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The Effects of Using Garlic Extract for Quail Hatching Egg Disinfection on Hatching Results and Performance

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ABSTRACT

This experiment was carried out to determine the effects of using garlic extract (*Allium sativum*) as an alternative to formaldehyde for the disinfection of hatching eggs on hatching traits and growth performance of quails. Four treatments, with 240 hatching eggs each, were evaluated: egg immersion in two different levels (2.5% and 5.0%) of garlic extract (garlic-1, garlic-2), formaldehyde fumigation (positive control), and eggs not submitted to disinfection (negative control). Mid-term and late embryomortality, pipped egg, and culled chick rates were not influenced by the ($p>0.05$). The highest ($p<0.05$) early embryo mortality rate was detected in the formaldehyde treatment (9.99%), and the lowest in garlic-2 treatment (2.68%). Hatchability of fertile eggs was the lowest in the formaldehyde group (83.78%), followed by the control (85.20%), garlic-1 (87.11%) and garlic-2 (88.72%) groups, respectively. The highest final body weight (5 weeks of age) was obtained in the garlic-2 group (304.1 g), followed by the control (294.13 g), formaldehyde (290.56 g), and garlic-1 (288.44 g), respectively, and the differences were statistically significant ($p<0.05$). Feed intake and feed conversion ratio were not influenced by the treatments ($p>0.05$). The results obtained for hatching traits and live performance parameters indicated that the immersion of eggs in garlic extract may be used as an alternative to formaldehyde fumigation for the disinfection of hatching quail eggs.

INTRODUCTION

In hatching eggs, microbial infections reduce hatchability and chick quality. Infection spreads in the hatchery and causes deaths in newly-hatched chicks due to omphalitis and yolk-sac infections (Pienaar *et al.,* 1995). Egg infection depends on pre- and post-laying conditions. Eggs are usually infected when microorganisms enter through the digestive tract of the hen, and are transferred to the blood, contaminating the oviduct, and consequently infecting the egg yolk (Gordon & Tucker, 1965). Several various microorganisms (*Streptococcus* spp. and coli-aerogenes bacteria) may infect the oviduct during artificial insemination (Harry, 1963), due to the contact of the cloaca with the nesting box or litter material during oviposition (Harry, 1963; Board, 1964; Williams *et al.,* 1968) or, most frequently, the microorganisms enter the egg through the eggshell pores (Cadirci, 1997).

In order to achieve high hatchability and to produce good-quality chicks, an effective hatching sanitation program is essential. The use of pre-hatching fumigation in breeding facilities and hatcheries aims at preventing the growth and reducing the numbers of microorganisms such as Salmonella, *Escherichia coli*, enterobacteria, molds, and yeasts on the eggshell surface (De Reu *et al.,* 2006).

Formaldehyde is typically used as a disinfectant of hatching eggs, but other chemicals, as hydrogen peroxide (Cox *et al.,* 2000), Virkon S (Gholami-Ahangaran *et al.,* 2016), propionic acid (Ibrahim *et al.,* 2014), and quaternary ammonium (Arhienowa *et al.,* 1980; Sacco *et al.,*1989) have been tested. Studies also report other methods for hatching egg disinfection, including ultraviolet light (Coufal *et al.,* 2003; Al-Shammari *et al.,* 2015), electrolyzed oxidizing water (Russell, 2003; Bialka *et al.,* 2004; Keita *et al.,* 2016), application of low and high frequency ultrasound (Yildirim*et al.,* 2015; Aygun & Sert, 2012; Al-Shammari *et al,* 2015), ozone (Hrcncar *et al.,* 2012), and glutaraldehyde surfactant solution (Proudfoot *et al.,* 1985). Natural products have been tested as alternatives to the use of formaldehyde, such as etheric thyme oil (*Origanum vulgare*), which has antimicrobial properties (Yildirim *et al.,* 2003; Baydar *et al.,* 2004;Copur *et al.,* 2010; Debes & Basyony, 2011), propolis (Aygun *et al.,* 2013) and allicin (Copur *et al.,* 2011).

Formaldehyde fumigation (FF) is an effective procedure for reducing the microbial contamination on the eggshell surface before hatching (Cadirci, 1997). However, it causes eyes and nose irritation, and has a pungent smell (Whistler & Sheldon, 1989). Due to the possible carcinogenic effects of formaldehyde fumigation, health protection agencies have recently carried out studies and the use of FF is being reconsidered according under Toxic Materials Control Act (C&EN, 1984).

Due to the growing demands for healthy and safe foods for human consumption in recent years, there has been a shift in the animal industry from synthetic compounds to natural and safer medicinal and aromatic plants. It was determined that active ingredients found in these plants have antibacterial, antifungal and antiviral effects. As a result, the number of studies on the use of natural products instead of synthetic materials for the disinfection of hatching eggs has increased (Copur *et al.,* 2010; Copur *et al.,* 2011; Aygun *et al.,* 2012; Aygun & Sert, 2012)

Studies performed to determine the antibiotic effects of garlic have focused on the use of unprocessed garlic, garlic extract, or on its active ingredient, allicin, as tablets. Garlic contains two powerful antibiotics: allicin and garlicin (Makaklı, 1980), which have antibacterial actions against Gram-positive and Gram-negative bacteria (Farbman *et al.,* 1993). The antibacterial properties of garlic against *Helicobacter pylori* have been established *invitro* (Salih & Abasıyanık, 2003). In the study of Astal & Younis (2003), garlic (*Allium sativum*) extract at a concentration of 750-1000 µg/mL showed very high antibacterial effect against Gram-positive bacteria, such as *Staphylococcus aureus*, *Staphylococcus saprophyticus*, *Streptococcus pneumonia* and *Streptococcus faecalis*, as well as against Gram-negative bacteria, including *Escherichia coli*, *Enterobacter cloacae*, *Klebsiella pneumonia*, *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Acinetobacter haemolyticus*.

In studies on garlic powder supplementation in poultry feeds, the effects of garlic on cholesterol levels in the egg yolk (Lim *et al*, 2006), plasma, and meat (breast and leg) and the level of microbial fermentation in the gastrointestinal tract (Choi *et al*, 2010) were studied. In broilers, the effects of garlic on the levels of lead in chicken tissues (Hanafy *et al.,* 1994), live weight (Al Homidan, 2005), performance and carcass traits (Raeesi *et al.,* 2010) were evaluated. In addition, in broilers, the effects of garlic on enteropathogen counts, ileal histological structure, and productive parameters (Peinado *et al*., 2012), antioxidant characteristics (Jakubcova *et al,* 2014) and ascites incidence (Varmaghany *et al.,* 2015) were studied. In turkeys, the effects on the parasitic disease histomoniasis (infectious enterohepatitis caused by *Histomonas meleagridis*) were evaluated by Hafez & Hauck (2006). In layers and quails, the effects of dietary garlic addition on egg weight, feed intake, feed conversion ratio, body weight gain, serum and egg yolk cholesterol levels and egg productivity (Chowdhury *et al.,* 2002 in layers; Yalcin *et al*., 2006 in layers; Yalcin *et al.,* 2007 in quails; Ao *et al.,* 2010 in layers; Canogullari *et al,* 2010 in quails), as well as on fecal bacterial load and on egg quality (Olobatoke & Mulugeta, 2011) were studied.

The objective of this study was to determine if garlic, due to its antibacterial, antifungal, antiparasitic, and antiviral properties, can be used as an alternative to formaldehyde fumigation for the disinfection of hatching eggs.

MATERIALS AND METHODS

The hatching eggs used in the present study derived from the breeder quail flock of the Mustafa Kemal University, Samandag Vocational College Quail Unit.

In this study, 960 hatching eggs were randomly divided in four treatments groups (240 each): control group (negative control), which eggs were not submitted to any disinfection procedure; formaldehyde fumigation group (positive control), which eggs were disinfected by formaldehyde fumigation, and two garlic-extract groups, garlic-1 and garlic-2, which eggs were disinfected with 2.5% and 5.0% garlic, respectively. Each treatment group was consisted of four replicates with 60 eggs each. Trial coincidence parcels were executed as per the trial pattern (60x4x4=960 eggs, 60 eggs per tray, 4 replicates per treatment, 4treatments).

Storage of hatching eggs

Hatching eggs were collected daily from the quail farm, and those that were not cracked, broken, or contaminated with fecal matter or litter were brought on the same day to the trial location, where they were stored for 7 days at 75% relative humidity and 16-18°C temperature.

Treatment of hatching eggs with garlic extract and formaldehyde

After 7 days of storage, eggs were immersed in garlic extract. The treatment of eggs with the garlic extract was performed using the dipping method. Hatching eggs were immersed in the extract for one minute, then left to dry for one minute at room temperature (25°C). The amount of active ingredient per egg and/or per 100 eggs was calculated to determine the standard solutions of 2.5 and 5% of garlic extract. Eggs were than immersed in 2 L of the mixture containing different doses of garlic extract (2.5% and 5.0%)

Eggs of the formaldehyde fumigation group (positive control) were fumigated for 20 minutes with the standard dose (79.86 mL formalin and 39.93 mg potassium per manganate/2m^3) or 2X the standard dose (USDA, 1985).

Hatching process

After disinfection, egg groups were transported to the hatchery unit. Each replicate of 60 eggs (four per replicate) were placed in egg trays. Each tray was placed into the two separate setters (garlic groups in one setter and formaldehyde and negative-control groups in another setter). Setter temperature and relative humidity were kept at 37.5°C and 55-60%, respectively, and 37.0°C and 70-80% in the hatcher.

Post-hatching processes

After incubation eggs were weighed to determine egg weight loss. Non-hatched eggs were counted to determine fertility rate (%)and hatchability rates (%) and broken to classify embryo mortality as early (0-6 days; black-eye and beak visible), mid-term (6 to 17 days; embryo with feathers, and with yolk out), or late (17 to 18 days; fully grown, with internalized yolk, and pipped eggs) mortality. The following formulas were used:

- Fertility rate: (number of fertilized eggs/number of eggs set) x100
- Egg weight loss: [(initial egg weight – egg weight measured on transfer day)/initial egg weight] x100
- Hatchability of set eggs: (number of hatched chicks /total number of eggs set) x100
- Hatchability of fertile eggs (%): (number of hatched chicks /number of fertilized eggs set) x100
- Early embryo mortality: (number of dead embryos on days 0-6 of incubation/number of fertilized eggs)x100
- Mid-termembryo mortality: (number of dead embryos on days 7-18 of incubation/number of fertilized eggs)x100
- Late embryo mortality: (number of dead embryos on 19-21 days of incubation/number of fertilized eggs)x100
- Culled chick rate: (number of culled chicks/number of saleable chicks)x100
- Contamination rate: (number of contaminated eggs/total number of eggs set) x100

Growth Performance

After the incubation period was completed, the chicks from each treatment group were weighed (between 144-161 chicks per treatment group), and chicks in each treatment group were divided in three replicates and housed in cages equipped with auto-heated brooder for the first two weeks, and then transferred to 5-tiered 20x50x100cm cages (three replicates of 50 chicks each per treatment), where they were kept until the end of the 5thweek of age. A lighting program of 24-h of natural+artificial light was applied. Quails were fed a commercial broiler chick feed, with 22% crude protein and 3000kcal/kg ME. Feed and water were provided *ad libitum*.

During the experiment, feed intake was measured once weekly per replicate, and body weight was measured once weekly per individual bird. Weekly feed intake and body weight gain were used to calculate feed conversion ratio. Weight measurements were taken using a scale sensitive to 0.1g.

Statistical Analysis

Statistical analysis of the experimental data was performed using the software package SPSS (oneway analysis of variance) (SPSS, release 18). Duncan multiple comparison test was applied to compare differences among treatments.

RESULTS AND DISCUSSION

The results of the effects of garlic extract use for hatching egg disinfection on early, mid-term and late embryo mortality, culled chick rate, contamination rate, and hatching parameters are given in Table 1.

The highest early embryo mortality rate was observed in the formaldehyde group (9.99%), and the lowest in the garlic-2 group (2.68%) ($p<0.05$). Mid-term and late embryo mortality rates were not statistically different ($p>0.05$) among treatments. Mid-term embryo mortality rates in the control, formaldehyde, garlic 1 and garlic 2 groups were 0.52%, 0.40%, 1.44% and 1.59%, respectively and late embryo mortality were 3.37%, 4.88%, 3.70% and 4.38%, respectively. The higher early embryo mortality in the eggs disinfected by formaldehyde fumigation compared with other groups may be attributed to the possible damage of the egg cuticle layer by formaldehyde. Formaldehyde is an effective antimicrobial compound that reduces the microbial load on the eggshell surface. However, it also has a toxic effect (Cadirci, 1997). The lower early embryo mortality rate in the garlic-1 and garlic-2 groups compared with the control group, which was not submitted to any disinfection process, may be due to the reduction of the microbial load on the eggshell surface by garlic. In fact, Ankri & Miralman (1999) reported that the active ingredient of garlic, allicin, is effective against many Gram-positive and Gram-negative bacteria, including *Escherichia coli*, and also has antifungal and antiviral activities. Similarly, Copur *et al*. (2011) reported that the use of allicin for the disinfection of hatching broiler eggs reduced the microbial load on the eggshell surface, consequently reducing early and late embryo mortality and improving hatchability.

The lowest culled chick rate was found in the formaldehyde group, with 0.52%, followed by garlic-2 (0.55%), garlic-1 (0.94%), and control (1.95%) groups; however, these differences were not statistically significant ($p>0.05$).

Among hatching parameters, hatchability of the control, formaldehyde, garlic-1 and garlic-2 groups were 79.46%, 75.11%, 76.44%, and 76.32%, respectively, and not statistically different ($p>0.05$). The better hatching performance observed in the control group compared with the other groups may be related to its higher fertility rate ($p<0.05$). The fertility rates of the control, formaldehyde, garlic 1 and garlic 2 treatments were 93.24 %, 89.62 %, 87.78 % and 86.08 % respectively.

The hatchability of fertile eggs was not statistically different ($p>0.05$) among treatments, with the lowest values determined in the formaldehyde group (83.78%), followed by the control (85.20%), garlic-1 (87.11%), and garlic-2 (88.72%). The numerically lower hatchability of fertile eggs obtained in the formaldehyde group may be explained with the higher early embryo mortality ($p<0.05$) observed in this group. This result supports the findings of Elibol *et al.* (2003), who observed that the use of a high concentration (4X; 1 m^3- 56mL formalin+28g $KMnO_4$) for the disinfection of chicken eggs reduced the hatchability of fertile eggs, which suggests that using formaldehyde for the disinfection of hatching eggs may have toxic effects on the embryos during incubation, increasing early stage mortality rates. However, our results are different from those obtained by Copur *et al.* (2010), who found that disinfecting chicken eggs with origanum etheric oil caused higher early and late embryo mortality compared with formaldehyde fumigation.

Table 1 – Hatching parameters according to treatmentgroups.

Parameters		Groups				SEM
		Control	Formaldehyde	Garlic 1	Garlic 2	
Fertility rate (%)		93.24[a]	89.62[ab]	87.78[ab]	86.08[b]	1.13
Hatchability of set eggs(%)		79.46	75.11	76.44	76.32	1.15
Hatchability of fertile eggs (%)		85.20	83.78	87.11	88.72	1.03
Embryonic mortality (%)	Early	7.52[ab]	9.99[a]	5.83[bc]	2.68[c]	0.83
	Mid-term	0.52	0.40	1.44	1.59	0.30
	Late	3.37	4.88	3.70	4.38	0.65
Pipped-eggs rate (%)		0.52	0.40	0.43	2.05	1.31
Contamination rate (%)		0.90	------	0.51	-------	1.91
Culling rate (%)		1.95	0.52	0.94	0.55	0.41

[ab]: Means with the different superscript within row are significantly different ($p<0.05$)

The average weekly body weight values of the birds derived from the eggs submitted to the different treatments during 5-week feeding period are given in Table 2. From week 3 to the end of the experiment (week 5), males and females were separately weighed. Weekly body weights were different ($p<0.05$) among treatment groups in all weeks, except for hatching weight. Despite not statistically analyzed, females were heavier than males from week 3, independently of treatment. In week 5, average body weight of the birds in the control, formaldehyde, garlic-1 and garlic-2 groups were 294.13, 290.56, 288.44, and 304.61 g respectively ($p<0.05$).

Wafar *et al.* (2017), studying the effects of octacosanol extracted from rice bran on quail performance, determined 155.22-186.76 g body weight on day 42, while Yasar & Gok (2015) obtained154.1-182.6 g on day 35. Guluwa *et al.* (2014) reported body weights of 103.81-149.24 gin 35-d-old quails. In the study of Kahksar *et al.* (2012) on the effects of essential thyme oil dietary supplementation on the performance and blood parameters of Japanese quails, obtained 159g body weight in the control group and 169 g the bird fed thyme essential oil. Alasahan *et al.* (2016) found an average body weight in 5-week-old quails of 215.77 g, whereas Bonos *et al.* (2010) reported 172-180.8 g. The body weight values reported in those studies for 5- and 6-week-old quails are lower than those obtained in the present study when quails were still 4 weeks old. In addition, 5-week body weight values were found to be considerably higher than those obtained in the aforementioned studies. This is may be attributed to the fact that the hatching eggs used in the present study derived from quail breeders selected for high body weight.

Table 2 – Weekly body weight (BW) results according to experimental group.

Week	Group	Sex	N	BW (g)	Male-female BW average
Beginning	Control	M,F	161	8.95±0.07	8.95±0.07
	Formaldehyde	M,F	144	8.87±0.07	8.87±0.07
	Garlic 1	M,F	146	8.69±0.07	8.69±0.07
	Garlic 2	M,F	150	8.79±0.03	8.79±0.03
1	Control	M,F	146	38.29±0.45	38.29±0.45^{b}
	Formaldehyde	M,F	133	40.42±0.44	40.42±0.44^{a}
	Garlic 1	M,F	134	39.65±0.42	39.65±0.42^{b}
	Garlic 2	M,F	124	38.48±0.44	38.48±0.44^{bc}
2	Control	M,F	145	92.92±0.88	92.92±0.88^{a}
	Formaldehyde	M,F	132	97.53±0.93	97.53±0.93^{b}
	Garlic 1	M,F	129	94.44±0.90	94.44±0.90^{a}
	Garlic 2	M,F	118	98.25±0.88	98.25±0.88^{a}
3	Control	M	72	161.56±2.38	
		F	73	164.98±2.00	163.45±1.53^{c}
	Formaldehyde	M	61	169.88±2.07	
		F	67	172.14±2.56	170.89±1.61^{a}
	Garlic 1	M	70	162.84±2.38	
		F	57	173.96±1.94	168.60±1.61^{ab}
	Garlic 2	M	58	162.39±2.79	
		F	59	167.70±2.95	164.89±2.03^{bc}
4	Control	M	71	229.77±2.51	
		F	73	237.15±2.24	233.77±1.70^{b}
	Formaldehyde	M	61	234.78±2.77	
		F	66	241.23±2.93	237.65±2.03^{ab}
	Garlic 1	M	70	227.09±2.87	
		F	57	242.37±2.64	235.08±2.05^{b}
	Garlic 2	M	57	236.15±2.23	
		F	59	250.43±2.69	242.79±1.86^{a}
5	Control	M	71	275.72±3.12	
		F	73	309.16±2.93	294.13±2.59^{b}
	Formaldehyde	M	61	276.33±3.13	
		F	66	308.93±3.73	290.56±2.84^{b}
	Garlic 1	M	68	267.73±3.47	
		F	57	307.67±3.92	288.44±3.25^{b}
	Garlic 2	M	58	283.59±3.16	
		F	58	329.28±4.43	304.61±3.50^{a}

^{ab}: Means in the same column with the different superscriptsin the same week are significantly different ($p<0.05$). M: male, F: Female

Independently of experimental group, body weight gain increased up to weeks 3 and 4 and started decreasing after week 4 (Table 3). Şeker *et al.* (2007) report that weight gain of quails increases until 5 weeks of age, and quickly decreases afterwards. Baylan *et al*. (2009) reported that the highest body weight gain in quails occurs between 3 and 5 weeks of age. In the present study, body weight gain values of the control, formaldehyde, garlic-1, and garlic-2 groups were 285.18, 281.69, 279.75, and 295.82 g, respectively. Bulus *et al.* (2013) fed Japanese quails with a commercial poultry feed with two different protein levels during the starter and finisher phases and reported an average body weight gain of 134.24 g at 5 weeks of age. Devarasetti *et al.* (2016), evaluating the effects of the dietary supplementation of baker's yeast as a probiotic on the performance of quails, reported that body weight gains during a 0 to 6-week period of 204.94, 219.96, and 255.95g for quails fed 0, 5, and 10% yeast, respectively.

Table 3 – Weekly body weight gain(BWG) andfeed intake(FI), cumulativefeed intake(CFI), and feed conversion ratio (FCR), according to treatment group.

Week	Group	N	BWG	FI	CFI	FCR
Initial	Control	161	----	----	----	----
	Formaldehyde	144	----	----	----	----
	Garlic 1	146	----	----	----	----
	Garlic 2	150	----	----	----	----
1	Control	146	29.34	47.57	47.57	1.62
	Formaldehyde	133	31.55	48.03	48.03	1.52
	Garlic 1	134	30.96	44.87	44.87	1.44
	Garlic 2	123	29.69	50.29	50.29	1.69
2	Control	145	54.63	100.57	148.14	1.84
	Formaldehyde	132	57.11	107.84	155.87	1.88
	Garlic 1	129	54.79	109.56	154.43	1.99
	Garlic 2	118	59.77	110.36	160.65	1.84
3	Control	145	70.53	150.35	298.49	2.13
	Formaldehyde	128	73.36	157.77	313.64	2.15
	Garlic 1	127	74.16	154.85	309.28	2.08
	Garlic 2	117	66.64	149.79	310.44	2.24
4	Control	144	70.32	207.46	505.95	2.95
	Formaldehyde	127	66.76	219.27	532.91	3.28
	Garlic 1	127	66.48	212.28	521.56	3.18
	Garlic 2	116	77.90	219.64	530.08	2.81
5	Control	143	60.36	254.65	760.60	4.21
	Formaldehyde	127	52.91	264.13	797.04	4.99
	Garlic 1	125	53.36	260.28	781.84	4.87
	Garlic 2	116	61.82	264.52	794.60	4.27

ab: Means in the same column with different superscriptsin the same week are significantly different (p<0.05)

Cumulative feed intake values at the end of the 5-week period of 760.60, 797.04, 781.84, and 794.60g and feed conversion ratios of 4.21, 4.99, 4.87, and 4.27 for the control, formaldehyde, garlic-1 and garlic-2 groups, respectively (Table 3). Both feed intake and feed conversion ratio were not significantly different (p>0.05) among treatment groups. Independently of treatment, FCR increased with age.

The values reported by Baylan *et al.* (1997) for cumulative feed intake (674.71g) and feed conversion ratio (3.32) of 5-week-old quails are lower than those found in the present study. Kahksar *et al.* (2012), evaluating the effects of the dietary addition of thyme essential oil on the performance of Japanese quails, reported 443 and 458g cumulative intake for the control and thyme-fed groups, respectively, which are lower than those found in the present study. On the other hand, the cumulative feed intake results of the present study are lower than those found by Kalio *et al.* (2016) in 35-d-old Japanese quails, of 951.45 g. The feed conversion ratio values obtained here are consistent with those reported by Wafar *et al.* (2017), of4.74-5.10 in 42-d-old Japanese quails, and by Cheong *et al.* (2016), who determined 4.49-5.10 in 5-week-old quails fed different levels of spirulina.

CONCLUSIONS

The use of garlic extract, a natural product, for the disinfection of hatching eggs does not have any negative effects on embryo development, hatching parameters or live performance of quails, and therefore, it may be used as an alternative formaldehyde fumigation.

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