

## Effect of separate calcium feeding and a limited feeding program on the metabolizable energy of the diet and on nitrogen, calcium and phosphorus retention in laying hens

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**Summary.** We first carried out an 8-day balance study with 8 laying hens fed *ad libitum* a low Ca diet (0.30 p. 100 Ca) and a maximum of 14 g/day/hen of seashells in order to study whether totally separate Ca feeding (SCF) would increase the corrected metabolizable energy (cME) value of the diet and the retention of nitrogen, calcium and phosphorus. These experimental birds (group 2) were compared to 7 control birds (group 1) fed *ad libitum* a normal Ca diet (3.7 p. 100 Ca).

During egg-forming (LO) days, the SCF treatment induced significant increases (table 2) in feed intake (121 vs 110 g/d) and in corrected metabolized energy (377 vs 342 Kcal/d). Neither the cME value nor N, P or Ca retention was significantly modified. During the non-egg-forming (L) days, the SCF treatment (table 4) considerably decreased Ca source intake (3.8 vs 11.8 g/d), resulting in a negative Ca balance and an increase in the cME value of the ingested feed mixture (2988 vs 2685 Kcal/kg). However, the difference was not significant (3087 vs 2984) when Ca intake was corrected. When all the types of days were pooled (L + O + LO), the recorded effects of SCF were about the same as during the LO days.

In a second trial, the amounts of feed offered were limited to 120 g/d/hen in control group 1 and to 108 g in group 2 (plus 12 g/d/hen of seashells). During LO days (table 3), only Ca retention increased (55 vs 48 p. 100) due to SCF, while the same response as in the first trial was recorded for ME and Ca metabolism during L days (table 4).

The results are first discussed from a methodological point of view to underline the importance of the laying rate controlling L and LO-day ratios during balance studies in laying hens. Then it is concluded that SCF might only be applied with a limited feeding program in order to avoid over-consumption of feed. When this is carried out, the relative calcium retention increases during LO days but the cME value of the diet and nitrogen retention does not. SCF tends to increase egg-shell quality but is probably unfavourable to calcium stores when the L days are numerous. It is concluded that it is probably better to leave 1 p. 100 of Ca as ground limestone than to use totally separate Ca feeding.

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### Introduction.

Many studies have shown (see review by Mongin and Sauveur, 1979) that when a laying hen is given a choice between a calcium source and the rest of its ration, the bird does not consume a diet of constant composition over a period

of time, *ie* the proportion of calcium ingested is related to the presence or absence of ovulation, the hour of the day and to shell secretion itself. Separate calcium feeding (SCF) is thus a method which permits the hen to adjust calcium intake to its needs, but it is not known whether digestibility and the retention of different nutrients in the ration are affected. Charles (1972) and Kuhl *et al.* (1977) have reported that dietary efficiency (the number of g of feed needed to produce 1 g of egg) could be improved by SCF but, to our knowledge, no other authors from Moran *et al.* (1970) to Sauveur (1981) found this to be true.

We know that the metabolizable energy (ME) of dietary lipid is decreased by the dietary calcium content (Sibbald *et al.*, 1961 ; Sibbald and Price, 1977) ; in the laying hen Tortuero and Centeno (1973) reported the slightly favourable effect of SCF on fat and cellulose digestibilities, while Moran *et al.* (1970) estimated that carcasses were leaner with SCF. According to Tortuero and Centeno (1973), a supply of 1.14 g/day of calcium in the form of SCF, supplementing a 3.86 p. 100 Ca diet, considerably increased nitrogen retention. These authors interpreted their results as indicating that SCF led to better separation of feed particles in the upper digestive tract and/or to higher enzyme activity.

In practice, it is very important to know the true ME value of a diet, especially since the technique of raising hens at high room temperature is becoming widespread and requires progressively concentrated feeds. The quantitative limitation of the diet during the laying period is also a major question but the margins of manipulation are very limited (Leclercq *et al.*, 1975).

To answer these questions and to redress the lack of information in the studies mentioned above, we carried out two trials to determine if : 1) SCF with *ad libitum* feeding would increase energy intake and retention of nutrients and – 2) if SCF would permit better metabolic utilization of the diet when the intake was limited and constant.

## Material and methods.

1. *Animals and diets.* — Balances were carried out with 15 Warren ISA hens which were 34 weeks old at the beginning of the trial. They were housed in individual cages in a building with controlled temperature ( $20 \pm 2$  °C) and humidity. The light/dark cycle was 14 h of light (from 9 to 23 h) and 10 h of darkness.

Two diets were given ; the control diet A (table 1) contained 10 p. 100 of crushed seashells (providing 34.6 p. 100 Ca) and 0.8 p. 100 of dicalcium phosphate ; its total calcium content was 3.72 p. 100. The second diet (experimental diet B) contained only 0.30 p. 100 Ca and was given as a supplement to seashells offered separately in the form of particles (diameter : 2-6 mm).

2. *Experimental procedure.* — For 3 weeks before the trials, 7 hens (group 1) were trained to eat control diet A and 8 hens (group 2) were trained to eat the Ca-deficient diet B and the seashells offered separately.

*Experiment 1.* — Balances were carried out for 7 days (see below) while the control birds were fed *ad libitum*. The experimental birds (group 2) received the

TABLE 1  
Diet composition (p. 100)

	Diet A (complete control diet)	Diet B (Ca-deficient diet)
Cornmeal .....	31.50	35.00
Wheatmeal .....	34.20	38.00
Soybean meal (50) .....	19.35	21.50
Tallow .....	3.33	3.70
Sodium chloride .....	0.20	0.22
Bicalcic phosphate .....	0.85	0.94
Trace minerals .....	0.09	0.10
Vitamin mixture .....	0.45	0.50
DL-methionine .....	0.03	0.04
Seashells .....	10.00	—
Total .....	100.00	100.00
<i>Chemical analysis</i>		
Dry matter (p. 100) .....	89.1	89.0
Gross energy (Kcal/kg) .....	3 590	3 998
Protein (N × 6.25) (p. 100) .....	15.45	17.11
Ca (p. 100) .....	3.72	0.30
P (p. 100) .....	0.53	0.60

Ca-deficient diet *ad libitum* and 14 g/d/hen of seashells (4.84 g of Ca) given separately.

*Experiment 2.* — At the end of experiment 1, the amount of feed offered to the experimental hens (group 2) was limited to 108 g/d/hen so that their energy intake equalled that of the control birds (group 1) then receiving 120 g/d/hen of feed. The amount of seashells offered to group 2 was also reduced to 12 g/d/hen (4.15 g of Ca). After the birds had been accustomed to these new conditions for one week, the balances were carried out for another 7-day period.

*Balance methodology* (fig. 1). — After having fasted (with water) the birds first for 24 h, we fed them for 7 days at 9 h at the beginning of the light period ; on day 8 they were fasted again for 24 h. The individual intakes of the diet and the seashells were assessed to 0.5 g by weighing the boxes every morning. Taking into account the observation of Mongin and Sauveur (1974) that no food is eaten during the night under the light/dark cycle we used, the recorded intakes were assumed to be totally representative of the previous day. The faeces of each hen were also collected every morning and subtracted from the intake of the previous day in order to calculate the daily balances, assuming that 10 h were enough to obtain quasi-total clearance of the intestine. The faeces collected after the last day of fasting were pooled with those of the day before when calculating the total balances. The eggs were collected from day 1 of refeeding to the last day of the fast (fig. 1).

### 3. Analysis.

*Sample preparation.* — Three samples of each diet and of the seashells were dried in an oven at 110 °C for 24 h before analysis. The faeces collected

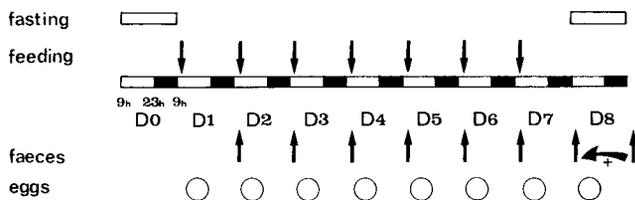


FIG. 1. — Diagram of the balance procedure used.

every morning were stored at  $-15^{\circ}\text{C}$  before 48-hour lyophilization. Each egg was weighed and then separated into white, yolk and shell with membranes. During the week, two « pools » were constituted for each hen, one of white and the other of yolks ; these were homogenized with an Ultraturax, lyophilized and weighed.

*Statistical analysis.* — The crude dry matter, total crude nitrogen ( $\text{N} \times 6.25$ ) and total calcium and phosphorus of each sample of the diets and the faeces were measured ; only the first three were measured in eggs. The gross energy (Kcal/g) was measured using a bomb calorimeter (Gallenkampft Q2572). To calculate the metabolizable energy of the diets, taking into account or not the ingested calcium source, we used two formulas :

$$\text{ME1} = \frac{\text{GE}_{\text{in}} - \text{GE}_{\text{ex}}}{Q_1} \text{ and } \text{ME2} = \frac{\text{GE}_{\text{in}} - \text{GE}_{\text{ex}}}{Q_2}$$

where  $Q_1$  = quantity of diet ingested (excepting the main Ca source),  $Q_2$  = total quantity ingested (including the Ca source),  $\text{GE}_{\text{in}}$  = gross energy ingested,  $\text{GE}_{\text{ex}}$  = gross energy of the excreta.

To correct ME in relation to nitrogen retention, we used the formula :

$$\text{cME} = \frac{\text{GE}_{\text{in}} - \text{GE}_{\text{ex}} - 8.22 (\text{N}_{\text{in}} - \text{N}_{\text{ex}})}{Q}$$

where  $\text{N}_{\text{in}}$  = ingested nitrogen and  $\text{N}_{\text{ex}}$  = nitrogen of the excreta. We used the coefficient of 8.22 employed by Hill and Anderson (1958), supposing that uric acid was the only form of nitrogen excreted.

Total nitrogen was measured by the Kjeldhal method. To assess Ca and P, samples were ashed in an oven at  $550^{\circ}\text{C}$  for 24 h, then redissolved in nitric acid and filtered. Ca was assayed by flame photometry (Eppendorf) and P by calorimetric method using a Technicon autoanalyzer.

## Results.

We used the terminology of Wood-Gush and Horne (1970) to designate the different successive days within the clutch : O = day with ovulation of the first egg of the clutch (day of pause), LO = day with ovulation and oviposition, L = day with oviposition of the last egg of the clutch (no ovulation). The LO days were the most numerous ( $> 80$  p. 100) because the hens used in the present study were at the onset of the laying period and thus presented very few days of pause.

In order to avoid possible bias due to differences in laying intensity or to the fasting periods, we first calculated only the data recorded during the LO days, excluding those of the first and last days of the balance period. The effects of SCF were tested by variance analysis using individual hen  $\times$  day data. We then calculated the « total » balances by adding the seven daily values of each hen to determine to what extent the capacity of the SCF hens to regulate their calcium intake affected the total retention of the other nutrients. Variance analysis was carried out with 7 or 8 values per group only.

Finally, after exclusion of the first and last days of balance periods, the values of each type of day (L, O and LO) were calculated separately using only hens presenting at least one L and one O day. The individual LO means of the same hens were compared by the paired t-test.

### 1. LO-day balances and total balances.

*Experiment 1 : ad libitum feeding.* — The means of intake, excretion and retention recorded during LO days are shown on the left-hand side of table 2. While the Ca source intake was the same in both groups of hens, the intake of the remaining ration, as well as dry matter excretion, were significantly increased by SCF (+ 11.0 and + 3.2 g/d/hen, respectively). In both groups this excretion represented 28 p. 100 of the ingested dry matter. Seashell intake with SCF (11.9 g) showed that the daily amount furnished (14 g) probably corresponded to *ad libitum* feeding.

The observed differences for energy and nitrogen balances between the two groups were similar to those reported for feed intake, that is ingested and excreted energy was highest in group 2. The difference between the ingested and excreted energy was also higher with SCF, but the value of the corrected ME of the diet, calculated from these data ( $cME_1$ ), did not differ significantly between the two groups during LO days. Taking ingested calcium into account when calculating obviously lowered the ME of the mixture considerably ( $cME_2$ ) but did not change the previous conclusion. The hens on SCF thus composed their own diet which had the same energy value as the control diet. The same remarks are true for nitrogen balance ; ingested and excreted N increased significantly ( $P < 0.01$ ) in group 2 compared to the control group, but nitrogen retention was not significantly different.

Total ingested Ca was the same in the two groups. Calcium retention tended to decrease slightly but not significantly in group 2. P balances were not significantly affected by SCF.

An examination of the total balances (table 2, right-hand side) confirms these results. The significance levels only differed slightly in some cases ; the  $ME_2$  value of the total diet (including the Ca source) appeared to increase significantly and Ca retention decreased by SCF due to the low consumption of seashells during L days (see below).

*Experiment 2 : feeding limited to 120 g/d/hen in the control group and to 108 g of Ca-deficient feed plus 12 g/d/hen of seashells in group 2.* — The restricted feeding program used in this experiment allowed us to actually obtain

TABLE 2

Experiment 1. — Effects of separate calcium feeding (SCF) on nutritional balance under ad libitum feeding conditions  
(Values of  $\bar{X}$  and  $\sigma/\sqrt{n}$ ) in g/day/hen, except where indicated

	L0 days only <sup>(1)</sup>				Whole balance period <sup>(2)</sup>			
	Control group 1		SCF group 2		Control group 1		SCF group 2	
	29	27	27	8	7	8	8	
Dietary intake without Ca-source	110.0 (2.7)	121.0 (2.6) *	110.2 (4.1)	122.0 (4.4) °				
Seashell intake	12.2 (0.3)	11.9 (0.4)	12.2 (0.4)	10.8 (0.7)				
Total intake	122.2 (3.0)	133.0 (2.8) *	122.5 (4.5)	132.8 (4.4)				
Seashell intake in p. 100 total intake	10.0	8.95	10.0	8.13				
Faecal dry matter	30.0 (1.0)	33.2 (1.1) *	30.7 (1.0)	33.0 (1.1)				
Gross energy ingestion (Kcal/d/hen)	438.7 (9.6)	483.7 (10.4) *	439.8 (14.7)	487.7 (17.6) °				
GE excretion (Kcal/d/hen)	85.4 (3.1)	94.9 (3.0) *	84.4 (2.5)	93.1 (4.1)				
Corrected metabolized En. (Kcal/d/hen)	342.2 (8.7)	376.3 (8.9) **	343.0 (13.8)	381.2 (13.6) °				
Corrected Metabolizable energy (Kcal/kg feed)	3100 (15) 2790 (14)	3106 (19) 2825 (20)	3110 (16) 2799 (14)	3124 (20) 2868 (27) *				
N ingestion	3.04 (0.07)	3.32 (0.07) *	3.03 (0.11)	3.34 (0.12) °				
N excretion	1.49 (0.05)	1.72 (0.06) **	1.52 (0.05)	1.69 (0.06)				
N retention	1.54 (0.07)	1.60 (0.07)	1.50 (0.11)	1.65 (0.12)				
N retention in p. 100 N ingestion	50.5 (1.7)	48.2 (1.8)	49.0 (2.5)	49.0 (1.5)				
Ca ingestion	4.54 (0.11)	4.49 (0.14)	4.55 (0.17)	4.11 (0.24) °				
Ca ingestion in p. 100 total intake	3.72	3.38	3.71	3.09				
Ca excretion	2.68 (0.14)	2.79 (0.16)	2.49 (0.16)	2.51 (0.20)				
Ca retention	1.86 (0.14)	1.70 (0.19)	2.06 (0.16)	1.60 (0.15) °				
Ca retention in p. 100 Ca ingestion	41.2 (2.6)	37.8 (3.8)	45.2 (2.8)	39.0 (3.0)				
P ingestion	0.647 (0.016)	0.726 (0.016) **	0.650 (0.02)	0.732 (0.02) *				
P excretion	0.463 (0.024)	0.503 (0.018)	0.484 (0.02)	0.500 (0.02)				
P retention	0.184 (0.025)	0.223 (0.022)	0.165 (0.03)	0.231 (0.03) °				
P retention in p. 100 P ingestion	28.6 (3.0)	30.5 (2.9)	25.0 (3.8)	31.5 (2.1)				

(1) Excluding the values of each hen on the first and last days of balance; (2) calculated by adding the seven daily values of each hen, whatever the day in the clutch, and then dividing by seven.

°  $0.05 < P < 0.10$ ; \*  $P < 0.05$ ; \*\*  $P < 0.01$ .

cME<sub>1</sub> = corrected metabolizable energy without Ca source; cME<sub>2</sub> = corrected metabolizable energy with Ca source.

Experiment 2. — Effects of separate calcium feeding (SCF) on nutritional balance under limited feeding conditions  
(Values of  $\bar{X}$  and  $\sigma/\sqrt{n}$ ) in g/day/hen, except where indicated

	L0 days only <sup>(1)</sup>		Whole balance period <sup>(2)</sup>	
	Control group 1	SCF group 2	Control group 1	SCF group 2
Number of data	29	27	7	8
Dietary intake without Ca-source	103.2 (0.8)	104.2 (0.6)	103.2 (1.0)	104.3 (0.7)
Seashell intake	11.5 (0.1)	11.3 (0.2)	11.5 (0.1)	10.2 (0.3)**
Total intake	114.7 (0.9)	115.5 (0.6)	114.7 (1.2)	114.5 (0.9)
Seashell intake in p. 100 total intake	10.0	9.78	10.0	8.91
Faecal dry matter	26.9 (0.9)	27.4 (0.7)	27.4 (0.8)	27.7 (0.5)
Gross energy ingestion (Kcal/d/hen)	411.8 (2.8)	416.6 (2.4)	411.8 (2.8)	416.6 (2.8)
GE excretion (Kcal/d/hen)	74.4 (2.4)	77.3 (2.1)	77.6 (2.0)	78.3 (1.5)
Corrected metabolized En. (Kcal/d/hen)	325.0 (3.8)	327.0 (2.7)	322.4 (3.2)	326.8 (2.0)
Corrected Metabolizable energy (Kcal/kg feed)	{ cME <sub>1</sub> 3146 (22) 2831 (19)	{ 3138 (17) 2831 (15)	{ 3122 (11) 2810 (10)	{ 3135 (10) 2855 (10)**
N ingestion	2.83 (0.02)	2.86 (0.02)*	2.83 (0.03)	2.86 (0.01)
N excretion	1.29 (0.05)	1.36 (0.04)	1.40 (0.07)	1.43 (0.04)
N retention	1.54 (0.05)	1.49 (0.04)	1.43 (0.05)	1.42 (0.04)
N retention in p. 100 N ingestion	54.2 (1.9)	52.2 (1.3)	50.7 (2.2)	49.8 (1.4)
Ca ingestion	4.27 (0.03)	4.22 (0.08)	4.27 (0.04)	3.86 (0.11)**
Ca ingestion in p. 100 total intake	3.72	3.65	3.72	3.37
Ca excretion	2.20 (0.07)	1.89 (0.09)*	2.15 (0.07)	2.02 (0.09)
Ca retention	2.06 (0.09)	2.33 (0.09)*	2.11 (0.04)	1.83 (0.15)
Ca retention in p. 100 Ca ingestion	48.2 (1.9)	55.2 (1.8)*	49.5 (1.2)	47.4 (3.2)
P ingestion	0.608 (0.006)	0.626 (0.004)	0.608 (0.006)	0.626 (0.004)
P excretion	0.371 (0.013)	0.374 (0.014)	0.407 (0.013)	0.403 (0.011)
P retention	0.236 (0.014)	0.252 (0.014)	0.201 (0.014)	0.222 (0.012)
P retention in p. 100 P ingestion	38.7 (2.3)	40.2 (2.2)	33.1 (1.8)	35.5 (1.8)

(1), (2), \*, \*\* : see table 2.

the same intake of calcium and of the rest of the ration in both groups during LO days (table 3, left-hand side). Faecal excretion no longer differed significantly.

As compared to the previous experiment (table 2), there was a large reduction in feed intake, especially in the SCF lot ( $-16.8$  vs  $-6.8$  g/d in the controls). The elimination of faecal dry matter was reduced by about the same proportions ( $-5.8$  and  $-3.1$  g/d, respectively) as those of energy and nitrogen. This control of ingested quantities led to a slight decrease in the absolute values of metabolized energy but increased relative nitrogen retention (53 vs 49 p. 100 in experiment 1); the  $ME_1$  value of the diet also increased (3142 vs 3103 Kcal). No variation in these parameters due to SCF was observed.

The mixture composed by the hens on SCF was slightly richer in Ca in this trial than in the previous one (3.7 vs 3.4 p. 100). However, owing to the limited feeding, daily Ca intake was slightly lower (4.2 vs 4.5 g/hen). The most interesting result in this respect was the reduction of excreted Ca observed in group 2 (1.89 g/d vs 2.20 g in group 1 and 2.79 in the same group 2 during experiment 1) in which SCF caused a significant improvement of calcium retention during LO days; this retention attained an average of 55.2 vs 48.2 p. 100 in the control group. The same observations were true for phosphorus; its excretion was reduced in this experiment compared to the previous one (0.37 vs 0.48 g/d/hen), resulting in a high increase in P retention in both groups. SCF had not effect at that level.

The results obtained on the whole balance period (table 3, right-hand side) were about the same as those reported above for LO days. As during experiment 1, the main variations concerned the effect of SCF on calcium balance and the  $ME_2$  value of the diet.

*2. Comparison of L, O and LO-day balances.* — Although we obtained very few data on L (oviposition of the last egg of the clutch) and O (ovulation of the first egg of the clutch) days, we report them here for the useful information they contain.

*Experiment 1* (table 4, left-hand side). — The recording of spontaneous Ca source intake confirmed the astonishing ability of the hen to almost totally suppress calcium intake during the L days when the Ca source was offered separately (3.8 g/d/hen of seashells vs 11.8 g in the controls). During L days, the ME value of the total diet (including the Ca source) was much higher with SCF (2988 Kcal/kg) than in the control group (2685 Kcal/kg). This result might be attributed mostly to the composition of the mixture composed by the hen since, with SCF, it contained no more than 1 p. 100 of calcium as against 3.7 p. 100 in the control diet. However, if the ME of the Ca-free diet is considered, it was still not significantly higher than diet A during the L days (3087 vs 2984 Kcal/kg).

Variations in nitrogen retention (in p. 100 of intake) in relation to the position of the egg in the clutch were similar to those described for energy, that is on L days and with *ad libitum* feeding (experiment 1), nitrogen retention tended to be better in birds on SCF than in the controls (49 vs 43 p. 100). The most characteristic results concern calcium balances since it is clear that Ca retention became negative in the SCF group during L days. On the contrary, during O and

Combined effects of separate calcium feeding (SCF) and of day within a clutch on ingestion and retention. Mean values in g/day/hen  
 Clutch days : L = oviposition only (last day of clutch) ; O = ovulation only (day of pause) ;  
 LO = ovulation and oviposition (middle of clutch)

Number of hens (1)	Control SCF	Experiment 1 (ad libitum feeding)				Experiment 2 (limited feeding)			
		L	O	LO	LO	L	O	O	LO
Dietary intake without Ca source	{ Control SCF	106.2 120.0	100.5 112.7	107.5 (2) 117.4 (2)	101.0 105.2	103.5 105.8	103.2 (2) 103.9 (2)		
Seashell intake	{ Control SCF	11.8 3.8***	11.2 12.7	11.9 12.6	11.2 2.8***	11.5 11.4	11.5 11.4		
Total intake	{ Control SCF	117.9 123.8	111.7 129.4	119.3 130.2	112.2 108.0	115.1 117.2	114.7 115.7		
Fecal dry matter	{ Control SCF	34.4 33.4	28.0 30.3	31.5 33.0	28.2 27.7	27.4 27.7	26.6 27.8		
cMet. Energy without Ca source (Kcal/kg)	{ Control SCF	2984 3087	3119 3082	3071 3124	3031 3093	3160 3192	3142 3130		
cMet. Energy with Ca source (Kcal/kg)	{ Control SCF	2685 2988**	2807 2764	2784 2808	2728 3017*	2844 2883	2831 2819		
N retention (%)	{ Control SCF	43.3 49.5	46.4 45.1	48.0 46.3	54.1 49.5	50.2 51.6	53.1 51.2		
Ca retention (g/d/hen)	{ Control SCF	1.89 - 1.50***	1.57 2.79	1.79 1.91	1.73 1.68***	1.72 2.11	2.11 2.39		
Ca retention (%)	{ Control SCF	41.1 < 0	36.9 63.3*	36.5 40.6	41.6 < 0	40.4 49.5	49.4 55.8		
P excretion (g/d/hen)	{ Control SCF	0.437 0.392	0.373 0.437	0.475 0.467	0.390 0.358	0.358 0.376	0.370 0.369		
P retention (%)	{ Control SCF	32.7 38.4	35.5 23.7	35.4 31.6	34.4 42.4	43.9 40.4	38.9 40.9		

(1) We used only hens presenting at least one L and one O day after exclusion of the first and last balance days. The individual means of the same hens were used for LO days. (2) The values for LO days may differ slightly from those in table 2 or 3 since we used fewer hens here.

\* P < 0.05 ; \*\*\* P < 0.01 ; comparison of control and SCF means for the same type of day. F-test performed on individual means.

Underlining indicates the values obtained with a same treatment and in a same experiment which did not differ significantly according to the paired t-test.

LO days Ca retention tended to be better in SCF than in control birds. Finally, phosphorus retention tended to vary inversely with calcium retention.

*Experiment 2* (table 4, right-hand side). — When the ingested amounts were limited, the SCF effects — low Ca intake and negative Ca balance during L days and increased ME of the mixture composed by the hens during those days — remained about the same.

3. *Egg characteristics.* — The effect of the treatments used on the main egg characteristics is shown on table 5. An unexpected effect of SCF was the slight increase in the weight of the egg-white in both experiments. Egg-shell weight and index also tended to increase in the SCF groups, but the differences between those groups and the controls were not significant.

At the bottom of table 5 we have presented a theoretical calculation of the calcium « yield » as the ratio between daily retained Ca and that secreted into the egg-shells actually produced during the balance period : shell Ca in the controls represented 82 and 78 p. 100 of the Ca retention during experiments 1 and 2, respectively. The symmetric figures of the SCF groups were 100 and 89 p. 100.

## Discussion.

The aim of the present nutritional balance studies was to determine whether the apparent digestibility of the main nutrients — energy, nitrogen, calcium and phosphorus — was affected in hens offered dietary calcium solely in the form of seashells separately from the rest of the ration.

A remark concerning the methodology used to obtain the balances is in order because the interpretation of the results presents problems particular to laying hens due to the occurrence of clutches. As mentioned above, L, O and LO days are distinguished according to whether there is oviposition, ovulation only, or both. During L days there is no egg-white secretion and very little shell secretion (only for several hours in the morning) ; it is thus assumed that the retention of some nutrients is modified on these days.

The two days of fast, one at the beginning and one at the end of the balance period, also lead to daily variations in retention which usually cancel one another (fig. 1) ; the highly positive balance of the first day of refeeding is supposedly compensated by the lower balance of the last day, including the faeces collected during the last fast. One difficulty in our study was that these two particular days might have corresponded to other particular days in the clutch and not necessarily to the LO days which are the most representative. Unless a study is carried out over long balance periods (which is materially impossible), there is no simple way to completely circumvent this difficulty.

We resolved this problem only partly by 1) first using only the LO days and eliminating the first or last balance days and then 2) by considering all the days recorded, except the first and last days of balance which were special due to their place in the clutch. With this method, our results on the ingested amounts entirely confirm those already obtained by some authors as Taylor (1970),

TABLE 5  
*Effects of separate calcium feeding (SCF) and limited feeding program on egg characteristics*  
 Values of  $\bar{X}$  and  $(\sigma/\sqrt{n})$

	Experiment 1 (ad libitum)		Experiment 2 (limited feeding)	
	Control (A) n = 50	SCF (B) n = 54	Control (A) n = 47	SCF (B) n = 53
Egg weight (g) .....	55.9 (0.4)	57.0 (0.5)	55.3 (0.5)	56.7 (0.4)
Egg-white weight (g) .....	35.7 (0.3)	37.1 (0.3)	34.9 (0.4)	36.4 (0.3)**
in p. 100 of total .....	63.9	65.1**	63.0	64.2
Egg-white dry matter (g) .....	3.76 (0.09)	4.06 (0.12)	3.64 (0.08)	3.98 (0.10)
Egg-yolk weight (g) .....	15.1 (0.1)	14.7 (0.2)	15.2 (0.1)	15.0 (0.1)
in p. 100 of total .....	27.0	25.8	27.5	26.5
Egg-yolk dry matter (g) .....	7.22 (0.13)	7.08 (0.21)	7.27 (0.10)	7.22 (0.10)
Egg-shell weight (g) .....	5.05 (0.09)	5.22 (0.10)	5.14 (0.07)	5.33 (0.09)
in p. 100 of total .....	9.0	9.2	9.3	9.4
Egg-shell index (g/100 cm <sup>2</sup> of surface) .....	7.41 (0.09)	7.54 (0.19)	7.62 (0.16)	7.72 (0.17)
Ca retained (g/7 days) <sup>(1)</sup> .....	14.43	11.21	14.80	12.85
Ca exported into egg-shells <sup>(2)</sup> .....	11.84	11.19	11.50	11.43
Ca exported in p. 100 Ca retained .....	82.0	99.9	77.7	89.0

<sup>(1)</sup> Estimated from the whole balance period, including L, O and LO days. <sup>(2)</sup> Calculated by (shell weight)  $\times$  0.373  $\times$  number of shells secreted during the whole balance period.

\*\* Significant difference ( $P < 0.01$ ) as compared to controls in the same experiment.

Mongin and Sauveur (1974), Sauveur and Mongin (1974) and Karunajeeva (1978) who reported that the intake of the basal diet (without Ca), if it was not limited, was always higher with SCF than in the controls. The total intake of the basal diet plus the Ca source was also higher with SCF, although overall Ca intake was lower with SCF due to the fact that this intake was almost zero on L days. These inter-group variations disappeared almost entirely when the intake was slightly limited. The amounts of faecal dry matter varied proportionally to the volumes ingested and were thus lower with limited intake.

The increased ingestion due to SCF caused an increase in metabolized energy (+ 34 Kcal/d/hen) and tended to augment the ME value of the complete diet during LO days ; however, this trend was not significant and totally disappeared when the ingested volumes were equalized (experiment 2).

The slight increase in the ME of the Ca-free diet ingested by the hens on SCF during the L days is interesting ; it could mean that the « useless » inevitable absorption of Ca by the control hens on L days caused a slight subutilization of the energy. This result is comparable to those obtained by Fedde *et al.* (1969), Yacowitz and Boyko (1962) and Sibbald and Price (1977) in the adult rooster ; these authors all showed that when dietary Ca content is elevated, fat ME decreases. Only Tortuero and Centeno (1973) reported that fat digestibility in the laying hen increased with the Ca supply. In the present study, we could not determine which of the carbohydrate or lipid fractions of the diet was affected by the level of ingested Ca, but it is clear that reducing ingested Ca appeared to enhance the ME of the diet.

Nitrogen retention (usually 50 p. 100) is difficult to change. Using a diet containing 3.83 p. 100 calcium plus 1.14 g/d/hen of Ca offered as granules, Tortuero and Centeno (1973) reported a 67 p. 100 nitrogen retention which was completely unexpected ; no trend of this type was found in our study since nitrogen retention during LO days was not improved by SCF. On the contrary, the slightly limited feeding in experiment 2 permitted a 4. p. 100 increase of that retention compared to the value obtained during *ad libitum* feeding.

A strange result in experiment 1 was the slightly higher value of nitrogen retention observed on L days with SCF compared to the controls (50 vs 43 p. 100). It thus seems that the reduced intestinal Ca content with SCF permitted better apparent protein digestibility (as for energy source) ; an alternative explanation would be that the rate of feed residue clearance was affected by ovulation. However, the variations observed in experiment 1 were not found again with the limited feeding program in experiment 2.

The present study confirming certain previous results on calcium metabolism and contributing new data, supports the well known fact that spontaneous Ca intake decreases on L days with SCF (Taylor, 1970). It also demonstrates that SCF slightly improves the Ca balance during O and LO days under limited feeding conditions. On the contrary, during L days the Ca balance was negative with SCF because the intake was very low ; therefore, the contribution of the L days to overall Ca balance is particularly important when the balances are carried out over several days.

It is interesting to compare these observations with those on egg-shell quality which is generally favourably affected by SCF (see review by Sauveur and Mongin, 1975). However, Roland (1978) recently found only 8 out of 24 American publications reporting a favourable effect of oyster-shell supply. These differences in response may be explained by many criteria, including ambient temperature and, to a lesser degree, animal age.

In the present study, shell improvement due to SCF was not statistically significant but it may be difficult to show this effect in young (34-week old) hens. Moreover, it is interesting to note that SCF did not seem to permit the hen to retain any more calcium than the control diet did because Ca export into the egg-shell represented 90 to 100 p. 100 of the retention vs 80 p. 100 in the controls. This would not confirm the interpretation of Moran *et al.* (1970), according to which SCF enables the hen to compensate eventual temporary Ca deficiencies. However, these authors did not use totally separate calcium feeding.

We found the following correlations (with 45 to 50 degrees of freedom per group) between egg and shell weights : Experiment 1 :  $r = 0.48$  (control group 1) and  $r = 0.68$  (group 2, SCF), Experiment 2 :  $r = 0.59$  (control group 1) and  $r = 0.69$  (group 2, SCF). It thus seems that SCF permits the hen to better adapt shell synthesis to egg size. This result and the small increase in egg-shell deposition recorded here tend to show that SCF could be useful during short-term periods, but would perhaps be unfavourable to calcium stores when L days become numerous. Another practical conclusion is that totally separate calcium feeding is probably not the best solution and that it would be preferable to leave 30 p. 100 of the total calcium as ground limestone included in the diet.

Finally, Meyer *et al.* (1973) showed that SCF was always less favourable to the first egg of the clutch than to the second or third. This unexplained result could originate in the highly negative Ca balance recorded during L days with SCF.

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**Résumé.** Effets d'une alimentation calcique séparée combinée ou non à un rationnement alimentaire sur l'énergie métabolisable du régime et la rétention d'azote, calcium et phosphore chez la poule.

Une première expérience de bilans a été réalisée pendant 8 jours sur 8 poules pour étudier si une alimentation calcique totalement séparée (ACS ; 14 g/j/poule de coquillages en particules offerts à côté d'un aliment B à 0,30 p. 100 Ca distribué *ad libitum*) affectait la rétention à court terme d'énergie métabolisable corrigée (EMc), d'azote, de Ca et de P. Ces animaux étaient comparés à 7 autres recevant *ad libitum* un aliment témoin A contenant 3,7 p. 100 Ca sous forme de poudre des mêmes coquillages.

Durant les jours de milieu de série (jours L0), l'ACS se traduit (tabl. 2) par des augmentations significatives de l'ingestion d'aliment sans le Ca (121 vs 110 g) et de la quantité d'énergie métabolisée (377 vs 342 Kcal). L'EMc du régime, les retentions relatives d'azote, de Ca et de P ne sont pas significativement modifiées. En l'absence de formation d'œuf (jours L), l'ACS entraîne (tabl. 4) une chute importante d'ingestion de la source calcique

(3,8 vs 11,8 g/j/p) et un bilan calcique négatif ; l'EM du mélange alimentaire est accrue par l'ACS (2 988 vs 2 685 Kcal/kg) mais la correction pour l'ingéré calcique rend cette différence non significative (3 087 vs 2 984). Sur le total des jours de bilan (L + O + LO), les effets de l'ACS sont voisins de ceux enregistrés durant les seuls jours LO.

Dans un deuxième essai, les quantités d'aliments offertes ont été limitées à 120 g/j/poule dans le lot témoin et 108 g d'aliment pauvre en Ca plus 12 g de coquillages dans le lot traité. Pendant les jours LO (tabl. 3), seule la rétention de Ca est alors améliorée par l'ACS (55 vs 48 p. 100). Pendant les jours L, les effets de l'ACS sur les bilans d'énergie et de calcium sont sensiblement les mêmes que durant le premier essai.

Au niveau de l'œuf (tabl. 5), l'ACS entraîne dans les deux essais une amélioration non significative des coquilles. L'exportation relative de Ca dans la coquille de l'œuf par rapport à l'ingéré est augmentée par l'ACS (90 à 100 p. 100 vs 80 p. 100).

Ces résultats sont discutés d'abord au plan méthodologique en soulignant l'importance de l'intensité de la ponte (gouvernant les proportions de jours L et LO) dans les expériences de bilan chez la poule. Il est conclu en outre que l'ACS ne devrait être utilisée qu'en présence d'un rationnement si l'on veut éviter une surconsommation d'aliment et qu'elle n'améliore globalement ni l'utilisation de l'énergie ni celle de l'azote de la ration. Cette technique risque d'être défavorable aux réserves calciques lorsque la proportion des jours de pause devient importante. Enfin, il est sans doute préférable de laisser 30 p. 100 du calcium sous forme de carbonate broyé mélangé au régime que de pratiquer une séparation totale de l'apport calcique.

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