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# TABLE EGGS STRUCTURE, FRESHNESS STATUS AND SHELL INTEGRITY TRAITS UNDER THE INFLUENCE OF CERTAIN ADDITIVES USED IN LAYING HENS DIET

**Cristina Gabriela RADU-RUSU, Ioan Mircea POP, Raluca Elena DONOSA, Daniel SIMEANU**

University of Agricultural Sciences and Veterinary Medicine, 3 Mihail Sadoveanu Alley, Iasi, Romania

Corresponding author email: ralucaelena2005@yahoo.com

***Abstract***

*Several nutritional approaches were used to warrant the quality and safety of hen table eggs throughout the last decades. Apart from most of the researches focusing on the usage of a wide variety of minerals sources in layers' nutrition, this study aimed to measure the effect of using six different feed additives on the eggs quality, hypothesizing in the beginning on the indirect effects that they might induce in hens' minerals metabolism, in electrolytic homeostasis or in improving the digestive organs capacity to uptake minerals form diet and to transfer them in egg structure. Seven groups comprising each 120 ISA Brown layers, aged 30 weeks, were formed and fed a conventional diet for peak of production at a daily intake of 120 g mashed feed (2750 Kcal/kg metabolizable energy, 17.5% crude protein). Control group (CG) received plain diet, without additives, while the other groups received different rates of additives supplementation: 0.03 % ascorbic acid (ASC group); 1% sodium bicarbonate (SOB group); 0.3% prebiotics as organic acids mixture (OAC group); 0.1% mannan oligosaccharide prebiotic (MOS group); 0.1% synbiotic mixture-probiotic, prebiotic and phytogenic compounds (SYN group); 0.2 % mycotoxins adsorbent (MYC group). The reasoning criteria were represented by: eggs structure (% of edible and non-edible parts); Haugh Index (HU) as freshness trait; eggshell integrity (% of whole eggs yield); eggshell defects and commercial loss (% of whole eggs yield); shell thickness (mm); shell breaking strength (Newton). All analyses were run for one-week eggs yield, using 280 eggs randomly collected from each group (40 eggs per day). Eggs structure varied between 25.17%-25.73% yolk proportion, 64.47-65.00% albumen proportion and 9.34-9.89% shell proportion, with certain levels of statistical significance between tested groups, in terms of shell participation in whole eggs structure. Haugh Index assessment indicated not significant improvement or decrease of freshness and commercial status of the eggs due to feed additives supplementation (88.33-90.38 H.U., commercial class AA). Usage of certain feed additives, such as prebiotics, probiotics, synbiotic and mycotoxins adsorbents significantly improved shell thickness, by 9.94 to 14.04% ($P < 0.05$), while ascorbic acid and sodium bicarbonate usage slightly but not significantly improved the same trait (3.80-4.39%). The most improved integrity trait was the shell breaking strength, subsequently to shell thickness increasing. Thus, it significantly differed versus control ($P<0.05$, 4.19-5.08% better resistance in ascorbic acid and sodium bicarbonate supplementation) and distinguished significantly ($P<0.01$, 10.3 to 14.2% improved). Overall, commercial loss due to shell quality issues decreased from 8.9% of whole yield to 5.0-6.8% in the hen groups whose diet was supplemented with studied experimental factors.*

***Key words****: table eggs, quality, shell integrity, feed additives.*

## INTRODUCTION

Certain feed additives, such as prebiotics as oligosaccharides were suggested to play a certain role in improving eggs quality and shell quality in particular (Youssef et al., 2013; Świątkiewicz et al., 2015; Bucław, 2016; Li et al., 2017). Organic acids used as dietary supplements, either in drinking water of hens or in feed, were reported to improve overall table eggs quality and shell strength (Grashorn et al., 2013; Khan and Iqbal, 2016). Probiotics containing lactobacilli and enterococci strains (Zhang et al., 2012; Abdelqader et al., 2013; Chung et al., 2015; Fathi et al., 2018) were found to improve eggs yield and eggs quality, mostly through their indirect actions on the hens gut beneficial microflora reinforcement and improvement of essential nutrients used in eggs synthesis. Other authors (Özek et al., 2011) signaled eggs quality improvement effects when mixtures of organic acids and essential oils were used in hens diet. Also, usage of polyvalent synbiotic feed additives are known to improve eggs yields and commercial qualities (Awad et al., 2009; Tang et al., 2015). It is known that

most of the feedstuffs have a certain level of mycotoxin contamination, which could interfere with the proper functioning of intestinal nutrient absorptions (Murugesan et al., 2015), leading to defective uptake of some elements essential in egg formation or with the normal reproductive tract functioning in hens, therefore it could affect eggs yield and, subsequently, certain eggs quality traits, such as shell structure (Filazi et al., 2017). Despite the fact that ascorbic acid provided standalone in diet could induce shell traits improvements especially under thermal stress conditions and at inclusion rated up to 250 mg/kg (Khan et al., 2012; Abidin and Khatoon, 2013), in certain situations, such as dietary synergies with certain minerals, the effects are controversial: either improvements of thickness and breaking strength in combination with Zinc (Karami et al., 2018) or decrease of shell density, due to metabolic acidosis installation in hens blood and disruption of acid-base metabolism (Torki et al., 2014). Sodium bicarbonate was one of the nutritional approaches in improving eggshell quality. In certain stressful conditions for laying hens, that affects the electrolyte balance and calcium uptake and transfer into shell by the oviduct uterus glands, such as thermal stress (ambient temperature above 30°C), $NaHCO_3$ seemed to play a protective role on the shell by 1%-19%, especially through improvements on the shell structure, rather than to the amount of calcium deposited in the shell (Balnave and Muheereza, 1997). Also, feed supplementation with 1-1.5% sodium bicarbonate induced 3-6% increase of shell thickness (Gongruttananun and Chotesangasa, 2005). The rate of improvement was higher as the exposure of hens to longer light programme, therefore to feed access and bicarbonate intake was longer throughout the day. Dietary sodium bicarbonate (3 ‰) significantly affected shell thickness, especially through an increase absorption rate of calcium in the gut (Abbas et al., 2019) or re-absorption in nephrons, to become more available for uterus transfer (Jiang et al., 2015). Under these circumstances, we proposed an overview of some comparable original research results issued from the usage of commercially available additives such as prebiotics, probiotics, synbiotic, mycotoxin inhibitors, vitaminic or mineral supplementations in laying hens diets on table eggs quality.

## MATERIALS AND METHODS

Seven groups comprising each 120 ISA Brown laying hens, aged 30 weeks, were formed and fed a conventional diet for peak of production at a daily intake of 120 g mashed feed (2750 Kcal/kg metabolizable energy, 17.5% crude protein, based on corn-wheat, soymeal) and a certain supplementation of additives supposed to affect eggs quality:

* CG = Control group, just plain diet;
* ASC group = + 0.03 % ascorbic acid;
* SOB group = +1% sodium bicarbonate;
* OAC group = +0.3% prebiotics as organic acids mixture;
* MOS group = +0.1% mannan oligosaccharide prebiotic;
* SYN group = +0.1% synbiotic mixture-probiotic, prebiotic and phytogenic compounds;
* MYC group = +0.2 % mycotoxins adsorbent.

No commercial brands of the used feed additives are specified because the purpose of the studies was not to differentiate among different suppliers of food additives, but among different types of additives, in terms of their components. In fact, not all additives categories were available from one single seller.

The reasoning criteria were represented by:

* eggs structure (% of edible and non-edible parts from whole weight);
* Haugh Index (HU) as freshness trait (U.H. = 100log (h-1,7 X $G^{0,37}$ + 7,57); Usturoi et al., 2014; measured via ORKA Food Egg tester);
* eggshell integrity (% of whole eggs yield);
* eggshell defects and commercial loss (% of whole eggs yield);
* shell thickness (mm, measured via micrometer; Igic et al., 2010);
* shell dynamic breaking strength (Newton; Hidalgo et al., 2008; measured via ORKA Egg Force reader instrument).

All analyses were run for one-week eggs yield, using 280 eggs randomly collected from each group (40 eggs per day).

Conventional statistical methods were applied to calculate the main descriptors (mean, standard deviation, coefficient of variation=, in accordance with the methodology described by Kaps and Lamberson, 2017. Then, percentage comparisons were run between groups, in order to run to which extent the experimental factors affected the analyzed traits and analysis of variance followed by post-hoc Tukey algorithm was conducted using the SingleFactorANOVA in MsExcel software.

## RESULTS AND DISCUSSIONS

Data related to eggs weight compounds dynamics (g) and, subsequently, to eggs structure (%), are presented in table 1. Thus, whole eggs weight varied between 63.27 g (CG group) and 65.40 g (sodium bicarbonate +1% supplementation). Out of this weights, the shell represented 5.91 g in CG (9.34% in egg structure), while, in comparison it reached higher values in experimental treatments: 6.19 g in ASC (9.70%, P<0.05), 6.39 g in OAC (9.77%, P<0.05), 6.38 g in MYC (9.82% of egg, P<0.01), 6.41 g in SYN (9.83% of egg, P<0.01), 6.44 g in MOS (9.86% of egg, P<0.01) and 6.35 g in SOB (9.89% of egg, P<0.01).

Eggs issued from control group had the highest yolk proportion (25.73%), while the lowest one was found in SYN group (25.17%, -2.19% vs.control). Albumen proportion in eggs structure varied between 64.93% in CG and 65% in SYN group. It seemed the synbiotic feed additive, through its cumulative effect of probiotic, prebiotic and phytogenic induced proper functioning of albumen secretory glands in the magnum area of hens oviduct, while those mobilising minerals from bloodstream and building the shell on the organic matrix in the uterus seemed to have higher value than in control hens, however moderate, in comparison with the other effects induced on shell formation by the other experimental treatments.

Table 1 – Dynamics of eggs structure in accordance with the experimental treatment (diet supplementation)

| Trait | Descriptor | Treatment | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | CG (regular diet) | ASC (+0,03% ascorbic acid) | SOB (+1% $NaHCO_3$) | OAC (+0.3% organic acids mixture) | MOS (+0.1% mannan oligosaccharides) | SYN (+0.1% synbiotic mixture: probiotic, prebiotic, phytogenic compounds) | MYC (+0.2 % mycotoxins adsorbent) |
| Whole egg weight | Mean (g) | 63.27 | 63.84 | 64.22 | 65.40 | 65.31 | 65.24 | 64.98 |
| | ±StDev (g) | 7.14 | 6.44 | 6.58 | 6.74 | 6.54 | 6.94 | 6.41 |
| | CV % | 11.28 | 10.09 | 10.24 | 10.31 | 10.01 | 10.63 | 9.87 |
| Shell weight | Mean (g) | 5.91 | 6.19 | 6.35 | 6.39 | 6.44 | 6.41 | 6.38 |
| | ±StDev (g) | 0.71 | 0.70 | 0.68 | 0.69 | 0.68 | 0.67 | 0.61 |
| | CV % | 12.08 | 11.38 | 10.66 | 10.82 | 10.63 | 10.47 | 9.63 |
| Yolk weight | Mean (g) | 16.28 | 16.41 | 16.47 | 16.61 | 16.59 | 16.42 | 16.53 |
| | ±StDev (g) | 1.83 | 1.75 | 1.72 | 1.73 | 1.76 | 1.67 | 1.66 |
| | CV % | 11.25 | 10.65 | 10.43 | 10.39 | 10.58 | 10.16 | 10.02 |
| Albumen weight | Mean (g) | 41.08 | 41.24 | 41.40 | 42.40 | 42.28 | 42.41 | 42.07 |
| | ±StDev (g) | 5.06 | 4.64 | 4.72 | 4.59 | 4.39 | 4.45 | 4.14 |
| | CV % | 12.32 | 11.26 | 11.39 | 10.82 | 10.39 | 10.49 | 9.84 |
| Eggs structure and percent differences between groups | Shell % | 9.34[a] | 9.70[b] *(P=0.036)* | 9.89[c] *(P=0.003)* | 9.77[b] *(P=0.021)* | 9.86[c] *(P=0.005)* | 9.83[c] *(P=0.006)* | 9.82[c] *(P=0.008)* |
| | *±% vs. CG* | - | +3.80 | +5.86 | +4.60 | +5.56 | +5.19 | +5.11 |
| | Yolk % | 25.73 | 25.70 | 25.65 | 25.40 | 25.40 | 25.17 | 25.44 |
| | *±% vs. CG* | - | -0.10 | -0.33 | -1.30 | -1.28 | -2.19 | -1.14 |
| | Albumen % | 64.93 | 64.60 | 64.47 | 64.83 | 64.74 | 65.00 | 64.74 |
| | *±% vs. CG* | - | -0.51 | -0.71 | -0.15 | -0.29 | +0.12 | -0.29 |

[ab] within the same row, significant differences, $0.01<P<0.05$
[ac] within the same row, distinguished significant differences, $0.001<P<0.01$

Related to eggs inner commercial quality and freshness status, data acquired in our research and presented in table 2 reveal that the usage of feed additives in all experimental treatments positively influence egg weight and mostly thick albumen height, because these two factors influence the Haugh index. However, regardless the feeding of hens, all eggs falled within the

highest AA commercial quality class, with Haugh Unit scores above 88 (Bhale et al., 2003). The differences between treatment groups and control group eggs varied within + 0.33% H.U. in MYC and +2.32% HU in SYN group, suggesting as well, that the magnum glands functioned better in hens group fed +0.1% synbiotic mixture).

In terms of shell development traits, it was found that the thicker gauge occurred as well in the SYN group, where the shell reached 0.390 mm (+14.04% vs. control, P<0.05), while he supplementation with ascorbic acid +0.03% and with sodium bicarbonate with 1% induced +3.8…+4.39% thickening of the shell. In the other groups, 0.2% dietary supplementation with mycotoxins adsorbent induced +9.94% shell thickening, +10.23% improvement in organic acids mixture 0.3% supplementation, respectively +12.87% improvement in MOS group. All these were significantly different from control and ASC, SOB groups.

Table 2 – Eggs freshness trait and shell quality parameters dynamics in accordance with the experimental treatment (diet supplementation)

| Trait | Descriptor | Treatment | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | CG (regular diet) | ASC (+0,03% ascorbic acid) | SOB (+1% $NaHCO_3$) | OAC (+0.3% organic acids mixture) | MOS (+0.1% mannan oligosaccharides) | SYN (+0.1% synbiotic mixture: probiotic, prebiotic, phytogenic compounds) | MYC (+0.2 % mycotoxins adsorbent) |
| Haugh Index | Mean (HU) | 88.33 | 89.72 | 88.68 | 89.18 | 89.21 | 90.38 | 88.62 |
| | ±StDev (HU) | 7.38 | 8.18 | 8.19 | 7.73 | 8.58 | 7.72 | 8.15 |
| | CV % | 8.36 | 9.12 | 9.24 | 8.67 | 9.62 | 8.54 | 9.20 |
| | *±% vs. CG* | - | +1.57 | +0.40 | +0.96 | +1.00 | +2.32 | +0.33 |
| Shell Thickness | Mean (mm) | 0.342[a] | 0.355[a] | 0.357[a] | 0.377[b] *(P=0.038)* | 0.386[b] *(P=0.025)* | 0.390[b] *(P=0.019)* | 0.376[b] *(P=0.047)* |
| | ±StDev (mm) | 0.02 | 0.02 | 0.02 | 0.02 | 0.03 | 0.02 | 0.02 |
| | CV % | 5.19 | 6.23 | 5.74 | 6.28 | 7.19 | 6.13 | 5.82 |
| | *±% vs. CG* | - | +3.80 | +4.39 | +10.23 | +12.87 | +14.04 | +9.94 |
| Dynamic shell breaking strength | Mean (N) | 40.12[a] | 41.80[b] *(P=0.041)* | 42.16[b] *(P=0.036)* | 44.69[c] *(P=0.006)* | 45.07[c] *(P=0.005)* | 45.82 *(P=0.003)*[c] | 44.25[c] *(P=0.008)* |
| | ±StDev (N) | 2.53 | 2.89 | 2.48 | 3.23 | 2.92 | 2.86 | 3.13 |
| | CV % | 6.31 | 6.92 | 5.89 | 7.23 | 6.48 | 6.24 | 7.08 |
| | *±% vs. CG* | - | +4.19 | +5.08 | +11.39 | +12.34 | +14.21 | +10.29 |

[ab] within the same row, significant differences, $0.01<P<0.05$

Shell thickening induced, as well, subsequently increasing effects of dynamic breaking strength, which varied from 40.12 N in control group to: 41.80 N in ASC group (+4.19%, P<0.05); 42.16 N in SOB group (+5.08%, P<0.05); 44.25 N in MYC group (+10.29%, P<0.01); 44.69 N in OAC group (+11.39%, P<0.01); 45.07 N in MOS group (+12.34%, P<0.01) and to 45.82 N in SYN group (+10.29%, P<0.01), eventually. Within these circumstances, it is interesting to follow the dynamics of eggshell faults occurrence due to the action of experimental factors (table 3).

In CG group (regular diet), there were found 25 eggs with unconformities out of the total 280 sampled eggs during the experimental weekly observation (8.93%). Out of these defects, 2.86% represented visible broken shells, 1.79% were found as micro breakages, 2.14% were eggs with rough shells, and 1.07 % each represented eggs with soft shells or with malformed shells. It is interesting to explore, as follow-up the real occurrence of microintegrity defects on the eggshell, knowing we used an ovoscopic method to identify micro breakages, while some other techniques, like ultrasound checking or resonance percution and analysis of acoustic response curve would be more accurate, as other studies reported (Hunton, 2005; Wang and Jiang, 2005).

Usage of different feed supplements induced modifications of shell thickness and breaking strength, which resulted in decreases by 24 – 44% of shell defects, compared to control group (table 3).

In the group receiving ascorbic acid +0.03%, the defects decreased to 6.79% from the total amount of analyzed eggs.

Table 3 – Proportion of shell defects in relation with the experimental treatment (diet supplementation)

| Egg production / defects | Treatment | | | | | | |
|---|---|---|---|---|---|---|---|
| | CG (regular diet) | ASC (+0,03% ascorbic acid) | SOB (+1% $NaHCO_3$) | OAC (+0.3% organic acids mixture) | MOS (+0.1% mannan oligosaccharides) | SYN (+0.1% synbiotic mixture: probiotic, prebiotic, phytogenic compounds) | MYC (+0.2 % mycotoxins adsorbent) |
| Total yield/week (pcs.) | 280 | 280 | 280 | 280 | 280 | 280 | 280 |
| Eggs with defects (pcs.) | 25 | 19 | 18 | 17 | 15 | 14 | 18 |
| *% out of total* | 8.93 | 6.79 | 6.43 | 6.07 | 5.36 | 5.00 | 6.43 |
| *±% vs. CG* | 100.00 | -24.00 | -28.00 | -32.00 | -40.00 | -44.00 | -28.00 |
| • broken shells (pcs.) | 8 | 6 | 5 | 5 | 5 | 4 | 5 |
| *% out of total* | 2.86 | 2.14 | 1.79 | 1.79 | 1.79 | 1.43 | 1.79 |
| • micro breakages (pcs.) | 5 | 3 | 4 | 3 | 3 | 3 | 4 |
| *% out of total* | 1.79 | 1.07 | 1.43 | 1.07 | 1.07 | 1.07 | 1.43 |
| • rough shells (pcs.) | 6 | 5 | 6 | 4 | 4 | 3 | 5 |
| *% out of total* | 2.14 | 1.79 | 2.14 | 1.43 | 1.43 | 1.07 | 1.79 |
| • soft shell/no shell (pcs.) | 3 | 2 | 2 | 2 | 2 | 3 | 2 |
| *% out of total* | 1.07 | 0.71 | 0.71 | 0.71 | 0.71 | 1.07 | 0.71 |
| • malformed shell (pcs.) | 3 | 3 | 1 | 3 | 1 | 1 | 2 |
| *% out of total* | 1.07 | 1.07 | 0.36 | 1.07 | 0.36 | 0.36 | 0.71 |

In organic acids supplemented eggs, the decrease of shell defects was of 32%, compared to control group (only 6.07% eggs with shell issues), while in both groups receiving sodium bicarbonate and mycotoxins adsorbent supplementations, the occurrence of shell defects decreased to 6.43%. Better protective effects related to shell integrity were observed in groups MOS and SYN, where the decrease reached 40-44 % compared to control group (5.0-5.4% eggs with shell unconformities, vs. 8.93% defects incidence). Despite the results sounds promising, it would be interesting to run in depth analysis of proteomics and electrolytes of the uterine fluid in each group in order to better understand and correlate the facts, because, apparently, the most resistant shells and the lowest defect rate appeared due to the synergistic effect of prebiotics, probiotics and phytogenics in SYN group and, meantime, the highest proportion of eggs with soft shells appeared in the same group, suggesting that the uterine environment could be in fact disturbed and forced to work above regular rhythmicity in such case. A follow-up of the research should be considered, knowing that 15%-20% of commercial losses due to shell integrity faults (Roland, 1988; Qiu et al., 2020), which could be prevented by hens nutrition.

## CONCLUSIONS

Dietary supplementation of regular laying hens feed with sodium bicarbonate, ascorbic acid, mixture of organic acids, prebiotics as oligosaccharides, synbiotics (mixture of probiotics-prebiotics-phytogenic compounds) or mycotoxin adsorbents induced significant effects on shell participation of whole egg structure, shell thickness and shell dynamic breaking strength.

Less eggs with shell unconformities were found in synbiotic +0.1% supplemented group, suggesting thus the positive synergistic effects of the three types of additives provided in the same product either on nutrients absorption in the intestine, re-absorption in kidneys tubular systems and blood to shell transfer in the uterus, the place where shell is synthetized.

However, studies should be followed up by an exhaustive approach on the uterine histological structure, fluid components and interactions in eggshell formations, because some aspects remain controversial (such as the occurrence of some soft-shelled eggs in the same group with the best dynamic breaking strength values).

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