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THE ROLE OF DIETARY CALCIUM IN THE CONTROL OF EGG PRODUCTION

A thesis presented in partial fulfilment
of the requirements for the degree of
Master of Agricultural Science
in Animal Science
at Massey University

Alan Michael Gibbins

1975

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ACKNOWLEDGEMENTS

In the presentation of this thesis I wish to acknowledge with thanks the supervision and encouragement given to me by Dr W.K. Smith and Mr M.R. Patchell. My gratitude extends also to all other staff members of the Poultry Research Centre and in particular, Miss Pat Hopkirk for assistance with experimental work. The manuscript was typed by Mrs Marie Eichstaedt to whom I express sincere thanks.

ABSTRACT

Dietary calcium restriction is studied as a method of controlling egg production. Attempts have been made to delay the onset of egg production in the fowl by feeding pre-laying diets deficient in calcium. Calcium restriction had no apparent effect on sexual development and did not delay the time of first oviposition.

Low calcium diets were used at a later date to halt egg production firstly just after peak production and secondly towards the end of the first laying year. Egg production was depressed markedly but never completely ceased, and remained at a low level until calcium restrictions were lifted whereupon a rapid rise returned egg production to levels comparable to egg production rates of non calcium restricted control hens.

Comparisons between egg production, egg weight, shell weight and a measure of shell quality (shell weight per unit surface area of egg) revealed trends towards improved shell production and shell quality following calcium restriction but little else. There was only a small number of significant differences. Egg production pauses induced by low dietary calcium were thought to be unsatisfactory as substitutes for force moulting.

Calcium restriction caused declines in food consumption and body weight. While food consumption returned to levels equivalent to food consumption of non calcium restricted hens after calcium restriction, body weight in general did not.

Calculations of the calcium loss from the body of calcium restricted hens via egg shell production show that extremely severe depletion occurs unless egg production is stopped or at least egg shell production is stopped. Such depletion of calcium has greatest effects on the skeleton and damage to the bones, particularly of the legs, may result. This is a condition which may predispose to a paralytic condition characteristic of extreme calcium deficiency.

INTRODUCTION TO THESIS

A considerable amount of calcium nutrition research involving egg production stock in the past has mainly focused upon the relationship between calcium and eggshell quality. In the early 1960's exploration of the effects of low calcium diets was made on the basis of claims that some antibiotics were more effective when administered during periods of calcium restriction.

The discovery that calcium may have a controlling influence on egg laying in the hen initiated considerable research into the use of low calcium diets to cause cessation of egg production. Since then a great deal of the experimentation on calcium restriction in laying hens has arisen from a search for a suitable alternative method of causing periods of reproductive inactivity which occur during moults. Withdrawal of calcium from the diet is known to cause severe egg production depression which continues as long as calcium restriction is enforced. Egg production returns to its normal level when adequate dietary calcium concentrations are reintroduced.

While the effect of calcium restriction on egg production is well known, there is little understanding of characteristics of hens or eggs during and after periods of calcium restriction. Effects of low calcium diets on parameters such as egg weight, shell weight, shell quality, body weight and food consumption must be thoroughly investigated as these are important practical measures of possible benefits of calcium restriction. The measurement of these physiological characters is the central theme to this thesis.

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CHAPTER ONE

Literature Review

1.1 INTRODUCTION

Physiological and nutritional aspects of egg laying are reviewed for two distinct periods of the hen's life. First is the pre-laying period which is generally considered to be the period prior to the first oviposition during which rapid development of the reproductive organs proceeds. The pre-laying period normally begins two to four weeks before the ovulation of the first ovum. Second is the period during and following a period of reproductive inactivity. Most of this section of the review is concerned with aspects of force moulting. Sections on calcium metabolism in the hen provide a satisfactory basis for discussion of low calcium diets and the effects of dietary calcium restriction.

1.2 PHYSIOLOGY OF THE HEN AT THE ONSET OF LAY

The time from hatching to about three to four weeks before sexual maturity is fundamentally a period of growth and development of all physiological systems except the reproductive system. The end of this period of growth heralds the beginning of a second phase of development which is geared to provide a rapid transition from the sexually non functional to the sexually functional state. In three to four weeks sexual development progresses at an extremely rapid rate leading to sexual maturity which is attained with the laying of the first egg.

Several specific changes are observed during the short period of time before and immediately after the onset of egg production.

1.2.1 Body Weight

Two to three weeks prior to the laying of the first egg there is a rapid surge in body weight. The increase is to the order of 400 to 500 g (Hurwitz and Bar, 1971). This is only seen when individual body weight records are kept, for it is not a time dependent change. Rather it depends on the precocity of the pullet. In flock recordings of body weight the physiological picture is obscured by the fact that some hens reach sexual maturity earlier than others. This leads to the false idea that body weight shows a relatively large gain two to three months after the beginning of egg production (Scott, Nesheim and Young, 1969).

Body weight resumes a gradual increase during the first month of egg production.

Increased body weight prior to the first egg is the result of very rapid development of the reproductive tract, increased skeletal mass due to intense mineral deposition and a general increase in all body stores. It is not difficult to appreciate the need for rapid development of the reproductive tract and increased skeletal deposition but it is difficult to find a reason for large increases in other body stores. It may be that body weight increase due to enlarged musculature and increased fat deposits is not necessary but is unavoidable because the hen's efficiency of absorption increases so much during the pre-laying period.

1.2.2 Food Consumption

Food consumption has been shown to decrease some six or seven days before the first oviposition (Meyer, Babcock and Sunde, 1970). The lowest level of food consumption is reached on the day of first oviposition or the day following the first oviposition. Food consumption returns to a high level some four to five days after the laying of the first egg. The experiments of Meyer, Babcock and Sunde (1970) show decreased consumption of diets containing levels of calcium varying from 0.4% to 1.5%. The level of calcium fed

during the pre-laying period had no consistent effect on the magnitude of the decreased food consumption. This produces a paradoxical situation for on the one hand, the hen absorbs, and has a need to absorb a large amount of mineral and deposits it in the skeleton but on the other hand a decreased food consumption occurs.

Experiments reported by Foster (1968) show that food consumption drops in a twelve day period immediately before the first oviposition. The average drop from twelve days before the first egg to three days before the first egg is reported for two experiments carried out in 1964 and 1965. The falls in consumption are 14.04 $\pm$ 2.35g/bird and 13.23 $\pm$ 2.69 g/ bird respectively. After laying of the first egg, food consumption increased so that by five to seven days after the first egg, it was at the same level as before the decrease was recorded.

Two possible explanations of the surprising fall in food consumption in the pre-laying period have been proposed by Foster (1968). The first is that pullets' appetite may be depressed while rapid anatomical changes particularly reproductive organs are taking place. The second suggests that a decreased energy requirement causes the decreased food consumption. He assumes that much of the body weight increase in the pre-laying period is due to increased oviduct and ovary weight and suggests that if the ovary and oviduct have developed to near their mature size before food consumption begins to drop, the observed interval of decreased food consumption could be explained by lowered food requirements.

1.2.3 Specific appetite for Calcium

During the pre-laying period the pullet has an increased requirement for calcium and increases the efficiency of absorption of calcium from the intestinal wall. Following the onset of lay absorption of calcium remains at a high level. Retention of up to 1.83g calcium / day has been reported by Hurwitz and Griminger (1961a). More recently Smith, Ballard and Biellier (1972) discovered an

increased appetite for calcium on days when hens ovulate or on "egg-forming" days. Conversely on days when hens do not ovulate their appetite for calcium decreases. Whether or not there is a different retention rate of calcium on ovulating days and non-ovulating days is unknown.

Calcium assimilated on non-ovulating days is mostly deposited in the skeleton. On days of ovulation, assimilated calcium and calcium resorbed from the skeleton are used in eggshell mineralisation.

1.2.4 Blood Calcium

At the time of reproductive activity some definite changes occur in female birds. Riddle, Rauche and Smith (1944) cited by Simkiss (1967) first detected the rise in blood calcium in pigeons. About 108 hours before the female ovulated blood calcium rose from the normal 9.3 mg % to over 20 mg % at the time of ovulation. Male birds in the same experiment did not show this increase.

The ionic calcium in the blood remains at a fairly constant level but non-ionic calcium fractions increase prior to ovulation, apparently due to the transport of yolk proteins to the ovary as calcium complexes (McDonald and Riddle, 1945). The findings of increases in lipid and protein phosphorus (McDonald and Riddle, 1945) support the idea that transfer of yolk material is occurring. Most of these changes can be induced artifically by administration of oestrogens into immature birds.

1.2.5 Skeleton

Preparation for egg laying is obvious in the skeleton during the pre-laying period. The most significant change is the formation of medullary bone. Medullary bone may form in most parts of the skeleton although it is most clearly seen in bones of the limbs. In fact, however, the ribs contain the highest percentage of medullary bone, 29% (Taylor and Moore, 1953).

The formation of medullary bone begins about two weeks before the onset of egg production. This corresponds with the increased retention of calcium and phosphorus during this period, (Common, 1933). Hurwitz (1964a) showed by the use of a radio isotope calcium - 45 that the mineral retained during the prelaying period is deposited in the bones. But he concluded that medullary bone calcium is derived largely from structural bone, not immediately from the diet.

The bone marrows exhibit intense osteoblastic activity during the pre-laying period (Simkiss, 1967). As the mineralisation of the first eggshell begins, osteoblastic activity changes to osteoclastic activity and medullary bone is resorbed. When eggshell secretion is completed, osteoclastic activity wanes and osteoblastic cells dominate. These changes are particularly noticeable in the pigeon (Bloom, Bloom and McLean, 1941) cited by Simkiss (1967).

The most prominent feature of medullary bone is its lability. Although it acts as a reservoir of calcium and phosphate for use in eggshell formation it only comprises a small part of the total skeleton. Medullary bone has no structural function in the skeletal system.

1.2.6 Formation of Medullary Bone

Medullary bone was first artificially induced by Zondek (1937) cited by Riddle, Rauche and Smith (1945) by injecting oestrogens into the cockerel. It was subsequently discovered that androgens are also necessary for medullary bone formation (Bloom *et al*, 1940; Bloom, McLean and Bloom, 1942, both cited by Taylor, Simkiss and Stringer, 1971). Hypercalcaemia was also shown to be induced by injections of oestrogens (Riddle and Dotti, 1936). The concept that hypercalcaemia and medullary bone formation are aspects of the same physiological mechanism (Clavert, 1942) has now been shown to be false. Medullary bone formation can be induced in birds by using mixtures of oestrogens and androgens in which the concentration of oestrogen is not too high to cause hypercalcaemia. Similarly, in the

absence of androgens, hypercalcaemia can be produced by oestrogens, but no medullary bone will form. Hypercalcaemia is related to formation of yolk proteins and requires only an oestrogenic stimulation, whereas medullary bone formation requires stimulation by both oestrogens and androgens (Simkiss, 1961).

Medullary bone does not occur to the same extent in all bones of the skeleton (Taylor and Moore, 1953). There appears to be a local factor involved in the distribution of medullary bone throughout the skeleton, for injection of sex hormones into male pigeons and drakes causes medullary bone formation at the point of injection (Benoit and Clavert, 1945).

An experiment involving the breaking of a metatarsus of laying and non-laying hens showed that blood supply is also an important factor in distribution of medullary bone. Taylor, Moore and Loosmoore (1958) cited by Simkiss (1967) demonstrated a small amount of medullary bone formation at the site of the metatarsal fracture where normally no medullary bone would be expected.

Thus, sex hormones, blood supply and a local factor are all important for the formation of medullary bone and its distribution throughout the skeleton.

1.2.7 Calcium kinetics during reproduction

The immediate source of calcium for the formation of medullary bone is structural bone of the skeleton, not the diet (Hurwitz, 1964b). Analysis of bones by Taylor and Moore (1954) showed that after hens had laid six eggs while consuming a low calcium diet, 38.4% of their skeletal calcium was removed from the body largely as eggshell. The medullary fraction of these bones was found to be relatively unchanged, but the cortical bone of the ribs, sternum, ilium, ischium, pubis, coccygeal vertebrae and fibula was up to 50% depleted.

Medullary bone did suffer a large fall in calcium/phosphorus ratio (Taylor and Moore, 1954). During eggshell calcification the blood ionic calcium decreases while inorganic phosphate increases (lowered calcium/phosphorus ratio). This similarity between medullary bone and blood suggests that the medullary bone is an extremely labile fraction of the skeleton.

Eggshell calcification places such a heavy demand for calcium on the skeleton that it can only be satisfied by an extremely labile source of calcium. But loss of medullary bone is replenished from cortical bone which becomes depleted if the dietary calcium is inadequate to replete it.

The mobilisation rate of medullary bone is much higher than cortical bone and Hurwitz (1965) calculates that medullary bone can be mobilised at least ten to 15 times faster than cortical bone and at least twice as fast as the bone ends.

1.2.8 Reproductive Organs

Remarkable changes in the size of the reproductive organs occur during the three stages of a reproductive cycle: developing, laying and regressing stages.

(i) Development of the ovary

There are two phases of ovarian growth the first beginning some five to six weeks before the onset of lay when there is an increase in ovarian weight from about 0.4g to about 2.0g (Amin and Gilbert, 1970). This growth is related not to follicular development but to an increase in weight of the cortex and medulla of the ovary. In the second phase, beginning two to three weeks before the onset of lay, rapid follicular development causes ovarian weight to increase to almost 40g. As the follicular hierarchy develops further, the mature ovarian weight of 50 to 60g is reached.

Since the oviduct begins to increase in size at the same time as the first phase of ovarian weight increase, Amin and Gilbert (1970) suggest that ovarian development is probably associated with an increase in ovarian steroid output, probably oestrogen, androgen and progesterone.

The first signs of increased steroid production by the ovary has been associated with an increase in follicle stimulating hormone (FSH) cell number and activity in the anterior pituitary. At this time few luteinising hormone (LH) cells are present. Thus, if the first ovarian activity is associated with FSH output alone, it may be that FSH alone causes only increased steroidogenesis. Both FSH and LH may be required for the development of ovarian follicles. In fact LH cells in the anterior pituitary increase in number just before follicular maturation and reach their peak number at the onset of egg production (Amin and Gilbert, 1970).

(ii) Development of the oviduct

In sexually immature pullets the oviduct is a narrow tube about 14 to 19 cm long. The oviduct of sexually active hens is some 80 cm long with five structurally distinct areas each associated with a specific process during transportation of the ovum from the ovary to the cloaca. Successively from the ovarian end of the oviduct the five regions are infundibulum, magnum, isthmus, uterus or shell gland, vagina.

Up till 16 weeks of age oviduct weight is in the vicinity of 0.2g but after 16 weeks of age an increase in weight is observed (Amin and Gilbert, 1970), reaching 40 to 45g in the laying hen.

Changes in hyperplasia and hypertrophy of the domestic fowl oviduct have recently been extensively researched. Hyperplasia and hypertrophy may briefly be described as increases in cell numbers and wet or dry mass per cell respectively. Yu and Marquardt (1974) found that differential growth patterns in weight, cell numbers and cell mass of the oviduct appear to be primarily associated with changes in protein secretion. Cell numbers and dry mass per cell of the magnum and isthmus increased most rapidly during developing and laying stages and decreased most rapidly during regression. Cell numbers and dry weight per cell of other regions of the oviduct not involved in protein synthesis and secretion did not change as extensively as in the magnum and isthmus.

In the mature oviduct the magnum and isthmus together comprise over half the length (Taylor and Hertelendy, 1960) and weight (Warren and Scott, 1935) of the oviduct. This illustrates the marked effect that protein synthesis has on the size of the oviduct.

Development of the oviduct is under the influence of sex hormones (Brant and Nalbandov, 1956) produced in the concurrently developing ovary.

1.2.9 Early Egg Production

Wide variation in the age at first egg causes all flock records of egg production and egg weight to be physiologically misrepresentative (Nijveld, 1968, cited by Hurwitz and Bar, 1971). Flock averages of egg production actually represent the ratio of laying to non laying pullets (Nijveld, 1968). In fact, individual pullets begin to lay immediately at a maximum rate followed by a slow decline (Hurwitz and Bar, 1971). Flock averages indicate an increase to a production peak and then a slow decline. The physiological picture for egg weight and eggshell weight shows

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large increases over the first 15 to 20 eggs reaching a peak at this stage (Hurwitz and Bar, 1971).

1.3 PHYSIOLOGY OF THE HEN DURING REPRODUCTIVE INACTIVITY

The ovary of a mature hen contains some 2500 oocytes visible to the naked eye and about 12000 visible with the aid of a microscope (Pearl and Schoppe, 1921, cited by Gilbert, 1971). Although only a few of these reach a stage of follicular growth and ovulation, the hen contains the necessary ova to lay for several years; much longer than is normally allowed.

The domestic fowl has the capacity for involution of its reproductive organs and this occurs naturally in response to shortening daylength at the end of the laying season. Moulting is the accompanying physiological phenomenon of loss and replacement of feathers.

To utilise the hen's potential egg laying ability a moult may be enforced by a number of methods, more commonly food and water starvation after which egg production ceases and involution of the reproductive organs follows. By the time feather loss and replacement is completed the hens are ready for a second laying period. The key to force moulting is a successful method of controlling egg production.

The withdrawl of the drug methallibure and legislation in Britain forbidding wilful water and food starvation of poultry combined with the inadequacy of other methods of force moulting have spurred research into the use of calcium restriction to control egg production. Low calcium diets have been considered a very promising alternative method of stopping egg production and a number of experiments have been conducted to examine reproductive pauses caused by calcium restriction (eg. Gilbert, 1973; Roland et al., 1973).

Although the effect of calcium restriction on egg production is clear, research of other physiological characteristics during reproductive pause periods has been inappreciable. A physiological discussion of reproductive inactivity in the hen is difficult because so little data is available but what is available can be compared with information about force moulted hens.

1.3.1 Involution of the Reproductive Organs

A conventional force moult causes involution of the reproductive organs but whether or not this occurs during reproductive pauses induced by calcium restriction appears to be a contentious issue. Nevalainen (1969) found that after four weeks of calcium restriction (0.13% calcium diet) there were marked decreases in ovary and oviduct weights. Urist (1959) suggests that ovarian regression is due to the failure to form calcium complexes in the liver for transportation of lipid and protein to the ovarian follicles. Nevalainen (1969) considers atrophy of the oviduct to be due to decreased oestrogen secretion resulting from lowered gonadotrophin stimulation during calcium deficiency, since exogenous oestrogen is capable of increasing oviduct weights in calcium deprived birds.

Not all hens consuming low calcium diets undergo involution of their reproductive organs. Douglas, Harms and Wilson (1972) fed 0.09% calcium diet to laying hens but regression of ovary and oviduct did not result. Upon necropsy it was found that the majority of hens had been internal layers. If involution occurs, regression of the reproductive organs appears to be a simple reversal of their development.

Ovarian regression is considered to involve follicular atresia and resorption of yolk material of the developing ova. A completely regressed or resting ovary of an adult hen weighs about 6.0 g (Romanoff and Romanoff, 1949) which is the weight of the medulla and cortex of a mature ovary (Amin and Gilbert, 1970). In other words, regression is the reversal of the follicular growth phase of ovarian development.

Yu and Marquardt (1974) studied hyperplastic and hypertrophic changes in the oviduct and found decreases in both cell numbers and dry weight per cell of the magnum and isthmus. They concluded that this resulted from the decreased protein synthesis of these oviduct regions. A regressed oviduct weighs some 4 to 6g and is 15 to 17 cm in length (Romanoff and Romanoff, 1949).

1.3.2 Body Weight

No significant differences were found by Nevalainen (1969) or Douglas, Harms and Wilson (1972) between body weights of calcium restricted and non calcium restricted hens. Using an even lower dietary calcium concentration (0.05%) than the above researchers, Roland et al., (1973) observed a significant reduction in body weight due to calcium restriction. Body weight changes during a forced moult are expected to decrease particularly if feed and water starvation is used to induce the moult.

1.3.3 Food Consumption

Roland et al., (1973) showed that calcium restriction caused reduced feed consumption. With 24 hours after the beginning of calcium restriction, food consumption decreased by about 30% and remained at the lowered level until calcium restrictions were lifted.

1.3.4 Egg Characteristics

In the second year the rate of egg production of force moulted hens never peaks as high as it did in the pullet year but it should reach 75 to 85% of that level. The decline in egg production in the second year is more rapid than in the first year so a shorter laying period is to be expected from force moulted flocks. Egg production following a reproductive pause caused by calcium restriction appears to continue at the level which would be expected if no calcium restriction had taken place (Gilbert, 1969),

although Douglas, Harms and Wilson (1972) and Mehring and Titus (1964) found that calcium restricted hens returned to a level of production 10% higher than control hens following the termination of calcium restriction.

Egg size of force moulted hens increases with increasing length of time in lay. The size of eggs at the beginning of the second period of egg production is the same as at the end of the first period of egg production. Since egg size is dependent on age, reproductive pauses are unlikely to have any effects on it. Gilbert (1972) came to this conclusion from observations of hens coming into lay after long periods of calcium restriction.

Egg quality traits improve after force moulting. They are better than when laying ceased and normally reach the same level as at the middle of the pullet year. But with the exception of egg size, the decline in egg quality is often more rapid than in the pullet year. Egg quality following a reproductive pause caused by calcium restriction has not been researched as far as is known.

If the use of low calcium diets is to gain approval as a tool for controlling egg production, the changes in production characteristics of the fowl will have to be at least as good as under more conventional methods of force moulting.

1.4 CONTROL OF CALCIUM METABOLISM

1.4.1 Parathyroid Hormone

The level of calcium in the blood is under the control of parathyroid hormone or parathormone (PTH). Calcitonin may counteract PTH's control over blood calcium but no proof of this has been documented for poultry.

Chickens respond to bovine parathyroid extracts (PTE) with a peak hypercalcaemia reached quickly and lasting a short time. Intravenous injections of PTE caused a significant rise in total

plasma calcium after 30 minutes, peaking after 1½ hours and returning to pre-injection levels in 2½ hours (Hertelendy, 1962 cited by Taylor, 1971). Candlish and Taylor (1970) report that an even more rapid response of plasma diffusible calcium to PTE was measured in ten minutes. The level of response plateaued and lasted for at least 15 minutes.

The nature of the hypercalcaemic response in birds has recently been studied by Kenny and Dacke (1974). Intravenous administration of PTH into Japanese quail and chicken caused complex hypercalcaemic responses. At least two underlying mechanisms were involved. It was suggested that the initial phase lasting 30 minutes or less resulted from the inhibition of bone accretion. The later phase was interpreted as being largely due to bone resorption.

Although the hypercalcaemic response to a standard dose of PTE is greater in the laying hen than the cock (Polin, Sturkie and Hunsaker, 1957; Polin and Sturkie, 1957, 1958), the rise in diffusible calcium is of the same magnitude in laying hens as in cocks (Urist et al., 1960). Taylor (1971) presumes that the discrepancy is due to the plasma lipophosphoproteins binding additional calcium as the ionic calcium or diffusible calcium rises.

There are three generally accepted effects of PTH in mammals:

1. Increases the rate of bone resorption
2. Increases urinary excretion of phosphate
3. Exerts a small increase on intestinal absorption of calcium.

Taylor (1971) assumes that in birds, PTH acts similarly although there is no evidence for this.

1.4.2 Calcitonin

The ultimobranchial glands of the fowl are the source of calcitonin (Copp, Cockcroft and Kueh, 1967), although MacIntyre (1967) cited by Simkiss and Dacke (1971) and Moseley et al., (1968) consider that calcitonin may be found in trace amounts in the thyroids. Low

and Brown (1968) cited by Simkiss and Dacke (1971) demonstrated fluctuation in the concentration of calcitonin in the blood in response to the calcium content of the diet. Five to six fold increases in secretion rates of calcitonin have been demonstrated in the hypercalcaemic fowl (Zeigler, Delling and Pfeiffer, 1970 cited by Simkiss and Dacke, 1971).

Removal of the ultimobranchial glands has no effect on plasma calcium but there is a decline in the ability of operated birds to compensate for injections of PTH. Plasma calcium remains elevated for much longer periods of time in these birds compared with sham-operated birds (Brown et al., 1969). Simkiss and Dacke (1971) suggest that the effect of calcitonin may be to prevent overshooting in the parathyroid regulation of plasma calcium levels. An important function of calcitonin may be to protect the skeleton from excessive resorption.

1.4.3 Bone Resorption

There are three types of bone cells responsible for mineral deposition and resorption from the bones. The osteoblasts are responsible for depositing new bone mineral while the osteoclasts resorb existing bone. The osteocytes which are embedded in lacunae within the bone mass have the ability to perform both functions of deposition and resorption (Belanger et al., 1963). Surface mineral of medullary and cortical bone is resorbed by osteoclasts in a process termed osteoclasis. Osteolysis is the resorption of bone mineral from within the bone tissue by the osteocytes.

1.4.4 Two Theories on Control of Bone Resorption

There are two opposing theories to explain the control of bone resorption.

1. Parathyroid Theory

As shell calcification begins the plasma ionic calcium falls. Hodges (1969) demonstrated a fall in plasma

calcium of 25 to 30% after four hours of shell calcification. After 10 to 12 hours at about the same level, plasma calcium rose again to around 20 mg/100 ml by the end of shell calcification. These measurements were made on shell gland venous blood.

The fall in blood calcium causes an increase in the secretion of PTH which stimulates resorption of bone mineral. At the end of shell calcification, plasma calcium rises, PTH output declines and the rate of bone resorption is reduced. Taylor (1971) admits that at present there is no real evidence for this theory but looks forward to a sensitive assay to measure blood PTH during egg laying.

2. Oestrogen Withdrawal Theory

Riddle, Rauche and Smith (1945) first advanced the theory that after ovulation, the level of circulating oestrogen falls and this withdrawal leads to bone resorption. The theory has many defenders (eg. Urist, 1967) but has been criticised by Taylor (1965). The evidence for this theory is almost non-existent, but it is easy to extrapolate from the fact that medullary bone is formed under the influence of oestrogen and androgen to the converse that is the destruction of medullary bone occurs when oestrogen is withdrawn.

But it is not only medullary bone which may be mobilised for eggshell calcification. When low calcium diets are fed the medullary bone is maintained while the amount of cortical bone decreases as the number of eggs laid increases (Taylor and Moore, 1954).

Taylor (1971) explains that medullary bone is more sensitive to small changes in PTH than is cortical bone and therefore under normal egglaying conditions and

adequate calcium dietary regime when only small elevations in PTH are made during shell calcification the response is from medullary bone only. But when large elevations of PTH occur as would happen during dietary calcium restriction, bone resorption occurs in both cortical and medullary bone.

The development of assays able to detect changes in blood plasma of oestrogen and PTH will provide some of the necessary evidence to support one or other of these theories.

1.5 ABSORPTION OF CALCIUM

Calcium absorption in the hen occurs almost exclusively in the duodenum and jejunum. Although the rate of calcium absorption is greater in the duodenum, the jejunum is the more important site of calcium absorption because the rate of passage of digesta is greater in this region (Hurwitz and Bar, 1966).

Wasserman and Taylor (1966) described a vitamin D dependent calcium binding protein (CaBP) in the intestinal mucosa of chicks, laying hens and rats. They suggested that CaBP may be involved in the transport of calcium across the intestinal wall. An anonymous author (1974) has briefly reviewed research into properties of vitamin D - dependent CaBP. Chick intestinal CaBP has a molecular weight of about 28,000 and is comprised solely of 242 amino acids. Calcium is bound to the CaBP at four sites.

Hurwitz and Bar (1969b) have demonstrated that calcium is absorbed down an electrochemical gradient in the duodenum and jejunum of laying hens when the dietary calcium concentration ranged from 0.6% to 3.9%. In the chick, absorption of calcium down the electrochemical gradient occurred when diets containing 0.71% and 1.11% calcium were consumed but when dietary calcium was 0.31% calcium absorption proceeded against the electrochemical gradient. Hurwitz and Bar (1969b) concluded that these results suggest that

in some situations active transport of calcium may be important. CaBP was the first macromolecular constituent of the intestine associated with intestinal calcium transport shown to respond to changes in dietary calcium (Morrissey and Wasserman, 1971), suggesting that CaBP may be involved in the vitamin D dependent calcium absorptive process and also with the process of adaptation (Nicolaysen, Eeg-Larsen and Malm, 1953).

Bar and Hurwitz (1972) observed an increase in CaBP at the onset of egg production to a level which was maintained as long as egg production continued. They also found that a long term calcium restriction (16 to 32 days) resulted in an elevation of CaBP. Changes in CaBinding activity were not associated with short term depletion periods or diurnal fluctuation (during shell formation) which lead to the conclusion that only some of the regulatory changes in the laying fowl could be explained on the basis of changes in the CaBP level.

These findings support earlier work by Nicolaysen, Eeg-Larsen and Malm (1953). They were first to recognise the capacity of the intestine to adapt to varying dietary concentrations of calcium by altering the absorptivity of the intestine. When the dietary calcium content is low, intestinal absorption increases and when it is high, intestinal absorption decreases.

Vitamin D administration to rachitic chicks produced an increase in the concentration of CaBP in the intestinal mucosa (Taylor and Wasserman, 1970). Melancon and De Luca (1970) suggested that mucosal brush border calcium dependent adenine triphosphatase (CaATPase) might be related to the adaption process, but although Taylor and Wasserman (1970) were able to produce changes in CaATPase after administration of vitamin D, no significant increase in the enzyme occurred during adaption to a low calcium diet.

Work by MacGregor, Hamilton and Cohn (1970) supports an hypothesis that vitamin D_3 works via protein synthesis. They

present evidence to show that the appearance of CaBP following vitamin D administration is due to de novo protein biosynthesis. This is supported by observations that the CaBP activity in the laying hen increases under long term dietary restriction (Bar and Hurwitz, 1972, 1973) and is highly and positively correlated with duodenal CaBP concentration.

A key to the understanding of calcium absorption and adaptation centres upon the unknown factor which recognises the calcium status of the diet and responds by adjusting the efficiency of absorption. Of the three sites which are more obvious, intestine, parathyroid and skeleton, Morrissey and Wasserman (1971) dismiss the first two but suggest that because a high inverse correlation ($r = -0.94$) was observed between skeletal mineralisation and rate of calcium absorption, a physiological relationship between the two parameters exists. This and the work by Bar and Hurwitz (1972, 1973) mentioned earlier, supports the hypothesis put forward by Nicolaysen, Eeg-Larsen and Malm (1953) that the adaptive process is dependent on the degree of mineralisation of the skeleton.

1.6 REGULATORY ROLE OF CALCIUM IN EGG LAYING

Taylor, Morris and Hertelendy (1962) proposed a theory which has been called the "pituitary cut-off" mechanism. It proposes that the decrease in egg production shown by hens fed low calcium diets is caused by depressed secretion of gonadotrophins from the anterior pituitary. Taylor (1965) suggests that the inhibition of FSH and LH release is mediated by the hypothalamic response to the fall in plasma calcium.

Roland et al., (1974) observed increased amounts of secretory material in adenohypophyses of calcium deficient hens which indicated that hormones were produced but not released. Hypothalamic extracts were demonstrated to be able to stimulate the reproductive system of calcium deficient hens so the conclusion that calcium is involved in the production or secretion of releasing factors from the hypothalamus was drawn.

Reduced secretion of gonadotrophins would lower the rate of growth of ovarian follicles and depress the production of the steroid hormone, oestrogen. Since yolk synthesis in the liver takes place under oestrogenic stimulus, a decreased synthesis of yolk protein would result and eventually egg production would decline. Oviduct regression may also follow since development and maintenance of this organ is under the control of sex hormones.

Another area where calcium may exert a regulatory role in egg laying involves transportation of yolk material to the ovary. Phosvitin, the characteristic protein of egg yolk is a phosphoprotein with great affinity for calcium. It is synthesised in the liver under influence of oestrogen and is transported (in combination with lipid material) to developing ovarian follicles. In the blood, phosvitin avidly binds calcium . An equilibrium exists between ionic and non diffusible calcium in the blood of the normal laying hen but in calcium deficiency the blood calcium available for phosphoproteins to bind is decreased.

Interference with yolk deposition does occur when low calcium diets are fed. Roland, Sloan and Harms (1972) first reported the increased incidence of yolk mottling and decreased yolk calcium in eggs laid by calcium deficient hens. It was hypothesised by McCready, Roland and Fry (1973) that calcium may be related to the development of yolk mottling and to vitelline membrane permeability. They suggest that reduced yolk protein and calcium values observed in eggs from calcium deficient hens may result from reduced or altered protein - lipoprotein transport to, or deposition in the yolk. Hens fed a calcium deficient diet produced egg yolks with altered lipoprotein composition when compared to control yolks, and slightly different vitelline membrane composition (McCready and Roland, 1973).

Phosphoproteins may bind some other cation in the blood when calcium is not available which could hinder the ability of the phosphoprotein to pass through the vitelline membrane which,

although disrupted in composition causing greater permeability, will not allow the passage of the phosphoprotein. The ability to selectively transport molecules across the membrane may be lost with the disruption caused by calcium restriction. As a result normally "unwanted" molecules may be deposited in the yolk.

It appears from the literature that the thyroid glands are involved in feather renewal and involution of reproductive organs during a moult. Two processes seem to be involved. Firstly a depressed thyroid hormone secretion can initiate a decline in the reproductive performance (Blivaiss, 1947), and secondly the same secretion rate can inhibit normal feather regeneration (Falconer, 1971).

Thyrotrophin releasing factor (TRF) is a hypothalamic neurosecretion responsible for the stimulation of Thyroid Stimulating Hormone (TSH) from the anterior pituitary. Falconer (1971) suggests that the thyroid changes associated with reproduction are mediated through hypothalamic regulation of TSH release from the pituitary.

A means of thyroid control over ovary regression can be suggested. The possibility exists that lowered plasma calcium may cause a depressed neurosecretion of TRF which in turn would depress TSH production by the pituitary. In this way thyroid hormone secretion would decrease with subsequent effects on reproductive rate. A severe reduction of thyroid hormone secretion may initiate ovarian regression.

1.7 CALCIUM NUTRITION BEFORE THE ONSET OF LAY

Mature male chickens have a daily calcium turnover of approximately 8 mg/kg body weight (Norris *et al.*, 1972). For a 2.5 kg white leghorn cockerel this amounts to a requirement of 20 mg calcium per day. Extrapolating to the female sex, a corresponding amount of calcium is expected to be required for maintenance only of adult hens in which reproductive activity is

completely absent. The reproductive activity of adult female hens causes a very much increased requirement for calcium.

Gilbert (1973) fed diets containing very low levels of calcium (mean calcium content was 0.05 ± 0.003%) to laying stock. Egg production declined rapidly and some hens stopped laying completely. One group of hens remained on the low calcium diet 39 weeks without symptoms of calcium deficiency. If a food intake of 100g is assumed, daily calcium ingestion for these hens would have been 0.05g or 50 mg which is 2.5 times the maintenance requirement suggested by Norris et al., (1972). While diets containing levels of calcium considered to be as low as possible using normal poultry feed ingredients have serious effects on egg production, they are more than adequate for maintenance alone. It is difficult to physiologically separate maintenance and production requirements in reality, but it is important to appreciate the wide difference in requirements for maintenance and production in adult hens.

The recommended daily nutrient requirements of calcium presented by the National Research Council (1960) rise with body weight from 0.25g/day at a body weight of 0.5 lb to 0.85g/day at 3.0 lb. When the daily food intakes of growing chicks are considered, the dietary calcium requirements are calculated to be 0.98% to 1.0% throughout the growing period.

Scott, Nesheim and Young (1969) recommend 1.0% calcium in the diet of chicks up till eight weeks of age and 0.6% calcium in the diet of growing chicks from eight to 20 weeks of age. Berg, Bearse and Merrill (1964) suggest that 0.4% calcium in the diet is adequate for growing pullets. Although calcium requirements are in fact lower than 1.0% calcium, most commercial growing rations contain that proportion of calcium.

During the pre-laying period, retention of 1g calcium/day was thought to be a maximum (Morgan and Mitchell, 1938). More recently Hurwitz and Griminger (1961a) report a calcium retention of 1.83g calcium/day for laying hens. Although these are not

pre-laying hens it demonstrates that a calcium retention rate in the hen can be much higher than originally thought. The extent to which the rate of retention of pre-laying pullets may be raised is not known but a diet of 1.0% calcium would not allow a rate of greater than 1.0g calcium/day. This calculation assumes a daily intake of 100g diet and 100% retention from the diet which is unlikely. For a calcium retention of greater than 1g/day there would have to be either a larger food intake or an increased calcium content of the diet.

Hurwitz and Bar (1971) consider mineral requirements during the pre-laying period to be higher than the commonly used 1.0 - 1.2% calcium and 0.7% phosphate diets allow. But large increases in the calcium content of the diet during the growing phase has been shown by Shane, Young and Krook (1969) to cause renal degeneration and mortality. They also suggested that a high calcium diet could cause hypoparathyroidism, (seemingly of a permanent nature) thus depressing the ability of the hen to produce parathyroid hormone (PTH) for mobilisation of skeletal calcium reserves.

Shane, Young and Krook (1969) fed a high calcium diet (3.0% Ca) from eight to 22 weeks of age which caused an increased level of mortality between 12 and 21 weeks of age or up till the expected beginning of the pre-laying period. Hurwitz and Bar (1971) observed no detrimental effects of feeding high calcium diet (4.1% Ca) from 16 weeks of age. From these two reports it may be hypothesised that the danger period for feeding high calcium diets occurs some time prior to 16 weeks of age. The work of Shane, Young and Krook (1969) does not indicate the age at which a high calcium diet no longer causes disruptive changes in the kidney. The earliest age at which a high calcium diet can be introduced is not known.

It is not detrimental to increase the calcium content of the diet to laying diet levels during the pre-laying period and it may be of some advantage for later egg production. Hurwitz

and Bar (1969a) proposed the theory that the ability of the hen to withstand the challenge of calcium deprivation is a function of the size of the hen's calcium reserves and therefore the greater the accumulation of skeletal calcium in the pre-laying period the better for subsequent egg production.

The size of pre-laying mineral stores was shown to be able to be manipulated by dietary mineral levels (Common, 1938; Hurwitz, 1964). Berg, Bearse and Miller (1947) concluded that the level of pre-laying dietary calcium had no statistical effect on egg production and shell quality, although a trend towards increase in shell thickness was apparent in a re-evaluation of the data (Hurwitz and Bar, 1971). No significant effects on shell quality were found by Hurwitz and Bar (1971) in their pre-laying mineral nutrition experiment. However, their data also showed a trend towards improved shell quality with high calcium feeding in the pre-laying period. They conclude that increased dietary calcium levels during the pre-laying period may be of limited benefit.

1.8 CALCIUM NUTRITION AFTER THE ONSET OF LAY

Following the onset of egg production, mineral nutrition influences productivity of the hen and the ability of the skeleton to maintain an adequate supply of calcium for egg shell formation. During the early production period the hen is in negative calcium balance (Morgan and Mitchell, 1938) which cannot be alleviated by high dietary calcium levels (Hurwitz and Griminger, 1960). During this period large quantities of skeletal calcium are required to supplement dietary calcium for eggshell production.

Meyer, Babcock and Sunde (1971) showed that pullets store calcium in the skeleton at a rapid rate for about three weeks reaching a peak the day before oviposition of the first egg. Mineral mass became depleted on the day of the first oviposition and a negative calcium balance continued for about three weeks. When a positive calcium balance was regained, a slow increase in

mineral reserves in the skeleton was observed. The level of dietary calcium during the growing period does influence the bone mineral mass in the subsequent laying period. Meyer, Babcock and Sunde (1971) fed 0.4, 0.7, 0.9, 1.2, 1.5% calcium from eight to 21 weeks of age after which a 0.4% calcium diet plus ad lib. oyster shell was fed. The bone mass of pullets was monitored by the method of Meyer, Babcock and Sunde (1968) and the results show that the new fed 1.5% calcium diet had the highest bone mass from before the onset of lay till after 40 weeks of egg production had passed.

A negative calcium balance lasting two to three weeks appears to be unavoidable. Pullets fed diets containing levels of calcium up to 3.54% were unable to avert negative calcium balance during the first few weeks of production (Griminger, 1961 cited in Meyer, Babcock and Sunde, 1971). Hurwitz and Griminger (1960) observed negative calcium balance after feeding 1.85% and 2.70% calcium diets to pre-laying pullets.

To obtain maximum productivity in a flock the introduction of a laying ration should be made not later than seven to 10 days after the first egg from the flock has appeared (Smith, 1973). This time may be delayed if there is a surplus amount of grower ration to be used up by providing oyster shell or granular limestone for those hens already ovulating. The mineral fraction of a laying ration must provide adequate calcium for maximum egg production. In effect it is necessary to provide enough calcium for the formation of one eggshell per hen per day.

It is generally understood that shell quality continues to improve as calcium intake increases but it is likely that there is an upper limit to the improvement gained from increases in calcium intake. Production depression has been observed when hens are fed 5.0% calcium laying ration (Hinners, Gholson and Ritchason, 1963; Scott, Hull and Mullenhoff, 1971). General effects of high calcium intakes have been reviewed by Davis (1959). High levels of calcium may depress the utilisation of other nutrients.

1.9 LOW CALCIUM DIETS

1.9.1 Low Calcium Diets fed during the pre-laying period

In many cases transition from growing diet to laying diet takes place after or towards the end of the pre-laying period. Within a flock, the more precocious hens receive 1% dietary calcium (growing diet) throughout the pre-laying period whereas the latest maturing pullets may receive 4% dietary calcium (laying diet) during their pre-laying period. Hurwitz and Bar (1971) fed pre-laying diets containing 4.1% and 1.3% calcium and found no significantly different effects on total bone calcium contents at any time during lay. However, increased dietary calcium and phosphorus tended to increase bone calcium during the pre-laying period (Hurwitz and Bar, 1971).

It is expected that a pre-laying diet containing an extremely low level of calcium (about 0.05%) would not allow extensive mineralisation as is normally the case. The extent of medullary bone formation during calcium restriction at this time is unknown. If medullary bone is formed at all it will be at the expense of cortical bone. This may during the pre-laying period, cause calcium deficiency symptoms such as leg paralysis. While no such effects may be noticeable the situation is potentially dangerous particularly when egg production begins.

At this point a rapid depletion may cause further loss of mineral from the cortical bone, resulting in calcium deficiency syndrome. On the other hand, egg production may cease almost immediately in an attempt to avoid further skeletal depletion, perhaps thereby avoiding calcium deficiency syndrome.

The use of a low calcium diet may be a useful tool for controlling egg production. Gilbert (1973) delayed the onset of full egg production until 23, 27, 31, 35 and 55 weeks. A low calcium diet (mean calcium content : 0.05 $\pm$ 0.003%) was fed from the age of 16 weeks until the above ages whereupon a normal layers ration was fed. He found that despite feeding a very low calcium diet, sexual development progressed apparently as normal.

All the birds came into lay at the same time but hens fed the low calcium diet laid no more than three eggs then production dropped to almost zero. When the normal laying ration was introduced egg production rose rapidly.

Gilbert (1973) found also that egg size was associated with chronological age, not with reproductive age. However, the first one or two eggs laid after delayed egg production tended to be small. Although no close analysis of eggsize was made in this experiment, eggsize appears to display the same change with egg number as that shown by Hurwitz and Bar (1971) at the normal onset of lay. It might therefore be concluded that delayed onset of full production involves nothing more complex than a time shift in the physiological status of the hen.

1.9.2 Low Calcium Diets fed during the production period

Feeding a low calcium diet during the production period has an immediate effect on egg production. Gilbert (1973) fed low calcium diets to hens just after peak production at 27 weeks of age. Egg production declined and reached almost zero after two weeks but resumed rapidly when the adequate calcium laying diet was reintroduced. Similar periods forcing an almost complete cessation of egg production were begun later in the production year at 47 weeks and again at 67 weeks of age. The same effects were shown. Mortality was not increased by low calcium treatments in Gilbert's (1973) experiments although skeletal defects or leg weaknesses were found in about 2% of the birds which had calcium restriction just after peak production. However, the condition corrected itself within 24 hours.

Eggshell calcification demands large quantities of calcium but when eggs are not being produced the hen's requirement for calcium is very low. When a thread is surgically placed in the uterus and soft-shelled eggs only are produced, egg laying can be maintained for a considerable period of time (about nine weeks) on only a 0.03% calcium diet (Gilbert, 1969). This helps to point

out the fact that very low calcium diets are not necessarily detrimental to the health of the hen as long as no eggshells are being produced. But the combination of a low calcium diet and continued production of eggs with shells is potentially dangerous, as the strength of the skeleton may be impaired.

1.9.3 Eggs laid during calcium restriction

Eggs produced by hens fed a calcium restricted diet become progressively thinner (Deobald et al., 1936; Tyler, 1945, 1946; Taylor and Moore, 1954; Donovan et al., 1960). Tyler (1945, 1946) reported that eggs continued to be laid until the thickness of the shells was about 60% of the normal value and then laying ceased. Taylor and Moore (1954) showed the decrease in shell calcium content becomes progressively greater with each successive egg laid when low calcium diets were fed. Egg production of hens fed calcium restricted diets will cease before shell-less eggs are produced (Deobald et al., 1936). Mehring and Titus (1964) found that shell-less eggs were not produced when a 0.20% calcium diet was fed. Recently, it has been shown that shell-less eggs are produced by hens consuming a low calcium diet (Roland et al., 1973).

1.9.4 Other ways to control egg production

A recent report shows that egg production can be controlled by feeding diets containing a low level of sodium (Whitehead and Shannon, 1974). It is suggested that egg production can be controlled by adjusting the dietary content of any nutrient for which the maintenance requirement is much less than the production requirement.

Several methods of inducing a forced moult have been used in the past. They all control egg production. Thorstensen (1969) reviews experimental methods of force moulting such as administration of progesterone, enheptin (2-amino-5-nitrothiazole), methallibure as well as the more practical methods which utilise lighting and nutritional restrictions or both.

CHAPTER TWO

Rationale for trial L45

There have been several reports in the literature of feeding low calcium diets (eg. Mehring and Titus, 1964; Eoff et al., 1962; Donovan et al., 1960; and others). But the level of calcium in the low calcium diets ranged from 0.45% to 1.5%. The feeding of such diets caused drops in egg production to the order of 60 to 70%. In some cases, skeletal defects became apparent after laying albeit at a low rate while consuming a low calcium diet (Mehring and Titus, 1964). Gilbert (1972) concluded from results of trials such as those mentioned above that if the aim was to inhibit egg production by the manipulation of dietary calcium content, then dietary levels of calcium would have to be much lower than those used previously. Rations containing calcium ranging from 0.02 to 0.06% were fed to hens before the onset of lay and at later stages in the productive year (Gilbert, 1972, 1973). As has already been discussed in a previous section, very severe and rapid drops in egg production were observed.

The experimental work carried out at the Poultry Research Centre is modelled on the work of Hurwitz and Bar (1971) and Gilbert (1973). As did Hurwitz and Bar (1971), measurements of body weight, food consumption, egg weight, shell weight were made but these parameters were measured following delayed onset of full egg production and after enforced egg production pauses which were shown by Gilbert (1973) to be produced by calcium restriction. Some interest was expressed by Gilbert (1973) that the use of extremely low calcium diets may be shown to be a method to control egg production. It may prove to be a useful method for enforcing "reproductive rests" instead of starvation induced forced moulting, particularly if it only affects egg production and not other physiological phenomena such as feather loss.

It appears that the onset of normal egg production can be delayed indefinitely by the use of extremely low calcium diets (Gilbert, 1973). The ability to delay the onset of full production could be a desirable management tool in certain situations. By delaying the onset of full production in a flock of pullets, a synchronisation of egg production may be possible when a laying diet is introduced. Hurwitz and Bar (1971) suggest the need for "re-evaluation of the dietary requirements at the onset of egg production." Such a synchronisation as mentioned above may allow better nutritional regimes at this period.

Circumstances which often arise on many poultry farms could be remedied by the use of low calcium diets. One such situation occurs if a flock of pullets are due to begin laying but their laying shed accommodation is not yet available. These birds could be fed a low calcium diet to hold them out of production, thereby avoiding many eggs laid on the floor in the rearing quarters. When the facilities in the laying shed allow the flock to be shifted there, the diet should be changed to a laying diet which would induce full egg production.

This experiment, L45, sets out firstly to confirm the effect of low calcium diets on egg production. Low calcium diets have been used to delay the onset of lay and later on in the laying year to enforce periods of decreased reproductive activity ("reproductive rests" or "production pauses") just after peak egg production and again towards the end of the laying year.

Few reports have appeared in the literature of the effects of extremely low calcium diets such as those used by Gilbert (1973) on body weight and food consumption of the hens. But characteristics such as egg weight and shell weight of the eggs produced following a period of enforced egg production pause have not been measured. These measurements were made in trial L45 at the following three stages in the laying year.

1. Following normal and delayed onset of lay in pullets

2. Following an enforced egg production pause in the middle of the production year

3. Following an enforced egg production pause towards the end of the laying year.

Particular interest was focused upon the production of eggshell at these times.

The objectives of trial L45 may be summarised as

1. To determine the nature of physiological changes that occur around the onset of lay and following enforced egg production pauses

2. To study egg shell production after the natural onset of lay, after a delayed onset of lay and following enforced egg production pauses.

CHAPTER THREE

Materials and methods

3.1 GENERAL

120 M-line pullets were randomly chosen and placed in single cages in the PRC cage laying shed which is semi-environmentally controlled. Water was provided by a nipple system and feed was provided in individual feeders which could easily be removed for weighing.

3.2 FEEDING

All rations were mixed by the university's Feed Processing Centre. Four rations were used:

Ration A	-	PRC grower ration
Ration B	-	Low calcium grower ration
Ration C	-	Adequate calcium layer ration
Ration D	-	Low calcium layer ration

Ration formulae are presented in Appendix IV.

3.3 EXPERIMENTAL DESIGN

The experiment consisted of three phases dealing with:

1. The onset of lay and delayed onset,
2. First enforced egg production pause,
3. Second enforced egg production pause.

3.3.1 Phase 1

In the first phase of the experiment, four treatments were imposed.

Treatment 1 (T1)

Normal grower ration up to 21 weeks of age followed by an adequate calcium layer ration. This was the Control treatment.

Treatment 2 (T2)

Normal grower ration up till age at first egg followed by an adequate calcium layer ration. The dietary change was made for each individual hen as soon as the first egg was laid.

Treatment 3 (T3)

Normal grower ration till 21 weeks of age, low calcium grower ration from 21 - 26 weeks of age followed by an adequate calcium layer ration.

Treatment 4 (T4)

Normal grower ration till 21 weeks of age, low calcium "layer" ration from 21 - 26 weeks of age followed by an adequate calcium layer ration.

Fig. 1 gives a schematic representation of Phase 1 treatments.

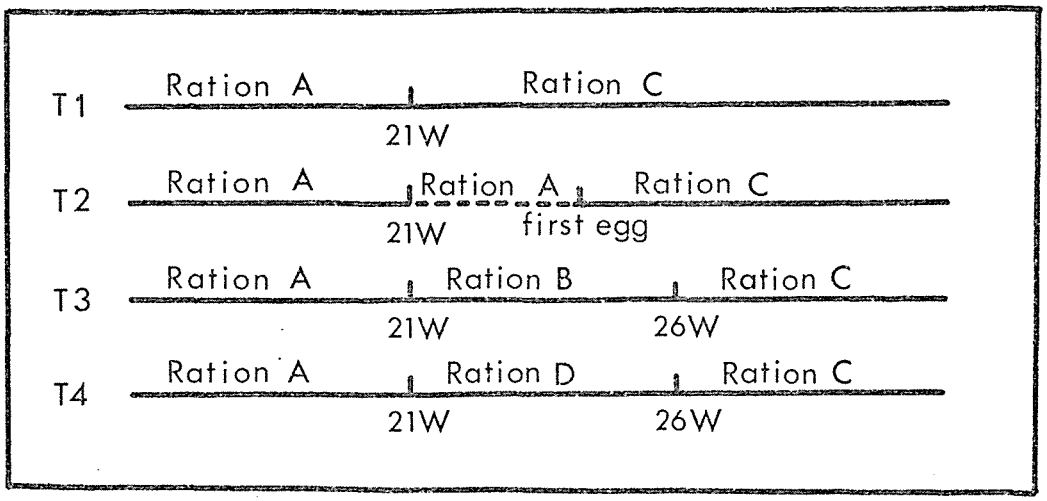


Fig.1 Phase 1 treatments.

At the conclusion of phase 1, a period of rest was allowed during which all hens were fed the adequate calcium laying diet (C). No experimental activity took place during this period of approximately one month's duration.

In order to remove any interdependence of treatments in phase 1 and phase 2, birds in T2, T3 and T4 in phase 1 were assigned as evenly as possible to phase 2 treatments 2, 3 and 4. (eg. of 30 hens in T2, phase 1, ten were assigned to T2, phase 2, ten were assigned to T3, phase 2 and ten were assigned to T4, phase 2) This rearrangement took place immediately phase 1 was completed so that there was a one month period to recover from the stress of shifting. T1 birds were not shifted, and they acted as an overall control group throughout the entire experiment.

3.3.2 Phase 2

In phase 2 egg production pauses were forced upon two groups, commencing when the hens were 38 weeks of age. There were four treatments:

Treatment 1 (T1)
Overall control, received adequate calcium layer diet throughout.

Treatment 2 (T2)
Second control group received adequate calcium layer ration throughout.

Treatment 3 (T3)
Low calcium layer ration fed for three weeks followed by adequate calcium layer ration.

Treatment 4 (T4)
Low calcium layer ration fed for five weeks followed by adequate calcium layer ration.

Fig. 2 gives a schematic representation of phase 2 treatments.

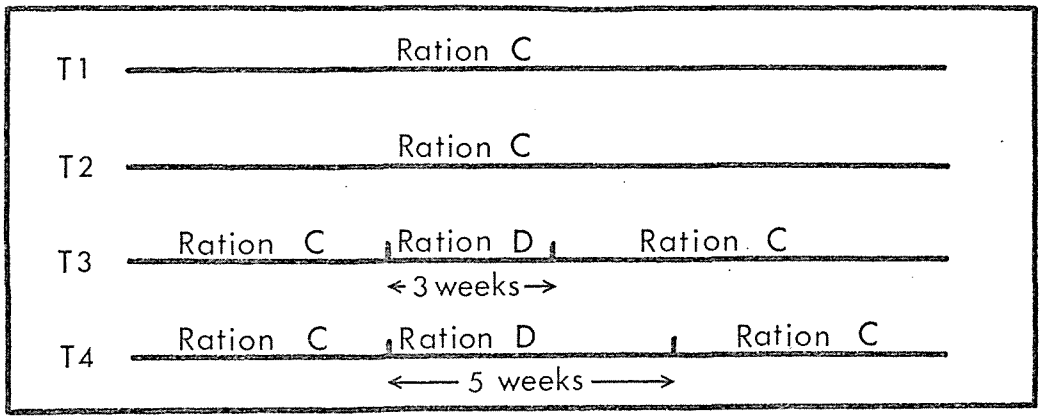


Fig. 2 Phase 2 treatments.

Immediately following the conclusion of phase 2 a rearrangement of birds in T2, T3 and T4 was undertaken in a similar fashion as at the end of phase 1 already described. Another rest period of about one month was allowed and no experimental activity took place during this period.

3.3.3 Phase 3

Phase 3 is a repetition of phase 2 except that it occurred at a later stage in the laying year commencing when the birds were 55 weeks of age. A slight difference between phase 3 and phase 2 was the duration of low calcium layer ration feeding. In phase 3, T3 hens received ration D for two weeks and T4 hens received ration D for five weeks. Otherwise, phase 3 and phase 2 were the same.

3.4 MEASUREMENTS

3.4.1 Body Weight

Initially all birds were weighed individually twice a week but half way through the second phase of the experiment the frequency of weighing was reduced to once per week. A set of "Salter" scales accurate to $\frac{1}{4}$ ounze was used for weighing all birds. Imperial weights were later converted to metric.

3.4.2 Food Consumption

Each individual feeder was weighed to the nearest gram on a Mettler Balance once per week and food consumption was calculated from the loss of weight of the feeder.

3.4.3 Egg Production, egg weight and shell weight

Daily egg production was recorded for each hen while experimentation was in progress. During periods of inactivity between experimental phases, eggs were counted so that the total number of eggs could be determined for the year.

Egg measurements involved weighing eggs then separating the shells from the egg contents. After carefully breaking the egg, the shells were washed in warm water to remove adhering albumen. All shells were dried overnight before weighing the next day. All egg weights and shell weights were measured to the nearest one hundredth of a gram using a Mettler Balance. Egg measurements were carried out on an individual hen basis. The first 25 eggs laid after calcium restriction were considered sufficient to show any differences attributable to low calcium feeding.

In phase 1 egg measurements were made for all 25 eggs for all treatments but because this presented such a time consuming daily routine, egg measurements were made on only some of the eggs in phases 2 and 3. Phase 2 and phase 3 eggs were collected for measurement four times a week for calcium restricted hens and only once a week for non calcium restricted hens.

3.5 STATISTICAL ANALYSIS

The time of reintroduction of adequate calcium layer diet was not the same for calcium restricted hens in T3 and T4 in phases 2 and 3. The time difference amounted to two weeks in phase 2 and three weeks in phase 3. It is not statistically correct to compare these two treatments with each other, nor is it correct physiologically since the ages at which measurements are made

following reintroduction of adequate calcium diet are different. To avoid age effects in phases 2 and 3, data from the low calcium treatments are separately compared with data from the control hens taken at the appropriate age.

In phase 1, age again exerts an effect since the onset of full egg production is delayed by two weeks. However, age effects are ignored in the analysis of phase 1 data because it is an aim of this experiment to compare egg parameters after the natural onset of full production with those after delayed onset of full production. Comparisons between T3 and T4 are legitimate in phase 1 since age does not affect this comparison.

Analysis of variance was performed on each of the first 25 eggs laid during the period of measurement in phase 1. For example an analysis of variance was carried out on the weight of the first eggs laid by all hens after their onset of full production. Similarly, the second, third and so forth up to the twentyfifth egg.

With much less data on eggs collected in phases 2 and 3, pooling of egg measurement data was thought necessary. Egg data were pooled in groups of five with an analysis of variance being performed on the pooled means. Plot size was 10 hens in phases 2 and 3 compared with one hen in phase 1.

In phase 1 body weight and food consumption measurements were grouped according to the physiological age of the pullets. The day on which a hen laid its first egg was set as day zero. Body weight and food consumption data were then manipulated for that hen so that weekly estimations were available for the weeks prior to and following the first egg. This was done for each hen with the aid of a computer. This manipulation was not necessary in phases 2 and 3.

Analysis of variance was used for weekly body weight and food consumption the same as for egg parameters in phase 1.

All other statistical tests are also analyses of variance. The 5% level of probability was chosen as the level at which the null hypothesis would either be accepted or rejected. Treatments were compared by the use of the Mean Significant Difference test.

Mention should be made of treatment comparisons involving T1. All comparisons with T1 are legitimate in phase 1 but this is not the case in phases 2 and 3. In the latter two phases there are two control treatments (1 and 2). T1 may be called an "overall" control treatment since it does not change during the entire experiment and hens in T1 receive only the control diet. T2 however is made up from a selection of hens from T2, T3 and T4 in the previous phase. This was done in order to remove the effects of the previous phase calcium restriction on the hens. For this reason, in phases 2 and 3, the true control treatment is T2. T1 was retained throughout so that some reference to non restricted birds could be available throughout the whole experiment.

3.6 COMPUTER PROGRAMS

Computer programs were written using the PL/I language for manipulation of data and statistical analysis. Calculations of surface area of eggs, shell weight per unit surface area and pooling of data from individual hens was incorporated into the programs. Since the onset of lay occurred at varying times in phase 1 all body weight and food consumption data had to be adjusted into weekly estimations from the day of the first oviposition for each hen.

Following the correct data manipulation, analysis of variance was performed. By using the computer an input of raw data could be completely processed and statistically analysed in a very short time.

CHAPTER FOUR

Results

4.1 THE ONSET OF LAY

While no statistical differences in the age at first egg were shown there was a slight tendency to later onset of lay in hens fed calcium restricted diets (Table 1).

	TREATMENT			
	1	2	3	4
Average age at first egg (days)	175.5	175.1	178.4	177.0

Table 1 Average age at first oviposition.

4.2 EGGS LAID DURING CALCIUM RESTRICTION

Feeding low calcium diets did not delay the onset of sexual maturity characterised by the laying of the first egg but it did depress subsequent egg production. Hens in T3 laid up to five eggs but generally about three eggs while consuming the low calcium diet and hens in T4 laid up to seven eggs but generally about four eggs before ceasing to lay. Some hens in both treatments laid no eggs while consuming low calcium diet. Table 2 shows the total number of eggs laid while low calcium diet was being consumed. If the number of eggs laid are divided by the appropriate number of hens in the treatment, averages of eggs per hen may be obtained.

	Phase 1		Phase 2		Phase 3	
	T3	T4	T3	T4	T3	T4
No. of eggs	66	74	180	196	134	184
No. of hens	29	28	27	24	28	26
Eggs/ hen	2.2	2.5	6.7	7.8	4.8	7.1
Length of calcium restriction (days)	*	*	21	35	14	35
Eggs/ hen/ day	*	*	0.32	0.22	0.34	0.20

Table 2 Eggs laid by hens fed low calcium diets.

* These values were not calculated because pullets came into lay at different days therefore the length of calcium restriction is variable.

In phases 2 and 3 similar averages can be calculated, but there was also a standard length of calcium restriction period for hens within each treatment so the eggs laid can be expressed in terms of the number of hens and the number of days of calcium restriction. Values calculated for the number of eggs laid per hen per day of calcium restriction show that in both phases 2 and 3, T3 hens laid one third of an egg per day or rather one egg every three days, whereas T4 hens laid approximately one fifth of an egg per day or rather one egg every five days.

The percentage of all eggs produced during calcium restriction which were shell-less have been calculated for both low calcium treatments, T3 and T4 in all three experimental phases. All groups laid 6 - 7% shell-less eggs while consuming low calcium diets except for T4 in phase 1 which only produced 2.7% of its eggs without a shell. This is less than half of all other groups and so far as can be seen, no obvious explanation why this should occur exists.

	% Shell-less Eggs			
Treatment	1	2	3	4
Phase 1	1.1	0	7.6	2.7
Phase 2	0	0.1	6.1	6.1
Phase 3	0.8	0.6	5.9	7.0

Table 3 Percentage of shell-less eggs laid by hens fed restricted calcium diets.

A similar calculation was made for eggs from the control groups where many fewer shell-less eggs were produced. The percentage of shell-less eggs of total eggs at this time ranged from 0% to 1.1%. These results suggest that under conditions of calcium restriction, some shell-less eggs may be produced. However, shell-less egg production by calcium restricted hens did not differ significally from that of non calcium restricted hens.

4.3 CALCIUM LOSS OF HENS FED LOW CALCIUM DIETS

The cumulative loss of calcium from T3 and T4 hens during calcium restriction is tabulated in Table 4. Appendix I contains details of calculations of calcium loss.

Egg	Cumulative Calcium Loss (g)					
	Phase 1		Phase 2		Phase 3	
	T3	T4	T3	T4	T3	T4
1	0.97	1.25	1.66	1.71	1.63	1.82
2	1.73	2.28	3.11	3.20	3.16	3.50
3	2.45	3.10	4.55	4.48	4.51	4.77
4	3.16	3.94	5.87	5.64	5.61	6.17
5	3.83	4.71	6.93	6.85	6.84	7.40
6		5.57	7.99	8.01	8.04	8.64
7		6.17	8.75	8.70	8.87	9.59
8			9.62	9.50	9.68	10.63
9			10.30	10.19		11.47
10				11.17		13.00

Table 4 Cumulative calcium loss of hens fed low calcium diets.

Since the total body calcium of a normal laying hen is to the order of 20 grams, calcium depletion such as has been observed in this experiment could lead to severe calcium deficiency problems. Some hens did experience paralysis as a consequence of depletion.

4.4 THE SKELETON

No objective measurements were made of the state of the skeletal system during calcium restriction but the legs of four hens which died during or following calcium restriction were dissected from the cadavers and preserved by deep freezing. While there was no analysis of the skeletal system, some of the femora

taken from the preserved leg samples were thawed, cleared of flesh, cut lengthways with a small fretsaw and ether extracted using a Soxhlet apparatus. Bone marrow was removed mechanically with a large needle. This exposed the remaining medullary bone. A typical bone from the calcium depleted bones was compared photographically to a bone from a normal laying hen. There are three important points to note about these bones (see plate I).

1. Medullary bone still exists in the calcium depleted bone

2. The cortical bone is much thinner in the depleted femur compared with the non-depleted bone

3. The proximal end of the depleted bone is structurally damaged. This was noticed in the femora of all calcium depleted hens dissected. The ball and socket joint of the hip was generally damaged, particularly the ball at the head of the femur which was often pitted. Its delicate nature was very obvious when dissecting since it was very easily broken off.

4.5 CALCIUM DEFICIENCY SYNDROME

No hens suffered three successive "bouts" of calcium deficiency syndrome and only one hen survived two successive "bouts" of calcium deficiency syndrome. Details of the incidence of calcium deficiency syndrome and mortality arising from it are shown in Table 5.

As well as leg paralysis in some birds the loss of a few feathers occurred.

The rubbing of the body over cage floor wires when hens are sitting down during calcium deficiency may have contributed to this feather loss.

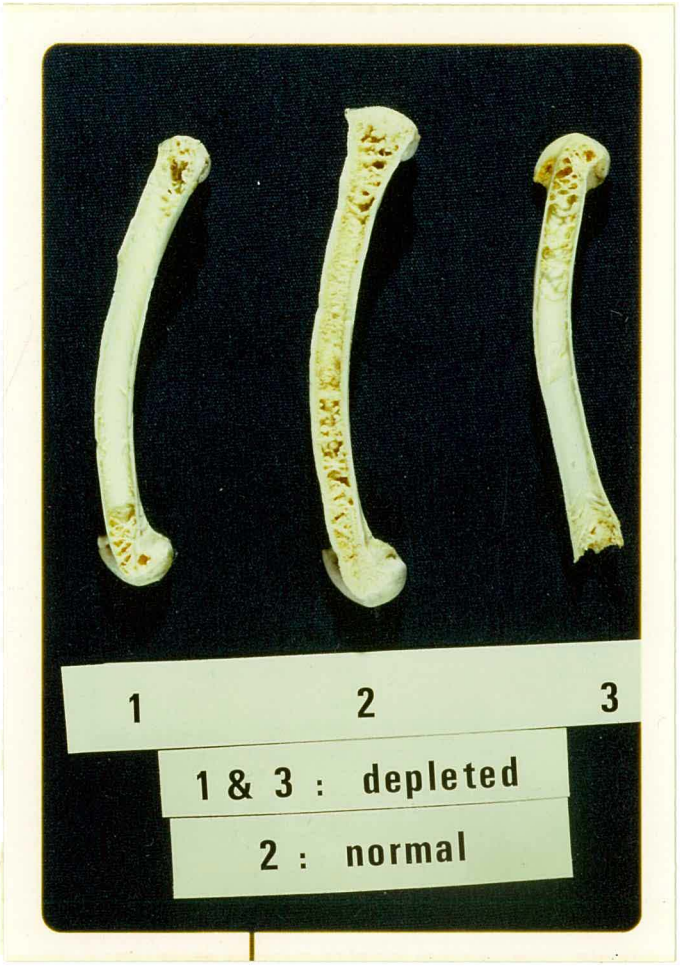


PLATE I Depletion of bone mineral in femora of calcium restricted hens.

	NUMBER OF HENS		
	Phase 1	Phase 2	Phase 3
Hens with CDS *	5	15	11
Hens dead due to CDS	0	4	0
Hens with CDS in the previous phase		2	6
Hens with CDS in the previous phase which had CDS in the present phase		2	1
Hens dying from second bout of CDS		2	0

* CDS Calcium deficiency syndrome

Table 5 Incidence of calcium deficiency syndrome during calcium restriction

4.6 PHASE 1 EGG PARAMETERS

4.6.1 Egg Production

Egg laying was not inhibited by calcium restriction but the rate of egg production was reduced markedly. Egg production of T3 and T4 hens reached almost 20% by the end of calcium restriction. Rates of egg production of calcium restricted or delayed hens and non calcium restricted hens were significantly different during the last week of calcium restriction and for one week after the dietary changeover from low calcium diets to adequate calcium layer diet at 26 weeks of age (fig. 3).

By the second week after dietary changeover egg production of the calcium restricted hens had reached a level not significantly different from non calcium restricted hens. Five

weeks after the dietary changeover from low calcium to adequate calcium diets, an isolated significant egg production difference occurred. T4 egg production was significantly less than all other treatments at this time. Although no other significant results have been revealed, egg production of calcium restricted hens was on average lower than that of non calcium restricted hens throughout phase 1.

4.6.2 Egg Weight

Significantly heavier eggs were produced by T3 and T4 hens compared to T1 and T2 hens. This by no means is the case for all 25 eggs measured but it did occur. T4 hens laid more eggs (five) which were significantly heavier than T1 or T2 eggs than did T3 hens (three).

This implies that T4 hens laid heavier eggs than T3 hens and although this is not shown statistically it is borne out visually on the graph of egg weight (fig. 4).

The differences between calcium restricted and non calcium restricted hens were expected since the onset of full egg production in T3 and T4 hens occurred three weeks later than in T1 and T2 hens. This means that their first eggs would be larger since egg size is dependent upon age, not egg number.

4.6.3 Shell Weight

From the 14th egg there were frequent and consistent significant treatment differences. Eggshells produced by calcium restricted hens in T3 and T4 were significantly different from eggshells produced by T1 and T2 hens. As well, T3 and T4 hens produced significantly different amounts of eggshell. Even T1 and T2 hens produce five eggshells of significantly different weights (fig. 5).

The superiority of T3 and T4 eggshells may be attributed to the fact that T3 and T4 eggs were significantly heavier than T1 and T2 eggs.

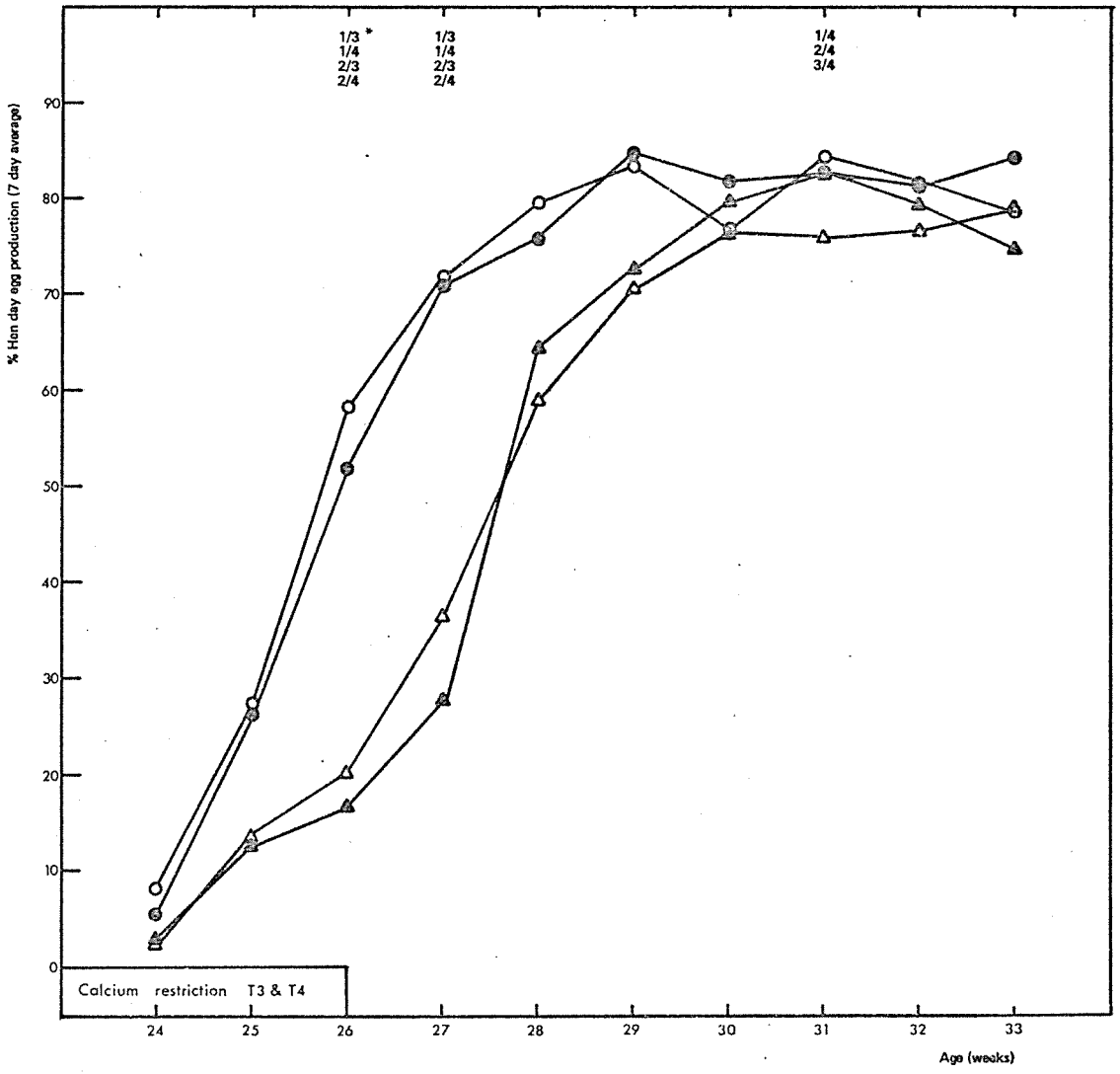


Fig. 3 Egg production in phase 1. Treatment 1 Treatment 3 Treatment 2 Treatment 4

* Statistically significant treatment differences

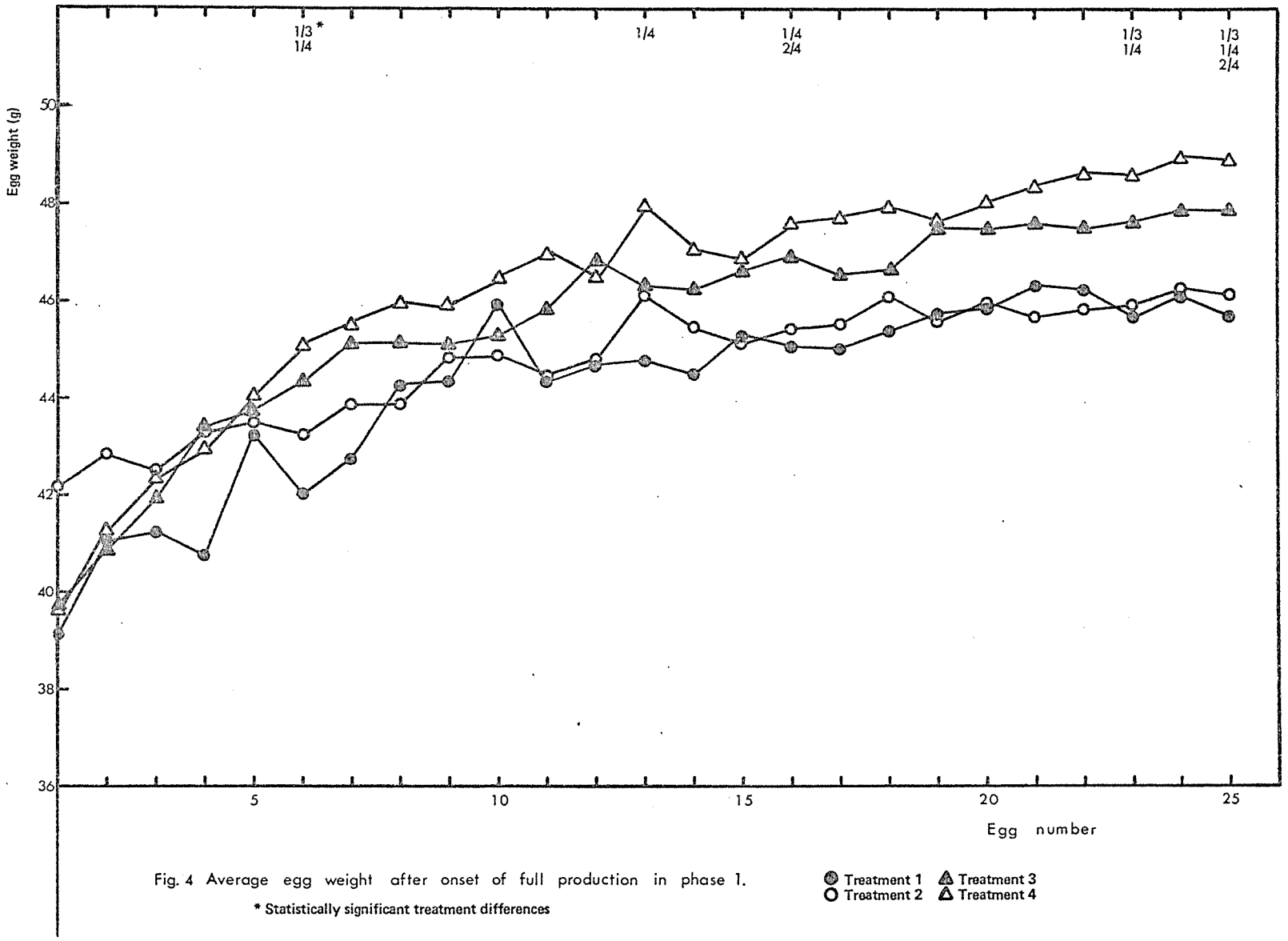


Fig. 4 Average egg weight after onset of full production in phase 1.

* Statistically significant treatment differences

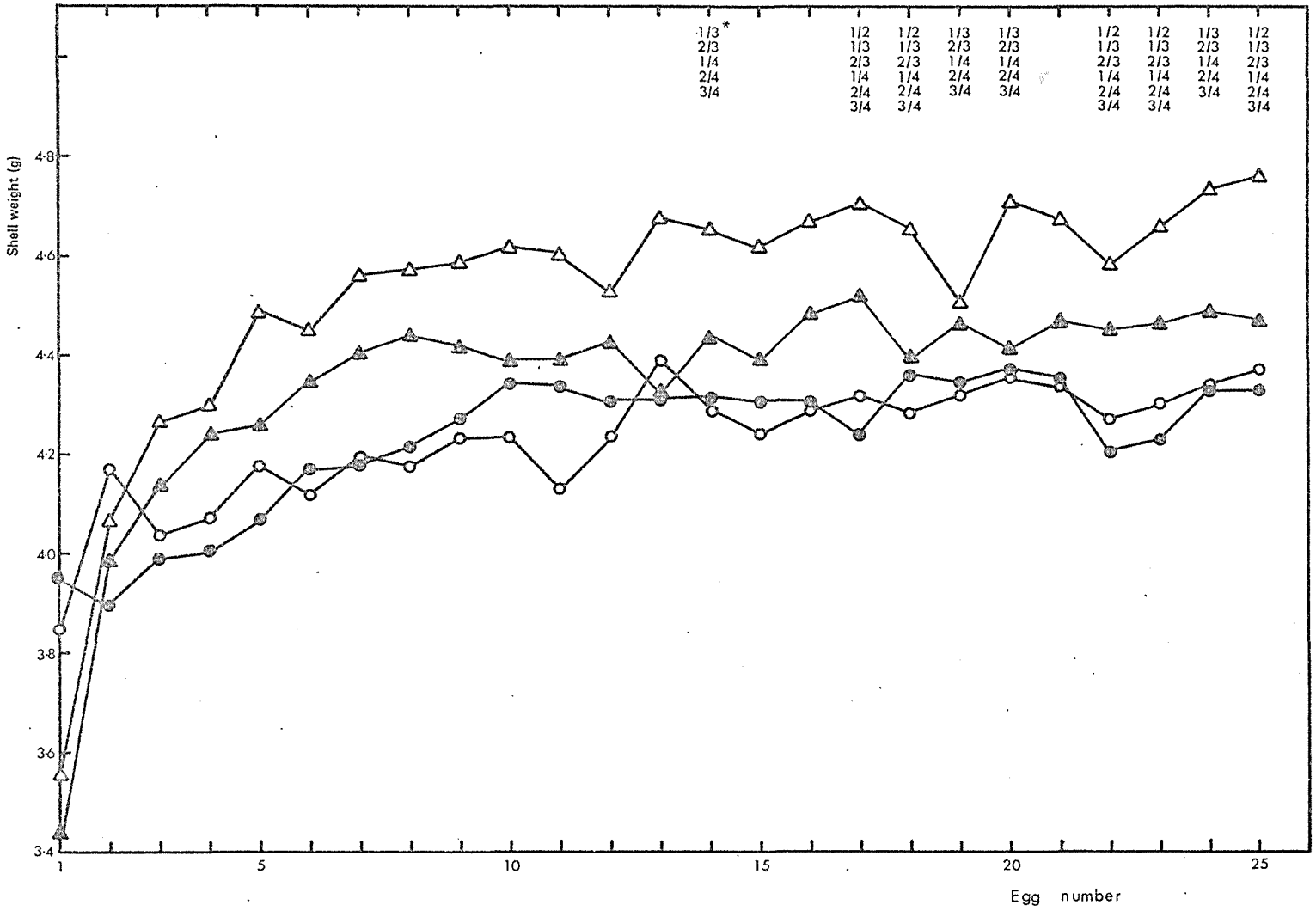


Fig. 5 Average shell weight after onset of full production in phase 1.

* Statistically significant treatment differences

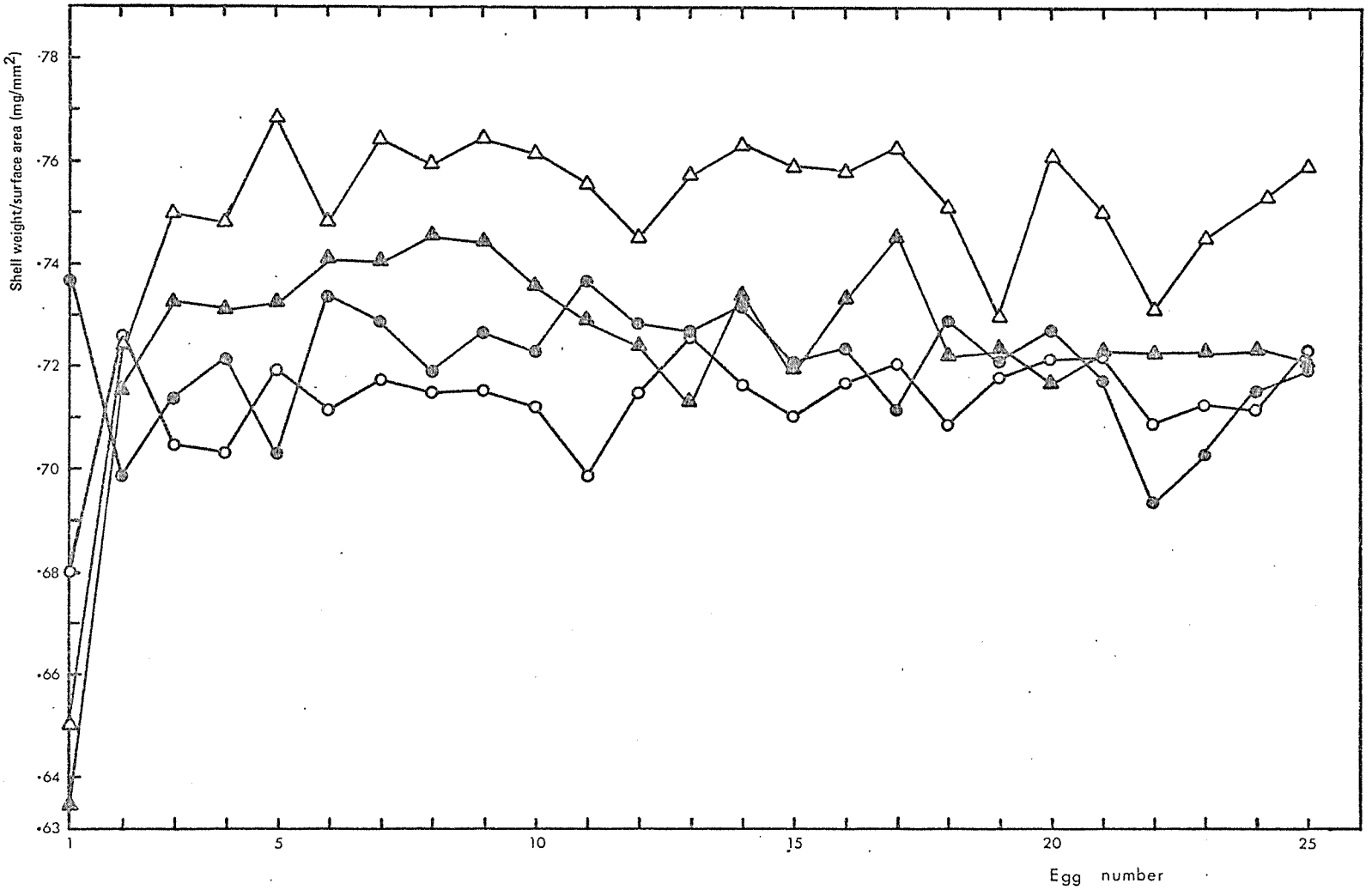


Fig. 6 Average shell quality (expressed as shell weight per unit surface are of egg) after onset of full production in phase 1. Treatment 1 Treatment 3 Treatment 2 Treatment 4

4.6.4 Shell Weight per Surface Area

T4 hens appeared to lay eggs with the best shell quality as measured by shell weight per unit surface area of egg. However no statistically significant differences between treatments were shown (fig. 6). A significant treatment by block interaction was seen for some eggs.

Little difference between T1, T2 and T3 was apparent although T3 hens seem to have produced slightly better quality eggshells.

4.7 PHASE 1 BIRD PARAMETERS

4.7.1 Body Weight

Two to four weeks before the onset of lay a very rapid increase in body weight was observed. The increase of some 400g may have been as much as a 30% body weight increase. Calcium restriction has no significant effect on the pre-laying body weight surge. As soon as the first egg was laid, body weight gain decelerated (fig. 7a).

As the hens fed low calcium diets continued producing eggs, body weight decreased. This continued for two weeks in T4 but in T3 the decline was interrupted by an inexplicable peak during the second week after the first oviposition. The average body weight loss by T4 hens was 40g.

When egg production of calcium restricted hens reached its lowest rate, body weight began to increase again. Following the lifting of calcium restrictions by changing to adequate calcium layer diet, there was a rapid return to egg production and body weight continued to rise at approximately the same rate as was observed in control treatments. Six to eight weeks after oviposition of the first egg, body weights of the hens which had been calcium restricted were still below that of the control hens although significant differences disappeared after the sixth week following first oviposition.

Body weight loss appears to have been greater in T4 hens than T3 hens but the differences were not significant at any time.

4.7.2 Food Consumption

Food consumption results (fig. 7b) show changes similar to body weight results.

The control treatment T1 had a dietary change well before the onset of lay so that by the time the first egg was laid, there was no drop in food consumption although the rate of increase of food consumption slowed down. In contrast to this, hens in T2 received a change in dietary regime at the time of laying their first egg. Some food intake depression occurred at this time, but after a week it followed the same slight rise as occurred in T1. However, T2 food consumption remained at a level of about 50g lower than T1 hens through the next six weeks at least but the difference was not significant.

With the change from a grower ration to the low calcium ration (whether grower or layer) there was no apparent drop in food consumption. Instead food intake continued to increase. When the first egg was laid food consumption decreased significantly by 80 to 100g in one to two weeks in T3 and T4. Two weeks after the onset of lay (when egg production had ceased in T3 and T4) there was a rapid rise in food intake so that by four weeks after the oviposition of the first egg, food intake was back to the same level as for hens in T1 and T2.

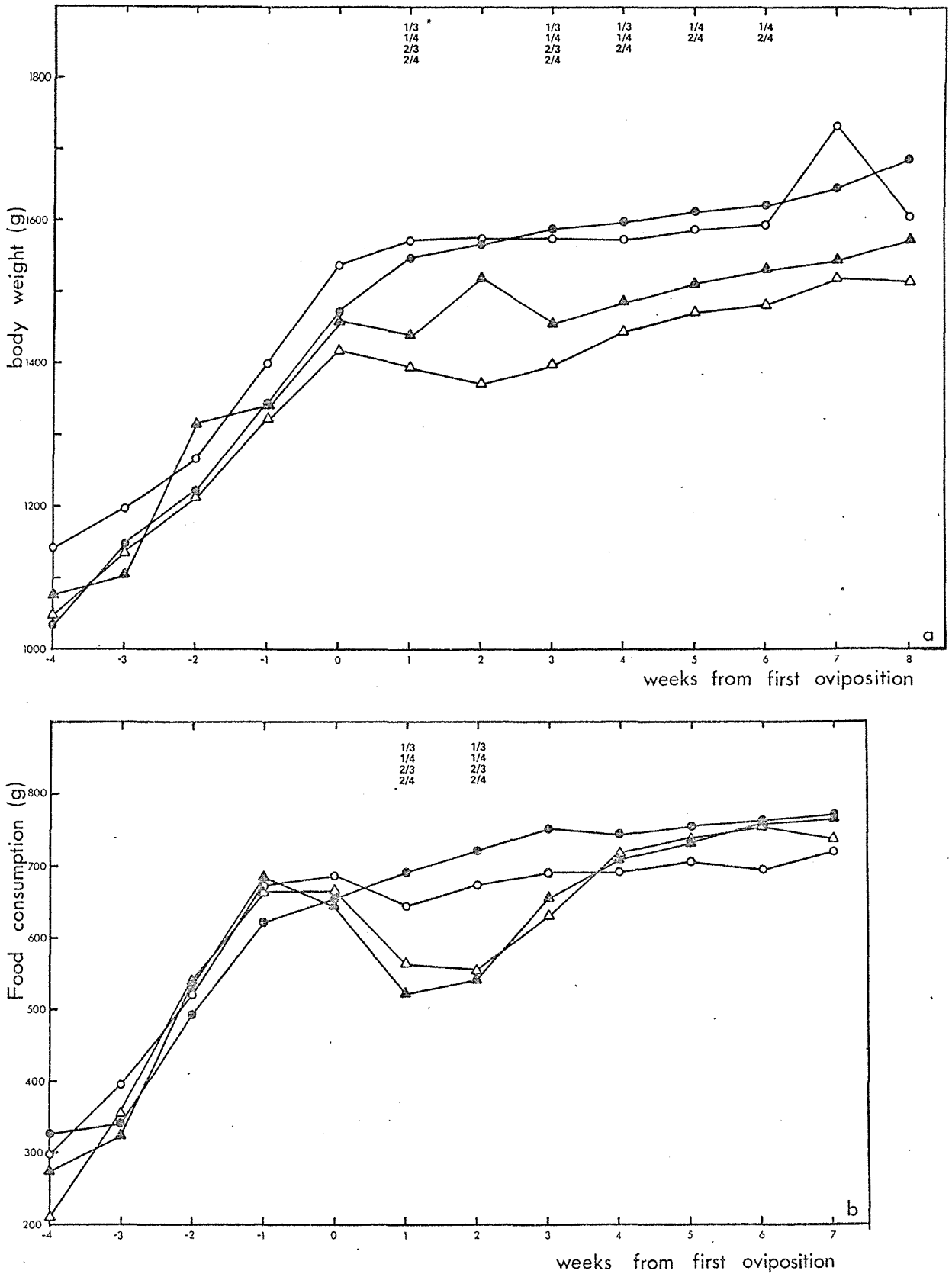


Fig. 7 Body weight and food consumption during phase 1.

Treatment 1 Treatment 3
Treatment 2 Treatment 4

* Statistically significant treatment differences

4.8 PHASE 2 EGG PARAMETERS

4.8.1 Egg Production

One week after the imposition of a low calcium diet egg production dropped significantly. The decline continued until adequate calcium diets were reintroduced whereupon a rapid increase in egg production was observed. In both calcium restricted treatments, egg production had reached a level not significantly different from non calcium restricted hens by three weeks after the reintroduction of the adequate calcium layer diet. Subsequently, no significant egg production differences were evident between calcium restricted and non calcium restricted hens (fig. 8).

4.8.2 Egg Weight, Shell Weight, Shell Weight per Surface Area

Mean egg weight, shell weight and shell weight per surface area of the 11th to 15th eggs produced by T1, T2 and T3 hens following reintroduction of adequate calcium laying diet were significantly different (fig. 9 a, b, c). The reason appears graphically to have been a decline in egg weight in T2 and only a very slight increase in egg weight in T1 in this egg group, rather than obvious superiority of T3 egg weight. This difference was reflected in eggshell weights and shell weight per surface area, hence the significant differences in these two parameters in this egg group.

The T4 comparisons with T1 and T2 revealed significant differences only in shell weight per surface area of the last two egg groups where T1 had a significantly lower shell weight per surface area than did T2 or T3 eggs (fig. 9 d, e, f). Again this was apparently the inferiority of T1 eggs rather than superiority of T3 eggs because no significant differences existed between T3 and T2 eggs.

It would be statistically improper to claim superiority of calcium restricted hens over non calcium restricted hens in terms of egg and shell producing abilities. But it would be unwise not

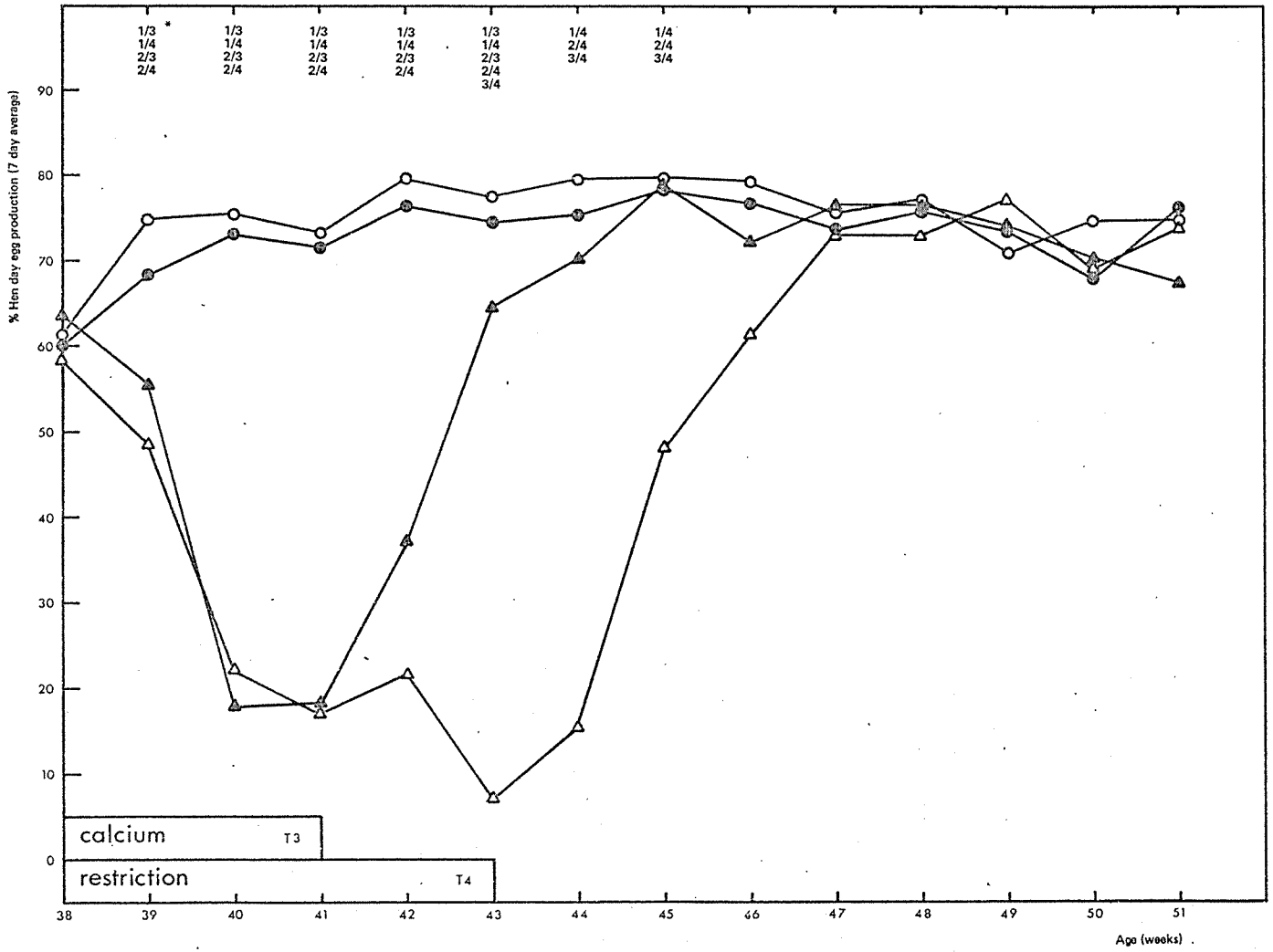


Fig. 8 Egg production in phase 2.

Treatment 1 Treatment 3
Treatment 2 Treatment 4

* Statistically significant treatment differences

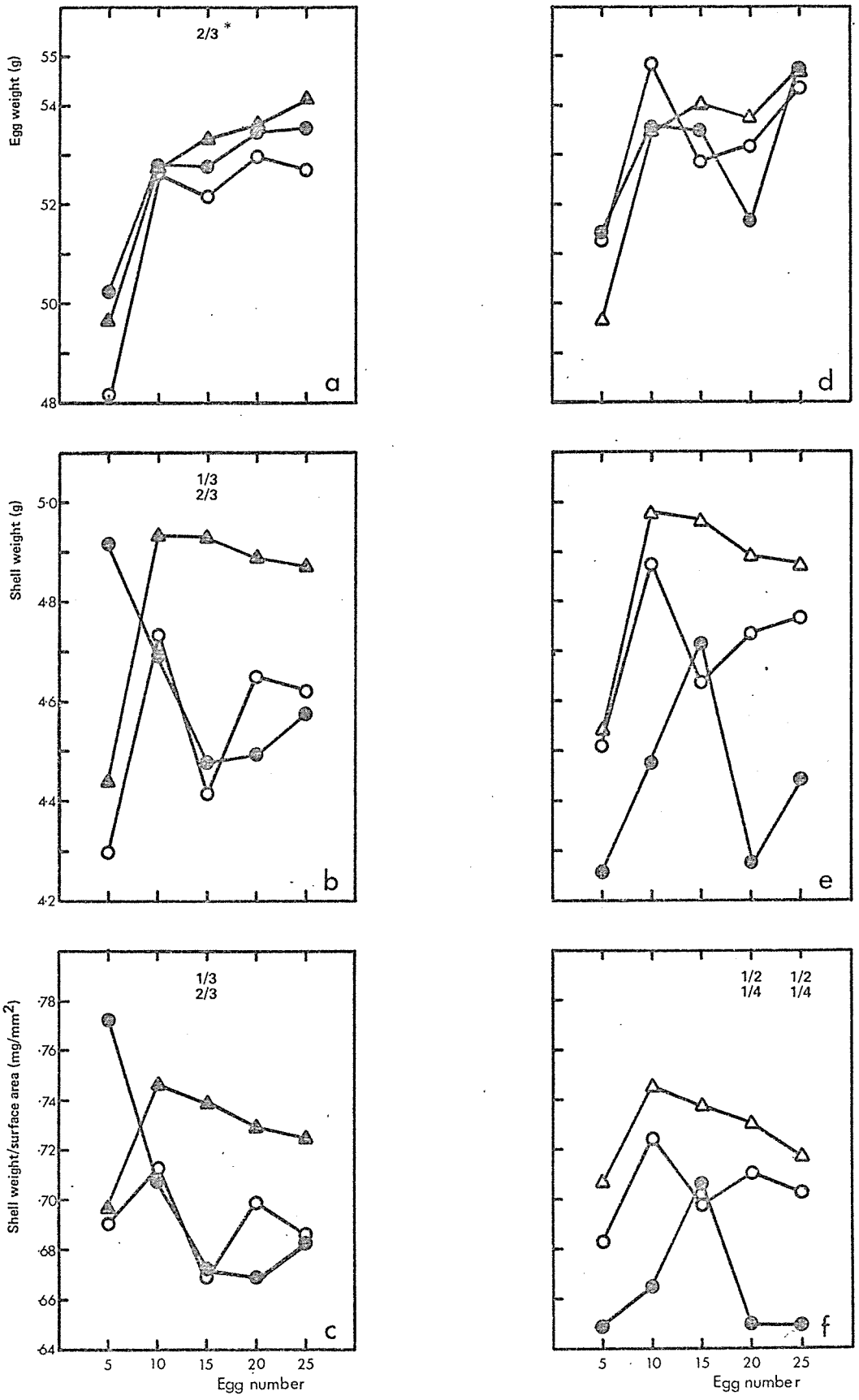


Fig. 9 Average egg weight, shell weight, shell quality (expressed as shell weight per unit surface area of egg) after reintroduction of adequate calcium laying diet in phase 2.

* Statistically significant treatment differences

Treatment 1 Treatment 3
Treatment 2 Treatment 4

to acknowledge the graphical trends which suggest that there may have been some improvement in egg parameters after calcium restriction when comparing calcium restricted and non calcium restricted hens.

4.9 PHASE 2 BIRD PARAMETERS

4.9.1 Body Weight

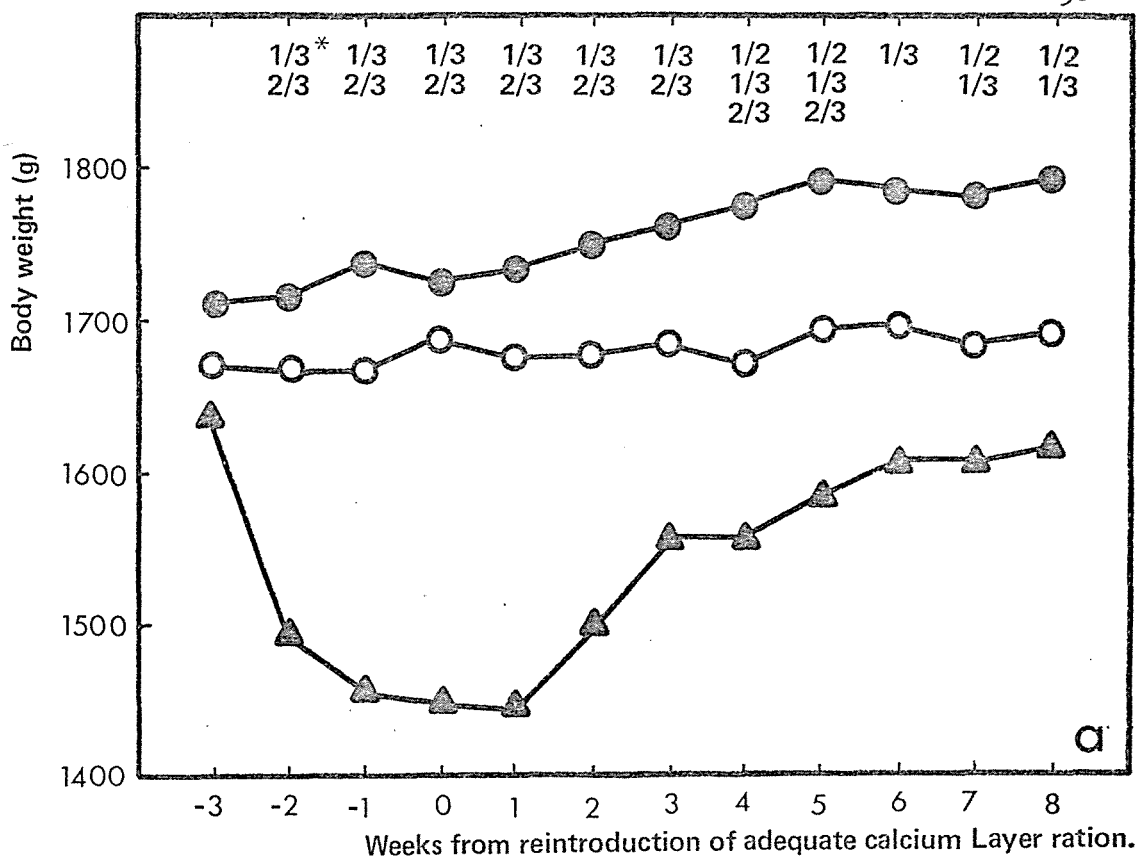
Body weights were significantly reduced by calcium restriction (fig. 10 a, b). Following the termination of calcium restriction, body weights rose but did not reach the weight of T2 hens. By five weeks after reintroduction of adequate calcium laying diet the body weights of T2 and T3 hens lost their significant difference. Body weights of T4 hens remained significantly different from those of T2 hens throughout phase 2 except for two isolated weeks where no significant differences between treatments were shown.

The body weight difference between T1 and T2 hens became significant four weeks and two weeks after the reintroduction of adequate calcium layer ration in the T3 and T4 comparisons respectively. This continued throughout phase 2 except for isolated weeks where significant treatment differences were absent.

Body weight differences between T1 and T2 hens were expected in phase 2 due to the shifting of stock in all treatment groups except T1 between phases. In phase 2 some hens in T2 previously (during phase 1) belonged to calcium restriction treatments. These hens began phase 2 with lighter body weights thereby causing the average T2 body weight to be less than the average T1 body weight.

4.9.2 Food Consumption

Food consumption of T3 and T4 hens was significantly depressed during calcium restriction but it rose to a level which is not significantly different from the control groups within three weeks after reintroduction of adequate calcium layer ration (fig. 11 a, b). However, food consumption of T3 hens was on average



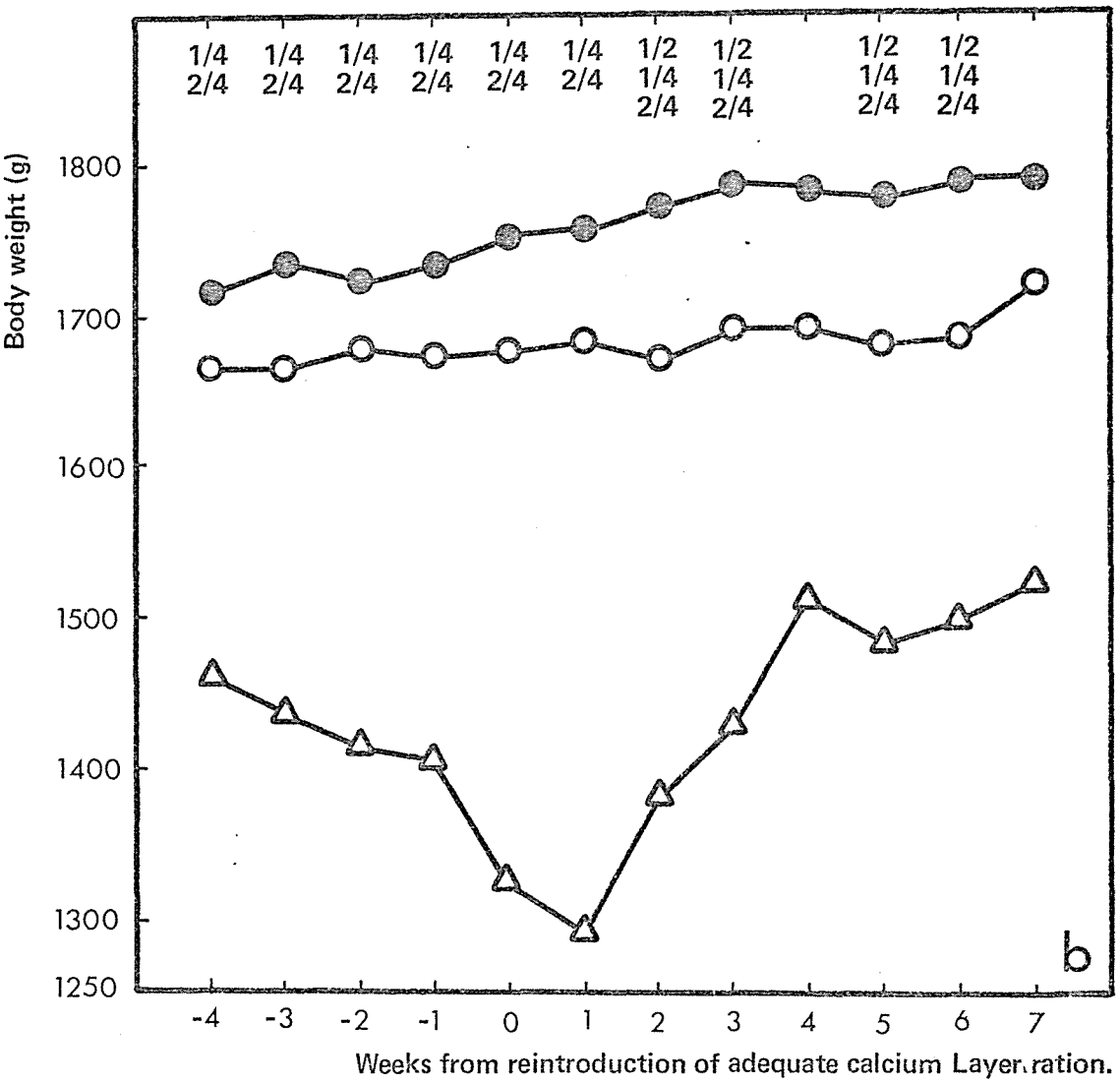


Fig. 10 Body weight during phase 2.

Treatment 1 Treatment 3
Treatment 2 Treatment 4

* Statistically significant treatment differences

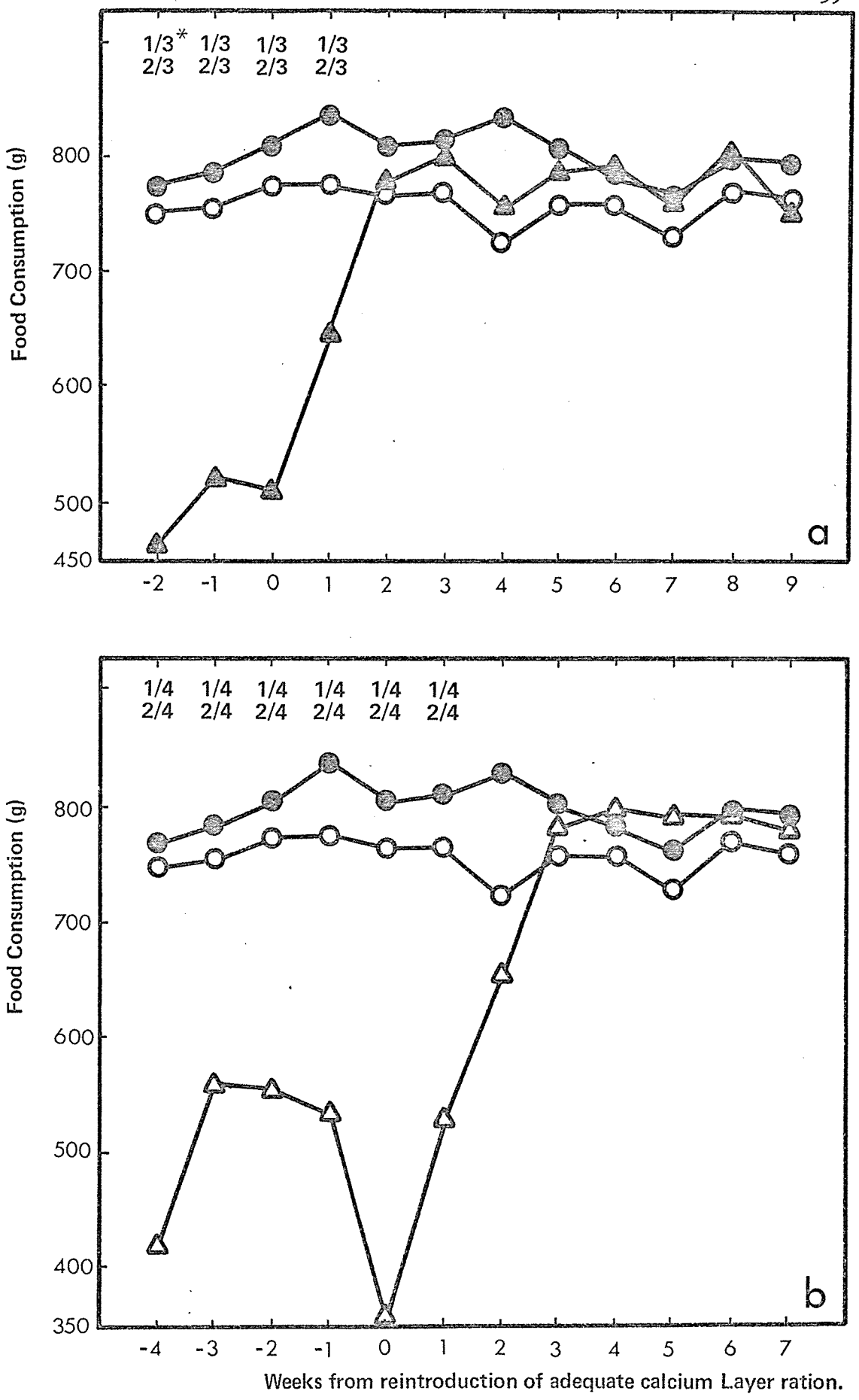


Fig. 11 Food consumption during phase 2.

Treatment 1 Treatment 3
Treatment 2 Treatment 4

* Statistically significant treatment differences

greater than that of T2 hens. Also, food consumption of T2 hens was less but not significantly less than that of T1 hens during all of phase 2. This probably reflected the body weight differences between these two groups.

4.10 PHASE 3 EGG PARAMETERS

4.10.1 Egg Production

Results were similar to those in phase 2. Both T3 and T4 hens decreased their egg production during calcium restriction, a significant drop was observed after one week's consumption of low calcium diet (fig. 12). A rapid rise in egg production followed the reintroduction of adequate calcium layer ration so that two weeks after the dietary changeover there were no significant differences between egg production of calcium restricted and non calcium restricted hens.

The last six to seven weeks of phase 3 revealed diverging egg production of calcium restricted and non calcium restricted hens. This was not a significant difference but it is of interest to note that what may be termed the "end of lay" production decline (at this stage hens were 65 - 68 weeks of age) was not as marked in calcium restricted treatments as it was in non calcium restricted treatments.

4.10.2 Egg Weight, Shell Weight, Shell Weight per Surface Area

Phase 3 results were similar to those in phase 2. There were no statistically significant results to prove the superiority of T3 calcium restricted hens over non calcium restricted hens (fig. 13 a,b,c). T4 calcium restricted hens produced some significantly better eggs.

Eggs laid by T4 hens were significantly heavier than eggs laid by T2 hens over two groups of eggs. Similarly shell weight and shell weight per surface area was significantly better in T4 than in T2 eggs for averages derived from 16th to 25th eggs laid following reintroduction of adequate calcium laying diet.

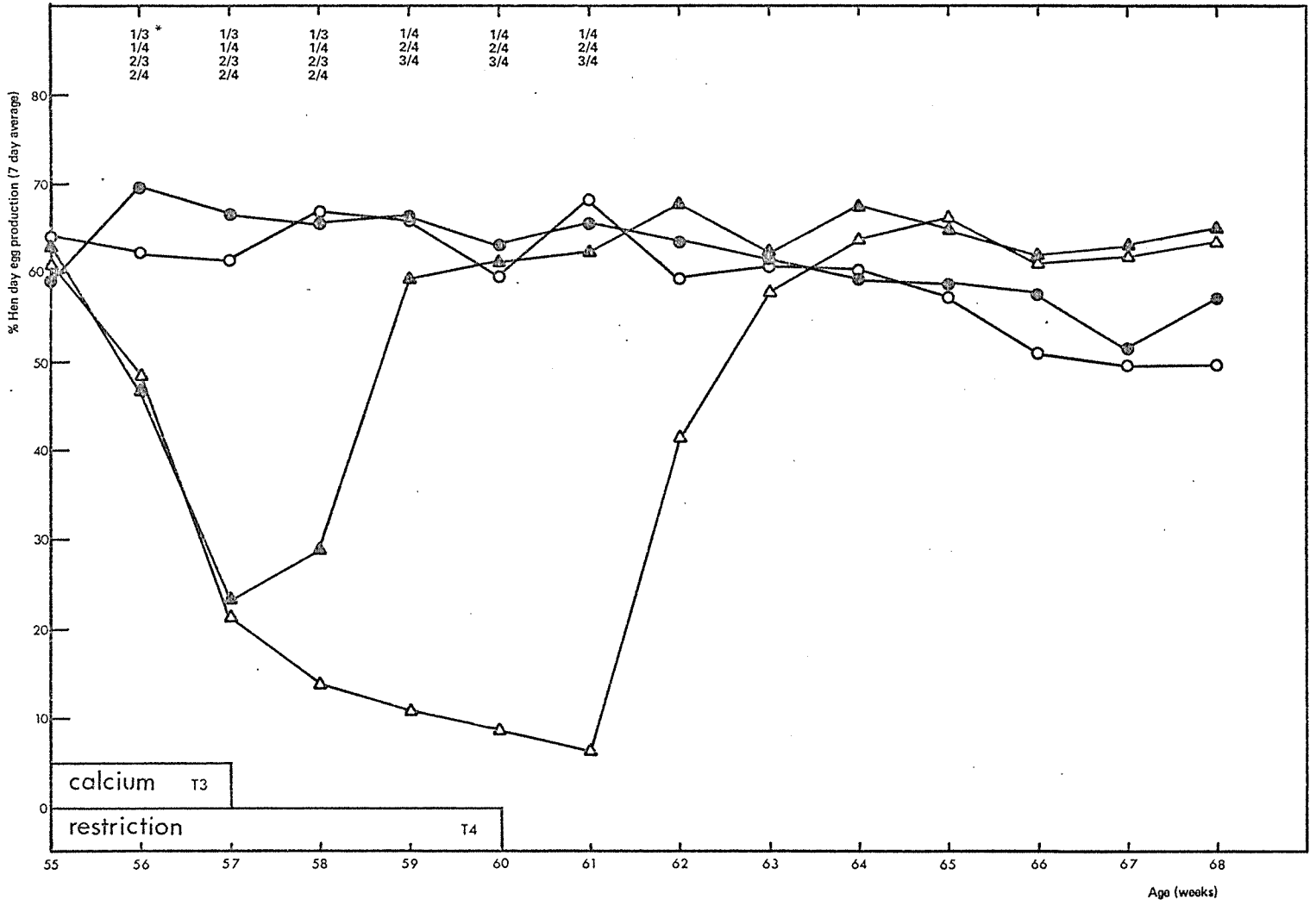


Fig. 12 Egg production in phase 3.

Treatment 1 Treatment 3
Treatment 2 Treatment 4

* Statistically significant treatment differences

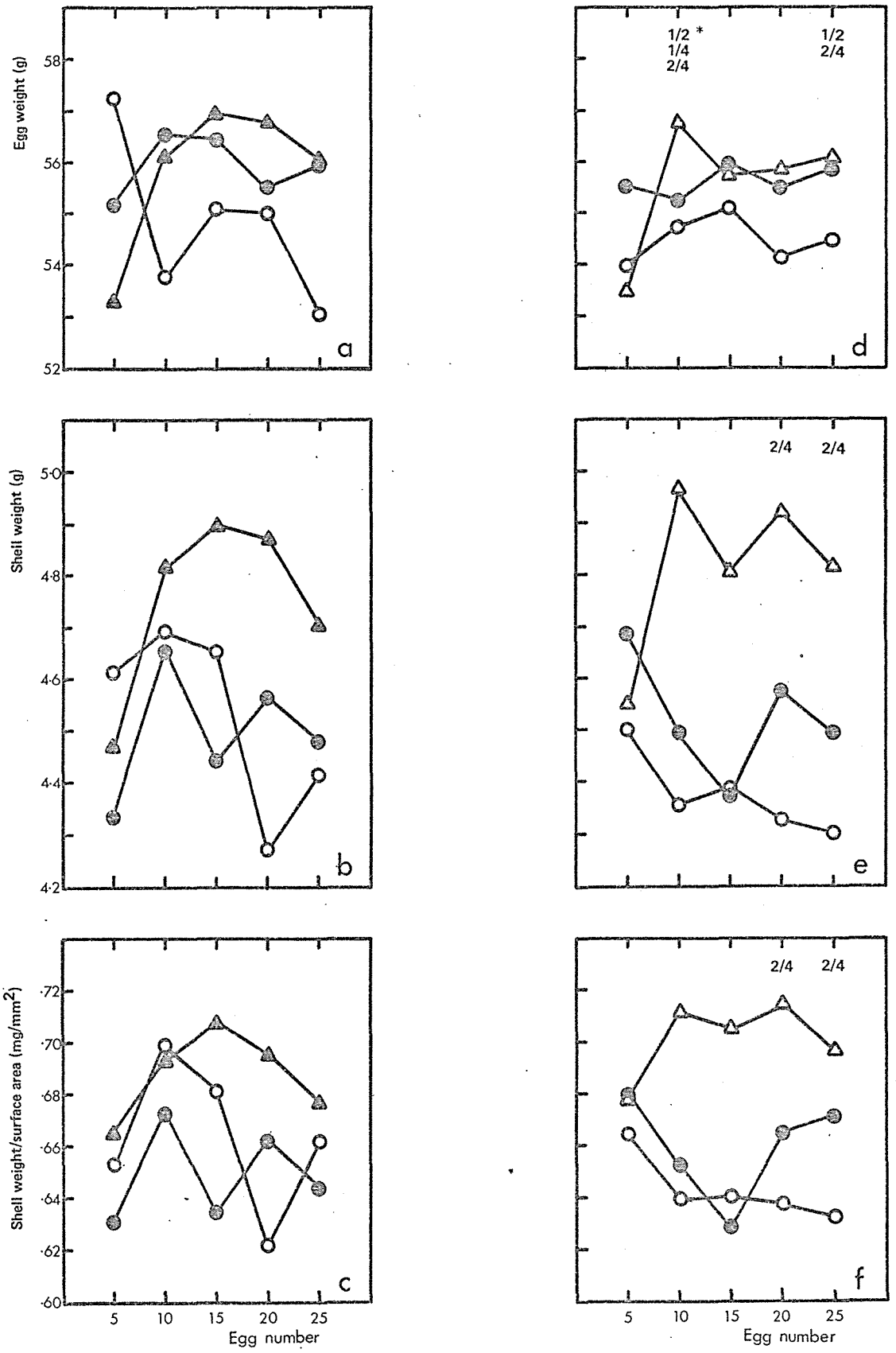


Fig. 13 Average egg weight, shell weight, shell quality (expressed as shell weight per unit surface area of egg) after reintroduction of adequate calcium diet in phase 3.

* Statistically significant treatment differences

Treatment 1 Treatment 3
Treatment 2 Treatment 4

4.11 PHASE 3 BIRD PARAMETERS

4.11.1 Body Weight

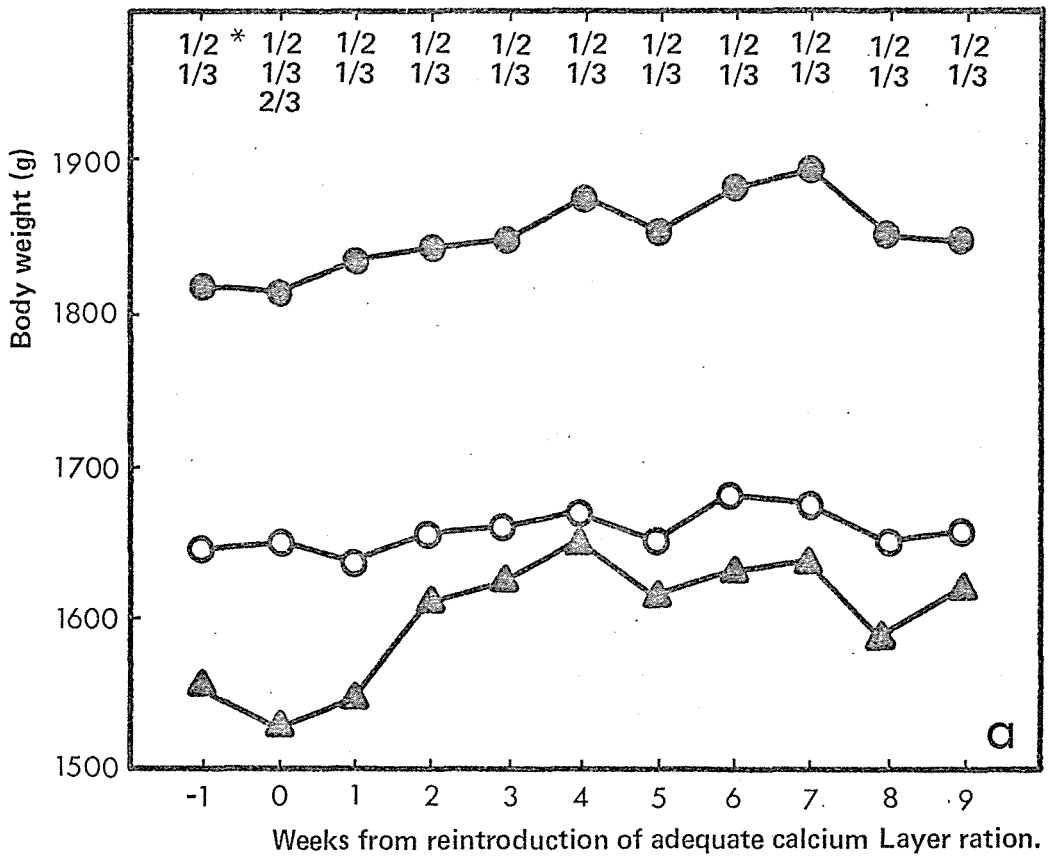
Body weight of T1 and T2 hens were significantly different throughout phase 3. This difference between the two control treatments is more severe than in phase 2 but the reasons for its occurrence are the same as in phase 2 (see section 4.9.1.).

Calcium restriction significantly depressed body weights (fig. 14 a,b). It appears that the longer the calcium restriction, the larger the body weight depression and the longer it takes for body weight to return to a level approaching that of non calcium restricted hens. Four weeks passed before the body weight of T4 hens lost its significant difference from T2 hens. But the body weight of T3 hens lost its significant difference from T2 hens within one week of reintroduction of adequate calcium layer diet. Body weights of T3 hens never returned to closer than 30g of T2 control body weights nor did body weights of T4 hens return to closer than 80g of the body weights of T2 hens.

4.11.2 Food Consumption

Food consumption was affected in exactly the same way in phase 3 as in phase 2. In both T3 and T4 there was a significant reduction in food consumption during calcium restriction but following the removal of calcium restrictions food consumption increased within two to three weeks to a level slightly above T2 hens. However, there are no significant food consumption differences after two weeks' consumption of adequate calcium laying diet (fig. 15 a,b).

A non significant food consumption difference between T1 and T2 occurred as in phase 2, in which T2 hens consumed about 50g/hen/week less than T1 hens throughout all of phase 2. Again, this reflects body weight differences between the two treatments. In contrast to body weight, food consumption differences between T1 and T2 hens were no greater in phase 3 than in phase 2.



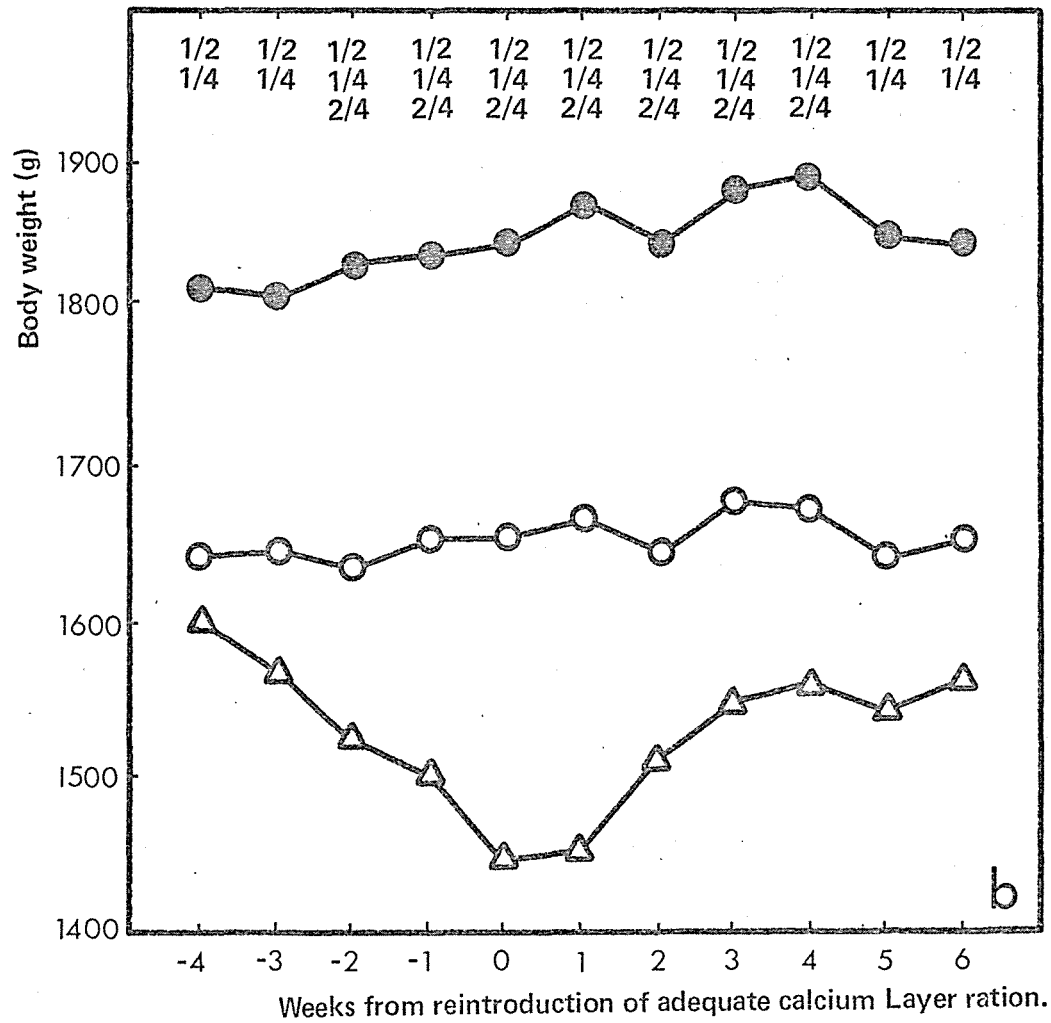
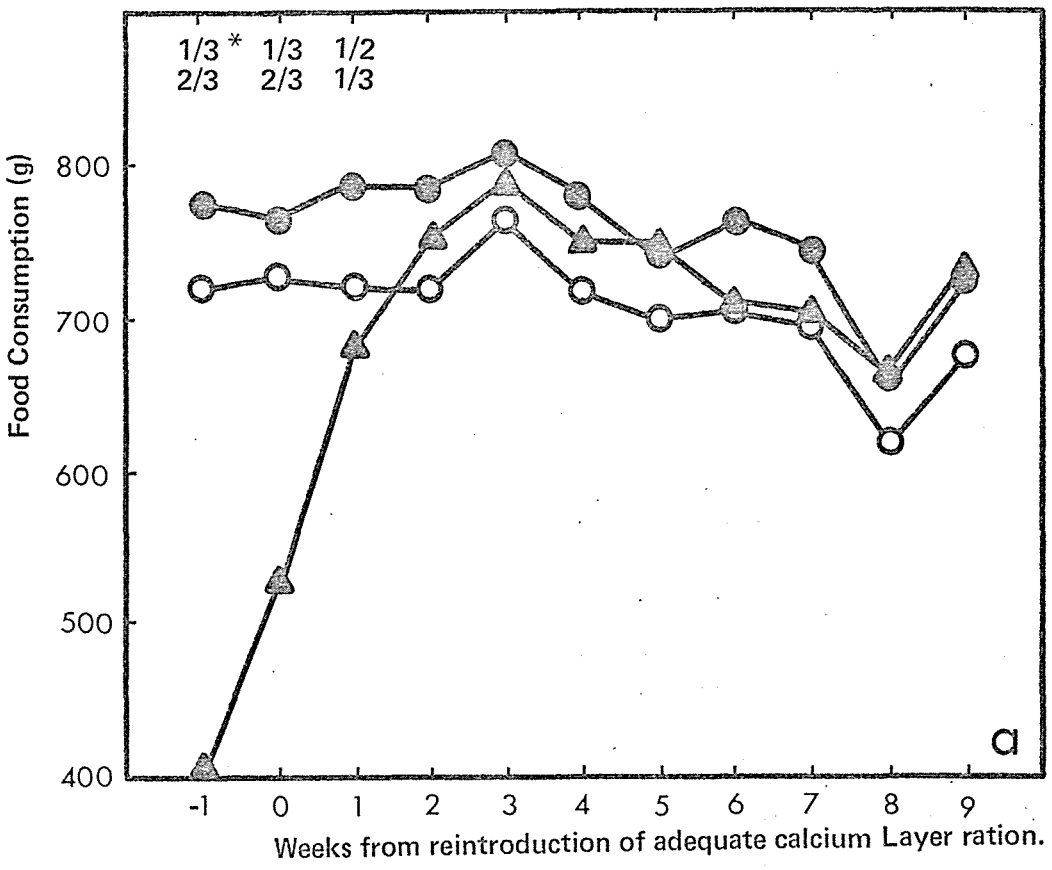


Fig. 14 Body weight during phase 3.

Treatment 1 Treatment 3
Treatment 2 Treatment 4

* Statistically significant treatment differences



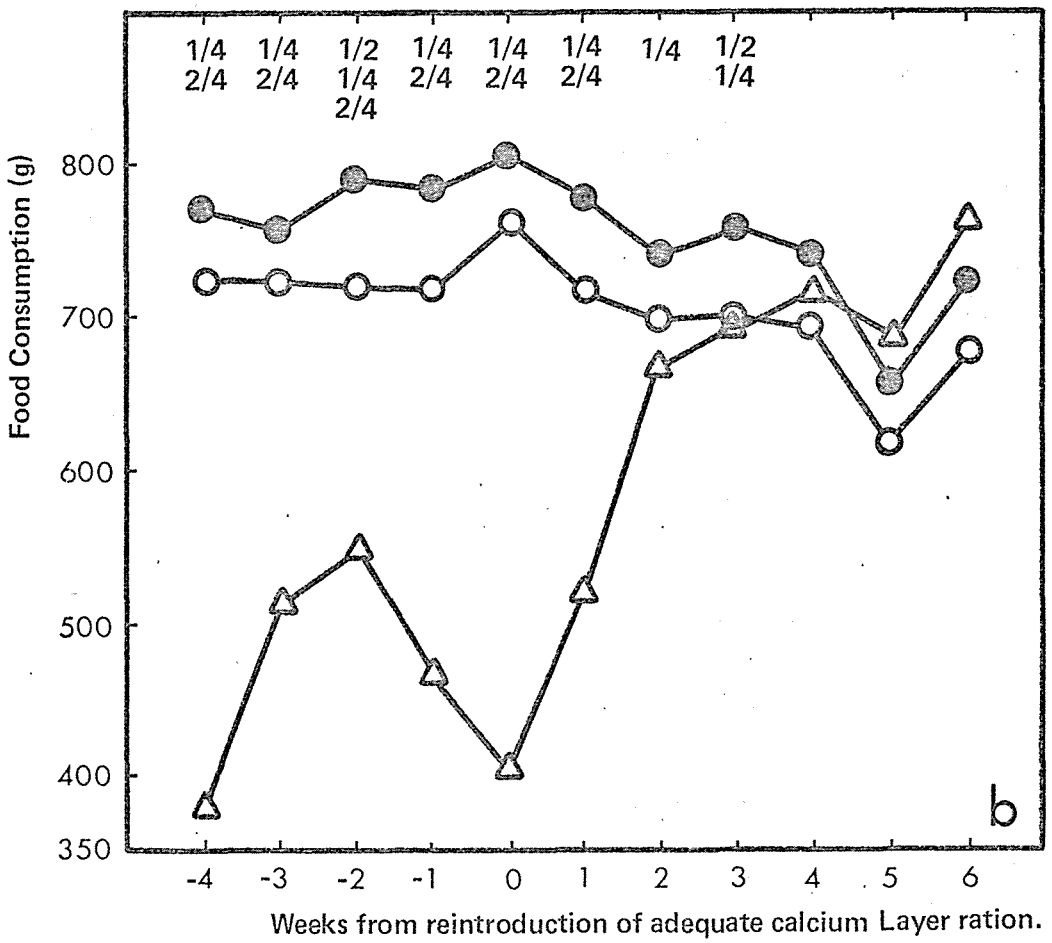


Fig. 15 Food Consumption during phase 3. Treatment 1 Treatment 3 Treatment 2 Treatment 4

* Statistically significant treatment differences

CHAPTER FIVE

Discussion

5.1 ONSET OF LAY

Feeding diets containing very low calcium contents does not delay the age of the pullet at the time of her first oviposition. This confirms earlier observations on age at first egg by Gilbert (1973) as does the pattern of egg production by calcium restricted pullets following their first oviposition. While not delaying the time of the first oviposition, calcium restriction certainly depresses subsequent egg production.

Low calcium diets have the capacity to seriously depress (but not completely inhibit) the onset of full egg production but do not appear to be able to inhibit the first ovulation. A logical conclusion may seem to be that although calcium is essential for the maintenance of a high rate of egg production, it is not necessary (except in trace amounts for maintenance) for the development of sexual organs and the beginning of ovarian activity.

5.2 EGGS LAID DURING CALCIUM RESTRICTION

With the large losses of calcium from the body, egg production may have stopped in some hens during phase 1 but this was by no means the general case since most hens continued laying through to the end of the period of calcium restriction, albeit at a low rate.

Earlier work by Gilbert (1973) found that during calcium restriction at the onset of lay, no more than three eggs were laid before egg production ceased. Phase 1 results agree with this observation when the eggs laid per hen is averaged over all hens in their respective treatments. But some individual hens laid no eggs during the period of calcium restriction and others laid up to five eggs. Of the hens in T3 which had begun egg production, most laid three eggs while consuming the low calcium diet. Most

T4 hens laid four eggs while eating low calcium diet but some hens laid up to seven eggs during calcium restriction.

The hens in T3 and T4 continued to lay longer while consuming a low calcium diet than did similar hens in the trial of Gilbert (1973). It has been suggested that the birds used in the present trial belong to a strain (M-line) which is particularly susceptible to cage layer fatigue. This has been shown in past generations of the strain (Patchell, 1975, pers. comm.). But the M-line layer is also an extremely "hardy" bird which will continue to lay at high rates no matter what form of stress is forced upon it. The genetics of the M-line strain may provide an explanation of why the birds in this experiment persisted to lay longer under calcium restriction than did Gilbert's (1973) birds.

During egg production pauses in phases 2 and 3, the average rate of egg production of T4 hens is lower than that for T3 hens. This arises from the fact that the periods of calcium restriction were longer for T4 hens than T3 hens thereby allowing more time for T4 hens to reduce their egg production. Egg production of T3 and T4 hens declined at the same rate during calcium restriction until a dietary change for T3 hens caused their egg production to rise.

Thus the severity of the effects on egg production of low calcium diets dependsnot only on the absence of an adequate level of calcium in the diet but also on the length of time that the diet is eaten. The length of the period of calcium restriction contributes to the decline in egg production but only down to a certain minimum level which was shown to persist through very long periods of calcium restriction (Gilbert, 1973).

The finding that shell-less eggs have been produced confirms recent observations of Roland, Sloan, Wilson and Harms (1973) but contradicts earlier work by Deobald et al., (1936) and Mehring and Titus (1964) who found shell-less eggs were not produced during calcium restriction. An explanation of this

contradiction may lie in the genetic makeup of the hens used in the earlier and more recent trials.

5.3 CALCIUM DEPLETION OF THE HEN DURING CALCIUM RESTRICTION

Calcium restriction causes extensive calcium depletion of the hen unless egg production can be stopped quickly enough by the hen. If egg production is not stopped or drastically reduced, calcium deficiency syndrome may result. Some hens reached 30% depletion of body calcium after laying seven eggs while consuming a low calcium diet in phase 1. These may be examples of hens which Taylor, Morris and Hertelendy (1962) suggest may be so highly bred for egg production that they have lost the ability to activate the pituitary cut-off mechanism. Calcium depletion of hens in phase 1 is less than that shown to occur by Taylor and Moore (1954) from hens of a comparable age but it is still an extremely large depletion. The degree of depletion at which calcium deficiency syndrome appears is not known but leg paralysis occurred in five birds during the period of calcium restriction in phase 1.

Calcium depletion in phases 2 and 3 was much more severe than in phase 1. Besides reaching a higher total depletion, calcium restricted hens in phases 2 and 3 lost larger amounts of calcium in each eggshell. If depletion of hens in phase 1 was extremely large, then in phases 2 and 3 it is incredible that hens could survive a rate of depletion which was somewhere near 50%. It is not surprising that calcium deficiency syndrome occurs during calcium restriction.

The severe depletion of the bones coupled with damage to a particular area of the bone could provide a more specific explanation of "cage layer fatigue". Perhaps an arthritis-like condition develops causing pain at the femur - ilium joint, resulting in the fatigued posture; sitting rather than standing.

Unless feed and water is within reach of the sitting hen the condition will be aggravated until the hen either dies through dehydration and emaciation or has to be culled.

The incidence of calcium deficiency was greater in phases 2 and 3 than in phase 1. It seems that the younger birds at the onset of lay were more able to avoid calcium deficiency than older hens. In section 4.3 calcium losses from the body are shown to be greater for calcium restricted hens in phases 2 and 3 than for similar hens of phase 1. This may be due to the fact that egg size is greater for older hens so calcium loss is larger due to greater shell formation. Calcium deficiency induced leg paralysis was more severe and more widespread in this trial than in the earlier work of Gilbert (1973). This may be explained by the increased genetic susceptibility to cage layer fatigue and the persistency of egg laying shown by the M-line strain in the face of calcium depletion (Patchell, 1975, pers.comm.) which is discussed in section 5.2.

5.4 PRE-LAYING DIETS

Phase 1 also contained a comparison between a blanket changeover from grower ration to layer ration at 21 weeks of age and a dietary changeover made for individual hens at the time of laying their first egg.

There was no difference in egg production or other egg parameter measurements following either dietary regime but food consumption of hens in T1 and T2 differed slightly although not significantly.

T2 hens lowered their food consumption by some 40g when they received the laying diet and actually consumed 40 - 60g/hen/week less than T1 hens for the next seven weeks. The initial depression may be explained by "acclimatisation" to the new diet and the fact that it did not increase to the same level as T1 hens may result from more efficient utilisation of food consumed. It would seem that the closer the dietary change from grower to layer ration is to the laying of the first egg, the more efficient is the food utilisation after the change. This could represent quite a large cost saving to commercial farmers. If oviposition of the first egg could be synchronised, then the full benefit of possible feed cost savings could be made.

It was hoped that low calcium diets may be able to synchronise the onset of lay but reproductive activity began regardless of the low calcium diets fed from 21 to 26 weeks of age.

Body weight does not appear to be affected by the timing of grower/layer diet changeovers.

5.5 EGG PARAMETERS

The evolvement of egg weight, shell weight and shell weight per surface area of eggs produced by T1 and T2 hens during phase 1 presents similar patterns to those shown by Hurwitz and Bar (1971). In both studies egg weight increases with egg number to approach a maximal value with the laying of the 15th to 25th egg. Shell weight and shell weight per surface area increased similarly but more rapidly indicating that for individual hens shell quality improves with the production of the first ten to 20 eggs but does not improve much subsequently. Results derived from T1 and T2 data during phase 1 support the conclusion of Hurwitz and Bar (1971) that on an individual hen basis, egg weight increases only during the first month of egg production.

There was little difference between all three experimental phases in the reaction of hens to reintroduction of adequate calcium laying diet after consuming low calcium diets. Only in phase 3 when the laying year was nearing an end and egg production of the control groups had declined to 50 to 60% was there any improvement in egg production of calcium restricted hens over non calcium restricted hens following the reproductive pause period. Earlier in the laying year, no improvement in egg production was seen to result from calcium restriction. The improved egg production of calcium restricted hens in phase 3 results from a slower rate of senescence, which is the declining egg production at the end of the laying year, than occurred in hens which were not reproductively rested by calcium restriction.

Force moulting is generally initiated during the senescent period, if it is to be used at all, so a comparison could be made between the improvement in egg production after a conventional force moult and after an enforced egg production pause as in phase 3. Egg production following a force moult would be expected to rise from 40 or 50% to 70 or 75%. Reproductive pause periods caused by calcium restriction are not, it is suggested, equal in effect to force moulting and the equation of one with the other in the past should not be promoted in the future.

Eggs laid by calcium restricted hens in phase 1 were heavier than eggs laid by non calcium restricted hens, but this was due to the age difference between the two groups of hens. Other than this there is only non proven evidence that calcium restricted hens may be able to produce heavier eggshells or better quality eggshells.

If it is true that superior eggshell production follows periods of calcium restriction, the reason for it may involve the parathyroid gland. During calcium restriction, blood calcium drops and this stimulates greater output of PTH from the parathyroid gland. There is evidence to show that the size of the parathyroid glands is increased during calcium restriction, (Hurwitz and Griminger, 1961b) but it is not known how long hyperactivity of the parathyroid glands persists after calcium restrictions are lifted. If increased PTH secretion does not persist for some length of time after calcium restriction, blood plasma calcium may rise to a higher level than it was before calcium restriction due to increased bone resorption. This would make more calcium directly available for eggshell mineralisation.

An interesting result from phase 1 experiments is the apparent superiority of T4 hens over T3 hens. The dietary regimes for both T3 and T4 were the same except for the type of low calcium diet fed from 21 weeks to 26 weeks of age. T3 hens received a low calcium grower ration while T4 hens received a

low calcium layer ration. It is reasonable to suggest that the difference between T3 and T4 egg parameters is attributable to the difference between the low calcium layer and low calcium grower diets. Both rations contained approximately the same levels of calcium and phosphorus (Appendix IV) and had similar calorie/protein ratios.

Evidence exists (section 4.2) to show that in phase 1, T4 hens produced more eggs while consuming low calcium layer diet than did T3 hens consuming low calcium grower diet. The production of more eggs during calcium restriction may have resulted from the fact that the low calcium layer ration contained higher levels of protein and energy than did the low calcium grower ration. With similar calcium intakes, T4 hens would in general have become more depleted of calcium than T3 hens (section 4.3). A longer period of calcium deficiency could result in a more extensive hyperactivity of the parathyroids which may be reflected in blood calcium levels subsequent to the reintroduction of adequate calcium diets. If this does occur higher blood calcium may be expected in more severely depleted hens than in less severely depleted hens after an adequate calcium diet is reintroduced, because of their greater PTH influence on resorption of bone mineral.

It would be of interest to study the level of blood calcium and PTH before and after calcium restriction to see whether any difference exists, and for how long the differences persist if at all. To test the sequence of events proposed above it is required to know whether a more severe calcium deficiency causes a greater depression of blood calcium than a less severe calcium deficiency. The case in discussion is an example. Was the blood calcium of T4 hens lowered to a greater degree than that of T3 hens? If so, then the proposed reaction of the parathyroids becomes more plausible.

A second possibility which could contribute to improved eggshell production after calcium restriction involves intestinal absorption of calcium. Adaptation of the intestine to low calcium

diets occurs by increasing the efficiency of absorption. Depending on the length and severity of restriction, increased absorption efficiency may persist even when calcium restriction is lifted and the new diet contains an adequate amount of calcium.

5.6 BIRD PARAMETERS

Body weight surged by 400g during a two to three week period before oviposition of the first egg. The increase which may be as much as a 30% body weight rise is caused by rapid attainment of the sexually functional state. The ovary and oviduct increase in size and the skeletal store of mineral expands. As well there is a general expansion in all body musculature and fat deposits. The pre-laying body weight increase occurred in all treatments and the magnitude of the body weight increase was also the same regardless of diet.

Similarly, a rapid food consumption increase occurred at the same time. Whether body weight increases due to increased food consumption at this time, or food consumption increases are necessitated by rapid development is not clearly understood. Rapid sexual development probably causes a physiological urge to eat which in turn allows such a rapid body weight increase.

After laying the first egg there is a drop in both food consumption and body weight of T3 and T4 hens. Eggs laid during the calcium restriction period contribute to the loss in body weight and are probably the main cause of body weight loss.

A change in diet from PRC grower ration to the low calcium diets at 20 weeks of age caused no drop in food consumption or just a small one, but when eggs were produced while consuming the low calcium diets, food consumption did decline rather rapidly. It is suggested that the reason for declining food consumption at this stage was due more to the inability of some hens to reach the feed troughs and water nipples rather than a depressed appetite. Inability to reach feed troughs arose when some hens suffered

calcium deficiency after laying too many eggs while consuming a restricted calcium diet. The paralytic condition shown by several hens (section 4.5) caused aggravation of the condition, and led to the catabolism of the hens' own body reserves for energy and protein. This contributed also to decreases in body weight.

It may not be a direct effect of inadequate calcium which causes decreased body weight and depressed food intake but rather a secondary effect via the calcium deficiency syndrome. If egg production could be stopped immediately by calcium restriction calcium deficiency syndrome may not eventuate and body weight and food consumption may not be affected. This leads to the question of what causes the depressed rate of egg production? It may be the pituitary cut-off mechanism suggested by Taylor, Morris and Hertelendy (1962) but on the basis of the foregoing perhaps a suddenly reduced intake of food and water may cause it.

Following an initial two week decline body weight and food consumption rise again. This begins at a time when egg production in the calcium restricted hens has reached its lowest level and has in some hens ceased. Unlike food consumption, body weight of the calcium restricted hens does not reach the level of the non calcium restricted hens after the adequate calcium layer diet is introduced. Calcium restriction has a more lasting effect on body weight than it does on food consumption. The reason may involve the rates of increase of egg production and body weight after calcium restrictions have ended. With the return to egg production, mobilisation of assimilated nutrients for production of eggs may take priority over growth of other body tissues. As egg production increases, more nutrient is required for producing eggs and less is available for body weight gain which therefore slows down. The more rapid is the return to full egg production, the more marked would be this effect. By the time egg production is fully recovered body weight increase would progress at the same rate in both non calcium restricted and previously calcium restricted hens. The hens were not held out of egg production for

long enough to see whether in fact body weight would have increased to the same level as that of non restricted hens before adequate calcium layer diet was introduced.

There is a relationship between the number of eggs laid while consuming a low calcium diet and severity of calcium depletion. If calcium deficiency syndrome (whether clinical or sub-clinical) depresses the ability of hens to eat then it is logical to expect that the more depleted birds decrease their food intake the most. As a result it would also be expected that these hens suffer the largest body weight losses. It is true in this experiment that the hens with the most severe calcium depletion suffered the greatest body weight losses. They remained the lightest birds after the first oviposition.

In phases 2 and 3 the most obvious conclusion is that the feeding of a low calcium diet causes food intake depression and loss of body weight. Although the level of food consumption of calcium restricted hens returned to the equivalent of non calcium restricted hens following reintroduction of the adequate calcium diet, body weight did not. There was a higher intake of food when calculated on a body weight basis for previously calcium restricted hens. This suggests either that the previously calcium restricted hens were inefficient food converters, they were more productive or perhaps they consumed larger quantities in order to replete the skeleton with calcium.

The latter is more likely since egg production was no greater in previously calcium restricted hens. Nor was egg weight, but the tendency towards heavier eggshell production by T3 and T4 hens may in part have been due to higher food consumption/body weight ratio of these hens. The first suggestion that previously calcium restricted hens were inefficient food converters is difficult to uphold at least for mineral nutrition which would be expected to be very efficient after a period of calcium depletion.

CHAPTER SIX

Concluding discussion

Extremely low calcium diets have marked effects on the rates of egg production when they are consumed by laying hens, but egg production is not inhibited completely by calcium restriction although this may occur in some hens. The consumption of pre-laying low calcium diets cannot delay the onset of egg production but it does have a marked effect on egg production once it has begun, and the onset of full egg production can be suppressed for as long as calcium restriction lasts.

The benefits of delaying the onset of full production depend on management situations. There may be times on commercial poultry farms when early egg production is undesirable. The main benefit of using low calcium diets at this time is the depression of egg production. The rate of egg production and egg weight of "delayed" hens are not superior to non delayed hens of the same age. If any improvement in shell production results from calcium restriction it is probably of little benefit except during the last half of the laying year, since eggshell quality at the beginning of the laying year is generally of a high standard.

Some benefit in reduced body weight may be gained but this could also be achieved by feed restriction during the rearing phase and if necessary during the laying phase.

One danger with using low calcium diets is the risk of calcium depletion causing calcium deficiency syndrome.

Egg production pauses enforced by calcium restriction have not been shown to cause better egg production or egg weight after the reintroduction of adequate calcium diets. But there is a tendency towards production of heavier eggshells and better eggshell quality by hens which have been calcium restricted. The benefit of this apparent improvement may be outweighed by the incidence of calcium deficiency syndrome during calcium restriction which may cause some mortality.

The reproductive pause induced by calcium restriction does not constitute a moult and involution of the reproductive tract does not always occur during a reproductive pause. While the use of low calcium diets is being researched as a possible substitute for starvation to induce moulting it should be remembered that the two processes are very different. Only if complete involution of the reproductive tract can be induced by calcium restriction could this method be claimed to be a real substitute for conventional force moulting. If involution does not take place during a reproductive pause, egg production following the pause cannot be considered to constitute a new laying "season". It is really the continuation of the same period of laying. Following this reasoning it is perhaps unreasonable to expect similar production characteristics from hens following a non-involutionary reproductive pause as from hens following a force moult where involution does occur. This and earlier discussion leads to the conclusion that enforced egg production pauses should not be substituted for force moulting.

But egg production can be stopped and restarted again by calcium restriction without any detrimental effects on subsequent egg production, egg weight and shell weight. These parameters return to levels at least as high as what would be expected from non calcium restricted birds of the same age. The only unsafe feature of enforced pauses caused by calcium restriction is the risk of calcium deficiency syndrome.

Decreases in body weight are observed during periods of calcium restriction but since food consumption of previously calcium restricted hens is just as high as non calcium restricted hens which did not show a body weight drop, the economic benefit of lower body weight is lost.

Earlier discussion of calcium depletion in the hen during calcium restriction suggests that calcium deficiency syndrome which is very likely the same condition as that commonly called caged layer fatigue is a self aggravating condition caused by the inability of hens to stand up and eat from their feed troughs coupled with the continuing drain of calcium from the body in eggshells.

Much more knowledge is required before a clear understanding of the role of calcium in avian reproduction control is gained. Many more details will undoubtedly be unfolded in the future, but it should be recognised that the calcium system in the reproducing hen is difficult to research because of its complexity. The skeleton, blood, diet, intestine, parathyroids and uterus all play a part in the calcium system and all are interdependent to some extent, so future research will require sophisticated techniques. Progress may be slow, but eventually an appreciation of the interaction of calcium and reproduction will be found.

Many diverse aspects of the role of calcium metabolism in avian reproduction have been presented in this thesis. A summary of the more salient points may provide valuable guidelines for further research and indeed for practical poultry farming. These guidelines are listed below.

1. The pre-laying period is characterised by preparation for egg laying

2. During the two to four week pre-laying period there is
   (i) a rapid body weight increase
   (ii) rapid sexual development
   (iii) intense mineralisation in the skeleton
   (iv) formation of medullary bone
   (v) a slight drop in food consumption prior to first oviposition.

3. Egg production starts at a high rate for individual hens.

4. Egg weight increases rapidly reaching a peak after 15 to 25 eggs have been laid.

5. Shell weight increases to a peak after 10 to 15 eggs have been laid.

6. Egg shells have high quality from the first two or three eggs.

7. Characteristics of individual early egg production of hens are obscured in flock records.

8. During early egg production the hen is in negative calcium balance which cannot be alleviated by increasing the intake of calcium.

9. Body weight and food consumption increase slowly after the laying of the first egg.

10. 1% calcium should not be exceeded for growing diets otherwise renal degeneration and mortality may result.

11. Higher calcium diets fed after 16 weeks have no detrimental effects.

12. In a flock, changeover from growing diet to laying diet should be made as soon as the very first egg appears.

13. Laying diet should contain sufficient calcium to allow for the formation of one egg shell per day (generally 3 to 4% calcium).

14. Low calcium diets do not delay the onset of egg laying but do seriously depress egg production.

15. Regression of reproductive organs is not necessarily induced by calcium restriction.

16. Low calcium diets cause body weight and food consumption depression.

17. Body weight never fully recovers after calcium induced production pauses but food consumption does.

18. Egg production and egg weight are not improved following periods of calcium restriction but shell production may be slightly better.

19. Low calcium induced production pauses should not be substituted for force moulting.

20. Extensive mineral depletion in the skeleton occurs during calcium restriction if egg laying continues.

21. Prolonged calcium deficiency can cause a calcium deficiency syndrome typified by leg paralysis.

22. Calcium deficiency syndrome may be a condition similar to cage layer fatigue.

APPENDIX I

CALCULATION OF CALCIUM LOSS OF HENS FED LOW CALCIUM DIETS

Successive eggs laid while consuming low calcium diet are expected to possess decreased amounts of calcium in their shells. To obtain an accurate estimation of the calcium content of an eggshell it is assumed that for successive eggs laid during calcium restriction the shell organic material contributes a larger fraction of the total eggshell weight or conversely the shell inorganic material contributes a smaller fraction of the total eggshell weight.

Rather than apply a constant ratio of shell inorganic to shell organic material to all eggs laid during calcium restriction, this ratio should decrease with successive eggs. To calculate the appropriate ratios the following steps were taken.

1. For the eggs laid during restricted calcium periods, subtract the weight of eggshell plus membranes from the total weight of the egg.

2. Do the same for eggs in the membrane determination. (see Appendix III)

3. Match the "low calcium" eggs to the appropriate average egg (on a weight basis) from the membrane determination.

4. Apply to the "low calcium" eggs the weight of eggshell organic matter from the membrane determination.

5. Express the membrane weight as a percentage of the shell weight and subtract the result from 100% to reveal the percentage of inorganic material in the shell.

6. Assume the inorganic material contains 98% $CaCO_3$ and 2% $MgCO_3$. The $CaCO_3$ molecule contains 40% calcium (derived from atomic weight analysis) therefore it follows that 40% of the 98% $CaCO_3$ in the shell is calcium. This means that 40 x 98 or 39.2% of the total shell inorganic weight is pure calcium.

7. To account for the organic phase in the eggshell, eggshell weight must be multiplied by the percentage inorganic shell and the result must be multiplied by 39.2% to find the amount of calcium in the shell.

APPENDIX II

EGG SURFACE AREA

Since the formula used for estimating surface area (A) is dependent on weight of the egg (W); ie.

$A = 4.67\ W^{\frac{2}{3}}$ . . . . (Mueller and Scott, 1940),

it is obvious that as W increases, A will increase.

Consider an egg weighing 45g with a normal shell weighing 10% of egg weight ie. 4.5g. Imagine a second egg the same as the first but with an eggshell weighing only 5% of the weight of the egg. By subtracting the weight of the eggshells from total weights the weight of egg contents are obtained.

For the First Egg, egg contents weight (45 - 4.5)g = 40.5g.

For the Second Egg, egg contents weight (45 - 2.25)g = 42.75g.

Although it is expected that egg surface area would in reality change very little, the formula $A = 4.67\ W^{\frac{2}{3}}$ will predict quite different surface areas as is seen in the following table (Table 6).

Egg Weight (g)	Surface Area predicted by $A = 4.67\ W^{\frac{2}{3}}$ (cm^2)
45	33.99
42.75	29.84

TABLE 6 Predicted egg surface area.

A correction factor has been devised in an attempt to rectify the error introduced by varying shell weights. The formula used to predict surface areas of eggs was $A = 4.67\ (W + (0.1 - \frac{S}{W})\ W)^{\frac{2}{3}}$ where A = surface area, W = egg weight, S = shell weight.

The correction factor $(0.1 - \frac{S}{W})\ W$ "normalises" each egg to the condition where the weight of the egg is adjusted to what it would be if the egg had a shell weighing 10% of egg weight. This was considered to be a "normal" egg.

APPENDIX III

MEMBRANE DETERMINATION

In order to obtain the best measure of mineral loss from hens via the eggshells an experiment to separate and measure the organic and inorganic fractions of eggshells was carried out. Two methods for separation of membranes from the eggshell were considered. The first method is a mechanical separation of shell membranes and shell mineral after boiling of the egg. In this method, eggshell mineral is carefully peeled away from the shell membranes (Smith, 1969). The second method involves dissolution of the inorganic phase of the eggshell in dilute HCl.

It was decided that the second method would be more accurate because a better separation of organic and inorganic phase is possible. With mechanical separation, the organic matrix plus mammillary cores are probably lost when removing the shell from the shell membranes. The method used is outlined below in a step fashion.

1. Select four eggs per weight range. Nine weight ranges were constructed, - 36 - 38g, 38 - 40g, 40 - 42g, 42 - 44g, 44 - 46g, 46 - 48g, 48 - 50g, 50 - 52g, 52 - 54g.

2. Identify eggs in their respective weight ranges.

3. Weigh all eggs accurately and record weights.

4. Break out eggs and wash carefully to remove adhering albumen.

5. Select the three "cleanest" breaks for each weight range and neglect all data on rejected egg.

6. Dry eggshells at 40°C for 24 hours then weigh.

7. Immerse shells in labelled beakers containing 3.5% HCl.

8. When the inorganic fraction of the shells have completely dissolved (1 - 2 days), remove the membranes and wash carefully.

9. Label watchglasses and weigh, then place washed membranes on watchglasses for drying.

10. Dry membranes at 40°C for 24 hours then weigh.

11. Label sintered glass crucibles and weigh (dry).

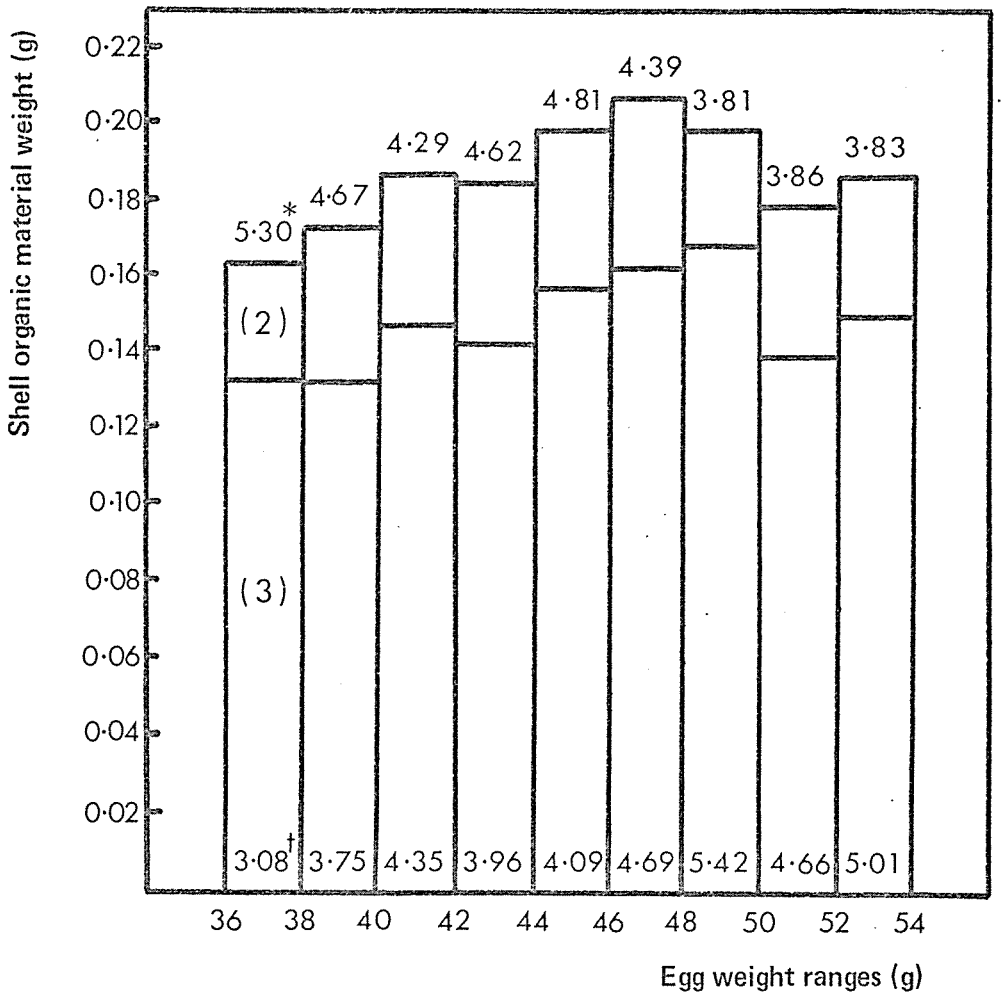
12. Filter remaining protein through sintered glass crucibles. Wash out beakers with distilled water and wash protein.

13. Dry and weigh remaining organic material.

14. Add together membrane weight and protein weight for each egg then obtain the average weight of organic material.

Results are presented histogramatically in fig. 16. The total shell organic material weight appears not to have increased greatly as egg weight increases, therefore it may be concluded that production of eggshell is relatively constant and is independent of egg size. It is assumed that age has no effect on the production of eggshell organic matter although there is no

foundation for such an assumption. The results gained from this short study compare well with earlier shell organic matter determinations (Stewart, 1935). Results of the determination showed total shell organic weight ranged from 0.16g to 0.21g. The average ratio of organic to inorganic shell contents was 4.383%. When shell organic material was expressed as a percentage of egg weight, a decline in the parameter occurred as egg weight increased (fig.17).



* Percent organic/inorganic shell contents

† Average shell weights

Fig. 16 Histogram of averages of 1. Total shell organic material weight
2. Shell membrane weight
3. Weight of remaining organic shell material.

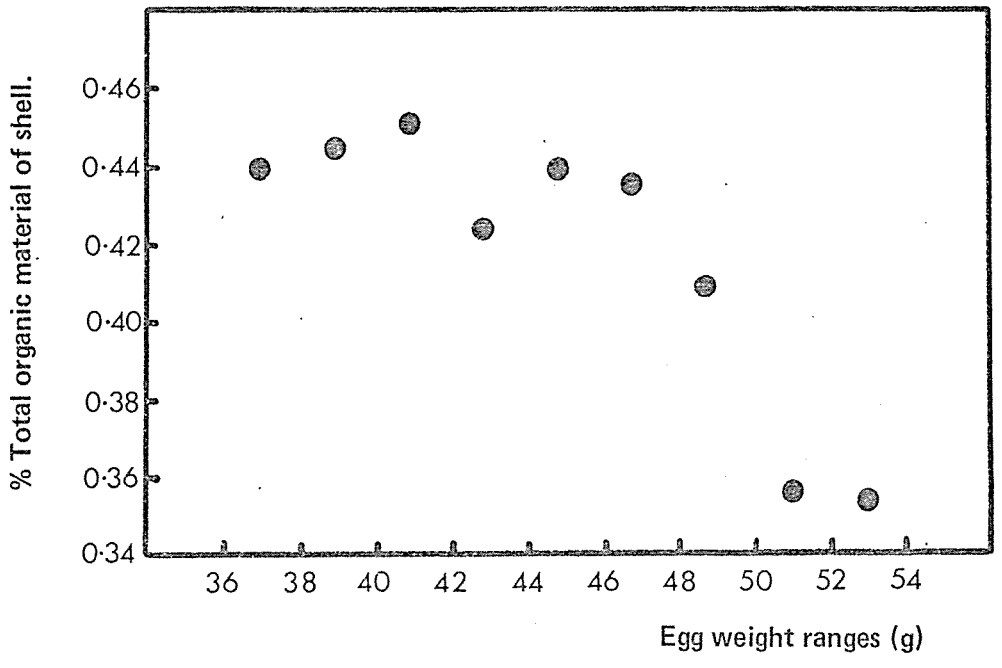


Fig. 17 Shell organic material expressed as a percentage of egg weight.

APPENDIX IV

RATION FORMULATIONS

Low calcium rations have been formulated employing vegetable protein sources since animal proteins contain too high a level of calcium. Attempts were made to use similar ingredients in the adequate calcium layer ration as in the low calcium layer ration in order to maintain similar levels of amino acids, but some meatmeal and tallow has been included to improve the protein and energy levels of the adequate calcium layer ration. The PRC grower ration was not formulated specially for this experiment. Ration formulae are presented in Table 7.

In the low calcium diets total calcium levels are in the vicinity of 0.06%. A considerable portion of this will be rendered unavailable to the hen by phosphorus present in plants as phytin. Unavailable complexes of calcium and phytin phosphates will be particularly important in the low calcium grower ration (Ration B), since this ration contains 12.55% bran which has a very high proportion of its phosphorus present as phytin.

Chemical analyses were made of all rations and these results are shown in Table 8. Protein levels are less than calculated protein levels.

Ingredient	Ration A PRC grower	Ration B Low calcium grower	Ration C Adequate Ca layer	Ration D Low Ca layer
Wheatmeal	-	30.00	23.60	41.15
Maizemeal	48.50	25.45	42.30	32.50
Barleymeal	30.00	-	-	-
Peas	-	15.00	10.00	12.00
Oats	-	15.00	-	-
Lupin	-	-	6.00	12.00
Bran	-	12.55	-	-
Pollard	6.2125	-	-	-
Patea Meatmeal	13.00	-	6.00	-
Tallow	-	-	2.00	-
Boneflour	1.50	-	1.50	-
Limestone	0.50	-	8.00	-
NaH_2PO_4	-	1.50	-	1.75
Salt	-	0.25	0.25	0.25
Methionine	-	-	0.10	0.10
Layer Premix	-	-	0.20	0.20
Grower Premix	0.25	0.20	-	-
Milpak	-	0.05[1]	0.05	0.05
Amprol Plus	0.0325			
	99.9950	100.00	100.00	100.00
Calc. ME (Kcal/lb)	1366.8	1275.3	1338.2	1380.2
Calc. Cr.Pr.%	15.74	13.48	14.675	14.93
Cal/Pr Ratio	87	94	91	93
Calc. % Ca	1.8129	0.0675	3.6215	0.0669
Calc. % P(Total)	0.8628	0.5925	0.5652	0.6242

[1]Included at a level for laying diets.

TABLE 7 Ration Formulae

Ration	Description	% Water	% Protein	% Fat	% Ash	% Crude Fibre	% Calcium	% Phosphorus
A	PRC Grower	9.54	14.78	4.40	*	2.77	2.25	1.10
B	Low Ca Grower	11.80	11.90	2.56	3.84	3.80	0.07	0.88
C	Adequate Ca Layer	9.95	13.06	5.10	11.60	2.83	4.12	0.63
D	Low Ca Layer	12.23	12.66	2.37	3.77	3.36	0.06	0.80

TABLE 8 Chemical Analyses of Experimental Rations.

* Analysis not made.

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