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This research was carried out by Wageningen Livestock Research, within the framework of the public private partnership "Voedsel-en voederveiligheid en valorisatie van nieuwe en wettelijk beperkte reststromen voor diervoeder" of the Dutch Topsector Agri&Food (LWV19091).

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Foreword

By 2050, the global human population is expected to have grown to around 9.5 billion people. Unless we change our patterns of production and consumption, we risk exhaustion of the planet's resources. A transition towards a circular agriculture system is needed, based on the principle of optimising the use of available biomass. Prevention and reduction of food waste and valorisation of side- and surplus streams are crucial elements in achieving circular agriculture. The waste streams of a supply chain can be the raw materials for another. The use of eggshells, as an alternative source of $CaCO_3$, may reduce the footprint related to the use of natural reserves of limestone. Dried eggshells from the breaker industry are already used as feed ingredient in laying hen diets. The residual material of hatched eggs of broiler breeders could also be an interesting waste stream for use in animal diets, but up to now it was not allowed to use eggshells from hatcheries in animal diets. The eggshells from hatcheries can be a valuable source of calcium, while removal of the eggshells can reduce the volume and improve the quality of the remaining organic waste stream. Schaffelaarbos, a Symrise company, Wageningen University & Research and broiler breeding companies worked together to develop processes to separate eggshells from other residual material and produce a calcium rich feed ingredient. The processes required after collection of the eggshells are largely similar to those used by Schaffelaarbos to produce the calcium product Calcolin from shells of eggs used in food products. Although this product is commercially available, the digestion and absorption of calcium and potential impact on phosphorous digestibility has not yet been determined. Therefore a study was conducted in which the pre-caecal digestibility of calcium from eggshells of different sources (broiler breeders or laying hens), process and particle size (coarsely broken or finely ground) was determined in male broilers. The pre-caecal Ca-digestibility of the different eggshell products was compared with limestone. This report describes the results of this study.

This study was part of the public-private partnership project LWV19091, entitled: 'Voedsel- en voederveiligheid en valorisatie van nieuwe en wettelijk beperkte reststromen voor diervoeder' of the Dutch Topsector Agri&Food.

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Summary

Prevention and reduction of food waste and valorisation of side- and waste streams are crucial elements in achieving circular agriculture. The use of eggshells, as an alternative source of $CaCO_3$, may reduce the footprint related to the use of the natural reserves of limestone. Dried eggshells of consumption eggs from the breaker industry are already used as feed ingredient in laying hen diets. The eggshells from hatcheries can, also be a valuable source of calcium, while removal of the eggshells can reduce the volume and improve the quality of the remaining organic waste stream. However, the digestion and absorption of calcium and potential impact on phosphorous digestibility is unknown. Therefore a study was conducted in which the pre-caecal digestibility of calcium from eggshells of different sources (broiler breeders or laying hens), process and particle size (coarsely broken or finely ground) was determined in male broilers.
The study was largely in line with the WPSA protocol for determination of pre-caecal P-digestibility in poultry and was conducted from 15 – 23 days of age with 360 one-day-old broiler chickens of the commercial Ross 308 strain in the research facility of Wageningen Bioveterinary Research, Lelystad, The Netherlands. The study compromised six treatments (see table) and each treatment had six replicate pens with ten broiler chickens per pen.

Treatment	Description	Test product	Inclusion (%)	n animals
1	Basal diet (BD)	--	--	(6 * 10 =) 60
2	BD + Limestone	Limestone	0.573	(6 * 10 =) 60
3	BD + Hatchery egg shells, coarse	Dried Hatchery Shell granulate	0.600	(6 * 10 =) 60
4	BD + Hatchery egg shells, fine	Dried Hatchery Shell powder	0.600	(6 * 10 =) 60
5	BD + Consumption egg shells, centrifuged	Dried Egg Shell (bench) granulate	0.600	(6 * 10 =) 60
6	BD + Consumption egg shells, pressed	Dried Egg Shell (SB) granulate	0.600	(6 * 10 =) 60

The diets were formulated to meet or exceed the CVB recommendations (CVB, 2018) for all nutrients except Ca and P. For the formulation of the basal diet, except soybean meal, ingredients with a very low P and phytate content were used. The main components were maize starch, soybean meal, sucrose, purified potato protein (Protastar®), oat hulls and egg white powder. The calculated (total) Ca- and P-content of the basal diet were 2.44 and 1.75 g/kg, respectively. The calculated Ca-content of the test diets was 4.54 g/kg with 2.1 g Ca per kg from the tested products and a calculated Ca:P ratio of 1.4:1 in all diets. Since the test products were not analysed prior to the diet formulation, we assumed that all eggshell products had the same Ca content (350 g/kg) and that the Ca-content of the limestone was 395 g/kg. Thus, the inclusion level was 0.6% for all eggshell products and 0.573% for limestone. Dietary Ca-levels were realised by replacing part of the Diamol in the basal diet by the test products. Additional mono sodium phosphate (MSP) was included to achieve a Ca:P ratio of 1.40 in the experimental diets. Titanium dioxide (5 g/kg) was used as indigestible marker. Feed and water were freely available and a day/night schedule of 18 hours light and 6 hours dark (18L:6D) was provided during the entire experimental period.
During the experimental period body weight gain (BWG), feed intake (FI), feed conversion ratio (FCR) and mortality were determined and at 23 days of age the chyme of the terminal ileum was collected and pooled per pen and analysed to determine pre-caecal Ca-digestibility. Furthermore, the left tibia bone of three birds per pen was collected at day 23 to determine the ash, Ca and P content. From the results, the following conclusions were drawn:

- Source, process and particle size of the eggshell products had no significant on performance of male broilers from 15 – 23 days of age. No significant differences in performance were observed between the eggshell products and fine limestone.
- Supplementation of limestone and the eggshell products to the deficient basal diet enhanced growth performance of the broilers, as indicated by a higher BWG and lower FCR.
- Supplementation of eggshell products and limestone to the deficient basal diet increased defatted tibia weight, tibia ash content, and tibia ash weight, but not P and Ca content within tibia ash.

- Estimated pre-caecal Ca-digestibility of the complete diets was 82% for granulated egg shells, 85% for finely ground hatchery egg shells and 87% for fine limestone.
- Estimated pre-caecal digestibility of the supplementary Ca from eggshell products was 96% for the granulated products, 103% for the finely ground hatchery shells and 105% for fine limestone. The high absorption is presumably mediated by the physiological requirements of the broilers for bone retention enabled by the increase in dietary P and Ca.
- No significant differences in pre-caecal Ca-digestibility were observed between the tested eggshell products in the same granulated form. Eggshell source and process did not significantly affect the pre-caecal Ca-digestibility.
- The results indicate a similar digestibility of Ca from CaCO3 in (finely ground) eggshells and fine limestone
- The eggshell products did not influence the pre-caecal P-digestibility of the diet in comparison to limestone.

In conclusion, all tested egg shell products are valuable sources of Ca with similar nutritional properties as fine limestone.

1 Introduction

1.1 General

In the context of PPP-project LVW19091, research has been conducted into residual streams that could potentially be used as ingredients of animal feed. One of these streams comprises the residual material of hatched eggs of broiler breeders. The eggshells can be a valuable source of calcium (Ca), while removal of the eggshells can reduce the volume and improve the quality of the remaining organic waste stream. Schaffelaarbos, a Symrise company, Wageningen University & Research and broiler breeding companies worked together to develop processes to separate eggshells from other residual material and produce a Ca rich feed ingredient. The processes required after collection of the eggshells are largely similar to those used by Schaffelaarbos to produce the calcium product Calcolin from shells of consumption eggs used in food products. Although this product is commercially available, the digestion and absorption of calcium and potential impact on phosphorous digestibility had not been determined. Therefore a study was performed in which the pre-caecal digestibility of calcium from eggshells of different sources (hatchery eggs of broiler breeders and consumption eggs of laying hens), process and particle size (coarsely broken or finely ground) was determined. The pre-caecal Ca-digestibility of the different eggshell products was compared with fine limestone, as the most commonly used Ca-source in poultry diets.

1.2 Study objective

The objective of this study was to determine the influence of source, processes and particle size of eggshells on pre-caecal calcium digestibility and growth performance in Ross 308 male broiler chickens in comparison to finely ground limestone as commonly used calcium source. Secondary, it was the objective to determine the influence of these calcium sources on the digestibility of dietary phosphorous.

2 Material and Methods

2.1 Experimental design

2.1.1 Time and location of the experiment

The experiment was conducted in the Research Facility of Wageningen Bioveterinary Research, Runderweg 4, Lelystad, The Netherlands according to Animal and Human Welfare Codes and Laboratory practice codes relevant in The Netherlands. The experimental protocol was approved by the Animal Welfare Body of Wageningen Research (IVD-WR), Wageningen, The Netherlands on June 3, 2022 (2019.D-0033.016).

Date of arrival broilers:	2 August 2022 (start pre-experimental period)
Date of start animal experiment:	17 August 2022 (start experimental period)
Date of end animal experiment:	25 August 2022 (end experimental period)

2.1.2 Pre-experimental period (0 – 15 days)

A total number of 380 day-old male Ross 308 broilers were obtained from a commercial hatchery. On the day of arrival of the birds at the experimental facility they were weighed as a group and placed in a floor pen (20 m^2) bedded with white wood shavings (2 kg/m^2). This pen was equipped with three drinking lines with each 14 drip cups (Impex, Barneveld, The Netherlands) and six Valenta feeding pans (ø 335 mm, VDL, Eindhoven, The Netherlands). During this period, all animals received a nutrient adequate broiler starter diet containing 214 g CP, 11.9 MJ $ME_{broiler}$, 10.0 g of Ca, and 7.3 g of total P and 4.7 g retainable P per kg (Appendix 1). Feed and water were *ad libitum* available during this pre-experimental period.

2.1.3 Experimental period (15 – 23 days)

At 15 days of age 360 healthy animals were selected, weighed and allocated to 36 pens (1.0 m × 1.2 m; surface 1.2 m^2, with 10 broilers per pen) all located in the same room (Figure 2.1). The allocation assured that the difference between the highest and lowest average broiler weight per pen did not exceed 3% of the average weight. The birds were placed on flexible plastic slatted floors to prevent intake of litter and faeces, which would disturb the determination of nutrient digestibility. Each pen contained a perch (length 90 cm), one Valenta feeding pan (ø 335 mm, VDL, Eindhoven, The Netherlands) and three drip cups (Impex, Barneveld, The Netherlands) (Figure 2.1). During the experimental period the broilers received one of six experimental diets, which were randomly assigned to the pens within each block. The pen and treatment allocation are included in Appendix 2.

Figure 2.1 *Top: overview of the experimental room. Bottom: overview of the experimental pens.*

2.1.4 Test products

The Ca-digestibility of four different eggshell products was determined, with pure limestone (Ca-content 395 g/kg) as a reference. Hatchery eggshells were tested at two different particle sizes: coarse (Dried Hatchery Shell granulate) and fine (Dried Hatchery Shell powder). The shells from consumption eggs were tested at two processing methods: centrifuged (Dried Egg Shell (bench) granulate), the commercially available product, and pressed (Dried Egg Shell (SB) granulate), a granulate of eggshells of consumption eggs that for various reasons had not been used as such and were returned from the breakers. Table 2.1 shows the characteristics of the test products as specified by the suppliers, Table 2.2 shows the analysed nutrient contents of the test products.

Table 2.1 *Characteristics of tested eggshell products (in g/kg) of hatchery eggs and consumption as specified by the suppliers.*

Identification	Batch nr	Origin	Particle Size	Ash	Moisture	Crude protein	Crude fat	Crude fiber	Ca
Dried Hatchery Shell granulate	500-02-220603-B1	RS 3 Hatchery by-product	0,5-1,5mm	939	10	55	<1	11	374
Dried Hatchery Shell powder	500-01-220603-B1	RS 3 Hatchery by-product	<0,2mm	939	10	55	4	11	376
Dried Egg Shell (bench) granulate	504-02-220607-R1	Consumption eggshells (centrifuged) - breaker	0,5-1,5mm	916	18	109	19	5	339
Dried Egg Shell (SB) granulate	504-02-220607-B1	Consumption eggshells (pressed) - Schaffelaarbos	0,5-1,5mm	883	18	75	14	7	356
Limestone	n.a.	Calcitec V / 40 S	D_{50}=6.0 µm	--	10	--	--	--	398

Table 2.2 *Analysed nutrient content of the tested eggshell products (in g/kg).*

Identification	Batch nr	Dry matter	Ash	Ca	P
Dried Hatchery Shell granulate	500-02-220603-B1	995	920	352	1.3
Dried Hatchery Shell powder	500-01-220603-B1	995	926	348	1.3
Dried Egg Shell (bench) granulate	504-02-220607-R1	988	911	340	1.1
Dried Egg Shell (SB) granulate	504-02-220607-B1	981	862	329	1.6
Calcium carbonate / limestone	n.a.	999	997	411	<0.1

In Appendix 3 the particle size distribution of the different test products is given.

2.1.5 Experimental diets

The diets were formulated to meet or exceed the CVB recommendations (CVB, 2018) for all nutrients except Ca and P. For the formulation of the basal diet, except soybean meal, ingredients with a very low P and phytate content were used. The main components were maize starch, soybean meal, sucrose, purified potato protein (Protastar®) and egg white powder. Oat hulls were used to ensure adequate fibre content required for normal functioning of the digestive tract. Diamol was used in the basal diet and replaced with the test products in the experimental diets. The raw materials were selected by Research Diet Services in Wijk bij Duurstede. All individual raw materials came from one representative homogeneous batch and were ground over a 3 mm sieve. Prior to the optimisation of the experimental diets, the raw materials were analysed for dry matter, protein, Ca and P. The calculated (total) Ca- and P-content of the basal diet were 2.44 and 1.75 g/kg, respectively. The calculated Ca-content of the test diets was 4.54 g/kg with 2.1 g Ca per kg from the tested products and a calculated Ca:P ratio of 1.4:1 in all diets. Since the test products were not analysed prior to the diet formulation, we assumed that all eggshell products had the same Ca content (350 g/kg) and that the Ca-content of the limestone was 395 g/kg. Thus, the inclusion level was 0.6% for all eggshell products of and 0.573% for limestone. Dietary Ca-levels were realised by replacing part of the Diamol of the basal diet by the test product. Additional mono sodium phosphate (MSP) was included to achieve a Ca:P ratio of 1.40 in the experimental diets. No coccidiostats or antimicrobial growth promoters were used in the diets. Diets were single pelleted using a 2.5 mm die without steam addition. The realised pellet temperature is included in Appendix 4. Titanium dioxide (5 g/kg) was used as indigestible marker.

The composition of the experimental diets is given in Appendix 5. Diets were produced by Research Diet Services, Wijk bij Duurstede, The Netherlands, and delivered in bags of 20 kg.

Table 2.3 *Description of dietary treatments with different calcium sources.*

Treatment	Diet	Description	Test product	Inclusion (%)	n animals
1	A	Basal diet (BD)	--	--	(6*10 =) 60
2	B	BD + Limestone	Limestone	0.573	(6*10 =) 60
3	C	BD + Hatchery eggshells, coarse	Dried Hatchery Shell granulate	0.600	(6*10 =) 60
4	D	BD + Hatchery eggshells, fine	Dried Hatchery shell powder	0.600	(6*10 =) 60
5	E	BD + Consumption eggshells, centrifuged	Dried Egg Shell (bench) granulate	0.600	(6*10 =) 60
6	F	BD + Consumption eggshells, pressed	Dried Egg Shell (SB) granulate	0.600	(6*10 =) 60

2.1.6 Light schedule

During the first three days of age, the room was continuously illuminated (24L:0D). From 3 to 23 days of age, a day/night schedule of 18 hours light and 6 hours dark (18L:6D) was provided. The light was switched off from 16:00h – 22:00h, to ensure steady state conditions at dissection and homogenous distribution of the feed intake during dissection at day 23. The light intensity was 20 Lux at bird level during the entire experimental period.

2.1.7 Temperature

The experimental room was heated to 33°C one day prior to the start of the experiment. The ambient temperature was gradually reduced to approximately 22.5°C at the end of the experimental period at 23 days of age (Table 2.4). The realised temperature and humidity during the pre-experimental (0 – 15 days) and experimental period (15 – 23 days) is included in Appendix 6.

Table 2.4 *Temperature schedule in the animal facility.*

Age (in days)	Setting temperature (˚C)
0	33
7	28
14	25
21	23
28	21

2.1.8 Vaccination

Day-old broilers were vaccinated at the hatchery against IB (Infectious Bronchitis, Poulvac IB primer, spray vaccination) and at 7 days of age against NCD (New Castle Disease; Nobilis ND Clone 30, spray vaccination) at the trial facility.

2.2 Observations and measurements

2.2.1 Growth performance

Body weight (BW) of birds per pen was determined at 0, 15 and 23 days of age. Cumulative feed intake per pen was determined from 0 – 15 days (pre-experimental period) and from 15 – 23 days of age (= experimental period) as feed supply from 0 – 15 days and 15 – 23 days minus remaining feed in the feeding pan on the last day of each period. Body weight gain (BWG) and feed conversion ratio (FCR) of broilers per pen were calculated from 0 – 15 days (pre-experimental period) and 15 – 23 days (experimental period). Body weight gain was determined as final BW – initial BW; FCR was calculated as (Total FI / (Total BWG + total BWG of dead and culled birds)); FI per animal was calculated as FCR * BWG.

Culling, mortality and health status, including probable causes of culling, illness or death, were recorded daily. In all cases of mortality or culling, the birds were weighed and the date of removal was recorded.

2.2.2 Dissection and sample collection

At 23 days of age the birds were euthanized by electrocution. Subsequently, the chest cavity and the abdomen were opened and the small intestine was ligated and removed from the bird. The contents of the terminal ileum (approx. 20 cm, Figure 2.2), with the ileum defined as the gut segment distal from Meckel's diverticulum to 2 cm proximal to the ileo-caecal junction, were collected from all birds in a pen. The ileal contents (digesta) were collected by flushing the gut segment with distilled water into a plastic container. The digesta of all animals per pen were pooled and immediately frozen and stored at -20°C until further analysis. The digesta were collected in the order of pen number. Digesta samples were freeze-dried and ground (0.5 mm) by NutriControl laboratory, Veghel, The Netherlands.

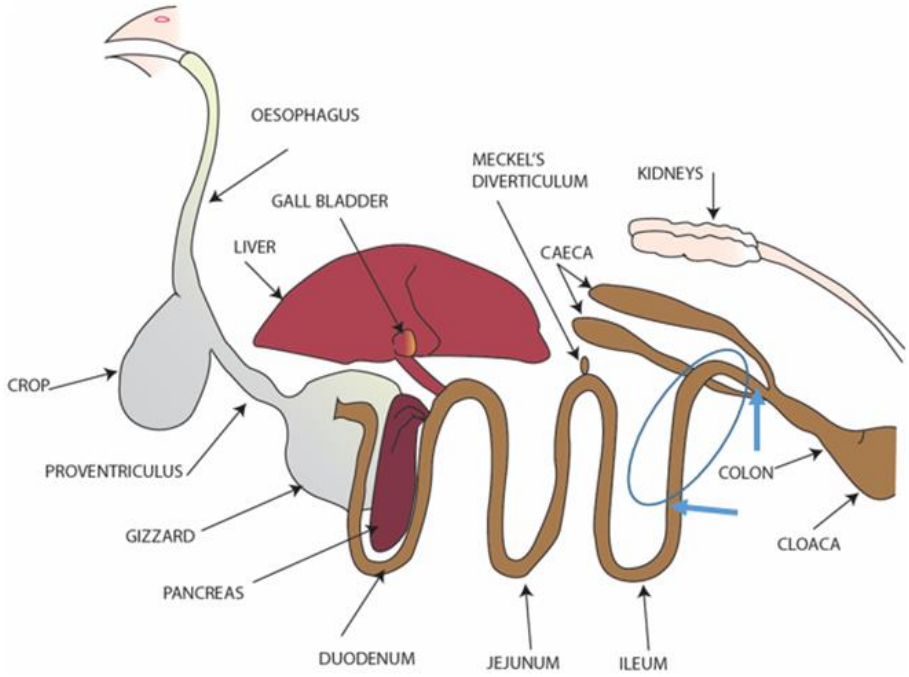


Figure 2.2 *Site of collection of the digesta from the distal part (approx. 20 cm) of the ileum. Adapted from Poultry Hub.*

From the first three animals of each pen, the left tibia bone was collected to determine the tibia ash, P and Ca content. The tibia bones were pooled per pen and stored in coded plastic bags at -20°C until further processing. At the lab, the tibias were cleaned from soft tissue after boiling each pooled tibia sample for 10 minutes in a closed 50 ml tube with demi water. Thereafter the bones were split longitudinally and the cartilage (bone marrow) was removed. After drying, the tibia bones were defatted with organic solvent, weighed and incinerated at 825°C to determine the ash content. The P and Ca content in the ash were subsequently measured using inductively coupled plasma optical emission spectrometry (ICP-OES). Based on the (fat-free) tibia weight and the determined ash content the total ash weight per tibia was calculated. The processing and analysis of the tibia bones was performed by the NutriControl laboratory, Veghel, The Netherlands.

2.2.3 Chemical analyses

Test products (eggshell products and limestone)

- All test products were analysed for dry matter, ash (550 °C), Ca and P.

Diets

- The starter diet (0 – 15 days) was analysed for dry matter, ash, crude protein (N*6.25), crude fat (HCl), crude fibre, total P and Ca.
- All experimental diets were analysed for dry matter, ash, crude protein, Ca, P and indigestible marker (TiO_2). The basal diet was also analysed for crude fat and crude fibre.

Digesta

- Ileal digesta were analysed for dry matter, ash, crude protein, Ca, P, and indigestible marker (TiO_2).

Tibia bones

- Defatted tibia bones were analysed for ash, P and Ca. Ash content of the tibia is expressed as ash in fat-free dry matter and the P and Ca content of the tibia as P and Ca in ash.

Diets, digesta, test products and tibia bones were analysed by NutriControl, Veghel, The Netherlands.

2.2.4 Digestibility calculations

Based on the analysed content in the experimental diets and digesta, the pre-caecal digestibility of dry matter, crude protein, Ca and P was calculated on pen basis using the following equation:

$$\text{Digestibility (\%)} = 100 - [100 \times (M_{Diet} \times \text{Nutrient}_{Digesta}) / (M_{Digesta} \times \text{Nutrient}_{Diet})]$$

Where: M_{Diet} and $M_{Digesta}$ are the analysed concentration of marker (TiO_2) in the diet and digesta (g/kg DM)
Nutrient_{Diet} and $\text{Nutrient}_{Digesta}$ are the analysed concentration of nutrient in the diet and digesta (g/kg DM)

The digestible Ca and P content in the diet was calculated using the following formula:

$$\text{Digestible nutrient content (g/kg)} = \text{digestibility of the nutrient (\%)} \times \text{nutrient}_{Diet} / 100$$

In addition, the digestibility of added Ca from the test products (and P from mono sodium phosphate) was calculated according to the classical method in which the digestible nutrient content of the basal diet is determined and subtracted from the digestible nutrient content in each of the experimental diets with one of the test products:

$$A = ((Y - b) / x) * 100$$

Where:
A = Ca digestibility test product
Y = digestible content of Ca in the test diet (g/kg)
x = Ca content originating from test product (g/kg)
b = digestible Ca in basal diet

2.3 Statistical analysis

A completely randomized block design consisting of six treatments and six blocks of 6 adjacent pens was used in this experiment. Performance, tibia bones and nutrient digestibility of the diets were statistically analysed by ANOVA using GenStat statistical software (19th edition, VSN International Ltd., Hemel Hempstead, UK), with pen as experimental unit, diet as fixed factor and block as random factor according in the statistical model:

$Y = \mu + block_i + diet_j + e_{ij}$

Where:

Y	=	Response parameter
μ	=	General mean
block	=	Effect of block (i=1..6)
diet	=	Effect of diet (j=1..6)
e	=	Error term

Nutrient digestibility of the tested products (Ca-sources: eggshells and limestone) was statistically analysed by ANOVA using test product as fixed factor and block as random factor according to the statistical model:

$Y = \mu + block_i + source_j + e_{ij}$

Where:

Y	=	Response parameter
μ	=	General mean
block	=	Effect of block (i=1..6)
source	=	Effect of test product (j=1…5)
e	=	Error term

The *P*-value of the treatment effect and the SEM (standard error of the least square means) were provided per response parameter. Treatment effects with a *P*-value ≤ 0.05 were considered statistically significant. Pairwise differences were determined with Fisher Unprotected t-test as multiple comparison test.

3 Results

3.1 Experimental diets

The results of dietary analysis in Table 3.1 show that the analysed values agree well with the calculated values and that the composition of the diets was as intended.

Table 3.1 *Analysed and calculated (in parentheses) nutrient content of experimental diets (g/kg).*

Treatment number	1	2	3	4	5	6
Description	**Basal diet**	**Limestone**	**Dried Hatchery Shell granulate**	**Dried Hatchery shell powder**	**Dried Egg Shell (bench) granulate**	**Dried Egg Shell (SB) granulate**
Dry matter	898 (911)	902 (911)	903 (910)	908 (910)	910 (910)	910 (910)
Ash	40 (46)	42 (47)	42 (46)	42 (46)	42 (46)	42 (46)
Crude protein	186 (185)	186 (185)	186 (185)	189 (185)	191 (185)	190 (185)
Calcium	2.51 (2.44)	4.74 (4.54)	4.58 (4.54)	4.80 (4.54)	4.54 (4.54)	4.52 (4.54)
Phosphorus	1.79 (1.75)	3.13 (3.25)	3.22 (3.25)	3.30 (3.25)	3.27 (3.25)	3.45 (3.25)
Ca/P	1.40 (1.40)	1.51 (1.40)	1.42 (1.40)	1.45 (1.40)	1.39 (1.40)	1.31 (1.40)
Titanium	3.14 (3.00)	3.10 (3.00)	3.10 (3.00)	3.04 (3.00)	3.00 (3.00)	2.97 (3.00)

3.2 Animal health and performance

Day-old broilers arrived healthy with an initial BW of 41 grams (Table 3.2). During the pre-experimental period the BWG, FI and FCR were in agreement with the Ross 308 performance objectives for male broilers (Aviagen, 2022).

Table 3.2 *Growth performance of broilers in the pre-experimental period (0 - 15 days of age).*

	This experiment	Ross 308, 2022
Body weight, day 0 (g)	41	44
Body weight, day 15 (g)	593	603
Body weight gain (g)	552	559
Mortality (%)	0.3	--
Feed conversion ratio	1.096	1.104
Feed intake (g)	605	617

Average BWG over the entire experimental period (15 – 23 days of age) of the broilers was slightly above the Ross 308 performance objectives (647 vs. 616 g), while the mean FI of the broilers was almost 5% higher than the Ross 308 performance objectives (855 vs. 817 g) (Table 3.3). Because both BWG and FI were higher, the FCR was similar to the performance objectives (1.324 vs. 1.326).
The mortality during the experimental period was 0.3%, which can be considered as relatively low mortality rate.

Growth performance during the experimental period is presented in Table 3.3. Broilers fed the basal diet (Treatment 1) had a lower BW and BWG and a higher FCR than broilers fed the diets supplemented with one of the test products. The poor performance of the birds fed the basal diet can be explained by the low Ca and P levels in this diet, well below the requirements of the broilers. No significant differences in BWG , final BW and FCR were observed between the treatments supplemented with one of the test products. Dietary treatment had no significant effect on the mortality rate of broilers.

3.3 Nutrient digestibility of the diets

Table 3.4 presents the pre-caecal digestibility (pcd) of dry matter (DM), crude protein (CP), Ca and P of the experimental diets. The DM digestibility of the diets varied between 80.0 and 81.6%. The basal diet had the lowest DM digestibility, but this value was only significantly lower than the diet supplemented with dried hatchery shell powder (Diet 4) and the diet supplemented with dried eggshell (bench) granulate (Diet 5), not with the other supplemented diets. The inclusion of the test products in the diet had no significant effect on the CP digestibility. The digestibility of Ca was significantly enhanced by the inclusion of all test products , but there were no significant differences in Ca-digestibility between diets supplemented with the different eggshell products. The highest Ca-digestibility was observed with the diet supplemented with limestone (Diet 2). The Ca-digestibility of this diet was significant higher than the diets supplemented with the eggshell products, with the exception of Diet 4, supplemented with dried hatchery eggshell powder. No differences in P-digestibility were observed between the diets supplemented with the test products, but the P-digestibility of the basal diet was significantly lower compared to all supplemented diets. The dietary P digestibility was enhanced by inclusion the test products plus an amount of mono sodium phosphate to assure a constant Ca:P ratio. The dietary P digestibility was not different among the eggshell products, but the P digestibility of the diet with limestone was lower than the diet with dried eggshell granulate (Diet 6).

3.4 Pre-caecal digestibility of the test products

The pre-caecal digestibility of Ca of the tested limestone and the different eggshell products are given in Table 3.5 and the variation in Ca- and P-digestibility within and among the test products is presented in Figures 3.1 and 3.2. No differences were observed in the pre-caecal digestibility of Ca between the different test products. The pre-caecal Ca-digestibility of all test products was high and ranged from 95 to 105%. Numerically, limestone had the highest pre-caecal Ca-digestibility of 105% and the benchmark eggshell product had the lowest pre-caecal Ca-digestibility of 95%. The benchmark eggshell product also had the highest variation in Ca-digestibility (Figure 3.1). Based on the estimated pre-caecal Ca-digestibility and the analysed Ca content of the test products, the digestible Ca (dCa) content of the test product (g/kg) was calculated and included in Table 3.5.

Table 3.3 *Growth performance of broilers per treatment with Ca from different eggshell sources, from 15 – 23 days of age (experimental period).*

Treatment number	1	2	3	4	5	6		
Description	**Basal diet**	**Limestone**	**Dried Hatchery Shell granulate**	**Dried Hatchery shell powder**	**Dried Egg Shell (bench) granulat**	**Dried Egg Shell (SB) granulate**	**F-prob**	**SEM**
Body weight day 15 (g)	599	593	589	588	598	595	0.347	4.0
Body weight day 23 (g)	1171 [b]	1251 [a]	1254 [a]	1240 [a]	1262 [a]	1264 [a]	<.001	11.4
Body weight gain day 15 – 23 (g)	573 [b]	658 [a]	665 [a]	652 [a]	665 [a]	669 [a]	<.001	8.3
Body weight gain day 15 – 23 (g/d)	71.6 [b]	82.2 [a]	83.1 [a]	81.5 [a]	83.1 [a]	83.6 [a]	<.001	1.03
Mortality (%)	0.0	0.0	0.0	3.3	0.0	1.7	0.551	1.55
Feed conversion ratio	1.435 [a]	1.304 [b]	1.291 [b]	1.312 [b]	1.291 [b]	1.312 [b]	<.001	0.0102
Feed intake day 15 – 23 (g)	822 [b]	858 [ab]	858 [ab]	855 [ab]	858 [ab]	878 [a]	0.032	10.6
Feed intake day 15 – 23 (g/d)	102.7 [b]	107.2 [ab]	107.2 [ab]	106.9 [ab]	107.2 [ab]	109.7 [a]	0.032	1.32

[a,b] Values without a common superscript per row differ significantly (P < 0.05).

Table 3.4 *Pre-caecal digestibility (%) of dry matter, crude protein, calcium and phosphorus of diets supplemented with different Ca-sources in broilers killed at 23 days of age[1].*

Treatment number	1	2	3	4	5	6		
	Basal diet	**Limestone**	**Dried Hatchery Shell granulate**	**Dried Hatchery shell powder**	**Dried Egg Shell (bench) granulat**	**Dried Egg Shell (SB) granulate**	**F-prob**	**SEM**
Dry matter	80.0 [b]	80.7 [ab]	80.7 [ab]	81.2 [a]	81.6 [a]	80.7 [ab]	0.028	0.31
Crude protein	82.7	81.9	81.7	82.5	83.4	82.7	0.348	0.58
Calcium	70.5 [d]	86.8 [a]	82.2 [bc]	85.7 [ab]	81.3 [c]	81.7 [c]	<.001	1.32
Phosphorus	71.1 [c]	81.3 [b]	82.0 [ab]	82.3 [ab]	82.5 [ab]	83.1 [a]	<.001	0.47

[a,b,c] Values without a common superscript per row differ significantly (P < 0.05).

[1] Results of pen 134, treatment 1 (Basal diet) were identified as outlier and excluded from the analysis

Table 3.5 *Pre-caecal calcium and phosphorus digestibility (%) and calculated available Ca content (g/kg) of the different Ca-sources in male broilers killed at 23 days of age.*

P- and Ca source	Limestone	Dried Hatchery Shell granulate	Dried Hatchery shell powder	Dried Egg Shell (bench) granulate	Dried Egg Shell (SB) granulate	F-prob	SEM
Pcd Ca (%)	105.3	96.7	102.5	94.8	95.8	0.104	3.10
Pcd P (%)	94.7	95.4	95.4	96.2	95.8	0.880	1.06
Available Ca (g/kg)	420	340	357	322	315		

Table 3.6 *Weight and composition of the defatted tibia-bones of male broilers killed at 23 days of age fed diets with the different P and Ca sources.*

Treatment number	1	2	3	4	5	6		
	Basal diet	Limestone	Dried Hatchery Shell granulate	Dried Hatchery shell powder	Dried Egg Shell (bench) granulate	Dried Egg Shell (SB) granulate	F-prob	SEM
Tibia defatted (g)	5.56 [b]	7.12 [a]	7.26 [a]	7.20 [a]	7.07 [a]	6.95 [a]	<.001	0.177
Ash (g/kg)	392 [b]	467 [a]	470 [a]	464 [a]	465 [a]	466 [a]	<.001	2.7
Total ash (g)	2.18 [b]	3.33 [a]	3.41 [a]	3.34 [a]	3.29 [a]	3.24 [a]	<.001	0.082
Calcium (g/kg)	376	374	373	372	374	372	0.597	1.6
Phosphorus (g/kg)	174	175	175	174	178	175	0.302	1.3
Ca/P ratio	2.16	2.14	2.13	2.14	2.10	2.13	0.144	0.015

[a,b] Values without a common superscript per row differ significantly (P < 0.05).

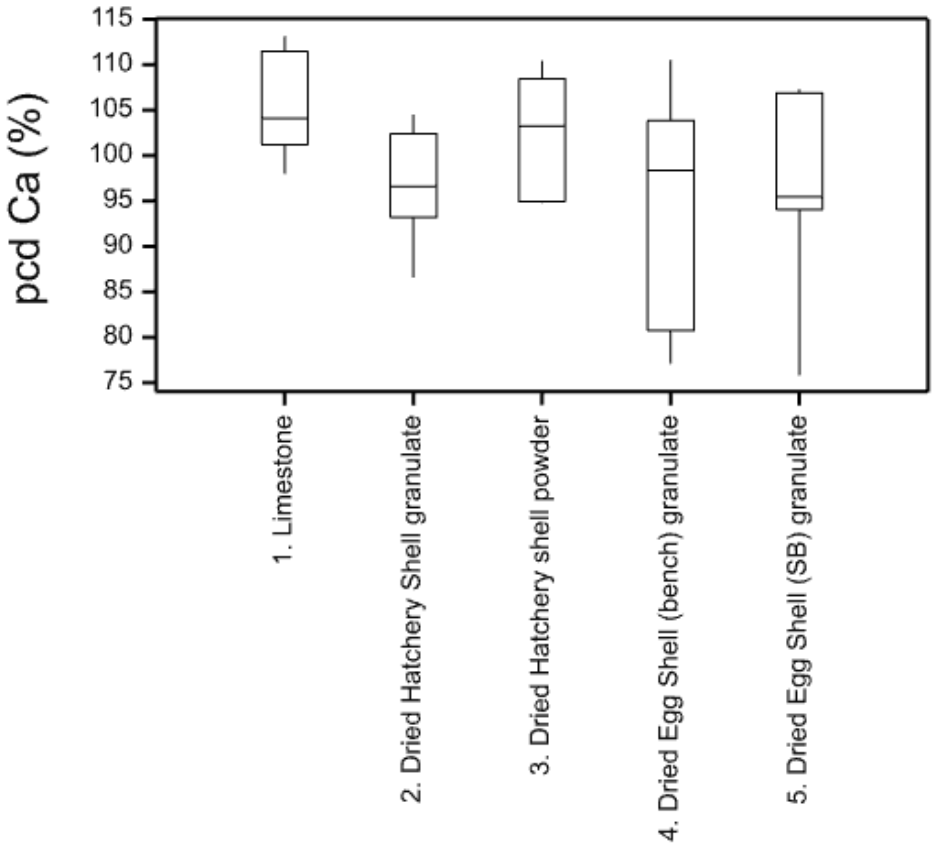


Figure 3.1 *Boxplot for pre-caecal Ca-digestibility (%) of the tested limestone (fine) and eggshell products as Ca sources.*

3.5 Tibia composition

Table 3.6 shows the effect of diet on weight and composition of the defatted tibia and the Ca:P ratio of the tibia-bone. Broilers fed the basal diet had a lower defatted tibia weight, tibia ash content and total ash weight compared to broilers fed diets supplemented with one of the test products. Inclusion of each test product enhanced weight and ash content of the tibia bones. No differences were observed in defatted tibia weight, tibia ash content and total ash weight between broilers fed diets supplemented with either of the test products (Ca sources). The mean Ca and P content in tibia ash was 374 and 175 g/kg, with a Ca:P ratio of 2.14. Dietary treatments did not significantly affect either of these characteristics, tibia Ca content, P content and Ca:P ratio.

4 Discussion

4.1 Eggshells

The aims of this study were to determine the digestibility of Ca from shells of hatchery eggs and consumption eggs, obtained via different processes, and the influence of these products on growth performance and digestibility of dietary P. High quality finely ground limestone was used as reference ingredient. Two products, dried hatchery shells granulate and powder were obtained as pilot product from a hatchery farm in the context of this project. These products were not commercially available when this experiment was conducted, but it was anticipated that hatchery egg shells would be a valuable source of Ca for animal diets. At present, eggshells of hatchery farms are removed for destruction and lost as ingredient in animal feed for food production. Nonetheless, eggshells can replace limestone as Ca source in animal diets and hence contribute to the circularity of animal production. In this study, these eggshells were used as granulate, i.e. in a finely broken form, or additionally ground using an 0.2 mm screen in a Retsch centrifugal mill, to determine the influence of particle size on the digestibility of Ca. As a third product, a granulate of dried shells from consumptions eggs was used, produced by centrifugation to remove the residual protein fraction. This product was already commercially available under the name Calcolin and used as Ca source in animal diets, but the digestibility of Ca was based on in vitro studies and had not been determined in vivo. The final product was a granulate of eggshells of consumption eggs that for various reasons had not been used as such and were returned from the breakers. These eggs were processed by pressing to separate the eggshells from the fluid contents, i.e. yolk and egg white. The contents are a valuable protein-rich product for various applications, while the shells are a potentially valuable Ca source.

4.2 Growth performance

The growth performance of birds fed the control diet was lower, i.e. lower BWG and higher FCR than all other treatments indicating that Ca and P limited the growth performance. This was expected since Ca and P content of the control diet were well below recommendation. Inclusion of all Ca sources equally enhanced BWG and feed efficiency. Hence, all supplemented diets allowed the birds to realize a growth performance in line with the Ross performance guide. No differences were observed between these dietary treatments. However, growth performance is not a sensitive read-out parameter for availability of Ca, therefore the pcd was determined.

4.3 Experimental model to determine digestibility

Nowadays the nutrient digestibility assays in poultry are based on the analysis of ileal digesta rather than of excreta, because of the variable and modifying effects of hindgut microflora and potential contamination with nutrient excreted in the urine (Ravindran et al., 1999). Ileal digestibility is the proportion of dietary Ca that is not recovered in the digesta at the terminal ileum. No generally accepted protocol to determine pcd of Ca has been published. Therefore we used the WPSA protocol for P digestibility (Rodehutscord, 2013). The principle makes use of a basal diet, limiting in Ca and P and one or two supplementary levels of Ca and P in a constant ratio. The recommended available P content of the basal diet should not exceed 1.5 g/kg, with a total Ca:P ratio of 1.3 to 1.4. The increase in absorbed Ca and P from the supplemented diet is divided by the increase in total Ca and P (from the test ingredient) to determine the digestibility of Ca and P from the test ingredient. Because the endogenous Ca and P losses from the digestive tract are accounted for in their excretion for the basal diet, the pcd calculated for the test ingredient by comparison of the basal and supplemented diets can be regarded as what is often called "true" or "standardised" pcd, i.e. corrected for basal endogenous losses. We considered the approach in the WPSA protocol suited to determine the pcd of both Ca and P since at each inclusion level the Ca:P ratio is kept constant to minimize the potential effects of an imbalance between these two minerals.

In the supplemented diets, 2.1 g Ca and 1.5 g P, with Ca:P ratio 1.4 was added. A similar approach, although using a higher dietary P level and lower Ca:P ratio was applied by Zhang and Adeola (2018) to determine additivity of Ca digestibility of limestone and dicalcium phosphate.

An alternative approach is based on the inclusion of the Ca source of interest, e.g. limestone or monocalcium phosphate as the main Ca source, and attributing the dietary Ca digestibility to this source. This so-called direct approach requires only one diet, since there is no comparison with a low Ca basal diet. Consequently, the influence of the basal diet and the potentially deviating digestibility of Ca from other sources in the basal diet, apart from the test source, is ignored, while it has been demonstrated that this can influence the observed digestibility (David et al., 2019). Moreover, in this approach, the basal endogenous Ca losses from the digestive tract are included in the undigested fraction, implicating that the obtained pcd reflects the apparent rather than the standardised digestibility. Hence we consider this approach as less preferred. In some studies an additional treatment with a Ca and/or P-free diet is included to determine the basal endogenous losses and subsequently calculate the standardised pcd of the test diets and ingredients (Anwar et al., 2016b). This precludes the latter disadvantage.

4.4 Realised digestibility

In the present study, Ca and P digestibility of the basal diet were both 71%. These results are largely in line with earlier studies of our institute. Bikker et al. (2016) and Van Harn et al. (2017) observed a pcd of P from 73 to 81% and of Ca from 66 to 70% using purified basal diets, similar to the present study. Calcium was largely derived from limestone, P from mono calcium phosphate. In their recent study, Zhang and Adeola (2018) reported for the basal diets a pcd of 66-69% for P and 56-62% for Ca, derived from potassium phosphate, dicalcium phosphate and limestone. The P content in the basal diet was substantially higher than in our study, 4.8 versus 1.8 g/kg, reflecting a higher phytate content that may have reduced Ca and P digestibility.
Apparent digestibility of the supplemented diets was between 81 and 87% for Ca and approximately 82% for P (Table 3.4). These results are in line with our earlier studies (Bikker et al., 2016; Van Harn et al., 2017) but relatively high compared to other studies. Walk et al. (2021) reviewed the literature and reported that in seven publications 55 values were reported for apparent digestibility of Ca in limestone, varying from 20 to 77%, with a mean of 53%. The standardised digestibility (i.e. corrected for basal endogenous losses) varied from 35 to 77% with a mean of 55%. Several dietary factors may have contributed to the large variation in the obtained Ca digestibility: dietary Ca content and Ca:P ratio, particle size of the Ca source, composition of the basal diet, dietary phytate content, presence of phytase (phytate degrading enzyme), and others. Dietary Ca content seems the major influencing factor. Several studies demonstrated that an increase in dietary Ca content and Ca:P ratio reduced the pcd of Ca. Anwar et al. (2016b) reported a decrease in true pcd of Ca from limestone from 65% to 49% with increasing Ca content from 6.7 to 11.0 g/kg and Ca:P ratio from 1.5 to 2.5. In the study of Hu et al. (2020) the pcd of Ca from fine limestone decreased from 62% to 43% with an increase of Ca content from 3.1 to 9.3 g/kg of diet. Walk et al. (2022) reported a linear decrease in apparent pcd of Ca from 79% to 50% when dietary Ca content was increased from 3.7 to approximately 9 g/kg. The higher absorption of Ca at a low dietary Ca content reflects the physiological mechanisms of the bird to regulate Ca absorption in relation to their requirements and dietary supply. The increased absorption is mediated by an increased release of the Parathyroid hormone (PTH) and synthesis of 1,25(OH)2D3 in the kidneys, resulting in increased Ca absorption from the small intestine and reabsorption of Ca from kidneys (Anwar et al., 2016). This was confirmed by Hu et al (2022) who reported an increase in Ca sensing receptors (CaSR) and Ca transporting proteins (CaBP-D28K, PMCA1) in the small intestine of broilers at a low dietary Ca content. With increasing Ca content, the expression of these genes was reduced, presumably indicating a shift from active to passive absorption of Ca. These results confirm the large impact of dietary Ca content on Ca digestibility. The high level of digestibility in our study can be largely explained by the relatively low dietary Ca content, as included on the basis of the WPSA protocol. These results indicate that the potential digestibility of Ca sources is relatively high, while the actual digestibility or absorption obtained in many studies is influenced by potential down regulation of the Ca absorption and possibly also from interactions between diet components. The influence of particle size of limestone on Ca digestibility is ambiguous. Anwar et al. (2016b) reported a 28%-units higher pcd of Ca in the coarse fraction compared to the fine fraction of limestone. In contrast, Hu et al.

(2020) reported a slightly higher Ca digestibility of coarse limestone at a low Ca level but a lower Ca digestibility at a higher inclusion level, compared to fine Ca. The later authors argued that large limestone particles may accumulate in the gizzard during the first few days of supply, thus causing an overestimation of the pcd of Ca. From these results it is difficult to conclude whether the fine particle size of limestone in our study had a beneficial effect on Ca digestibility or not.

The pcd of P was 71% for the basal diet and enhanced to 81-83% in the supplemented diets, reflecting the high digestibility of P in mono sodium phosphate. The P digestibility among supplemented diets was similar, with a small difference between the limestone diet and the diet with dried hatchery shell (SB) granulate (Table 3.4).

4.5 Digestibility of egg shells

The Ca digestibility of diets supplemented with egg shells was 81-82% for the three granulated products and 85% for the finely ground dried hatchery shells, compared to 87% for fine limestone (Table 3.4). These results indicate that the origin of the eggshells, from consumption eggs or hatchery eggs and the processes involved did not significantly influence the Ca digestibility. The fine grinding of hatchery egg shells did not cause a significant difference between the granulate and powder of this product. Nonetheless, the difference suggests that a small increase of 3-4% in digestibility can be realised by the additional grinding step. The digestibility of fine limestone diets was approximately 5%-units higher than the granulated egg shell diets. The similar pcd of fine limestone and fine hatchery eggshells suggests that the difference between limestone and granulated eggshells may be (partly) explained by particle size of the products. Hence, these results indicate an overall high digestibility of egg shells, close to the digestibility of fine limestone. The similarity of the products is confirmed by the absence of a difference in tibia ash weight and tibia composition.
The digestibility of the supplemented Ca was determine by difference of the basal diet and the supplemented diets. The results in Table 3.5 indicate a high digestibility of approximately 96% for Ca in the granulated products and 103 and 105% for finely ground hatchery shells and limestone, respectively. Hence, these results confirm a potentially high digestibility of these sources when Ca (and P) is supplied below requirements. A question is whether a digestibility above 100% of supplemented Ca is physiologically possible. This is addressed in the next paragraph.

4.6 Absorption of supplementary calcium

The digestibility of Ca and P of the supplemented Ca sources and monosodium phosphate was determined from the difference in digestibility between the supplemented diets and the basal diet. The digestibility (absorption) of supplemented Ca was between 95 and 105%, while P digestibility of supplemented mono sodium phosphate was 95% (Table 3.5). These values are relatively high, while in particular the pcd of Ca above 100% is remarkable. Nonetheless, it can be argued that this result is within physiological limits. The Ca and P digestibility of test products is calculated as the additional absorption of Ca and P when a test product is added, divided by the Ca and P added via the test products. Hence, when the inclusion of the test products additionally enhanced the absorption of Ca from the basal diet, this is (arithmetically) regarded as derived from the test products and would result in a digestibility coefficient close to or above 100%. Thus, the question is whether the simultaneous increase in P from mono sodium phosphate could stimulate the absorption of Ca from the basal diet to facilitate bone retention in the birds. This would depend on the retention of Ca and P in different body components. Results in Table 3.3 indicate an increase in BW of 573 and 662 for respectively the basal and supplemented diets, reflecting a 15.5% increase in BW. Results in Table 3.6 indicate tibia ash weight of 2.18 and 3.32 g, reflecting a 52% increase in supplemented diets. Assuming that tibia represent the overall effect in bone development, these results indicate the majority of additionally retained Ca and P in supplemented diets was retained in bone with a ratio of 2.13 (Table 3.6). When Ca and P from Ca-sources and mono sodium phosphate were both 95% digested, the ratio of extra pre-caecal digestible Ca and P would be 1.40, which is lower than the ratio of 2.13 as retained in bone. Therefore it seems justified to conclude that the simultaneous increase in Ca and P in a ratio of 1.40 may stimulate the absorption of Ca from the basal diet and/or reduce the urinary excretion of absorbed Ca. The increase in P intake facilitates additional bone retention and would reduce the plasma Ca level.

This would stimulate PTH production and synthesis of vitamin $1,25(OH)_2D_3$ to enhance absorption and reduce excretion of Ca. The results of Hamdi et al. (2015) and Anwar et al. (2016a) confirm that this mechanism may play a role. In both studies, an increase in inorganic P supply to a diet, at a constant Ca content enhanced Ca digestibility. Therefore, we conclude that the results of the present study, including the high absorption of the supplemented Ca is not an artifact but is caused by the regulation of Ca and P absorption by the birds.

5 Conclusions

In this broiler study the growth performance and pre-caecal (ileal) calcium digestibility of calcium from eggshells of different sources (broiler breeders and laying hens), process and particle size (coarsely broken or finely ground) was determined and compared with fine limestone. The test products were added to a basal diet using monosodium phosphate to assure a constant Ca:P ratio in all treatment diets. Fine limestone was used as a reference. From the results can be concluded that:

- Source, process and particle size of the eggshell products had no significant on performance of male broilers from 15 – 23 days of age. No significant differences in performance were observed between the eggshell products and fine limestone.
- Supplementation of limestone and the eggshell products to the deficient basal diet enhanced growth performance of the broilers, as indicated by a higher BWG and lower FCR.
- Supplementation of eggshell products and limestone to the deficient basal diet increased defatted tibia weight, tibia ash content, and tibia ash weight, but not P and Ca content within tibia ash.
- Estimated mean pre-caecal Ca-digestibility of the complete diets was 82% for granulated egg shells, 85% for finely ground hatchery egg shells and 87% for fine limestone.
- Estimated pre-caecal digestibility of the supplementary Ca from eggshell products was 96% for the granulated products, 103% for the finely ground hatchery shells and 105% for fine limestone. The high absorption is presumably mediated by the physiological requirements of the broilers for bone retention enabled by the increase in dietary P and Ca.
- No significant differences in pre-caecal Ca-digestibility were observed between the tested eggshell products in the same granulated form. Eggshell source and process did not significantly affect the pre-caecal Ca-digestibility.
- The results indicate a similar digestibility of Ca from $CaCO_3$ in (finely ground) eggshells and fine limestone
- The eggshell products did not influence the pre-caecal P-digestibility of the diet in comparison to limestone.

References

Adedokun, S. A., & Adeola, O. 2013. Calcium and phosphorus digestibility: Metabolic limits. The Journal of Applied Poultry Research, 22, 600-608.

Amerah, A. M., Plumstead, P. W., Barnard, L. P., & Kumar, A. 2014. Effect of calcium level and phytase addition on ileal phytate degradation and amino acid digestibility of broilers fed corn-based diets. Poultry Science, 93, 906-915.

Anwar, M. N., Ravindran, V, Morel, P. C. H., Ravindran, G., & Cowieson, A. J. 2016a. Apparent ileal digestibility of calcium in limestone for broiler chickens. Animal Feed Science and Technology 213, 142-7.

Anwar, M. N., Ravindran, V., Morel, P. C. H., Ravindran, G., & Cowieson, A. J. 2016b. Effect of limestone particle size and calcium to non-phytate phosphorus ratio on true ileal calcium digestibility of limestone for broiler chickens. British Poultry Science, 57, 707-713.

Aviagen, 2019. Ross Nutrition Specifications 2019. Aviagen publication 0419-AVNR-035.

Aviagen, 2022. Ross 308 / Ross 308 FF Performance Objectives 2022. Aviagen publication 0822-AVNR-157.

Bikker, P., Spek, J. W., Emous, R. A. van, & Krimpen, M. M. van. 2016. Precaecal phosphorus digestibility of inorganic phosphate sources in male broilers, British Poultry Science, 57, 810-817, DOI: 10.1080/00071668.2016.1222604

CVB, 2018. CVB Table Booklet Feeding of Poultry; Feeding standards, feeding advices and nutritional values of feed ingredients for poultry. CVB-series no 61.

CVB, 2021. CVB Feed Table 2021. Chemical composition and nutritional values of feedstuffs.

David, L.S., Abdollahi, M.R., Ravindran, G., Walk, C.L., Ravindran, V., 2019. Studies on the measurement of ileal calcium digestibility of calcium sources in broiler chickens. Poult. Sci. 98, 5582–5589.

Hamdi, M., Solà-Oriol, D., Davin, R., % Perez, J. F. 2015. Calcium sources and their interaction with the different levels of non-phytate phosphorus affect performance and bone mineralization in broiler chickens. Poultry Science 94, 2136–2143. http://dx.doi.org/10.3382/ps/peu061.

Hu, Y.X., Bikker, P., Duijster, M., Hendriks, W.H. Baal, J. van, & Krimpen, M.M. van 2020. Coarse limestone does not alleviate the negative effect of a low Ca/P ratio diet on characteristics of tibia strength and growth performance in broilers, Poultry Science, 99, 4978-4989, https://doi.org/10.1016/j.psj.2020.06.037.

Hu Y.X., van Baal J., Hendriks W.H., Duijster M., van Krimpen M.M., & Bikker P. 2022. Mucosal expression of Ca and P transporters and claudins in the small intestine of broilers is altered by dietary Ca:P in a limestone particle size dependent manner. PLoS ONE 17(9): e0273852. https://doi.org/10.1371/journal.pone.0273852

Ravindran, V., Hew, L. I., Ravindran, G., & Bryden, W. L. .1999. A comparison of ileal digesta and excreta analysis for the determination of amino acid digestibility in food ingredients for poultry. British Poultry Science, 40(2), 266-274.

Reid, B. L., & Weber, C. W. 1976. Calcium availability and trace mineral composition of feed grade calcium supplements. Poultry Science 55, 600-605.

Rodehutscord, M., 2013. Determination of Phosphorus availability in Poultry. Poult. Sci. 69:687-698.

Schaffelaarbos, 2021. Nutrient matrix Calcolin. Version 1, February 2021.

Zhang, F., & Adeola, O. (2018). True ileal digestibility of calcium in limestone and dicalcium phosphate are additive in diets of broiler chickens. Poultry Science, 97(12), 4290-4296.

Van Harn, J., Spek, J.W., Vuure, C.A. van, & Krimpen, M.M. van, 2017. Determination of pre-cecal phosphorus digestibility of inorganic phosphates and bone meal products in broilers. Poultry Science 96 1334-1340.

VSN International, 2019. Genstat for Windows 19th Edition. VSN International, Hemel Hempstead, UK. Web page: Genstat.co.uk.

Appendix 1 Composition of starter diet

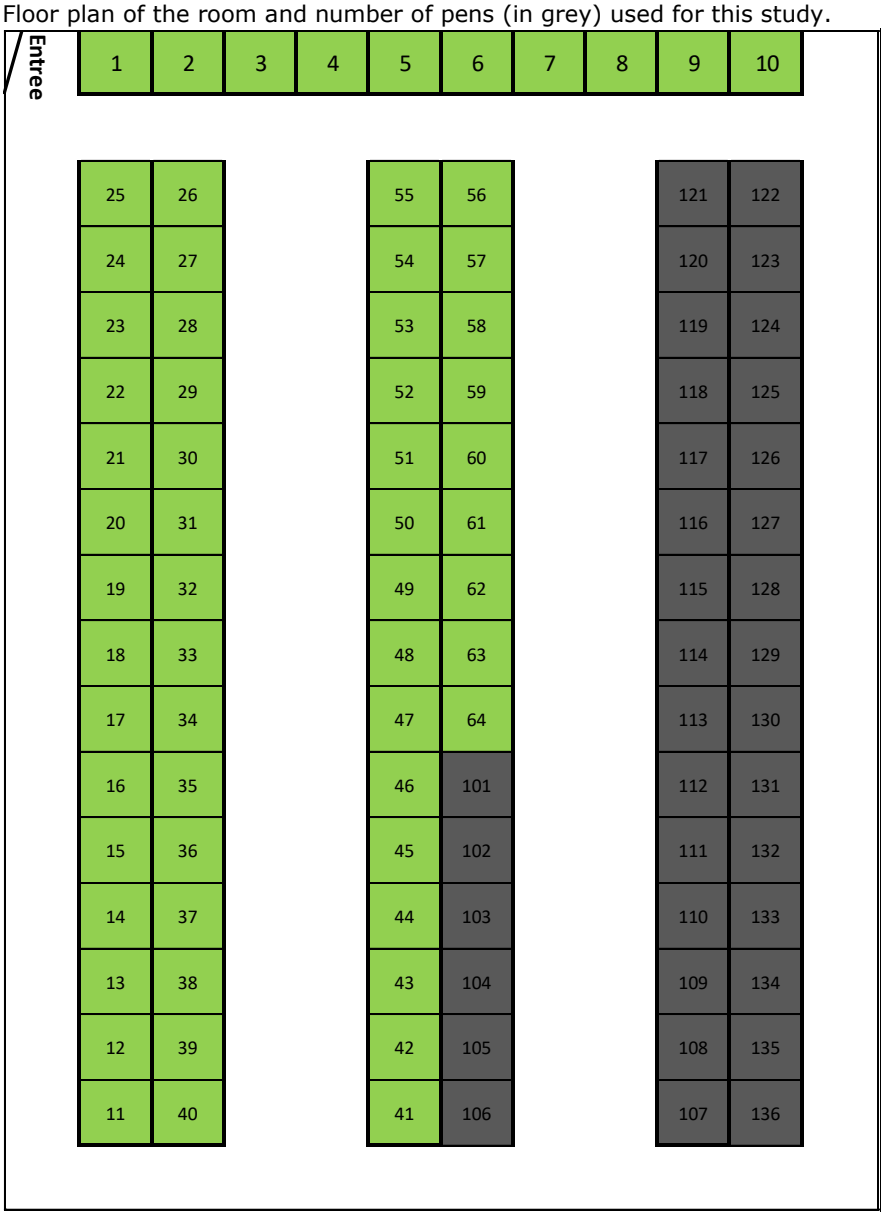
Table A1.1 *Ingredients and nutrient composition of starter diet, with analysed nutrient content in parenthesis.*

Ingredients	**(g/kg)**
Corn	325.8
Wheat	250.0
Soybean meal	330.0
Soybean oil	36.0
Limestone fine	16.0
Monocalcium phosphate	15.0
Palm oil	10.0
Premix broilers[1]	5.0
Salt	1.9
Sodium bicarbonate	2.9
L-Lysine HCL	2.5
DL-Methionine	3.1
L-Threonine	1.1
L-Valine	0.4
RonoZyme WX	0.1
Clinacox (chem. anticoccidial)	0.2
Total	1000
Calculated content (g/kg)	
$ME_{broiler}$ (MJ/kg)	11.91
Crude protein	214 (225)
Crude fat	66 (69)
Crude fibre	27 (24)
Ash	65 (58)
Starch	369
Dig. lysine	11.6
Dig. methionine	5.8
Dig. methionine + cysteine	8.7
Dig. threonine	7.6
Dig. tryptophan	2.2
Dig. valine	8.9
Calcium	10.0 (10.2)
Phosphorous	7.3 (6.9)
Retainable P	4.7
Potassium	9.2
Sodium	1.6
Chloride	2.0
Magnesium	1.7

[1] Premix contributed per kg of diet: 12000 IU vitamin A, 2400 IU vitamin D3, 50 IU vitamin E, 1.5 mg vitamin K3, 2 mg vitamin B1, 7.5 mg vitamin B2, 35 mg niacin amide, 12 mg d-pantothenic acid, 3.5 mg vitamin B6, 25 µg vitamin B12, 200 µg biotin, 460 mg choline chloride, 1 mg folic acid, 80 mg Fe (as FeSO4•7H2O), 85 mg Mn (as MnO), 12 mg Cu (as CuSO4•5H2O), 60 mg Zn (as ZnSO4•H2O), 0.8 mg I (as KI), 0.15 mg Se (as Na2SeO3•5H2O) and 125 mg anti-oxidant.

Appendix 2 Floor plan of the treatments

Floor plan of the room and number of pens (in grey) used for this study.



Allocation of the treatment to the pens

Treatment	Diet	Description	n animals	Pen number
1	A	Basal diet (BD)	(6 * 10 =) 60	104,107,113,119,127,134
2	B	BD+Limestone	(6 * 10 =) 60	106,111,117,122,130,136
3	C	BD+Dried Hatchery Shell granulate	(6 * 10 =) 60	102,108,116,121,125,132
4	D	BD+Dried Hatchery shell powder	(6 * 10 =) 60	105,112,114,124,128,135
5	E	BD+Dried Egg Shell (bench) granulate	(6 * 10 =) 60	103,109,115,123,126,133
6	F	BD+Dried Egg Shell (SB) granulate	(6 * 10 =) 60	101,110,118,120,129,131

Appendix 3 Particle size distribution

Table A3.1 *Particle size distribution of the different test products*

Identification	Batch nr	Dv 10	Dv 50	Dv 90
Dried Hatchery Shell granulate	500-02-220603-B1	435	884	1742
Dried Hatchery Shell powder	500-01-220603-B1	2	61	1182
Dried Egg Shell (bench) granulate	504-02-220607-R1	119	588	1183
Dried Egg Shell (SB) granulate	504-02-220607-B1	331	672	1258
Calcium carbonaat / limestone	n.a.	1	9	21

Dv 50 – the size in microns at which 50% of the sample is smaller and 50% is larger. This value is also known as the Mass Median Diameter (MMD) or the median of the volume distribution. The v in the expression Dv 50 shows that this refers to the volume distribution. This can be replaced by s for surface, l for length or n for number distributions; Dv 10 – the size of particle below which 10% of the sample lies.; Dv 90 – the size of particle below which 90% of the sample lies.

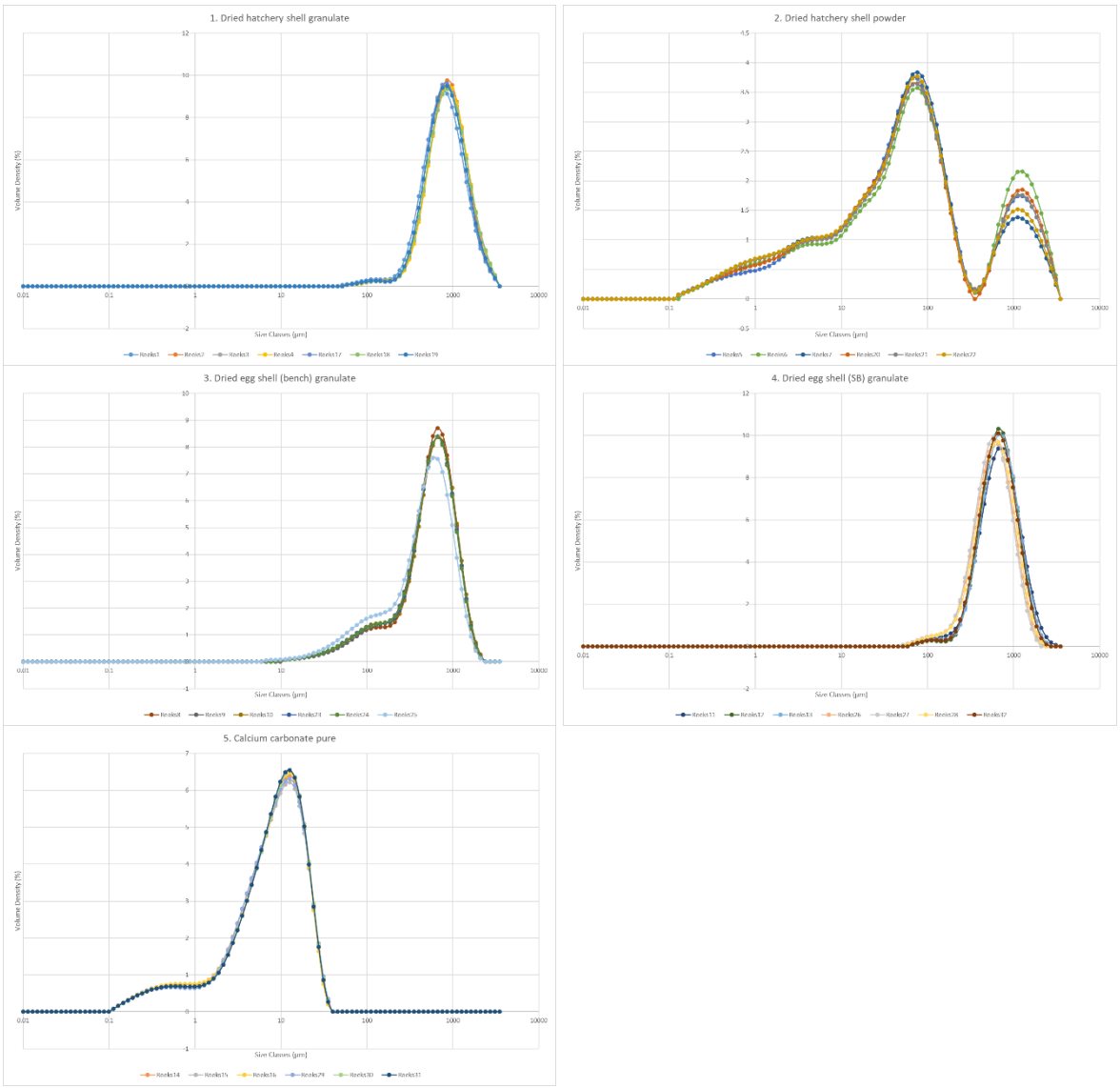


Figure A3.1 *Graphical representation of the particle size distribution of the different test products.*

Appendix 4 Pelleting temperatures

Table A4.1 *Temperature of the experimental diets immediately after pelleting.*

Diet	Description	Pellet Temperature (°C)
A	Basal diet (BD)	68
B	BD+Limestone	70
C	BD+Dried Hatchery Shell granulate	70
D	BD+Dried Hatchery shell powder	70
E	BD+Dried Egg Shell (bench) granulate	69
F	BD+Dried Egg Shell (SB) granulate	69

Appendix 5 Composition experimental diets

Table A7.1 *Ingredients and nutrient contents (analysed in parenthesis) of experimental diets.*

		Diet A Basal diet (BD)	Diet B BD+ Limestone	Diet C BD+Dried Hatchery Shell granulate	Diet D BD+Dried Hatchery shell powder	Diet E BD+Dried Egg Shell (bench) granulate	Diet F BD+Dried Egg Shell (SB) granulate
Maize starch A9262		48.000	48.000	48.000	48.000	48.000	48.000
Soybean meal (Hipro) A9269		11.500	11.500	11.500	11.500	11.500	11.500
Sugar		10.000	10.000	10.000	10.000	10.000	10.000
Potato protein A9329		9.500	9.500	9.500	9.500	9.500	9.500
Egg white powder A9345		5.360	5.360	5.360	5.360	5.360	5.360
Oat hulls A9355		7.000	7.000	7.000	7.000	7.000	7.000
Soya oil		2.698	2.698	2.698	2.698	2.698	2.698
Arbocel		2.000	2.000	2.000	2.000	2.000	2.000
Limestone (fine)		0.318	0.318	0.318	0.318	0.318	0.318
Magnesium oxide		0.170	0.170	0.170	0.170	0.170	0.170
MCP Yara A9352		0.303	0.303	0.303	0.303	0.303	0.303
Potassium chloride		0.177	0.177	0.177	0.177	0.177	0.177
Premix broilers 5 g/kg		0.500	0.500	0.500	0.500	0.500	0.500
Potassium carbonate		0.257	0.257	0.257	0.257	0.257	0.257
Titanium dioxide		0.500	0.500	0.500	0.500	0.500	0.500
DL-Methionine		0.118	0.118	0.118	0.118	0.118	0.118
L-Arginine		0.129	0.129	0.129	0.129	0.129	0.129
Sodium bicarbonate		0.469					
Diamol		1.000	0.272	0.245	0.245	0.245	0.245
Mono sodium phosphate			0.625	0.625	0.625	0.625	0.625
Limestone (fine)			0.573				
Dried Hatchery Shell granulate				0.600			
Dried Hatchery shell powder					0.600		
Dried Egg Shell (bench) granulate						0.600	
Dried Egg Shell (SB) granulate							0.600
Total		**99.999**	**100.000**	**100.000**	**100.000**	**100.000**	**100.000**
Nutrients (g/kg)							
Calcium (Ca)	g	2.44	4.54	4.54	4.54	4.54	4.54
Phosphorous (P)	g	1.75	3.25	3.25	3.25	3.25	3.25
Inositol P	g	0.63	0.63	0.63	0.63	0.63	0.63
Magnesium	g	1.30	1.33	1.33	1.33	1.33	1.33
Sodium	g	1.98	1.98	1.99	1.99	1.99	1.99
Potassium	g	5.88	5.88	5.89	5.89	5.89	5.89
Chloride	g	2.00	2.00	2.00	2.00	2.00	2.00
Diet electrolyte balance (dEB)(meq)	kg	180	180	180	180	180	180
Dry matter	g	911	911	910	910	910	910
Ash	g	46	47	46	46	46	46
Crude protein	g	185	185	185	185	185	185
Crude fat	g	33	33	33	33	33	33
Crude fibre	g	37	37	37	37	37	37
Starch	g	420	420	420	420	420	420
Sugars	g	119	119	119	119	119	119
NSP	g	116	115	115	115	115	115
LYS	g	12.26	12.26	12.26	12.26	12.26	12.26
MET	g	5.33	5.33	5.33	5.33	5.33	5.33
CYS	g	3.16	3.16	3.16	3.16	3.16	3.16
M+C	g	8.50	8.49	8.49	8.49	8.49	8.49
THR	g	8.52	8.52	8.52	8.52	8.52	8.52
TRP	g	2.51	2.51	2.51	2.51	2.51	2.51
ILE	g	9.20	9.20	9.20	9.20	9.20	9.20
ARG	g	11.92	11.91	11.91	11.91	11.91	11.91
PHE	g	10.35	10.35	10.35	10.35	10.35	10.35
HIS	g	4.21	4.21	4.21	4.21	4.21	4.21
LEU	g	15.78	15.78	15.78	15.78	15.78	15.78
TYR	g	8.13	8.13	8.13	8.13	8.13	8.13
VAL	g	10.76	10.76	10.76	10.76	10.76	10.76
ALA	g	8.89	8.89	8.89	8.89	8.89	8.89
ASP	g	20.63	20.63	20.63	20.63	20.63	20.63
GLU	g	23.84	23.84	23.84	23.84	23.84	23.84
GLY	g	7.82	7.82	7.82	7.82	7.82	7.82
PRO	g	8.30	8.30	8.30	8.30	8.30	8.30
SER	g	9.93	9.93	9.93	9.93	9.93	9.93
Sum of AA	g	181.56	181.55	181.55	181.55	181.55	181.55
MEbroiler (MJ)	MJ	12.88	12.88	12.88	12.88	12.88	12.88
Dig. LYSp	g	10.88	10.88	10.88	10.88	10.88	10.88
Dig. METp	g	4.97	4.97	4.97	4.97	4.97	4.97
Dig. CYSp	g	2.63	2.63	2.63	2.63	2.63	2.63
Dig. M+Cp	g	7.60	7.60	7.60	7.60	7.60	7.60
Dig. THRp	g	7.49	7.49	7.49	7.49	7.49	7.49
Dig. TRPp	g	2.22	2.21	2.21	2.21	2.21	2.21
Dig. ILEp	g	8.24	8.24	8.24	8.24	8.24	8.24
Dig. ARGp	g	10.90	10.89	10.89	10.89	10.89	10.89
Dig. PHEp	g	9.35	9.35	9.35	9.35	9.35	9.35
Dig. HISp	g	3.79	3.79	3.79	3.79	3.79	3.79
Dig. LEUp	g	14.30	14.30	14.30	14.30	14.30	14.30
Dig. TYRp	g	7.36	7.36	7.36	7.36	7.36	7.36
Dig. VALp	g	9.63	9.63	9.63	9.63	9.63	9.63

[1] Premix contributed per kg of diet: 12000 IU vitamin A, 2400 IU vitamin D3, 50 IU vitamin E, 1.5 mg vitamin K3, 2 mg vitamin B1, 7.5 mg vitamin B2, 35 mg niacin amide, 12 mg d-pantothenic acid, 3.5 mg vitamin B6, 25 µg vitamin B12, 200 µg biotin, 460 mg choline chloride, 1 mg folic acid, 80 mg Fe (as FeSO4•7H2O), 85 mg Mn (as MnO), 12 mg Cu (as CuSO4•5H2O), 60 mg Zn (as ZnSO4•H2O), 0.8 mg I (as KI), 0.15 mg Se (as Na2SeO3•5H2O) and 125 mg anti-oxidant.

Appendix 6 Temperature and humidity

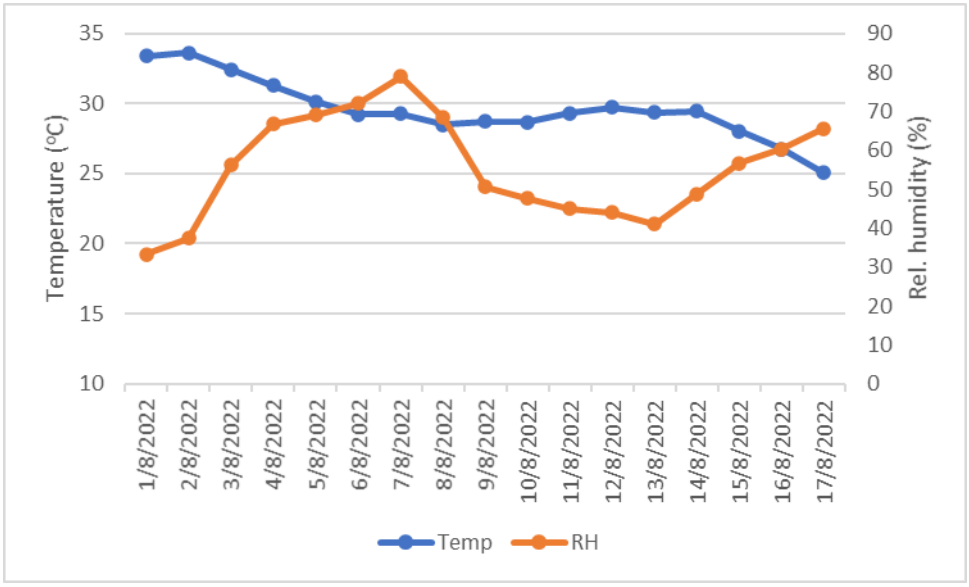


Figure A6.1 *Realised indoor temperature and relative humidity during pre-experimental period (0 – 15 days).*

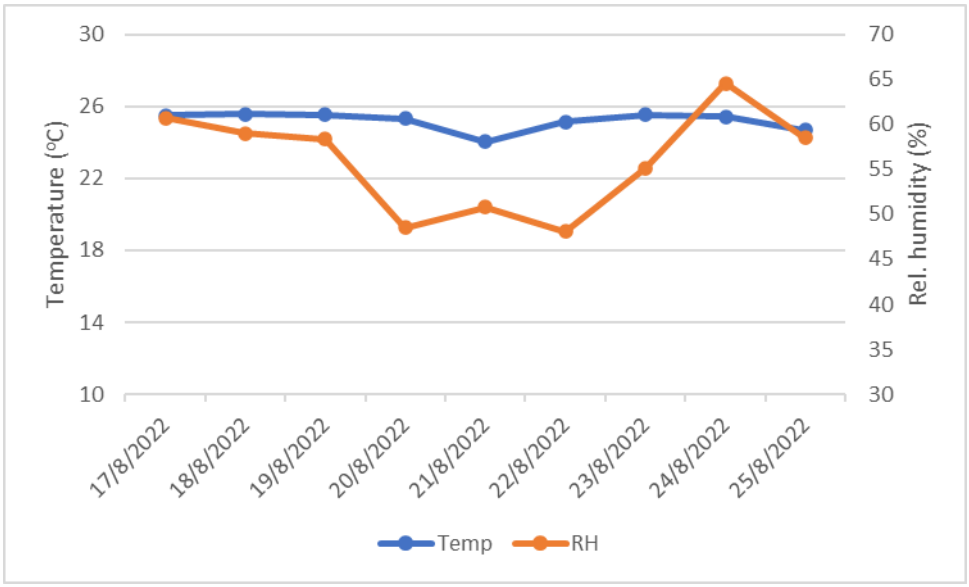


Figure A6.2 *Realised indoor temperature and relative humidity during experimental period (15 – 23 days).*

To explore the potential of nature to improve the quality of life

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