

Some More Consequences of the Intermittent Feeding of Particulate Calcium to Laying Hens

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Introduction

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

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is not provided, egg production is poor and calcium balances on days of production are negative (Taylor and Jones, unpublished).

Where supplemental calcium is provided, there is considerable debate as to the superiority of the source used. Miller and Sunde (1975), in a review of the literature, indicated that calcium was better provided by oyster shell compared to limestone as measured by traditional shell quality parameters and when both sources were ground. When large particles were provided, no difference between sources was observed. Mannion and Reichmann (1984) concluded that

differences between sources could be ascribed to particle size. Although differences in layer performance with different particle sizes have been demonstrated, the reasons propounded for any differences observed (ie changes in shell quality) may be incorrect or misguided. Increasing the particle size per se is not important but rather particle area, which, if considered, may account for observations indicating that shell grit is superior to limestone. However, increasing the surface area by decreasing the particle size may result in the particle passing through the gut more rapidly (Roland *et al.*, 1972). Large particles of shell or limestone may provide calcium to the bird for up to 4 d after ingestion (Sloan, 1976).

The skeletal system accounts for approximately 98% of the bird's bodily calcium. Prior to reaching sexual maturity, the hen begins to store calcium for egg shell production. This calcium is stored as medullary bone which is formed in the marrow spaces 10-14 days prior to the commencement of egg production (Taylor *et al.*, 1971) and may eventually comprise 4-5 g of the skeletal calcium. The major source of calcium for shell formation, however, is that obtained directly from the diet after passive and/or active absorption from the more acidic segments of the intestinal tract (Hurwitz and Bar, 1965).

The absorption of calcium is regulated by the bird's hormonal system as well as dietary concentrations of other nutrients, such as phosphorus and vitamin D. These considerations have been extensively reviewed and will not be considered here, but it is worth noting that of the dietary calcium, 78% is absorbed and 70% retained by the bird (Mueller *et al.*, 1964) although later work by Taylor and Kirkley (1967) produced a lower (57%) value. This discrepancy may be related to total calcium intake by the bird. Approximately 15 min after absorption, dietary calcium has been found as egg shell (Driggers and Comar, 1949). Dietary calcium may remain the sole source of calcium until 4-5 hr after shell formation commences, whereupon medullary bone is mobilized (Taylor *et al.*, 1971) following hormonal intervention to maintain blood calcium concentration.

There is debate as to the effectiveness of the provision of plasma calcium from these different sources as measured by shell quality and it seems that dietary calcium may be superior. Scott *et al.* (1971) stated that dietary calcium produced better shell quality than bone calcium. Earlier work by Taylor and Hertelendy (1962) and recent studies (Keshavarz *et al.*, 1993) support this theory. Naturally, once Ca^{2+} is in the plasma, the immediate source is unimportant. It is possible that in some situations (dietary calcium insufficiency) the bird will attempt to conserve medullary bone (Clunies *et al.*, 1992). Where dietary calcium is adequate, 60-75% of the shells' calcium is supplied by dietary sources (Driggers and Comar, 1949) although Tyler (1954) suggested that when shell formation normally occurs (late afternoon, evening and early morning), dietary calcium constitutes a greater proportion of the shell.

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

The study of calcium nutrition in layers has largely been empirical, although experiments using radioactive tracers have provided some illumination as to the mechanisms involved in calcium metabolism with respect to egg production. Indeed, Boorman *et al.*, (1989) stated that "much of the work aimed at redressing problems (shell quality) has been essentially empirical . . . (this) is less likely to be successful in identifying remaining problems and in ameliorating new problems".

The work presented here uses the practice of intermittent feeding of particulate calcium to elucidate current problems of calcium metabolism in layer hens.

Experiments and Discussion

These experiments were undertaken using 104 SIRO-CB (New Hampshire x Australorp) layers that had been trained to choice-feed from 8 weeks of age. From point of lay, the hens had been held in two-bird cages in an uninsulated shed on the poultry farm at the University of New England. The hens were fed whole wheat (100 g/kg crude protein, 12.4 MJ/kg ME), mixed with a protein-rich concentrate mash with no added calcium (422 g/kg crude protein, 10.9 MJ/kg ME) in one feed trough extending across half the cage front. A mixed sea-shell grit was freely available in an identical trough across the other half of the front of the cage.

Where appropriate, data were analysed by repeated measures analysis of variance (using the Greenhouse-Geisser correction factor where necessary) using the general linear models procedure of SAS (SAS Users Guide, SAS Institute, 1989, Cary, NC). Where necessary, significant means were separated by paired t-tests. Organ, feed and bone data in Experiment 2 were analysed by oneway analysis of variance using Minitab (Version 7, copyright Minitab Inc.).

At 37 weeks of age, the birds were randomly allocated to one of three dietary treatments viz; Tr1- Shell grit available *ad libitum* (n=17); Tr2- Shell grit freely available every second day (n=18); Tr4- Shell grit freely available every fourth day (n=17). The three treatments were allocated at random within adjacent groups of three cages along the line of cages. Daily grit consumption was recorded for the next 6 weeks. Grit consumption (Table 1) showed a highly significant ($P<0.01$) treatment x time interaction. The hens on Treatment 2 displayed a lower grit consumption early in the experiment but this increased later and differences in grit intake between treatments were not significant by the end of the experiment.

A similar experiment was undertaken using the same hens and treatments (re-randomized) and commenced when the hens were 46 weeks of age. At 56

Table 1 Daily grit consumption (g/bird) by hens offered *ad libitum* grit daily (Tr1), every second day (Tr2) or every fourth day (Tr4) in Experiment 1. (LS Means \pm SE)

Treatment	Week				
	1	2/3 γ	4	5	6
1	7.6 \pm 0.44	8.2 \pm 0.36 _a	7.6 \pm 0.36 _a	7.3 \pm 0.32 _a	6.8 \pm 0.38
2	7.5 \pm 0.44	6.5 \pm 0.36 _b	5.6 \pm 0.36 _b	6.3 \pm 0.32 _b	7.1 \pm 0.38
4	8.1 \pm 0.46	6.1 \pm 0.38 _c	7.3 \pm 0.38 _c	8.1 \pm 0.33 _c	6.9 \pm 0.39

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

γ Values for weeks 2 and 3 were averaged

weeks of age, at lights off on the last day of this experiment, 3 birds per treatment, randomly selected from a group of birds that had laid each day for the previous 5 days, were killed by cervical dislocation. At this time, hens on Treatments 2 and 4 had not had access to shell-grit for 24 hr and 72 hr respectively. Contents of the digestive organs were removed and weighed and wet weights of the organs recorded. Gut contents were washed and separated and then oven-dried for 48 hrs whereupon calcium particles were removed and weighed. The right femur of each bird was cleaned by boiling and careful scraping, fat removed by solvent (X-55) extraction for 12 hrs and the remaining bone ashed for 12 hrs at 600°C. At each stage, amounts of bone remnants were recorded. Samples were prepared for mineral analysis by Inductively Coupled Plasma (ICP) spectrophotometry using the method of Anderson and Henderson (1986) modified by performing a final 1 in 10 dilution.

The pattern of grit consumption by the hens in the subsequent experiment (Experiment 2) was similar (Table 2), such that a highly significant ($P<0.01$) treatment x time interaction was observed. The hens on Treatment 2 displayed a lower grit consumption early in the experiment (week 3) with the difference

between treatments becoming non-significant from week 4. Hens on Treatment 4 appeared to overcompensate for the period of denial by consuming more grit than non-restricted hens during weeks 1 and 9.

Total calcium consumption per hen (Table 3), calcium from both shell-grit and the protein concentrate, showed a highly significant ($P<0.01$) time x treatment interaction. As the proportion of protein concentrate consumed was similar across the treatments, the significant effects were due to the differences in grit intake.

Neither hen weight, crop, proventriculus or gizzard weights, nor their relative proportions of body weight, or total feed contents of these organs were significantly different across the treatments (Taylor, unpublished).

Grit contents of crop (Tr 1=6.1±0.64g; Tr 2=0; Tr 3=0) and gizzard (Tr 1=6.1_a; Tr 2=5.0_{ab}; Tr 3=2.9_b; LS Means ± 0.91) were significantly different ($P<0.05$) across treatments. The proportion of fat-free femur and ash content of fat-free femur were significantly different ($P<0.05$) but calcium and phosphorus content of femur ash were not significantly different across the treatments (Table 4).

Table 2 Daily grit consumption (g/bird) by hens offered *ad libitum* grit daily (Tr1), every second day (Tr2) or every fourth day (Tr4) in Experiment 2. (LS Means ± SE in parentheses)

Tr	W e e k									
	1	2	3	4	5	6	7	8	9	10
1	5.9 _b (0.36)	6.6 (0.31)	7.3 _a (0.32)	6.8 (0.34)	7.0 (0.44)	6.7 (0.32)	6.3 (0.31)	6.5 (0.36)	6.2 _b (0.42)	6.2 (0.30)
2	5.2 _b (0.38)	6.3 (0.33)	5.8 _b (0.34)	6.9 (0.36)	5.7 (0.46)	6.7 (0.34)	5.8 (0.32)	6.7 (0.38)	6.0 _b (0.45)	6.2 (0.31)
4	7.3 _a (0.37)	6.7 (0.32)	7.3 _a (0.33)	7.5 (0.35)	6.6 (0.45)	7.4 (0.33)	6.6 (0.31)	6.5 (0.37)	7.5 _a (0.43)	5.8 (0.31)

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

Table 3 Total daily calcium consumption (g/bird) by hens offered *ad libitum* grit daily (Tr1), every second day (Tr2) or every fourth day (Tr4) in Experiment 2. (LS Means ± SE in parentheses).

Tr	W e e k									
	1	2	3	4	5	6	7	8	9	10
1	3.0 _b (0.16)	3.5 (0.13)	3.7 _a (0.13)	3.5 (0.14)	3.4 (0.16)	3.5 (0.13)	3.3 (0.13)	3.3 (0.15)	3.3 _{ab} (0.17)	3.3 (0.12)
2	2.7 _b (0.17)	3.4 (0.13)	3.3 _b (0.13)	3.6 (0.15)	3.2 (0.17)	3.6 (0.14)	3.2 (0.14)	3.5 (0.16)	3.2 _b (0.18)	3.2 (0.13)
4	3.5 _a (0.15)	3.4 (0.13)	3.7 _a (0.13)	3.8 (0.14)	3.3 (0.16)	3.7 (0.13)	3.4 (0.13)	3.4 (0.15)	3.7 _a (0.17)	3.1 (0.12)

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

Table 4 Bone results from hens offered *ad libitum* grit daily (Tr1), every second day (Tr2) or every fourth day (Tr4) in experiment 2. (LS Means).

Tr	Solvent extract (fat-free femur) (%)	Ash (fat-free femur) (%)	Calcium (bone ash) (%)	Phosphorus (bone ash) (g)
1	88.6 _b	35.1 _a	45.7	20.8
2	97.3 _a	39.1 _{ab}	45.1	20.4
4	96.3 _a	40.1 _b	45.0	20.4
SE	1.77	1.01	2.17	1.06

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

At 63 weeks of age, a final experiment was commenced when 48 hens, identified as the best layers from the previous experimental studies, were placed in single bird cages and 4 randomly allocated treatments were imposed. The treatments ($n=12$) were as for Experiment 1 with a another treatment (here designated Tr 3), shell grit freely available throughout every third day. The experiment was run in three periods of 5 days at hen ages of 63, 66/67 and 70 weeks respectively. Calcium and phosphorus balances were determined at 70 weeks of age for all hens that laid for 5 consecutive days. Values for these balances were calculated from intakes of feed and grit and ICP analysis of excreta. Egg-shell calcium was included in the calcium excretion values.

The birds during Experiment 3 showed a highly significant ($P < 0.01$) treatment effect in grit consumption at 70 weeks of age only, and this was also reflected in total calcium intake (Table 5).

Calcium and total phosphorus intakes, excretion and balance are presented in Table 6. There were no significant differences across treatments for any measurement but calcium balance approached significance ($P=0.057$).

Table 5 Daily calcium from grit (g/bird) and total calcium intakes (in brackets) by hens offered *ad libitum* grit daily (Tr1), every second day (Tr2), every third day (Tr3) or every fourth day (Tr4) at 63, 66/76 and 70 weeks of age in Experiment 3 (LS Means).

Tr	Weeks					
	63		66/67		70	
1	2.7	(3.7)	2.2	(3.2)	2.9 _a	(3.5) _a
2	2.7	(3.6)	1.9	(2.9)	2.8 _{ab}	(3.7) _a
3	2.4	(3.4)	1.9	(3.0)	2.2 _{ab}	(3.0) _{ab}
4	1.9	(2.7)	2.1	(3.1)	2.0 _b	(3.1) _b
SE	0.30	(0.31)	0.27	(0.29)	0.27	(0.16)

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

Table 6 Calcium and total phosphorus intakes, excretion and balances (g) at 70 weeks of age of hens offered *ad libitum* grit daily (Tr1), every second day (Tr2), every third day (Tr3) or every fourth day (Tr4) in Experiment 3 (LS Means).

Tr	Calcium			Phosphorus		
	Intake	Excretion	Balance	Intake	Excretion	Balance
1	12.6	7.2	5.4	2.0	2.7	-0.7
2	11.7	8.3	3.4	3.2	3.4	-0.1
3	13.5	7.4	6.1	3.3	3.3	0
4	7.7	5.9	1.8	2.7	2.9	-0.2
SE	1.85	1.31	1.01	0.35	0.19	0.21

The effect of non-laying on the first day of the grit provision cycle on calcium intake and egg production is presented in Table 7. The low number of non-laying birds made an analysis on treatment inappropriate and the figures given only provide an overall indication of the effects.

It appears from these trials that the laying hen has the capacity to rapidly adjust her intake of particulate calcium when denied access to calcium for fixed periods. The lower intake of grit during some weeks by the hens on Tr2 in the first two experiments may possibly be due to a temporary improvement in calcium retention, this effect being modified as calcium from the gizzard was reduced over time which led to a slightly higher intake each week. This improvement in calcium retention may be possible as no significant differences in either feed intake, proportions of feed constituents consumed or egg production were recorded for hens on any treatment. A negative phosphorus balance indicates that bone is being mobilized to provide calcium (Shafey, 1993). The hens on Treatment 2 although in negative phosphorus balance, may not have been losing as much as those fed *ad libitum* calcium daily. What is interesting is that there is a large turnover of bone calcium under normal conditions and intermittent feeding of particulate calcium may allow most of the egg-shell calcium to be provided by dietary calcium.

The total calcium intake by the birds in Experiment 1 was similar to that recommended by the NRC (1994) for brown egg layers but the total calcium

intake by the hens in Experiment 2 was lower than these guidelines. The lower intake was in line with the lower production later in lay. Egg production was not significantly different across treatments in Experiments 1 or 2 and was 94 % in Experiment 1 and 85 % in Experiment 2. It appears that these choice-fed hens finely adjust calcium intake by physiological means acting on a cognitive calcium memory.

The relatively large particle size of the grit used in these experiments may allow for longer term availability of calcium for absorption as it is metered from the gizzard Scott *et al.* (1971). This in turn may allow prolonged synthesis of medullary bone when shell formation is not occurring (Taylor, 1970) so providing a greater reserve for future shell synthesis. The fat-free femur weight and ash content indicated that hens given daily access to the grit may have had lower medullary bone formation than those offered grit intermittently. The phosphorus balance data indicated that this bone may also turn over more rapidly when birds have constant access to grit. The hens on Treatments 2 and 4 which consumed double or triple the quantity of grit, may have stimulated medullary bone formation due to greatly raised blood Ca^{2+} immediately following consumption of this quantity of grit. Whitehead *et al.* (1994) noted an increase in medullary bone in hens fed on shell grit compared with those given limestone flour. However, there appears to be a limit to the capacity of the gizzard reserve of calcium to meet calcium requirements for shell formation and hence medullary bone calcium is then

Table 7 Effect of laying or non-laying on day 1 of the grit provision cycle on day 1 and 4 day LS Mean calcium (Ca) intakes from grit and protein concentrate of hens offered *ad libitum* grit daily (Tr1), every second day (Tr2), every third day (Tr3) or every fourth day (Tr4) at 63, 66/76 and 70 weeks of age in Experiment 3 (LS Means \pm SE in brackets).

	63 Weeks		66/67 Weeks		70 Weeks	
	non-layer (n=9)	layer (n=39)	non-layer (n=9)	layer (n=39)	non-layer (n=8)	layer (n=40)
Ca intake (g) (from grit) day 1	0.4 (0.30) a	2.3 (0.14) b	1.4 (0.35) a	2.6 (0.16) b	1.4 (0.31) a	2.7 (0.14) b
Ca intake (g) (from conc. Y) day 1	1.0 (0.10)	0.9 (0.05)	1.1 (0.14)	1.0 (0.06)	0.8 (0.14)	1.0 (0.06)
Egg production (%) day 1	0 a	100 b	0 a	100 b	0 a	100 b
Egg production (%) 4 day mean	61.1 (5.34) a	91.7 (2.57) b	61.1 (6.01) a	86.5 (2.89) b	62.5 (5.18) a	93.6 (2.34) b

Values across week columns with different subscripts are significantly different ($P < 0.05$).
Y calcium from protein concentrate.

utilized. This is evinced by the poorer shell parameter results obtained from hens subjected to Treatment 3 after 3 days without access to grit (data not presented). This **finding** is similar to those of Taylor and Hertelendy (1962), Scott et al. (1971) and Keshavarz et al. (1993) who noted decreases in shell quality related to medullary bone mobilization.

The shell grit (380 g calcium/kg) was apparently identified as the sole calcium source and the protein concentrate (40 g calcium/kg) was not selected for in Experiments 1 and 2. This is similar to the proposal of Hughes (1972) that at a 1% level of dietary calcium, feed intake may be regulated by calcium, rather than energy appetite, but a low level (0.2%) may result in feed intake being independent of dietary calcium. It appeared that the protein concentrate consumption increased as shown in Experiment 3 where a non-significant trend in increasing protein concentrate intake was found in birds on Treatments 3 and 4.

The capacity of the laying hen to rapidly adjust the intake of her calcium to account for periods of denial is remarkable in that she rapidly adjusts her intake of calcium to a close multiple of the time of denial by the relative daily intake of those birds allowed *ad lib.* access. Consideration was made to extend the period of deprivation in Experiment 2 to determine the period of time that the bird would attempt to compensate for this denial. It was considered that as shell quality subsequently deteriorated from three days of denial and a non-significant trend to lower egg production was apparent, then, from a practical point of view, it was deemed inappropriate to extend this denial. In the final period of Experiment 3 a relationship was found between energy metabolizability of the diet selected by the hens and calcium intake and this is currently being pursued. The use of two different calcium isotopes to determine rates of calcium absorption and fluxes into and out of the skeleton is being pursued.

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