Improving the incubation results by sealing the eggshell of cracked hatching eggs with surgical tape

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ABSTRACT In broiler breeder production, up to 2% of hatching eggs are rejected because of cracked or broken shells. Eggs with cracks give a reduced hatchability and a lower chick quality and cause economic loss. The main goal of this study was to determine the effect of sealing egg-shell cracks with surgical tape on hatching parameters. A total of 3,000 eggs from a 34 weeks old Cobb 500 broiler breeder flock was used in the experiment. Six hundred intact eggs represented a positive control. Other eggs were artificially cracked by the operator either on the first day of storage (1,200 eggs) or on the fourth day of storage (1,200 eggs). In both groups, cracks on 600 eggs were sealed by the adhesive surgical tape while the other 600 eggs remained untreated and were used as a negative control. Within each experimental group, eggs were assigned

randomly to 4 setter trays representing 4 replicates of 150 eggs. The egg weight loss during incubation was the highest (P < 0.01) in groups of nonsealed cracked eggs. The egg weight loss in sealed groups was higher compared to the control group (P < 0.01). Percentage of egg contamination was not different between groups. Embryonic mortality was higher in non-sealed groups in all stages of embryonic development (P < 0.01) compared to groups of sealed cracked eggs and the control group. Hatching percentage was significantly lower in non-sealed groups (P < 0.01) compared to sealed groups and positive control. No significant difference in hatching parameters was observed between sealed groups and positive control, indicating that surgical tape can be used for sealing cracks on the eggshell to support embryonic survival.

Key words: incubation, cracked egg, sealing, surgical tape

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INTRODUCTION

An important quality criterium for a good quality hatching egg is an intact shell. However, in broiler breeder production, up to 2% of hatching eggs are rejected because of cracked or broken shells (Narahari et al., 2000; Salahi et al., 2011; Butcher and Nilipour, 2018).

Shell damage can occur on a breeder farm in the laying nest, during collection and storage (Pilotto et al., 2015), but also during transport and handling of eggs in a hatchery (Anderson et al., 1969; Campo and Ruano, 1992; Nazareno et al., 2013). Some eggshell breakage may be complete crack, star crack, or hairline cracks (Khabisi et al., 2012). Hairline cracks usually occur when eggs hit some inflexible surface, whereas star cracks are more likely to occur due to the collision between eggs (Gupta, 2008). Salahi et al. (2011) found that of the 1.3% of cracked eggs, 0.7% had a star crack, and 0.6% had a hairline crack.

The incubation of cracked eggs significantly reduces the hatchability rate, result in lower chick quality, and cause economic loss (Barnett et al., 2004; Salahi et al., 2011; Rayan and Badri, 2017). Higher embryo mortality and lower chick quality may be due to greater water loss and microbial contamination of hatching eggs (Barnett et al., 2004; Rayan and Badri, 2017; Jabbar et al., 2019). Due to the relatively large number of cracked eggs in broiler breeder production, researchers have tried to find an effective method of sealing the cracked shells to increase their incubation potential. Studies examining the effectiveness of adhesive resin, cellophane tape, and insulation tape (Narahari et al., 2000)nail polish and molted or paraffin (Gulcihan Simsek and Gurses, 2009; Gholami et al.,

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2018) have shown that it is possible to reduce water loss during incubation, and to reduce contamination of eggs as well. Narahari et al. (2000) applied synthetic adhesive resin, pasting cellophane tape or insulation tape over the cracked part of the shell, and improved the hatchability of cracked eggs. The same effect was reported by Gulcihan Simsek and Gurses (2009) who used nail varnish to treat hairline cracks on hatching eggs. Gholami et al. (2018) found that the method of sealing cracked eggs with colorless varnish improved hatchability and was more efficient than the treatment with melted paraffin.

The main goal of this study was to determine the efficacy of sealing eggshell cracks with surgical tape and the effect on hatching parameters. Surgical adhesive tape has good fixing properties and good permeability to water vapor and gases. The hypothesis is that it mimics better the shell parameters than solid tape or nail polish, allowing the normal flow of oxygen, carbon dioxide and moisture to go through the shell. In particular, the passage of carbon dioxide and oxygen can have a significant difference compared to methods that seal of the crack completely.

MATERIALS AND METHODS

Egg Collection and Experimental Design

A total of 3.000 Cobb 500 broiler breeder eggs was used in the experiment. The breeder flock was 34 weeks of age and was kept under the standard management conditions according to the breeding company's recommendations (Cobb, 2020). All collected eggs used for the experiment had a clean and undamaged shell. All eggs used in the trial were collected on the same day, packed, and transported to a commercial hatchery. Eggs were not disinfected on the farm or during transport. Eggs were divided into 5 groups of 600 eggs each. Control group consisted of eggs with intact eggshells. Other eggs were artificially cracked either on the first day of storage (1,200 eggs) or on the fourth day of storage (1,200 eggs). In both groups, cracks on half of the eggs (600 eggs) were sealed by adhesive surgical tape immediately after breaking, and the other half (600 eggs) remained untreated and was used as a negative control, resulting in 5 treatment groups of 600 eggs each. Each treatment group was divided over 4 setter trays of 150 eggs each. Each setter tray was considered an experimental unit, resulting in 4 replications of 150 eggs within each treatment.

Cracking and Sealing the Eggshell

Artificial cracking of the eggshell was made by a quick but controlled impact of a metal spoon on the surface of the shell over the longitudinal side of the egg. Egg shell membrane remained intact. Sealing of cracked eggs was done using surgical adhesive Microtape manufactured by Tosama (Domžale, Slovenia). The average size of the crack was 1 to 2 cm (Picture 1). The length of the tape is



Picture 1. Crack on the shell.

determined for each egg to cover the crack + 1 cm on both sides (Picture 2).

Storage, Incubation and Hatching

Eggs from all groups were stored for four days at 15° C and 65% relative humidity. After storage the trays were randomly divided over incubator trolleys.



Picture 2. Crack sealed by surgical tape.

Preheating of eggs lasted for 12 h at 25°C and 65% relative humidity. After that, the eggs were incubated in a Pas Reform setter, HOM series, with a capacity of 115,200 eggs, and hatched in the TIROS hatcher (Pas Reform, The Netherlands) with a capacity of 19,200 eggs. Incubation was done under standard hatchery operating procedures used by the hatchery and recommended by the incubator manufacturer. Eggs were candled on the 13th d of incubation, and data about the clears have been recorded. The clears have not been removed from the trays in order to be able to calculate the moisture loss. The breakout has been conducted after the hatch, when the number of infertile and early deads has been established.

During the first ten days of incubation, the air temperature was 38°C, and the relative humidity was 47%. The eggshell temperature ranged from 37.6 to 38°C. The eggs were turned every 60 minutes. At the second phase of incubation, from the 11th to the 18th day, the air temperature was 37.4°C, and the eggshell temperature ranged from 38.7°C to 39.3°C. The turning of eggs was stopped on the 12th day of incubation as per the standard operating procedure in this hatchery.

The eggs were transferred to hatchery baskets at 18th day, The initial air temperature was 36.8° C, and relative humidity 47%. At the peak of hatching, the highest relative humidity was 80%, and the hatching process was completed with 55% relative humidity and air temperature of 36.5° C.

Measurements

At the first day of incubation initial egg weight was measured on full tray basis. Egg weight was measured again at 18th day of incubation during transfer to the hatcher baskets, and weight loss was calculated as:

Egg weight loss

= ((egg weight before setting – egg weight at 18 th day of incubation)/egg weight before setting) \times 100.

After the hatching the number of first and second grade chicks was determined. Grading of chicks was done by individual visual inspection according to the method described by Tona et al. (2004). First grade chicks are clean and dry, with completely closed navel, properly developed body without deformities, lesions and inflammation, and chicks that do not meet these criteria were classified as culls (unhealed navels, red hocks, splayed legs, or obvious abnormalities). Body weight was determined on randomly selected 30 chicks per group by individual measurement on a technical scale Kern EMV 200-2 (Kern & Sohn, Balingen, Germany) with an accuracy of ± 0.01 g.

After the chicks were removed from the hatcher, the remaining unhatched eggs were individually broken to record the infertile eggs and to assess the approximate day of embryonic death. Clear eggs with no sign of any cell or membrane development were classified as infertile. Eggs that had visible elements of cell or membrane development were classified as early mortality. Eggs that showed a dark content and emit an odor were classified as contaminated. Eggs containing build up gases (exploders) were classified as contaminated as well. Contaminated eggs were classified as initially fertile eggs. Based on the morphological characteristics, dead embryos were classified either as early (1-7 d), middle (8-14 d), or late mortality (15-21 d + pipped). Fertility, percentages of contaminated eggs, embryonic mortality, and culled chicks were calculated based on the set egg number.

Statistical Analyses

All data were analyzed using General Lineal Model of STATISTICA 14 (TIBCO Software Inc, Palo Alto, CA, 2019). The analysis was done as a complete random design with five groups and four replicates. The setter tray was considered the experimental unit. Percentage data were subjected to arc sine transformation prior to analyses. The homogeneity of variance was tested using Levene tests. One-way ANOVA was used to determine the effects of treatment.

The overall model used was:

 $Yij = \mu + Ai + \varepsilon ij$

Where Yij is the dependent variable, μ is the overall mean, Ai is the effect of the treatment (control, cracked on first day sealed, cracked on first day nonsealed, cracked on fourth day sealed, cracked on fourth day non-sealed), and Eij is the error.

LS means were separated using Fisher LSD post hoc test. Differences between treatments were considered significant at P < 0.05.

RESULTS AND DISCUSSION

Effect of sealing the eggshell of cracked eggs on egg weight loss is shown in Table 1. The egg weight loss during incubation was higher (P < 0.01) in groups of nonsealed cracked eggs compared to the groups of sealed cracked eggs and the control group. Significantly higher weight loss of cracked eggs was expected because damaged eggshell alters the water balance and gas exchange between the embryo and the environment (Solomon, 2010). This is in line with the results reported by other authors (Barnett et al., 2004; Rayan and Badri, 2017; Jabbar et al., 2019). Differences in the egg weight loss during incubation can be attributed to the type and size of the crack (Salahi et al., 2011; Khabisi et al., 2012), shell quality, and egg weight (Molenaar et al., 2010). In addition, higher weight loss (P < 0.01) was found in the group of non-sealed eggs that were cracked on day 4 of storage compared to the group that was cracked on day 1 of storage. Egg weight loss during incubation was calculated, but eggs also lose

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Table 1. Effect of sealing the eggshell of hatching eggs by surgical tape¹ on egg weight loss during incubation.

Traits		Cracked on the 1st day		Cracked on the 4th day			
	Control (intact)	Sealed	Non-sealed	Sealed	Non-sealed	SEM^2	<i>P</i> -value
Initial weight, g	63.31	62.86	63.00	63.66	63.36	0.09	0.056
Weight on 18th day, g	57.71 ^A	55.68^{B}	50.98°	56.37^{AB}	49.11 ^D	0.77	< 0.001
Weight loss, g	$5.60^{\mathbf{D}}$	7.19°	12.02^{B}	7.28°	14.25^{A}	0.76	< 0.001
Weight loss, $\%$	8.85^{D}	$11.44^{\rm C}$	19.10^{B}	$11.44^{\rm C}$	22.50^{A}	1.21	< 0.001

^{A-D}Means within a row lacking a common superscript differ (P < 0.01).

¹Surgical adhesive tape Microtape (Tosama, Domžale, Slovenia).

²SEM, standard error of means (n = 4).

weight during storage. It can be assumed that the increased moisture loss during storage in eggs cracked on the first day affected the moisture loss during incubation. Similar results were reported by Meijerhof (1994) who found that when eggs lose more weight during storage, they reduce weight loss during incubation, but the exact mechanism is not clear.

The egg weight loss did not differ between groups of sealed cracked eggs (P > 0.05), but the weight loss was higher in the sealed cracked eggs compared to control group (P < 0.01). However, although the egg weight loss was higher in groups of sealed eggs, it was still within normal limits. According to Molenaar et al. (2010) weight loss in the range of 6.5 to 14.0% of the initial egg weight has no detrimental effect on the hatchability and quality of day-old chickens.

It can be stated that sealing cracked eggs with surgical tape successfully prevented excessive water loss as the covering of the crack reduced the intensity of water diffusion during incubation. This effect is in accordance with the findings of researchers who managed to prevent excessive weight loss by sealing the cracked shells using varnish (Gulcihan Simsek and Gurses, 2009; Gholami et al., 2018) or plain and insulating adhesive tape (Narahari et al., 2000).

Effect of the sealing the eggshell of cracked eggs by surgical tape on fertility, egg contamination, embryo mortality and percentage of second grade chicks are summarized in Table 2.

Egg fertility in the group of nonsealed eggs cracked on the fourth day was lower compared to other groups (P < 0.05). Because the cracks were caused artificially in the hatchery, fertility rate will not have been influenced by the applied treatments. This means that the indications that cracked eggs had lower fertility compared to the eggs with intact shells, as is supported by the results of Narahari et al. (2000), Gulcihan Simsek and Gurses (2009), Khabisi et al. (2012) and Rayan and Badri (2017) are probably caused by a misinterpretation of very early embryonic death. This is supported by the Barnett et al. (2004) who reported similar fertility rates of eggs with intact and cracked shells.

Percentages of contaminated eggs were not significantly different between groups (P > 0.05), which is not in line with the findings of other researchers who stated that microbial pathogens can easily invade cracked eggshell and significantly decrease hatchability

(Barnett et al., 2004; Gil de los Santos et al., 2007; Gulcihan Simsek and Gurses, 2009; Salahi et al., 2011; Khabisi et al., 2012). However, it is important to emphasize that in present research the eggshells were artificially cracked in the hatchery in the environment with much lower bacterial load compared to the farm. Also, the cracks were sealed very soon after cracking, so the possibilities for contamination were significantly reduced. In production conditions the percentage of contamination can vary depending on the type of crack, and it is higher in hairline cracks compared to star cracks (Khabisi et al., 2012). Salahi et al. (2011) found that as the crack length increases, so does the percentage of contaminated eggs. Gulcihan Simsek and Gurses (2009) did not manage to reduce egg contamination by sealing the shell with nail varnish whereas washing cracked eggs even led to increased contamination (Gil de los Santos et al., 2007).

The embryonic mortality was higher in nonsealed groups compared to sealed groups and the control group in all stages of embryonic development (early (P <0.01), middle (P < 0.01), and late (P < 0.01)). The results showed that most embryos in non-sealed groups died at an early stage. Other authors have also reported that overall mortality is significantly higher in cracked than whole eggs (Gil de los Santos et al., 2007; Salahi et al., 2011; Khabisi et al., 2012; Rayan and Badri, 2017). Higher early mortality in cracked eggs was observed Salahi (2011),also by \mathbf{et} al. Khabisi et al. (2012) and Gholami et al. (2018) but Barnet et al. (2004) and Gulcihan Simsek and Gurses (2009) found higher middle and late embryonic mortality in cracked eggs. Salahi et al. (2011) emphasized the importance of crack size and found that increasing the crack size by 1 cm increased early embryonic mortality by 11.23%.

In the present research embryonic mortality in the groups of sealed eggs did not differ from the control group (P > 0.05), which confirms the positive effect of sealing the shell with surgical tape. Similar results were presented by Narahari et al. (2000) who reported reduced embryonic mortality by sealing the eggshell by adhesive resin or insulation adhesive tape. Gulcihan Simsek and Gurses (2009) got the same effect by applying nail varnish. On the other hand, Gholami et al. (2018) found that molted paraffin, uncolored and colored nail varnish have different effects on

Table 2. Effect of sealing the eggshell of cracked hatching eggs	by surgical tape ¹ on fertility	, egg contamination, eml	oryo mortality, per-
centage of culled chicks, hatchability and chick weight.			

Traits	Control (intact)	Cracked on the 1st day		Cracked on the 4th day			
		Sealed	Non-sealed	Sealed	Non-sealed	SEM^2	<i>P</i> -value
Fertility of set eggs, %	97.00^{a}	97.67^{a}	96.00^{a}	96.99^{a}	$93.50^{\rm b}$	0.42	0.021
Contaminated eggs, $\%^3$	0.00	0.17	0.67	0.33	0.00	0.11	0.293
Embryo mortality, ^{%4}							
Early	3.83^{B}	5.67^{B}	$39.00^{\rm A}$	3.17^{B}	37.36^{A}	3.92	< 0.001
Middle	0.84°	0.83°	11.50^{B}	0.84°	16.67^{A}	1.60	< 0.001
Late	2.00^{B}	$3.50^{\mathbf{B}}$	16.67^{A}	2.84^{B}	15.83^{A}	1.62	< 0.001
Culls, $\%^5$	0.67^{B}	$0.67^{\mathbf{B}}$	4.67^{A}	0.83^{B}	$3.50^{\mathbf{A}}$	0.45	< 0.001
Hatchof set $\%^6$	89.50^{A}	$86.83^{\mathbf{A}}$	22.83^{B}	88.50^{A}	20.00^{B}	7.57	< 0.001
Hatch of fertile, $\%^7$	92.26^{A}	88.93^{A}	23.79^{B}	91.22^{A}	21.35^{B}	7.72	< 0.001
Chick weight, g	43.4^{A}	42.1^{AB}	41.9^{AB}	42.9^{A}	$40.1^{\mathbf{B}}$	0.32	0.003

 $^{\rm a,b}{\rm Means}$ within a row lacking a common superscript differ (P<0.05).

 $^{\rm A-C}{\rm Means}$ within a row lacking a common superscript differ (P < 0.01).

¹Surgical adhesive tape Microtape (Tosama, Domžale, Slovenia).

²SEM, standard error of means (n = 4).

 $^{3}\mathrm{Eggs}$ with a visible onset of infection calculated based upon the number of set eggs.

⁴Early (1-7 d), middle (8-14 d) or late mortality (15-21 days + pipped) calculated based upon the number of set eggs.

⁵Culled chicks (unhealed navels, red hocks, splayed legs, or obvious abnormalities) calculated based upon the number of set eggs.

⁶Percentage of first grade chicks calculated based upon the number of set eggs.

⁷Percentage of first grade chicks calculated based upon the number of fertile eggs.

embryonic mortality, for example, early mortality was reduced by applying both types of varnishes, whereas molted paraffin significantly increased late mortality compared to the untreated group.

The percentage of second grade chickens was higher (P < 0.05) in nonsealed groups compared to other groups in the experiment. There were no significant differences between the sealed groups and the control group, which means that sealing the cracks by surgical tape had positive effect on the number of first grade chicks.

In the research reported by Barnett et al. (2004) and Khabisi et al. (2012) no significant differences in the percentage of second-grade chicks between intact and cracked eggs were found. However, Salahi et al. (2011) and Rayan and Badri (2017) reported that the quality of chicks was significantly lower in groups hatched from the eggs with cracked shell. Gholami et al. (2018) established that sealing the shell of cracked eggs reduces the percentage of second-grade chicks, but Gulcihan Simsek and Gurses (2009) did not find that effect.

Hatching percentage and number of first grade chickens differed between groups (P < 0.01). In groups with cracked shells, a very small number of chickens hatched which means that incubation of cracked eggs is not justified. However, if the cracked shell was quickly sealed with surgical tape, the hatching percentage was at the level of the control group.

The average weight of chicks was lower in the group hatched from nonsealed eggs broken on 4th day (P < 0.01) compared to group of sealed eggs broken on the same day and the control group. This is in line with the fact that in this group the highest egg weight loss during incubation was found.

Similar to these results, Barnett et al. (2004) and Salahi et al. (2011) found lower chick weight in cracked eggs compared to chicks hatched from intact eggs. In the work of Khabisi et al. (2012) the weight of chickens hatched from eggs with hairline cracks was significantly lower compared to eggs with star-type cracked shells and control group. Positive effect of sealing the cracked shell with epoxy glue and adhesive tapes on the weight of the chicks was reported by Narahari et al., (2000). On the other hand, Rayan and Badri (2017) did not establish the effect of cracked shell on body weight of chicks. Gholami et al. (2018) did not find any difference between in body weight of chicks hatched from intact or sealed cracked eggs.

From the obtained results it can be concluded that surgical tape can be used for sealing cracks on the eggshell because it positively affects embryonic survival, prevents water loss during incubation and protects the embryo from contamination. However, this was proven in conditions where the challenge of contamination was relatively small. In practical conditions with much higher bacterial load, sealing of the cracked shell should be done as quickly as possible, to avoid contamination already occurring. Based on the results of this experiment, sealing cracked eggs with surgical tape, preferably immediately after collection but also after cracks occurring during handling in the hatchery, can support the embryonic survival rate. The reason for this result is not solely due to avoiding bacterial contamination or avoiding excessive moisture loss but might be related to gas exchange for the embryo as well.

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Ethical Statement: According to the Annex 4 of the Rulebook on the conditions for entry in the register for

inspections on animals, the content and manner of keeping that register, the welfare training program experimental animals, the application form for approval for conducting experiments on animals, the manner of care, treatment and deprivation of life of experimental animals, as well as content and manner of keeping records on keeping, reproduction, trade, and conducting animal experiments (Annex 4, Official Gazette of RS No. 39/10), trials on hatching eggs as such does not require Ethical approval of the Ministry.

DISCLOSURES

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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