Calcium solubilization and retention in the gastrointestinal tract in chicks (*Gallus domesticus*) as a function of gastric acid secretion inhibition and of calcium carbonate particle size

BY F. GUINOTTE, J. GAUTRON AND Y. NYS*

Station de Recherches Avicoles, INRA Centre de Tours, 37380 Nouzilly, France

AND A. SOUMARMON

Inserm Unité 10, Hôpital Bichat, 170 boulevard Ney, 75877 Paris Cedex 18, France

(*Received 20 January 1994 – Revised 12 May 1994 – Accepted 7 June 1994*)

In chicks, immature pullets and laying hens, the inhibition of gastric acid secretion by omeprazole, an H^+,K^+-transporting ATPase (*EC* 3.6.1.36) inhibitor, greatly increased proventricular and gizzard pH values. Consequently, gizzard soluble Ca concentration deceased and the insoluble Ca fraction increased. Inhibition of acid secretion increased duodenal pH values in immature pullets and laying hens but not in chicks. Duodenal soluble and ionic Ca concentrations were lowered by gastric acid inhibition in chicks and to a larger extent in immature pullets and laying hens. The use of Ca of coarse particle size increased the gizzard insoluble Ca fraction in chicks and pullets. However, it did not influence its soluble Ca fraction in chicks but tended to reinforce the negative effect of omeprazole on soluble Ca in the gizzard and duodenum of chicks and laying hens. Coarse particles of Ca led to an increase in gizzard and duodenal soluble Ca at the end of eggshell calcification in laying hens. An enhancement in the level of Ca in the diet from 10 to 36 g/kg increased gizzard soluble Ca and duodenal soluble and ionic Ca concentrations in immature and adult hens. Intestinal Ca retention and bone mineralization was unaffected by gastric acid inhibition in chicks but were largely diminished by the use of coarse particles of Ca. Gastric acid inhibition was associated in laying hens with decreased Ca retention to a small extent and with reduced eggshell quality. These observations confirm that gastric acid secretion is of importance for $CaCO_3$ solubilization but question its role as a prerequisite for intestinal Ca retention in chicks and even in hens fed on a high Ca diet.

Calcium: Particle size: Omeprazole: Gastric acid secretion: Chicken

It is generally accepted that gastric acid secretion is a prerequisite for $CaCO_3$ solubilization before its intestinal absorption in the ionic form (Ivanovitch *et al.* 1967). Nevertheless, in humans, a previous report has pointed out the lack of effect of a high intragastric pH on dietary Ca absorption (Bo-Linn *et al.* 1984) and partial gastrectomy does not affect Ca absorption (Nilas *et al.* 1985). In rapidly growing chicks the high requirement of Ca for skeletal growth, and in hens for shell formation, is supplied as $CaCO_3$ in the diet in a form such as limestone or marine shells (Guinotte, 1992). Moreover, laying diets include coarse particles of Ca in substitution of 50–66% of fine particles to improve eggshell quality (Guinotte, 1992). These increases in amount and size of $CaCO_3$ in poultry, when compared with other species, might reveal a prominent role of gastric acid secretion in Ca solubilization and might affect the acid secretion as suggested by a higher *in vitro* activity of H^+,K^+-transporting ATPase (*EC* 3.6.1.36) in hens receiving coarse particles of Ca (Guinotte *et al.* 1993). In humans, such an increase in Ca input (Behar *et al.* 1977) and

* For reprints.

coarse particle size of dietary ingredients (Low, 1990) leads to an enhancement in gastric acid secretion. In chicks the soluble fractions of Ca in gizzard and intestine are related to their corresponding pH, but not in laying hens (Mongin, 1976*a*). Moreover, hens are characterized during the period of shell formation by a large amount of soluble Ca in the duodenum and jejunum (Nys & Cabrera-Saadoun, 1986) despite high intestinal pH values (Mongin, 1976*b*), and by an associated increase in Ca retention (Itoh, 1967). The soluble Ca enhancement resulted from an increase in gastric acid secretion at the onset of the dark period (Mongin, 1976*a*; Nys & Cabrera-Saadoun, 1986) induced by crop dilatation (Ruoff & Sewing, 1971) due to the consumption of a large amount of feed just before night (Mongin, 1976*a*).

The aim of the present studies was to evaluate, in birds, the importance of gastric acid secretion for Ca solubilization as a function of sexual maturity, Ca level in the diet and $CaCO_3$ particle size. The consequences of gastric acid secretion inhibition, using an H^+,K^+-transporting ATPase inhibitor were studied on Ca solubilization, intestinal retention and biological efficiency in chicks and hens.

MATERIALS AND METHODS

Animals and diets

Shaver male broiler chicks (Saint Loup d'Ordon, 89330 Saint Julien du Sault, France) were raised in individual wire cages in an air-conditioned building. They were exposed to 16 h light (8 h dark) from 04.00 to 20.00 hours and room temperature was 28°. They were given free access to a diet including (g/kg): maize 300, wheat 300, soya beans 280, according to recommendations of the Institut National de la Recherche Agronomique (INRA, 1989; g/kg: protein 222, fibre 23, Ca 10, available P 4·6, cholecalciferol 37·5 μg, metabolizable energy (ME) 12·7 MJ). In Expt 1 (Table 1), 6 g Ca/kg were provided by ground limestone in a granulated diet. In Expts 2 (Tables 2 and 3) and 3 (Table 6), seashells (Calcialiment; ZI de la gare, 22690 Pleudihen sur Rance, France) were used as the only Ca supplement (10 g/kg) and were offered under two particle sizes (fine < 0·5 mm or coarse > 1·18 mm) in a flour diet. At the beginning of the experimental procedure, chicks were not offered feed for 12–14 h and, thereafter, had access for 2·5 h to 10 g feed in Expt 1 at 9 d of age and to 60 g feed for 3 h at 3 weeks of age in Expt 2. Individual records of feed consumption were kept.

In Experiments 4 and 5 respectively (Table 4), twenty-four brown immature pullets and sixty laying hens (Institut de Sélection Animale, 69427 Lyon, France) were individually caged. At 16 weeks the immature pullets were submitted to a cycle of 14 h light (10 h dark)/d from 06.00 to 20.00 hours similar to the hens. Before the experiment, pullets were fed on a standard diet (g/kg: protein 156, 11·85 MJ ME) containing 10 g Ca/kg as fine particles. Then, from 15 weeks, they received a flour diet containing (g/kg): wheat 470, maize 220, soya beans 110, formulated according to laying hens recommendations (g/kg: protein 167·5, fat 48, fibre 20, Ca 36, including 32 from sea shells, available P 3·3, cholecalciferol 37·5 μg and 12 MJ ME). The sixty laying hens were fed until 41 weeks on a layer diet and accustomed to one of the experimental layer diets during 1 week. Two particle sizes of the seashells were used as Ca source (fine particles < 0·5 mm or coarse particles > 1·18 mm). Birds were provided with free access to feed and water until the beginning of the experimental procedure (16 and 42 weeks). At that time they were feed-restricted during 14 h and had access to feed for 2 h. Birds were killed by an intravenous nembutal (Sanofi, Paris, France) injection.

In Expt 6 (Table 7) a balance study was carried out for 4 d on 30-week-old hens laying daily as previously described (Guinotte & Nys, 1991). Hens were fed on the layer diet (35 g Ca/kg) with coarse particles of seashells.

Treatments

In Expt 1, groups of chicks were injected once with one of the five doses of omeprazole (Ab Hässle, Mölndal, Sweden; 0, 2·5, 5, 7·5 and 10 μmol/kg body weight; *n* 6 chicks/group) during the last 30 min of the feed consumption period and were killed 1·5 h later. Additional groups were injected with 10, 50 or 100 μmol omeprazole/kg body weight and were killed at various time intervals as indicated in Fig. 1. In Expt 2 the experimental design was a 2 × 2 × 2 factorial arrangement of the treatments; 3-week-old chicks were either treated or not treated with omeprazole (50 μmol/kg body weight), supplied with two particle sizes of Ca and sampled 3 or 6 h after omeprazole injections. In Expt 3 a 72 h Ca balance study was carried out as previously reported (Guinotte *et al.* 1991) on sixteen replicates of two chicks which had been previously randomly assigned to one of the four experimental treatments. The experimental design was a 2 × 2 factorial arrangement of treatments with diets containing fine or coarse particles of Ca for 18 d and omeprazole (100 μmol/kg body weight) or vehicle injection for three consecutive days.

In Expts 4 and 5 respectively, twenty-four immature pullets and twenty-four laying hens were randomly assigned to the four experimental treatments. The experimental design was similarly a 2 × 2 factorial arrangement of treatments with or without gastric acid inhibition by omeprazole (30 μmol/kg body weight) or placebo and two particle sizes of the seashell Ca. Birds were killed 4 h after omeprazole injections, i.e. 3·5 h after the end of the meal. Only hens which had laid an egg 0·5–5 h after the start of the light period were used. The time-course between oviposition and slaughtering did not exceed 8 h and preceded the calcification of another egg. However, to investigate a possible role of eggshell calcification during the night, ten additional hens receiving either fine or coarse particles of Ca were assayed before the end of eggshell calcification. Eventually, the effect of a lower Ca intake (10 g Ca/kg from fine particles) was investigated in two groups of five pullets and five hens submitted to a similar procedure. In Expt 6, eight hens were treated daily for 5 d, during the balance study, with omeprazole (100 μmol/kg body weight) or vehicle.

Omeprazole powder was dissolved in polyethylene glycol (molecular weight 400; PEG 400; Merck, Schuhardt, 8011 München, Germany) and a weak $NaHCO_3$ buffer solution (0·56 g/l) and injected intramuscularly in the breast muscles.

pH and calcium measurements

pH and ionic Ca measurements were carried out in the digestive tract immediately after sampling. Proventriculus, gizzard and duodenum were clamped to avoid bolus contamination from one part to another. pH was measured with an Ingold mini electrode (30 mm long and 3 mm wide; 440 M3; Messtechnick AG, CH-8902 Urdorf, Switzerland) connected to a pH meter with an automatic printer (Bioblock, 67403 Illkirch, France). The pH electrode was gently introduced in each section where pH was recorded twice and the mean was calculated. Between each gastrointestinal section the electrode was rinsed with distilled water. Recalibration with buffer solutions (pH 1, pH 4·01 and pH 7·00; Radiometer Copenhagen, 2880 Bagsvaerd, Denmark) was carried out every fourteen chicks to avoid any drift of the electrode. Time-course of recording did not exceed 2 min per chicken.

Gizzard contents were poured out into centrifugation tubes (13 ml for chicks, 50 ml for pullets and hens) and 5 ml distilled water were mixed with gizzard contents in order to extract soluble Ca. A duodenal loop was isolated. Distilled water (1 ml) was injected through the lumen in order to pour out its contents into centrifugation tubes. After a few minutes decantation, ionic Ca was assayed on a Corning 634 ISE pH and ionic Ca analyser (Ciba Corning Diagnostics Ltd, Halstead, Essex).

Duodenal and gizzard sample tubes were centrifuged at 4° for 20 min at 11500 and

8500 *g* (model PR20; Jouan, 44805 St Herblain, France) respectively. Gizzard and duodenal supernatant fractions were frozen (−20°) until further determinations. Dry matter contents from gizzard were determined and Ca contents were assayed by absorptiometry (Varian atomic absorption spectrometer, model AA-475; Varian Techtron Pty Ltd, Springvale, Victoria, Australia). The gizzard insoluble fraction of Ca was expressed as a concentration (mmol/g pellets dry matter) or as the total amount per gizzard. Soluble Ca concentrations were assayed in duodenal and gizzard supernatant fractions containing additional water by complexometry and fluorimetry (Corning 940 Ca analyser; Ciba Corning Diagnostics Ltd).

Blood was sampled in five hens fed on fine or coarse Ca and was assayed immediately for ionized Ca (Corning 634 ISE, Ciba Corning Diagnostics Ltd). After centrifugation (10 min at 2000 *g*; Beckman J6, Beckman, 93220 Gagny, France) plasma Ca was determined on a Corning 940 Ca analyser.

Tibia mineralization and eggshell quality

In Expt 3, tibias sampled in chicks which had been treated on three consecutive days with omeprazole were tested for ultimate stress (Guinotte *et al.* 1991). Eggshell quality variables (eggshell weight and eggshell index) were assayed as previously described (Guinotte & Nys, 1991) on eggs layed by hens used in the Ca balance study.

Statistical analysis

Data were submitted to one-, two- or three-way analysis of variance depending on the factorial arrangement of the treatments. Splitting of the data was carried out for analysis when standard errors were not of similar magnitude. Differences between means were tested by paired *t* test or Tukey's multiple-range test. Pearson correlation coefficients were calculated among pH and Ca content variables. The calculations were performed using the Systat software program (Wilkinson Leland, Systat inc., Evanston, IL, USA).

RESULTS

pH values in the diets were 6·3, 6·3, 6·1, 5·9, 6·1 respectively for Expts 1, 2, 3, 4, 5.

Effect of omeprazole on pH and calcium contents of the gastrointestinal tract

In chicks, feed consumption was not different between experimental groups treated with omeprazole (overall mean 7·9 (SD 1·3) and 33·8 (SD 3·5) g in Expts 1 and 2 respectively). Similarly, in immature pullets (26 (SD 13) g) and laying hens (23 (SD 9) g), feed consumption was not affected by omeprazole. Gizzard dry matter was higher in laying hens injected with omeprazole compared with control (0·79 (SD 0·57) *v.* 1·68 (SD 0·41) g, $P < 0{\cdot}001$). No changes were observed in chicks (0·63 (SD 0·30) and 0·92 (SD 0·45) g in Expts 1 and 2 respectively) or in pullets (2·28 (SD 0·83) g).

Gizzard pH values increased as a function of omeprazole doses in chicks (Table 1) but duodenal pH changed little. The increase in gizzard pH and the duration of acid secretion inhibition were in proportion to the level of omeprazole injected in chicks (Fig. 1). A dose of 50 μmol omeprazole/kg body weight enhanced proventricular and gizzard pH and increased crop pH slightly. Duodenal pH remained unchanged (Table 2). In immature pullets, omeprazole treatment increased pH values in proventriculus (2·38 (SD 0·82) *v.* 5·98 (SD 0·34), $P < 0{\cdot}001$), gizzard and duodenum (Table 4). Similarly, laying hens' pH values were increased 4 h after the injection of 30 μmol omeprazole/kg body weight in the proventriculus (1·84 (SD 0·74) *v.* 5·75 (SD 0·62), $P < 0{\cdot}001$), in the gizzard and in the duodenum (Table 4).

Soluble Ca in gizzard contents decreased linearly with pH enhancement until pH 5·62 in chicks sampled 1·5 h after the treatment (Table 1). A variation of 3·18 pH units led to a

Table 1. *Expt. 1. Effects of omeprazole on gizzard and duodenal pH values and on levels of soluble and insoluble calcium in the digestive contents of chickens at 9 d of age**

(Mean values and standard deviations for six chicks per treatment)

Omeprazole (µmol/kg body wt)	Gizzard pH	Ca in gizzard contents: Soluble (mmol/l) Mean	SD	Insoluble (mmol/g dry matter)	Insoluble (mmol/ gizzard)	Duodenal pH	Ca in duodenal contents: Soluble (mmol/l)	Ionic (mmol/l) Mean	SD
0	2·56^{c}	6·73^{a}	3·0	0·227	0·089^{b}	6·30^{a}	6·27^{a}	5·58^{a}	2·0
2·5	3·22^{c}	5·08^{ab}	2·4	0·303	0·115^{b}	5·91^{b}	3·47^{b}	2·94^{b}	1·7
5	4·54^{b}	2·36^{b}	1·6	0·296	0·158^{ab}	6·39^{a}	1·26^{c}	0·65^{c}	0·8
7·5	5·62^{a}	1·21^{bc}	0·4	0·370	0·189^{ab}	6·40^{a}	1·08^{c}	0·30^{cd}	0·1
10	5·74^{a}	0·99^{c}	0·4	0·304	0·253^{a}	6·30^{a}	0·99^{c}	0·23^{d}	0·1
SEM	0·55			0·18	0·06	0·17	0·98		
Statistical significance of effect of dose: *P*	< 0·001	< 0·001		NS	< 0·001	> 0·01	< 0·001	< 0·001	

a, b, c, d Mean values with unlike superscript letters were significantly different by one-way ANOVA ($P < 0·05$).
NS, not significant.
* Chicks were injected intramuscularly with omeprazole and killed after 1·5 h. They were fed on a 10 g fine particles of Ca/kg diet (for details of diet and procedures, see pp. 126–127).

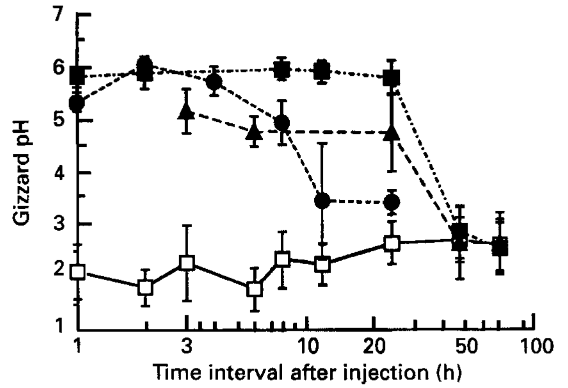

Fig. 1. Inhibition kinetics of four doses of omeprazole on gizzard pH values in chicks: (□), 0; (●), 10; (▲), 50; (■), 100 μmol omeprazole/kg body weight. Values are means and standard deviations represented by vertical bars for six chicks per dose. For details of procedures, see pp. 126–127.

Table 2. *Expt 2. The effect of omeprazole, particle size and sampling time on digestive tract pH values in 4-week-old chicks**

(Mean values and standard deviations for five chicks per treatment)

			pH				
				Proventriculus			
Omeprazole (μmol/kg body wt)	Ca particle size (mm)	Sampling time (h)	Crop	Mean	SD	Gizzard	Duodenum
0	< 0·5	3	5·21^{bc}	2·15^{bc}	1·1	2·26^{b}	6·28
50	< 0·5	3	4·98^{bc}	5·13^{a}	0·6	5·14^{a}	6·25
0	> 1·2	3	4·75^{c}	1·59^{bc}	0·6	2·59^{b}	6·34
50	> 1·2	3	5·13^{bc}	4·88^{a}	0·7	5·30^{a}	6·40
0	< 0·5	6	5·18^{bc}	1·48^{c}	0·3	1·77^{b}	6·55
50	< 0·5	6	6·49^{a}	2·78^{b}	1·1	4·76^{a}	6·62
0	> 1·2	6	5·83^{abc}	1·62^{bc}	0·5	2·47^{b}	6·36
50	> 1·2	6	6·09^{ab}	5·21^{a}	1·0	5·60^{a}	6·62
SEM			0·59			0·52	0·19
Statistical significance (P) of effect of							
Omeprazole			0·03	< 0·001		< 0·001	NS
Particle size			NS	0·08		0·005	NS
Sampling time			< 0·001	0·01		NS	0·002
Omeprazole × particle size			NS	0·01		NS	NS
Omeprazole × time			0·07	NS		NS	NS
Particle size × time			NS	0·02		NS	NS
Treatment × particle size × time			0·04	0·05		NS	NS

a, b, c Mean values with unlike superscript letters were significantly different by three-way ANOVA with interaction ($P < 0·05$).

NS, not significant.

* Chicks were injected intramuscularly with omeprazole and were fed on a 10 g Ca/kg diet (for details of diet and procedures, see pp. 126–127).

reduction of 85 % in gizzard soluble Ca and increased 2·8-fold total insoluble Ca. This enhancement in pH decreased by 84 and 96 % duodenal soluble and ionic Ca respectively. Omeprazole (50 μmol/kg body weight) decreased gizzard soluble Ca by 38 % at 3–6 h after the injection (Table 3), duodenal soluble and ionic Ca by 52 and 63 % respectively, and increased greatly the insoluble gizzard fraction. Similarly, gizzard and duodenal soluble Ca concentrations were lowered in pullets and hens (Table 4) treated with the gastric acid

Table 3. *Expt. 2. The effect of omeprazole, particle size and sampling time on digestive tract calcium in 4-week-old chicks**

(Mean values and standard deviations for five chicks per treatment)

Omeprazole (μmol/kg body wt)	Ca particle size (mm)	Sampling time (h)	Ca content: Gizzard: Soluble (mmol/l) Mean	SD	Gizzard: Insoluble (mmol/g dry matter) Mean	SD	Gizzard: Insoluble (mmol/gizzard) Mean	SD	Duodenum: Soluble (mmol/l) Mean	SD	Duodenum: Ionic (mmol/l) Mean	SD
0	< 0·5	3	5·71	2·4	0·056[d]	0·1	0·07[d]	0·1	5·98[ab]	5·9	3·24	4·9
50	< 0·5	3	3·62	1·7	0·154[c]	0·1	0·22[c]	0·1	2·36[ab]	0·8	1·42	1·3
0	> 1·2	3	6·31	5·0	0·342[b]	0·2	0·39[c]	0·1	3·39[a]	0·8	2·83	1·5
50	> 1·2	3	2·66	0·9	2·270[ab]	2·6	1·66[b]	1·4	2·18[b]	0·7	0·91	0·4
0	< 0·5	6	3·11	3·4	0·032[d]	0·1	0·03[d]	0·1	1·86[a]	2·0	0·80	0·8
50	< 0·5	6	4·23	1·9	0·298[bc]	0·1	0·21[c]	0·5	2·34[b]	0·5	1·37	0·3
0	> 1·2	6	5·93	1·7	0·865[b]	0·9	0·58[bc]	0·5	6·82[ab]	6·0	5·85	6·4
50	> 1·2	6	2·72	1·5	2·940[a]	0·7	3·13[a]	0·9	1·82[a]	0·5	0·99	0·3
Statistical significance (*P*) of effect of †												
Omeprazole			0·02		0·002		< 0·001		0·02		0·04	
Particle size			NS		< 0·001		< 0·001		NS		NS	
Sampling time			NS		NS		< 0·05		NS		NS	

[a, b, c, d] Mean values unlike superscript letters were significantly different by a three-way ANOVA with interactions ($P < 0·05$).

NS, not significant.

* Chicks were injected intramuscularly with omeprazole and were fed on a 10 g Ca/kg diet (for details of diet and procedures, see pp. 126–128).

† The interactions between treatments were not significant with the exception of those between omeprazole and particle size for gizzard insoluble Ca ($P < 0·01$) and between omeprazole, particle size and sampling time for gizzard insoluble Ca ($P < 0·05$) and duodenal soluble Ca ($P = 0·04$).

https://doi.org/10.1079/BJN19950014 Published online by Cambridge University Press

Table 4. *Expts 4 and 5. The effect of omeprazole, particle sizes and calcium level on pH and calcium content in the digestive tract of immature pullets and laying hens**

(Mean values and standard deviations for five or six birds per treatment)

					Ca in gizzard contents						Ca in duodenal contents			
					Soluble (mmol/l)			Insoluble (mmol/gizzard)			Soluble (mmol/l)		Ionic (mmol/l)	
Omeprazole (μmol/kg body wt)	Sampling time (h)	Particle sizes (mm)	Ca level (g/kg)	Gizzard pH	Mean	SD	Insoluble (mmol/g dry matter)	Mean	SD	Duodenal pH	Mean	SD	Mean	SD
Immature pullets														
0	4	< 0·5	10	2·76^{c}*	4·8^{b}	3	1·90	0·42^{c}	0·4	6·33	10·18^{b}	7	7·35^{b}	6
0	4	< 0·5	36	3·82^{b}	20·2^{a}	12	3·18	3·92^{b}	2·0	6·29	22·49^{a}	10	21·27^{a}	11
30	4	< 0·5	36	5·90^{a}	4·0^{b}	3	3·15	4·16^{b}	1·4	6·59	4·66^{b}	8	1·89^{c}	1
0	4	> 1·2	36	3·94^{b}	17·2^{a}	6	4·46	11·22^{ab}	10	6·18	22·51^{a}	2	19·94^{a}	11
30	4	> 1·2	36	5·61^{a}	4·7^{b}	3	4·42	17·26^{a}	9·5	6·60	1·85^{c}	1	0·97^{c}	1
SEM				0·45			1·68			0·23				
Laying hens														
0	4	< 0·5	10	2·12^{b}	5·60^{bc}	3·6	1·50	0·54^{d}	0·8	5·85^{b}	3·96^{bc}	2·6	4·29^{b}	1·7
0	4	< 0·5	36	2·45^{b}	9·21^{ab}	4·3	3·52	2·88^{bcd}	2·7	5·96^{b}	8·36^{ab}	4·6	8·00^{ab}	5·2
0	12	< 0·5	36	2·47^{b}	5·66^{bc}	4·1	1·95	1·61^{cd}	2·0	5·67^{b}	8·01^{ab}	4·6	5·45^{b}	3·8
30	4	< 0·5	36	6·17^{a}	2·19^{c}	0·5	4·42	8·59^{a}	3·6	6·75^{a}	1·46^{c}	0·6	0·84^{c}	0·4
0	4	> 1·2	36	2·88^{b}	14·24^{a}	5·1	2·62	1·70^{bcd}	1·1	5·98^{b}	13·05^{a}	7·5	11·25^{a}	5·4
0	12	> 1·2	36	2·50^{b}	15·46^{a}	5·8	2·76	3·92^{abc}	2·6	5·87^{b}	12·58^{a}	7·5	10·73^{a}	6·1
30	4	> 1·2	36	6·21^{a}	1·96^{c}	0·4	3·56	5·27^{ab}	3·8	6·61^{a}	1·38^{c}	0·3	0·72^{c}	0·4
SEM				0·60			1·87			0·29				
Statistical significance (*P*) of effect of														
Sexual maturity†				0·02	0·009		NS	< 0·001		NS	< 0·001		0·003	
Omeprazole				< 0·001	< 0·001		NS	< 0·001		< 0·001	< 0·001		< 0·001	
Particle size				NS	NS		NS	NS		NS	0·09		NS	
Calcium level‡				0·02	0·005		0·12	0·06		NS	0·01		0·008	
Sexual maturity × omeprazole§				< 0·001	NS		NS	0·002		< 0·01	0·007		0·008	

a, b, c, d Mean values with unlike superscript letters were significantly different by a three-way ANOVA (sexual maturity, omeprazole, particle size) or a two-way ANOVA (sexual maturity, Ca level or sampling time, particle size) with interactions ($P < 0·05$).

NS, not significant.

* Immature pullets and laying hens were injected intramuscularly with omeprazole (for details of diets and procedures, see pp. 126–128).

† Statistical analysis was carried out with experimental groups at 36 g Ca/kg.

‡ Analysis was carried out with experimental groups receiving 10 or 36 g Ca/kg at < 0·5 mm without omeprazole treatment.

§ Interactions were not significant except between sexual maturity and particle size ($P < 0·05$) for gizzard insoluble Ca. There was no effect of the sampling period.

Table 5. *Expts 2, 4 and 5. Correlation coefficients between gizzard pH and pH or calcium contents in the digestive tract of chicks, pullets and laying hens*†

	Gizzard pH		
Expt no....	2	4	5
	Chicks	Pullets‡	Laying hens‡
n...	37	22	20
pH			
Proventricular	0·88***	0·92***	0·92***
Duodenal	0·18	0·59**	0·80***
Gizzard Ca			
Soluble (mmol/l)	−0·28	−0·79***	−0·66**
Insoluble (mmol/g DM)	0·55**	0·19	0·67***
Duodenal Ca (mmol/l)			
Soluble	−0·35*	−0·79***	−0·63**
Ionic	−0·31	−0·83***	−0·63**

DM, dry matter.
* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.
† For details of procedures, see pp. 126–128.
‡ Birds were fed on 36 g Ca/kg diet.

inhibitor when fed on 36 g Ca/kg. Gizzard soluble Ca decreased by 77 and 82 % in pullets and laying hens respectively, 4 h after the treatment. Duodenal soluble and ionic Ca concentrations showed a reduction of higher magnitude (Table 4). The concentration of insoluble Ca in the gizzard (mmol/g dry matter) was not affected by omeprazole but the total content of insoluble Ca was higher in the gizzard in laying hens treated with the inhibitor.

Effect of the physiological stage, particle size and calcium intake on pH and calcium solubility

In chicks, feed consumption was decreased by the use of coarse particles (38 *v.* 29 (SD 3·5) g, $P < 0.001$) but the amounts of dry matter in the gizzard were similar (1·33 (SD 0·44) g). Gizzard dry matter increased in immature pullets fed with coarse particles of calcium (1·53 *v.* 3·04 (SD 0·82) g, $P < 0.001$). It was not modified in laying hens.

Gastrointestinal pH and Ca contents did not differ significantly between chicks, pullets and hens when they were fed on a 10 g Ca/kg diet. A larger level of Ca in the diet enhanced gizzard pH in pullets (Table 4) without altering proventricular (1·79 (SD 0·47) *v.* 1·80 (SD 0·42)) or duodenal pH. In hens the Ca intake did not change gizzard or intestinal pH values. Omeprazole inhibition of gizzard pH was larger in laying hens than in pullets (interaction between sexual maturity and treatment, Table 4). Duodenal pH values were not modified by the inhibitor.

Soluble and total insoluble Ca of the gizzard increased with Ca level (Table 4). Soluble and ionic Ca fractions in the duodenum showed a similar pattern. These effects were more pronounced in immature pullets than in laying hens.

Gizzard pH was higher in chicks fed on coarse particle size of Ca (Table 2) but crop, proventricular and duodenal pH values were not influenced by the size of the particles. This observation of an increased gizzard pH associated with coarse Ca was not confirmed in pullets and laying hens (Table 4). The use of coarse particles of Ca maintained in chicks a higher concentration of insoluble Ca in the gizzard 6 h after the feed intake (Table 3), and increased soluble Ca in the duodenum at this time ($P = 0.04$) compared with chicks fed on fine Ca. Moreover, the inhibition of acid secretion reinforced the increase in gizzard concentration of insoluble Ca ($P < 0.001$) and the decrease in duodenal soluble Ca ($P = 0.04$) associated with the particulate size of the Ca source in chicks (Table 3). In older birds, coarse particles of Ca have less influence on the amount of soluble Ca in gastrointestinal contents (Table 4). In pullets fed on coarse $CaCO_3$, concentrations of gizzard soluble or insoluble Ca were similar but the total content of insoluble Ca was higher ($P < 0.01$). Soluble Ca in gizzard ($P < 0.01$) and ionic Ca in duodenum ($P = 0.07$) tended to be larger in laying hens receiving coarse particles of Ca compared with hens fed on a fine particle Ca source at the end of eggshell calcification (Table 4). As a consequence, large particles tended to reinforce the decrease in gizzard and duodenal soluble Ca elicited by omeprazole. Results from blood samples confirmed the positive effect of particle size on soluble Ca. The ionized blood Ca was increased by the use of coarse seashells (0·98 (SD 0·09) *v.* 1·17 (SD 0·15) mmol/l, $P < 0.05$) at the end of eggshell formation but blood total Ca was not significantly affected (2·10 (SD 0·53) *v.* 2·47 (SD 0·83) mmol/l, $P > 0.05$).

Relationship between pH and calcium solubility

Proventricular and gizzard pH values were highly correlated in chicks treated with increasing levels of omeprazole (*r* 0·96, *n* 28) and in chicks, pullets and laying hens injected with high doses of the H^+,K^+ ATPase inhibitor (Table 5). The relationship between gizzard and duodenal pH values was low in chicks (Expt 1: *r* 0·31, *n* 28; Expt 2: *r* 0·18, *n* 37) in contrast to those observed in immature and laying hens (Table 5).

Gizzard pH was negatively correlated in chicks treated with low levels of omeprazole (Expt 1) with gizzard soluble Ca (*r* −0·83; $P < 0.001$), duodenal soluble Ca (*r* −0·74; $P < 0.001$) and ionic Ca (*r* −0·76; $P < 0.001$) and was positively associated with total insoluble Ca in the gizzard (*r* 0·43; $P < 0.05$). These relationships were lower in chicks fed on coarse particles of Ca in Expt 2 (Table 5). In pullets and laying hens, gizzard and duodenal soluble Ca concentrations were also dependent on gizzard pH (Table 5). Intestinal pH and soluble Ca were not correlated in the duodenum of chicks, but were in those of pullets (*r* −0·54; $P < 0.01$) and laying hens (*r* −0·49; $P < 0.05$).

A high correlation was observed between gizzard soluble Ca and duodenal soluble Ca in pullets (*r* 0·79; $P < 0.001$) and laying hens (*r* 0·9; $P < 0.001$) and to a lower extent in chicks (Expt 1: *r* 0·74; $P < 0.001$; Expt 2: *r* 0·44, $P < 0.01$). Similarly, the correlations between gizzard soluble Ca and duodenal ionic Ca were high in pullets (*r* 0·76, $P < 0.001$) and laying hens (*r* 0·9, $P < 0.001$) and in chicks (Expt 1: *r* 0·71, $P < 0.001$; Expt 2: *r* 0·74, $P < 0.001$). Ionic and soluble Ca levels were highly correlated in the duodenum (chicks: Expt 1, *r* 0·96; Expt 2: *r* 0·67; pullets: *r* 0·97; hens: *r* 0·90; $P < 0.001$). Finally, gizzard insoluble Ca was negatively correlated with its soluble fraction only in the first experiment in chicks (*r* −0·51, $P < 0.01$) but correlation coefficients were not significant in the second experiment, in pullets (*r* 0·22) and in laying hens (*r* 0·33). Moreover, duodenal soluble and ionic Ca were not correlated with gizzard insoluble Ca concentrations in chicks, pullets and laying hens.

Effect of proventricular acid secretion inhibition on calcium balance in laying hens and chicks

In chicks, feed consumption was impaired at high level of omeprazole (Table 6). As a consequence, chick body weight diminished. Faecal dry matter (29·5 *v.* 26·5 (SD 3·34) g/cage per d) and Ca excretion were lower in chicks injected with omeprazole but coarse seashells enhanced Ca excretion. Total Ca retention (0·71 *v.* 1·51 (SD 0·32) g/cage in chicks fed on

Table 6. *Expt 3. The effect of omeprazole and calcium particle size on calcium retention and bone breaking strength in chicks**

(Mean values for eight observations per treatment and sixteen tibias per treatment)

Omeprazole (μmol/kg body wt)	Ca particle size (mm)	Wt gain (g/cage)	Feed consumption (g/cage per d)	Ca excretion (g/cage per d)	Ca retention (%)	Tibia ultimate stress (N)
0	< 0·5	198^{a}	100^{a}	0·62^{b}	48·2^{a}	56·2^{a}
100	< 0·5	173^{b}	81^{b}	0·55^{b}	43·5^{a}	53·9^{a}
0	> 1·2	174^{b}	90^{ab}	0·78^{a}	21·5^{b}	49·9^{ab}
100	> 1·2	145^{c}	79^{b}	0·60^{b}	29·8^{b}	44·2^{b}
SEM		17	9·5	0·10	7·7	8·4
Statistical significance of effect of						
Omeprazole		< 0·001	< 0·001	0·002	NS	0·06
Particle size		< 0·001	NS	0·007	< 0·001	< 0·001
Omeprazole × particle size		NS	NS	NS	0·04	NS

NS, not significant.

a, b, c Mean values with unlike superscript letters were significantly different by two-way ANOVA with interaction ($P < 0·05$).

* For details of diets and procedures, see pp. 126–128.

Table 7. *Expt 6. Effects of omeprazole on calcium retention and eggshell quality in laying hens**

(Mean values for eight hens per treatment and thirty-one eggs per treatment)

Omeprazole (μmol/kg body wt)	Particle sizes (mm)	Ca			Eggshell quality	
		Intake (mmol/hen)	Excretion (mmol/hen)	Retention (%)	Shell wt (g)	Shell index (g/dm^{2})
0	< 0·5	393^{b}	193^{b}	51^{ab}	5·68^{a}	7·96^{a}
0	> 1·2	572^{a}	265^{a}	54^{a}	5·85^{a}	8·23^{a}
100	> 1·2	422^{b}	241^{a}	43^{b}	4·46^{b}	6·27^{b}
SEM		58	53	7·2	0·54	0·60
Statistical significance of difference: P		< 0·001	0·055	0·03	< 0·001	< 0·001

a, b Mean values with unlike superscript letters were significantly different by one-way ANOVA.

* For details of diet and procedures, see pp. 126–128.

coarse and fine Ca respectively, $P < 0·001$) or retention calculated as percentage of Ca ingestion was lower in groups of chicks injected with placebo or omeprazole and fed on coarse particles of Ca compared with those fed on fine Ca. Conversely, omeprazole had no influence on Ca retention in chicks (Table 6). Weight gain and tibia ultimate stress decreased in chicks fed on particulate Ca. Bone strength was diminished by the feeding of coarse particles of Ca and tended to be lower in chicks injected with H^{+},K^{+} ATPase inhibitor ($P = 0·06$).

Laying hens treated for 5 d with 100 μmol omeprazole/kg and fed on coarse particles of Ca showed a lower feed intake (Table 7). The intestinal retention of Ca was decreased as a consequence of inhibition of acid secretion by omeprazole but was not affected by the size of the Ca particles. The shell quality was markedly lowered in hens treated with omeprazole (Table 7).

DISCUSSION

The present study demonstrates that Ca solubilization in the upper gastrointestinal tract of chicks is directly related to proventricular acid secretion and largely decreased when acid secretion is inhibited by omeprazole. However, a drop in gizzard and duodenal soluble Ca slightly lowered intestinal Ca retention in laying hens fed on a 35 g Ca/kg diet and did not modify Ca retention in chicks receiving a lower Ca diet (10 g/kg).

The increase in proventricular and gizzard pH values and the duration of the acid inhibition were in proportion to the dose of the H^{+},K^{+} ATPase inhibitor but the amount of omeprazole producing total inhibition for 24 h in chicks was markedly higher than the effective dose in human (Walt *et al.* 1983), rat or dog (Larsson *et al.* 1983). This discrepancy could be a consequence of the 26-fold higher concentration of protons observed in chicks (Long, 1967) compared to humans, or of a lower affinity of omeprazole for the avian H^{+},K^{+} ATPase due to the lack of intracellular canaliculi in the oxynticopeptic cells. The antisecretory effect of omeprazole confirmed *in vivo* the involvement of the H^{+},K^{+} ATPase in the process of acid secretion in birds as shown in mammals. The existence of H^{+},K^{+} ATPase activity in proventricular extract *in vitro* (Guinotte *et al.* 1993) supports this hypothesis. Proventricular acid secretion is collected in the gizzard, as reflected by the high correlation between their pH values (Table 5), and induces Ca solubilization. Inhibition of proventricular acid secretion by omeprazole resulted in large increases in gizzard pH values and concomitant lowering in soluble Ca. However, the correlation between gizzard pH and gizzard or duodenal soluble Ca fluctuated with time, levels and particle sizes of Ca in the diet and with the physiological stage of the birds, suggesting altered duration of contact between acid solution and $CaCO_3$.

In chicks treated with omeprazole the concentration of soluble Ca decreased in gizzard by 95, 37 and 26% and the pH increased to 5·7, 5·1 and 4·8 respectively when sampled 1, 3 and 6 h after the treatment (Table 2). In the first experiment gizzard soluble Ca was impaired by 65% and the pH enhanced to 4·5, 1·5 h after the treatment (Table 1). Therefore, changes in gizzard pH explained only partly those in soluble Ca concentration. Gastrointestinal transit time and the amount of Ca in digestive contents are likely to explain differences in Ca solubilization efficiency. In chicks, a great amount of Ca is rapidly solubilized in the gizzard as suggested by the high correlation between gizzard pH and duodenal soluble Ca 1·5 h after treatment and by the lower values observed thereafter, at the 3 and 6 h sampling periods (Table 5). Moreover, the fall in duodenal ionic Ca decreased from 95 to 43%, 1 and 3 h after gastric acid inhibition respectively. On the contrary, in pullets and hens treated with omeprazole gizzard (80 and 76%) and duodenal (79 and 83%) soluble Ca concentrations largely dropped 4 h after the treatment. As a consequence, correlations between gizzard pH and soluble or ionic Ca in the duodenum were maintained at a higher level in pullets and hens than in chicks. In chicks the high transit rate observed in the digestive tract (van der Klis *et al.* 1991) contributed to the reduction in gizzard soluble Ca 3–6 h after the mean (Table 4) relative to concentrations observed earlier (Table 1). In laying hens the transit of intestinal contents is slower (Akahori *et al.* 1971) and gizzard hyperactivity is associated with reduced intestinal motility (Roche *et al.* 1983). Consequently, gizzard soluble Ca supplied for a longer period the duodenal content with soluble Ca as shown by the close relationship between gizzard and duodenal soluble and ionic Ca (Table 5).

The absence of correlation between duodenal ionic Ca and gizzard or duodenal pH and its lower correlation with gizzard soluble Ca in chicks than that of laying hens, on the one hand, and the lack of modification in intestinal Ca retention in chicks following inhibition of acid secretion, on the other hand, suggest a subsequent Ca solubilization in the distal intestinal tract of chicks. Of $CaCO_3$, 37 % is solubilized *in vitro* at pH 6 after 2 h of reaction (Bo-Linn *et al.* 1984). *In vivo*, $CaCO_3$ could be dissolved during its passage through the jejunum which is slightly acidic and its absorption might be facilitated by the occurrence of intestinal reflux content in chicks (Sklan *et al.* 1978).

High Ca level in the diet increased gizzard pH due to the buffering action of carbonates. Nevertheless, it enhanced the amount of soluble Ca in the gizzard and duodenum of pullets to a higher extent than in laying hens (Table 4). The higher proton concentration observed in the gizzard supernatant fraction in immature pullets compared with hens (Nys & Cabrera-Saadoun, 1986) and the slightly higher H^+,K^+ ATPase activity observed in pullets (Guinotte *et al.* 1993) might explain this observation. Moreover, disappearance of soluble Ca is slower in pullets because of a lower intestinal Ca absorption. The levels of insoluble Ca in the gizzard tended to increase with the Ca consumption and were similar in immature pullets and laying hens. There is, therefore, no evidence that sexual maturity is associated with any stimulation of acid secretion as confirmed by the absence of increase in H^+,K^+ ATPase activity in hens compared with pullets (Guinotte *et al.* 1993).

The substitution of fine Ca by a coarse source largely increased the amount of insoluble Ca in the gizzard content in chicks (Table 3). The soluble:insoluble Ca in the gizzard was 11-fold higher in chicks fed on a fine Ca compared with those fed on a particulate Ca. The insoluble Ca level in the gizzard content increased with the lateness of the sampling period and was 3·5-fold higher 6 h after the meal than that in the diet, suggesting a selective accumulation of Ca. Such a retention in the gizzard of particle Ca larger than 0·8 mm has been demonstrated in birds by Rao & Roland (1990). The duration of the stay of coarse particles in the gizzard doubled compared with fine particles (Gonalons & Moretto, 1989). Moreover, frequency and amplitude of gizzard contractions (Roche *et al.* 1983) increase when chicks are fed on a coarse particle food or granulated diet and the feed transit slows down. These changes elicit opposite consequences in young and adult birds. In chicks, coarse-particle Ca decreased intestinal Ca retention and bone mineralization (Table 6) in agreement with previous observations (Guinotte *et al.* 1991). These results suggest insufficient solubilization process of the large particles in chicks, possibly because of a faster digestive transit (van der Kliss *et al.* 1991) and a lower proventricular liquid secretion compared with hens (Mongin, 1976*b*). In laying hens the proventricular liquid secretion doubled following the dilation of the crop associated with the increase in feed intake at the end of the day (Mongin, 1976*b*). The insoluble Ca fraction in the gizzard plays the role of a Ca reservoir during eggshell calcification when there is an increase in gastric acid secretion (Mongin, 1976*b*; Nys & Cabrera-Saadoun, 1986). Then, coarse Ca particles are slowly dissolved compared with fine particles (Guinotte *et al.* 1991) and maintain a high level of the insoluble fraction 12 h after feed withdrawal (Table 4). Consequently, gizzard soluble Ca, duodenal ionic Ca (Table 4) and blood ionic Ca values were greater 12 h after the feed intake in laying hens fed on coarse particles than those receiving fine Ca. The retention of coarse Ca particles in the crop and gizzard (Rao & Roland, 1990) and their relative insolubility (Guinotte *et al.* 1991) allow, therefore, a constant metering of Ca during the period of shell calcification, throughout the night, when there is no food intake. It explains the beneficial effects of coarse-particle Ca on eggshell quality and bone mineralization observed in laying hens (Guinotte, 1992).

Negative effects of acid secretion inhibition by omeprazole on intestinal Ca retention were limited to a low extent in hens fed on a diet with a high level of Ca (Table 7). The lower Ca retention was associated with a depressed eggshell quality in agreement with the reduction induced by another gastric acid inhibitor, cimetidine (Wyatt *et al.* 1990). On the other hand, the inhibiting effect of omeprazole on osteoclast H^+,K^+ ATPase activity (Tüüklanen & Väänanen, 1986) reduced bone resorption and might decrease the supply of Ca for shell formation.

The inhibition of acid secretion largely lowered Ca solubilization in the upper intestine but did not affect intestinal Ca retention in chicks or elicit lower effects in laying hens (−9%) than expected from the drop in duodenal ionic Ca (−90%). This observation questions the importance of gastric acid secretion for intestinal Ca absorption and suggests the existence of compensating factors such as intestinal solubilization as discussed previously. Distal Ca absorption by the colon has been reported in rats (Petith & Schedl, 1976) and humans (Grimstead *et al.* 1984). In humans, increased acid secretion is associated with larger calcium solubility *in vivo* (Pak & Avioly, 1988). In gastrectomized subjects, Ca absorption from $CaCO_3$ (Recker, 1985) and bone mineralization are reduced after a long period of asecretion (Nilas *et al.* 1985). Conversely, Bo-Linn *et al.* (1984) did not observe deleterious effects in the short term, of large increased gastric pH values on Ca absorption in humans, as observed presently in birds.

Many thanks to C. Leterrier and P. Constantin for their enthusiasm during these studies and their excellent technical help.

This work was supported by a CIFRE (Contrat Industrie Formation Recherche) grant n°120/87 with the following memberships: Calcialiment (ZI de la gare, 22690 Pleudihen sur Rance, France), Institut National de la Recherche Agronomique (INRA Centre de Tours, Station de Recherches Avicoles, 37380 Nouzilly, France) and the Association Nationale pour la Recherche et la Technologie (ANRT, 16, Avenue Bugeaud, 75116 Paris, France).

REFERENCES

Akahori, F., Matsura, M. & Arai, K. (1971). Studies on the movement of the alimentary canal. VI. Physiological values in growing female chicks and quails. *Bulletin of Azabu University of Veterinary Medicine* **22**, 25 Abstr.

Behar, J., Hitchings, M. & Smith, R. D. (1977). Calcium stimulation of gastrin and gastric acid secretion: effect of small doses of calcium carbonate. *Gut* **18**, 442–448.

Bo-Linn, G. W., Davis, G. R., Buddrus, D. J., Morawski, S. G. & Santana, C. (1984). An evaluation of the importance of gastric acid secretion in the absorption of dietary calcium. *Journal of Clinical Investigation* **73**, 640–647.

Gonalons, E. & Moretto, M. (1989). Intestinal motility and absorption of nutrients in the fowl. In *Proceedings of the VIIIth European Poultry Symposium on Poultry Nutrition*, pp. 13–27 [World's Poultry Science Association, editors]. Lloret del Mar, Spain: World's Poultry Science Association.

Grimstead, W. C., Pak, C. Y. K. & Krejs, G. J. (1984). Effects of 1,25-dihydroxyvitamin D3 on calcium absorption in the colon of healthy humans. *American Journal of Physiology* **247**, G189–G192.

Guinotte, F. (1992). Efficacité biologique de diverses sources de carbonate de calcium chez la poule pondeuse et le poulet de chair en croissance (Gallus domesticus). Rôle de la sécrétion acide du proventricule dans la solubilisation et l'utilisation digestive de ces sources (Biological efficiency of various calcium carbonate sources in hens and boilers (Gallus domesticus). Role of proventricular acid secretion in their solubility and intestinal retention). Doctoral thesis, Université de Paris VII.

Guinotte, F., Gautron, J., Soumarnon, A., Robert, J. C., Peranzi, G. & Nys, Y. (1993). Gastric acid secretion in the chicken: effect of histamine H_2 antagonists and H^+,K^+ ATPase inhibitors on gastrointestinal pH and of sexual maturity, calcium carbonate level and particle size on proventricular H^+,K^+ ATPase activity. *Comparative Biochemistry and Physiology* **106A**, 319–327.

Guinotte, F. & Nys, Y. (1991). Effects of particle size and origin of calcium sources on eggshell quality and bone mineralization in egg laying hens. *Poultry Science* **70**, 583–592.

Guinotte, F., Nys, Y. & de Monredon, F. (1991). The effects of particle size and origin of calcium carbonate on performance and ossification characteristics in broiler chicks. *Poultry Science* **70**, 1908–1920.

INRA (1989). *L'alimentation des animaux monogastriques: porc, lapin, volailles.* Paris: Institut National de la Recherche Agronomique.

Itoh, H. (1967). Ca metabolism in laying hens. 5. Ca balance and mobilisation of Ca sources for eggshell formation. *Japanese Journal of Zootechnical Science* **38**, 507–514.

Ivanovitch, P., Fellows, H. & Rich, C. (1967). The absorption of calcium carbonate. *Annals of International Medicine* **66**, 917–923.

Larsson, H., Carlsson, E., Junggren, U., Olbe, L., Sjöstrand, S. V., Skanberg, I. & Sundell, G. (1983). Inhibition of gastric acid secretion by omeprazole in the dog and rat. *Gastroenterology* **85**, 900–907.

Long, J. F. (1967). Gastric secretion in unanesthetized chickens. *American Journal of Physiology* **212**, 1303–1307.

Low, A. G. (1990). Nutritional regulation of gastric secretion, digestion and emptying. *Nutrition Research Reviews* **3**, 229–252.

Mongin, P. (1976*a*). Composition of crop and gizzard contents in the laying hen. *British Poultry Science* **17**, 499–507.

Mongin, P. (1976*b*). Ionic constituents and osmolality of the small intestinal fluids of the laying hen. *British Poultry Science* **17**, 383–392.

Nilas, L., Christiansen, C. & Christiansen, J. (1985). Regulation of vitamin D and calcium metabolism after gastrectomy. *Gut* **26**, 252–257.

Nys, Y. & Cabrera-Saadoun, M. C. (1986). Daily changes in acid secretion, intestinal soluble calcium and study on carbonic anhydrase activity in pullets and laying hens laying shell-less or calcified eggs. *VIIth European Poultry Conference*, pp. 1037–1041 [M. Larbier, editor]. Paris: World's Poultry Science Association.

Pak, C. Y. C. & Avioly, L. V. (1988). Factors affecting absorbability of calcium from calcium salts and food. *Calcified Tissue International* **43**, 55–60.

Petith, M. M. & Schedl, H. P. (1976). Intestinal adaptation to dietary calcium restriction: in vivo caecal and colonic transport in the rat. *Gastroenterology* **71**, 1039–1042.

Rao, K. S. & Roland, D. A. Sr (1990). Retention pattern of various sized limestone particles in gizzard of commercial leghorn hens. *Poultry Science* **69**, 185 Abstr.

Recker, R. R. (1985). Calcium absorption and achlohydria. *New England Journal of Medicine* **313**, 70–73.

Roche, M., Ruckebush, Y. & Achard, F. (1983). Effects of mash diet and pellets upon digestive motor functions in domestic hens and guinea hens. *Canadian Journal of Animal Science* **63**, 663–669.

Ruoff, H. J. & Sewing, K. F. (1971). The role of crop in the control of gastric acid secretion in chickens. *Naunyn-Schmiedebergs Archiv für Pharmakologie* **271**, 142–148.

Sklan, D., Shachaf, B., Baron, J. & Hurwitz, S. (1978). Retrograde movement of digesta in the duodenum of the chick: extent, frequency and nutritional implications. *Journal of Nutrition* **108**, 1485–1490.

Tüüklanen, J. & Väänanen, H. K. (1986). Omeprazole, a specific inhibitor of H^+,K^+ ATPase, inhibits bone resorption in vitro. *Calcified Tissue International* **38**, 123–125.

van der Klis, J. D., Verstegen, M. W. A. & de Witt, W. (1991). Absorption of mineral and retention time of dry matter in the gastrointestinal tract of broilers. *Poultry Science* **69**, 2185–2194.

Walt, R. P., Gomes, M. de F. A., Wood, E. C., Logan, L. H. & Pounder, R. E. (1983). Effect of daily omeprazole on 24 hours intragastric acidity. *British Medical Journal* **287**, 12–14.

Wyatt, C. L., Jensen, L. S. & Rowland, G. N. (1990). Effects of cimetidine on eggshell quality and plasma 25-hydroxycholecalciferol in laying hens. *Poultry Science* **69**, 1892–1899.

Printed in Great Britain