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Can Propolis, the Natural Disinfectant of Bees, be Used As an Effective and Healty Disinfectant for Hatching Eggs?

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ABSTRACT

The aim of this study was to investigate how the incubation parameters and microbial activity of the eggshell were affected by the disinfection of breeding quail eggs with water-based propolis extract in two different concentrations, 0.1% formalin, and distilled water prior to storage. A total of 480 hatching eggs of *Coturnix coturnix* (Japanese quail) were used and they were divided into a total of 5 groups (1 control and 4 separate treatment groups). Following the disinfection, the eggs were stored as 3 groups with different periods of storage, namely 7, 14 and 21 days before the incubation. In order to determine any microbiological activity, 100 eggs were used and aerobic-mesophilic bacteria counts were performed in a total of 5 groups on day 0, week 1, week 2 and week 3 for *E. coli*, yeast and mold and *Staphylococcus aureus*. It was observed that the eggs stored for 3 weeks lost more weight in the pre-development period as compared to those stored for 1 and 2 weeks (P<0.01). Egg weight loss rates had a negative effect on hatching (P<0.01). Total amount of aerobic-mesophilic bacteria was low in the propolis group, medium in the 0.1% formalin group and high in the water and control groups (P<0.05). Furthermore, it was found that the total amount of aerobic-mesophilic bacteria was higher in the shells of the eggs stored for 3 weeks as compared to those with a storage time of 1 and 2 weeks, which pointed to a statistically very significant relationship between the storage time and the total number of aerobic-mesophilic bacteria (P<0.01). On the other hand, yeast and mold growth varied according to treatment groups and storage time. The results suggest that propolis use does not have any negative effect on the incubation performance, to the contrary it keeps the microbial load in check during periods of storage and that it is safe to use in hatcheries.

Key words: Disinfectant, Egg, Hatchability, Propolis, Quail.

INTRODUCTION

As the first and most important stage of raising poultry, incubation management is an essential factor in profitability (Hulet, 2007). With today's high-capacity incubators, even the smallest problems during the hatching process may cause significant financial loss (Cadirci, 2009; Kamanlı *et al.* 2009). Bacterial contamination in eggs begins before ovulation and continues thereafter. The shell of a freshly formed egg contains 300 to 500 bacteria on average. The bacterial load of the egg may change rapidly depending on the environment of ovulation and post-ovulation storage conditions. In particular, eggs laid on the ground can get contaminated with feces and this number may rise to 20.000 to 30.000 in one hour (Elibol *et al.* 2003). This causes a drop in hatchability rates by increasing the number of embryo deaths. Moreover, it is a cause of omphalitis in chicks, leading to poor survival rates and developmental retardation (Cadirci, 2009; Ernst *et al.* 1980). Even though reducing the mechanical contamination of eggs has a positive effect on the outcome of incubation, it is required to disinfect the eggs to improve hatchability and maximize the number of healthy chicks (Cadirci, 2009; Ibrahim *et al.* 2018). Fertilized egg supply from different breeders and transfer of incubated chicks between farms are also factors facilitating spread of disease, further stressing the importance of disinfecting eggs (Elibol *et al.* 2003).

Disinfection process employs oxidized water and chemicals such as sodium hydroxide, phenols and hydrogen peroxide (Baylan *et al.* 2015). Another method most commonly used in the disinfection of hatching eggs, but at the same time subject to most heated debates is formaldehyde fumigation. Formaldehyde is an excellent anti-microbial agent, but also a toxic chemical and can damage the embryo. In recent years, alternative natural products are used to wipe, spray or immerse eggs in order to control microbial contamination and reduce or eliminate the dependence on synthetic pesticides (Baylan *et al.* 2015; Cadirci, 2009). Some studies show that formaldehyde is a cause of respiratory, neurological, reproductive and digestive systems diseases in humans, as well as of some types of cancer (Lam *et al.* 2018).

Propolis is a glue-like sticky substance collected by honey bees from leaves, buds, branches and tree barks. Its color ranges from dark yellow to brown and bees use it

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to polish and disinfect hive interiors and honeycomb cells as a protection against microbial infections. It contains an abundance of vitamins and minerals and has antiseptic, anti-inflammatory, antioxidant, antibacterial, antimycotic, antifungal, antiulcerative, anticarcinogenic and immunomodulatory effects. Because of these properties, it is used both for research and treatment in various medical fields (Akpinar *et al.* 2015; Aygun and Sert, 2013; Aygun *et al.* 2012; Sarıçoban and Yerlikaya, 2016).

In light of this information, in this study hatching quail eggs were immersed in 5% and 10% propolis, in 0.1% formalin and purified water in separate groups before the incubation and stored for three different lengths of time in order to assess the influences of these factors on the hatch outcomes and shell microorganism counts. The propolis extract used in the study was supplied by a manufacturer employing the method of dissolving organic propolis in water. It is known that propolis is traditionally dissolved in alcohol in such studies and no literature was found on the use of water-based propolis.

MATERIALS AND METHODS

Hatching Eggs Used

The study used hatching eggs collected for 2 days from the multi-level hatching cages in the Poultry Unit of the Atatürk University Food and Livestock Application and Research Center. In each cage were 1 male and 3 female breeding quails (*Coturnix coturnix japonica*) all at the same age (18 weeks) and fed *ad libitum* with bird food containing 20% protein and 2900 kcal/kg metabolic energy.

Preparing the Solutions for Treatment

Fanus Gıda® supplied the 10% water-based organic propolis extract used in the study. This product was mixed with sterile distilled water to obtain an extract in the ratio of 5%. Since water was used as solvent in these two experiment groups, the effect of water was evaluated with distilled water in another group. Moreover, a formalin solution of 37% was mixed with sterile water to obtain a 0.1% formalin solution.

Experimental Applications

A total of 480 fresh *Coturnix coturnix* (Japanese quail) eggs were used in the study. These were divided into 5 treatment groups as control, distilled water, 5% propolis, 10% propolis, and 0.1% formalin and the eggs in each group were randomly distributed further into 3 groups in an environment of 15 to 18°C temperature and to be collected after 7, 14, and 21 days respectively. 32 eggs were used in each subgroup. Weight loss was calculated on the basis of weekly egg weighing according to storage period. Following the storage, eggs were pre-warmed in a room at 25°C for 6 hours and incubated in a preliminary development machine (with a temperature of 37.5°C, a humidity 65% and turning the eggs at 1 hour intervals) for 15 days. At the end of the 15th day, the eggs were weighed again and transferred to the output section (temperature 36.5°C, humidity 75%, no turning).

Once the hatched chicks dried, they were counted and weighed. Eggs that did not hatch were broken to identify the unfertilized and the ones with a dead embryo, the times of death were also specified for the latter. The eggs were evaluated in 3 groups according to the death of embryos: early term (1-6 days), medium term (7-14 days) and late term (15-18 days). In order to determine the outcome of incubation, 132 eggs found to be unfertilized were left out during the evaluation and (early-, medium- and late-term) embryo mortality was calculated as hatchability (number of chicks/number of fertilized eggs) and percentage (%).

Microbial Analyses

For the identification of microbial activity 100 eggs were used in addition to the eggs used in hatching, in a total of 5 groups (4 different treatments and 1 control) and total counts of aerobic-mesophilic bacteria, *E. coli*, yeast and mold and *S. aureus* were achieved on day 0, in week 1, week 2 and week 3.

Preparing the dilution

A dilution of 1/10 was prepared by soaking 1 egg in 50 ml sterile ringer water. Other dilutions were prepared with the dilution method (Kurt *et al.*, 1996).

Total aerobic-mesophilic bacteria count

Plate Count Agar (PCA, Merck) medium was used to count the microorganisms in this group and plates were evaluated following an incubation of 72+1 hours at 30 ± 1°C (Harrigan and McCance, 1976).

Yeast and mold count

Rose Bengal Chloramphenicol Agar (RBC, Merck) medium was used for the yeast and mold count. After incubating the petri dishes for 5 days at 21°C, the microorganism count was performed (Harrigan and McCance, 1976).

S. aureus count

Baird Parker Agar (BPA, Merc) medium was used for counting this microorganism. The count was performed following the incubation of petri dishes at 30°C for 24-48 hours (Harrigan and McCance, 1976).

E. Coli count

Tryptone Bile X-glucuronide Agar (Merck 1.16122) was seeded with a suitable dilution of 0.1 with the spread plate technique, followed by a 48-hour incubation at 44°C. Blue-green colonies were evaluated (Halkman and Sagdas, 2005).

Statistical Analysis

The study was carried out according to the fully randomized block trial plan. SPSS software package was used to analyze the data obtained in the study (SPSS and Inc.). One-Way ANOVA was used to calculate egg weight loss, chicks' hatching weight and microbial analyses; treatment groups

and the storage times were calculated with the Logistic Regression method; Chi-Square was used for embryonic deaths and finally, the T-test procedure was applied to find the effect of egg weight loss to hatching.

RESULTS AND DISCUSSION

Egg weight loss

Egg weight loss during the incubation period was not affected by the treatment method (P>0.05). However, the eggs stored for 3 weeks lost more weight in the pre-development period than those stored for a 1- and 2-week period and therefore they weighed less prior to the transfer to hatching (P<0.01, Table 1). Some researches on the subject it was found that egg weight loss during the storage (Aygun and Sert, 2013; Aygun *et al.* 2012; Akpinar *et al.* 2015) and incubation periods (Aygun and Sert, 2013; Shahein and Sedeek, 2014) were lower in the group with propolis as compared to other treatment groups.

Weight loss rates of hatching and non-hatching eggs were 0.172 ± 0.04 and 0.147 ± 0.01 g, respectively and it was found that the rate of weight loss had a negative effect on hatching (P<0.01, Table 2). Moreover, it was found that the greater the weight loss, the smaller was the hatching ratio. Study showed that eggs stored for 3 weeks were 4 times less hatchable (P<0.01). Suggested cause is that extended storage times affected internal quality of the egg. Various other studies support this result and suggest that long-term storage affects the internal quality of eggs negatively. Further weight loss during incubation resulted in a higher number of late-stage embryonic deaths in quail eggs, in addition to reducing hatchability (Jones and Musgrove, 2005; Lacin *et al.* 2008; Toplu *et al.* 2007). In another study, eggs stored for 1, 2, 3 and 4 weeks had a hatchability of 60.2%, 57.9%, 42.4% and 16.2% respectively and it was reported that the longer was the storage time, the lower was hatchability (Wilson, 1984).

Table 1: Variance analysis results on the egg weight on day 0, egg weight prior to hatching and the weight loss rates of eggs.

		N	Egg weight (g) before storage	Egg weight (g) after incubation	Egg weight loss (g)
Treatment	Control	65	11.18±0.14	9.402±0.131	0.161±0.004
	Water	71	11.33±0.13	9.581±0.120	0.155±0.004
	5% propolis	67	11.64±0.14	9.834±0.131	0.156±0.004
	10% propolis	74	11.67±0.14	9.809±0.126	0.159±0.004
	0.1% formalin	71	11.50±0.13	9.706±0.122	0.156±0.004
Storage Time	1 week	159	11.47±0.85	9.790±0.79[a]	0.147±0.002[a]
	2 weeks	113	11.57±0.101	9.805±0.94[a]	0.153±0.003[a]
	3 weeks	76	11.35±0.125	9.405±0.116[b]	0.173±0.003[b]
				P	
Treatment			0.069	0.110	0.718
Storage Time			0.419	0.012	0.0001

Table 2: Variance analysis results on the weight loss rates of eggs as per hatching status.

Chick hatching status	N	Egg weight loss rate	Sig.
0	99	-0.172±0.04[b]	0.0001
1	249	-0.147±0.01[a]	

Table 3: Logistics regression results for treatment groups and storage times as per hatchability.

		β	S.E.	Exp (B)
Treatment	Control			1
	Water	0.248	0.412	1.281
	5% propolis	-0.502	0.394	0.605
	10% propolis	-0.492	0.385	0.611
	0.1% formalin	0.357	0.419	1.429
Storage Time	1 week			1
	2 weeks	-0.137	0.296	0.872
	3 weeks	-1.328	0.308	0.265
	Constant	1.406	0.330	4.081
		P		
Treatment		0.080 NS		
Storage Time		0.0001 ***		

Table 3 shows the logistic regression results of treatment groups and storage times as per hatching. Results show that treatment was not a significant factor affecting chick output or hatchability (P>0.05). However, a highly significant correlation was found between the storage period and hatchability and it was determined that a 3-week storage decreased hatchability almost 4 times (P<0.01, Table 3). In some studies using water (Simsek and Bayraktar, 2005), propolis (Aygun *et al.* 2012) and other disinfectants (Bailey *et al.* 1996) it was determined that the treatment method did not affect hatchability. In other studies using propolis in different percentages, it has been reported that the treatment method affects hatchability (İbas, 2018; Shaheen and Sedeek, 2014).

Hatchability

Table 4 shows hatchability (number of chicks/number of fertilized eggs) by treatment group and storage time. Upon comparison of hatchability between 1- and 2-week storage and treatment groups, it was found that in both storage times, control groups returned the lowest rate of hatchability.

No correlation was observed between treatment methods and embryo deaths (P>0.05, Table 5). Similarly, in two different studies examining how disinfection of hatching quail eggs with propolis in different percentages and the other treatment method affected the microbial load and incubation parameters, reported no difference between applications in terms of embryonic mortality rates (Aygun *et al.* 2012; Aygun and Sert, 2013). However, in some other studies, propolis groups were found to be effective on embryo death (Shahein and Sedeek, 2014; İbas, 2018).

In the current study, the highest rate of embryonic death was observed in eggs stored for 3 weeks, but the storage period did not affect embryonic deaths (P>0.05). In another study, early-term embryonic death rate in eggs stored for 14 days was found higher as compared to eggs stored for 7 days and it was concluded that extended storage time caused an increase in early-term embryonic deaths (Aygun and Sert, 2013).

Neither storage time nor treatment method is a factor in the hatching weight of chicks (P>0.05, Table 6). Toplu *et al.* (2007) reported that storage time had no statistically significant effect on chick hatching weight. This study is similar to the current one in its conclusion that storage time has no significant effect on chick hatching weight. On the other hand, Shahin and Sedeek (2014) reported that the highest weights were found in the chicks which were administered 14% propolis.

Microbiological analysis

Microbiological analysis of the egg shells found no trace of *Escherichia coli* or *Staphylococcus aureus*. These two species of bacteria develop under circumstances of poor hygiene, especially the bodily hygiene of employees. This shows that eggs are collected and stored under hygienic conditions. Furthermore, yeast and mold growth did not vary

Table 4: Hatchability (number of chicks/number of fertilized eggs) (%) as per treatment group and storage time.

		Storage Time		
		1 week	2 weeks	3 weeks
Treatment	Control	72.41	61.30	46.15
	Water	79.31	77.27	70.00
	5% propolis	73.52	70.00	30.76
	10% propolis	80.55	66.00	38.46
	0.1% formalin	83.87	76.95	58.82

Table 5: Embryonic death outcome by treatment group and storage time.

		Death Period			
		Eearly Period	Medium Period	Last Period	Total
Treatment	Control	7 (%41.2)	3 (%17.6)	7 (%41.2)	17 (%17.2)
	Water	8 (%47.0)	2 (%11.8)	7 (41.2)	17 (%17.2)
	5% propolis	10 (%41.7)	4 (16.6)	10 (41.7)	24 (%24.2)
	10% propolis	11 (%42.3)	2 (7.7)	13 (%50)	26 (%26.2)
	0.1% formalin	4 (%26.7)	2 (%13.3)	9 (%60)	15 (%15.2)
	Total	40 (%40.4)	13 (%13.1)	46 (%46.5)	99 (%100)
Storage Time	1 week	13 (%37.1)	6 (%17.2)	16 (%45.7)	35 (%35.3)
	2 weeks	9 (%33.3)	3 (%11.1)	15 (%55.6)	27 (%27.3)
	3 weeks	18 (%48.6)	4 (%10.8)	15 (%40.6)	37 (%37.4)
	Total	40 (%40.4)	13 (%13.1)	46 (%46.5)	99 (%100)
			P		
Treatment			0.991 NS		
Storage Time			0.649 NS		

Table 6: Variance analysis results on weight at hatching as per treatment group and storage time.

		N	Chick weight
Treatment	Control	48	7.629±0.135
	Water	54	7.529±0.110
	5% propolis	43	7.917±0.159
	10% propolis	48	7.697±0.146
	0.1% formalin	56	7.726±0.115
Storage Time	1 week	124	7.834±0.072
	2 weeks	86	7.686±0.087
	3 weeks	39	7.578±0.141
		P	
Treatment		0.357	
Storage Time		0.187	

Table 7: Variance analysis results of the microbial load in egg shells as per treatment groups and storage times.

		Log total	Log yeast and mold
	Control	2.770±0.170^{a}	0.744±0.104
	Water	2.311±0.170^{ab}	1.040±0.104
	5% propolis	1.369±0.170^{d}	1.025±0.104
	10% propolis	1.634±0.170^{cd}	1.160±0.104
	0.1% formalin	1.947±0.170^{bc}	0.965±0.104
Storage Time	0. day	1.890±0.152^{ab}	0.957±0.093
	1 week	2.224±0.152^{a}	1.187±0.093
	2 weeks	2.220±0.152^{a}	0.906±0.093
	3 weeks	1.690±0.152^{b}	0.896±0.093
		P	
Treatment		0.035	0.097
Storage Time		0.000	0.078

according to treatment group or storage time (P>0.05) and the total amount of aerobic-mesophilic bacteria was low in the 5% and 10% propolis groups, medium in the 0.1% formalin group and high in water and control groups (P<0.05). A highly significant relation was found between storage time and the total number of aerobic-mesophilic bacteria as shown by the findings that the shells of the eggs stored for 3 weeks contained a higher rate of total aerobic-mesophilic bacteria than those stored for 1 week and 2 weeks (P<0.0001, Table 7). Some researches argued that the best results in the total aerobic-mesophilic bacteria, coliform and *Staphylococcus* counts on eggshell surface were obtained with propolis and that this safe, non-toxic product was a good alternative to keep microbial load in check in the storage and incubation periods (Shahein and Sedeek, 2014; Aygun and Sert, 2013; Aygun *et al.* 2012). A separate study, considering the bacteria count on the 18th day of incubation, no significant bacteria growth was reported in the eggs disinfected with chloride, 6% propolis and 9% propolis, however the bacteria growth was found to be at significant levels in the group disinfected with 3% propolis and that it was in fact at pre-disinfection levels. As a result, it was interpreted that the efficacy was not sustainable in the disinfection with 3% propolis (İbas, 2018).

CONCLUSION

As the use of high-capacity incubators become more widespread, poultry hatcheries provide eggs from different breeders and send the chicks produced to various enterprises themselves. This requires the development and administration of an effective disinfection procedure to prevent transfer of disease from farm to farm, to maximize the number of chicks hatched, and increase profitability. Substances used in egg disinfection should be effective on microorganisms, but even more important than that they should not have any detrimental or harmful effects on human and animal health. Based on the findings of this study, the use of propolis as a non-toxic substance is proposed for the disinfection of hatching eggs. Propolis has no negative effects on hatchability, it keeps the microbial load in check during storage and can be safely in hatcheries used.

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