusion of Experiments 4 and 5 (Chapters 4 and 5) all the hens were returned to the commercial feeding regime (see Chapter 3).

After two weeks, at 61 weeks of age, 48 hens, identified as the best layers, were moved to a room, 3.9 m long x 6.5 m wide x 3.5 m high at the apex. The room was insulated internally with sprayed polystyrene foam and was a discrete part of an experimental laying shed on the University of New England campus. The shed was constructed of tubular, mild steel frames lined with corrugated, galvanised iron.

The room was locked to prevent unauthorized access and unwanted disturbance to the hens. The room had two windows on opposite sides of its long north-south axis which provided good ventilation without direct disturbance to any individual birds.

The hens were held in conventional two-bird laying cages (46 cm long x 30.5 cm wide x 44 cm high) in banks of six cages. The banks of cages were supported in two 3-tier and one 2-tier metal frames. The 3-tier banks of cages were placed against the western wall of the room, the 2-tier bank against the northern wall. A common external polythene drinker trough was attached to the back of each bank of 6 cages. The water trough held approximately 6.5 l of water and was emptied and refilled daily. Two galvanised sheet steel trays, lined with clear, heavy gauge plastic, to catch all excreta, were placed under each row of 6 cages. Two galvanised steel feed troughs were fitted across the outside of each cage front. Whole wheat and the protein concentrate mash were supplied in one trough (13 cm long x 19 cm wide x 10 cm deep). The calcium source was offered in the other trough (13 cm long x 11 cm wide x 10 cm deep).

Hens were placed in the cages adjacent to those hens that they had been in close contact with in the layer house in an attempt to minimize social disturbance.

The hens were allowed *ad libitum* access, until 63 weeks of age, to the shellgrit (Section 3.2.5, Chapter 3) which for this experiment was sorted by hand so that only flat pieces of shell, greater than 4 mm in diameter (see Treatment 1, Experiment 4, Chapter 4) were available.

When the hens were 63 weeks of age, four treatments were imposed and allocated within each group of four adjacent cages in a randomised block design. The four treatments, all comprising 12 hens, were;

Tr1. Shellgrit available *ad libitum.*

Tr2. Shellgrit available *ad libitum* every second day.

Tr3. Shellgrit available *ad libitum* every third day.

Tr4. Shellgrit available *ad libitum* every fourth day.

For Treatments 2, 3 and 4 the shellgrit trough was placed on the cage front at lights on (0530 hr) and removed at lights out (2130 hr). When not in use the shellgrit trough was placed in the egg roll-out area in front of and below the cage. The hens had no access to the shellgrit with the trough in this position. Eggs were collected at 0900 h daily except during the experimental periods. The pattern of shellgrit presentation was maintained throughout the course of the experiment regardless of whether data were being recorded.

On the advice from a consultant statistician, the experiment was run in three separate periods of 5 days as follows;

Period	Dates	Hen age (weeks)
1	8-12 Jan 95	63
2	1-5 Feb 95	66 / 67
3	25 Feb-1 Mar 95	70

This allowed for simplicity of data collection using the common starting point of all treatments receiving shellgrit on the one day at the commencement of each experimental period.

The sequence of the 4 treatments was thus;

Tr	Day				
	1	2	3	4	5
1	O	O	O	O	O
2	O	X	O	X	O
3	O	X	X	O	X
4	O	X	X	X	O

X = access to shell-grit denied. O = grit available.

The wheat and protein concentrate were offered as the 70:30 ratio of the original feed formulation; 175 g wheat and 75 g protein concentrate. A wire grid (Section 3.4, Chapter 3) was used to reduce wastage of feed but the volume of feed presented allowed the hens to peck to the bottom of the feed trough. This avoided the problem of poor accessibility to the protein concentrate identified in Experiment 5, Chapter 5.

Shellgrit was provided at 50 g per bird for each day of access.

At lights on, feed was separated using a sieve with a 2 mm diameter wire grid. Large fractions of the protein concentrate mash were removed from the wheat by hand. The amounts of wheat and protein concentrate eaten and shellgrit intake were recorded. Fresh feed and shellgrit were then placed in the appropriate troughs.

During the experimental periods, eggs laid late the previous evening were noted and removed at lights-on. New eggs were then collected hourly from lights on until 1600 hr. Eggshell quality measurements were only recorded for the fresh eggs. Egg production was recorded on a hen day percentage (HD%) basis.

Excreta were collected immediately after feed and shellgrit were replaced. After removal of feed, feather and as much fomites contamination as possible, the excreta were weighed immediately after placement in pre-weighed tin foil trays. The trays were placed in a drying oven at 65°C for 23 hr. Dry weight of excreta was recorded the following morning. Water was placed in tin-foil trays at three locations around the room to give an indication of relative levels of evaporation of moisture from the excreta over 24 hr. Excreta were coarse, then fine milled (3 mm and 1 mm screen respectively). Sub-samples were prepared for mineral analysis by ICP spectrometry using the method of Anderson and Henderson (1986) modified by a final sample dilution of 1:10 in deionised water. The modification was found necessary by preliminary analysis which showed that calcium contents of some excreta samples were above the range of the ICP spectrometer. Excreta samples for the analyses were taken for each treatment from 3 birds that had laid eggs on 7 consecutive days prior to and during data collection periods 1 and 2, but from all hens that had laid eggs on 7 consecutive days during period 3.

Calcium and phosphorus balances were determined from intakes of wheat, protein concentrate and shellgrit and the output of excreta and eggshells. Calcium and phosphorus contents of eggshells were determined by ICP analysis (as above).

Gross energy (GE) content of feed and excreta samples, from the same birds selected for the mineral analyses in period 3, were determined using a bomb calorimeter (CP500, Digital Data Systems (Pty) Ltd). This was done as the consistency of excreta in period 2 displayed marked differences across treatments and it was felt that perhaps feed digestibility may have varied across treatments.

For each period, the total feed, wheat, protein concentrate, shellgrit intake and egg production results and excreta water contents from day 1 were compared with the 4-day period means. This was designed to test the effect of non-laying on day 1 on shellgrit intake and to determine if there were any subsequent effects from the level of shellgrit intake.

Results for day 1 and the 4-day means (above) were compared using the General Linear Models procedure of SAS.

Regression analysis was performed on egg production versus either total feed, shellgrit, calcium or protein concentrate intake using Minitab. Multiple regression analyses (Minitab) were also performed on shellgrit and the components of calcium intake to remove variables that were highly correlated with others.

Multiple regression analyses were performed on egg results (EW, SG, SP and ST) versus shellgrit and the components of calcium intake. Eggshell parameters were then compared with egg production. total feed, wheat, crude protein and shellgrit calcium intakes using the regression procedures of Minitab.

6.3 Results

The egg production (HD%) of the hens (Table 6.1) was generally excellent during the experiment and exceeded the breeding company's production targets of 80.0, 77.6 and 75.8 % for hens of these ages. Egg production did not differ (P>0.05) across the treatments (analysed separately for each period).

Approximately 4 % of the eggs laid for the duration of the experiment had eggshell cracks, breaks and malformations (data not presented) which were evenly distributed across the three treatments. Most of the cracks were "star" and "straight" cracks and these were rarely apparent in the earlier experiments.

Table 6.1. Experiment 6: Egg production (HD%) of hens offered *ad libitum* shellgrit daily (Tr1), every second day (Tr2), every third day (Tr3) or every fourth day (Tr4) in periods 1-3 (LS Means).

Tr	Period		
	1	2	3
1	91.7	75.0	89.6
2	89.6	83.3	93.8
3	85.4	81.3	87.5
4	77.	83.3	83.3
SE	5.69	5.50	5.42

One hen on Treatment 1 died on the second last day of the experiment and her data were removed from the analyses of the final experimental period. Veterinary inspection of this hen could not attribute death to a specific cause.

No differences (P>0.05) in total feed consumption by the hens (Table 6.2) were found across the treatments.

Table 6.2. Experiment 6: Total feed intake (g/d per bird) of hens offered *ad libitum* shellgrit daily (Tr1), every second day (Tr2), every third day (Tr3) or every fourth day (Tr4) in periods 1-3 (LS Means).

Tr	Period		
	1	2	3
1	113	116	112
2	104	117	116
3	107	114	114
4	104	111	110
SE	5.3	2.6	2.9

Protein concentrate intakes, as a percentage of total feed intake, and total crude protein intakes were not significantly different (P>0.05) across treatments (Table 6.3). Hens on the three intermittent shellgrit supply regimes (Treatments 2, 3 and 4) tended to eat a greater percentage of protein concentrate than those given daily access to shellgrit (Treatment 1).

Table 6.3. Experiment 6: Protein concentrate intake (%) and, in brackets, total crude protein intake (g/d per bird) of hens offered *ad libitum* shellgrit daily (Tr1), every second day (Tr2), every third day (Tr3) or every fourth day (Tr4) in periods 1-3 (LS Means).

Tr	Period					
	1		2		3	
1	20.7	(18.3)	21.0	(18.8)	18.5	(17.4)
2	22.5	(17.4)	22.1	(19.5)	21.0	(18.9)
3	23.8	(18.9)	24.6	(19.8)	21.8	(18.9)
4	22.7	(17.2)	22.8	(18.7)	23.1	(18.4)
SE	2.21	(0.92)	2.10	(0.75)	2.42	(0.84)

Shellgrit consumption and calcium intake by the hens showed highly significant ($P<0.01$) treatment effects in period 3 only. These are presented in Table 6.4. A trend to lower shellgrit and thus, lower calcium intakes was apparent in the hens on Treatments 3 and 4 compared with the hens given daily access to the shellgrit (Treatment 1).

Table 6.4. Experiment 6: Shellgrit and, in brackets, calcium intakes (g/d per bird) of hens offered *ad libitum* shellgrit daily (Tr1), every second day (Tr2), every third day (Tr3) or every fourth day (Tr4) in periods 1-3 (LS Means).

Tr	Period					
	1		2		3	
1	7.2	(3.7)	5.9	(3.2)	7.1_{a}	$(3.5)_{a}$
2	7.1	(3.6)	5.0	(2.9)	7.3_{a}	$(3.7)_{a}$
3	6.2	(3.4)	5.0	(3.0)	5.4_{b}	$(3.0)_{b}$
4	4.9	(2.7)	5.4	(3.1)	5.6_{b}	$(3.1)_{b}$
SE	0.79	(0.31)	0.71	(0.29)	0.34	(0.23)

Values within columns with different subscripts are significantly different ($P<0.05$).

Egg weights (Table 6.5) did not differ significantly (P>0.05) across the treatments for all 3 periods. There was a significant (P<0.05) time effect on egg weight in period 2 and a highly significant (P<0.001) time effect in period 3. Egg weights tended to become lighter over the 4 d of both periods. Egg weights were lighter than the breeders' production targets of 64.4-64.8 g for hens of these ages. However, very few eggs were under the commercially viable weight of 55 g (3.8 %, 7.2 % and 4.5 % for periods 1, 2 and 3 respectively).

Table 6.5. Experiment 6: Egg weights (g) of hens offered *ad libitum* shellgrit daily (Tr1), every second day (Tr2), every third day (Tr3) or every fourth day (Tr4) in periods 1-3 (LS Means ± SE).

Tr	Period		
	1	2	3
1	60.8± 0.86	61.2± 1.05	62.0± 1.01
2	60.7± 0.86	60.3± 1.00	62.5± 1.01
3	61.3± 0.86	60.5± 1.00	60.9± 1.01
4	59.6± 0.86	59.7± 1.00	60.7± 1.01

Egg SG (Table 6.6) was not significantly different (P>0.05) across treatments for periods 2 and 3. There was a significant (P<0.05) time x treatment effect for SG in period 1, within the 4 days of measurement, and a significant (P<0.05) time effect in period 3 with SG increasing slightly each day.

Egg SW (Table 6.7) and egg ST (Table 6.8) did not differ (P>0.05) across treatments for periods 2 and 3, and similarly with egg SG, there were highly significant (P<0.001) time x treatment effects in period 1 within the 4 days of measurement.

Table 6.6. Experiment 6: Specific gravity of eggs produced by hens offered *ad libitum* shellgrit daily (Tr1), every second day (Tr2), every third day (Tr3) or every fourth day (Tr4) in periods 1-3 (LS Means ± SE).

Tr	Period					
	1				2	3
	d 1	d 2	d 3	d 4		
1	1.080± 0.0021	1.078± 0.0016	1.080 ± 0.0014_{a}	1.080 ± 0.0018_{ab}	1.082± 0.0015	1.080± 0.0019
2	1.080± 0.0027	1.078± 0.0022	1.078 ± 0.0019_{ab}	1.075 ± 0.0024_{bc}	1.080± 0.0014	1.078± 0.0019
3	1.084± 0.0031	1.081± 0.0025	1.079 ± 0.0022_{a}	1.083 ± 0.0027_{a}	1.079± 0.0014	1.078± 0.0019
4	1.078± 0.0027	1.075± 0.0022	1.073 ± 0.0019_{b}	1.070 ± 0.0024_{c}	1.078± 0.0014	1.076± 0.0019

Values within columns with different subscripts are significantly different ($P<0.05$).

For simplicity, data are not presented where significant time effects only were found as all treatments displayed similar trends.

Table 6.7. Experiment 6: Shell weight, as a percentage of egg weight, of eggs produced by hens offered *ad libitum* shellgrit daily (Tr1), every second day (Tr2), every third day (Tr3) or every fourth day (Tr4) in periods 1-3 (LS Means ± SE).

Tr	Period					
	1				2	3
	d 1	d 2	d 3	d 4		
1	8.2± 0.25	8.4± 0.16	8.4 ± 0.17_{a}	8.4 ± 0.19_{a}	8.5± 0.20	8.4± 0.21
2	7.9± 0.31	8.2± 0.20	8.1 ± 0.21_{ab}	7.8 ± 0.24_{b}	8.2± 0.19	7.9± 0.21
3	8.5± 0.31	8.5± 0.20	8.0 ± 0.21_{ab}	8.6 ± 0.24_{a}	8.2± 0.19	8.0± 0.21
4	8.1± 0.31	8.1± 0.20	7.5 ± 0.21_{b}	7.2 ± 0.24_{b}	8.0± 0.19	8.0± 0.21

Values within columns with different subscripts are significantly different ($P<0.05$).

Table 6.8. Experiment 6: Eggshell thickness (μm) of eggs produced by hens offered *ad libitum* shellgrit daily (Tr1), every second day (Tr2), every third day (Tr3) or every fourth day (Tr4) in periods 1-3 (LS Means ± SE).

Tr	Period					
	1				2	3
	d 1	d 2	d 3	d 4		
1	332± 11.9	335± 6.5	339± 8.2 **a**	342± 10.2 **a**	345± 7.5	340± 7.3
2	331± 14.1	324± 7.7	327± 9.7 **ab**	320± 10.9 **ab**	335± 7.2	329± 7.3
3	335± 14.1	338± 7.7	321± 9.7 **ab**	340± 10.9 **a**	327± 7.2	320± 7.3
4	326± 14.1	320± 7.7	303± 9.7 **b**	292± 10.9 **b**	323± 7.2	321± 7.3

Values within columns with different subscripts are significantly different ($P<0.05$).

Room temperatures varied little within each experimental period and are presented in Table 6.9.

Table 6.9. Experiment 6: Mean ambient room temperatures (°C) during periods 1, 2 and 3.

Temperature	Period		
	1	2	3
Maximum	27.5	28.0	28.0
Minimum	17.5	19.5	17.0

Calcium and phosphorus balances and water contents of excreta were only determined for period 3. Calcium and phosphorus intakes, excretion and balance are presented in Table 6.10. There were significant ($P>0.05$) differences across treatments for calcium intakes and balances but calcium excretion did not differ ($P>0.05$). Phosphorus intakes, excretion and balances were similar across the treatments. Mean eggshell calcium and phosphorus contents were 37.0 and 0.9 % respectively.

Table 6.10. Experiment 6: Calcium and phosphorus intakes, excretion and balances (g), during period 3, of hens offered *ad libitum* shellgrit daily (Tr1), every second day (Tr2), every third day (Tr3) or every fourth day (Tr4) (LS Means ± SE).

Tr	Period 3					
	Calcium			Phosphorus		
	Intake	Excretion	Balance	Intake	Excretion	Balance
1	14.3±	13.3±	1.0±	3.2±	2.4±	0.8±
(n=5)	1.15_{bc}	0.96	0.60_{b}	0.27	0.37	0.16
2	16.0±	14.8±	1.2±	3.9±	3.2±	0.7±
(n=5)	1.15_{ab}	0.96	0.60_{b}	0.27	0.37	0.16
3	17.5±	13.4±	4.1±	4.0±	3.5±	0.5±
(n=7)	0.98_{a}	0.81	0.51_{a}	0.23	0.32	0.13
4	12.0±	13.5±	1.5±	3.7±	3.4±	0.3±
(n=4)	1.29_{c}	1.08	0.67_{c}	0.31	0.42	0.18

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

Excreta water content (Table 6.11) displayed a highly significant (P<0.01) time x treatment effect with Treatment 3 displaying a continually higher excreta moisture content than the other three treatments over the 4 day period. On day three, Treatments 1, 2 and 4 had a substantial drop in excreta water content.

Table 6.11. Experiment 6: Excreta water content (%), during period 3, of hens offered *ad libitum* shellgrit daily (Tr1), every second day (Tr2), every third day (Tr3) or every fourth day (Tr4) (LS Means ± SE).

Tr	Period 3			
	Day 1	Day 2	Day 3	Day 4
1	76.0_{a}	75.7_{ab}	45.6_{a}	76.0_{a}
2	76.8_{a}	75.2_{a}	55.1_{a}	74.6_{a}
3	79.8_{b}	78.3_{b}	71.3_{b}	79.9_{b}
4	75.7_{a}	74.2_{a}	49.1_{a}	79.0_{b}
SE	1.05	1.14	5.11	1.21

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

The form of the excreta varied noticeably across treatments. Excreta from hens on Treatment 1 were solid, those from Treatment 2 relatively so, then the consistency and appearance dramatically altered with loose, paler excreta for hens on Treatments 3 and 4 respectively. Excreta from hens on Treatment 4 appeared to be in an almost liquid state and uniformly pale green which became more noticeable with each successive day as the shellgrit was withheld.

Over each 24 hr in period 3, evaporative loss from the water placed around the room, averaged 20 %.

The possible influence of total feed intake on water content of excreta was then examined by dividing total feed intake (g) of the hens by excreta moisture content (g). This result (data not presented) produced similar across-treatment differences (similar to excreta water content (%), Table 6.10) but only on days 1 and 3.

Apparent metabolizable energy (AME) of the feed (MJ/kg) did not differ (P>0.05) across treatments but the effect of time on AME of the diet was highly significant (P<0.01) (Fig. 6.1) with AME for all 4 treatments altering over the four days of period 3.

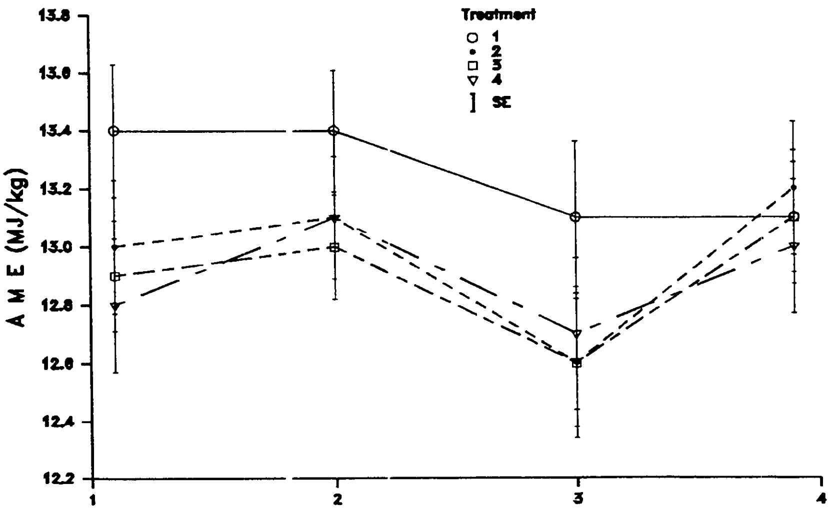

Figure 6.1. Apparent metabolizable energy of the diet (MJ/kg), during period 3, of hens offered *ad libitum* shellgrit daily (Tr1), every second day (Tr2), every third day (Tr3) or every fourth day (Tr4). Vertical error bars represent standard errors of the LS Means.

The effect of non-laying on the first day of the shellgrit provision cycle on shellgrit intake and egg production is presented in Table 6.12. The low number of non-laying birds made an analysis on treatment inappropriate and the figures are given to provide an overall indication of the effects. There was no relationship (P>0.05) between laying or non-laying on total feed, wheat or crude protein intake of the hens or their excreta water content (data not presented) however, crude protein intake tended to be higher in laying hens.

Table 6.12. Experiment 6: Effect of laying or non-laying on day 1 of the shellgrit provision cycle on day 1 and 4 day mean egg production and shellgrit intake of hens (LS Means ± SE).

Measurement	Period 1		Period 2		Period 3	
	non-layer n=9	layer n=39	non-layer n=9	layer n=39	non-layer n=8	layer n=40
Grit intake (g) day 1	1.0± 0.80 **b**	6.1± 0.37 **a**	4.5± 0.91 **b**	6.7± 0.39 **a**	3.7± 0.81 **b**	7.0± 0.37 **a**
Grit intake (g) 5 day mean	5.8± 0.95	6.5± 0.45	4.6± 0.81	5.5± 0.39	7.8± 0.70 **a**	5.8± 0.32 **b**
Egg production (%) day 1	0 **b**	100 **a**	0 **b**	100 **a**	0 **b**	100 **a**
Egg production (%) 4 day mean	61.1± 5.34 **b**	91.7± 2.57 **a**	61.1± 6.01 **b**	86.5± 2.89 **a**	62.5± 5.18 **b**	93.6± 2.34 **a**

Values across rows within each period with different subscripts are significantly different (P<0.05).

Results of the regression analyses performed between egg production and feed intake data are presented, sequentially for each measurement for periods 1, 2 and 3, in Table 6.13. Multiple regression analyses led to the removal of shellgrit and protein concentrate calcium intake as variables from the regression analyses as both were highly correlated with either shellgrit calcium or crude protein intake. Hen day egg production was related to shellgrit calcium intake in periods 1 and 2 only, and total calcium intake in all 3 periods. Egg production was related to total feed intake only during period 1. There was an inverse relationship between total feed and crude protein intakes in all periods. Wheat intake was inversely related to total calcium intake in period 3. These inverse relationships indicate that more protein concentrate was eaten in this period. This may be corroborated by the positive correlation of total calcium intake and crude protein intakes in periods 2 and 3.

Table 6.13. Experiment 6: Regression matrix indicating relationships between intakes of feed constituents and relationships between intakes of feed constituents and egg production, sequentially for periods 1, 2 and 3, of hens offered *ad libitum* shellgrit daily (Tr1), every second day (Tr2), every third day (Tr3) or every fourth day (Tr4).

	Total feed intake (g/d)	Wheat intake (%)	Crude protein intake (g/d)	Shellgrit calcium intake (g/d)	Total calcium intake (g/d)
Egg production (HD%)	*** NS NS	NS NS NS	NS NS NS	* * NS	* * *
Total feed intake (g/d)		NS NS NS	*** ** *	NS NS NS	NS NS NS
Wheat intake (%)			*** *** ***	NS NS NS	NS NS *
Crude protein intake (g/d)				NS NS NS	NS * **
Shellgrit calcium intake (g/d)					*** *** ***

Multiple regression analyses performed between egg results (EW, SG, SP and ST) and shellgrit and calcium intake data for periods 1, 2 and 3 resulted in shellgrit and total calcium intakes being removed from the equations as both were highly correlated (r>0.95) with shellgrit calcium intake. The intake of protein concentrate calcium was similarly removed as it was highly correlated (r>0.96) with crude protein intake. Eggshell parameters were compared with egg production, feed constituents and shellgrit calcium intakes for periods 1, 2 and 3 and the results are presented in Table 6.14. Egg production was related to egg weight, SG, SP and ST during period 2. Higher egg production resulted in lower values for these measurements. During period 3, intakes of wheat and calcium from shellgrit were positively related to SG, SP and ST. Crude protein intake was negatively correlated with SP and ST which may be due to a non-significant (P>0.05) trend for egg weights and therefore, size, to be greater with higher crude protein intake.

Table 6.14. Experiment 6: Regression matrix indicating relationships between egg production and intakes of feed constituents and egg weight and eggshell quality measures, sequentially for periods 1, 2 and 3, of hens offered *ad libitum* shellgrit daily (Tr1), every second day (Tr2), every third day (Tr3) or every fourth day (Tr4).

	Egg weight (g)	Specific Gravity	Eggshell Percentage	Eggshell Thickness
Egg production (HD%)	NS * NS	NS * NS	NS * NS	NS * NS
Total feed intake (g/d)	NS NS NS	NS NS NS	NS NS NS	NS NS NS
Wheat intake (g/d)	NS NS NS	NS NS *	NS NS *	NS NS *
Crude protein intake (g/d)	NS NS NS	NS NS NS	NS NS *	NS NS *
Shellgrit calcium intake (g/d)	NS NS NS	NS NS **	NS NS **	NS NS **

The slightly hotter period 2 produced a problem with a greater rate of feed wetting by hens dribbling crop contents. A number of feed measurements were removed from the analyses for individual days as the protein concentrate matted with the wheat and could not be adequately separated even after oven drying. It was found that birds with a propensity to do this got worse in warmer weather and the number of birds involved increased. With slightly cooler (and noticeably drier) conditions in period 3 the problem was not as pronounced. Between the two measurement periods, half of the birds had their feed troughs raised 8 cm for 5 d in warm conditions and the problem was largely alleviated.

6.4 Discussion

The hens in these trials were laying extremely well (HD%), exceeding the breeders' standards throughout this experiment which may explain why egg weights were below the breeders' target for average egg weight. However very few eggs were under the commercially important threshold weight of 55 g. The rate of production indicates that the selection of the higher producing hens for this experiment had satisfied the aim of using heavy producers. This ensured that the requirement for calcium was maximised so that any effects of the intermittent feeding of shellgrit were highlighted.

The 4 % level of reject eggs was approximately four times greater than for production during the course of Experiments 1 and 2 (Chapter 3). The greater proportion of the cracks were "star" and "straight" cracks and these were rarely apparent in the earlier experiments. These types of cracks, called "impact cracks" (Belyavin *et al.*, 1987) are due to damage as the egg strikes the cage wire of the floor upon oviposition. Both Hunton (1987) and Oosterwoud (1987) emphasised the importance of the effective mass of the wire floor in producing cracked eggs. Oosterwoud (1987) stated that housing was more important than nutrition as a problem to be addressed in reducing the number of cracked eggs. Belyavin *et al.* (1987) stated that good egg shell quality measures may indicate good eggshell strength but not necessarily the likelihood of a shell cracking. It is possible the difference in the wire diameter of the cages in Experiment 6 may have led to the increase in eggshell cracks over the earlier experiments. A wire diameter x shell thickness interaction may be indicated in this result as eggshell thickness throughout this experiment was approximately 20 µm less than from eggs collected during Experiment 1.

The results of eggshell quality measurements indicated that all measures of shell quality deteriorated after three days of calcium denial for hens given access to their calcium source every fourth day (Tr4). The hens given shellgrit every third day produced eggshells which did not differ significantly from those laid by hens given continual access (Tr1) to shellgrit. As no differences in egg production or times of lay were evident across the treatments, different oviposition interval was not an explanation for lower shell quality measurements (Belyavin *et al.*, 1987) for hens on Treatment 4.

The differences in eggshell quality occurred during period 1 only and it is possible that the hens required more than one exposure to the treatments to adjust to the intermittent supply of shellgrit. This may have been especially so with the hens on the intermittent shellgrit treatments that had not previously been exposed to an intermittent regime of calcium supply. The low intake of shellgrit and, hence, calcium by the hens on Treatment 4 supports this point.

During period 2, hens on all 3 intermittent shellgrit treatments tended to consume less shellgrit but had similar calcium intakes as the hens on daily access (Tr1) to shellgrit. This appeared contradictory but the lower intakes of shellgrit and calcium of hens on Treatments 3 and 4 during period 3 indicated the possibility of a change in feeding habits.

Birds on Treatment 4, and to a lesser extent those on Treatment 3, displayed increasing activity and apparent agitation from late on the second day after shellgrit withdrawal in this experiment. This activity was also noted and described in Section 3.7. Wood-Gush and Kare (1966) found that calcium deficient birds were more active than replete birds. It was noted that the shellgrit was eaten avidly by hens on Treatments 3 and 4, for a short period, usually less than 30 s, after the trough was placed before the hen. This behaviour occurred from the first period of shellgrit withdrawal and did not alter throughout the trial as occurred during Experiment 2. This may indicate that these hens were calcium deficient and were replenishing calcium loss as suggested by Hughes (1984). However, the behaviour generally ceased before any possible effect of calcium absorption could have occurred. This replenishment, if it occurred, would be contradictory to the results of day 1 and 4 day mean calcium intakes. The shortfall in calcium intake by day 1 non-layers was not made up by subsequent calcium intake over the four days during periods 1 and 2. However, the low number of non-laying hens may have provided confounding results.

The recorded calcium intakes were variable and this may be due in part to the short measurement times in each period to the presence of limestone (described below). As with total feed intakes, the effects of temperature, humidity and egg laying differences on any one day may show little correlation with the intakes of any feed constituent. This was exemplified in the one-week trial carried out during Experiment 2. For this reason daily results were not presented for any measurement during each period when only a significant time effect was found.

There may have been a greater stress on the calcium metabolism of the high producing hens in Experiment 6, compared with the earlier experiments, especially as they were laying in hot weather (mean maximum room temperatures above 27 °C). Sauveur and Picard (1987) found that temperatures above 25°C reduced shell weight with the mamillary and palisade layer thicknesses decreased by 15-16 %.

A measure of the longer-term effect of supplying calcium to the hens intermittently may be indicated by determining the rates of decline of eggshell quality across the treatments from the first experiment (Section 3.6.1, Chapter 3) to Experiment 6. These results are presented in Table 6.15. From 37 to 70 weeks of age egg weight increased and eggshell quality measures decreased as would be expected (Mannion and Reichmann, 1984). The rate of decrease was greater for the eggs from hens given calcium on an intermittent basis ie the longer the interval the greater the decline.

Table 6.15. Experiment 6: Percentage change in egg weight and eggshell quality measurements between Experiments 1 and 6 of hens offered *ad libitum* shellgrit daily (Tr1), every second day (Tr2) or every fourth day (Tr4).

Tr	Egg weight	Specific Gravity	Eggshell Percentage	Eggshell Thickness
1	1.4	-0.2	-8.7	-5.6
2	1.4	-0.3	-10.6	-8.7
4	1.0	-0.4	-11.6	-8.3

There was a tendency for intakes of protein concentrate to be higher when shellgrit was provided intermittently during all three periods but actual protein intakes for hens were significantly higher ($P<0.05$) over those on Treatment 1 in period 3. This indicates that some hens on Treatments 3 and 4 may have begun to target the protein concentrate as an alternative calcium source. This was surprising as no indication of this had been detected in Experiments 1 and 2 when the hens were laying at a higher rate, or in Experiment 5 when the hens were provided with varying quantities of the protein concentrate.

After the completion of the experiments for this thesis, the protein concentrate was used for further work and was wet-mixed before being pelleted. It was moved for air drying into direct sunlight and whilst still wet, small reflections were noted. The pellets were broken up and small fragments of dark limestone were found (determined to be limestone as the mineral dissolved completely in HCl). Large bone fragments were also found which were not noticed in previous batches of the protein concentrate. It seems likely that some of the hens on intermittent shellgrit provision had detected the limestone and/or bone fragments and were actively seeking these amongst the protein concentrate. The particles had passed through the 2 mm mesh screen of the sieve used for separating the feed ingredients and so were not noticed in the dry mix previously. This observation explains why the third batch of protein concentrate, used exclusively for Experiment 6, gave a mean calcium figure of 44.1 g/kg upon ICP analysis. The previous 2 batches (used in Experiments 1-5) gave figures of 36.9 g/kg of calcium (Table 3.3, Chapter 3). The commercial supplier confirmed that the limestone had been added at the feed mill.

The limestone content of the protein concentrate confounds the results for feed and shellgrit intakes but accentuates the idea that the laying hen is adept at investigating her feed sources. It is assumed that not all of the hens were substituting the limestone as a calcium source but the presence of the limestone, for those hens that had found it, may have been confusing. This is indicated by mean shellgrit intakes and hence, calcium intakes being reduced in hens on Treatments 3 and 4 even though they were laying at similar rates to hens on Treatments 1 and 2.

Calcium intake of the hens was regarded as adequate in this experiment as eggshell quality was reasonable overall, and egg production was excellent for hens of this age. Even though lower amounts of shellgrit were eaten by the hens on Treatments 3 and 4, these hens may have retained more calcium as a lower calcium intake leads to a greater retention rate (Summers *et al.*, 1976; Rao and Roland, 1989 and 1990). However, the presence of limestone in the concentrate confounds this result.

The calcium balance estimates in period 3 indicate that the hens on Treatments 1, 2 and 3 were in positive balance, but those on Treatment 4 were eating insufficient shellgrit to balance losses. This did not result in lower production but produced a trend towards lower eggshell quality. As no increased excretion of phosphorus was detected in the hens on Treatment 4, losses due to mobilisation of bone for calcification may have been minimal. It was not possible to determine balances on a daily basis as there was no method (other than serial slaughter) of estimating the quantity of shellgrit, and thus, calcium, remaining in the digestive tract of the hen at any one time. If a non-destructive method was possible then the rate of loss would allow rates of calcium retention and bone calcium losses to be determined for each day of the period of shellgrit denial. Furthermore, the amount of limestone possibly eaten by any of the hens with the protein concentrate must be considered again as a confounding factor in these results.

Water content of excreta during period 3 was consistently higher from hens on Treatment 3, especially on day 3 when a sudden fall in excreta moisture was experienced in hens on Treatments 1, 2 and 4. Even though temperatures were similar for each day, a weather change overnight between the second and third days produced much lower humidity on the third day. Most of the hens may have then reduced water intake as evaporative cooling by the birds would have been more effective on this day. The drying effect on the excreta must be considered to have affected excreta water content but no discernible difference in the evaporation of the water samples was recorded and the hens on Treatment 3 produced excreta as wet as on the other three days in this period. Hens on Treatment 3 had their shellgrit provided on this day and may have required more water to deal with the calcium load as suggested by Leeson and Summers (1991) and Leeson (1993). The influence of the trend to higher protein concentrate intake by the hens fed intermittent calcium on the water content of excreta produced a significant ($P<0.05$) time x treatment effect (data not presented). This may suggest that the salt content of the protein concentrate was not causing differential water intakes across the treatments but rather that calcium intake *per se* was influencing water consumption. This adds support to the contention of Leeson and Summers (1991) and Leeson (1993) that wet excreta is a problem with high calcium diets. This may have serious implications for the intermittent feeding of particulate calcium to layers in hot weather.

The trend to a lower AME of the diet consumed by hens on Treatments 2, 3 and 4, compared with those on daily calcium supply, was reversed on the final day of period 3. The reason for this is unknown. The AME experiment was conducted as it was regarded as possible that the production of more pale, liquid excreta, with each successive day of shellgrit denial, by the hens on Treatment 4, was influenced by a lack of calcium possibly affecting enzyme function. Calcium is required as a co-factor for trypsin and α-1,4-glucosidase in the intestine (Larbier and Leclercq, 1992). The possibility exists that hens on a complete compounded diet may suffer a similar reduction in dietary metabolizability if feed intake is not sufficient to supply the birds' calcium requirement. Such a problem may occur, as noted by Parkinson and Almond (1995), with weight loss and poor performance, due to pre-peak inappetence, in small-bodied Leghorns. Even though the differences between AME for Treatments 1 and 2, 3 and 4 were small, on an industry scale there would be a significant loss in feed efficiency.

Total feed intakes were similar to those for which the diet was originally formulated. The total feed intakes were similar across the treatments and were much lower than those encountered in the earlier experiments conducted throughout the colder winter months. This supports the statement of Johnson (1983) that climate is more important in the long, rather than in the short term, as a regulator of feed intake in the mature layer.

It was apparent that the position of the troughs used in this experiment caused the head and extended neck of the hens to be below crop level when feeding thereby allowing liquid to return along the oesophagus. Bolton (1965) stated that the crop was an important site of starch digestion and was more than a storage organ. Sturkie (1976b) noted the regurgitation of proventricular, gizzard and duodenal contents, including enzymes, into the crop. Duke (1989) described fully co-ordinated contractions of the proventriculus, gizzard and crop which caused a flushing of chyme back and forth. Gizzard pressures are greater in birds grown on whole grain (Duke, 1989) and gizzard development is poorer in hens fed on mash feeds (Cumming and Ball, 1995). Cumming (1984) found that the problem of fluid dribbling was greater with hens fed on whole grain. The flushing of chyme may have reached the crop due to gizzard pressures in the whole grain reared hens in this experiment. If this is so, enzyme contents in this chyme may explain the rapid decomposition of feed in the feed troughs of hens that frequently lost fluid. Perhaps the back-flushing of chyme into the crop is natural occurrence in hens with a strongly developed digestive tract as it would begin the digestive process of all feed items in the crop with enzymes from further down the digestive tract.

The problem of feed wetting may be exacerbated in hot weather as the hen drinks more water. Raising the feed troughs seemed to largely prevent this loss of fluid from the hens. It was noted that the hens, feeding in the troughs, were picking up food from below the level of their feet which may not happen so readily in a natural situation.

The different intakes of shellgrit on day 1 of each shellgrit provision cycle by layers and non-layers highlights several points. Firstly, a non-laying hen eats less calcium than a layer but this difference in intake was negated in period 3 where day 1 non-layers ate more shellgrit with subsequent provision of shellgrit. Secondly, the demand for calcium is anticipatory, as suggested by Mongin and Sauveur (1979a) as non-layers did not replace shellgrit, and hence, calcium, used for subsequent eggshells in periods 1 and 2. However, the low number of non-layers and the low mean of 4-day egg production by these hens may simply be indicative of birds with low, or falling production towards the end of the laying cycle.

A low intake of calcium by non-layers on the day of shellgrit provision creates a potential problem for hens on an intermittent calcium supply, as subsequent eggshells may suffer due to a low calcium supply from the gut. This would then require the hen to supply eggshell calcium from skeletal reserves (Mongin and Sauveur, 1984; Boorman *et al.*, 1989). When calcium became available from the gut there may then be a competing demand for the calcium absorbed for both skeletal calcium accretion and eggshell formation for any one egg.

The results of regression analyses of feed intakes (Table 3.17) indicate that greater intakes of calcium are required for higher levels of egg production. It appears that the change in protein concentrate intake of hens exposed to the intermittent calcium regimes affected the results during the course of Experiment 6. Increasing crude protein intake, indicative of protein concentrate intake, changed from having no initial relationship with total calcium intake to being closely related during the final period. This suggests that the presence of limestone in the protein concentrate compromised the results for this experiment. Another interpretation may be that the change from significant relationships, found between egg production, feed and shellgrit and calcium intakes in the earlier experiments, was due to changes in individual requirements, such as for calcium caused by bird age (Belyavin *et al.*, 1987) or protein and energy (Forbes and Shariatmadar , 1994) with age.

In period 2, egg production was negatively correlated with egg weight and all shell quality parameters as may be expected. The reasons for these results only occurring in period 2 are unknown. Temperatures and feed intakes were not greatly different in this period compared with the other periods.

In period 3 eggshell quality measures were positively correlated with wheat and shellgrit calcium intake. Wheat intake was negatively correlated with crude protein intake and, hence egg size, and so it follows that better measures of shell quality are associated with greater wheat intakes ie greater egg size indicates that less wheat and more protein concentrate is eaten. Eggshell percentage and eggshell thickness were negatively correlated with crude protein intake which, along with the trend to greater egg weight with increasing crude protein consumption, indicates that larger eggs with their greater surface area result in thinner shells (Mannion and Reichmann, 1984).

Specific gravity was not related to crude protein intake unlike the other eggshell quality parameters. Meyer *et al.* (1973) found that the palisade layer of the shell could vary in extent and that penetration of it into the roots of the mamillary layer appeared important in determining shell strength and so, the density of this layer could vary. The unknown contribution of limestone to the calcium intake of the hens and the generally higher intakes of calcium by the hens on Treatments 1 and 2, may have been the reason for the significant relationships between eggshell results and feed and calcium intakes in period 3.

It appears that the laying hen can rapidly adapt to the supply of an identifiable particulate calcium source on an intermittent basis. A number of potential problems such as polydipsia and lower dietary metabolizability were detected. More detailed experimental work is required to examine the behavioural and reproductive capacity of the laying hen to manage the intermittent supply of calcium. Nevertheless, the presentation of particulate calcium to hens on an intermittent basis may provide a method of allowing the even distribution of calcium in any form of feeding system to be closely monitored by direct observation at the time of feeding.

Chapter 7

General discussion

The successful rapid adaptation by the laying hen to the intermittent supply of her calcium source is due to her complex nervous system allowing the formation of a memory of the source of her calcium supply and the amount required to maintain physiological well-being.

The hen can adjust her calcium intake to a multiple of the amount eaten by *ad libitum* supplied hens by the number of days of withdrawal of the calcium source. This has been displayed for up to four days by the hens used in these experiments (Chapters 3 and 6). With the hens considered as a flock, this appears to occur with as few as one or two exposures to novel times of access to their calcium source. The rapidity with which the hen makes adjustments to calcium intake probably indicates the importance placed by the hen on shell formation and quality to achieve reproductive success. Some hens take longer to adjust accurately with the lengthening of the period of calcium withdrawal.

The apparent ability to memorise the correct amount of calcium eaten supports the claim by Rogers (1995) that the laying hen has complex cognitive abilities allowing it to make complex decisions and that the cognitive capacity is dependent on environmental stimulation throughout development. This further supports the suggestion by Cumming *et al.* (1987) that the laying hen should by exposed during rearing to all the feedstuffs it may encounter during lay and adds weight to the idea (Mastika and Cumming, 1987) that the laying hen is well adapted to the use of choice-feeding as it has adequate time in which to learn to regulate its feed intake accurately. Similarly, the view of Forbes and Shariatmadari (1994) that the "poor" results obtained by some choice-feeding trials may be due to a lack of training is supported by the experiments presented in this thesis.

The hens in the trials reported in this thesis were laying extremely well compared to the breeders' standards with egg production being superior at all times. It is apparent that the choice-fed laying hen can regulate her calcium, energy and protein intakes to match the highest production rates obtained under compounded feeding systems. This regulation was achieved so that the intakes of calcium and protein were similar to the recommendations of the NRC (1994) for laying hens of this type. Energy intake was adjusted in the longer term to account for changing environmental temperatures. The production levels achieved in the experiments reported in this thesis do not support the suggestion by Hughes (1979; 1984) that only hens selected under choice-feeding regimes may match the very highest production rates or food efficiencies (Appleby *et al.*, 1992) of hens given compounded feeds.

Eggs were of lower weight than the breeders' suggestions but as there is no current economic gain in producing large eggs in Australia, it is advantageous to produce greater numbers rather than size.

The presence of extraneous limestone in the protein concentrate resulted, in the final experimental period, in variations in protein concentrate and shellgrit intakes which affected total calcium intakes (Chapter 6). Egg production was unaffected and total feed intakes were similar across the treatments. This may provide support for the suggestion by Hughes (1972), supported by Mongin and Sauveur (1979a) that calcium and feed intakes are regulated separately. No significant differences in feed intakes occurred across the treatments even though hens receiving calcium every fourth day were eating approximately 24-30 g of shellgrit on the day of access. The results of shellgrit and calcium intakes concur with the suggestion by Taylor (1970) that hens can accurately regulate the intake of a particulate calcium source and that calcium intake meets the anticipated requirements of eggshell formation (Hughes, 1972; Mongin and Sauveur, 1979a).

The relatively large particle size of the shellgrit used in these experiments may have allowed for longer term availability of calcium for absorption as it is meted from the gizzard as suggested by Scott *et al.* (1971). This was supported by the calcium balance indicating that only hens denied access to their calcium source for four days were in a negative balance over the experimental period (Chapter 6). Eggshells of lower quality were produced by hens only after access to their calcium source was withheld for more than 3 days (Chapters 3 and 6). Scott *et al.* (1971) suggested that dietary calcium produced better shell quality than bone calcium and Sauveur (1992) found that hens offered a recognisable calcium source which could be eaten in response to eggshell formation produced stronger shells and this was associated with decreased bone mobilisation. Even though the mobilisation of bone mineral is rapid in response to dietary calcium insufficiency, perhaps it may be regarded as a reserve to prevent hypocalcaemic catastrophes as suggested by Hurwitz (1987) and not as a major reserve of calcium for constant daily eggshell formation. Perhaps this may explain the increasing problem of osteopenia in modern egg production systems with calcium supplied in a ground form to birds with increasingly limited appetites.

In the longer term, the effect of supplying calcium to the hens intermittently was to cause a greater reduction in all measures of eggshell quality. The rate of decrease was greater for the eggs from hens given calcium on an intermittent basis; the longer the interval the greater the decline. Hence, from a practical point of view the intermittent supply of calcium could not be recommended if the maintenance of optimum eggshell quality was required.

The shape of the shellgrit affected its intake by the hens (Chapter 4). The rejection of cone-shaped shells was due, apparently, to shape *per se* and not palatability. However, the hens, having targeted the shellgrit as their calcium source, will eat the cones if nothing else is available. This has implications for experiments where comparisons between particulate calcium sources are made as no literature considers this aspect of particulate calcium intake.

Variation in the level of protein concentrate presented to the hens (Chapter 5) indicated that the hens given protein concentrate at 20 % of total feed presentation were effectively deprived of access to the amount of protein voluntarily consumed by those hens given feed constituents at the rate of the original feed formulation (30 % protein concentrate). This caused a reduction in calcium intake in the hens on the lower level of protein concentrate inclusion and this may be due to the hens not associating, in the short-term, any calcium deficiency with limited access to the protein concentrate. However, as no adverse production effects were apparent during this short trial perhaps the overall calcium intake was sufficient. This may imply that the hens eating slightly more calcium, on the treatments with greater protein concentrate intakes, were effectively overconsuming calcium. Further testing of the longer term consequences of protein deprivation is warranted. This experiment showed that it is imperative to maintain good management standards when attempting to manipulate, for ostensibly good reasons, proportions of feedstuffs presented to choice-fed hens.

Hens given access to shellgrit every 3 or 4 days displayed increased activity which was due to their being thwarted in attempts to gain access to the shellgrit (Chapters 3 and 6). This may have indicated that the birds were becoming calcium deficient after 2 days denial of access to their calcium source as Wood-Gush and Kare (1966) found that calcium deficient birds were more active than replete birds. Unlike the birds of Wood-Gush and Kare (1966) the hens in the experiments in this thesis did not eat more feed when denied access to their calcium source. This increasing activity may be linked to hormonal involvement in the maintenance of plasma calcium levels as there is a relationship between the ovulatory increase in oestradiol and calcium intake (Mongin and Sauveur, 1979a) and calcium deprivation leads to increasing appetitive behaviour (Wood-Gush and Kare, 1966). With each succeeding day of eggshell formation and reduced gut calcium supply, the maintenance of plasma calcium levels and calcium for eggshell formation is increasingly drawn from medullary bone supplies (Hurwitz, 1987).

The greater fat-free proportions and ash contents of the femurs (Chapter 3) of hens given shellgrit intermittently compared to those allowed free access should be examined further as few birds were used in this experiment. Medullary and cortical bone were not separated in this experiment and it may be that cortical bone was being used to maintain medullary bone volumes as found by Clunies *et al.* (1992). However, the result is of particular interest given the finding of Whitehead (1994) that particulate shell produced a greater proportion of medullary bone in hens than a ground calcium source.

The results of gut measurements taken from sacrificed hens (Chapter 3) are of interest for several reasons. The different proportions of feed constituents eaten prior to lights out by the hens indicated that the diurnal pattern of feed intake may be altered by providing the shellgrit on an intermittent basis. The 6.1 g of shellgrit found in the crop and similar content in the gizzard of hens fed Treatment 1 is of interest in that the regulation of shellgrit intake may have a volume component. Furthermore, the differences in development of the gizzard, in complete and choice-fed, whole-grain reared pullets (Cumming and Ball, 1995) may have a profound influence on the regulation of calcium supply in the gut. This is due to a greater retention time in, and hence, more efficient utilisation of calcium from, larger gizzards produced by whole-grain rearing of birds (Karunajeewa, 1977).

The similar and lower quantity of shellgrit in the crop of hens with either a soft-shelled or no egg present in the oviduct respectively, compared with those containing a hard-shelled egg, may indicate that a late time of ovulation may reduce calcium intake and this may affect subsequent shell formation. Mongin and Sauveur

(1979a) found a peak of calcium intake 8-12 hr after oviposition at the start of the next eggshell calcification event. If the oviposition is later in the day, the stimulus for this calcium intake may coincide with the dark period and birds do not normally eat in the dark (Appleby *et al.*, 1992).

Water content of excreta (Chapter 6) is of concern as calcium intake *per se* may influence water consumption. This adds support to the contention of Leeson and Summers (1991) and Leeson (1993) that wet excreta is a problem with high calcium diets. A high water content of excreta is problematical due to storage, ammonia and disposal problems of wet excreta. The results of this experiment were not conclusive and warrant further investigation.

The trend to a lower AME of the diet consumed by hens on intermittent shellgrit provision compared with those on daily calcium supply requires further investigation. The possibility exists that hens on a complete compounded diet may suffer a similar reduction in dietary metabolizability if calcium levels in a complete ration, or feed intake, are not sufficient to supply the birds' calcium requirement. Such a problem may occur as weight loss and poor performance, due to pre-peak inappetence, in small-bodied Leghorns was noted by Parkinson and Almond (1995). Even though the differences between AME for all treatments were small, on an industry scale there would be a significant loss in feed efficiency.

The different intakes of shellgrit on day 1 of each shellgrit provision cycle by layers and non-layers (Chapter 6) highlights a potential flaw in the method of supplying a calcium source to the hen on an intermittent basis. If, as suggested by Mongin and Sauveur (1979a), the demand for calcium is anticipatory of laying, non-laying hens, on the day of their calcium provision, may not eat enough calcium to allow for dietary calcium supply for calcification of subsequent eggs produced in the calcium withholding cycle. As so few non-laying hens were available for these comparisons in this experiment, this potential difficulty requires thorough examination.

Other, more detailed experimental work is required to examine the behavioural and reproductive capacity of the laying hen to manage the intermittent supply of calcium. This includes the comparison of complete rations versus choice-feeding during rearing to determine the ability of the hen to learn to accurately regulate calcium intake under both systems. This would include comparing complete ration reared birds with and without a clearly identifiable calcium source, and with and without calcium included in the ration itself.