Effects of propolis on eggshell microbial activity, hatchability, and chick performance in Japanese quail (*Coturnix coturnix japonica*) eggs

A. Aygun,*[1] D. Sert,† and G. Copur‡

*Faculty of Agriculture, Department of Animal Science, and †Faculty of Engineering and Architecture, Department of Food Engineering, Konya University, Konya 42060, Turkey; and ‡Faculty of Agriculture, Department of Animal Science, Mustafa Kemal University, Hatay 31034, Turkey

ABSTRACT Propolis is a sticky resin produced by worker honeybees from substances collected from plants, and it has strong antibacterial and antifungal properties. The purpose of this study was to establish the effects of propolis on egg weight loss, hatchability, chick performance, and to control microbial activity naturally occurring on eggshells. A total of 750 fresh eggs was randomly divided into 5 groups. Eggs from the first group were sprayed with ethyl alcohol (70%, A), the second group was sprayed with benzalkonium chloride (B), and the third, fourth, and fifth groups were sprayed with propolis at 3 doses: 5, 10, and 15%. Eggs sprayed with propolis had lower egg weight loss than eggs from groups A and B ($P < 0.001$). Bacterial activity was reduced significantly in all propolis groups. There were no significant differences between treatments for hatchability, embryonic mortality, BW gain, and relative growth. Results of the present study indicated that propolis could be an alternative hatching egg disinfectant versus a chemical disinfectant, without adverse effects on hatchability and performance of quail chicks.

Key words: propolis, eggshell microbial activity, hatchability, relative growth, sanitizer

2012 Poultry Science 91:1018–1025
http://dx.doi.org/10.3382/ps.2011-01944

INTRODUCTION

Numerous infectious organisms can colonize the egg before and after laying. In addition to surface contamination, a freshly laid egg is wet and warm, and the cuticle is immature and some pores may be open, thus susceptible to penetration by microorganisms (Board, 1966; Williams et al., 1968; Mayes and Takeballi, 1983; Bruce and Drysdale, 1994).

When laid, the number of bacteria on the shell range from 300 to 500. This number may increase up to 20,000 or 30,000 bacteria rapidly in one hour after the egg was laid (North and Bell, 1990). Typical contaminants are *Salmonella*, *Pseudomonas* (Jones et al., 2004), coliforms and *Escherichia coli* (Singh et al., 2009). Yeast and mold have also been monitored as well (Jones et al., 2011).

Lactobacillus spp. and *Micrococcus* spp. from the ova, *Salmonella* ssp. (*Salmonella* Enteritidis and host-specific *Salmonella* Gallinarum and *Salmonella* Pullorum) are able to translocate to the ova via the blood from the alimentary canal (Gordon and Tucker, 1965). Harry (1963) suggested the possibility of direct infection of the ovarian tissue itself; however, infection of the ova can occur in the oviduct as well. Although *Micrococcus*, *Streptococcus*, and *coli*-aerogenes organisms are the dominant contaminants (Harry, 1963), *Staphylococcus aureus*, *Salmonella* spp., and *Pasteurella* spp. have also been isolated from the oviduct (Mayes and Takeballi, 1983).

Egg contamination occurs most frequently after oviposition and contaminants may be divided into pathogens (e.g., *Salmonella* Enteritidis) or spoilage bacteria (e.g., *Aeromonas*, *Enterobacter*, *Proteus*, and *Pseudomonas*). Some infectious organisms can pass through the eggshell in contact with feces or bedding. Therefore, sanitation is essential in successful hatching egg production. Several sanitation methods are available. Fumigation, spray application, UV light, and washing with appropriate sanitizer are common practices (Adler et al., 1979; Arhienbuwa et al., 1980; Proudfoot et al., 1985; Kuhl, 1989; Sacco et al., 1989; Whistler and Sheldon, 1989; Coufal et al., 2003). Sanitation practices depend on the size of operation, history of the disease problems at the site, and the capacity of the equipment. In the case where hatching eggs were not sanitized before incubation, excessive bacterial contamination and subsequent growth can lead to decreased hatchability, poor chick quality, and poor growth/per-

Received October 14, 2011.
Accepted December 10, 2011.
[1]Corresponding author: aaygun@selcuk.edu.tr

formance (Scott and Swetnam, 1993) and to increased mortality (Reid et al., 1961).

Human health, environmental concerns, and consumers' demand for residue-free food require evaluation of alternative, low-risk control methods. Natural biologically active compounds from plants are generally thought to be more acceptable and less hazardous than synthetic compounds and represent a rich source of potential disease-control agents. As a result, increased interest is being shown in developing alternative methods to control microbial contamination reducing or eliminating reliance on synthetic pesticides. One such method involves the use of plant-derived-products, such as plant essential oils and propolis, which possess microbicidal effects.

Propolis is a sticky gummy resinous substance collected by worker honeybees (*Apis melifera*) from young shoots and buds of certain trees and shrubs (Greenaway et al., 1990; Schmidt, 1997), and it has strong antibacterial, antifungal, and antiviral properties (Ghisalberti, 1979; Krell, 1996; Bankova et al., 2000). Bees also use it to cover the inside of the hive and mix it with beeswax during the building of combs to protect the colony and larvae from pathogenic microorganisms, such as *Bacillus subtilis*, *B. alvei*, *Proteus vulgaris*, and *P. galangin* (Ghisalberti, 1979). In addition, propolis has considerable antibiotic effects on *Salmonella*, *Staphyloccus aureus*, *P. vulgaris*, and *Esherichia coli* (Powers, 1964). Recently, investigations have indicated that the interest for natural preservatives has increased. The use of propolis as an alternative preservative agent has been considered safe by consumers (Ghisalberti, 1979). Due to the antibacterial effects, propolis is used for protection of various agricultural products during storage. Copur et al. (2008) stated that covering table eggs with propolis improved interior egg quality during storage. The composition of propolis varies with the origin of the plant species. Bees tend to collect these resins from a large variety of trees and shrubs to take advantage of the antibacterial, antifungal, and antiviral effects (Krell, 1996).

Keeping all of these points in consideration, an attempt has been made in the present study to find out the practical applicability of propolis to control microbial activity naturally occurring on the eggshell and to determine its effect on hatchability, embryonic mortality, and performance of quail (*Coturnix coturnix japonica*) chicks.

MATERIALS AND METHODS

Breeder Flock

In total, 750 fresh eggs (unwashed, feces-free) were obtained from Japanese quail (*Coturnix coturnix japonica*; 13 wk of age) that were raised on the Research and Application Farm of Agricultural Faculty, Selcuk University (Konya, Turkey). The quail were housed in battery cages (1 male:2 female) and a photoperiod of 16L:8D. The quails were fed a breeder diet containing 2,901 kcal of ME/kg and 20% CP (Table 1). Food and water were provided ad libitum.

Table 1. Composition of the diet

	Amount	
Item (%, unless noted)	Grower	Breeder
Ingredient		
Corn, yellow	47.00	43.50
Soybean meal, 45%	40.30	29.50
Sunflower meal, 28%	6.80	7.00
Barley	—	7.78
Limestone	1.15	5.43
Dicalcium phosphate	0.90	1.32
Salt	0.30	0.30
Vitamin premix[1]	0.15	0.15
Mineral premix[2]	0.10	0.10
DL-Methionine	—	0.15
Vegetable oil	3.30	4.77
Total	100	100
Calculated value		
CP	24.10	20.00
ME (kcal/kg)	2,912	2,901
Ca	0.80	2.50
Available P	0.30	0.35
Sodium	0.15	0.15
Lysine	1.29	1.02
Methionine + cystine	0.78	0.81
Threonine	0.92	0.75
Tryptophan	0.40	0.30

[1]Vitamin premix supplied per kilogram of diet: vitamin A, 8,000 IU; vitamin D_3, 2,200 IU; vitamin E, 13 IU; vitamin K_3, 2.67 mg; vitamin B_1, 2.5 mg; vitamin B_2, 4.67 mg; vitamin B_6, 3.33 mg; vitamin C, 33 mg; calcium D-pantothenate, 6.67 mg; nicotine acid, 17 mg; D-biotin, 0.03 mg; folic acid, 0.67 mg; and vitamin B_{12}, 0.01 mg.

[2]Mineral premix supplied per kilogram of diet: Cu, 5 mg; Fe, 60 mg; Mn, 100 mg; Zn (ZnO), 60 mg; Se, 0.15 mg; Co, 0.50 mg; and choline, 125 mg.

Preparation of Solutions

Propolis was collected from honey bees in Hatay (Turkey), in 2010 and extracted according to the method suggested by Krell (1996). A 5% propolis solution was prepared by mixing 950 mL of 70% ethyl alcohol and 50 g of propolis; a 10% propolis solution was prepared by mixing 900 mL of 70% ethyl alcohol and 100 g of propolis. A 15% propolis solution was prepared by mixing 850 mL of 70% ethyl alcohol and 150 g of propolis. Solutions were kept in a container, the top was sealed, and it was shaken twice daily for one week. Each solution was filtered (coarse filter) separately and was kept in a clean, dark bottle at 4°C until use.

Application of Solutions

Eggs were collected between 1100 h and 1700 h and were stored at 22°C overnight before application of disinfectant. The eggs were randomly divided into 5 groups of 150 eggs. Because the propolis was dissolved in ethyl alcohol, the first group was sprayed with ethyl alcohol (70%; A) to determine whether there was any synergistic effect. The second group was sprayed with benzalkonium chloride solution (B). The third, fourth,

Table 2. Media and incubation conditions used in microbiological analysis

Microorganism	Medium[1]	Incubation conditions		Reference
		Temperature (°C)	Time (d)	
Total aerobic mesophilic bacteria	Plate count agar (5% NaCl was added)	37	2	APHA, 1993
Mold-yeast	Potato dextrose agar (pH 3.5; acidified with 10% tartaric acid)	20	5	Pitt and Hocking, 1985
Coliform bacteria	MacConkey's broth	37	2	WHO, 1993
Escherichia coli	MacConkey's broth	44	1–2	APHA, 1993
Staphylococcus ssp.	Baird Parker agar (supplemented with 5% egg yolk-tellurite)	37	2	IAEA, 1970
Salmonella ssp.	Selenite cystine broth and	37	1	ISO, 1993
	bismuth sulfite agar	37	1	

[1]Merck, Darmstadt, Germany.

and fifth groups were sprayed with propolis at 3 doses: 5 (**P5**), 10 (**P10**), and 15% (**P15**). Benzalkonium chloride is widely used as a sanitizer and disinfectant for hospital, livestock, and water treatment (Klimek and Bailey, 1956; Russell et al., 1992; Tebbs and Elliott, 1993; Gradel et al., 2005; Thomas et al., 2005). The solutions were taken (15 mL of each solution) and sprayed onto the egg, using a hand sprayer, to cover the whole surface. After applications, the eggs were allowed to dry at 22°C for 10 min. A total of 74 eggs from each treatment was numbered and weighed at the beginning and on d 14 of incubation to calculate the moisture loss. Eggs containing dead embryos and unfertile eggs were excluded from the calculation percentage of egg weight loss.

Incubation Management

Eggs were incubated in a commercial incubator (Çimuka Incubator Co., Ankara, Turkey) with a dry-bulb temperature of 37.5°C and 60 to 65% RH until d 14 of incubation when incubator conditions were changed to 37.2°C and 75% RH at the Department of Animal Science, Agriculture Faculty, Konya, Turkey. Eggs were turned through 90° once every 2 h.

Microbiological Analyses

The 5 eggs per group were taken for microbiological analysis at 1, 7, and 14 d of incubation and immediately placed on sterile stomacher bags containing 50 mL of sterile PBS (pH 7.2). A whole-egg washing technique was used to recover the shell-associated microorganisms for estimating the total aerobic mesophilic bacteria, coliforms, *Salmonella* ssp., *Staphylococcus* ssp., and mold-yeast. Serial dilutions were made in PBS and then were inoculated into sterile Petri plates (Gentry and Quarles, 1972; Jones et al., 2002). Colonies were measured as log cfu/egg.

Media and incubation conditions used in microbiological analysis are shown in Table 2. Coliforms were determined with the most probable number technique. The incubated tubes that showed a yellow tint (acid production) and gas were considered to be positive. Confirmation of *E. coli* was carried out by indole, methyl red, Voges-Proskauar, incubation temperature, and citrate (IMVIC) tests. Suspected *Staphylococcus* ssp. colonies were tested for coagulase activity and confirmed by other biochemical reactions.

Hatching

Between 360 and 416 h of incubation, transferred eggs were checked individually every 8 h, and hatched chicks were recorded. After 17.5 d of incubation, all hatched chicks were removed from each hatch basket.

At d 17.5 of incubation, unhatched eggs were opened to establish the stage of embryonic mortality. The stages of embryonic mortality were classified as follows: d 1 to 9 (black-eye visible and embryo without feathers), d 10 to 17 (embryo with feathers and embryo with yolk out), and d 17 to 18 (full-grown embryo dead and with yolk subtracted). Fertility was calculated as the percentage of set eggs. The hatchability was calculated as both set eggs set and the fertile eggs.

Chick Performance Procedure

After 17.5 d of incubation, 52 chicks per group (13 chicks/pen) were randomly selected to growth to determine their performance for 10 d. Chicks were weighed and identified with a leg ring number. Chicks were raised (4 pens/group) in different pens with 13 chicks per 0.29 m^2. During the 10 d of growing, a grower diet (2,912 kcal of ME/kg and 24.1% CP) was provided ad libitum (Table 1). Room temperature was set at 33°C. The photoperiod was 23L:1D. At the end of 10 d, all chicks were individually weighed (without leg ring). For each chick, the BW of d 1 (BW1) and the BW of d 10 (BW10) were used to calculate the relative growth (RG), where RG was defined as RG = 100 × (BW10 – BW1)/BW1.

Statistical Analysis

Fertility, hatchability of set, hatchability of fertile eggs, and embryonic mortality were phenotypes of interest and analyzed using binary logistic regression

Table 3. Effects of hatching egg sanitizers on microbial activity of eggs during incubation (log cfu/egg)

		Incubation (d)[2]													
		1					7				14				
Group[1]	n[1]	T	C	Sa	St	M	T	C	Sa	St	M	T	C	Sa	St	M
A	5	5.35[a]	3.81[a]	2.87[a]	4.85[a]	5.67[a]	5.89[a]	4.24[a]	3.25[a]	5.59[a]	6.41[a]	6.82[a]	5.05[a]	3.88[a]	6.60[a]	7.23[a]
B	5	3.79[b]	3.34[b]	2.07[b]	3.27[b]	4.10[b]	4.43[b]	3.71[b]	2.45[b]	3.93[b]	4.84[b]	5.28[b]	4.41[b]	3.11[b]	5.06[b]	5.61[b]
P5	5	3.42[c]	2.81[c]	1.71[c]	2.75[c]	3.44[c]	3.88[c]	3.19[c]	2.05[c]	3.32[c]	3.87[c]	4.85[c]	3.74[c]	2.46[c]	4.12[c]	4.36[c]
P10	5	3.14[d]	2.04[d]	1.38[d]	2.42[d]	2.62[d]	3.62[d]	2.39[d]	1.54[d]	2.95[d]	3.06[d]	4.36[d]	2.94[d]	1.86[d]	3.58[d]	3.37[d]
P15	5	2.21[e]	1.16[e]	1.16[e]	2.11[e]	2.43[e]	2.53[e]	1.36[e]	1.35[d]	2.61[e]	2.76[e]	3.13[e]	1.86[e]	1.48[e]	3.26[e]	3.29[d]
Pooled SD		0.0356	0.0479	0.0513	0.0279	0.0410	0.0530	0.0484	0.0657	0.0361	0.0217	0.0430	0.0351	0.0616	0.0407	0.0691

[a–e]Means within a column with different superscripts differ significantly ($P < 0.001$).

[1]A: 70% ethyl alcohol, B: benzalkonium chloride, P5: 5% propolis, P10: 10% propolis, P15: 15% propolis.

[2]T: total aerobic mesophilic bacteria, C: coliform, Sa: *Salmonella* ssp., St: *Staphylococcus* ssp., M: mold-yeast.

analysis. The BW at d 10 and BW gain were analyzed using GLM by including the BW at d 1 as a covariate. Bacterial activity, egg weight loss, hatching time, and relative growth were analyzed via one-way ANOVA. The least significant difference test was applied to detect statistically significant differences between groups. All analyses were carried out by using Genstat (Payne et al., 2003).

RESULTS AND DISCUSSION

Microbiological Activity

Application of different disinfection solutions significantly ($P < 0.001$) affected total aerobic mesophilic bacteria values (Table 3). Surface flora of hatching eggs decreased ($P < 0.001$) with the increased propolis concentration. The microbial count at incubation periods of 1 d decreased from 5.35 to 2.21 log cfu/egg within the P15 propolis treatment. The decreased rate of microbial loads continued parallel with progressive incubation durations. The lowest and the highest total aerobic mesophilic bacterial counts of all storage periods were determined in the P15 treatment and in the alcohol control treatment, respectively. Fasenko et al. (2009) found that spraying eggs with electrolyzed oxidizing water resulted in the reduction of total aerobic bacteria counts. Kuo et al. (1997) evaluated different UV (254 nm) treatment times (0, 15, and 30 min) at an intensity of 620 $\mu W/cm^2$ and also different intensities (620, 1,350, and 1,720 $\mu W/cm^2$) at a treatment time of 15 min. For all UV treatments, a 2-log reduction of cfu of aerobic bacteria per eggshell was observed. The visibly clean eggshell surfaces initially contained 5.0 log cfu aerobic bacteria per eggshell. Favier et al. (2001) found a reduction of 1.6 log on uninoculated, clean eggs after a UV exposure for > 25 min (254 nm; 4,573 $\mu W/cm^2$).

Significant ($P < 0.001$) differences were also found in values of coliform bacteria, which were entitled as a fecal contamination indicator in foods, among study groups with respect to the type of disinfectant. Although the microbial inhibition of coliforms in the control group (alcohol treatment) was circa 10%, this value in the P5 treatment group increased to 26%. Microbial inhibition raised to 70% with the increased propolis concentrations. The lowest coliform count (1.16 log cfu/egg) was obtained from the P15 treatment. The increasing coliform count was obtained during the incubation period. Although the increase of the coliform count was 0.7 log units from the P15 treatment, it was 1.2 log units in the alcohol-treated group. Cox et al. (1994) stated that the number of total bacteria and coliform bacteria were significantly reduced by washing the eggs with a sanitizing solution. Effective washing and sanitizing of hatching eggs at the breeder farm can eliminate or reduce an assortment of bacteria, a result which in turn may increase hatchability and may reduce the number of new hatched chicks contaminated with human foodborne pathogens, such as *Salmonella* (Cox et al., 1994).

Table 4. Effects of hatching egg sanitizers on percentage of egg weight loss during incubation (%)

Group[1]	n	Egg weight loss
A	72	10.58[a]
B	70	10.41[a]
P5	73	9.73[b]
P10	72	9.28[b]
P15	70	9.21[b]
Pooled SD		1.33

[a,b]Means within a column with different superscripts differ significantly ($P < 0.001$).

[1]A: 70% ethyl alcohol, B: benzalkonium chloride, P5: 5% propolis, P10: 10% propolis, P15: 15% propolis.

The colony count of *Salmonella* ssp. was reduced ($P < 0.001$) by disinfection treatments. Application of propolis in all incubation periods diminished the *Salmonella* count. *Salmonella* values of the alcohol-treated group were higher than the other treatment groups. *Salmonella* counts at the beginning and at the end of the incubation period were observed to be 2.87 and 3.88 log cfu/egg, respectively. *Salmonella* inhibition of the P15 treatment at the incubation beginning and end increased from 60 and 62%, respectively. *Salmonella* counts after 7 d of storage were not different between the P10 and P 15 treatments.

Staphylococcus ssp. counts in all incubation periods decreased with the higher propolis concentrations. Although the inhibition rate at the beginning of incubation for the P5 treatment was 43%, it increased to 56% in the P15 treatment. At d 14 of incubation, *Staphylococcus* ssp. obtained a 23% reduction from treatment B, while this rate of treatment P15 was 50% lower than treatment A. This result confirmed that obtained by Alencar et al. (2007) and Rahman et al. (2010), who stated that propolis has antimicrobial activity against *Staphylococcus aureus*.

Although *E. coli*, *S. aureus* (inoculated eggs), and *S. linens* or *S. equorum* (major flora on clean eggs) require a comparable amount of energy to be deactivated by UV (6,600 μJ for *E. coli* and 5,720–6,600 μJ for *Staphylococcus* sp., respectively; Srikanth, 1995), our study showed that the propolis addition was clearly more effective on coliforms and *Staphylococcus* ssp.

Mold-yeast counts of A- and B-treated groups at the beginning of incubation were 5.67 and 4.10 log cfu/egg, respectively. This count decreased with increasing propolis concentrations and it reached 2.43 log cfu/egg in P15. The inhibition rate increased to 55% at the end of incubation. The P15 treatment group was 1.07 log units lower than the P5 treatment for mold-yeast at 14 d of incubation. The difference between P 10 and P 15 treatments was not significant at the end of incubation. This result agrees with the findings of Longhini et al. (2007), who showed that propolis has antifungal activity.

Egg Weight Loss

The results of egg weight loss during the incubation of 0 to 14 d of embryonic development are given in Table 4. The rates of egg weight loss significantly varied between 9.21 and 10.58% among all groups. The egg weight losses of all propolis treatment groups were lower compared with those of the A and B groups. This might be explained by minimization of water loss through occluded egg pores after propolis treatment. There were no significant ($P > 0.05$) differences in egg weight loss among propolis groups. Egg weight loss is an important parameter for incubation. Geng and Wang (1990) reported that too-fast moisture loss was disadvantageous for the normal embryonic development.

Fertility, Hatchability, and Embryonic Mortality

The sanitizer's effect on hatchability and embryonic mortality are shown in Table 5. Treatments had no effect on fertility, hatchability of set (B, 92.59%; A, 97.04%; P5, 97.78%; P10, 97.04%; P15, 93.33%), or hatchability of fertile eggs (B, 94.02%; A, 97.75%; P5, 98.52%; P10, 98.49%; P15, 94.73%). Propolis did not negatively affect the hatchability of eggs. These results agree with Yıldırım and Ozcan (2001) and Copur et al. (2010), who found no significant differences using oregano oil and formaldehyde as disinfectants for hatchability. Similarly, no significant differences were observed between electrolyzed oxidizing water and the control group for hatchability (Fasenko et al., 2009). On the other hand, Yıldırım et al. (2003) stated that there were significant differences between oregano oil and formaldehyde treatments on hatchability. There

Table 5. Effects of hatching egg sanitizers on fertility, hatchability, and embryonic mortality (%)

					Embryonic mortality (% of fertile eggs)		
Group[1]	n	Fertility	Hatchability of set eggs	Hatchability of fertile eggs	1 to 9 d	10 to 17 d	17 to 18 d
A	135	99.26	97.04	97.75	1.51	0.20	0.00
B	135	98.52	92.59	94.02	2.99	0.40	1.48
P5	135	99.26	97.78	98.52	0.00	0.00	1.48
P10	135	98.52	97.04	98.49	0.74	0.20	0.00
P15	135	98.52	93.33	94.73	4.53	0.20	0.00
P-value		0.649	0.792	0.649	0.549	0.525	0.144

[1]A: 70% ethyl alcohol, B: benzalkonium chloride, P5: 5% propolis, P10: 10% propolis, P15: 15% propolis.

Table 6. Effects of hatching egg sanitizers on BW at d 10, relative growth, and BW gain

Group[1]	n	BW d 1 (g)	BW d 10 (g)	Relative growth	BW gain (g)
A	51	7.98	40.21	404.51	32.23
B	51	7.89	41.14	422.05	33.25
P5	52	7.99	40.88	412.16	32.89
P10	52	7.90	40.99	419.46	33.09
P15	52	7.85	41.17	425.49	33.32
P-value		0.381	0.808	0.313	0.721
Pooled SD		0.418	4.428	55.07	4.328

[1]A: 70% ethyl alcohol, B: benzalkonium chloride, P5: 5% propolis, P10: 10% propolis, P15: 15% propolis.

was also a significant decrease in hatchability when formaldehyde fumigation was used at longer durations and higher concentrations (Williams and Gordon, 1970; Cadırcı, 1997). We did not find a significant increase in embryonic mortality at any stage of incubation among all treatment groups. These findings do agree with Copur et al. (2010) and Elibol et al. (2003), who stated that disinfectants had no increasing effect on early embryonic mortality. Conversely, Yıldırım et al. (2003) reported a significantly higher mortality rate after formaldehyde treatment compared with oregano oil treatment. The use of P15 may be toxic for the embryo, especially at 1 to 9 d of incubation.

Spread of Hatch

Hatching began at 368, 377, 377, 377, and 385 h of incubation duration in B, A, P10, P15, and P5 groups, respectively (Figure 1). Hatching ended in all groups at 416 h of incubation. The lowest hatching was observed in group B (56%) at 385 to 392 h of incubation. No significant differences were found among P15 (92%), A (89%), and P5 (83%) groups, but P15 had a significantly higher hatching percentage than that of P10 (82%) at 385 to 392 h of incubation. There were no significant differences among groups at other hatching times. A narrow hatch window (spread between early and late hatched chicks) is important for commercial breeders, because there will be homogeneity for chicks. A spread in the hatching period will increase the number of chicks that will be delayed access to water and food. Extended hatching causes chicks to dehydrate, reduces posthatch performance, and depresses immune response (Becker, 1960; Pinchasov and Noy, 1993; Casteel et al., 1994; Bigot et al., 2003; Gonzales et al., 2003).

Chick Performance

The effect of hatching egg sanitizer on BW on d 10, relative growth, and BW gain are shown in Table 6. There were no significant differences between treatments in terms of BW, relative growth, and BW gain. Propolis did not have a negative effect on performance traits. The results of this study agree with the findings of Copur et al. (2010), who reported no significant differences with respect to BW and BW gain following oregano oil or formaldehyde treatment. Likewise, Fasenko et al. (2009) showed that application of electrolyzed oxidizing water did not affect BW in broilers.

Mortality

No significant differences were observed among treatments for growth performance during d 10 (data not

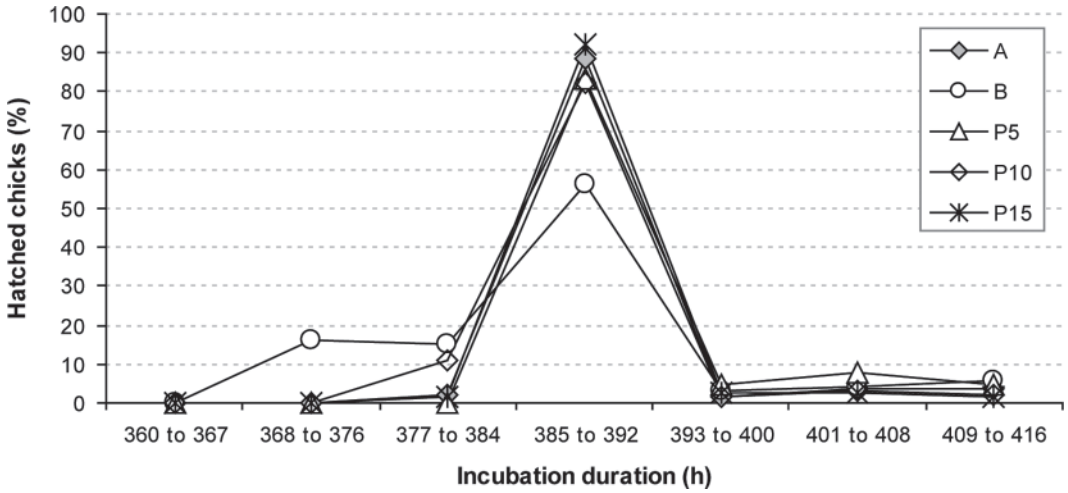

Figure 1. Effects of hatching egg sanitizers on spread of hatch. A: 70% ethyl alcohol, B: benzalkonium chloride, P5: 5% propolis, P10: 10% propolis, and P15: 15% propolis.

shown). Mortality in the A and B groups was 1.92%, whereas there was no death in all P groups.

Conclusion

The results of this study demonstrated that propolis could be used effectively to make a reduction in microbial activity on the surface of quail hatching eggs. Propolis did not negatively affect hatchability, embryonic mortality, or relative growth. Based on the results of this study, propolis may provide an effective, safe, and nontoxic natural hatching egg sanitization.

ACKNOWLEDGMENTS

The authors are grateful to O. Olgun (Department of Animal Science, Faculty of Agriculture, Selkuk University, Konya, Turkey) for his assistance during the experiment.

REFERENCES

Adler, H. E., A. J. DaMassa, and W. F. Scott. 1979. Studies on egg disinfection. Poult. Sci. 58:799–806.

Alencar, S. M., T. L. Oldoni, M. L. Castro, I. S. Cabral, C. M. Costa-Neto, J. A. Cury, P. L. Rosalen, and M. Ikegaki. 2007. Chemical composition and biological activity of a new type of Brazilian propolis: Red propolis. J. Ethnopharmacol. 113:278–283.

APHA (American Public Health Association). 1993. Standard methods for the examination of dairy products. R. T. Marshall, ed. 16th ed. Washington, DC.

Arhienbuwa, F. E., E. Adler, and A. D. Wiggins. 1980. A method of surveillance for bacteria on the shell of turkey eggs. Poult. Sci. 59:28–33.

Bankova, V. S., S. L. Castro, and M. C. Marcucci. 2000. Propolis: Recent advances in chemistry and plant origin. Apidologie 31:3–15.

Becker, W. A. 1960. The storage of hatching eggs and the posthatching body weight of chickens. Poult. Sci. 39:588–590.

Bigot, K., S. Mignon-Grasteau, M. Picard, and S. Tesseraud. 2003. Effects of delayed feed intake on body, intestine, and muscle development in neonate broilers. Poult. Sci. 82:781–788.

Board, R. G. 1966. The course of microbial infection of the hen's egg. J. Appl. Bacteriol. 29:319–341.

Bruce, J., and E. M. Drysdale. 1994. Trans-shell transmission. Pages 63–91 in Microbiology of the Avian Egg. 1st ed. R. G. Board and R. Fuller, ed. Chapman and Hall, London, Glasgow, UK.

Cadırcı, S. 1997. The effect of fumigation regimens on shell structure and embryo viability. MSc Thesis. Glasgow Univ., UK.

Casteel, E. T., J. L. Wilson, R. J. Buhr, and J. E. Sander. 1994. The influence of extended posthatch holding time and placement density on broiler performance. Poult. Sci. 73:1679–1684.

Copur, G., M. Arslan, M. Duru, M. Baylan, S. Canogullari, and E. Aksan. 2010. Use of oregano (*Origanum onites* L.) essential oil as hatching egg disinfectant. Afr. J. Biotechnol. 8:2531–2538.

Copur, G., O. Camci, N. Sahinler, and A. Gul. 2008. The effect of propolis eggshell coatings on interior egg quality. Arch. Geflugelkd. 72:35–40.

Coufal, C. D., C. Chavez, K. D. Knape, and J. B. Carey. 2003. Evaluation of a method of ultraviolet light sanitation of broiler hatching eggs. Poult. Sci. 82:754–759.

Cox, N. A., J. S. Bailey, M. E. Berrang, R. J. Buhr, and J. M. Mauldin. 1994. Automated spray sanitizing of broiler hatching eggs. 3. Total bacteria and coliform recovery after using an egg spraying machine. J. Appl. Poult. Res. 3:234–237.

Elibol, O., A. Uysal, and S. Ertas. 2003. The effect of preincubation fumigation with different formaldehyde fumigation concentrations and periods on hatchability of chicken eggs. J. Agric. Sci. 9:9–12. (In Turkish, with English abstract)

Fasenko, G. M., E. E. O'Dea Christopher, and L. M. McMullen. 2009. Spraying hatching eggs with electrolyzed oxidizing water reduces eggshell microbial load without compromising broiler production parameters. Poult. Sci. 88:1121–1127.

Favier, G. L., M. E. Escudero, and A. M. S. de Guzma'n. 2001. Effect of chlorine, sodium chloride, trisodium phosphate, and ultraviolet radiation on the reduction of *Yersinia enterocolytica* and mesophilic aerobic bacteria from eggshell surface. J. Food Prot. 64:1621–1623.

Geng, Z. Y., and X. L. Wang. 1990. Relationship of hatchability and the percentage of egg weight loss and shell pore concentration during incubation. Chin. J. Anim. Sci. 26:12–14. (In Chinese, with English abstract)

Gentry, R. F., and C. L. Quarles. 1972. The measurement of bacterial contamination on eggshells. Poult. Sci. 51:930–933.

Ghisalberti, E. L. 1979. Propolis: A review. Bee World 60:59–84.

Gonzales, E., N. Kondo, E. S. Saldanha, M. M. Loddy, C. Careghi, and E. Decuypere. 2003. Performance and physiological parameters of broiler chickens subjected to fasting on the neonatal period. Poult. Sci. 82:1250–1256.

Gordon, R. F., and J. F. Tucker. 1965. The epizootology of *Salmonella menston* infection of fowls and the effect of feeding poultry food artificially infected with *Salmonella*. Br. Poult. Sci. 6:251–264.

Gradel, K. O., L. Randall, A. R. Sayers, and R. H. Davies. 2005. Possible associations between *Salmonella* persistence in poultry houses and resistance to commonly used disinfectants and a putative role of mar. Vet. Microbiol. 107:127–138.

Greenaway, W., T. Scaysbrook, and F. R. Whatley. 1990. The composition and plant origins of propolis. A report of work at Oxford. Bee World 71:107–118.

Harry, E. G. 1963. The relationship between egg spoilage and the environment of the egg when laid. Br. Poult. Sci. 4:63–70.

IAEA (International Atomic Energy Agency). 1970. Microbiological specifications and testing methods for irradiated food. Technical reports series No: 104, Vienna, Austria.

ISO (International Organization for Standarization). 1993. Microbiological-general guidance on methods for the detection of *Salmonella*. ISO 6579, 3rd ed. ISO, Geneva, Switzerland.

Jones, D. R., K. E. Anderson, P. A. Curtis, and F. T. Jones. 2002. Microbial contamination in inoculated shell eggs: I. Effects of layer strain and hen age. Poult. Sci. 81:715–720.

Jones, D. R., K. E. Anderson, and M. T. Musgrove. 2011. Comparison of environmental and egg microbiology associated with conventional and free-range laying hen management. Poult. Sci. 90:2063–2068.

Jones, D. R., P. A. Curtis, K. E. Anderson, and F. T. Jones. 2004. Microbial contamination in inoculated shell eggs: II. Effects of layer strain and egg storage. Poult. Sci. 83:95–100.

Klimek, J. W., and J. H. Bailey. 1956. Factors influencing the rate of killing of *Escherichia coli* exposed to benzalkonium chloride. Appl. Microbiol. 4:53–59.

Krell, R. 1996. Value-added products from beekeeping. FAO Agricultural Services Bulletin No: 124. Food and Agriculture Organization of the United Nations, Rome, Italy.

Kuhl, H. Y. 1989. Washing and sanitizing hatching eggs. Int. Hatchery Pract. 3:29–33.

Kuo, F. L., S. C. Ricke, and J. B. Carey. 1997. UV irradiation of shell eggs: Effect on populations of aerobes, molds, and inoculated *Salmonella* Typhimurium. J. Food Prot. 60:639–643.

Longhini, R., S. M. Raksa, A. C. P. Oliveira, I. Terezinha, E. Svidzinski, and S. L. Franco. 2007. Obtenção de extratos de própolis sob diferentes condições e avaliação de sua atividade antifúngica. Braz. J. Pharmacognosy. 17:388–395. (In Portuguese, with English abstract)

Mayes, F. J., and M. A. Takeballi. 1983. Microbial contamination of the hen's egg: A review. J. Food Prot. 46:1092–1098.

North, M. O., and D. D. Bell. 1990. Commercial Chicken Production Manual. 4th ed. Chapman and Hall, New York, NY.

Payne, R. W., D. A. Murray, S. A. Harding, D. B. Baird, and D. M. Soutar. 2003. GenStat for Windows Introduction. 7th ed. VSN International, Hemel Hempstead, UK.

Pinchasov, Y., and Y. Noy. 1993. Comparison of posthatch holding time and subsequent early performance of broiler chicks and turkey poults. Br. Poult. Sci. 34:111–120.

Pitt, J. I., and A. D. Hocking. 1985. Fungi and Food Spoilage. Academic Press, New York, NY.

Powers, J. J. 1964. Action of anthocyanias and related compounds on bacterial cells. Pages 59–75 in Proc. Fourth Int. Sym. Food Microbiology. N. Molin, ed. Goteborg, Sweden.

Proudfoot, F. G., D. M. Nash, and H. W. Hulan. 1985. Effects of glutaraldehyde surfactant solution on the hatchability of the hen's egg. Poult. Sci. 64:2400–2402.

Rahman, M. M., A. Richardson, and M. Sofian-Azirun. 2010. Antibacterial activity of propolis and honey against *Staphylococcus aureus* and *Escherichia coli*. Afr. J. Microbiol. Res. 4:1872–1878.

Reid, W. M., T. A. Maag, F. M. Boyd, A. L. Kleckner, and S. C. Schmittle. 1961. Embryo and baby chick mortality and morbidity induced by a strain of *Escherichia coli*. Poult. Sci. 40:1497–1502.

Russell, A. D., W. B. Hugo, and G. A. J. Ayliffe. 1992. Principles and Practice of Disinfection, Preservation, and Sterilization. 2nd ed. Blackwell Publishing, Oxford, UK.

Sacco, R. E., P. A. Renner, K. E. Nestor, Y. M. Saif, and R. N. Dearth. 1989. Effect of hatching egg sanitizers on embryonic survival and hatchability of turkey eggs from different lines on eggshell bacterial populations. Poult. Sci. 68:1179–1184.

Schmidt, J. O. 1997. Bee products: Chemical composition and application. Pages 15–26 in Bee Products: Properties, Applications, and Apitherapy. A. Mizrahi and Y. Lensky, ed. Plenum Press, New York, NY.

Scott, T. A., and C. Swetnam. 1993. Screening sanitizing agents and methods of application for hatching eggs I. Environmental and user friendliness. J. Appl. Poult. Res. 2:1–6.

Singh, R., K. M. Cheng, and F. G. Silversides. 2009. Production performance and egg quality of four strains of laying hens kept in conventional cages and floor pens. Poult. Sci. 88:256–264.

Srikanth, B. 1995. The basic benefits of ultraviolet technology. Water Cond. Purif. December, 26–27. http://aquvair.com/Basic%20Benefits%20of%20UV%20-WCP%20Reprint.pdf.

Tebbs, S. E., and T. S. J. Elliott. 1993. A novel antimicrobial central venous catheter impregnated with benzalkonium chloride. J. Antimicrob. Chemother. 31:261–271.

Thomas, L., A. D. Russell, and J. Y. Maillard. 2005. Antimicrobial activity of chlorhexidine diacetate and benzalkonium chloride against *Pseudomonas aeruginosa* and its response to biocide residues. J. Appl. Microbiol. 98:533–543.

Whistler, P. E., and B. W. Sheldon. 1989. Biocidal activity of ozone versus formaldehyde against poultry pathogens inoculated in a prototype setter. Poult. Sci. 68:1068–1073.

WHO (World Health Organization). 1993. Guidelines for Drinking-Water Quality. Vol. 1. Recommendations. 2nd ed. WHO, Geneva, Switzerland.

Williams, J. E., L. H. Dillard, and G. O. Hall. 1968. The penetration of patterns of *Salmonella* Typhimurium through the outer structures of chicken eggs. Avian Dis. 12:445–466.

Williams, J. E., and C. D. Gordon. 1970. The hatchability of chicken eggs fumigated with increasing level of formaldehyde gas before incubation. Poult. Sci. 49:560–564.

Yıldırım, I., and M. Ozcan. 2001. Use of oregano and cumin essential oils as disinfectant on hatching Quail eggs. J. Anim. Res. Inst. 11:61–63.

Yıldırım, I., M. Ozcan, and R. Yetisir. 2003. The use of oregano (*Origanum vulgare* L.) essential oil as alternative hatching egg disinfectant versus formaldehyde fumigation in quails (*Coturnix coturnix japonica*) eggs. Rev. Med. Vet. (Toulouse) 154:367–370.