CHAPTER 3

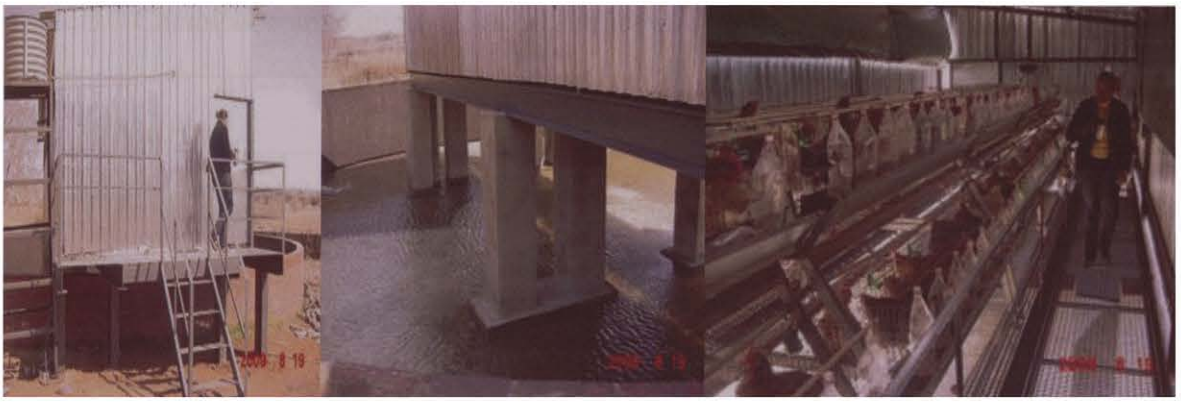
Materials and methods

3.1 Experimental Site and Housing

The study was conducted at the Animal Production Institute of the Agricultural Research Council (ARC) Irene, in Gauteng Province of South Africa. Chickens were housed in two facilities equipped with cages. The control house (Fig 3.1) had open sides at the top and the bottom for ventilation. The experimental house (Fig 3.2 a-d) was built on top of a concrete dam filled with water and had open sides on top and an open thick mash floor underneath allowing chicken manure to fall directly into the dam.

Figure 3.1 Control house

a.



b.

c. d.

Figure 3.2 a. Chicken house constructed over fish dam
b. Front view
c. Dam underneath the chicken house
d. Inside view

3.2 Experimental Animals

A total of 320 pullets of 8 different layer breeds were used. The two lines of indigenous breeds (i.e. Potchefstroom Koekoek and Ovambo) were purchased from ARC-Irene (Poultry Breeding Section; Fowls for Africa). The commercial breeds used were two lines of Hyline (i.e Hyline-Silver and Hyline-Brown) purchased from Almur Smith and Magalies Eggs, respectively. The dual purpose breeds were New Hampshire and Black Australorp purchased from ARC-Irene, Poultry Breeding Section. Unfortunately, it was not possible to get all the hens from the different breeds at point of lay. Therefore, data were only collected from after peak starting at 48 weeks of age for a total period of five months. Upon arrival at the research site, the chickens were examined and any obviously sick or dehydrated birds were culled. Chickens were then individually placed in wire cages, measuring about 25 cm (breadth) x 38 cm (length) x 40 cm (height) which was part of a two-tiered unit consisting of 32 cages. A lighting program of 16 hours was used. Chickens were provided with 150g/day of a standard layer mash (Table 3.1) (Alzu® Feeds) and water was given *ad libitum* throughout the entire trial. The chickens were adapted to the experimental conditions for at least a month before commencement of the experiment.

Table 3.1 Chemical composition of the experimental diet as stated on the label (Alzu, 2009)

Nutrient	g/kg
Protein (min)	160.0
Lysine (min)	7.5
Fibre (max)	70.0
Moisture (max)	120.0
Phosphorous (min)	5.2
Calcium (max)	42.0
Calcium (min)	35.0
Chemical composition of analysed feed	
Crude Protein g/kg	161.7
Calcium g/kg	43.3
Total Phosphorus g/kg	6.0
Potassium g/kg	7.7

3.3 Experimental Design, Treatments and Care of the Birds

Three hundred and twenty laying hens were randomly assigned to either the conventional (control) layer house or the treatment house constructed over a dam. The design used for the study was a randomized block design in each treatment. The houses were blocked in five blocks with one replicate per treatment (breed) in each of the blocks. Each replicate comprised of four hens, individually caged in adjacent cages (Table 3.2).

Table 3.2 Experimental design

Replicates	Dam house								Control house								
	Commercial breeds				Dual purpose breeds		Indigenous breeds		Commercial breeds				Dual purpose breeds		Indigenous breeds		
	HB	HS	LB	LS	BA	NH	OV	PK	HB	HS	LB	LS	BA	NH	OV	PK	
1	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	
2	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	
3	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	
4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	
5	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	
Hens/breed	**20**	**20**	**20**	**20**	**20**	**20**	**20**	**20**	**20**	**20**	**20**	**20**	**20**	**20**	**20**	**20**	**Total**
Total/ house								**160**	**Total/ house**							**160**	**320**

HB: Hyline-Brown, HS: Hyline-Silver, LB: Lohman-Brown, LS: Lohman-Silver, BA: Black Australop, NH: New Hampshire, OV: Ovambo, PK: Potchefstroom Koekoek

3.4 Measurements

3.4.1 Egg production parameters and mortalities

Eggs were collected twice daily at 8am and 12pm and labeled clearly according to replicate, breed and housing system.

Figure 3.3 Collection of eggs from different laying hens

3.4.1.1 Egg weight

All eggs were daily weighed individually on an electronic Richter scale (Hocking *et al.*, 2003; Singh *et al.*, 2009).

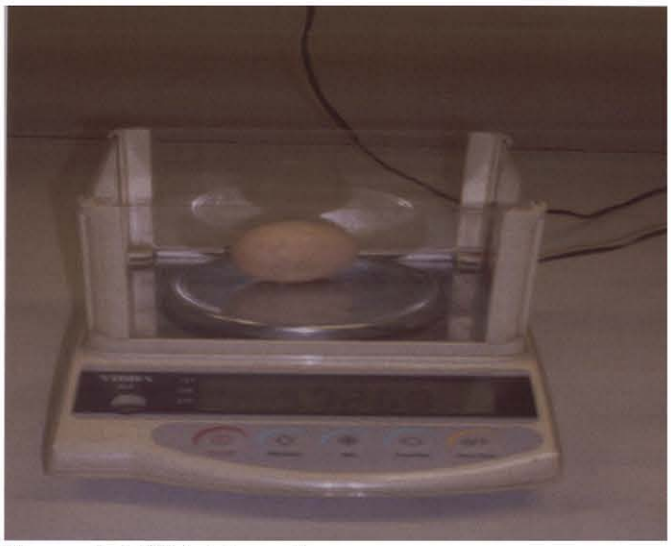


Figure 3.4 Weighing of eggs

3.4.1.2 Body weight

Each bird was weighed at least three times during the trial period i.e at the beginning of the trial (at 48wks of age), in the middle of the trial (3 months after the start of the trial) and on the last day of the trial (5 months after the start of the trial). All birds were individually weighed on an electronic scale in kilograms, calibrated to three decimal places.

3.4.1.3 Feed intake

Daily intake of feed was recorded for each replicate by calculating the difference of feed offered and feed remaining in the feeder. Monthly feed intake and feed conversion ratio were calculated and also that of the entire experimental period.

3.4.1.4 Feed conversion ratio

Feed conversion ratio (FCR) of laying hens was observed daily and recorded as a gram of eggs produced per gram of feed consumed for each month. The following formula was used:

$$\text{FCR} = \frac{\text{Total feed intake for a month}}{\text{Total mean egg weight for a month}}$$

3.4.1.5 Hen day production %

Hen-day production percentage was calculated as described by Mussawar *et al.* (2004), using the following formula:

$$\text{Hen-day production \%} = \frac{\text{Number of eggs produced per day}}{\text{Number of live hens on that day}} \times 100$$

3.4.2 Mortalities

Mortalities were recorded and sent to the Poultry Reference laboratory, Faculty of Veterinary Sciences, University of Pretoria, Onderstepoort to determine the cause of death.

3.4.3 Physical characteristics of eggs

3.4.3.1 Albumen height

Albumen height was recorded for six months period, starting during adaptation and for every second month of the trial period. All eggs produced on that day were each broken on a flat mirrored table and the albumen height was measured using the Mutotuyo gauge (Figure 3.5 & 3.6).

Figure 3.5 Measuring albumen height

3.4.3.2 Haugh unit

Haugh unit was recorded for six months starting during the adaptation and for every second month of the trail period. Records of eggs used for measuring albumen height and its shelled egg weights were used to calculate Haugh unit as described by Doyon *et al.* (1986) using the following formula:

$$HU = 100 \log (H - 1.7w^{0.37} + 7.6)$$

Where

HU = Haugh unit

H = Height of the albumen (mm)

W = Weight of the shelled egg (g)

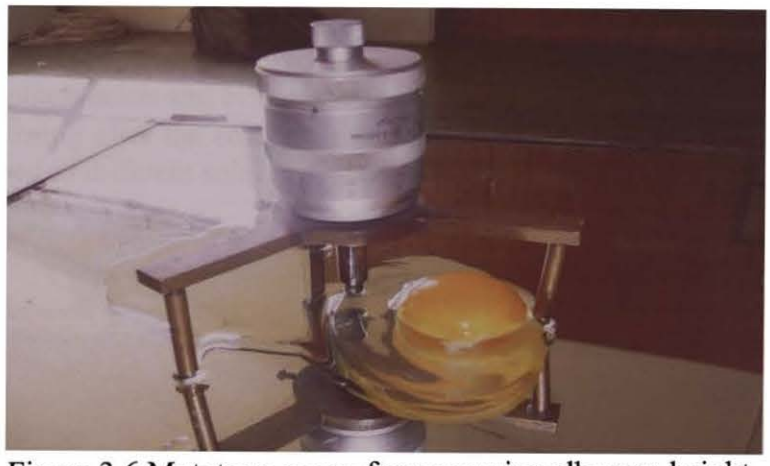


Figure 3.6 Mututoyo gauge for measuring albumen height

3.4.3.3 Specific gravity

Specific gravity was measured for six months starting during the adaptation and for every second month of the trail period. All eggs produced for that day used for this measurement. Specific egg gravity was determined by immersing the eggs in saline solution with densities ranging between 1.075 and 1.080 and between 1.085 and 1.090. Specific gravity was always determined within 24 hours of collection as recommended by Butcher and Miles (2003). Prior to placing the eggs in salt solutions, the solutions were re-stirred and re-checked to verify their densities of saline concentration. The temperature of solutions was also measured before starting, to ensure constant temperature of the solution. Sampled eggs were placed in a medium sized wire basket with a minimum capacity of 5-10 eggs (depending on egg sizes) and immersed into solutions with increasing concentration of salt, from the weakest to the strongest solution (Figure 3.7). Every egg that floated in the weakest to the strongest solutions was removed from the solution and placed in the plastic fillers and all the floaters for every solution were recorded according to the different breeds, houses and specific gravity.

Procedure for measuring specific gravity

In preparing the salt solutions the appropriate amount of salt needs to be dissolved in the appropriate amount of water.

- The salt was dissolved in water first. The solutions were stirred thoroughly using a magnetic stirrer.
- A 4000ml cylinder was filled with the solution and the hydrometer was placed in the cylinder.
- Specific gravity was determined. If the reading was too high, the hydrometer was removed from the cylinder. A small amount of water was added to the solution in a cylinder and bestirred to dilute the saline concentration and the specific gravity was determined.
- This was repeated until the desired density of specific gravity was obtained. If the reading was too low, a small amount of saline was added to the solution and bestirred, and then

specific gravity was determined again. The small amount of saline was repeatedly added to the solution until the desired density was obtained. Once the desired densities were obtained the solutions were stored in a cool room overnight in buckets with lids to minimize contamination and evaporation of the solutions.

- The next morning after the collection of eggs, specific gravity was rechecked and eggs were immersed in different solutions, starting with the weakest to the strongest.

Figure 3.7 Measuring specific gravity of eggs: An egg floating on top of the salt solution

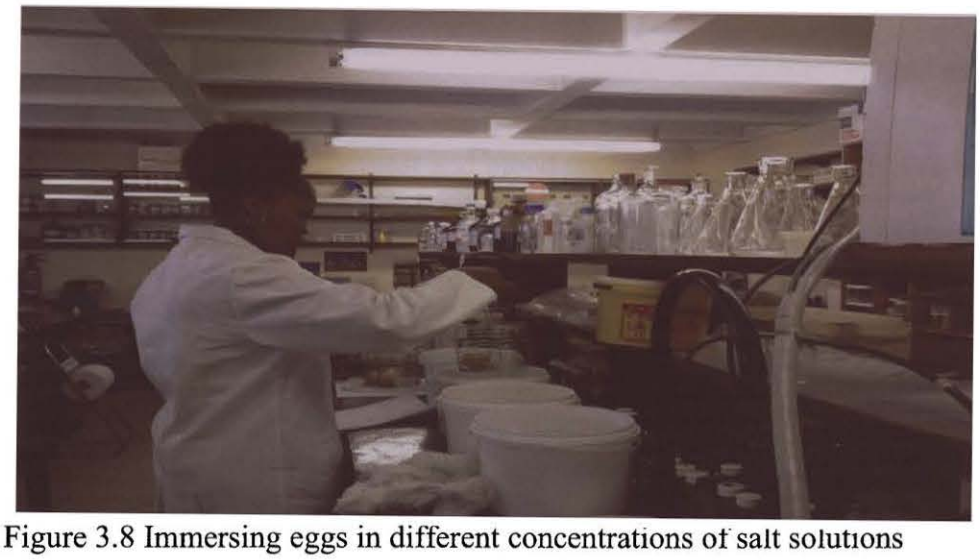


Figure 3.8 Immersing eggs in different concentrations of salt solutions

3.4.3.4 Egg shell strength

Egg shell strength was measured for six months starting during the adaptation and for every second month of the trail period. All eggs produced on that day from both housing systems were used to measure egg shell strength. Breaking eggshell strength of uncracked eggs was measured with an Instron apparatus (model 1011, Instron Ltd, Bucks, UK) (Rodriguez-Navarro *et al.*,

2002; Phosa, 2009). A constantly increasing force was applied on an egg facing up until it broke, depending on its shell strength. The applied force that was necessary to crush the eggshell of each egg was recorded in newtons (N) (Figure 3.9).

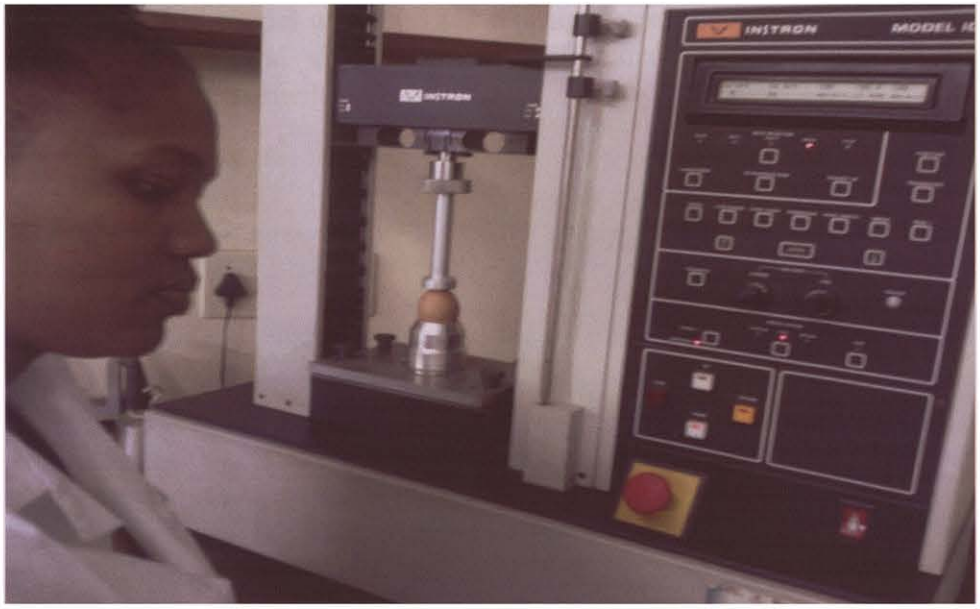


Figure 3.9 Measuring egg shell strength using Instron 10111

Procedures for breaking egg shell strength and Instron apparatus calibration were used as described by Phosa (2009). The Instron was calibrated each time before use according to standard procedures as described by the manufacturer.

3.4.3.5 Meat and Blood spots

Haugh unit eggs were used to identify meat and blood spots. All eggs produced on that day were from all the treatments were broken on a flat mirrored table (Figure 3.10). The eggs were visually examined for meat and blood spots and the total number of meat and blood spots was recorded for each treatment. Both types of spots were treated as single traits as described by Honkatukia (2010), solely as an indication of visual faultiness that might occur.

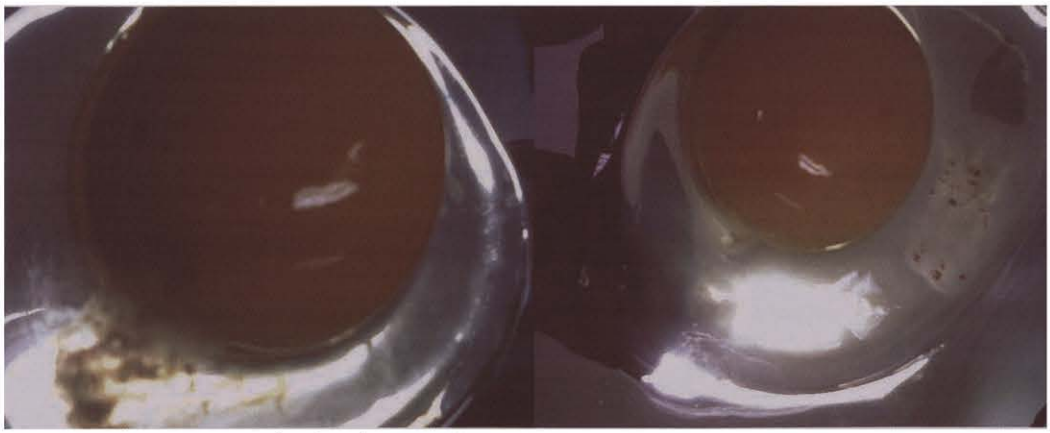


Figure 3.10 Meat and Blood spots (MBS)

3.5 Statistical analysis

Repeated data were analysed statistically as a randomized block design with the GLM model (Statistical Analysis System, 2011) for the average effect over time. Repeated Measures Analysis of Variance with the GLM model was used for repeated period measures. Means and standard deviations were calculated and significance of difference ($P<0.05$) between means was determined by Fischers test (Samuels, 1989).