ARTICLE DOI: 10.5504/BBEQ.2011.0027 FB

USE OF ALLICIN AS AN ALTERNATIVE HATCHING EGG DISINFECTANT VERSUS FORMALDEHYDE FUMIGATION IN BROILER HATCHING EGGS

Gulsen Copur[1], M. Arslan[2], M. Baylan[1], S. Canogullari[3]
[1]Mustafa Kemal University, Faculty of Agriculture, Department of Animal Science, Hatay, Turkey
[2]Mustafa Kemal University, Faculty of Agriculture, Field Crops Department, Hatay, Turkey
[3]Mustafa Kemal University, Samandag Vocational School, Samandag, Hatay, Turkey
Correspondence to: Gulsen Copur
E-mail: gulsenankara@gmail.com

ABSTRACT
Allicin, diallyl thiosulfinate, has a strong antibacterial activity against a wide range of gram-negative and gram-positive bacteria. The purpose of this study was to determine the possible use of allicin as a formaldehyde alternative on broiler breeder hatching eggs. The lowest microbial counts on the eggs were obtained from formaldehyde followed by allicin. Microbial counts slightly decreased with the increasing allicin concentrations. Compared to the positive control formaldehyde, allicin treatment lowered the early and late embryonic mortality, and feed conversion ratio, but increased the discarded chick rate, pipping rate and hatchability of the fertile eggs. Allicin concentrations had no significant effect either on hatching or chick growth and development after hatching. These results imply that allicin had a potential as a hatching egg disinfectant since allicin had no detrimental effect on the developing embryo.

Biotechnol. & Biotechnol. Eq. 2011, **25**(2), 2494-2498

Keywords: Allicin, egg disinfectant, hatching egg

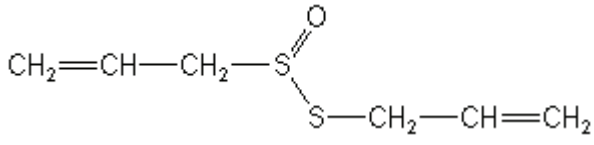
Introduction

Hatching eggs are infected by numerous infectious organisms before and after laying. Among them *Esheria coli, Proteus* spp., *Enterobacter* spp., *Pseudomonas* spp., *Klebsiella* spp., *Staphylococcu*s spp., *Streptococcus* spp., *Clostridium* spp., *Bacillus cereus*, *Salmonella typhimurium* and *Enterococcus*, are the most common bacteria that have been isolated from hatching eggs. They can enter the egg from an infected reproductive tract of a hen. Also they can penetrate through the eggshell, if the egg is contaminated with fecal material. Dirty nests and cages can serve as sources of contamination to eggs (6, 15, 30). Microbial contamination of hatching eggs causes poor hatchability and chick performance.

Sanitation is essential for successful hatching egg production. Several methods are available for sanitizing hatching eggs. Fumigation, spray application, UV light and washing with appropriate sanitizers are the common applied practices for sanitation (1, 3, 17, 19, 22, 29). An effective hatchery sanitation program is critical to achieve a high level of hatchability and ensure the production of high quality chicks. The most common and arguably effective method of reducing the microbial contamination on hatching eggs is the pre-incubation fumigation of eggs with formaldehyde. Formaldehyde fumigation (FF) however – besides being an excellent anti-microbial agent – is a toxic chemical, and as such, can seriously damage the dormant embryo if the fumigation is carried out improperly (7). In addition, FF is an irritant for the eyes and nose and has a lingering noxious odor (29). Most importantly, recent actions by the protection agency regulate the use of FF under the toxic substances control act due to its suspected carcinogenicity (11).

Allicin is one of the most biologically active compounds of garlic which shows more bacteriostatic than bactericidial activity against gram-positive and gram-negative bacteria (9). Several investigations have been conducted on the antimicrobial effect of various species and their derivates (24). Garlic allicin represents a rich potential source of alternative and environmentally acceptable control agents for infectious organisms due to their antimicrobial properties. Experiments demonstrated that a wide range of microorganisms have been shown to be sensitive to allicin (9, 10, 12, 18, 20, 21).

The chemical responsible for the antibacterial and antifungal activity in garlic is diallyl thiosulfinate (common name, allicin) (27):



Allicin is not found in intact plants but is formed by the action of the enzyme alliin alkyl-sulfenate-lyase (EC 4.4.1.4) on the non-protein amino acid S-allylcysteine S-oxide (alliin). The transformation of alliin into the biologically active allicin molecule upon crushing of a garlic clove is extremely rapid, being complete in seconds. The enzyme responsible for the

lysis is alliinase, or alliin-lyase (E.C.4.4.1 4), a pyridoxal 3-phosphate-dependent glycoprotein consisting of two subunits 17, 81. Alliinase is present in unusually high amounts in garlic cloves: at least 10% of the total protein content (10 mg/g fresh weight). However, by crushing or cutting the garlic cloves, the barriers between these compartments are broken and the alliin lyase catalyzes the beta elimination of alliin to yield pyruvate, ammonia, and allylsulfenic acid, two molecules of which react spontaneously to form allicin (2, 5, 8, 9, 26). Pure allicin is a volatile molecule that is poorly miscible in aqueous solutions and which has the typical odor of freshly crushed garlic (5).

The purposes of this study were to determine the practical applicability of the garlic extract allicin to control the microbial activity naturally occurring on eggshells, and to determine its effects on hatching parameters, and growth and development of chicks after hatching.

Materials and Methods

In this study, commercial allicin powder tablets (Allimax Nutraceuticals US, Chicago) were used. The daily collected hatching eggs of 54-week-old Ross-308 broilers were obtained from a commercial company located in Antakya, Turkey. The eggs were collected twice a day (early in the morning, 9 a.m.; and late in the afternoon 4 p.m.). Dirty and faecally contaminated, cracked eggs and eggshells with visible defects were discarded. After collection, the eggs were stored for 1 day at about 15-18°C and 75 % relative humidity (RH) prior to the experiment.

In the study, a total of 1200 eggs were used. The eggs were divided into four groups to investigate the effects of sanitizers on the hatching results. The first group consisted of non-treated eggs (negative control). The second group was treated with formaldehyde (triple strength formaldehyde gas (3X=119.8 ml formalin: 59.9 g potassium permanganate/m^3) (28) for 20 minutes at 24°C (positive control). Triple strength formaldehyde is used commercially on hatching eggs (28). The third and fourth groups were treated with allicin at two doses, 3600 mg/L and 7200 mg/L with 300 eggs in each group, in a plastic container 35 × 20 × 15 cm in size.

The eggs were disinfected by immerging in the solution containing allicin at room temperature. After disinfection, five eggs from each treatment group were immediately placed on sterile plastic bags. The treated and packed eggs were kept at 18°C in a temperature controlled room. Following their transfer to the microbiology laboratory, all eggs were handled aseptically with new disposable gloves for each egg. A whole egg washing technique was used to recover the shell associated micro-organisms for estimating the total bacteria, coliforms and fungi and mold counts of five eggs per treatment. Dilutions were prepared (10^{-1}-10^{-3}), and then were inoculated into sterile Petri dishes. The total bacteria, coliforms and fungi were incubated at 37°C for 48 h. Total aerobic bacteria were counted by using Plate Count Agar (Merck), and coliforms and *E. coli* were counted according to FDA(14). Potato Dextrose Agar (Merck) was used to count fungi and molds. Colonies were measured as cfu/mL.

The eggs were incubated at 37.8°C and 55-60% RH until the 18th day of incubation. After that the incubator conditions were changed to 37.2°C and 70-80 % RH for the actual hatching process. After hatching all chicks were counted and the eggs that did not hatch were broken to macroscopically inspect their contents to determine the true fertility rate and estimate the time of death for the non-hatched fertile eggs (%). The hatchability of the fertile eggs (%) was determined by discounting all truly infertile eggs and dividing the number of chicks hatched by the total number of fertile eggs. In addition to these parameters, the early embryonic mortality EEM (%), middle embryonic mortality (MEM) (%), late embryonic mortality LEM (%), pipped (%), discarded chicks (%) and contamination (%) rates were determined.

After hatching, the chicks in the different treatment groups were separately raised in a poultry house. Each of the 4 treatments had 13 chicks and each treatment was replicated 3 times. The chicks were fed by starter feed (24% CP and 3000 kcal ME/kg) and chick diets (22% CP and 3100 kcal ME/kg), between 0 and 10 days and between 10 and 28 days, respectively. The chickens were fed by chicken diets (20% CP and 3200 kcal ME/kg) and finisher diet (19% CP and 3200 kcal ME/kg) between 28 and 35 days and between 35 and 42 days, respectively. Water was provided *ad libitum*. Forty-two-day-old chickens were slaughtered in each treatment. The initial body weight (g), body weight (g), body weight gain (g), total feed consumption (g) and feed conversion ratio (%) were determined.

The obtained data from the experiment were analyzed by one-way ANOVA using a randomized complete block design, using the general linear models procedure in the Statistical Analysis System (23) (SAS Institute, 1996). Each treatment was replicated 3 times. The microbial counts were transformed to Log_{10} prior to statistical analysis. The means of the measured parameters were compared using Fisher's protected least significance difference (LSD) at $P < 0.05$.

Results and Discussion

Allicin application did not significantly reduce the microbial activities on the surface of the egg shell. The lowest bacteria and yeast/mold counts were obtained from formaldehyde followed by allicin treatment (**Table 1**). The highest bacteria and yeast/mold counts were obtained for the negative control treatment. Allicin had no significant effect on bacteria, yeast and mold count. The bacteria, yeast and mold count did not significantly decrease with the increasing doses of allicin (**Table 2**).

TABLE 1

The anti-microbial effect of allicin disinfection and formaldhyde fumigation on the microbial count of hatching eggshell surface

Treatment	Microbiological determinations (cfu/egg, geometric mean Log_{10})*	
	Total Bacteria	Yeast and mold
Negative control	1.516	0.646
Positive control Formaldhyde	1.224	0.548
Allicin	1.241	0.570
LSD (0.05)	N.S.	N.S.

*Counts expressed as logarithms (base 10) of number per egg

The early embryonic mortality, middle embryonic mortality and late embryonic mortality, discarded chick rate, pipped rate, contamination rate, hatchability of set and hatchability of fertile eggs were not statistically significantly different among treatments (**Table 2**). The lowest (3.16%) and the highest (4.55%) early embryonic mortality were obtained from the positive control formaldhyde and negative control treatment, respectively. When middle embryonic mortality was considered, allicin applications were slightly lower than the negative and positive control formaldehyde treatments. The positive control formaldehyde treatment resulted in the lowest late embryonic mortality (3.87%), followed by allicin treatments. Discarded chicks rate values slightly varied among treatments. Accept for allicin-1 (3600 mg/L), negative control, allicin-2 (7200 mg/L) and positive control formaldehyde treatments had the

TABLE 2

The effect of allicin doses on the hatchability of fertile eggs and embryonic mortality stages (%)

Treatment Dose (mg/L)	EEM	MEM	LEM	DCR	Pipped	CR	HFE	H
Negative control	4.55	0.35	4.91	0.69	0.34	1.40	87.71	84.67
Allicin-1	3.49	0.34	4.08	0.69	0.69	0.92	89.60	86.67
Allicin-2	3.51	0.34	4.21	0.70	0.34	0.81	90.02	86.33
Positive control Formaldhyde	3.16	0.35	3.87	0.69	0.34	0.70	90.84	84.00
LSD 0.05	N.S.	N.S	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

EEM: early embryonic mortality; MEM: middle embryonic mortality; LEM: late embryonic mortality; DCR: discarded chicks rate; CR: contamination rate; HFE: hatchability of fertile egg; NS: not significant; H: Hatchability of set eggs

TABLE 3

The effect of allicin doses on the growth performance and feed consumption of chicks

Treatment dose (mg/L)	Chick count (n)	Initial body weight (g)	Total feed consumption (g)	Body weight gain (g) (0-42 days)	Feed conversion ratio (%) (0-6 week)	Body weight (g) (At 42 days)
Negative control	39	45.66	3663.33	2268.33	1.56	2314.00
Allicin-1	39	45.77	3819.78	2436.22	1.56	2436.22
Allicin-2	39	45.77	3880.67	2464.00	1.56	2464.00
Positive control Formaldhyde	39	45.56	3917.00	2460.33	1.55	2506.90
LSD 0.05		N.S.	N.S.	N.S.	N.S.	N.S.

NS: not significant

TABLE 4

The effect of allicin doses on the slaughter and carcass parts weights

Treatment dose (mg/L)	Slaughter weight (g)	Carcas (g)	Legs (g)	Wing (g)	Breast (g)
Negative control	2302.33	1859.67	513.67	222.00	574.67
Allicin-1	2410.11	1899.67	521.00	212.44	588.33
Allicin-2	2423.56	1923.56	529.89	217.77	595.89
Positive controlFormaldhyde	2521.67	1948.00	534.33	210.66	612.33
LSD 0.05	N.S.	N.S.	N.S.	N.S.	N.S.

N.S.: not significant

same pipping rate (0.34%). The contamination rate values varied between 0.70% and 1.40%. The negative control treatment had the highest contamination rate, and the positive control formaldehyde, the lowest one. The hatchability of fertile eggs varied between 87.71% and 90.84%. The negative control group was the lowest hatchability value of fertile eggs (87.71%) compared to allicin-1 (89.60%), allicin2 (90.02%) and positive control formaldehyde (90.84%) treatments.

The initial body weight, total feed consumption, body weight gain between 0-42 days, body weight gain at 42 days and feed conversion ratio were not significantly affected by allicin-1, allicin-2, negative control and positive control formaldehyde treatments (**Table 3**). The initial body weights varied between 45.66 and 45.77 g. The highest and the lowest values of the total food consumption were considered were obtained from the negative control and positive control formaldehyde treatments, respectively.

There were no statistically significant differences between allicin-1, allicin-2, negative control and positive control formaldehyde treatments with respect to slaughter weight, carcass, legs, wings and breast weights (**Table 4**). The highest slaughter weight was obtained from the positive control formaldehyde with 2521.67 g, while the lowest one was obtained from the negative control treatment with 2302.33 g. Compared to the negative control, the total feed consumption, body weight gain, and body weight were slightly higher in allicin and positive control formaldehyde treatments.

Increased allicin concentration had no significant effect on slaughter weight, carcass weight, legs weight, wings weight and breast weight. When allicin concentration was compared, only slight slaughter weight, carcass weight, legs weight and wings weight differences occurred.

The number of total bacteria, yeast and molds on the surface of the egg shell were not reduced by the allicin treatment. The pure form of allicin has antibacterial activity against a wide range of gram-negative and gram-positive bacteria (9, 21), *Candida* spp, *Cryptococcus neoformans*, and *Helicobacter pylori*[15], antifungal and antiviral activity (2). Garlic products and garlic oil are effective against *Salmonella typhi*, *Staphylococcus aeureus*, *Esheria coli*, *Bacillus cereus*, and a mixed lactic culture consisting of *Lactobacillus delbrueckii* subsp. *Bulgaricus* and *Streptococcus thermophilus*, *Staphylococcus albus*, *Listeria monocytogenes, Aspergillus niger*, *Acari parasitus*, *Pseudomonas aeruginosa* and *Proteus morganni* (12, 18, 20).

Compared to other natural products such as essential oils, allicin did not remarkably reduce the microbial count on the egg shell. More success on the reduction of microbial counts was obtained from essential oil treatments of the hatching eggs (13, 31).

The eggshells of hens are perforated with many pores from 9 to 35 μm in diameter (25). It is known that pathogenic bacteria present on the surface of the egg may contaminate the egg shell and penetrate the egg through the shell pores (4). When microorganisms pass through the membranes of hatching eggs, there is no effective way to eliminate them or prevent their further invasion of the egg contents or developing embryo. Therefore, harmful microorganisms must be removed or destroyed as rapidly as possible on the surface of the hatching egg.

Allicin treatment improved the hatchability of fertile eggs and decreased the early, late embryonic mortalities and contamination rate. The improved hatchability of fertile eggs may be a direct result of decreased microbial contamination of the eggs (**Table 2**). Although hatching egg disinfection is often helpful to reduce contamination on the egg shell surface, it is not the only solution and special attention should be paid to producing microbe-free eggs that do not need to be disinfected. Less microbial contamination could also aid in the production of cleaner and healthier chicks (16).

In this study, however, the microbial count was not significantly different between 3600 and 7200 mg/L allicin treatment. On the other hand, further studies are needed to determine the effects of higher concentrations of allicin on hatching eggs. The field studies showed that feed conversion ratios did not increase with the increased allicin doses. Body weight at 42 days, body weight gains at 42 days and total feed consumptions increased with the positive control formaldehyde and allicin treatments.

Conclusions

Allicin is a biologically active compound in garlic which presents a rich potential source of alternative and environmentally acceptable control agents for infectious organisms due to their antimicrobial properties. Allicin applications decreased the microbial counts on the egg surface. Allicin treatment improved the hatchability of the fertile eggs and decreased the contamination rate, early and late embryonic mortality rates. It had no adverse affect either on hatching or chick parameters after hatching. Our findings showed that allicin could be considered as a potential compound that could be used as a hatching egg disinfectant.

Acknowledgements

This paper was supported by BAP, Mustafa Kemal University, Project No: 07 B 0301.

REFERENCES

1. **Adler H.E., DaMassa A.J., Scott W.F.** (1979) Poult. Sci., **58**, 799-806.
2. **Ankri S. and Mirelman D.** (1999) Microbes and Infection, 125-129.
3. **Arhienbuwa F.E, Adler E., Wiggins A.D. (**1980) Poult. Sci., **59**, 28-33.
4. **Barbour E.K., Nabbut N.H., Hinners S.W., Al-Nakhli H.M.** (1985) Vet. Q., **7**, 39-43.
5. **Block E.** (1985) Sci. Am., **252**, 94-99.

6. **Board R.G., Ayers J.C., Kraft A.A., Forsythe R.H.** (1964) Poult. Sci., **43**, 584-595.
7. **Cadirci S.** (1997) The Scottish Agricultural College, Poultry Science Department, the University of Glasgow for the Degree of Master of Science.
8. **Caporaso N., Smith S.M., Eng R.H.K.** (1983) Antimicrob. Agents Chemother., **23**, 700-702.
9. **Cavallito C.J. and Bailey J.H.** (1944) J. Am. Che. Soc., 66, 1944-1952.
10. **Cellini L., Di Campli E., Masulli M., Di Bartolomeo S., Allocati N.** (1996) Med. Micrbiol., **13**, 273-277.
11. **Chemical and engineering news** (1984) Chem. Eng. News, **62**, 8.
12. **Conner D.E. and Beuchat L.R.** (1984) J. Food Sci., **49**, 429-434.
13. **Copur G., Arslan M., Duru M., Baylan M., Canogullari S., Aksan E.** (2010) African Journal of Biotechnology, **8**, 2531-2538.
14. **FDA** (1998) Ch. 4. Bacteriological Analytical Manual, 8th Ed Revision A.
15. **Harry E.G.** (1963) Br. Poult. Sci., **4**, 91-100.
16. **Harry E.G. and Gordon R.F.** (1966) Veterinarian, **4**, 5-15.
17. **Kuhl H.Y.** (1989) Int. Hatchery Pract., **3**, 29-33.
18. **Kumar M. and Barval J.S.** (1998) J. Appl. Microbiol., **84**, 213-215.
19. **Proudfoot F.G., Nash D.M., HuIan H.W.** (1985) Poult. Sci., **64**, 2100-2402.
20. **Rahman M.S., Al-Sheibani H.I., Al-Riziqi M.H., Mothershaw A., Guizani N., Bengtsson G.** (2006) Int. J. Food Prop., **9**(3), 503-513.
21. **Rode H., de Wet P.M., Cywes S.** (1989) South African Journal of Science, **85**, 462-464.
22. **Sacco R.E., Renner P.A., Nestor K.E., Saif Y.M., Dearth R.N.** (1989) Poult. Sci., **68**, 1179-1184.
23. **SAS Institute SAS/STAT Software** (1996) Chances and enhancements through release 6.11., SAS Inst. Inc., Carry, NC.
24. **Shelef L.A., Syotti E.K., Bulgarelli M.A.** (1984) J. Food Sci., **49**, 737-740.
25. **Smeltzer T.J., Orange K., Peel B., Runge G.** (1979) Aust. Vet. J., **55**, 592-593.
26. **Stoll A. and Seebeck E.** (1949) Helv. Chim. Acta., **32**, 197-205.
27. **Stoll A. and Seebeck E.** (1951) Adv. Enzymol., **11**, 377-400.
28. **USDA** (1985) National poultry improvement plan and auxiliary provisions. Animal and Plant Health Inspection Service. Veterinary Service, Miscellaneous Publication No. 91-40. USDA, Hyattsville, MD.
29. **Whistler P.E. and Sheldon B.W.** (1989) Poult. Sci., **68**, 1345-1350.
30. **Williams J.E., Dillard L.H., Hall G.O.** (1968) Avian Dis., **12**, 445-466.
31. **Yildirim I., Ozsan R., Yetisir R.** (2003) Revue Med. Vet., **154**(5), 367-370.