Chapter 1

Introduction

Traditionally, and again more recently, calcium has been supplied supplementally to the diet of laying hens in the form of particulate limestone, marble or shell chips. This is so the bird does not consume diet merely to satisfy the increased calcium requirement that accompanies eggshell formation (Morris and Taylor, 1967; Taylor, 1970). Mannion and Reichmann (1984) noted that on egg-forming days, the bird consumed 12-20 g more diet (3.2 % Ca) when calcium was not provided supplementally and which these authors ascribed to a distinct calcium appetite. This result may also be obtained by dietary dilution by the added limestone and the subsequent need for the bird to consume more of this lower density diet. In restricted feeding systems, typical of those for broiler breeders and where supplemental calcium is not provided, egg production is poor and calcium balances on days of production are negative (Taylor, R.D. and Jones, G.P.D., unpublished).

Where supplemental calcium is provided, there is considerable debate as to the superiority of the source used. Miller and Sunde (1975), in a review of the literature, indicated that calcium was better provided by oyster shell compared to limestone as measured by traditional shell quality parameters and when both sources were ground. When large particles were provided, no difference between sources was observed. Mannion and Reichmann (1984) concluded that differences between sources could be ascribed to particle size. Although differences in layer performance with different particle sizes have been demonstrated, the reasons propounded for any differences observed (ie changes in shell quality) may be incorrect or misguided. Increasing the particle size *per se* is not important but rather particle area, which, if considered, may account for observations indicating that shellgrit is superior to limestone. However, increasing the surface area by decreasing the particle size may result in the particle passing through the gut more rapidly (Roland *et al.*, 1972). Large particles of shell or limestone may provide calcium to the bird for up to 4 d after ingestion (Sloan, 1976).

The commercial laying hen has demonstrated appetites for energy, protein and calcium. Compounded layer diets are balanced with respect to energy as it is generally

considered that the energy appetite of the bird is dominant. The current practice of feeding compounded diets has been shown to be inefficient where environmental or disease status alters the bird's energy metabolism (Walker and Farrell, 1976; Mastika, 1987) which then may result in protein and calcium insufficiency. Similarly, the metabolic requirements of the layer for each of the dietary constituents varies with individual and age. Calcium requirement is generally greatest 8-12 hr after the previous oviposition (Hughes, 1972; Mongin and Sauveur, 1974; Mongin and Sauveur, 1979a) which corresponds with the formation of the shell of the next egg.

The ARC (1975) emphasised that a generous excess of particulate calcium must be allowed in any diet with limited calcium incorporated within it as rationing is not accurate. The NRC (1994) suggested that in the quest to use less feed and with the low feed intake in small-bodied layers developed for the modern layer industry the high levels of calcium carbonate or calcium phosphate required in complete diets to meet suggested calcium requirements may cause problems with dietary dilution (as mentioned above) and unpalatability.

Morris and Taylor (1967) found that the requirement for calcium in layers caused a far greater difference in feed intakes between layers and non-layers than any other nutrient. Taylor (1970) suggested that perhaps the ability of the hen to determine its own calcium intake should be utilised. Taylor (1972) claimed that the ability of the layer to regulate calcium intake, with an *ad libitum* shellgrit supply, saved feed compounders the worry of determining the amount of limestone to add to a diet and that it was pointless to establish a calcium requirement in terms of g/kg or g/MJ of diet. This is especially so under Australian conditions where poor environmental control in poultry houses leads to the regular under- and over-consumption of nutrients in compounded feeding systems. Summers *et al.* (1976) supported these claims and stated that the use of an absolute value for calcium intake, based on a percentage in the diet, was useless due to variable feed intakes by the hen.

Karunajeewa (1987) indicated that layers had effectively been free-choice fed until the 1950s but this method of feeding was abandoned in favour of the ease of automated feeding of complete diets. Summers and Leeson (1979) found little change in dietary presentation since the advent of automation due to equipment needs rather than good nutrition and that choice-feeding, or the separate provision of the major dietary ingredients ie energy, protein and calcium, should be re-examined. Not only would cost savings be incurred due to the reduced mixing and grinding of feeds but the

hen would be more feed efficient as the overconsumption of certain dietary ingredients, as stated above, would be largely eliminated. Similarly, better dietary utilisation may accrue through improved gizzard function due to the feeding of whole grains, which induces greater muscular development (Duke, 1989), and calcium in grit form, which has a longer retention time in these larger gizzards (Karunajeewa, 1977). Poorly developed gizzards in birds on mash feeds has been recently reported by Cumming and Ball (1995).

One particular difficulty for compounders of complete diets is presented by flocks prior to, or at, point-of-lay when individual birds in the flock are at various stages of sexual development. The skeletal system accounts for approximately 98% of the bird's bodily calcium and prior to reaching sexual maturity, the hen begins to store calcium for egg shell production. This calcium is stored as part of medullary bone which is formed in the marrow spaces 10-14 days prior to the commencement of egg production (Taylor *et al.*, 1971) and may eventually comprise 4-5 g of the skeletal calcium. The proportion of medullary bone may vary with the strain of hen as Clunies *et al.* (1992) found a low (< 1 g) medullary bone store in their experimental laying hens.

Hurwitz (1970) suggested that successful rearing involved maximising this skeletal calcium reserve. This may be especially important in sexually maturing birds as Whitehead and Wilson (1992) pointed out that even short-term marginal nutrition may lead to structural bone loss which is not regenerated during the period the hen remains in lay. Roland (1986a) found that, in the pullet to layer phase, a low calcium diet leads to over-consumption of feed in the more developed birds and this effectively acts similarly to force-feeding to produce a fat hen more prone to Fatty Liver and Haemorrhagic Syndrome.

There is debate as to the effectiveness of the provision of plasma calcium from diet or bone sources as measured by shell quality and it seems that dietary calcium may be superior. Scott *et al.* (1971) suggested that dietary calcium produced better shell quality than bone calcium. Earlier work by Taylor and Hertelendy (1962) and recent studies (Keshavarz *et al.*, 1993) support this theory. Naturally, once Ca^{2+} is in the plasma, the immediate source is unimportant. It is possible that in some situations (dietary calcium insufficiency) the bird will attempt to conserve medullary bone (Clunies *et al.*, 1992). Where dietary calcium is adequate, 60-75% of the eggshells' calcium is supplied by dietary sources (Driggers and Comar, 1949) and Tyler

(1954) suggested that when shell formation normally occurs (late afternoon, evening and early morning), dietary calcium constitutes a greater proportion of the shell.

A better knowledge of the fluxes of calcium, from dietary source to blood then bone and/or shell are critical if shell quality, and to a lesser extent, egg production are to be effectively manipulated by management. At present, we possess a general understanding of these mechanisms but little specific knowledge. This is exacerbated when calcium separation in feeding systems occurs (Belyavin, 1994) and when the particle size of supplemental calcium supplies varies.

It has become commonplace, when describing the importance of calcium nutrition research in laying hens, to emphasise the level of waste or downgrading of eggs. For example, 5-10 % downgrading of eggs occurs in Australia, the bulk of which comes from thin and cracked shells (Karunajeewa, 1977). Between 6-7 % egg downgrades occur in the UK of which 90-95 % are due to cracks (Spackman 1987). This level of downgrading is often claimed to be so expensive as to warrant more research into improving shell quality by nutritional means.

However, Oosterwoud (1987) found that the low correlation of laboratory measures of eggshell strength to the percentage of cracks in the commercial sphere suggested that the major area to be addressed in this issue was housing, more than nutrition, as damage was largely due to impact of the shell with the cage floor or other eggs. Solomon (1990) pointed out that the efforts of geneticists and nutritionists had not reduced egg downgrading and that perhaps research is misdirected in relation to the needs of improvement of eggshell quality.

Rogers (1995) suggested that complex cognitive abilities may occur in many types of birds as they possess a complex nervous system forming a multitude of memories. Rogers (1995) further stated that cognitive capacity depends on stimulation throughout development and this supports the suggestion by Cumming *et al.* (1987) that the choice-fed layer should be exposed during rearing to all the feedstuffs it may be presented with during lay. This exposure from a young age may allow the developing bird to form precise discriminatory abilities based on "positive post-ingestional feedback" giving a reinforcement of the behaviour of correct food selection as hypothesised by Hughes (1979).

Perhaps then, it would be better to examine the effects of variation in calcium provision to the laying hen using the inherent ability of the hen to determine her requirement. Mongin and Sauveur (1979a) suggested that a calcium appetite was due to a preference for a property of a dietary component and so, was best described in terms of learning. Hughes (1979) postulated that calcium is a key element for hens due to the demand for rapid shell production in the progenitors of domestic stock. It would be sensible for the species to have developed a mechanism for calcium discrimination through natural selection instead of each bird learning anew. This could be initiated by calcium demand and lead to appetitive behaviour triggering calcium recognition after ingestion of different feedstuffs.

The use of the cognitive abilities of the hen developed by exposure to various feed ingredients during rearing using the choice-feeding system may lead to reduced egg downgrades. Certainly, a reduction in egg downgrades has not been achieved through the standard compounding of complete feeds.

An experimental programme, designed to examine the ability of the choice-fed reared laying hen to determine her calcium intake, using the practice of intermittent feeding of coarse shellgrit, is described in the chapters following the literature review.

Chapter 2

Literature review

2.1 Calcium and the commercial laying hen

Soares (1987), in a symposium introduction, expressed doubt as to the ability of any other animal class to absorb, transport and metabolize more calcium per unit of body weight than avians. This emphasises the crucial role of calcium in the reproductive success of birds. The level of human interest in the subject of avian calcium metabolism is largely derived from the intensive utilisation of birds in food production.

There are many excellent reviews of calcium metabolism in laying hens. Mannion and Reichmann (1984) and Hurwitz (1987) reviewed the subject with practical reference to eggshell quality The latter emphasised that a major factor in egg production was the control of calcium metabolism in relation to its effect on egg quality. Boorman *et al.* (1989) reviewed mineral and in particular, calcium metabolism, with respect to eggshell formation and quality as did Tullet (1987) with respect to eggshell (quality) faults.

Leeson and Summers (1991) and Larbier and Leclercq (1992) reviewed the role of calcium metabolism as part of the whole subject of poultry management. The British Agricultural Research Council (ARC) and U.S. National Research Council (NRC) committees both reviewed the subject with reference to the latest available research and then provided recommendations for the different rates of calcium inclusion in diets for birds to optimise production and eggshell quality.

The ARC (1975) stated that the inclusion of all calcium within the diet was more easily justified on the grounds of convenience than nutrition and that "it is only practicable to provide for the full estimated requirements of laying hens for calcium if all of it is incorporated in the diet". This implies that calcium must be provided daily and in a ground form. The NRC (1994) found that the quest to use less feed for egg production had progressed beyond decreasing the amount of feed required for maintenance. With the development of increasingly small-bodied layers improved feed

efficiency had occurred. There were several points emphasised such as the re-evaluation of calcium requirements for pullets due to research into the change in the metabolism of medullary bone before maturity and, with apparent frustration at the plethora of conflicting results, a plea for research to be directed at clarifying the amounts and sizes of particulate calcium that would result in consistent improvement in eggshell strength.

The NRC (1994) recommendations were 3.25 g/d for white egg layers and 3.6 g/d for brown egg layers. The latter recommendation appears to be derived largely from extrapolation from the figure for Leghorn-type strains, based on the greater body size of brown egg layers, as little research appears to have been directed to the requirements of the calcium needs of larger strains.

Calcium is involved in many metabolic processes such as enzyme and complement activation, blood clotting, skeletal muscle contraction, hormone secretion, action as a secondary messenger like c-AMP and involvement in the neuro-muscular system. The role of calcium was detailed in an extensive review by Larvor (1983). In the laying hen, calcium is extremely important due to the requirement for reproduction.

The following review will introduce calcium metabolism using, as a framework, the integrating model of Hurwitz *et al.* (1987). In particular it will concentrate on the role of dietary calcium through methods of supplying dietary calcium to laying hens, feeding behaviour, feed intake, the fate of calcium in the gut. The thrust of the review is directed towards the use of particulate calcium in the feeding of the commercial laying hen. Some aspects of choice-feeding will also be considered as this was the preferred feeding method selected for the experiments to be conducted for this thesis.

2.2 Calcium metabolism and its control systems

An integrated model of calcium metabolism was detailed by Hurwitz *et al.* (1987). Their model attempts to describe and quantify the effects of dietary calcium levels on the system of plasma calcium regulation. The reader is directed to the diagram provided by these authors which provides a succinct overview of the proposed feedback control mechanisms of plasma calcium levels.

The system of calcium homeostasis proposed by Hurwitz *et al.* (1987) has three control sub-systems: bone, kidney and intestine and three endocrine systems: parathyroid glands (secreting parathyroid hormone-PTH), the ultimobranchial gland (secreting calcitonin) and the kidney 25-hydroxycholecalciferol-1α-hydroxylase which converts vitamin D3 to its active metabolite. The calcium binding-proteins (Cabp) and the sex hormones are also involved in calcium homeostasis.

These systems have been reviewed extensively as detailed in the following sections.

2.2.1 Bone

Bone formation, physiology, maintenance and cellular activity in avians was reviewed by Taylor *et al.* (1971) and Mueller (1976). More recently reviews have described the rapid advances in bone cell biology due largely to the application of molecular biology techniques in research (Thomson and Loveridge, 1992). Loveridge *et al.* (1992) reviewed the biology, biochemistry and endocrinology of bone growth and metabolism and detailed the bone matrix proteins which act as nucleators or inhibitors of bone crystal growth. Thomson and Loveridge (1992) described osteoblast and osteocyte development and function and the interaction of the cells and calciotropic hormones (PTH, calcitonin, vitamin D3, growth hormone and oestradiol) with the plethora of recently discovered locally-derived growth factors. Watkins (1992) described the fatty acid requirements for the formation of eicosanoids, including prostaglandins, which are involved in the local regulation of bone metabolism and emphasised the close interaction of bone formation and resorption processes.

Along with the intensive research into the role of bone in calcium metabolism and its interaction with egg production, has emerged concern over the escalating economic losses due to bone fragility in laying hens. Riddell (1992) defined the three terms describing poor bone structure in layers viz; osteoporosis, osteomalacia and osteopenia (the general term for bone loss). Whitehead and Wilson (1992) and Whitehead (1994) provide good reviews of bone loss in layers. Beckett (1992) suggested that osteopenia is the result of genetic, nutritional and environmental manipulation by scientists which has resulted in producing a small-bodied, earlier maturing, feed efficient bird laying 40 % more eggs than birds 40 years ago with, consequently, great demands on its bone metabolism. Roland and Rao (1992) claimed osteopenia caused 15-30 % of hen mortality in the US. Battery cage housing of layers

is implicated in exacerbating the problem (Lanyon, 1992) partly by reducing the mechanically induced stimulus to bone strength provided by exercise (Fleming *et al.*, 1994). Inducing greater medullary bone volume by feeding particulate calcium (Whitehead, 1994), may be of practical value in alleviating some bone fragility problems. Wilson (1991) found that the breaking force of the radius was greater in the non-laying hen, rather than in the laying hen, as there was more medullary bone present.

2.2.2 Kidney

Kidney function was reviewed by Sturkie (1976a) and Wideman (1992). Wideman's (1992) review indicates that the kidney co-regulates inorganic phosphorus and calcium metabolism by excreting them when in excess or retaining them when in deficit. PTH secretion in response to low plasma calcium acts on the kidney to decrease calcium and increase phosphorus excretion and to accelerate activation of vitamin D3. The effects of the active metabolite of vitamin D3 on renal calcium and phosphorus transport are uncertain. Acid-base balance, kidney damage associated with disease (such as Infectious Bronchitis) or mycotoxins cause variations in renal calcium and phosphorus transport and there is circumstantial evidence for age and strain differences in renal function. Shane and Young (1967) and Wideman *et al.* (1993) indicated that kidney damage problems may be due to feeding high calcium pre-layer diets to pullets.

2.2.3 Intestine

The intestine will be dealt with in the section on calcium and the avian gut.

2.2.4 Parathyroid hormone

Parathyroid hormone (PTH) is a single chain 84 amino-acid polypeptide produced exclusively by cells within the parathyroid gland (Price and Russell, 1992). The parathyroid gland is actually four smaller glands attached to or near the posterior pole of the thyroid gland (Ringer and Meyer, 1976). Secretion of PTH is modulated by plasma calcium in a sigmoid-like mode and stimulated intracellularly through c-AMP mediation by hormones such as catecholamines, dopamine and secretin (Hurwitz, 1990). The rapid action of PTH is uneconomical as it involves a net loss of calcium

(Hurwitz, 1990). The direct effect of PTH is to stimulate bone resorption via an increase in c-AMP production in the target bone cells. PTH stimulates osteoclasts via an intermediate osteoblast cell type as osteoclasts themselves have no PTH receptors; this may be by osteoblasts synthesizing collagenase to degrade the non-mineralized osteoid layer allowing access to the matrix by the osteoclasts (Price and Russell, 1992).

Miller (1992) suggested PTH may have functions in the movement of minerals in the uterus as it is produced in this organ.

There is a circadian rhythm of PTH caused by changes in dietary (hence plasma) phosphorus (Frost *et al.*, 1991).

2.2.5 Calcitonin

Calcitonin is the principal hormone inhibitory to bone resorption (Price and Russell, 1992) produced in the C-cells of the ultimobranchial glands which are paired organs 1-3 mm long located posterior to the parathyroids (Ringer and Meyer, 1976). It is a polypeptide (Larvor, 1983) with a 32 amino-acid sequence (Hurwitz, 1990) and molecular weight of about 3,000 (Hurwitz, 1987). Both calcitonin and PTH determine the amount of calcium and P built into, retained and released from bone (Ali, 1992). The activity and formation of the osteoclasts, which are the target cells (having specific receptors), are inhibited upon treatment with calcitonin, mediated by a dose-dependent increase in c-AMP (Price and Russell, 1992).

2.2.6 Vitamin D

Hurwitz (1992) reviewed the homeostatic role of vitamin D in avians in detail. He indicated the importance of the commercial availability of this steroid hormone for the industrialisation of the poultry industry by allowing indoor farming. Norman (1987) reviewed the mode of action of all the vitamin D metabolites and highlighted the conceptual advances and large database on the vitamin D endocrine system stemming largely from studies in avian species. Hurwitz (1990) found that during prolonged perturbation of calcium metabolism vitamin D3 dominates the control of calcium metabolism, as it has a slower response time than PTH, and this may be beneficial to overall calcium economy in the bird.

Tsang and Grunder (1993) discussed vitamin D3 action in the uterus of the hen and Mannion and Reichmann (1984) concluded that vitamin D3 was the key factor in determining eggshell quality. Frost *et al.* (1991) indicated that there was a circadian rhythm for vitamin D3 in layers.

2.2.7 Calcium binding-proteins (Cabp)

Hurwitz (1970) discussed Cabp in a review of calcium metabolism. Cabp are found in the intestine, uterus (and eggshell) and in the epithelial cells of the kidney. In these organs, an increase in Cabps are associated with increased calcium transfer. The role and function of the many Cabps was reviewed by Mannion and Reichmann (1984) who indicated that the vitamin D metabolites broadly control Cabp synthesis and action and that there is little direct relationship between the Cabps and eggshell formation. However, Kang *et al.* (1993) found duodenal and uterine Cabp levels to be highest in hens laying good eggshells and lower in those laying bad eggshells and suggested that eggshell quality is largely determined by the quantity of Cabp produced by the individual hen.

2.2.8 Sex hormones

The role of the sex hormones is summarised (Mannion and Reichmann, 1984) as follows. The activity of the ovary and production of ovarian steroids are controlled by the hypothalamus and pituitary gland. Direct feed-back to these centres, via plasma calcium levels, possibly occurs as ovulation is halted in severe calcium deficiency. Oestrogen, interacting with androgens, stimulates turnover and net formation of medullary bone in the pre-lay pullet even under dietary calcium restriction. Testosterone, progesterone and oestradiol provide maximum stimulation of 25-OH-D3-1α-hydroxylase in the kidney during each ovulation cycle.

Evaluating the role of sex hormones in calcium homeostasis and determining whether they affect shell quality is difficult due to problems in experimental assay and the large non-physiological doses often used. Whitehead *et al.* (1993) found egg weight responses in layer hens to the feeding of fats. Moderately saturated, 18 carbon fatty-acids enhanced oestrogen metabolism. These authors indicated that nutritional regulation of hormonal metabolism by dietary fat in the layer could have wide implications and warrants study by nutritionists.

2.3 Calcium and the avian gut

2.3.1 Crop

The crop is more than a storage organ as some absorption may occur due to the prominence of blood vessels in the full crop (Bolton, 1965). Larbier and Leclercq (1992) indicated that the rate of crop throughput depended upon food particle size and particle moisture content. Sturkie (1976b) reported the regurgitation of proventricular, gizzard and duodenal contents into the crop. The contractions of these organs were fully co-ordinated and resulted in chyme flushing in both directions between them (Duke, 1989) indicating that the acid breakdown of calcium carbonate may begin in the crop.

2.3.2 Proventriculus

The proventriculus produces and releases pepsinogen, hydrochloric acid (HCl) and mucus (Duke, 1989). The secretion of HCl in the proventriculus was important to solubilize 7-8 g of calcium carbonate eaten daily by the hen and to maintain pH of 1-2 (Larbier and Leclercq, 1992). Hill and Tyler (1954) suggested that calcium carbonate was dissolved less readily when cereals were present, but below pH 3.5 nearly all would be. More water and HCl were secreted in the proventriculus during eggshell calcification (Mongin and Sauveur, 1984). Persson *et al.* (1991) found that an increase in serum gastrin induces hypocalcaemia and proposed that this was due to the release of "gastrocalcin", a hypothetical calciotropic peptide hormone, which was formed in the proventricular mucosa and released upon food intake. The hypocalcaemia was due to increased bone uptake of calcium but calcitonin was not involved in causing this post-prandial hypocalcaemia. The phenomenon does not occur in proventricularized chicks.

2.3.3 Gizzard

The gizzard has a thick, coarse lining called koilin to cope with the demands of mechanical digestion of food. Duke (1989) found that gizzard pressures were greater in birds in the past due to the feeding of whole grains which induced greater muscular development. However, the advent of compounded and finely ground feeds has led to the poor muscle development of gizzards in birds (Hill, 1971; Cumming and

Ball, 1995). Karunajeewa (1977) found that in larger gizzards there was an increase in the retention time of feed so giving more efficient utilization of calcium especially in grit form.

2.3.4 Intestine

Sturkie (1976b) reported the sequence of calcium absorption in the segments of the avian intestine with the duodenum > jejunum > ileum. Sunahara *et al.* (1989) found that calcium was absorbed sequentially in the jejunum > ileum > colon when the insoluble marker Cr_2O_3 was used but colon > proximal duodenum when using the soluble marker polyethylene glycol. This conflict has still to be clarified. With high dietary calcium absorption is largely by simple diffusion, but with low dietary calcium there is active transport. Calcium absorption is higher during peak shell deposition but with no difference in early or late calcification when the rate of calcium deposition is lower. Duke (1989) found that in birds the enterogastric reflex exists with gastric motility regulated by the chemical nature and volume of duodenal contents. Stillmak and Sunde (1971) found that high magnesium (Mg) levels in dolomitic limestone caused cathartic action in the gut and increased feed throughput which lowered calcium absorption. Duke (1989) found that diurnal rhythms also occur. Moreover, changes in illumination are anticipated in that the frequency and amplitude of contractions decrease before lights-out. The activity of the gut of mature laying hens displays less change between light and dark periods and this is believed to be due to calcium requirements. The retention of dietary calcium rises about 10 d prior to the initiation of lay (Mueller, 1976) and this may be initiated by the increase in plasma 17-β oestradiol stimulating the activity of alkaline phosphatase in the intestinal mucosa (Qin and Klandorf, 1993). Wu *et al* (1993) found traces of the mRNA that encodes calcium binding-protein (Cabp) but no Cabp in the jejunal tissue of immature or point-of-lay pullets but large amounts of both in layers particularly in the villus tip enterocytes.

Nys *et al.* (1992) indicated that eggshell formation caused the predominant Cabp, the 28 kiloDalton (28 kDa) Cabp, to increase due to more effective translation by the Cabp mRNA. This may be influenced by calcium level *per se* but not by increased vitamin D3. Wasserman *et al.* (1992) found that the enhanced absorption of calcium in calcium deficient chicks was due to increased levels of both Cabp and the enterocyte basolateral membrane ATP-dependent calcium pump epitope. Guinotte *et*

al. (1993) found H^+/K^+-ATP-ase activ ty was not affected by dietary calcium levels but increased when coarse, rather than fine, calcium particles were fed to pullets or layers.

Larbier and Leclercq (1992) concluded that calcium absorption from the jejunum was 45 or 17 % depending on whether or not egg formation was occurring and suggested a mean utilisation of dietary calcium of 50 %.

Nemere (1992) suggested that the critical question in transcellular calcium transport was to identity the carrier, which is needed to minimize toxicity due to high intracellular calcium concentrations. In a review of proposed carriers Nemere (1992) directed attention to the endosomal-lysosomal pathway.

Dunn *et al.* (1993) assayed Cabp 28 kDa levels using SDS-PAGE and ^{45}Ca binding to Western blots and found that dietary aluminium (Al) may interfere with the body's ability to regulate intestinal Cabp 28 kDa levels. This may affect other tissues containing substantial levels of Cabp 28 kDa such as the uterus in hens.

2.4 Radioactive tracers in calcium studies

The study of calcium nutrition in layers has largely been empirical but experiments using radioactive tracers, usually ^{45}Calcium (^{45}Ca), have provided some illumination as to the mechanisms involved in avian calcium metabolism.

Driggers and Comar (1949) found ^{45}Ca in one eggshell 15 min after intubation of the hen with ^{45}Ca labelled $CaCl_2$ and 30-35 % of the labelled calcium was generally found in eggs laid 24 hr after dosing. They detected appreciable quantities of ^{45}Ca in the bones and blood of hens after 21 days of heavy production after a single administration of ^{45}Ca. Tyler (1954) found no isotope in the eggshell up to 2 hr after feeding ^{45}Ca labelled feed and suggested that calcium absorption was not particularly fast. These two studies are contradictory but may indicate differences in the stage of eggshell development or relative levels of dietary calcium available to the hens.

Mueller *et al.* (1964) fed 44-week-old pullets ^{45}Ca and calculated that there was 20-25 g of total body calcium. Hurwitz (1965), using either a single dose of ^{45}Ca or a long-term $^{45}CaCl_2$ labelled diet in hens, described a sequence of rates of calcium turnover in various bone segments. He calculated the calcium turnover rate in

medullary bone segments to be 10-15 times greater than the cortical segments and suggested that the cortical bone may be utilized by hens during severe calcium depletion. Clunies *et al.* (1993) used ^{45}Ca to study the dynamics of body calcium compartments during a single ovulatory cycle and found that bone ends continue to lose ^{45}Ca 18-24 hr post-oviposition but medullary bone does not. They suggested that perhaps mechanisms involved in bone mobilization are not easily stopped.

Farmer *et al.* (1986) intubated hens in the morning or evening with ^{45}Ca mixed with fine granular limestone and confirmed the finding of Bar and Hurwitz (1975) that calcium absorption was almost doubled during eggshell formation.

Hurwitz (1968) suggested that the presence of a slowly exchanging fraction in the protein-bound calcium in the blood may impose serious limitations on studies of calcium kinetics in the layer.

2.5 Phosphorus

Calcium and phosphorus metabolism are intimately connected.

Roland (1986a), in a review of papers where intake of phosphorus was specified, indicated that the actual requirement by layers was uncertain as there were various feed intake and production responses in hens fed a range of phosphorus levels. Summers *et al.* (1976) indicated that the layer absorbs very little phosphorus irrespective of dietary level. Hopkins *et al.* (1989) noted that NRC recommendations for phosphorus had consistently decreased since 1944. Roland and Rao (1992) reviewed the perceived problem of low phosphorus levels in feeds and found that 5-15 % of the layers in any flock may receive marginal levels (100-200 mg/d) due simply to their appetite. Low phosphorus intake was exacerbated by overestimation of phosphorus levels or availability in feedstuffs and overestimates of feed intake in the hens which may be disturbed by short-term effects such as temperature (Rao and Roland, 1992). Cost often precluded a sufficient safety margin for extra phosphorus inclusion in feeds. Sauveur (1991) suggested the separate, or "intermittent", feeding of high phosphorus feed in the morning and high calcium feed in the afternoon as a means of reducing the requirement for dietary P.

Rao and Roland (1992) reviewed the effects on dietary P levels of the high P availability from animal sources and the problems of determining P availability from phytate P found in plant sources. Martin (1995) reviewed the role of phytase in wheat and found that phytate hydrolysis continues from the crop to the proximal duodenum. Hill and Tyler (1954) found that if little hydrolysis of phytate occurred in wheat before it reached the proventriculus then only 50 % of the total phosphorus would be dissolved. Ravindran (1995) suggested that of the 1.9-2.9 g/kg of total phosphorus in wheat, 61-78 % is present as phytate but much of which is available due to the high phytase level in wheat. He suggested that high calcium intakes may inhibit phytase activity.

Mannion and Reichmann (1984) found that the nature of the relationship between calcium and phosphorus in the layer was unclear. Rao and Roland (1992) indicated that feed intake, not the calcium:phosphorus ratio, was the problem in relation to low phosphorus intake in layers. If calcium, phosphorus and vitamin D3 are adequate in the diet, the calcium:phosphorus ratio is of little practical significance (Larvor, 1983). Larbier and Leclercq (1992) claimed that the concept of calcium:phosphorus ratios was redundant in layer nutrition but in 1993, Keshavarz and Nakajima were still using the term and suggesting a calcium:phosphorus ratio of 27.5:1 was not a problem in layers.

Mongin and Sauveur (1979b) studied inorganic phosphorus concentrations in the plasma and urine of layers during eggshell formation. They found that the peak in plasma phosphorus level was reduced if shellgrit was provided to the hens, as bone mobilisation to supply calcium for eggshell calcification was reduced. Boorman *et al.* (1989) concurred with this and further advised supplying particulate calcium to improve eggshell quality for hens with poor bone mobilising capacity. However, Roland and Rao (1992) suggested that, with the growing problem of osteopenia in layer strains, low dietary phosphorus may be more involved than calcium.

2.6 Calcium and the feeding laying hens

2.6.1 Historical background

With the importance of the debate as to the best sources and methods of presentation of calcium to laying hens it is surprising that there has been minimal development of practical methods for supplying calcium to hens.

Pliny the Elder described the high rates of lay of hens in Roman farming and differences in egg size with age and clutches and, according to Rackham (1940), he did not mention anything recognisable as a calcium supplement being supplied for the benefit of egg production. He did point out that a hen may lay as many as 60 consecutive eggs and then drop dead from exhaustion, and it is of interest to speculate as to whether this may have been due to calcium deficiency. Wood-Gush (1959) reviewed poultry farming methods from antiquity to the 19th century but did not mention calcium.

A field study in India of red jungle fowl, one of the reputed progenitors of modern hens, by Collias and Collias (1967) does not mention calcium in the dietary intake. Several studies of feral poultry likewise do not mention calcium in descriptions of dietary constituents (McBride *et al.*, 1969; Savory *et al.*, 1978 and Duncan *et al.*, 1978). Savory *et al.* (1978) found grit in excreta from feral fowls but it was apparently insoluble grit. Duncan *et al.* (1978), in describing these same fowl, state that the birds were apparently selecting an adequate diet as determined from an average production of 28-30 eggs per bird during the breeding period. However, a housed control group laid 58 eggs per bird so a nutrient deficit may have been involved. The wild birds were subject to predation by mink which may possibly explain the difference in production.

Watkins *et al.* (1977) stated that for nearly a century the optimum particle size of calcium for layers had been a controversial subject and Roland (1986b) found that in the late 1800s, it was common practice for concentrated forms of calcium to be provided for hens to maintain optimum shell quality.

With the development of the poultry industry through the 20th century layers were effectively fed free-choice with calcium supplied as either limestone chips or some form of shellgrit until the 1950s when all-mash feeding systems were introduced. This occurred because of the ease of automated feeding of a single feed to hens in cages (Karunajeewa, 1987). Summers and Leeson (1979) indicated that there had been little change in dietary presentation since automation due to the needs of the feeding equipment rather than the nutritional requirements of the bird. This supported the view of Miller and Sunde (1975) who found that small mills were not equipped or preferred not to add coarse calcium to diets.

Taylor (1970) took both blame and credit for, and reversed his earlier encouragement of, calcium inclusion to the full requirements in the diet itself.

2.6.2 Feed intake in layers

To enable an animal to selec an adequate diet, Lepkovsky (1967) suggested that it required sensory informatic n about food in the external environment, information about internal requirements and integration of this information to direct food-seeking behaviour to the most favourable choice. Appetite and feed intake are controlled by the interaction of internal processes which can be loosely classified as physiological, neurological or behavioural factors. All of these factors are described in the review by Forbes (1995) whilst behavioural and some physiological factors are reviewed by Rogers (1995).

Feed intake (how much is eaten) and feed choice (what is eaten) are subtly different even though part of the same general topic and are intertwined in the following sections.

Physiological factors

Olfaction has an important role in feed intake in the chicken (Rogers, 1995). Lindenmaier and Kare (1959) found that chickens possessed taste buds morphologically similar to mammalian taste buds. The chicken secretes a small quantity of saliva and holds food in the mouth for a short time so it was generally believed that the chicken had little need for a well developed sense of taste (Kare *et al.*, 1957). However, Lindenmaier and Kare (1959) observed chicks savouring new foods suggesting that novel food items were treated with caution and tasted before ingestion. Rogers (1995) indicated the role of taste in feeding in birds and suggested that it was used mainly for avoiding distasteful objects.

Rogers (1915) decided that the controlling factor for feed intake was crop distension but Fisher and Weiss (1956) found that feed intake was unaffected by surgical removal of the crop. When chickens were trained to eat their daily food ration in 2 hr, the crop became larger and heavier to hold more food (Lepkovsky *et al.*, 1960). Wood-Gush and Gower (1968) suggested that crop distension was involved in food intake, as greater distension was tolerated in birds after a period of food deprivation. Duncan *et al.*, (1970) suggested that an "activating" mechanism, dependent on crop distension, may halt or initiate feeding.

The role of circulating nutrients in the control of food intake is a contentious issue. Kare and Beily (1948) found great differences in NaCl tolerance and its dietary effects on chicks. Gleaves *et al.* (1968) indicated that as dietary energy level increased food intake was reduced. Duncan *et al.* (1970) agreed that circulating nutrient levels rose after feeding but argued that the elapsed time between eating and consequent changes in the concentrations of circulating nutrients is such that other factors must control food intake immediately.

Neurological factors

The hypothalamus has long been considered to be the centre of appetite control as it receives thermal, metabolic, cortical, hormonal and neurogenic information describing the nutritional state of the animal (Morley, 1980). Lepkovsky (1973) indicated that body adipose tissue stores provided a set point for hypothalamic control of appetite and tested the validity of this proposal by demonstrating that force-fed obese cockerels refused feed until fat levels decreased to normal levels.

Polin and Wolford (1973) postulated that peripheral receptors in the crop regulated food intake via neural connections to the hypothalamus with hormones influencing the set point at which the receptors operate. They found that testosterone decreased, and oestrogen increased, body fat levels.

Morley (1980) developed a peptidergic hypothesis of appetite control whereby food intake is regulated by a balance of interacting peptides and mono-amines acting to influence a network of appetitive behaviours on a backdrop of interneurons in the hypothalamus.

A detailed review of this area of research was provided by Forbes (1995).

Behavioural factors

Duncan *et al.* (1970), studying birds in a Skinner box, found a diurnal feeding rhythm, with a marked decrease in food intake towards the end of the day. Each rhythm was characteristic of one individual bird. Savory (1976) found that fowls preferred eating more towards the end of the day and suggested that this was to ensure an adequate overnight store of food in the crop. Feral fowls were observed to do

likewise (Savory *et al.*, 1978). Savory (1979) reviewed feeding behaviour in birds. Different feeding patterns in layers and non-layers may reflect the requirements of egg formation. The layer fills her crop in the evening, the non-layer usually eats more in the morning under artificial lighting but eats most in the evening under natural light or with an artificially simulated dusk. This suggests that all birds prefer to avoid a food deficit at night but many cannot predict days end under artificial lighting. In the layer there is a low morning intake as behaviour is more directed towards laying. Afternoon feed intake is due largely to calcium demand. Changes in calcium and food intake during egg formation are probably hormonally controlled. A diurnal feeding pattern may be primarily due to the bird's ability to predict the end of the photoperiod but in layers, late feeding is a direct consequence of the timing of oviposition and/or egg formation which are themselves synchronised by the light/dark cycle.

Activity levels of chicks increase when they are food-deprived with little individual variation in activity patterns between birds (Campbell *et al.*, 1966). Wood-Gush and Guiton (1967) and Duncan (1970) described escape behaviours and redirected pecking when layers were thwarted in their attempts to feed. Hughes and Wood-Gush (1973) reported an increase in spontaneous activities in calcium-deficient birds in cages. The main activity was "air pecking" performed in a similar way by each bird. This stereotyped behaviour was thought to be a means by which birds reduce anxiety when unable to satisfy a physiological need.

Feeding behaviour of the bird is influenced by factors other than those concerned with its internal state (Wood-Gush, 1971). Visual cues are important in influencing feed intake. Rogers (1995) reviewed this area in detail and emphasised the effect of brain asymmetry in feeding behaviour in birds.

Ross *et al.* (1962) noted that birds consumed more feed when presented with larger piles but added that these larger piles may allow the bird easier access to the food as small piles are quickly scattered. Wood-Gush and Gower (1968) found that fowls ate more pellets than mash and suggested that pellets resembled the natural grain or insect foods that the wild progen tors and domestic fowls (until recently) normally consumed. Gentle (1979) found an innate preference of day-old chicks to peck at round more than angular objects and 3 day-old chicks to peck more at solid hemispheres than flat discs. This was supported by Rogers (1995). Wood-Gush (1971) in a review of feeding behaviour in fowls said that shape preferences were important in determining the intake of grains with an order of preference of wheat >

maize > rye > barley > oats. This preference was based on shape alone and did not differ if the various grains were ground and reconstituted in the shape of the other grain types.

Feed intake in fowls is stimulated by social factors as feeding increased in chicks when a model hen made pecking movements (Tolman, 1967). Tapping sounds caused a further increase in feeding. Tolman and Wilson (1965) suggested that a specific kind of behavioural interaction, only occurring when interaction amongst chicks is unrestricted, produces social facilitation of feeding. Savory (1975) doubted whether social facilitation of feeding occurred at all, and Savory and MacLeod (1980) found a temporary increase in feeding activity but not daily food intake with groups of chicks.

Duncan *et al.* (1970) found that feeding in fowls was not random but concentrated into bouts constituting distinct "meals" depending on the individual bird. Masic *et al.* (1974) compared the feeding behaviour of male broilers and layers and found a similar pattern of feed intake throughout the day. Both responded to stimuli such as lights on/off and renewal of feed supplies but broilers spent half as long eating twice the amount of feed as layers. The layers spent much time manipulating food but not eating. Classen and Urrutia (1980) found that female broilers exhibited greater social facilitation than males as indicated by more birds eating together during feeding bouts.

2.6.3 Calcium and feed intake

Morris and Taylor (1967) claimed their's was the first report of a relationship between daily feed intake and egg production, citing previous balance experiments where hens were fed below appetite in order to minimise variations in feed intake. They indicated that the relative difference in calcium required by the layer on egg-forming and non-laying days was far greater than for any other nutrient. Taylor and Kirkley (1967) using colostomised hens found feed intakes of 112 versus 78 g on laying and non-laying days. The feed intake differences may have been greater as, on 7 of the 10 laying days, the hens ate the allocated 120 g of food so may have been effectively feed-restricted. They found little difference in excretion of minerals on laying or non-laying days, suggesting greater dietary absorption on laying days but considered that mineral excretion may have been modified by colostomy.

Taylor (1970) indicated that the greater feed intake on egg-forming days was due to the need for calcium by the hen as hens fed on a low calcium diet with particulate calcium free-choice did not increase food intake.

Hughes (1972) suggested that calcium intake is controlled by a hormonal or neural response to ovulation and is regulated on an hour-to-hour basis by the maximal "anticipated" need governed by the requirements of eggshell formation. In a review of specific appetites in layers, Hughes (1979) cited unpublished work by Savory, C.J. in which calcium-deficient layers developed a strong preference for a calcium supplement in 15 min but calcium-replete birds required several hours. The suggestion by Hughes (1979) as to why a specific appetite would be of value to birds was that calcium may be regarded as a key requirement in birds as the progenitors would have had a large calcium requirement in a short period during the breeding season. It was postulated that it would be highly adaptive of the species to have developed, through natural selection, an innate appetite for calcium triggered when the need for calcium arose and a "positive post-ingestional feedback" reinforcing correct behaviour in selecting the correct food.

Mongin and Sauveur (1979a) attempted to describe a calcium appetite in terms of a learned preference for some property of a food. They concurred with the suggestion by Hughes (1972) that calcium and feed intakes were regulated separately. The calcium appetite is unique in birds as it "anticipates" need and the layer does not need to be calcium deficient to display an ability to identify calcium (Mongin and Sauveur, 1979a). They also suggested three components of calcium intake in layers; the maintenance requirement of immature hens, the requirement linked to ovarian activity in the week prior to laying or on non-egg forming days and the demands of eggshell formation.

Hurwitz and Bar (1965) found that calcium consumption was stimulated at or before the initiation of eggshell deposition and this concept was refined by Mongin and Sauveur (1974) who showed that an increase in calcium intake during the afternoon is modulated by ovulation time. Mongin and Sauveur (1974) further suggested that the diurnal rhythm provided a stronger stimulus for calcium intake but was amplified at the time of eggshell formation. These authors reported over-consumption of feed in fowls given a low calcium diet and advised giving hens adequate calcium in the diet plus a separate calcium supplement. Leeson and Summers (1979) supported this advice after a long-term study with layers on a split-diet (offered both high energy, high protein and

low calcium and low energy, low protein and high calcium). Hens on the split-diet ate 7 % less feed than those on a conventional ration as conventionally fed birds initially ate more feed to meet their calcium requirement. The difference in feed intake was reduced as the laying period progressed and egg production declined resulting in a decrease in calcium intake.

However, some previous experience of separate, identifiable calcium sources may be required by birds as Leeson and Summers (1978) found that the timing of introduction of a split diet was crucial, as feed intake was not reduced if self-selection was introduced at point of lay. They reasoned this to be an adaptive response which supports the finding by Bray (1978) that pullets would only alter an established feeding pattern to adjust calcium intake if a diet was extremely deficient (0.42 %) or superadequate (4.22 %) in calcium.

Simkiss (1975) suggested that for calcium intake to be increased at the time of eggshell calcification, an integrated control system which could "anticipate" calcium requirements over time was required. Lobaugh and Mueller (1978) found a link between plasma calcium concentration and the neural regulation of calcium intake via a sensory apparatus located in the upper neck or head which is distinct from receptors regulating food consumption. Lobaugh *et al.* (1981) used calcium infusion and continuous PTH infusion in chicks to reduce calcium intake without altering feed consumption. Oestrogen injection increased food intake in the chicks but the calcium appetite was unaffected. They suggested that two conditions would have to be met if it were to be shown that calcium appetite and the nervous system play a role in the calcium homeostasis of birds. Firstly, calcium appetite and endocrine control must be integrated and secondly, calcium intake must give a rapid response to a change in calcium status. Both conditions were satisfied as calcium intake was reduced when calcium or PTH infusion occurred and this happened within 150 min of calcium infusion once birds had learned to choose a calcium supplement.

The use of the word "anticipate" by Hughes (1972), Mongin and Sauveur (1979a) and Simkiss (1975), in relation to calcium intake for subsequent eggshell formation, is descriptive of the "priming" effect of the ovulatory surge of oestrogen. The oestrogen surge leads to calcium intake several hours later but prior to the beginning of eggshell calcification.

Classen and Scott (1980) trained layer pullets to consume a calcium supplement and found that, over the rearing period and prior to point of lay, the calcium needs of the birds altered radically. The pullets ate 2.0 % calcium 19 d before laying, 2.88 % on the day of ovulation and this increased to 9.07 % on the day of first oviposition. Scott and Balnave (1986) found a marked change in dietary preference in choice-fed pullets which increased their intake of the protein supplement 2 weeks prior to laying and this intake was maintained post-maturity. However, some of the dietary calcium was included in the protein supplement and this may have confounded the result as the birds may have associated the protein supplement with satisfying calcium needs.

Wood-Gush and Kare (1966) and Hughes and Wood-Gush (1971) studied the preferences of fowls for various calcium compounds and found that calcium carbonate was preferred by the birds. Wood-Gush and Kare (1966) found that the birds could distinguish between calcium salts in solution as they avoided calcium lactate, so taste could be more important than nutritional need. These authors reasoned that a true specific hunger is not formed by calcium deficient birds but an intense form of "appetitive behaviour" leads to the correct nutrient being learned and recognised after a period of time which was determined by the degree of deprivation and the palatability of a calcium-rich food. Conversely, Hughes and Wood-Gush (1971) found a preference for calcium lactate in food in calcium deficient birds and believed this was due to the strong taste acting as a cue for the birds. These authors concluded that fowls have a learned preference for calcium which is reinforced by post-ingestional effects of food consumption. It appears that the flavour of calcium lactate was enhanced in solution which supports the suggestion by Kare and Pick (1960) that the greater sensitivity of birds to flavours in solution could cause practical problems for the use of additives in drinking water.

Calcium-deficient birds also explore odd items in the environment to a greater extent than replete birds (Hughes and Wood-Gush, 1971) and these may be potential food sources that will satisfy the calcium requirement. Rogers (1995) described the complex cognitive capacities of birds with their elaborate nervous system allowing them to make complex decisions due to their ability to form a multitude of memories. She suggested that this cognitive capacity depends on environmental stimulation throughout development. El Boushy *et al.* (1989) described the acceptability of feed for birds in terms of a memory of "bad feed" affecting subsequent intake. These authors used the description "bad feed" to describe feed that may be contaminated with, for instance, fungal toxins.

Joshua and Mueller (1979) found that social facilitation was important in encouraging broilers to consume a calcium supplement. Birds housed as a group adjusted their calcium intake to levels within established requirements and continued to do this after being housed singly. However, birds initially housed individually but allowed visual contact with others did not develop this skill even when colour cues were provided. Summers and Leeson (1979) found that some birds had difficulty in selecting feed constituents correctly from 3 troughs as an item in the middle trough was eaten less. They quoted Hurnik J.F. (unpublished) who found, conversely, that birds correctly selected nutrients wher positions of the troughs were changed daily.

Larbier and Leclercq (1992) accepted the concept of a specific appetite for calcium in birds as a practical reality. They emphasised that it was essential for calcium to be supplied separately to layers at the end of the day, or in feed restriction or with compounded diets, the feed should be given at the end of the day.

2.7 Supplying dietary calcium

2.7.1 Modern methods

Larbier and Leclercq (1992) outlined some of the major scientific advances in the field of poultry nutrition that have led to the modern method of feeding calcium to laying hens. These advances included the discovery of calcium and phosphorus requirements of hens in 1910; the concept of a calcium:phosphorus balance in 1934; the development of pelleting of feeds and the first NRC tables in 1944 and, in 1955, the use of analogue computers for diet formulation based on "least cost".

The current standard feeding method for laying hens is to provide a complete feed in the form of a mash. As the majority of laying hens are caged this feed is provided by various methods of conveyance including belts, chains and augers. Mannion and Reichmann (1984) emphasized the importance of supplying an adequate and continuous supply of dietary calcium but added that providing supplemental calcium to diets adequate in calcium was not common. The current method of providing the hen with calcium, generally as a ground limestone, is being questioned. The use of particulate calcium supplements is being suggested in many practical production guides such as that of Larbier and Leclercq (1992). However, in its most recent edition, the major advisory body, the NRC (1994) makes recommendations based around provision of calcium as part of a complete diet. The NRC (1994)

advises providing 3.25 g/d for white egg layers and 3.6 g/d for brown egg layers. Summers *et al.* (1976) supported the use of absolute values to describe intakes of calcium as using dietary percentages was inappropriate due to variable feed intakes by birds. Leeson *et al.* (1993) questioned the suggested calcium recommendations for brown egg layers, as the figures were often based on extrapolation from Leghorn strains, and suggested that heavier birds required less calcium. The NRC (1994) cautioned against excess dietary calcium on the grounds that it interferes with the availability of P, Mg, Mn and Zn and that high levels of calcium carbonate and calcium phosphate may cause unpalatability and dietary dilution problems.

Keshavarz and Nakajima (1993) claimed that there was no scientific justification for the common commercial practice of phase feeding of calcium (increasing calcium contents of feeds to adjust for lower feed intakes as the bird ages). This supported the suggestion by Härtel (1989) that the phase feeding of calcium was unjustified as the relative effects of both calcium and phosphorus on production were almost constant over time.

Taylor (1970) emphasised that the modern feeding system for laying birds was one of convenience for the poultryman and compounder and does not consider the needs nor the abilities of the hen. Harwitz (1970) reviewed the feeding of layers and concentrated on the difficulties of calcium provision to a flock at point of lay. His recommendations, and those of Leeson (1993), in dealing with this problem were made within the current standard practice of providing complete diets to these birds. In other words, using particulate calcium was not suggested as an alternative method.

2.7.2 Supplying particulate calcium

Fussell (1961) suggested that calcium would be best provided as a fixed amount in the feed based on hen size and net energy of the feed. However, the subject of supplementation of calcium in particulate form to laying hens has led to considerable debate as to the superiority of the calcium source used. Roland (1986b) stated that concentrated forms of calcium were provided to layers in the late 1800s to maintain optimum shell quality and it was not until 1970 that the debate about the best source of calcium gained momentum. This probably commenced with the admission by Taylor (1970) that he had altered his earlier opinion that the hen could not be trusted to balance her calcium intake and that the hen would be better provided with a source of shellgrit for efficient egg production.

Sources of calcium vary but Muir *et al.* (1976) found that forms of calcium carbonate, such as limestone, oyster, clam or eggshell and aragonite allowed for similar egg production in layers. Brister *et al.* (1981) found that aragonite was a good substitute for other calcium sources.

The optimum particle size of calcium for layers had been a controversial subject for almost a century (Watkins *et al.*, 1977). Miller and Sunde (1972) found that shell strength was better with large rather than ground calcium sources, but, when ground, oyster shell was better than limestone. These conclusions were supported in later reviews by Miller and Sunde (1975) and Roland (1986b). A review by Mannion and Reichmann (1984) showed an advantage in eggshell quality of shellgrit over limestone but particle size was a confounding factor as larger particles were better than ground supplies of the same type. The general recommendation by most authors was to supply a ground calcium source and to substitute various proportions in a coarse form such as half-ground half-coarse (Sanford, 1974), one-third to two-thirds coarse (Roland, 1986b) and three-quarters coarse (Kuhl *et al.*, 1977) to improve or maintain shell quality. In some reports improvements in shell quality in response to the use of coarse shellgrit were only reported in summer (Roland and Harms, 1973) or in warm weather (Karunajeewa, 1977). Holder and Sullivan (1973) found that using larger particle sized limestone resulted in improved shell quality as determined by fewer cracked eggs.

Scott and Mullenhoff (1970) and Scott *et al.* (1971) suggested that the improvement in egg shell strength by providing coarse oyster shell was due to a constant 'metering' of dissolved Ca from the accumulated shell in the gizzard during the night which maintained a higher level of blood calcium in agreement with Keshavarz *et al.* (1993). Keshavarz and Nakajima (1993) found a positive improvement in egg specific gravity (SG) with the addition of particulate calcium even to hens on high calcium feed which supports a residence time theory. Burnell *et al.* (1990) suggested that large particles of calcium (2.3 mm dia.) partially blocked the exit from the gizzard and therefore spent a longer time in an acidic environment where the particles may dissolve more completely. Small particles of calcium may pass through the gut quickly (Roland *et al.*, 1972) and Rao *et al.* (1992) concluded that there was a minimum size of 1.01 mm for limestone which allowed for its selective retention in the gizzard of commercial layers. Sloan (1976) found that large particles of shell or limestone may provide calcium to the bird for up to 4 d after ingestion.

In many experiments with particulate calcium sources there is a lack of detail regarding the sizes of calcium sources used and ill-defined references to "pullet" and "hen" sized sources (Roland *et al.*, 1972 and 1974) are made. Such size groupings of calcium sources were shown to vary considerably both between and within sources (Watkins *et al.*, 1977).

Sauveur (1992) suggested that in relation to eggshell formation the hen, when offered a recognizable calcium source, can eat calcium according to a discontinuous schedule which decreases bone mobilization and increases shell strength.

Intermittent lighting patterns may be more important for improving eggshell strength than the calcium source by allowing hens access to dietary calcium during the period of maximum eggshell calcification (Sauveur, 1991) and faster movement of calcium through the gut due to increased activity at night (Grizzle *et al.*, 1992)

Many experiments with particulate calcium sources are conducted using layers which appear to be naive with unground calcium sources (Tyler, 1955; Roland *et al.*, 1974; Keshavarz *et al.*, 1993). A particular example of this was the experiment of Roland *et al.* (1972) when 66 week old hens were dosed with shellgrit, by tube into the crop, after failing to eat the required quantity (0.5 g) of limestone chips to allow the experiment to continue.

Many authors discuss the solubility of the calcium sources used in experiments and appear to equate *in vivo* solubility with *in vitro* measurements (Cheng and Coon, 1990b; Keshavarz *et al*, 1993). These authors apparently take no account of, and do not discuss, the transit time of calcium and the mechanical contribution of the gizzard to calcium availability in the gut of the layer. For example, Rao and Roland (1989) found that the *in vitro* solubility of limestone was one-third less for large (2-5 mm) compared with a fine (0.5-0.8 mm) limestone. They failed to mention mechanical activity of the gizzard when, *in vivo*, the large limestone was 20-30 % more solubilized than the small limestone and more calcium was retained by the hens. Later, this failure was reinforced when Rao and Roland (1990) stated that *in vitro* solubility had no relevance to *in vivo* solubility and that classic *in vitro* solubility may not simulate actual calcium source solublization.

In a series of experiments Cheng and Coon (1990a,b,c,d) compared the solubilities of limestones, marble and oyster shell of different sizes. They found little

effect on eggshell quality, blood or gu: pH, bone ash volume or strength with different calcium sources. They found that bone ash concentration was a good measure of calcium status and suggested that, at a calcium intake of 4.5 g/d, bone deposition was still proceeding and so NRC recommendations of 3.75 g d were inadequate. Optimum solubility of calcium sources to maximise bone ash concentration and egg SG was claimed to be 11-14%. The suggestion by these authors was to mix the right proportion of limestones of different sizes to obtain this solubility. However, this would add substantially to the cost of calcium provision and again these authors did not mention the effect of gizzard action on calcium source solubility and appeared to equate *in vitro* solubility with that *in vivo*. Cheng and Coon (1992) appeared to contradict their earlier recommendations by suggesting that if calcium intake was adequate then substitution of some dietary calcium with a less soluble particulate limestone would have no effect on eggshell quality.

The abovementioned authors are actually referring to rates of solubilization of particulate calcium in acid solutions (as detailed in each individual experiment) when they use the terms "solubility" and "solubilized".

The experiment of Rao and Roland (1990) is suspect as they made note of accumulated limestone remaining on top of the feed at higher levels of provision. They say this was separated to determine limestone intake and not calculated by using remaining feed weight. However, they stated earlier that limestone was to be separated from the other feed ingredients to determine actual intake.

2.7.3 Practical reasons for using large particulate calcium

Taylor (1970) stated that since the introduction of complete diets for layers, the level of calcium provision had steadily increased but with no fall in the numbers of cracked eggs or any indications of improvements in eggshell strength. This was supported by Solomon (1990) who reported that no reduction in egg downgrading had occurred in the intervening years despite the efforts of geneticists and nutritionists and asked if the wrong criteria had been applied in efforts to improve shell quality. However, Daghir (1988) suggested that the separate feeding of calcium sources was the most effective way of reducing the effects of temperature on eggshell quality.

Morris (1972) found little interest by nutritionists in improving the efficiency of calcium utilization as it had little direct dietary cost. A high level of dietary calcium

inclusion was practiced to maximize eggshell thickness. Taylor (1970) further indicated that feed intake was higher on low than on high calcium diets and that high calcium diets depressed feed intake to the point of weight loss in layers. This early report was recently supported by the NRC (1994) which mentioned the low feed intakes of modern small-bodied layer strains and cautioned nutritionists about high calcium levels in feed causing dietary dilution and unpalatability problems. This may explain some of the problems, raised by Parkinson and Almond (1995), of weight loss and poor egg production due to pre-peak inappetence especially in low bodyweight layer strains. These authors found that the problem was unresponsive to traditional nutritional approaches.

The above problems may be due in part to calcium provision by conventional methods. As the hen appears to possess the innate ability to determine the crucial point for calcium intake (Hughes, 1979; Mongin and Sauveur, 1979a; Savory, 1979) and can efficiently regulate the intake of shellgrit (Taylor, 1970), this ability could be utilised by the industry.

A further problem of modern feed distribution is one of ingredient separation in mash feeds in bulk tankers and farm bins (Portsmouth, 1970). Miller and Sunde (1975) found that when coarse calcium was provided the hen could consume calcium effectively but with a fine source separation problems were greater and the hen could only eat the amount provided. An insufficient calcium supply may occur due to feed ingredient separation in complete feeds in mash form (Anon., 1989). Belyavin (1994) reviewed the problem of feed ingredient separation which occurs from the time of delivery of feed into bulk bins and in all types of feed distribution equipment. The problem is not only due to calcium settlement in feed lines. It also occurs because the proportion of dietary phosphorus increases and dietary protein decreases along the feed line. Performance problems such as low egg production or weak eggshells are caused mainly by the distortion of nutrient ratios. Belyavin (1994) suggested that reversing chains to maximize feed mixing or providing corner hoppers were expensive rectification methods.

Both Cumming *et al.* (1987) and Belyavin (1994) highlighted the problem of selective feeding by birds fed on conventional diets. Larger particles are generally selected out of mash and crumbled feeds so causing nutrient variations in the feed along the length of the distribution system. The use of a large particle size calcium source allows the hen to select calcium which is in a form that is clearly identifiable by

the bird. Providing large particulate calcium would allow the manager to quickly check the distribution and relative quantity of calcium in the feeding system.

Roland and Rao (1992) emphasised the difficulties associated with supplying calcium to pullets at point-of-lay. Roland *et al.* (1985) raised this point and the problem of overconsumption of feed by early maturing pullets on low calcium pre-lay diets. The overconsumption of feed by these birds was equated with force-feeding and the consequent excess body-fat deposition by Roland (1986a).

Mongin and Sauveur (1979b) found that plasma phosphorus levels were reduced due to lower bone resorption during eggshell formation when calcium was provided as a separate source. Boorman *et al.* (1989) suggested that providing a separate source of calcium obviates the need for birds to mobilize bone reserves and had positive implications for the unwanted accumulation of excess dietary phosphate in the excreta of hens. Sauveur (1991) reviewed phosphorus supply to the layer and the use of different types and sizes of particulate calcium. Two silos were used in experimental and field trials in which two different daily feeds were supplied to layers; a morning feed (8 am to 3 pm) with 1.5 % calcium and 0.63 % available phosphorus, and an evening feed (3-9 pm and 7-8 am) with 5.3 % calcium and 0.5 % available phosphorus which lowered the phosphorus requirement of the hens.

2.8 Laying hens and choice-feeding

The commercial laying hen has demonstrated appetites for energy, protein and calcium. Compounded layer diets are formulated with respect to the expected energy intakes based on bird weight and egg production level with the requirements for protein and minerals expressed per unit of ME (Chwalibog and Baldwin, 1995). The current practice of feeding compounded diets has been shown to be inefficient where environmental or disease status alters the bird's metabolism (Mastika, 1987; Walker and Farrell, 1976). Lower intakes may then result in protein or calcium insufficiency. The metabolic requirements of the layer for each of the dietary constituents varies with individual, age, environment and stage of lay. The calcium requirement is affected by laying status and is generally greatest 8-12 hr after the previous oviposition (Hughes, 1972; Mongin and Sauveur, 1979a).

The capacity of the domestic fowl to select a balanced diet has been reported for over 60 years since the early work of Funk (1932) and Graham (1932). Wood-

Gush (1971) emphasised the problems encountered in the study of dietary selection when noting that most natural plant and animal feedstuffs contain a range of nutrients and that a balanced diet could be obtained by random selection by the bird. Emmans (1977) stated that the ability of the hen to regulate its diet is not well defined, meaning that it had not been much studied at that time, but that the evidence was sufficient for the exploitation of such an ability. Emmans (1975 and 1978) suggested three reasons for using choice-feeding as a practical feeding method for layers; nutrient requirements vary between and within flocks; diet formulations based on the average hen penalises good producers and oversupplies poor ones and cereals are the bulk of the ration so energy costs of grain milling are saved.

In his review of choice-feeding Karunajeewa (1987) stated that layers had effectively been free-choice fed until the 1950s when this feeding method was abandoned in favour of the all-mash system. The all-mash feeding system allowed for simple automated feeding of birds in cages. Summers and Leeson (1979) considered that there had been little change in dietary presentation since automation and that the feeding method was mainly due to the needs of the equipment rather than nutrition. They suggested a re-examination of grain feeding systems with a view to saving costs by eliminating the grinding and mixing of grains. McIntosh *et al.* (1962) found no difference in the efficiency of utilization of whole, ground or pelleted wheat in 0-15 week old pullets. This contrasts with the generally held view that pelleting improves energy utilization of feed (Hussar and Robblee, 1962; Douglas *et al.*, 1990); however there is an energy cost of pelleting to be considered. Karunajeewa (1987) found that layers 20-52 weeks of age on whole grain gave earlier and better production and had a lower feed conversion ratio (FCR) than those on a complete feed.

Hughes (1984) reviewed the principles underlying choice-feeding behaviour in fowls with specific reference to production experiments. He suggested that three main factors influence feed intake; feed composition, palatability and variety. He criticised feed intake models, which account for metabolic requirements only, as far too simplistic due to the importance of social, learning, palatability and feed positioning interactions. He emphasised that it was surprising how much of the ancestral capacity of the bird to regulate its diet remained, considering the many generations of selection under a feeding regime based on compounded diets. He hypothesised that perhaps the very best production may only be possible with choice feeding with birds selected using that feeding method.

Cumming (1984; 1992) and Cumming *et al.* (1987) discussed the practical aspects of choice-feeding of layers and emphasised the need to expose the layer, in the rearing period, to all the feedstuffs she would encounter during lay. Mastika and Cumming (1987) indicated that layers learn to choice-feed better than broilers as they have more time to learn. Cumming (1992) indicated that a major problem for egg producers was that the heat-stressed layer was forced to eat excessive amounts of protein and energy to satisfy her calcium need when offered a complete ration.

Cumming *et al.* (1987) highlighted a potential problem in breeder production where males are forced to eat a high calcium diet with no account of the consequences, and Hurwitz (1968) found that cockerels on a breeder diet were hypercalcaemic. The provision of feed to feed-restricted broiler breeder hens in the morning was examined by Farmer *et al.* (1983) who found that the hens suffered a low calcium concentration in the gut during the active time of shell calcification but the afternoon feeding of the hens improved gut calcium levels. Larbier and Leclercq (1992) recommended that breeders be fed in the afternoon. However, in hot climates it is often inappropriate to feed broiler breeders during the afternoon or evening due to the heat load incurred by the hens. Taylor (1994) found that providing heavily feed-restricted broiler breeders with coarse (>10 mm diameter) shellgrit *ad libitum* resulted in a significant improvement in egg specific gravity and a trend to larger eggs. The hens used had been reared on a compounded crumble and were exposed to the shellgrit at 19 weeks of age. They adapted remarkably well to the shellgrit and their feed (standard breeder formulation but with the cereal fraction (sorghum) as whole grain and no added calcium source) Taylor, R.D. and Jones, G.P.D. (unpublished) had found this strain and another low-producing broiler breeder strain were in a negative calcium balance after peak production when fed a compounded diet containing 30 g/kg of calcium.

Mastika and Cumming (1987) and Forbes and Shariatmadari (1994) reviewed the practical aspects of choice feeding and concluded that many of the poor results obtained from choice-feeding experiments were due to inadequate "training" and the consequent limited experience of the birds. The latter authors found that little experimental work had been directed at determining optimal training methods.

2.9 Summary and conclusions of literature review

The commercial layer has demonstrated the capacity to balance its intake of the major dietary components energy, protein and particularly calcium when offered suitable choices of feeds. Compounded layer diets are formulated with respect to expected energy intakes of birds as the energy appetite of the bird is generally considered, in commercial practice, to be dominant. The current practice of feeding compounded diets has been shown to be inefficient where environmental or disease status alters the bird's energy metabolism which then may result in calcium insufficiency. The metabolic requirements of the layer vary with individual, age, environment and the demands of eggshell formation on a daily basis. This is particularly so of the calcium requirement which is greatest 8-12 hr after the previous oviposition and corresponds with the formation of the next eggshell. Traditionally, and to a limited extent more recently, calcium has been provided supplementally to the diet in the form of particulate limestone, marble or shell chips so that the bird does not consume feed merely to satisfy this increased calcium requirement.

Where supplemental calcium is provided, there is considerable debate as to the superiority of the source used. Miller and Sunde (1975) indicated that calcium was better provided by oyster shell compared to limestone as measured by traditional eggshell quality parameters when both sources were ground. When large particles were provided, no difference between sources was observed. Mannion and Reichmann (1984) concluded that differences between sources could be ascribed to particle size. Although differences in layer performance with different sized particles have been demonstrated, the reasons propounded for these differences (ie changes in eggshell quality) may be misguided. Increasing the particle size *per se* is not important but rather particle area, which, if considered, may account for observations indicating that shellgrit is superior to limestone. However, increasing the surface area by decreasing the particle size may result in the rapid passage of particles through the gut (Roland *et al.*, 1972). Large particles may provide calcium to the bird for up to 4 d after ingestion. Consideration of *in vitro* solubility rates without accounting for the mechanical effect of the gizzard is remiss.

There is increasing advice by nutritionists to provide ground calcium in the diet plus a particulate source. Provision of two separate forms of calcium to the hen would be both costly and inconvenient to the compounder and/or the producer. The ability of the hen to accurately manage its calcium requirement is apparently distrusted

by nutritionists as highlighted by recommendations to provide several forms of calcium to the hen. Perhaps this distrust is based on the comments by the proponents of the concept that the calcium appetite exists that although most hens select calcium well, some do not so well and a few not at all (Taylor, 1970; Hughes, 1979).

The manipulation of dietary calcium content and form appears to be based around attempts to maintain or improve eggshell quality. However, eggshell quality has not been improved by either the increasingly sophisticated knowledge of dietary constituents and feed formulation nor with modern feed provision equipment. Taylor (1970) indicated that dietary manipulation had not reduced the numbers of eggshell cracks and the situation had not changed when Solomon (1990) noted that nutrition and genetics had not affected the level of egg downgrades.

The knowledge about the metabolic aspects of the role and fluxes of calcium in the laying hen has expanded dramatically. This has been particularly so in relation to bone metabolism with the utilisation of molecular biological techniques. However, there has been a noticeable slackening in research into bird behaviour since the 1970s. This is highlighted by Forbes and Shariatmadari (1994) who suggested that no work has been devoted to determining the optimal training methods to allow maximum efficiency of feeding in the bird.

In her recent review, Rogers (1995) indicated that the laying bird has highly complex cognitive abilities developed by experience. This highlights the problem, raised by Taylor (1970), of inadequate training of the bird in modern production systems. This was reinforced by Forbes and Shariatmadari (1994) who suggested that most poor results of choice-feeding trials were due to the hens being given little prior experience of feedstuffs and different feeding methods.

Studies were devised in which the ability of the choice-fed trained laying hen to accurately identify its calcium source could be determined. The capacity of the laying hen to adjust the amount of shellgrit consumed when this calcium source was removed over fixed periods of one, two or three days was also studied. No studies have apparently been conducted into this aspect of calcium intake regulation in the laying hen. There may be practical benefits to be gained by harnessing any calcium intake regulatory abilities of laying hens. These benefits may include more efficient use of feeds and improvements in eggshell quality.

Chapter 3

Effects of the intermittent supply of shellgrit calcium on the performance of choice-fed laying hens

3.1 Introduction

The determination of the calcium requirements of laying hens and methods of providing adequate calcium to optimize production, feed efficiency and shell quality appear to be based around the perceived need of the hen to have calcium available daily. This has been so whether calcium is supplied *ad libitum* as a discrete source or in various proportions of incorporation in a compounded feed with or without supplemental provision. Indeed, to provide for the full calcium requirements of laying hens, ARC (1975) recommendations stated that to provide the calcium requirement it is only practicable if all calcium is incorporated in the diet and, as laying hens are generally full-fed, this implies daily provision of calcium. NRC (1994) recommendations do not contradict this but acknowledge that research is required into the use of larger particle sizes of calcium especially in relation to the improvement of eggshell strength. This may, in part, be due to the separation of feed constituents in complete feeds as was acknowledged by Portsmouth (1970). The problem of calcium separation in complete feeds in all types of feeding systems in modern poultry housing was recently reviewed by Belyavin (1994).

The use of large particulate calcium may offer one possibility in addressing this problem. The provision of large sized particulate calcium allows the bird to consume its daily calcium requirement without the encumbrances of its energy and/or protein appetites. Cumming *et al.* (1987) queried how often it was necessary to present granular calcium to the laying hen. It is possible that particulate calcium may not be required each day due to retention of the particles in the gut.

Sauveur (1991) provided two dietary calcium levels to laying hens on an intermittent basis within each day and found favourable responses in eggshell quality to the intermittent supply of high and low calcium diets. However, it appears that the

responses of laying hens to the supply of a separate calcium source on an intermittent basis, extending over more than one day, have not been determined. The ability of the hen to accurately regulate her calcium intake, when it is interrupted over extended periods of time, is worthy of investigation.

The experiments presented in this chapter were designed to examine the responses of free-choice fed laying hens to the supply of particulate calcium in the form of shellgrit, provided either daily or every second or fourth day. Experiment 1 was a preliminary investigation into the capacity of the laying hen to regulate the intake of a particulate calcium source when her usual daily supply was altered to provision once every two or four days. The effects of the intermittent calcium supply on eggshell quality were also monitored. Experiment 2 was designed to investigate this capacity over a longer period and to determine if the intakes of wheat and protein concentrate by choice-fed hens were altered by the intermittent supply of particulate calcium. The effects of temperature on feed intake by these hens was also investigated to determine if feed intakes, altered in response to changing temperatures, affected calcium consumption in any way. Experiment 3 was undertaken as a preliminary investigation of the effects of the intermittent supply of particulate calcium on digestive organ and bone physiology and metabolism of laying hens.

3.2 General materials and methods

3.2.1 Layer stock

The strain used was the SIRO-CB (New Hampshire x Australorp), a commercial medium-sized hybrid brown egg layer, supplied by Hyline Australia, High St., Maitland, N.S.W., 2322 Australia.

3.2.2 Management

Flock rearing history

The birds were hatched on 25.10.1993 at the Hyline Australia commercial hatchery at Toowoomba, Qld., where they were beak trimmed at day old. Table 3.1 details bird management prior to the onset of lay.

Table 3.1 Management of the birds prior to the onset of lay.

Period	Activity
Day old	Transferred by road to Tamworth, N.S.W. for commercial cage rearing. Birds fed a commercial starter crumble.
3 weeks old	Beak topped.
5 weeks old	Transferred by road to "Laureldale", Armidale, N.S.W., the University of New England poultry layer farm. Floor rearing on hardwood sawdust. Commercial starter crumble including coccidiostat. Natural lighting provided (12 h d).
7 weeks old	Commercial pullet developer crumble including coccidiostat.
10 weeks old	Choice-feeding commenced on the flock using whole wheat (100 g/kg crude protein) and a protein concentrate, supplied by Fielders Stockfeeds, Tamworth, N.S.W.
16 weeks old	Access to calcium commenced by *ad libitum* provision of limestone chips 2-4 mm in diameter distributed throughout the feed.
18 weeks old	Transferred to laying cages. Pullets found to be overweight and time-restriction feeding was applied until 23 weeks of age.
20 weeks old	Layer ration supplied by choice-feeding using whole wheat (100 g/kg crude protein) and a protein concentrate (422 g/kg crude protein) supplied by Koombi Stock Feeds, Tamworth, N.S.W. Particulate calcium as limestone chips 2-4 mm in diameter distributed throughout the feed.
24 weeks old	Natural light supplemented by introducing fluorescent lighting, increasing by 20 min/d at weekly intervals (over 9 weeks), until the pullets were provided with 16 hours light per day (h d) from 0530 to 2130 h.

Vaccination history

Table 3.2 provides details of the flock vaccination programme.

Table 3.2 Vaccination history of the flock.

Period	Vaccination
Day-old	Marek's disease (MD) (standard Herpes Virus Turkey).
21 days old	Poulvac A3-Infectious Bronchitis (IB) by in-contact method.
14 weeks old	Avian Encephalomyelitis (AE) and IB (Vic S strain) both by in-contact method.

Laying history

The flock commenced laying at 20 weeks of age and peaked at 30 weeks of age. Basic commercial management by University farm staff involved thrice weekly feeding (Monday, Wednesday and Friday) where the three feed ingredients (wheat, protein concentrate mash and limestone chips) were supplied in a single feed trough. Each day eggs were collected, an inspection for dead or sickly birds performed and temperature maxima and minima, at hen height in the centre of the shed, recorded.

The birds were managed by University staff until 36 weeks of age whereupon management was provided by the author, from 4th July 1994, during the series of experiments reported in this thesis.

3.2.3 Housing

Shedding

The birds were housed for the duration of Experiments 1-3 in an uninsulated laying shed. The shed is aligned east-west on its long axis. The north and south walls are slatted for 60 cm from the ground and for 60 cm from the eaves with a solid wall between. Each end wall is solid. No curtains or louvres are used so airflow is uncontrolled.

The cages were laid out as a double bank on each side of the shed which provided access along each outer wall and through the middle of the shed.

Cages

The birds were caged in pairs in conventional wire mesh laying cages (47 cm long x 46 cm wide x 43 cm high) in the inner rows of cages of the double bank of cages on both sides of the central aisle. Extending from the western end of the shed, 38 cages were used on the northern side and 32 on the southern side. A common external nipple drinker line, above and between the double bank of cages, supplied 3 nipples per 2 cages. A drip tray under the nipple drinkers removed all excess water to drains at the eastern end of the shed. Two identical galvanized steel feed troughs (13 cm long x 22 cm wide x 10 cm deep) were fitted across the outside of each cage front. Wheat and protein concentrate mash were supplied in one trough; the calcium source in the other.

3.2.4 Feed

For all experiments described in this thesis, hens were choice-fed on whole grain wheat (100 g/kg crude protein) and a commercially formulated and mixed protein concentrate mash (422 g/kg crude protein and 40.1 g/kg calcium).

Feed constituents

The protein concentrate ingredients and analysis are shown in Table 3.3.

Formulation was provided by Applied Nutrition (PO Box U40, UNE, Armidale, NSW 2351) and feed supplied by Koombi Stock Feeds, Tamworth, NSW, 2340. The feed formulation was based on the protein concentrate supplying the equivalent of 30% of total feed intake of a compounded ration consumed at a rate of 110 g per bird per day (exclusive of calcium) and 10.88 MJ metabolizable energy (ME) /kg.

The layer premix (included at the recommended rate of 1.5 g/kg) provided the vitamin and mineral specifications as per Table 3.4. A yolk pigment, Carophyll gold, was included at 35 mg/kg premix.

Table 3.3 The composition and analysis of the layer protein concentrate mix used in all experiments (g/kg except as specified).

Raw Material	Incorporation Level
Soybean Meal (48%, Expeller)	460.0
Meat Meal (50%)	352.5
Wheat Millrun	143.6
Sunflower Oil	15.0
Salt	11.8
DL-Methionine	7.3
Koombi Layer Mineral and Vitamin Premix	7.2
Lysine Mono-HCl	2.6
Analysis[1]	
Dry Matter	917 (925)
AME (MJ/kg)	10.88
Crude Protein	422 (399)
Fat (Ether Extract)	71.1 (100)
Crude Fibre	44.7
Calcium	40.1 (36.9)
Phosphorus-Total	23.2 (19.3)
Ca:P	1.7:1
Lysine	25.4
Meth. + Cyst.	17.1
Methionine	12.3
Linoleic Acid	15.0

[1]Calculated ('as is basis') except those in parentheses which were determined.

Table 3.4 Koombi layer premix vitamin and mineral mix (mg/kg except as specified)

Vitamin		Mineral	
A (IU/kg)	5000	Mn	50
D3 (IU/kg)	1500	Zn	50
E	10	Cu	3
K	1	Co	0.3
B1	0.5	Fe	25
B2	3	Se	0.15
Niacin	10	I	0.5
Pantothenic Acid	5		
Pyridoxine	2		
B12	0.003		
Biotin	0.05		
Choline	100		

Proximate analysis was performed on each delivery of protein concentrate to monitor quality. Mineral analysis was also performed on each batch of protein concentrate by an Inductively Coupled Plasma (ICP) Emission Spectrometer (ARL 3560 B ICP Analyser, Fisons Instruments, 4/149 Arthur St., Homebush West, NSW 2140) using the method of Anderson and Henderson (1986).

3.2.5 Calcium source

The particulate calcium used was a commercially available mixed sea-shell grit, collected on the north coast of NSW. This shellgrit was washed with tap water, sun-dried and then sieved. Only shellgrit that was retained by a 4 mm sieve was used.

Total calcium intakes, calculated on a daily basis, were determined from shellgrit and protein concentrate intakes. The calcium content of shellgrit was determined by ICP spectrometric analysis.

3.2.6 Feed measurements

Wheat and protein concentrate were weighed and mixed in the desired proportions (70:30) in plastic containers before being poured into the feed troughs and mixed again by hand. Sufficient feed was provided to allow for 2 d intake by the two hens per cage.

To determine feed consumption, the feed remnants were poured from each feed trough into a plastic container and the trough was thoroughly cleaned with a metal paint scraper. The feed remnants were then separated by sieving through a 2 mm sieve which held back the wheat fraction. Any larger remnants of the protein concentrate were picked out of the wheat by hand. The separate feed constituents were weighed in plastic containers on a portable electronic scale.

Shellgrit intake was determined by weighing the remaining shellgrit in the pre-weighed shellgrit trough.

3.2.7 Egg measurements

Daily egg collection involved egg pick-up by a very experienced egg collector to monitor variation in egg weight and any eggshell malformations. The egg collector had had 4 yr commercial experience in the pick-up and hand grading of broiler breeder and duck breeder eggs. The method of egg pick-up was alternated from day 1 of each experiment. Firstly, the eggs were picked up sequentially from cage number 1. Secondly, the eggs from each treatment, beginning with Treatment 1, were picked up in turn. Cracked, broken and malformed eggshells were recorded at pick-up.

Egg measurements, where recorded, were performed as indicated below.

Egg weights (EW) (g) were recorded at intervals of a maximum of 2 h post-oviposition to minimize the effects of evaporation.

Specific gravity (SG) was determined immediately after weighing using the Archimedes Method as outlined by Pym (1969).

Percentage shell weight (SW) was determined as shell weight (g)/egg weight (g) x 100. Shell weight was taken after each egg had had a line drawn in pencil around its equator, was broken out and the shell washed in warm, soapy water. The shell was then oven-dried at 105 °C for 24 hr, removed and allowed to cool for 1 hr before weighing.

Shell thickness (ST) was determined from the mean of three micrometer measurements (μm) of shell pieces, taken from evenly distributed points at the previously marked equator, of the oven-dried shells.

3.2.8 Statistical analysis

The statistical evaluation of the data was performed by repeated measures analysis using the Greenhouse-Geisser correction factor (where necessary) within the General Linear Models procedure of SAS (SAS Institute, 1989).

Where appropriate, significant Least Squares (LS) Means were separated using paired-sample t-tests and are presented in tables with appropriate standard errors (SE).

Where significant treatment x time interactions were found, treatment differences are only differentiated by subscripts at the appropriate time to avoid confusion.

Other analyses, where required, are specified within each experiment.

The following descriptors are used in the text and tables to indicate statistical significance.

NS	Not Significant $P>0.05$
*	$0.01<P\leq0.05$
**	$0.001<P\leq0.01$
***	$P\leq0.001$

3.2.9 Behavioural and other observations

Behavioural observations were noted at all times when work was performed in the shed. These observations included hen response to the opening and closing of shellgrit trough covers, hen response to the provision of feed, and bird interaction and activity in general.

Other observations included monitoring bird condition and appearance, the colour and consistency of excreta and the form of excreta coning under the cages. Appearance of feed and shellgrit remnants was noted daily. Temperature maxima and minima at hen height in the centre of the shed and outside weather conditions were noted daily.

3.3 Materials and methods

3.3.1 Experiment 1

The first experiment commenced in early July 1994 and continued for 6 weeks. At 36 weeks of age the birds were allowed 1 week to adjust from their normal method of choice-feeding (wheat, protein concentrate and particulate calcium in 1 trough) to wheat and protein concentrate in 1 trough and shellgrit in a second trough.

At 37 weeks, 3 treatments were imposed and allocated within each group of 3 adjacent cages in a randomised block design. The treatments were;

Tr1. Shellgrit available *ad libitum*. (17 replicates).
Tr2. Shellgrit available *ad libitum* every second day. (18 replicates).
Tr4. Shellgrit available *ad libitum* every fourth day. (17 replicates).

In Treatments 2 and 4 a metal cover on the shellgrit trough was opened or closed as required after lights out at 2130 h. Eggs were collected at 0900 h daily and production was recorded on a hen week percentage (HW%) basis. Total feed and shellgrit consumption were determined weekly. At the end of the fifth and sixth weeks of the experiment, feed remnants were separated to record wheat and protein consumption. From the second week, detailed egg measurements (see Section 3.2.7) were taken from eggs collected once weekly over 4 periods (0600-0800, 0800-0930, 0930-1100 and 1100-1200 h), and egg weight, SG, SW and ST were measured.

Regression analysis was performed on egg production versus various bird measurements such as total feed, shellgrit, calcium or protein concentrate intake for example, using the regression analysis procedures of the statistical package Minitab (Version 7, copyright Minitab Inc.).

The relationships between temperature and feed intake and time and egg dependent variables (EW, SG, SW and ST) were analysed using the reduction in sums of squares technique (Snedecor and Cochran, 1980). Where slopes of the treatments were non-significantly different the data were analysed using the General Linear Models Procedure of SAS (SAS Institute, 1989).

3.3.2 Experiment 2

This experiment commenced 4 weeks after the completion of Experiment 1. Between the two experiments the birds were subjected to standard management, for this farm, where their calcium source was mixed with the whole wheat and protein concentrate in one feed trough. Shellgrit, 2-4 mm in diameter, was substituted for the limestone chips used for standard management. This shellgrit was used simply to make use of shellgrit that had been sieved from the experimental supply.

The birds were then allowed 1 week to re-adjust to the provision of the shellgrit in the separate feed trough.

At 46 weeks of age the same experimental treatments were imposed as in Experiment 1 but the hens were re-located to treatments so that as many hens as possible were facing a new regimen of calcium provision. Wheat and protein concentrate were provided at a ratio of 80:20 in Experiment 2. This was a response to the buildup of protein concentrate in the feed troughs that occurred as Experiment 1 progressed into winter. A 2.5 cm wire grid was placed in the wheat and protein concentrate trough to reduce feed wastage due to "flicking" that had been noted during Experiment 1. Access to shellgrit by the hens on Treatments 2 and 4 was allowed by opening the metal covers on the troughs at lights on at 0530 h. Feed and shellgrit were weighed at lights on to give full weekly consumption figures.

In this experiment feed and protein concentrate were sieved and weighed back weekly, shellgrit consumption was measured after each period of access and eggs were collected daily at 0900 h. Total daily crude protein intakes were determined from protein concentrate and wheat consumption.

The relationship between temperature and feed intake was analysed using the reduction in sums of squares technique (Snedecor and Cochran, 1980). Where slopes of the treatments were non-significantly different the data were analysed as previously. Within this experiment, daily feed intake was recorded during week 3 (hens 48 weeks of age) to determine the relationship, if any, of temperature with feed intake in the short term. The above statistical analysis was employed.

Again, regression analysis was performed on egg production versus either total feed, shellgrit, calcium or protein concentrate intake for example, using the regression analysis procedures of the statistical package Minitab (Minitab Inc.).

3.3.3 Experiment 3

At 56 weeks of age, at lights off on the last day of Experiment 2, 3 birds per treatment, selected on the basis of having laid each day for the previous 5 days, were killed by cervical dislocation. Hens on Treatments 2 and 4 had not had access to shellgrit for 24 h and 72 h respectively.

Body weights were recorded and the crop, proventriculus, gizzard and both femurs removed from each hen. The oviduct was opened and the egg status (absence or presence of an egg and whether soft- or hard-shelled) was determined for each hen. Contents of the digestive organs were removed and weighed and wet weights of the organs recorded.

Gut contents were washed and separated then oven dried for 48 h whereupon calcium particles were removed and weighed.

The right femur of each bird was cleaned of excess flesh by boiling and careful scraping. Fat was removed by solvent (X-55) extraction for 18 h using Soxhlet apparatus. Fat-free bone weight was recorded after oven drying at 105°C for 12 h. The oven-dried bone was then ashed for 12 h at 600°C. Samples of ash were prepared for mineral analysis by ICP spectrometry (see Section 3.2.4). The method was modified by performing a final 1:10 dilution of the sample in de-ionised water to allow for the high calcium content of bone ash.

The statistical evaluation of the data was performed using the General Linear Models procedure of SAS (SAS Institute, 1989).

3.4 Results

3.4.1 Experiment 1

The egg production by the he ıs (Table 3.5) was excellent during the course of this experiment and exceeded the bree ding company's target peak production levels of 92.7-90.7 % for birds of this age (see Hyline Australia SIRO-CB production manual). Egg production (HW%) was not signi icantly different (P>0.05) across the treatments. There was a highly significant (P<0.01) time effect whereby production dropped in week 5 but improved in week 6.

Table 3.5. Experiment 2: Egg pı oduction (HW%) of hens offered *ad libitum* shellgrit daily (Tr1), ev ery second day (Tr2) or every fourth day (Tr4) (LS Means ± SE).

Tr	Week					
	1	2	3	4	5	6
1	95.3± 2.14	100.4± 1.65	97.5± 1.58	95.4± 2.07	90.3± 2.44	100.0± 2.64
2	95.6± 2.08	98.1± 1.60	97.3± 1.53	92.2± 2.01	85.6± 2.37	101.2± 2.56
4	95.3± 2.14	95.9± 1.65	99.6± 1.58	95.9± 2.07	89.4± 2.44	94.2± 2.64

For the duration of the experiment the total of eggshell cracks, breaks and malformations (data not presented) v ere under 1 % and evenly distributed across the three treatments.

There were no mortalities r corded during this experiment. Feather cover of all the hens was excellent and the feathers were in good condition. No apparent differences in colour, consistency or coning of excreta under the cages was evident. The hens appeared to reject cone sha ped shells.

Feed consumption (g/d per bird) by the hens was not significantly different across the three treatments (Tr 1=137.9±2.74, Tr 2=137.1±2.66 and Tr 4=137.1±2.74)

although there was a highly significant time effect (P<0.001) (Fig. 3.1) with feed intake increasing with falling temperatures and decreasing during the last week of the experiment as temperatures rose. Hence, mean weekly feed intake was regressed against mean weekly temperature (Fig 3.2). The relationship of weekly feed intake to temperature approached significance (P=0.06). However, the narrow temperature range may have precluded a clear effect of temperature on feed intake.

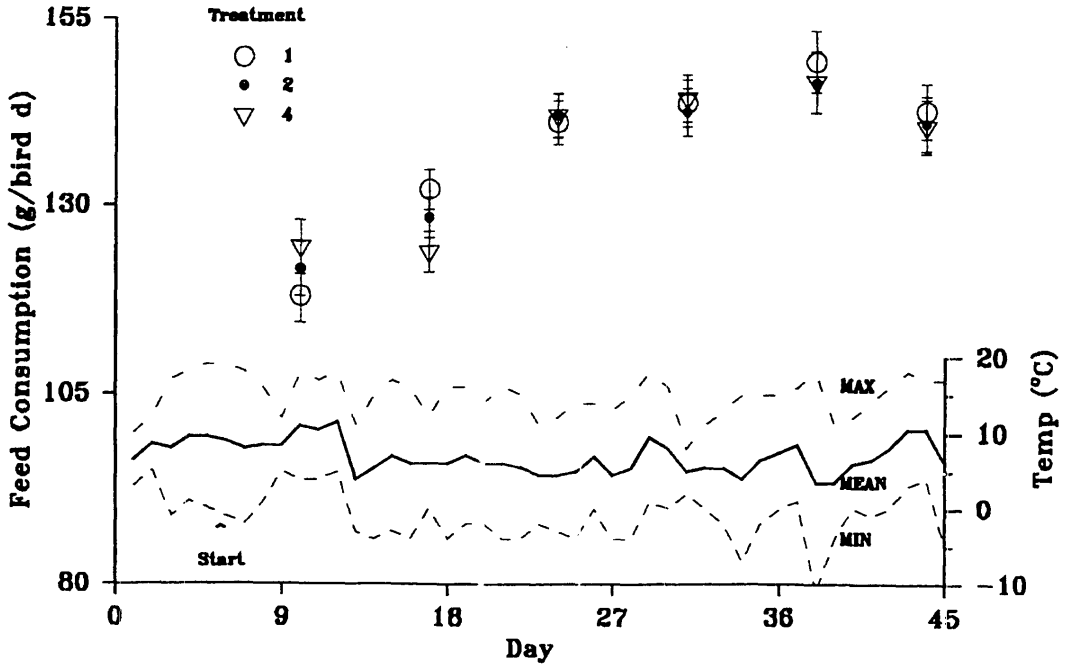

Figure 3.1 Mean weekly feed intake of hens and temperature means, maxima and minima in Experiment 1. Vertical error bars represent standard errors of the LS Means.

An initial inspection of the mean feed intake of the hens by covariate analysis using temperature as the covariate, showed that treatment intercepts and slopes of the regression equations were not significantly different (P=0.993 and P=0.995 respectively) so the treatment means were pooled to determine the regression of mean temperature against mean weekly feed intake. The very low temperatures experienced during Experiment 1 (Fig. 3.1) and the relationships of minimum and maximum temperatures as well as the difference between the two temperatures with feed intake were also regressed. Feed intake was significantly (P=0.037) related to minimum temperature but not to the maximum temperature nor temperature difference (P=0.143 and P= 0.150 respectively).

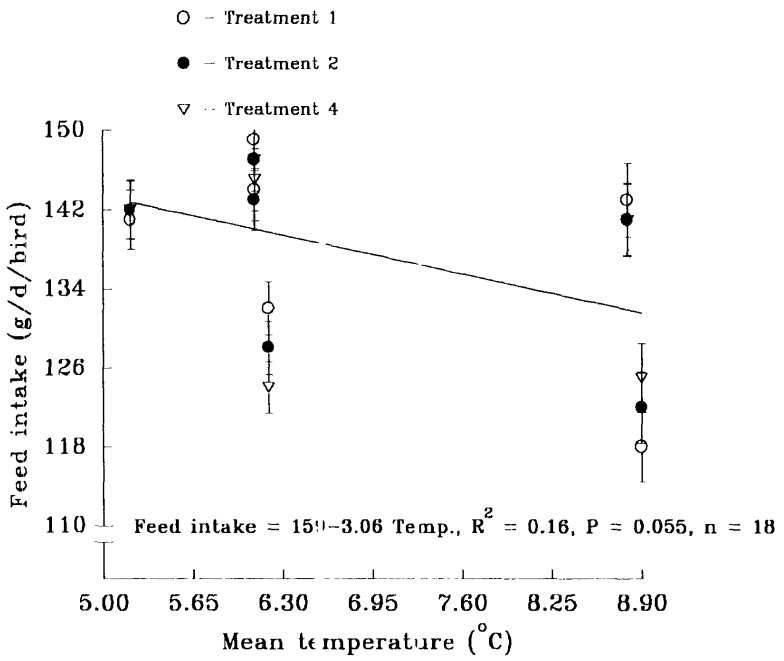

Figure 3.2 The relationship between mean weekly temperature and mean weekly feed intake of layers in Experiment 1. Vertical error bars represent standard errors of the LS Means.

Wheat intake as a proportion of total feed intake was not significantly different (P>0.05) across treatments and averaged 77% ± 0.3 and 84% ± 0.6 of total feed intake by the hens for weeks 1-5 and week 6 respectively. These figures were produced from the fifth week of the experiment in response to observation of the feed remnants which indicated that protein concentrate was accumulating in the feed troughs. The initial ratio (70:30) of wheat to protein concentrate proved to be inappropriate as the experiment progressed, possibly due to the low temperatures encountered, as indicated by the relative intake of each constituent by the birds. The ME of the wheat was not deemed to be low as determined in subsequent broiler trials.

Daily shellgrit consumption (Table 3.6) showed a highly significant (P<0.01) treatment x time interaction. The treatment effect was not significant (NS) but showed a strong trend towards significance (P=0.06). Shellgrit consumption for weeks 2 and 3 was combined because results were not obtained at the appropriate time on the last day of week 2. This would have caused the results of either 'week' to be biased so it was decided to combine the results. The hens on Treatment 2 displayed a lower (P<0.05) shellgrit consumption early in the experiment but this increased and became non-significantly different from the other two treatments by the end of the experiment.

Table 3.6. Experiment 1: Daily shellgrit consumption (g/d per bird) by hens offered *ad libitum* shellgrit daily (Tr1), every second day (Tr2) or every fourth day (Tr4) (LS Means ± SE).

Treatment	Week				
	1	2/3	4	5	6
1	7.6±0.44	8.2±0.36 $_{a}$	7.6±0.36 $_{a}$	7.3±0.32 $_{a}$	6.8±0.38
2	7.5±0.44	6.5±0.36 $_{b}$	5.6±0.36 $_{b}$	6.3±0.32 $_{b}$	7.1±0.38
4	8.1±0.46	6.1±0.38 $_{b}$	7.3±0.38 $_{a}$	8.1±0.33 $_{a}$	6.9±0.39

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

Total calcium consumption per hen during week 6 was calculated from the calcium content of shellgrit (see Section 3.6.2) as well as the protein concentrate consumed. (The remaining protein concentrate was only separated from the wheat remnants at the end of that week). The calcium content of the wheat (0.05 %) was ignored for these calculations. There was no significant difference (P>0.05) across the treatments and mean calcium intake was 3.7±0.14 g/d per bird. As total feed and the proportion of feed constituents consumed were not affected by treatment, calcium intake was due largely to the differences in shellgrit intake and would have been marginally less for hens on Treatment 2 during weeks 2/3 and 5 and hens on Treatment 4 during weeks 2/3 (from shellgrit intakes shown in Table 3.6).

No significant effects on egg production by total feed intake, percentage wheat (and, therefore, protein concentrate intake), shellgrit or total calcium intake were found by regression analysis.

The results obtained from the measurement of egg production and quality parameters presented are based on the elapsed time from last access to shellgrit (Table 3.7).

Egg weights did not differ (P>0.05) across treatments or times of collection (Table 3.8). Mean egg weight (60.0 g) was marginally lower than the breeders specifications (60.1-60.5 g) for the strain used.

Table 3.7. Experiment 1: Availability of shellgrit, on the day of egg collection for shell quality measurements each week, to hens offered *ad libitum* shellgrit daily (Tr1), every second day (Tr2) or every fourth day (Tr4).

Tr	Week				
	2	3	4	5	6
1	Available	Available	Available	Available	Available
2	Available	Available	Off	Off	Available
4	Off 1 day	Off 3 days	Off 2 days	Off 2 days	Available

Table 3.8. Experiment 1: Egg weights (g) from hens offered *ad libitum* shellgrit daily (Tr1), every second day (Tr2) or every fourth day (Tr4) (LS Means ± SE).

Treatment	Week				
	2	3	4	5	6
1	60.9±0.9	59.2±0.6	60.4±0.8	60.1±0.9	61.2±0.7
2	59.8±0.8	59.4±0.4	59.6±0.7	60.0±0.7	59.0±0.5
4	60.3±0.8	59.5±0.6	59.4±0.9	59.8±0.7	59.3±0.8

Data obtained for the egg quality parameters SG, SW and ST are presented in Tables 3.9, 3.10 and 3.11 respectively. Eggs produced by the hens fed Treatment 4 on the third day after shellgrit was withdrawn (Table 3.7 week 3) showed significantly poorer SG, SW and ST ($P<0.05$, $P<0.01$ and $P<0.01$, respectively) although egg weight was not affected (Table 3.8). Time of collection affected SG, SW and ST during week 3 where slopes of the Treatment means were significantly different ($P<0.05$). Specific gravity during week 6 (Table 3.9) was significantly greater ($P<0.05$) for eggs produced by hens on Treatment 2 (1.083) than Treatment 4 (1.080) but was not different to that obtained of hens fed Treatment 1 (1.082). Shell thickness was greater for eggs produced under Treatment 1 compared to Treatment 4 (access to shellgrit every fourth day) but not when access was provided every second day (Treatment 2, Table 3.11).

Table 3.9. Experiment 1: Specific gravity of eggs from hens offered *ad libitum* shellgrit daily (Tr1), every second day (Tr2) or every fourth day (Tr4) (LS Means ± SE).

Treatment	Week				
	2	3	4	5	6
1	1.082± 0.0010	1.082 ± 0.0007 **a**	1.082± 0.0010	1.080± 0.0011	1.082 ± 0.0010 **ab**
2	1.083± 0.0009	1.081 ± 0.0009 **a**	1.081± 0.0008	1.079± 0.0010	1.083 ± 0.0008 **a**
4	1.081± 0.0008	1.076 ± 0.0008 **b**	1.080± 0.0009	1.080± 0.0009	1.080 ± 0.0009 **b**

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

Table 3.10. Experiment 1: Shell weight (% EW) of eggs from hens offered *ad libitum* shellgrit daily (Tr1), every second day (Tr2) or every fourth day (Tr4) (LS Means ± SE).

Treatment	Week				
	2	3	4	5	6
1	9.2±0.14	9.1±0.10 **a**	9.3±0.16	8.9±0.15	9.1±0.17
2	9.3±0.13	9.0±0.12 **a**	9.0±0.12	8.7±0.14	9.0±0.14
4	9.0±0.12	8.3±0.10 **b**	8.9±0.14	8.8±0.13	8.8± 0.17

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

Table 3.11. Experiment 1: Shell thickness (µm) of eggs from hens offered *ad libitum* shellgrit daily (Tr1), every second day (Tr2) or every fourth day (Tr4) (LS Means ± SE).

Treatment	Week				
	2	3	4	5	6
1	366±5.1	356±4.0 **a**	359±5.4 **a**	355±5.4	367±5.5
2	363±4.6	360±5.0 **a**	350±4.1 **ab**	345±4.8	362±4.5
4	358±4.3	337±4.3 **b**	343±4.9 **b**	348±4.5	355±5.3

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

3.4.2 Experiment 2

The egg production of these hens (Table 3.12) during this experiment again generally exceeded the breeding company's target production levels for hens of this age (89.0-84.5 %). Egg production (HW%) tended (P=0.058) to decrease when shellgrit was supplied less than daily. The time effect was highly significant (P<0.001) as would be expected during the course of a 10 week experiment. The sudden decline in egg production across all treatments during week 5 seems related to decreased feed intake (see Fig. 3.3) and the increased ambient temperatures during this week.

Table 3.12. Experiment 2: Egg production (HW%) of hens offered *ad libitum* shellgrit daily (Tr1), every second day (Tr2) or every fourth day (Tr4) (LS Means ± SE).

Tr	Week									
	1	2	3	4	5	6	7	8	9	10
1	84.1± 2.07	92.1± 2.68	92.1± 2.04	91.7± 2.83	83.8± 2.95	89.7± 3.49	92.1± 3.39	84.9± 3.24	88.9± 2.93	79.4± 2.60
2	82.9± 2.26	88.1± 2.94	91.4± 2.24	92.4± 3.01	78.1± 3.23	83.3± 3.82	94.8± 3.71	85.7± 3.55	92.4± 3.21	79.5± 2.85
4	81.1± 2.13	83.2± 2.76	87.4± 2.10	92.0± 2.92	77.7± 3.03	81.9± 3.59	80.7± 3.48	74.0± 3.33	87.8± 3.02	75.2± 2.67

Total eggshell cracks, breaks and malformations (data not presented) were under 1 % and evenly distributed across the three treatments.

Two hens died during the course of Experiment 2; one each on Treatments 2 and 4. Subsequent data were calculated for the single hen remaining in these cages. The dead hens were subjected to veterinary post-mortem and were determined to have died from Fatty Liver and Haemorrhagic Syndrome (R.B. Cumming, pers. comm.). The dead hens stopped laying 18 (Tr4) and 21 (Tr2) d before dying. Hens in cages where egg production declined for more than a few days were palpated daily and no egg was detected in the hens that died. A number of hens stopped laying during the course of Experiment 2 then resumed laying after varying periods of time; from 10-35 d. There was no moult apparent in these hens. There was no consistent pattern to this cessation of lay as each treatment group suffered to a similar extent. (See Section

3.6). It was considered possible that failure to consume the shellgrit may have led to a cessation of lay in these hens. A check of shellgrit intakes by hens in the affected cages showed that shellgrit intakes fell, from generally mean intakes, after the afflicted hen stopped laying then rose again when she recommenced laying.

Again, feather cover of all the hens was excellent and the feathers were in good condition. No apparent differences in the colour of excreta under the cages was evident. As the experiment progressed and temperatures increased, excreta consistency became softer and coning lower under the hens fed Treatment 4. Cone shaped shells appeared to be largely rejected by the hens as occurred during Experiment 1.

Feed consumption by the hens (Fig. 3.3) did not differ significantly across the three treatments and, as with egg production, there was a highly significant time effect ($P<0.001$).

Mean weekly feed intake was again regressed against mean weekly temperature (Fig. 3.4) and weekly feed intake was found to be highly related to temperature ($P<0.001$).

An initial analysis of the mean feed intake of the hens by covariate analysis using temperature as the covariate, showed that treatment intercepts and slopes of the regression equations were not significantly different ($P=0.652$ and $P=0.543$ respectively) so the treatment means were pooled to determine the regression of mean temperature against mean weekly feed intake. The temperatures (Fig. 3.3) during Experiment 2 were increasing over those experienced in Experiment 1 as winter receded. The influence of minimum, maximum and difference between the two temperatures on feed intake was also determined. Feed intake was significantly ($P<0.001$) associated with minimum and maximum temperatures but not with the temperature difference ($P=0.837$).

After allowing for temperature, feed intakes tended to be higher in Experiment 2 than Experiment 1 even though egg production was lower. Bodyweight increases may have been involved in this effect, however, the hens were not weighed during these experiments.

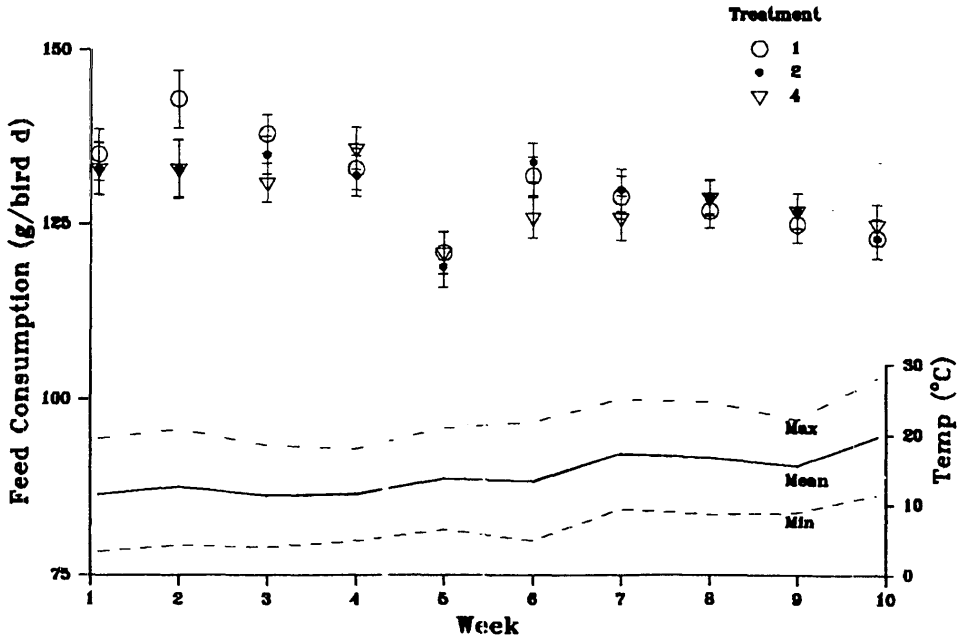

Figure 3.3 Mean weekly feed intake of hens and weekly temperature means, maxima and minima in Experiment 2. Vertical error bars represent standard errors of the LS Means.

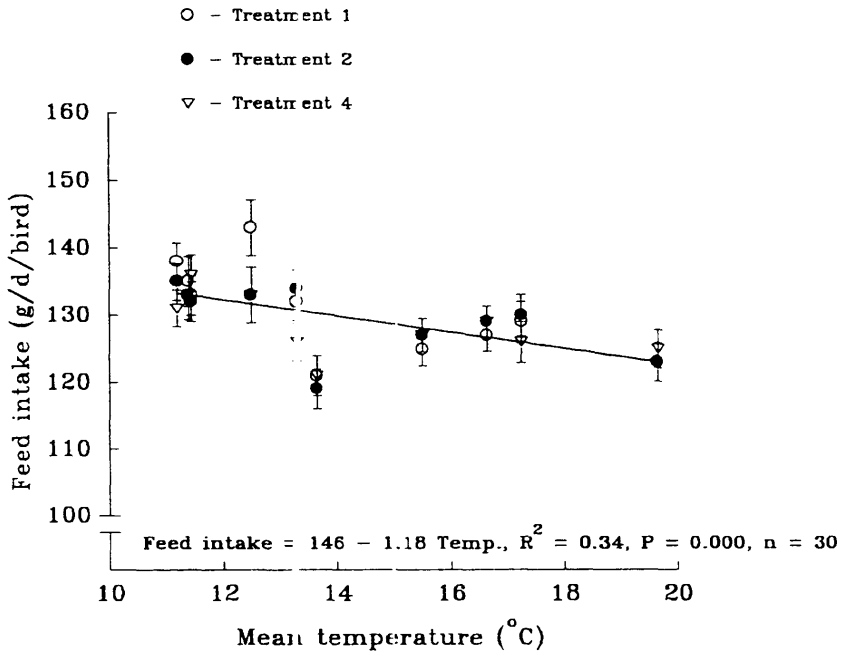

Figure 3.4 The relationship between mean weekly temperature and mean weekly feed intake of layers in Experiment 2. Vertical error bars represent standard errors of the LS Means.

Some evidence was found that suggested that daily feed intakes were not closely related to daily temperatures so mean daily feed intake for the 7 days of week 3 (Fig. 3.5) was regressed against mean daily temperature. There was no effect (P=0.473) of daily temperature on feed intake for the 7 days of week 3 as shown by the regression equation (Fig. 3.6).

Again, an initial analysis of mean daily feed intake of the hens, as related to temperature, for the three treatments by covariate analysis using temperature as the covariate, showed that treatment intercepts and slopes of the regression equation were not significantly different (P=0.568 and P=0.429 respectively) so the treatment means were again pooled to determine the regression of mean daily temperature against mean daily feed intake. The relationships of temperature minimum, maximum and difference between the two temperatures to feed intake were also regressed. Feed intake was not related to temperature minimum or maximum (P=0.186, P=0.736 respectively) but the temperature difference tended (P=0.059) to be associated with daily feed intake. The feed intakes at 16°C (Fig. 3.6) may affect the overall regression result but they are included as no obvious peculiarities in the experiment occurred on this day.

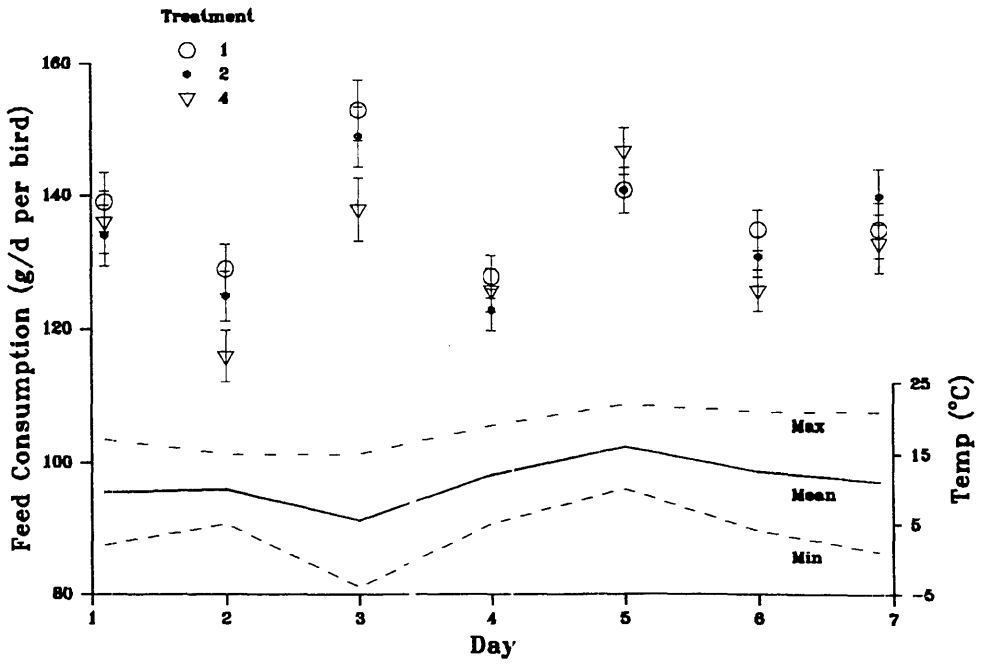

Figure 3.5 Mean daily feed intake of hens and daily temperature means, maxima and minima in week 3 of Experiment 2. Vertical error bars represent standard errors of the LS Means.

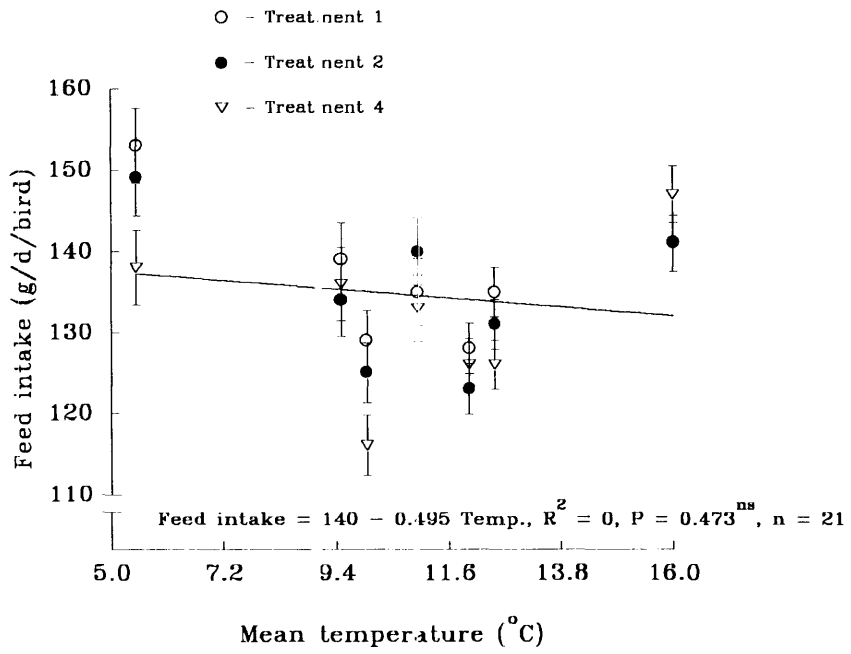

Figure 3.6 The relationship between mean daily temperature and mean daily feed intake of layers in week 3 of Experiment 2. Vertical error bars represent standard errors of the LS Means.

Wheat intake (Table 3.13) as a proportion of total feed intake was not significantly different (P>0.05) across treatments and was 83% ± 0.3.

Table 3.13. Experiment 2: Wheat intake (% of total intake excluding shellgrit) of hens offered *ad libitum* shellgrit daily (Tr1), every second day (Tr2) or every fourth day (Tr4) (LS Means ± SE).

Tr	Week									
	1	2	3	4	5	6	7	8	9	10
1	83.2± 0.41	82.6± 0.39	82.9± 0.33	83.4 ± 0.31	83.3 ± 0.44	82.9± 0.34	82.9± 0.39	83.1± 0.26	83.1± 0.37	82.9± 0.26
2	82.6± 0.43	82.8± 0.41	82.9± 0.36	83.1 ± 0.3?	83.5± 0.47	82.3± 0.37	82.8± 0.41	82.5± 0.28	82.9± 0.39	82.7± 0.28
4	82.6± 0.41	82.7± 0.39	82.8± 0.33	83.0 ± 0.31	83.2± 0.44	82.8± 0.34	82.2± 0.39	82.3± 0.26	82.2± 0.37	82.6± 0.26

Crude protein intake (Table 3.14) was not significantly different (P>0.05) across the treatments. There was a highly significant (P<0.001) time effect.

Table 3.14. Experiment 2: Crude protein intake (g/d per bird) of hens offered *ad libitum* shellgrit daily (Tr1), every second day (Tr2) or every fourth day (Tr4) (LS Means ± SE)

Tr	Week									
	1	2	3	4	5	6	7	8	9	10
1	19.2± 0.78	24.8± 0.96	23.6± 0.72	22.1± 0.73	20.2± 0.79	22.8± 0.76	22.2± 0.84	21.4± 0.56	21.1± 0.71	21.3± 0.59
2	19.9± 0.83	24.0± 1.02	23.5± 0.76	22.8± 0.78	19.7± 0.84	24.2± 0.81	22.9± 0.89	23.0± 0.59	22.0± 0.76	21.8± 0.63
4	19.8± 0.78	23.1± 0.96	22.6± 0.72	23.1± 0.73	20.4± 0.79	21.8± 0.76	22.5± 0.84	22.9± 0.56	22.5± 0.71	21.8± 0.59

Shellgrit consumption (Table 3.15) showed a highly significant (P<0.01) treatment x time interaction but differences were not significant across treatments. The hens on Treatment 2 displayed a lower shellgrit consumption early in the experiment (weeks 1 and 3) but it was not different to the birds on continuous access thereafter. This consumption increased during the experiment and shellgrit intakes did not differ (P>0.05) at the end of the experiment relative to the control birds.

Table 3.15. Experiment 2: Shellgrit intake (g/d per bird) of hens offered *ad libitum* shellgrit daily (Tr1), every second day (Tr2) or every fourth day (Tr4) (LS Means ± SE).

Tr	Week									
	1	2	3	4	5	6	7	8	9	10
1	5.9 ± 0.36 **b**	6.6± 0.31	7.3 ± 0.32 **a**	6.8± 0.34	7.0± 0.44	6.7± 0.32	6.3± 0.31	6.5± 0.36	6.2 ± 0.42 **b**	6.2± 0.30
2	5.2 ± 0.38 **b**	6.3± 0.33	5.8 ± 0.34 **b**	6.9± 0.36	5.7± 0.46	6.7± 0.34	5.8± 0.32	6.7± 0.38	6.0 ± 0.45 **b**	6.2± 0.31
4	7.3 ± 0.36 **a**	6.7± 0.32	7.3 ± 0.33 **a**	7.5± 0.35	6.6± 0.45	7.4± 0.33	6.6± 0.31	6.5± 0.37	7.5 ± 0.43 **a**	5.8± 0.31

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

Hens on Treatment 4 appeared to overcompensate for the period of shellgrit denial by consuming more shellgrit during weeks 1 and 9 although during the other measurement periods, no differences between Treatments 1 and 4 were observed.

Calcium content of the shellgrit was determined to be 37.9 % by ICP analysis.

Calcium consumption (Table 3.16) per hen (from shellgrit and protein concentrate) showed a highly significant time x treatment interaction ($P<0.01$). As total feed and the proportion of feed constituents consumed were not affected by treatment the significant effects were due largely to the differences in shellgrit (37.9 g/kg calcium) intake (see Table 3.15) rather than protein concentrate (36.9 g/kg calcium) intake. Calcium intake from the protein concentrate was low (0.88 ± 0.013 g/d per bird).

Table 3.16. Experiment 2: Total calcium intake (g/d per bird) of hens offered *ad libitum* shellgrit daily (Tr1), every second day (Tr2) or every fourth day (Tr4) (LS Means ± SE).

Tr	Week									
	1	2	3	4	5	6	7	8	9	10
1	3.0 ± 0.16 $_{b}$	3.5± 0.13	3.7 ± 0.13 $_{a}$	3.5± 0.14	3.4± 0.16	3.5± 0.13	3.3± 0.13	3.3± 0.15	3.3 ± 0.17$_{ab}$	3.3± 0.12
2	2.7 ± 0.17 $_{b}$	3.4± 0.13	3.3 ± 0.13 $_{b}$	3.6± 0.15	3.2± 0.17	3.6± 0.14	3.2± 0.14	3.5± 0.16	3.2 ± 0.18$_{b}$	3.2± 0.13
4	3.5 ± 0.15 $_{a}$	3.4± 0.13	3.7 ± 0.13 $_{a}$	3.8± 0.14	3.3± 0.16	3.7± 0.13	3.4± 0.13	3.4± 0.15	3.7 ± 0.17 $_{a}$	3.1± 0.12

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

Results of the regression analyses performed between egg production and feed intake data are presented in Table 3.17. Hen week production was related to total feed, shellgrit and calcium intake.

Table 3.17. Experiment 2: Regression matrix indicating the statistical significance levels for relationships between intakes of feed constituents and relationships between intakes of feed constituents and egg production of hens offered *ad libitum* shellgrit daily (Tr1), every second day (Tr2) or every fourth day (Tr4).

	Total feed intake (g/d)	Wheat intake (%)	Crude protein intake (g/d)	Shellgrit intake (g/d)	Shellgrit calcium intake (g/d)	Protein concent. calcium intake (g/d)	Total calcium intake (g/d)
Egg production (HW%)	* *	* *	* *	* *	* *	* *	* * *
Total feed intake (g/d)		* *	* * *	* *	* *	* * *	* * *
Wheat intake (%)			* * *	NS	NS	* * *	NS
Crude protein intake (g/d)				*	*	* * *	* * *
Shellgrit intake (g/d)					* * *	NS	* * *
Shellgrit calcium intake (g/d)						NS	* * *
Protein concent. calcium intake (g/d)							NS

3.4.3 Experiment 3

Neither hen body weight, crop, proventriculus and gizzard weights, nor their relative proportions of body weight, differed significantly ($P>0.05$) between sacrificed hens from the three treatments (Table 3.18). There were no significant differences ($P>0.05$) in total feed contents of either crop or gizzards of the hens (Table 3.19). However, there were significant ($P<0.05$) differences in the percentage of wheat in total feed content of the crop with hens on Treatment 1 having less ($P<0.05$) wheat

than hens on either Treatment 2 or 4. Hens on Treatment 1 had 6.1 g of shellgrit in their crops whilst those on Treatments 2 and 4 had none. Gizzard shellgrit contents differed significantly ($P<0.05$) between Treatments 1 and 4 with more shellgrit in the hens on Treatment 1. Hens on Treatment 2 had an intermediate amount of shellgrit between the other two treatments which did not differ significantly ($P>0.05$) to the other treatments.

Table 3.18. Experiment 2: Body weight, digestive organ wet weights and digestive organs as a percentage of body weight of hens offered *ad libitum* shellgrit daily (Tr1), every second day (Tr2) or every fourth day (Tr4) (LS Means).

Tr	Hen weight (g)	Crop weight (g)	Crop weight (%)	Prov. weight (g)	Prov. weight (%)	Gizzard weight (g)	Gizzard weight (%)
1	2366	7.0	0.2	7.3	0.2	36.0	1.5
2	2663	6.7	0.2	8.7	0.3	35.7	1.3
4	2269	7.0	0.2	7.7	0.3	35.3	1.5
S E	139.9	0.24	0.03	0.53	0.04	1.53	0.08

Table 3.19. Experiment 2: Crop and gizzard feed contents, wheat and shellgrit content of the crop and shellgrit content of the gizzard of hens offered *ad libitum* shellgrit daily (Tr1), every second day (Tr2) or every fourth day (Tr4) (LS Means).

Tr	Crop feed content (g)	Gizzard feed content (g)	Crop wheat content (%)	Crop shellgrit content (g)	Gizzard shellgrit content (g)
1	77.7	17.7	81_{a}	6.1_{a}	6.1_{a}
2	70.0	19.3	95_{b}	0_{b}	5.0_{ab}
4	56.7	23.0	93_{b}	0_{b}	2.9_{b}
S E	10.75	2.07	1.8	0.64	0.91

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

Femur weights and fat-free, solvent extracted femur weights were not significantly different (P>0.05) across the treatments (Table 3.20). However fat-free femur as a percentage of femur weight and ash as a percentage of fat-free femur weight did differ (P<0.05) across the treatments with Treatment 1 lower than Treatments 2 and 4. The calcium and phosphorus content of femur ash and calcium to phosphorus ratio did not differ significantly (P>0.05) across treatments.

Table 3.20. Experiment 2: Femur weights, ash, calcium and phosphorus concentrations measured from hens offered *ad libitum* shellgrit daily (Tr1), every second day (Tr2) or every fourth day (Tr4) (LS Means).

Tr	Femur weight (g)	Fat-free femur weight (g)	Fat-free femur (%)	Femur ash weight (g)	Femur ash (%)	Femur ash Ca (%)	Femur ash P (%)	Femur ash Ca : P
1	5.7	5.1	88.5 ^{a}	2.0	35.1 ^{a}	45.7	20.8	2.2
2	5.9	5.7	97.2 ^{b}	2.3	39.1 ^{b}	45.1	20.4	2.2
4	5.0	4.8	96.4 ^{b}	2.0	40.1 ^{b}	45.0	20.4	2.2
S E	0.35	0.38	1.77	0.11	1.01	2.17	1.07	0.01

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

The hens were also categorised according to egg status irrespective of treatment; whether they were empty or had a soft- or hard-shelled egg in the uterus. No significant differences were obtained from this procedure other than for shellgrit content of the gizzard where hens holding hard-shelled eggs had significantly more (P<0.05) shellgrit (7.3±1.5 g n=2), than those with soft-shelled eggs (2.6±0.8 g n=4) and those that were empty (3.4±1.0 g n=3). There were no differences (P>0.05) between hens that were not shell forming or held soft-shelled eggs.

3.5 Discussion

The hens in these trials were laying extremely well, both productively (HW%) and by egg weights, and exceeded the breeder's standards in Experiments 1 and 2.

It appears from Experiments 1 and 2 that the free-choice fed laying hen has the capacity to rapidly adjust its intake of particulate calcium when denied access for

fixed periods. This adjustment occurred during the first week of Experiment 1 and in the case of Treatment 4 occurred on the first possible occasion when calcium was supplied. This finding was confirmed in the second experiment although the hens on Treatment 4 ate more shellgrit during the first week than hens on Treatments 2 and 4.

The lower intake of shellgrit during some weeks by the hens on Treatment 2 in both experiments may be due to a temporary improvement in calcium retention, this effect being modified as calcium from the gizzard was reduced over time which led to a slightly higher intake each week. This improvement in calcium retention is possible as no significant differences in either feed intake, proportions of feed constituents consumed or egg production were recorded for hens on any treatment. Summers *et al.* (1976) found that calcium retention was related to the level in the diet and Rao and Roland (1989 and 1990) found that the amount of calcium retained was directly related to intake, with calcium deficient hens achieving a greater retention with a lower intake. In the experiments reported here, the lower shellgrit intake and thus, calcium intake, may have led to body stores of calcium being progressively depleted which then led to more shellgrit being eaten.

The total calcium intake (3.7 g/d per bird) in Experiment 1 was close to the 3.6 g/d per bird recommendation set by the NRC (1994) guidelines for brown egg layers but the calcium intake by the hens in Experiment 2 (3.3 g/d per bird) was lower than recommendations. However, the lower intake in Experiment 2 is in line with lower production later in lay (Table 3.5 Experiment 1 versus Table 3.12 Experiment 2). The shellgrit and calcium intake figures (Tables 3.6, 3.15 and 3.16) and the relationship of egg production to shellgrit and calcium intake (Table 3.17) support the later work of Taylor (1970) who found that layers efficiently regulate the intake of calcium grit. However, the data are at variance with the finding by Tyler (1955) that voluntary limestone intake was excessive in layers and bore no relationship to egg production. Tyler (1955) conceded that the results in his experiments may have been due to the hens having no previous exposure to a grit source.

As proposed by Scott *et al.* (1971), a relatively large particle size of shellgrit, as used in these experiments, may allow for longer term availability of calcium for absorption as it is meted from the gizzard. This in turn may allow prolonged synthesis of medullary bone when shell formation is not occurring (Taylor, 1970) so providing a greater reserve for future shell synthesis. This suggestion is supported by the fat-free femur weights (Table 3.19) which were significantly lower in hens on Treatment 1.

These birds presumably did not have the need to produce as much medullary bone as they had continual access to a calcium source and a large quantity of shellgrit continually present in the gut as shown by the amount of shellgrit found in the crop and gizzard of those hens slaughtered. Cunies *et al.* (1992) found that hens on a 4.5 % calcium diet had less medullary bone than those hens on a 3.5 % calcium diet. The hens on Treatments 2 and 4, even though consuming double or triple the quantity of shellgrit on the days when shellgrit was available, may have reached a threshold level of calcium content of the gut. This may have stimulated formation of medullary bone in response to falling levels of calcium in the intestine and circulatory system. Alternatively the large volume of calcium in the gizzard may have saturated the intestinal absorptive mechanism, after grinding and solubilization in the gizzard, and this may have stimulated medullary bone formation. Cheng and Coon (1990c) found that bone deposition proceeding at an undiminished rate in hens consuming 4.5 g d of calcium. In their model of calcium regulation, Hurwitz *et al.* (1987) found that high levels of calcium intake resulted in the normal feedback control mechanisms of calcium absorption being overwhelmed. As the calcium content of the femur ash was similar across the treatments (Table 3.19), the extra bone, as indicated by both the greater proportion of fat-free femur and proportion of femur ash of hens on Treatments 2 and 4, appeared to have been present as mineralised bone rather than osteoid. Whitehead (1994) found an increase in the proportion of medullary bone in hens that were given particulate calcium sources. In the experiments reported here, it should be noted that the cortical and medullary sections of the femur were not separated.

There appears to be a short term limit to the ability of the decreasing gizzard reserve of shellgrit and the capacity of the bone calcium reserve of birds to meet the calcium requirements for shell formation. This was evinced by the poor shell quality (lower SG, SW and ST) of eggs from hens (Treatment 4) after 3 days without access to shellgrit. These hens also displayed a tendency to produce eggshells of lower quality than those of the other two groups of hens at all times. Hens on Treatment 2 produced shells similar to those produced by hens subjected to Treatment 1. In Experiment 1, the supply of shellgrit in the gizzard appeared to be reduced quickly, as shell quality of the eggs of the hens on Treatment 4 deteriorated on the third day after shellgrit withdrawal. This is confirmed by the data obtained from the sacrificed hens in Experiment 3 where shellgrit level in the gizzard of the hens fed on Treatment 4 was reduced to 2.9 g (Table 3.18) even though the birds had consumed 23.2 g of shellgrit (four times the mean daily intake of shellgrit Table 3.15, week 10, Treatment 4), after shellgrit was withdrawn. Perhaps this small quantity of shellgrit did not provide adequate calcium levels for intestinal absorption and bone mobilisation of calcium was

stimulated to provide for eggshell calc fication. Both Mongin and Sauveur (1984) and Sauveur (1992) found that improved eggshell quality was associated with higher levels of calcium available from the gut. There was a large variation between individuals as Treatment 2 hens, with 5.0 g of shel grit in the gizzard, did not have a significantly different level of shellgrit to those hens on Treatment 4 and therefore care must be exercised with these results as only three hens per treatment were killed. Similarly, care must be exercised when examining the results of the analysis of shellgrit content of the gizzard from the hens according to egg status, as only two hens, one each from Treatments 1 and 2, had hard-shelled eggs in the oviduct.

However, the finding that shellgrit was still present in the gizzard, after shellgrit had been withheld for 4 d, confirms the finding of Roland *et al.* (1972) who found particles from "hen-sized" limestone or oyster shell still present in the gizzard of hens 4 d after dosing with the calcium source. Roland *et al.* (1972) and Rao and Roland (1990) found that fine particles of calcium (called "pullet-sized") passed rapidly through the gut of the layer and calcium was not retained as well as from coarser calcium sources. Rao and Roland (1990) defined small limestone as 0.5-0.8 mm and large limestone as 2-5 mm in diameter.

The indication of a lower overall production by the hens on Treatments 2 and 4 may be partially explained by some birds not laying for periods of up to 35 d. These birds displayed lethargy and pale combs. The deaths of two birds during this trial affected production results in both Treatments 2 and 4 as they had both been found to have stopped laying 18 and 21 days before dying. The deaths of these hens on these particular treatments is attributed to chance, as all three treatments had a similar number of poorly birds. Veterinary examination of the dead hens showed a heavy accumulation of abdominal fat and haemorrhages of the liver. It can be characteristic of apparently healthy, heavy producing hens with a high energy intake to die from Fatty Liver and Haemorrhagic Syndrome (Couch, 1956) as detailed by (Peckham, 1984), and this tends to occur at an earlier age in choice-fed hens than birds on complete diets (R.B. Cumming, pers. comm.). During the dissection of hens in Experiment 3, it was noted that all the hens had a full complement of developing ova and reasonably large accumulations of abdominal fat and fatty, pale brown livers. The hens were selecting a high energy diet, ie. a high proportion of wheat (see Table 3.13), which is attributed to the cold temperatures that occurred throughout these experiments. This is a good measure of the success of choice feeding as a feeding system as the hen can increase her energy intake in the form of whole grain but still maintain a relatively constant crude protein intake (see Table 3.14). However, the

deaths of hens on Treatments 2 and 4 only, may indicate that the intermittent feeding of the calcium source may cause a calcium deficiency in some birds. Calcium deficiency was implicated as a cause of Fatty Liver and Haemorrhagic Syndrome by Roland *et al.* (1985).

The shell grit (379 g calcium/kg) was apparently identified as the sole calcium source by the hens. The protein concentrate (36.9 g calcium/kg) was not, apparently, selected as an alternative calcium source by the shellgrit-deprived hens as the proportion of protein concentrate in the total feed intake was not significantly greater on days when the hens were deprived of calcium. This is at odds with the proposal of Hughes (1972) that a 1% level of dietary calcium may still control feed intake, but a very low level (such as the 0.2% in his trial diet) may result in feed intake being independent of dietary calcium. As the intermittent feeding of shellgrit in the experiments described in this thesis is a confounding factor, no true comparison with other results can be made.

There is certainly a contribution of calcium, largely from the animal protein ingredients of the protein concentrate, to total calcium intake. This is also true for wheat, but the contribution of wheat to total calcium intake was ignored as the amounts were small (less than 0.1 g d) and less than the error involved in the total calcium intake determination. However, it would appear that the hens were selecting the protein concentrate principally for its protein content, so avoiding an excess intake of protein as shown in pigs (Kyriazakis *et al.*, 1990). The crude protein intake of the hens during Experiment 2 (Table 3.14) was in accord with NRC (1994) recommendations for brown egg layers and with the hypothesis of Emmans (1978) that hens in a two-feed system will avoid excess nutrient intake.

The results of regression analyses of feed intakes (Table 3.17) indicate that greater intakes of energy, crude protein and calcium are required for higher levels of egg production. It appears that wheat, or energy, consumption is not related to shellgrit and calcium intakes but shellgrit intake is related to protein intake. This may therefore indicate that energy and calcium intakes are regulated separately. Hughes (1972), supported by Mongin and Sauveur (1979a), hypothesised that food and calcium intakes were separately regulated. Crude protein and calcium intake are related because both are required in higher amounts during egg formation but this does not imply that the regulatory mechanism is the same for both.

Birds on Treatment 4 displayed increasing activity and apparent agitation from late on the second day after shellgrit withdrawal in both experiments. This activity was particularly apparent when the metal cover was removed from the shellgrit troughs of the hens on Treatment 2, or when feeding all the hens. It is possible that this agitation was due to the hens identifying the experimenter and anticipating calcium provision. However, the hens were regularly observed from outside the shed apparently without the hens being aware of human presence and at times other than normal feeding hours. The increased activity of hens on Treatment 4 was still noticeable. Hen activity and agitation were most apparent as escape behaviours whereby the hens would push at the wire gate, look into the corners, front and back of the cage and generally attempt to push their way through the cage wire or attempt to stretch across to the feed or shellgrit troughs of neighbouring cages. Most of the hens on Treatment 4 pecked at the metal cover in the shellgrit trough at these times. These observations are similar to the findings of Wood-Gush and Guiton (1967) who elicited escape behaviours in laying hens by thwarting them in their attempts to feed. The escape behaviour in their hens disappeared after a few episodes of thwarting. Duncan (1970) thwarted hungry brown leghorns by placing their feed under a perspex cover. He found that the hen would attempt to escape when unable to feed and that this escape behaviour did not diminish after 13 exposures to the treatment and non-aggressive pecking at the covered food source increased with more exposure. The observations made of the hens in Experiments 1 and 2 confirm Duncan's (1970) results and it was considered that the hens may have eaten more feed when thwarted in their attempts to gain access to the shellgrit. However, the feed intake data (Figs. 3.1 and 3.3) suggest that hens exposed to intermittent access to the shellgrit did not eat more feed.

It was noted that the shellgrit was eaten avidly, for a very short time (as little as 20 s) when the trough was opened on the fourth day. This behaviour occurred from the first period of shellgrit withdrawal and did not alter throughout the trial. This prompted the change to opening the shellgrit trough at lights-on in Experiment 2, as it had been found in Experiment 1 that some hens ate shellgrit in the dark after the grit trough was opened after lights-out. This occurred despite opening the trough as late as 4 h after lights-out when the hens were firmly settled.

The significantly ($P<0.05$) lower wheat content of the crops of birds subjected to Treatment 1 is inexplicable, without further experimentation. However, it lends support to the idea that the protein concentrate is not regarded as a calcium source by the hens on Treatments 2 and 4. This does not mean that the wheat should be

considered as being selected as a calcium source by the hens. The different proportions of feed constituents eaten prior to lights out indicates that the diurnal pattern of feed intake may be altered by providing the shellgrit on an intermittent basis. There was no difference in total feed content of the crop across treatments, although there was a trend towards decreasing content with increasing length of shellgrit deprivation. This, the egg status of the hens, and in association with so few hens being used, probably confounds any sensible interpretation of these results.

Feed wastage, mainly by beak raking and flicking of the feed, normal bird foraging behaviours as described by Appleby *et al.* (1992), was noted in Experiment 1. The provision of approximately 2.5 d feed every second day to prevent overfilling of the feed trough, and a wire grid placed on the feed, reduced this to an insignificant level (determined by visual inspection of the floor). The use of a wire grid on the feed is one of a number of commercial practices employed to reduce feed wastage (Appleby *et al.*, 1992). The grid may have played a role in the protein concentrate consumption pattern not being altered in Experiment 2. The grid may have prevented the hens from searching for the protein concentrate which was noticed to aggregate in the bottom of the trough a few hours after feeding. This was possibly due to the trough being shaken by normal bird movement in the cage.

The 6.1 g of shellgrit found in the crop of hens fed Treatment 1 (Table 3.18) may indicate that these hens are eating more of their calcium requirement in the period from late afternoon to lights out, as Taylor (1970) stated that calcium grit intake was highly correlated with egg formation and Mongin and Sauveur (1974) found layers ate more oyster shell in the afternoon. Savory (1979) found that layers ate more in the evening than non-layers and that the afternoon appetite was due to the need for calcium. This intake of shellgrit found, was similar to the total daily shellgrit intake of 6.2 g and it is remarkable that the crop and gizzard contents of shellgrit of the hens on Treatment 1 was similar in both organs.

Mongin and Sauveur (1979a) showed that layers displayed a higher evening intake of calcium than non-layers and that calcium and feed intake were regulated separately. This supports the results found in Experiments 1 and 2 as hens did not eat more feed when the shellgrit was withheld for 1 or 3 d (Figs. 3.1 and 3.3). Also, the results of regression analysis of wheat and calcium intakes (Table 3.17) indicate little relationship between energy and calcium intake. The hens with hard-shelled eggs in the oviduct had more calcium in the gizzard than those that were not forming eggs or

had soft-shelled eggs. Again, these results must be viewed with caution as very few hens were examined.

The patterns of intakes of feed and shellgrit were not measured within each day as the experience of this author is that any disturbance to feed troughs leads to feed intake which is not indicative of the normal pattern of intake of undisturbed birds.

The capacity of the laying hen to rapidly adjust her intake of calcium to account for periods of denial is remarkable. In these experiments, hens rapidly adjusted their intake of calcium by a multiple of the number of days of denial such that calcium intake was similar to the weekly mean intake of those birds allowed *ad libitum* access to shellgrit. Extending the period of deprivation in Experiment 2 to see how long the bird would attempt to compensate for this denial was considered. However, as shell quality subsequently deteriorated from three days of denial, and a non-significant trend to lower egg production was apparent, then, from a practical point of view, it was pointless to extend this denial. A more detailed examination of the effects of withholding calcium for fixed periods from individual hens was subsequently undertaken.