Eggshell quality and calcium utilization in feed of hens in repeated laying cycles

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ABSTRACT: Hens of the laying hybrid Shaver Starcross 288 were used in two consequential experiments. The first experiment (for 10 months) was conducted on hens at the age from 18 to 60 weeks (1^{st} cycle; $n = 50$) and moulted hens after 10 months laying from 70 to 110 weeks of age (2^{nd} cycle; $n = 50$). For the second experiment (for 8 months) in the 1^{st} cycle 18 weeks old hens were bought ($n = 60$) and hens from the first experiment after moulting were used in the 2^{nd} and 3^{rd} cycle ($n = 32$ and $n = 28$). During the experiments eggshell qualitative parameters and calcium utilisation (%) were determined. The results indicate that the percentage of eggshell decreased with the increasing number of cycles. The significant correlation of the eggshell proportion and the age of hens was higher in long, advanced cycles ($P < 0.05$). The shell strength in both experiments decreased slightly in relation to the age of hens and repeated laying cycles. The shell deformation had an opposite trend to the shell strength, i.e. it increased with the increasing laying. The average shell thickness showed a decrease in the repeated laying cycles, more significant in shorter cycles ($P < 0.01$). The specific shell gravity maintained balanced values both in cycles and by the age of hens. The shell density in all cycles in both experiments showed a decreasing tendency in relation to the age of hens. In dietary calcium utilization for the shell formation a significant decrease was recorded in the second cycles after moulting ($P < 0.05$).

Keywords: laying hens; forced moulting; repeated laying cycle; eggshell quality; calcium

Hen eggs as compulsory foodstuff deserve an increased attention, mainly their eggshells. The organization of eggshell microstructure is determined by genetic, physiological and external factors. Eggshell microstructural characteristics can inform us about biological and physicochemical processes affecting its formation (Rodriguez-Navarro, 2007). For eggshell formation the hen needs to take in 2.5–3.5 g of calcium from the feed in the calcite form and for this reason it is necessary to provide 3.4–3.8% $CaCo_3$ (Tullet, 1987) in the feed. Adequate dietary levels of calcium have to be provided during rearing, transition and laying periods but a further increase in the calcium level or the use of Ca step-up phase feeding system are poorly efficient (Nys 1999). The hen eggshell consists of 94% of $CaCO_3$, 1% of $MgCO_3$, 1% of $Ca_3(PO_4)_2$ and 4% organic substances mostly of albuminous character (Nys-Gautron, 2007). The shell calcium content is 28.2–41.2% and phosphorus content is 0.102%. Calcium in the shell underlie its fragility and brittleness and phosphorus its resiliency and elasticity. This corresponds to the phosphorus and calcium contents in different eggshell layers. Calcium is concentrated in the eggshell palisade layer. Phosphorus is a part of phosphoproteins which create a network organic matrix in the mammillary layer consisting of collagen fibrils leading to the eggshell surface (Nys et al., 2001).

The eggshell during laying shows different dynamics of its properties. It is given generally that

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the egg weight, that means also its components, increases with the ageing of hens. The dynamics of the changes in eggshell properties from the aspect of its quality is usually reduced to firmness because it conditions the biological and market value of eggs.

The dynamics of the eggshell properties during the day was described by Ledvinka et al. (2000), Tůmová and Ebeid (2005), Tůmová et al. (2007, 2009), who analysed the properties of eggs and eggshells in different hen hybrids during the laying cycles.

At the end of the laying cycle egg production and egg quality are reduced. In order to increase the production and effectiveness of consumer egg production the so called forced moulting is induced (Wakeling 1977; Swanson and Bell, 1984). During moulting the hens have time to go out of production, their reproductive bodies regenerate (Donalson et al., 2005) and an increase in egg production and quality is possible during the subsequent laying cycles (Koch et al., 2005). With the increasing laying the eggshell quality decreases and also its thickness, strength and specific weight, the percentage of cracked and deformed eggshells increases (Bell, 1998) and also the total proportion of nonstandard and broken eggs increases (Zimermann et al., 1987). The natural or forced moulting can cause an improvement in the eggshell quality and prolongation of the production period of hens (Berry and Brake, 1991). The mineral nutrition during and after the period of forced moulting with regard to the eggshell quality was a subject of interest for several authors (Gilbert et al., 1981; Barbosa et al., 1999; Koch et al., 2005). Moulting can positively affect the eggshell strength. This fact is more expressive when hens are moulted at a higher age (Koch et al., 2005). In the studies of several authors a positive effect on the eggshell quality was observed during the first 3-6 months of laying after moulting. The development of the eggshell properties such as strength, thickness, specific weight, and percentage of nonstandard and broken eggs is of the same tendency as during the 1st laying cycle (Scheideler and Al Batshan, 1997).

In the experiment (Lichovníková and Zeman, 2008) the rate of calcium intake deposited in the eggshells was higher in cage systems compared with a floor system depending on the age of laying hens. Řezáč et al. (2000) recorded that the plasma calcium level was higher in hens with cracked eggshells than in hens with unbroken eggshells but this difference was not significant. The results of Bar et al. (1999) suggest that hens forming thick eggshells had significantly higher eggshell weight, average eggshell thickness and lower rate of breakage (cracked, broken or shell-less eggs) in comparison with hens forming thin eggshells.

The purpose of this study is to investigate the changes in eggshell quality and in the utilization of the main eggshell component – calcium in the hen feed for repeated laying cycles.

MATERIAL AND METHODS

Animals, Diets and Treatments

The first experiment was conducted on young hens of the laying hybrid Shaver Starcross 288 at 18 weeks of age (1st cycle; $n = 50$) and moulted hens after 10-month laying (2nd cycle; $n = 50$). For the second experiment in the 1st cycle 18 weeks old hens were bought ($n = 60$) and in 2nd and 3rd cycle hens from the first experiment after moulting were used ($n = 32$ and $n = 28$).

During rearing hens were housed in a three-stage cage battery for laying hens. Hens received standard feed mixture *ad libitum* containing 16.3% proteins, 11.11 kcal ME, 3.96% of calcium (Ca) and 0.8% of phosphorus.

The birds were moulted when they finished the first experiment by methods of restriction of water (1 day), feed (6 days) and light (15 days). In the 1st period windows in the hall were lightproof, access to feed and water was restricted for 24 hours. In the 2nd period – after 24 hours – water supply was renewed and the daylight length was regulated to 6 hours at the intensity 2 W/m^2. Layers were left without feed. This regime lasted for 6 days. In the 3rd period – on the 8th day – low-protein (nitrogen substances 9%) feed mixture was administered to layers, composed of 50% of maize grits, 50% barley grits and with the addition of 1.5% feed limestone in a total quantity of 60 g per hen and day. This regime lasted until 15 day of re-feathering. In the 4th period, which began on the 15th day, the daylight length was adjusted to 16 hours at the intensity of 5 W/m^2 and feeding the complete feed mixture HYD 10 started.

The course of moulting, efficiency, egg quality and standard in the first experiment for the 10-month laying period (1st and 2nd cycle) as well as in the second experiment for the 8-month laying period (1st; 2nd and 3rd cycle) was determined. Feed consumption was measured once a month.

Eggs of laying hens were collected regularly in 14-day intervals (n = 30 per cycle) and were analyzed immediately after collection. In the first experiment 1 200 eggs and in the second experiment 1 440 eggs were analysed.

Sample Analysis

In the eggshell quality analysis eggshell content (%), eggshell strength (N/cm^2), eggshell deformation (µm), average eggshell thickness (µm), egg-

Table 1. Changes in eggshell qualitative parameters in repeated laying cycles

Parameter of eggshell quality	Experiment	Laying cycle	Statistical parameters		
			mean ± SEM	correlation coefficient r	regression equation $y = a + bx$
Percentage of egg weight (%)	1	1st cycle	8.89 ± 0.471	–0.777*	$y = 9.60 - 0.172x$
		2nd cycle	8.83 ± 0.481	–0.324	$y = 9.50 - 0.079x$
	2	1st cycle	8.93 ± 0.211	–0.376	$y = 9.38 - 0.035x$
		2nd cycle	8.65 ± 0.421	–0.789*	$y = 9.37 - 0.177x$
		3rd cycle	8.68 ± 0.388	–0.735	$y = 9.38 - 0.153x$
Egg shell strength (N/cm^2)	1	1st cycle	24.60 ± 2.463	–0.953***	$y = 28.90 - 1.075x$
		2nd cycle	23.10 ± 1.748	–0.943***	$y = 29.00 - 0.750x$
	2	1st cycle	22.95 ± 2.409	–0.917**	$y = 27.09 - 1.181x$
		2nd cycle	23.64 ± 2.386	–0.989***	$y = 28.02 - 1.262x$
		3rd cycle	22.41 ± 2.734	–0.907**	$y = 27.35 - 1.328x$
Deformation (µm)	1	1st cycle	30.12 ± 2.760	0.789*	$y = 26.01 + 1.03x$
		2nd cycle	29.06 ± 1.923	0.714*	$y = 26.52 + 0.64x$
	2	1st cycle	30.93 ± 2.157	–0.117	$y = 31.40 - 0.135x$
		2nd cycle	30.34 ± 1.065	0.633	$y = 29.07 + 0.360x$
		3rd cycle	30.79 ± 1.353	0.347	$y = 29.91 + 0.251x$
Average egg-shell thickness (µm)	1	1st cycle	419 ± 34.078	–0.684*	$y = 462 - 10.46x$
		2nd cycle	407 ± 18.166	–0.539	$y = 425 - 4.54x$
	2	1st cycle	411 ± 54.923	–0.938**	$y = 482 - 23.06x$
		2nd cycle	415 ± 54.173	–0.739	$y = 479 - 21.40x$
		3rd cycle	396 ± 54.281	–0.811*	$y = 488 - 23.55x$
Eggshell specific gravity (g/cm^3)	1	1st cycle	1.79 ± 0.096	–0.084	$y = 1.795 - 0.004x$
		2nd cycle	1.80 ± 0.104	–0.451	$y = 1.884 - 0.022x$
	2	1st cycle	1.83 ± 0.092	–0.001	$y = 1.827 - 0.052x$
		2nd cycle	1.86 ± 0.061	–0.398	$y = 1.907 - 0.013x$
		3rd cycle	1.82 ± 0.120	0.617	$y = 1.697 + 0.040x$
Density of egg shell (mg/cm^2)	1	1st cycle	73.23 ± 2.760	–0.805*	$y = 81.61 - 1.61x$
		2nd cycle	76.66 ± 1.923	–0.318	$y = 78.96 - 0.573x$
	2	1st cycle	73.37 ± 2.157	–0.015	$y = 75.46 - 0.025x$
		2nd cycle	73.46 ± 1.065	–0.765	$y = 80.03 - 1.312x$
		3rd cycle	75.58 ± 2.87	–0.732	$y = 79.87 - 1.231x$

*P < 0.05; **P < 0.01; ***P < 0.001

shell specific gravity (g/cm^3) and eggshell density (mg/cm^2) were evaluated. All these parameters were detected using routine methods (Arpasova et al. 2007). The strength of the eggshells was measured manually by a destructive method with the use of an egg crusher (Veit Electronics, Czech Republic).

The feed and the eggshell were analysed for calcium content with an atomic absorption spectrophotometer (VARIAN, Varian Australia Pty. Ltd.) every 60 days of experiments. The calcium amount deposited in the eggshell during the experimental periods for each experiment and cycle was calculated on the basis egg weight and egg production of hen/day, eggshell weight ratio and calcium content in the feed and eggshell.

Statistical Analysis

Statistical analysis was done using *t*-test and one-way analysis of variance (ANOVA) with the post hoc Duncan's multiple comparison tests by Statgraphics. Correlation coefficient (r) and regression equation were calculated by Statgraphics.

The regression equation expresses changes in the parameter shell quality in the individual times (months) of the observed period during the laying cycle period:

$$Y = a + bx$$

where:

a = weighted mean is the basic value

b = regression coefficient = increase or decrease in the parameter during the given month

x = the month of the laying cycle period

RESULTS

The statistical characteristics and the changes in the eggshell properties in laying cycles are given in Table 1. In the first experiment (10 months) 237.42 and 204.02 eggs were obtained in the 1st and 2nd cycle, respectively. During the 8-month laying period in the second experiment 180.1; 171.6 and 165.6 eggs were obtained in the 1st, 2nd and 3rd cycle, respectively.

The eggshell percentage ratio of the analysed egg weight decreased with the increasing number of cycles in the experiments. In the 1st cycle of the second experiment it amounted to 8.93%, in the 2nd cycle 8.65% and in the 3rd cycle 8.68%.

Significant correlation coefficients of eggshell strength were obtained in both experiments. In the 1st cycle of the first experiment 24.60 N/cm^2; in the second experiment in the 1st cycle 22.95, 2nd cycle 23.64 and 3rd cycle 22.41N/cm^2 with a negative decrease of correlation from $r = -0.649^*$; $r = -0.950^{***}$ and $r = -0.925^{***}$.

Eggshell deformation showed an opposite tendency to eggshell strength: on the average of all eggs it represented 30.22 µm, in the 1st cycle 30.12 µm and in the 2nd cycle 29.06 µm. In the second experiment the values 30.93, 30.34 and 30.79 µm were obtained. A significant increase ($P < 0.01$) in shell deformation with increasing egg production was found out in the 1st and 2nd cycle of the second experiment.

Eggshell thickness in both experiments was 409 µm, with depression in repeated cycles. In the first experiment in the 1st cycle 419 µm and in the 2nd cycle 407 µm; and 411, 415 and 396 µm in the respective cycles of the second experiment. During the laying period the thickness decrease was significant $r = -0.299$ and $r = -0.416$ in the 1st experiment; $r = -0.817^{**}$, $r = -0.717^{**}$ and $r = -0.625^*$ in the respective cycles of the 2nd experiment.

Specific eggshell gravity (g/cm^3) of all eggs amounted to 1.82g/cm^3 on average when in the particular cycles it was 1.79 and 1.80g/cm^{-3} in the 1st experiment and 1.83; 1.86 and 1.82 in the 2nd experiment, respectively. During the laying period the relation between egg specific gravity and the age of hens was not significant.

Eggshell density (mg/cm^2) amounted to 74.80 mg per cm^2 on the average of all eggs. After moulting the correlation coefficient in the first experiment was $r = -0.672^*$ and $r = -0.665^*$; in the 2nd experiment there was no significant dependence on the age of hens.

The utilization of calcium from the feed by hens in repeated laying cycles in both experiments is shown in Table 2. The production of egg mass per feeding day ranged in the experiments and cycles from 43.08 g/day in the first experiment in the 2nd cycle to 45.40 g/day in the second experiment in the 1st cycle. In the second cycles of the laying period a decrease in egg mass production in g/day is quite evident. In the course of the laying period except for the 1st cycle in the first experiment, the decrease in egg mass production in g/day is marked $r = -0.936^{***}$ (first experiment, 2nd cycle), $r = -0.638^*$ and $r = -0.619^*$ in the 2nd and 3rd cycle in the second experiment. Eggshell production in g/day and

Table 2. Dietary calcium utilization in repeated laying cycles (hen/day)

Parameters		1st experiment		2nd experiment		
		1st cycle	2nd cycle	1st cycle	2nd cycle	3rd cycle
Feed consumption (g/day)	$\bar{x}$ ± SEM	118 ± 16.19	117 ± 4.88	110 ± 4.32	116 ± 1.98	118 ± 1.68
	r	0.650*	0.250	0.853***	0.425	0.491
Ca consumption in feed (g/day)	$\bar{x}$ ± SEM	4.582 ± 0.518	4.616 ± 0.203	4.350 ± 0.310	4.598 ± 0.083	4.648 ± 0.098
	r	0.709*	0.249	0.853***	0.712*	0.453
Egg mass production (g)	$\bar{x}$ ± SEM	45.34 ± 6.89	43.08 ± 8.44	45.40 ± 3.91	44.90 ± 3.93	45.00 ± 3.84
	r	0.524	–0.936***	–0.487	–0.638	–0.619*
Eggshell production (g)	$\bar{x}$ ± SEM	4.026 ± 0.645	3.850 ± 0.793	3.91 ± 0.333	3.93 ± 0.537	4.045 ± 0569
	r	0.530	0.160	–0.487	–0.649*	–0.620*
Output of calcium in eggshell (g/per hen/day)	$\bar{x}$ ± SEM	1.411 ± 0.110	1.343 ± 0.276	1.369 ± 0.117	1.375 ± 0.187	1.416 ± 0.199
	r	0.532	0.155	–0.488	–0.638*	–0.619*
Ca shell: Ca intake ratio (%)	$\bar{x}$ ± SEM	30.65 ± 4.293	29.09 ± 5.990	31.62 ± 5.270	29.91 ± 4.170	30.45 ± 4.140
	r	–0.504	0.159	–0.736*	–0.713*	–0.707

$^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; *r* = correlation coefficient

Ca content in it during the 10-month laying period (the first experiment) shows a positive insignificant trend in the laying. In shorter cycles (8 months), in the second experiment, the tendency is negative, that means the decrease expressed as a correlation coefficient with the values of the eggshell production $r = -0.487$, –0.649* and –0.620* and Ca values in the eggshell –0.488, –0.638* and –0.619*.

Feed consumption increased significantly (110 to 118 g) with the increasing age of hens in the 1st cycles of both experiments. A similar tendency was observed in Ca intake from the feed by hens.

The calculated utilisation of Ca from feed for the eggshell formation ranged from 31.62% to 29.09% within the experiments and cycles. In the second cycles of the laying period in both experiments a decrease of Ca utilisation was identified. During laying a common decrease in Ca utilization by hens was significant in shorter laying cycles (se-cond experiment, 1st and 2nd cycle $r = -0.736^*$ and $r = -0.713^*$).

DISCUSSION

In the forced moulting of hens a certain regeneration of nutrient supplies and in parallel detoxification of the organism occur, i.e. getting rid of noxious metabolites, of body fat depositions and restoration of the skin cover. This is how a hen prepares itself for a future reproductive cycle (Roland, 1984).

The dynamics of changes in the eggshell properties in hens is related to physiological processes of mineral metabolism before, during and after moulting. During the pre-laying period young hens create reserves of mineral substances especially of calcium, which is stored in bones and other tissues. Therefore the bone density and strength increase before sexual maturity (Gilbert et al., 1981).

The percentage of eggshell in our experiment decreased with the increasing number of cycles. These results are in agreement with Kim et al. (2005), in whose experiment the older moulted hens exhibited heavier egg weights than the younger unmoulted ones ($P < 0.05$), whereas the eggshell percentage and egg production of the younger birds were significantly higher than those of the older birds. In the experiment of these authors shell weight, shell percentage and shell thickness were positively correlated with each other, whereas egg weight was negatively correlated with shell percentage. Faitarone et al. (2008) reported that the eggs from an experimental group in which hens were two days under fasting and restricted feeding had the higher eggshell percentage as compared to an experimental group in which hens were three days under fasting and consequently *ad libitum* feeding and to a group with one day of fasting and restricted feeding as well. Bell (2003) indicated that the most substantial differences in performance between laying cycles are caused by the egg weight.

During the laying period the Ca reserves in medullar bones decrease and at insufficient intake of Ca through the feed the eggshell quality is deteriorated, incidence of nonstandard and broken eggs is increased and also shell-less eggs can be laid (Gilbert et al., 1981; Bell, 1998). These findings agree with the results of our experiments in which the shell strength decreased slightly in relation to the age of hens and repeated laying cycles.

No significant differences among the groups were observed in specific gravity and shell breakage value in the study of Petek et al. (2008) and in eggshell strength values in the study of Landers et al. (2005). On the contrary, in our experiment a significant correlation was recorded with a decrease in eggshell strength in both experiments.

The improvement in eggshell quality after forced moulting is a rather short and transient process. Verheyen and Decuypere (1991) recorded also the ability of a concrete hybrid to maintain the strong shell for a laying period. Relative improvement in shell quality compared to the end of the previous laying year was less expressed in both evaluated hybrids, although the absolute mean of shell deformation values and the proportion of broken eggs after forced moulting were equal for both hybrids. In our experiment no expressive differences were recorded among the particular laying cycles. The shell deformation increased with the increasing laying.

In the experiment of Aksoy et al. (1997) high and significant correlations were detected between egg weight and shell weight ($r = 0.67$, $P < 0.01$), specific gravity and shell thickness ($r = 0.62$, $P < 0.01$), specific gravity and % egg shell ($r = 0.55$, $P < 0.01$), and shell thickness and % egg ($r = 0.53$, $P < 0.01$). These authors reported significant ($P < 0.01$) increases in egg weight and eggshell weight in the post-moulting period. While egg weight and shell weight increased with age, shell thickness and specific gravity decreased. Similarly in our experiment the average shell thickness showed a decrease in the repeated laying cycles.

The fasting and feeding regimes applied during the induced moulting in the experiment of Mushtaq-Ul-Hassan et al. (2000) did not show any significant effect on post-moulting shell thickness. On the contrary, in the experiments of Koch et al. (2005) or Mushtaq-Ul-Hassan et al. (2001) significantly different shell thickness was observed compared with the control group. Alike Faitarone et al. (2008) reported specific gravity, eggshell thickness and eggshell percentage significantly influenced by moulting. In experimental groups in which hens were under the short-term fasting with subsequent restricted feeding an increase in eggshell thickness was found compared to an experimental group with applied fasting and subsequent *ad libitum* feeding, in which hens produced thinner eggshells due to the high egg production of the hens in this treatment.

Generally, the higher gravity value is related to thicker eggshell, which is a desirable characteristic in the egg industry (Keshawarz and Quimby, 2002).

Bar et al. (2001) showed that induced moulting resulted in increases of egg production, egg mass per housed or surviving hen, numbers of intact eggs and shell quality. Alike Wu et al. (2007) reported that hens moulted by feed withdrawal had significantly higher egg production and egg mass during the later stage of the post-moulting production period compared with hens moulted by no-salt diet. There was no significant difference in egg specific gravity due to the moulting method.

These results are not in agreement with our results which did not demonstrate any distinct effect of moulting on either egg mass or eggshell quality in the 2nd laying cycle or in the 3rd laying cycle. Feed and calcium consumption per feeding day increased during our experiment, an opposite tendency was shown by the production of egg matter. These results are in agreement with the results of Bell (1998) and Ledvinka et al. (2000). The utilisation of calcium is irregularly decreased in the laying cycle period by the age of hens. Lichovníková and Zeman (2008) presented the ratio of calcium in the eggshell to calcium intake (%) 47.6% in the cages to 38.7% on deep litter at the beginning of the laying cycle period and 44.2% to 38.9% at the end of the laying cycle period. In our experiment the calcium utilisation was found to be lower (30.65% in the 1st cycle and 29.09% in the 2nd cycle in the first experiment and 31.62; 29.91 and 20.45% in the 1st, 2nd and 3rd cycle in the second experiment).

The exact mechanisms that affect the eggshell quality during forced moulting have not been fully explained yet. The egg weight can be lower immediately after forced moulting for a short period and this corresponds to the lower Ca deposition for eggshell formation. In the second phase of moulting hens can take more feed in order to increase egg production. The dietary Ca content higher by 3.5–4.5% showed no significant effect on the eggshell quality. After moulting these indicators increase and remain at a higher level during 4 to 6 months of laying, almost the same as it was before moulting. After moulting the calcium metabolism in hens, and especially its absorption in the intestine, calcium transport to the oviduct for the shell synthesis in the uterus and also calcium deposition in medullary bones are improved. A correct complex diet, especially a mineral diet during moulting and at the start of a new reproductive cycle, decides on the effectiveness of the consumer egg production (Roland and Brake, 1982). It can be stated that the above information has been confirmed by our observations.

The results indicate that the percentage of eggshell decreased with the increasing number of cycles. The significant correlation of the eggshell proportion and the age of hens was higher in long, advanced cycles. In both experiments the shell strength decreased slightly in relation to the age of hens and repeated laying cycles. The shell deformation showed an opposite trend than the shell strength, i.e. it increased with the increasing laying. The average shell thickness showed a decrease in the repeated laying cycles, more significant in shorter cycles. The specific shell gravity presented balanced values in cycles as well as by the age of hens. The shell density in all cycles in both experiments showed a decreasing tendency in relation to the age of hens. Calcium utilization from feed for the shell formation showed a clear decrease in the second cycles after moulting.

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