Effect of hen age, environmental temperature, and oviposition time on egg shell quality and egg shell and serum mineral contents in laying and broiler breeder hens

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ABSTRACT: The aim of the study was to evaluate egg shell quality characteristics, mineral content in the egg shell, and serum mineral concentration during the egg formation process as influenced by oviposition time and ambient temperature at the beginning and end of the laying cycle in laying hens and broiler breeders. Egg shell quality deteriorated significantly at the higher temperature and was lower in broiler breeders than in laying hens. The Ca (P = 0.047) and P (P = 0.018) contents of the egg shell were significantly higher at the higher temperature. The highest (P = 0.028) shell Ca content (352 g/kg) was in eggs laid in the morning, but the highest P (P = 0.030; 1.43 g/kg) and Mg (P = 0.001; 3.88 g/kg) contents were in eggs laid in the afternoon. Broiler breeder egg shells contained significantly more P (P = 0.004) and Mg (P = 0.001) than did those from laying hens. Serum Ca and P levels remained constant throughout the day whereas serum Mg and Zn levels decreased, the rate of decrease in Zn content being the same in all treatments, but with the amounts being greater in laying hens than broiler breeders. The results demonstrate that shell quality characteristics are more severely affected by different factors implemented in this trial compared to the shell mineral composition and especially compared to the serum mineral content.

Keywords: egg; mineral composition; serum minerals

INTRODUCTION

Hens lay eggs in sequences, the length of which varies over the age of the hen both within and between the various types of chicken (Johnston and Gous 2007). In commercial laying hens the sequences are long, while in broiler breeders they are mostly short, of 3–4 eggs (Gumulka and Kapkowska 1996). In both types, the laying pattern is similar: the first egg in the sequence is laid in the morning and the last is laid late in the afternoon. Ovulation times may be estimated from oviposition times. Internal ovulations take place at random, further disrupting oviposition sequences. All these events are influenced by the age of bird, the strain, level of nutrition, and other environmental factors (Johnston and Gous 2006, 2007). Lewis et al. (2004) reported that in commercial crossbred laying hens the mean oviposition time was by about 1 h earlier in comparison with broiler breeders. Zakaria et al. (2005) reported that as a flock was ageing, the eggs were laid later in the day. Oviposition time may also be affected by heat stress which reduces reproductive performance of laying hens by interrupting egg production (Franco-Jimenez et al. 2007). In this case, the effect of heat stress is connected with the disruption of hormones responsible for ovulation (Novero et al. 1991).

Egg shell quality is an important economic factor for producers, being affected by time of oviposition (Pavlovski et al. 2000; Tumova and Ebeid 2005; Gumulka et al. 2010), age of hen (Silversides and

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Scott 2001; Campo et al. 2007), genotype (Silversides and Scott 2001; Tumova et al. 2007), and environmental temperature (Franco-Jimenez et al. 2007; Sahin et al. 2009; Ajakaiye et al. 2011). Most of these factors do not act independently: interactions are described between oviposition time and age (Campo et al. 2007; Tumova and Ledvinka 2009), between oviposition time and genotype (Tumova et al. 2007, 2009), and between genotype and temperature (Franco-Jimenez et al. 2007). Egg shell quality may depend also on its trace mineral content: Waddell et al. (1991) found that eggshells from laying hens fed less than the recommended level of magnesium (400 mg Mg/kg food) were thinner than those receiving sufficient magnesium. In all cases, magnesium was incorporated into the developing shell only in regions of high organic content. They suggested that magnesium distribution would alter with age, and Cusack et al. (2003) confirmed this. However, heat stress increases mineral excretion (El Huseiny and Creger 1981) and decreases serum mineral content (Sahin et al. 2009).

It seems clear that a combination of many factors may positively or negatively affect all aspects of egg quality. The objective of the study was to examine egg quality parameters with special attention being paid to egg shell characteristics, mineral content in the shell, and serum mineral concentration as influenced by oviposition time and ambient temperature in laying hens and broiler breeders at the beginning and end of their laying cycle.

MATERIAL AND METHODS

Design of the experiment. In total, 96 birds were used in the experiment which lasted 7 weeks. Birds were placed into four environmental chambers with controlled temperature. In two chambers the temperature was maintained at 20°C and in the other two, at 28°C. In each chamber 24 birds were housed in individual cages, 12 of which were Lohmann Brown laying hens, six being 22 weeks of age, and the other six being 83 weeks old at the beginning of the experiment. The remaining 12 birds in each chamber were Cobb 500 broiler breeder hens, six of which were 36 weeks old, and the other six 64 weeks of age at the beginning of the experiment. The lighting regime used consisted of 14 h light and 10 h darkness, with lights being turned on at 05:00 h and off at 19:00 h. Laying hens were given a commercial laying feed *ad libitum* (174 g/kg crude protein (CP), 11.65 MJ metabolizable energy (ME), 32.6 g/kg Ca, 4.8 g/kg P, 2.3 g/kg Mg, and 168 ppm Zn). Broiler breeders received 160 g of a high protein broiler breeder feed daily (174 g/kg CP, 11.31 MJ ME, 32.8 g/kg Ca, 5.0 g/kg P, 2.3 g/kg Mg, and 180 ppm Zn).

Blood and egg sampling. The objective here was to measure mineral contents in the serum from each hen during the shell formation process and then to analyze the egg that was subsequently laid. In order to collect blood samples during the time of shell formation, the time of oviposition had to be predicted. This was done by collecting eggs and recording the egg production at 2-hour intervals for three days prior to blood sampling so that the ovulation sequence of each bird could be determined. Hens were divided into three groups according to their expected oviposition times, namely: morning (those laying before 07:30 h), midday (those laying from 07:30 till 11:30 h), and afternoon (hens laying from 11:30 till 15:30 h), and blood samples were collected from three hens of each strain from each chamber and from each group at 7:30 h, 11:30 h, and 15:30 h. Over a period of 3 weeks, blood samples were taken from 12 birds per strain, age, temperature, and oviposition time, being 288 samples in total. Selected birds were bled from a wing vein with a frequency of no less than once a week.

Eggs for shell quality measurements were collected on the day of blood collection from the birds sampled.

Blood and egg analyses. Biochemical parameters analyzed in serum were calcium, phosphorus, magnesium, and zinc. Minerals were determined photometrically by a Libra S 22 spectrophotometer (Biochrom Ltd., Cambridge, UK) using standard commercial kits (Randox Laboratories Ltd., Crumlin, UK).

Eggs were weighed individually on the day of collection. Egg shell strength was measured using a tensometer (Loadtech Loadcells (Pty) Ltd., Centurion, South Africa). The shell weight was determined by a method described by Skrivan et al. (2013). Shell thickness was evaluated by taking the mean of three measurements in the equatorial region using a micrometer (Mitutoyo UK Ltd., Andover, UK). After completing the physical measurements, shells were analyzed for calcium, phosphorus, magnesium, and zinc content. Calcium and phosphorus were determined by a method described by Englmaierova et al. (2013). AOAC

(2005) procedures were used to determine CP, magnesium, and zinc in feed mixtures and egg shells. Mineral elements were analyzed using a Varian ICP spectrophotometer (Varian, Inc., Palo Alto, USA).

Statistical analysis. Data collected during the experiment were analyzed using the general analysis of variance in GenStat (Version 12.0, 2009) software with four-way interactions between age, temperature, time of oviposition, and hen type. The change in the serum mineral content over the three collection periods during the day to the factors investigated in the trial was measured using simple linear regression (GenStat software), the groups being age, oviposition time, strain, and temperature.

RESULTS

In only one case (temperature × oviposition time influencing the Mg content of the shell) a statistically significant interaction between two factors that influenced the physical or chemical composition of the egg shell was detected. Otherwise there were insignificant 2-, 3- or 4-way interactions in any of the variables measured on the egg shell in this experiment, hence only the main effects are displayed in Tables 1 and 2, together with the *P*-values for each of the main effects and interactions. Egg shell quality measurements are given in Table 1. These include shell weight, which was lower (P = 0.027) in eggs produced at 28°C than

Table 1. Main effects of strain, age, temperature, and oviposition time on eggshell quality with *P*-values for main effects and interactions

Factor	Treatment	Shell weight (g)	Shell thickness (mm)	Shell strength (kg/cm^2)
Strain	layer	7.05	0.31^a	3.74^a
	breeder	7.40	0.30^b	3.13^b
	SEM	0.19	0.006	0.14
Age	young	6.76^b	0.31	3.60^a
	old	7.74^a	0.31	3.33^b
	SEM	0.19	0.006	0.14
Oviposition time	07:30	7.32	0.30	3.55
	11:30	7.11	0.30	3.38
	15:30	7.19	0.32	3.46
	SEM	0.24	0.007	0.18
Temperature (°C)	20	7.45^a	0.32^a	3.61^a
	28	6.96^b	0.30^b	3.32^b
	SEM	0.19	0.006	0.14
Strain		0.103	0.005	0.001
Age		0.001	0.873	0.031
Oviposition		0.769	0.487	0.163
Temperature		0.027	0.002	0.020
Temperature × age		0.166	0.652	0.775
Temperature × oviposition		0.358	0.486	0.940
Age × oviposition		0.750	0.404	0.645
Temperature × strain		0.345	0.387	0.707
Age × strain		0.372	0.302	0.208
Oviposition × strain		0.882	0.481	0.097
Temperature × age × oviposition		0.234	0.987	0.304
Temperature × age × strain		0.879	0.407	0.568
Temperature × oviposition × strain		0.760	0.976	0.763
Age × oviposition × strain		0.626	0.509	0.192
Age × oviposition × temperature × strain		0.951	0.807	0.837

^{a,b}statistically significant differences ($P \leq 0.05$) within columns are indicated by different superscripts

Table 2. Main effects of strain, age, oviposition time, and temperature on shell calcium, phosphorus, magnesium, and zinc contents, with *P*-values for main effects and interactions

Factor	Treatment	Ca (g/kg)	P (g/kg)	Mg (g/kg)	Zn (ppm)
Strain	layer	344	1.17[b]	3.32[b]	4.82
	breeder	350	1.36[a]	3.98[a]	4.23
	SEM	0.32	0.007	0.008	0.355
Age	young	346	1.30	3.55[b]	4.30
	old	347	1.20	3.68[a]	4.84
	SEM	0.32	0.007	0.008	0.423
Oviposition time	07:30	352[a]	1.20[b]	3.56[b]	4.88
	11:30	344[b]	1.21[b]	3.51[c]	4.08
	15:30	342[b]	1.43[a]	3.88[a]	4.69
	SEM	0.40	0.009	0.009	0.448
Temperature (°C)	20	343[b]	1.18[b]	3.55	4.65
	28	351[a]	1.34[a]	3.67	4.46
	SEM	0.32	0.007	0.008	0.352
Strain		0.282	0.004	0.001	0.104
Age		0.662	0.351	0.025	0.357
Oviposition		0.028	0.030	0.001	0.098
Temperature		0.047	0.018	0.109	0.525
Temperature × age		0.532	0.686	0.229	0.566
Temperature × oviposition		0.091	0.959	0.008	0.132
Age × oviposition		0.549	0.897	0.857	0.625
Temperature × strain		0.337	0.358	0.114	0.731
Age × strain		0.091	0.764	0.083	0.163
Oviposition × strain		0.833	0.293	0.158	0.292
Temperature × age × oviposition		0.300	0.788	0.184	0.750
Temperature × age × strain		0.778	0.510	0.322	0.458
Temperature × oviposition × strain		0.129	0.907	0.495	0.858
Age × oviposition × strain		0.440	0.985	0.469	0.304
Age × oviposition × temperature × strain		0.919	0.535	0.617	0.709

[a,b]statistically significant differences ($P \leq 0.05$) within columns are indicated by different superscripts

at 20°C, and which increased ($P = 0.001$) with hen age. At the higher temperature, shell thickness decreased ($P = 0.002$) compared to that at 20°C and the eggs laid by the commercial layers had thicker ($P = 0.005$) shells than those from broiler breeders. Egg shell strength ($P = 0.020$) declined at the higher temperature and with advancing age ($P = 0.031$) and was lower in broiler breeders ($P = 0.001$) than in commercial layers.

Of the minerals measured in the egg shell, only Zn content was unaffected by the evaluated factors (Table 2). Calcium content was higher ($P = 0.047$) in shells of eggs laid at the higher temperature and was the highest in eggs laid in the morning ($P = 0.028$). Similarly, shell P content was higher ($P = 0.018$) in eggs laid at 28°C but unlike Ca, it was higher ($P = 0.030$) in eggs laid in the afternoon. Phosphorus content was also affected by hen type, with lower ($P = 0.004$) amounts being deposited in shells from laying hens than from broiler breeders. Advancing age increased ($P = 0.025$) the Mg content of the shell as did the time of oviposition, with the highest ($P = 0.001$) content being in the shells of eggs laid in the afternoon while the lowest Mg content was in the shells of eggs laid at midday. The Mg content was lower ($P = 0.001$) in shells of eggs laid by commercial layers than in those from broiler breeders. A significant two-way interaction ($P = 0.008$) was measured between temperature and oviposition time: Mg content was lower in

Table 3. Main effects of strain, age, oviposition time, and temperature on serum Ca, P, Mg, and Zn concentrations at three collection times during the day, with regression coefficients, residual mean squares (RMS), and degrees of freedom (d.f.)

Factor	Treatment	Serum Ca (mmol/l)			Serum P (mmol/l)			Serum Mg (mmol/l)			Serum Zn (µmol/l)		
		07:30	11:30	15:30	07:30	11:30	15:30	07:30	11:30	15:30	07:30	11:30	15:30
Strain	layer	4.39	4.18	3.98	1.95	1.80	1.88	1.53	1.45	1.46	88.0	81.0	76.5
	breeder	3.95	3.81	3.54	1.75	1.88	1.85	1.35	1.26	1.24	66.5	63.7	51.8
Regression coefficient			0.045 (± 0.063)			0.007 (± 0.035)			–0.045 (± 0.020)			–6.11 (± 2.22)	
RMS			1.384 (523 d.f.)			0.427 (519 d.f.)			0.126 (484 d.f.)			289 (129 d.f.)	
Age	young	4.11	4.09	3.85	1.76	1.80	1.75	1.45	1.32	1.34	80.8	72.6	69.5
	old	4.24	3.94	3.73	1.93	1.88	1.97	1.43	1.41	1.36	76.1	74.7	64.6
Regression coefficient			0.045 (± 0.063)			0.008 (± 0.035)			–0.045 (± 0.020)			–6.11 (± 2.22)	
RMS			1.418 (524 d.f.)			0.422 (519 d.f.)			0.135 (485 d.f.)			392 (130 d.f.)	
Oviposition time	07:30	4.18	4.01	3.90	1.77	1.88	1.88	1.51	1.41	1.40	83.4	75.4	77.0
	11:30	4.22	4.01	3.90	1.93	1.79	1.81	1.38	1.29	1.30	74.1	74.1	57.5
	15:30	4.11	4.07	3.31	1.88	1.84	1.90	1.41	1.37	1.35	76.7	69.1	61.1
Regression coefficient			0.045 (± 0.063)			0.008 (± 0.035)			–0.045 (± 0.020)			–6.11 (± 2.22)	
RMS			1.418 (523 d.f.)			0.427 (519 d.f.)			0.135 (485 d.f.)			378 (130 d.f.)	
Temperature (°C)	20	4.22	3.98	3.79	1.76	1.88	1.92	1.45	1.32	1.34	78.2	74.8	70.5
	28	4.14	4.08	3.80	1.93	1.79	1.79	1.43	1.41	1.36	80.5	72.0	63.6
Regression coefficient			0.045 (± 0.063)			0.008 (± 0.035)			–0.045 (± 0.020)			–6.11 (± 2.22)	
RMS			1.418 (523 d.f.)			0.427 (520 d.f.)			0.135 (485 d.f.)			411 (131 d.f.)	

shells of eggs laid at 20°C with the lowest value in eggs laid at midday, whereas at 28°C the lowest Mg content was in eggs laid in the afternoon.

The factors investigated in this trial had only minor effects on serum mineral contents during the time of shell formation (Table 3) as measured at three times during the day. Serum Ca content remained constant throughout the day, being unaffected by all factors other than strain: laying hens had higher serum Ca contents than broiler breeders (4.37 vs 3.98 mmol/l). Serum P concentrations also remained constant throughout the day, with overall mean contents differing only with age: in this case, higher serum P contents occurred in older hens (1.98 vs 1.76 mmol/l). Serum Mg content decreased (–0.045 ± 0.020 mmol/l at each sampling after the early morning sampling) with the slope remaining the same for all factors, although the constant term differed significantly between the strains. Mean serum Mg content was higher in laying hens than in broiler breeders (1.57 vs 1.37 mmol/l). In the case of serum Zn contents, significant differences in mean content were evident between strains (laying hens having a mean content of 94.3 ppm and broiler breeders of 73.7 ppm) and between oviposition times (89.7, 81.3, and 80.5 μmol/l for the three times, respectively) but in all cases the change in content over time remained the same (–6.11 ± 2.22 μmol/l for each sampling).

DISCUSSION

Egg shell parameters are mainly related to ambient temperature, and heat stress caused all shell characteristics to deteriorate. Poorer egg shell quality in birds under heat stress may be related to the lower feed consumption observed (Tumova and Gous 2012a) resulting in a lack of minerals for egg shell formation and acid-base imbalance. Reduced shell weight in eggs at higher ambient temperatures has been reported by Roberts (2004), Franco-Jimenez et al. (2007), and Ogutunji and Alabi (2010) who also reported lower egg shell thickness and strength at high temperatures. Age is the other main factor which negatively affects shell quality. In this experiment shell weight increased and shell strength declined with age. These two characteristics are negatively correlated and relate to egg size and shell surface area (Tumova and Ledvinka 2009). There were also significant differences in egg shell thickness and strength between laying hens and broiler breeders. This result was presumably the result of contrasting egg sizes in layers and broiler breeders which corresponds with our previous findings in laying hens where we detected correlations between egg size and thickness (r = 0.85) and between shell thickness and strength (r = 0.47) (Tumova and Ledvinka 2009).

Shell strength is one of the most important external quality parameters of an egg, usually dependent on egg shell proportion and thickness. Differences in egg shell physical parameters are dependent, among other factors, on the rate and extent of mineral deposition in the egg shell. Shell calcium content was higher in eggs from hens kept at the higher temperature, this result corroborating with that of Cusack et al. (2003) who explained this by demonstrating that calcium carbonate precipitation is more rapid at higher temperatures. It is possible that the higher calcium deposition rate at the high temperature caused larger crystals to form resulting in poorer egg shell strength: Ahmed et al. (2005) stated that material with smaller crystal size is more solid and is therefore consistent with stronger shells. This assumption may be supported by the shell index measurement which incorporates crystal size and shell density. In this experiment, the shell index was lower in eggs laid at the higher temperature (9.9 at 20°C vs 9.5 at 28°C) (Tumova and Gous 2012b). Time of oviposition was the second main factor affecting shell Ca content with the highest shell Ca content measured in eggs laid in the morning. This situation may be explained by the dynamic model of Ca flow (Kebreab et al. 2009) which shows higher rates of Ca deposition during the dark period. The effect of temperature on the shell P content was similar to that with Ca, the P content being significantly higher in eggs laid at the higher temperature. It can be assumed that the shell P content is closely related to the Ca content, as described by Kebreab et al. (2009).

The shell P content was the highest in eggs laid in the afternoon which is in agreement with Hester (1986) who also determined a higher P content in the afternoon eggs. Dennis et al. (1996), Cucsak et al. (2003), Ogawa et al. (2004) assume that P possibly continues to be deposited until calcification is terminated. We could think of no explanation for the difference in the shell P content in eggs from broiler breeders and laying hens.

Shell Mg content was unaffected by ambient temperature but it was significantly higher in

eggs from older hens as reported by Waddell et al. (1991), who suggested that magnesium distribution would also alter with increasing bird age, by Cusack et al. (2003), who revealed a greater increase in magnesium content in the outer region of egg shells from older birds, and by Yair and Uni (2011), who assumed that layer age-related changes occur in shell mineral composition. This higher Mg content in older hens is the more interesting because there were no differences in egg shell thickness between young and old birds. Ogawa et al. (2004) described a linear increase in the shell Mg content throughout the period during which the egg remained in the uterus which corresponds with the significantly higher shell Mg content in eggs laid in the afternoon. These observations support the role played by Mg in birds of prolonging the time of the egg forming in the shell gland which results in thicker shells. As with the shell P content, the shell Mg content was higher in broiler breeders than in laying hens. However, in spite of this, the shells of broiler breeders were weaker than those of laying hens, but there are many factors other than Mg content that influence shell strength. Afternoon eggs contained more Mg compared to eggs laid earlier which corresponds with Hester (1986). The author suggested that eggs laid in the afternoon are more efficient in absorbing and utilizing minerals. None of the evaluated factors affected shell Zn content, which was very low in all eggs. Skrivan et al. (2005) revealed that shell Zn content was marginally influenced by diet and that the retention of dietary Zn in egg shell was in the range of 0.28–0.67%.

Serum Ca content remained constant throughout the day (Table 3), these results being in accordance with Clunies et al. (1993) who reported constant plasma-Ca concentrations 1–6, 6–12, 12–18 or 18–24 h post oviposition. Similarly, Gunaratne and Boorman (1996) could measure no significant trends in plasma Ca during the day. The serum Ca content was the same at both ages which is in contrast with Brackpool et al. (1996), Suchy et al. (2004), Gyenis et al. (2006), and Pavlik et al. (2009) who demonstrated a gradual decrease in plasma Ca with age. However, differences in serum Ca between laying hens and broiler breeders were the same as reported by Suchy et al. (2004) who described highly significantly lower plasma Ca levels in broiler breeders than in egg type hens. Serum P content also remained constant throughout the day (Table 3) irrespective of treatment, although older birds had higher serum P contents than younger hens. Higher plasma P levels have been reported by Suchy et al. (2004) in laying hens at the end of the laying cycle but these results are in contrast to those of Eren et al. (2004) and Pavlik et al. (2009) where plasma P content was higher at the beginning of the laying cycle. Gunaratne and Boorman (1996) and Boorman and Gunaratne (2001) reported a negative relationship between plasma P level and egg shell weight in the early eggs in the sequence, but we did not take into account the sequence number of eggs in our trial.

Serum Mg levels decreased at the same rate during the day (–0.045, Table 3) irrespective of treatment, but the content was higher in younger hens. Pavlik et al. (2009) described plasma Mg levels increasing till 47 weeks of age and subsequently decreasing to 75 weeks of age, our results corroborating with this report. Serum Zn contents also decreased during the day irrespective of treatment (Table 3) but in this case the levels were significantly different between strains. Broiler breeders had lower Zn levels compared to laying hens (73.7 vs 94.3 μmol/l) and the Zn content decreased with oviposition time (89.7, 81.3, and 80.5 μmol/l, respectively) but this could be due to the confounding effect with the time of collection. Nys et al. (2001) pointed out that Zn is a component of the carbonic anhydrase enzyme which is crucial for supplying carbonate ions during the egg shell formation. Inhibition of this enzyme results in a lowered bicarbonate ion secretion and, consequently, a greatly reduced egg shell weight.

In conclusion, these results demonstrate that shell quality characteristics are more severely affected by the different factors implemented in this trial compared to shell mineral composition and especially compared to serum mineral content. The data collected also confirmed the relationships between shell quality, shell mineral composition, and serum mineral concentration. It appears that time of oviposition plays an important role not only in egg quality, which is widely described in literature, but also in shell mineral composition.

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