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Short Communications

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Efficacy of three sealing methods on hatchability of micro-cracked eggs from broiler breeder hens¤

Eficacia de tres métodos de sellado de huevos con micro grietas sobre la incubabilidad de gallinas reproductoras de pollos de engorde

Eficácia de três metodos de selagem de microfissuras em ovos na incubadora provenientes de galinhas reprodutoras de frangos de corte

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Abstract

Background: Shell fragility of hatching eggs can have negative implications on the economic performance of hatcheries. **Objective:** To determine the efficacy of sealing eggshell micro cracks with either coloured or uncoloured nail varnish, and molten paraffin on hatchability, embryonic mortality (EM) and hatched chick weight (CW). **Methods:** Eggs (n= 576) with micro-cracks were assigned among four groups (n=144 each group) for a 21 d incubation period. One group was untreated (CE). In the other groups, the micro cracked area of eggshells was sealed with uncolored nail varnish (NV), colored nail varnish (CV), or molten paraffin wax (MP). A positive control group of un-cracked eggs (UE) was also included (n= 144). **Results:** The eggshell sealant treatments allowed normal conductance related to egg weight loss after 18 d of incubation (11.45%), and chick weights were normal among treatment groups (44.7 g). Hatchability and embryonic mortality in the early and late incubation periods of the NV group was similar to UE (84.02 vs 86.11% for hatchability, 6.95 vs 10.42% for EM on days 1-10, and 2.08 vs 1.39% for EM on days 18-21 respectively; $p>0.05$). The CV group had lower hatchability than the NV (77.77 vs 84.02% respectively; $p<0.05$), whereas MP showed similar hatchability compared to the CE group (59.72 vs 72.92%, respectively $p>0.05$). **Conclusions:** Application of uncoloured nail varnish on shell micro-cracks improves egg hatchability.

Keywords: *Eggshell, embryonic mortality, hatching, incubation, micro fissure repairing, poultry.*

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Resumen

Antecedentes: la rotura de la cáscara es un problema que afecta a los huevos para incubación, reduciendo la eficiencia económica de la producción. **Objetivo:** determinar la eficacia del sellado de micro grietas de huevos utilizando parafina fundida, esmalte de uñas transparente, o esmalte de color sobre la incubabilidad, mortalidad embrionaria (EM) y peso de los pollitos (CW). **Métodos:** los huevos (n= 576) con micro fisuras fueron asignados a uno de cuatro grupos durante un período de incubación de 21 días. Un grupo se mantuvo sin tratar (CE), en los otros grupos se selló el área micro-agrietada de la cascara con esmalte incoloro de uñas (NV), esmalte de color (CV), o parafina fundida (MP). Tambien se incluyó un grupo de huevos no agrietados (UE, n= 144). **Resultados:** los tres tratamientos aplicados a la cascara permitieron obtener una pérdida de peso normal de los huevos a 18 d (11,45%), asi como un peso normal de los pollitos (44,7 g). El grupo NV presentó incubabilidad y EM similares a UE durante el periodo de incubación (84,02 vs 86,11% incubalidad y 6,95 vs 10,42% EM entre los días 1-10; y 2,08 vs 1,39% EM entre los días 18-21 respectivamente; p>0,05). El grupo CV tuvo incubabilidad inferior a la de NV (77,77 vs 84,02% respectivamente; p<0,05), mientras que el grupo MP mostró incubabilidad similar a la obtenida por el grupo CE (59,72 vs 72,92%, respectivamente p>0,05). **Conclusiones**: la aplicación de esmalte incoloro de uñas en las micro-grietas de la cascara mejora la incubabilidad de los huevos.

Palabras clave: *cascara de huevo, eclosión, incubación, mortalidad embrionaria, producción avícola, reparación de micro fisuras.*

Resumo

Antecedentes: a quebra da casca do ovo é um dos problemas mais importantes durante a incubação, reduzindo a eficiência económica da produção. **Objetivo:** determinar a eficácia da selagem de microfissuras na casca de ovo com verniz de unhas, transparente ou colorido, e parafina fundida na eclosão, mortalidade embrionária (EM), e peso dos pintos ao nascimento (CW). **Métodos:** ovos (n= 576) apresentando microfissuras foram divididos em quatro grupos (n= 144) e colocados a incubar por um período de 21 dias. Um dos grupos não foi tratado (CE). Os ovos dos restantes grupos foram selados com verniz de unhas transparente (NV), verniz de unhas colorido (CV) ou parafina fundida (MP). Foi ainda incluido um controlo positivo constituído por ovos sem microfissuras (UE) (n= 144). **Resultados:** em todos os grupos tratados, observou-se uma perda de peso normal após 18 dias de incubação (11,45%) assim como um peso vivo normal dos pintos ao nascimento (44,7 g). Observou-se uma taxa de eclosão e mortalidade embrionária similar dos períodos iniciais e finais de incubação entre os grupos NV e UE (84,02 vs 86,11% para eclodibilidade, 6,95 vs 10,42% para EM entre os dias 1-10, e 2,08 vs 1,39% para EM entre os dias 18-21 respectivamente; p>0,05). O grupo CV evidenciou uma taxa de eclosão menor do que a do grupo NV (77,77 vs 84,02% respectivamente; p<0,05). No entanto, o grupo MP apresentou uma eclodibilidade similar à observada no grupo CE (59,72 vs 72,92%, respectivamente p>0,05). **Conclusões:** o uso de verniz de unhas transparente é um método apropriado para incrementar a eclodibilidade de ovos com microfissuras.

Palavras-Chave: *casca de ovo, incubação, mortalidade embrionária, produção de aves, reparação de fissuras, taxa de eclosão.*

Introduction

World production of eggs increased by 23% from 2004 to 2013. This increase involved many of the least developed countries (+68% increased egg production) (FAO, 2016). Considering that eggs are an important and relatively inexpensive source of protein, vitamins and minerals, and that the world population is rapidly growing (Oluwafemi *et al*., 2015; FAO, 2016) further increase of egg production can be expected in the coming years.

Cracked eggs is one of the most important problems decreasing the economic efficiency of producers (Mazzuco and Bertechini, 2014). Kingori (2012) reviewed that more than 5% of eggs are not available for consumers, and 0.5-6% have eggshell damage. According to Narahari *et al*. (2000), about half of the eggs rejected for shell defects shows micro-cracks, while the eggshell membrane remains undamaged. With regard to hatching, damaged eggshell alters the water balance and gas exchange between the embryo and the environment, favouring bacterial penetration (Solomon, 2010). Little information is available on the hatchability of eggs with micro-cracks and the subsequent health of chicks (Moosanezhad Khabisi *et al*., 2012). In a comparison of micro cracked eggs with normal eggs, Barnett *et al*. (2004) observed a

32% reduction of hatchability and increased chick mortality associated with micro cracked eggshells.

A potential method for improving hatchability consists in sealing the eggshells with substances that reduce the impact of micro-cracks on embryo development. To this purpose, Narahari *et al*. (2000) proposed the use of adhesive resin, cellophane tape, and insulation tape.

We hypothesised that sealing micro-cracks on eggshells (which retain undamaged eggshell membranes) using nail varnish or molten paraffin might improve hatchability. Additionally, coloured nail varnish should be tested for a possible negative impact of colour additives on embryonic development, embryonic mortality (EM) and hatching weight of the chick (CW). Therefore, the aim of this study was to determine the efficacy of sealing eggshell micro cracks with either coloured or uncoloured nail varnish or molten paraffin on hatchability, embryonic mortality (EM) and hatched chick weight (CW).

Materials and Methods

Ethical considerations

The study was conducted in agreement with the rules set by the Animal Ethics Committee of the Islamic Azad University, Rasht Branch, Iran, and with Directive 2010/63/EU.

Experimental design and treatments

Seven hundred and twenty (720) eggs (144 uncracked eggs, UE; and 576 eggs with micro-cracks) from Ross 308 broiler breeder layers (48 weeks old) were individually weighted. Broiler breeder hens were immunized against encephalomyelitis, EDS, salmonella, and Gambaro. Eggs (n = 576) with micro-cracks on the eggshell, but with undamaged eggshell membranes, were distributed among four groups (n = 144 in each group) for a 21 d incubation period. Groups were balanced for the proportion and location of micro-cracks on the eggshell. Group 1 remained untreated (CE) and was used as a negative control. In Group 2, micro-cracked areas were sealed with uncolored nail varnish (NV). In Group 3, micro-cracked areas were sealed with colored nail varnish (CV). In Group 4, micro-cracked areas were sealed with molten paraffin (MP). In Group 5, uncracked eggs (UE) served as a positive control. Within each experimental group, eggs were assigned randomly to four setter trays and placed on hatcher trays within each group at transfer.

Incubator management and measurements

After sealing, eggs were incubated for 21 d in a commercial incubator (Jamesway Hatchery Company Inc. PS500 Multi-Stage Controller, Toronto, CA). The incubation conditions were: 37.7 °C and 70-75% relative humidity from d 1 to d 18 of incubation. On the 18th d of incubation, the eggs were transferred into a hatcher (Jamesway Hatchery Company Inc. PS500 Multi-Stage Controller, Toronto, CA) and incubated at 36.7 °C and 75-82% relative humidity from d 19 to d 20 of incubation. On the 21st d of incubation, the temperature was decreased hourly from 36.7 to 36.1 ºC.

Eggs were candled and embryonic mortality (EM) recorded on the 10th d of incubation, during transfer to the hatcher, and at the end of the incubation period. During transfer to the hatcher, egg weight (EW) was also recorded, and weight loss was calculated as: EW at transfer/initial EW × 100. The number of contaminated and pipping eggs were recorded during the incubation period. After completing hatching, individual chick body weight, number hatched, dehydrated and culled chicks were recorded, and chick weight/egg weight ratio was calculated. Number of chicks with red hocks and black cord was recorded and expressed as a percentage of viable chicks, while hatchability, EM, and cull chicks were expressed as a percentage of the number of eggs set.

Statistical analyses

The statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS, 2008), and the R software (R core team, 2015). The normality of data distribution and homogeneity of variance were tested using the Shapiro-Wilk and Levene tests, respectively. The hatcher tray was considered the experimental unit. A one-way ANOVA was used to determine the effects of treatment (UE, CE, NV, CV, MP) on variables. When ANOVA assumptions were violated, robust one-way ANOVA was conducted, and also a robust post hoc test was

applied using the WRS2 package (Mair *et al.*, 2016). For multiple comparisons, Bonferroni adjustments were made. Data is presented as mean ± SEM.

Results

Initial EW was similar among experimental groups (65.40 ± 0.22; p=0.464; data not shown). Compared to UE, non-treated CE showed lower weight and higher weight loss ($p<0.05$) at 18th d of incubation (Table 1). All three eggshell treatments (NV, NP, and MP) had similar EW, EW loss at transfer, and CW at hatch compared to UE ($p>0.05$; Table 1). Regarding hatchability, only the NV group showed similar hatchability compared with that of UE ($p>0.05$). The MP eggshell treatment had hatchability similar to non-treated CE ($p>0.05$; Table 1). Chick weight and CW/EW ratio were lower ($p<0.05$) for the negative control (CE) compared with the positive control (UE) at hatch (Table 1). Chick weights and CW/EW ratios were similar among UE and NV, CV, and MP groups ($p>0.05$; Table 1).

The early incubation period (d 1 to 10) presented the highest EM (Table 2). Moreover, early EM was greater ($p<0.05$) in the negative control (CE) compared with the positive control (UE) (Table 2). Within the treated groups, NV presented the lowest early EM (Table 2). However, MP and NV groups had higher mid EM (from d 11 to d 17 d of incubation) than the UE group ($p<0.05$; Table 2). Although the lowest mid EM was found in the UE group, the mid EM did not differ ($p>0.05$) among eggshell treated groups. No significant differences were found for EM among UE, CE, NV and CV during the hatch period (from d 18 to d 21 of incubation), but EM in the MP group was higher compared with all other groups ($p<0.05$). Contaminated eggs were only found in CV (n = 4) and MP (n = 14) groups (data not shown). The percentage of cull chicks was similar among experimental groups ($p>0.05$; Table 2). Only one pipped egg was noted in the MP group (data not shown). Chicks with signs of dehydration were only found in the CE (n = 9) and in the MP group (n = 2; data not shown). The percentage of chicks with red hock and/or black cord was the lowest in UE, NV and CV groups ($p<0.05$), while the MP group presented an intermediate percentage of red hock and/or black cord compared with the low and high extremes in UE and CE groups, respectively (Table 2).

Table 1. Effect of three eggshell micro-crack sealing methods [uncolored nail varnish (NV), colored nail varnish (CV), molten paraffin (MP)] on egg weight (EW) at transfer, EW loss, hatchability, chick weight (CW) and CW/EW ratio. Data are expressed as mean ± standard error (n = 20).

Trait	Uncracked eggs	Non-sealed cracked eggs	NV	CV	MP	p-value
EW at transfer (g)	59.2±0.41^{a}	55.6±0.17^{b}	57.9±0.33^{a}	58.3±0.36^{a}	58.1±0.18^{a}	<0.001
EW loss at transfer (%)	9.62±0.26^{a}	14.66±0.49^{b}	11.74±0.65^{a}	11.56±0.57^{a}	11.05±0.72^{a}	<0.001
Hatchability (%)	86.11±1.13^{a}	72.92±4.30^{bc}	84.02±2.08^{a}	77.77±0^{b}	59.72±6.46^{c}	0.002
CW (g)	45.2±0.32^{a}	43.4±0.29^{b}	44.6±0.24^{ab}	44.6±0.50^{ab}	45.0±0.26^{ab}	0.019
CW/EW (%)	68.92±0.32^{ac}	66.61±0.29^{b}	67.97±0.54^{abc}	67.63±0.53^{ab}	69.87±0.43^{c}	0.001

Means with different superscript letters ($^{a, b, c, d}$) within the same row differ ($p<0.05$).

Table 2. Effect of three eggshell micro-crack sealing methods [uncolored nail varnish (NV), colored nail varnish (CV), molten paraffin (MP)] on embryonic mortality (EM) and chick traits. Data are expressed as mean ± standard error (n = 20).

Trait	Uncracked eggs	Non-sealed cracked eggs	NV	CV	MP	p-value
EM 1-10d (%)	10.42±1.33^{ab}	16.57±1.13^{c}	6.95±1.79^{a}	13.20±0.69^{b}	15.97±2.86^{bc}	0.048
EM 11-17d (%)	0.69±0.69^{a}	7.64±2.86^{b}	4.17±0.80^{b}	2.08±1.33^{ab}	4.17±0.80^{b}	0.059
EM 18-21d (%)	1.39±0.80^{a}	1.39±0.80^{a}	2.08±0.69^{a}	2.08±0.69^{a}	6.94±1.39^{b}	<0.001
Cull (%)	1.39±0.80	1.39±1.39	2.78±1.13	2.08±0.69	2.78±1.13	0.701
RHBC (%)	0.83±0.83^{a}	11.59±1.67^{c}	1.64±0.95^{ab}	0±0^{a}	5.82±0.85^{b}	<0.001

RHBC = chick with red hock and/or black cord.

Means with different superscript letters ($^{a, b, c}$) within the same row differ ($p<0.05$).

Discussion

The average initial EW was similar to that suggested by Ross (2016; 65.1 g), as a performance objective of Ross 308 hens at 44 weeks of age. The average weight loss of eggs at transfer, 11.45 ± 0.35%, was consistent with the 12% recommended by Ross (2009). No differences among UE and treated eggshell groups were found regarding initial EW. Thus, all the sealed eggs showed normal weight, and normal conductance weight loss during incubation. Considering that the CE group had higher EW loss, it can be hypothesised that NV, CV, and MP were able to control the evaporative water losses within a normal range, suggesting that the sealed surface was not wide enough to affect normal water vapour conductance. These results appear to be reflected in CW. Traldi *et al*. (2011) explained that CW is highly influenced by egg weight. Our findings are in close agreement with Narahari *et al*. (2000), who applied different eggshell treatments (synthetic adhesive resin, cellophane or insulation tape) to cracked eggs. The CW/EW ratio was similar among the positive control (UE) and NV, CV and MP groups; and the average value (68.49 ± 0.40) was in line with the proportion (67%) proposed by Ross (2009). The UE average hatchability was comparable to that recommended by Ross (2016) as a performance objective (88.6%) of Ross 308 hens around 44 weeks of age. It is important to note that only the NV group showed similar hatchability to the positive control, while MP hatchability was similar to the CE group. Lower hatchability in CE eggs are likely due to a gas-exchange reduction and/or altered humidity conditions, or also to increased bacterial contamination. Considering the EW results previously discussed, it might be hypothesised that hatching reduction was mainly due to increased bacterial penetration in eggs. The highest level of contamination and high early EM found in the MP group seems to support this hypothesis. Barnett *et al*. (2004) reported that the embryo can withstand a relatively wide range of humidity in the first phases of development. However, a reduction of gas and water conductance in the MP group can not be precluded. Indeed, MP showed numerically higher chicks with red hock and black cord (string naval associated with temperature variations within the incubation/hatching cabinets) than NV, CV and UE. Moreover, red hock is due to protracted push on the eggshell (Wilson, 2004), which might result in an increase of carbon dioxide concentration and/or decreased moisture loss inside the egg (Visschedijk, 1968; Van de Ven *et al*., 2011). Gulcihan Simsek and Gurses (2009) sealed hairline cracked egg with nail polish and reported that hatchability was reduced by 24.9% compared to intact eggshells, which had higher hatchability compared to cracked eggshells. Gulcihan Simsek and Gurses (2009) explain that these differences were mainly due to late EM (from d 18 to d 21 of incubation), and that nail polish was not dangerous for the embryo development. It is not stated in their article if they used colored or uncolored nail polish. The influence of early EM (from d 1 to d 10 of incubation) has an effect on hatchability. Jull and Lee (2011) explained that early incubation is a critical period due to important events associated with organogenesis. Conversely, in the late incubation period (from d 18 to d 21) only the MP group showed EM higher than the positive control group. This could be due to the higher and more protracted bacterial contamination in the MP group, in agreement with Gulcihan Simsek and Gurses (2009). Nascimento *et al.* (1992) showed that bacterial penetration is not related to eggshell pore numbers, and could be independent from changes in gas and water conductance of the embryo. Our results are generally in agreement with the study of Narahari *et al.* (2000), who reduced EM by covering eggshell cracks with either resin, cellophane or insulation tape.

In conclusion, sealing micro-cracked eggshells with NV or CV are effective to numerically improve hatchability, and to reduce EM in micro-cracked eggs. Improved hatchability (15.2%) was found when micro-cracked eggshells were sealed with uncolored varnish, which seems to facilitate embryonic development similar to un-cracked eggs.

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Conflict of interest

The authors declare they have no conflicts of interest with regard to the work presented in this report.

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