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Isolated or associated experimental contamination of albumen and egg yolk for *Salmonella* Enteritidis and *Escherichia coli* – influence of temperature and storage time

[*Contaminação experimental de albúmen e gema de ovos por* Salmonella *Enteritidis e* Escherichia coli *isoladas ou associadas – influência da temperatura e tempo de armazenagem*]

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ABSTRACT

The behavior of *Salmonella* Enteritidis (SE) and *Escherichia coli* in albumen and yolk of chicken eggs submitted to two different temperatures, 8°C and 30°C, when separately inoculated or in the form of mixed cultures was studied. There was no difference in the behaviour of the tested agents even when inoculated together. Even under high temperature, *E. coli* did not multiply in the albumen, demonstrating the inhibition power for that microorganism, while SE increased its population up to three logarithmic cycles. In egg yolk, SE demonstrated psychrotrophic characteristics.

Keywords: chicken, egg quality, bacterial growth, temperature, storage

RESUMO

Avaliou-se o comportamento de Salmonella *Enteritidis (SE) e* Escherichia coli *em albúmen e gema de ovos de galinha, submetidos a duas diferentes temperaturas, 8 e 30°C, quando inoculadas isoladamente ou na forma de culturas mistas. Não houve diferença no comportamento dos agentes testados mesmo quando inoculadas em conjunto. Mesmo sob temperatura alta,* E. coli *não se multiplicou no albúmen, demonstrando o poder inibidor de seus constituintes para esse microrganismo, enquanto a SE aumentou sua população em até três ciclos logarítmicos. Em gema de ovo, SE demonstrou características psicrotróficas.*

Palavras-chave: galinha, qualidade do ovo, multiplicação bacteriana, temperatura, armazenagem

INTRODUCTION

Salmonella spp. is the most important etiologic agent for human gastroenteritis all over the world (Guidelines…, 1983) and, in recent years, *S.* Enteritidis (SE) has been the most prevalent serotype in Brazil (Tavechio et al., 2002; Nunes et al., 2003).

Different enteric pathogenic bacteria such as *Salmonella* spp. and *Escherichia coli* can contaminate the components of the eggs leading to food poisoning. These genera can contaminate eggs separately or together.

Temperature, pH, and antimicrobial substances are limiting growth factors in the albumen (Yadav and Vadehra, 1977; Tranter and Board, 1982; Tranter and Board, 1984; Baron et al., 1997a; Gast and Holt, 2001). However, there are no limiting growth factors in the egg yolk so, when the environmental factors are favourable,

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bacteria can easily grow (Tranter and Board, 1982).

Few studies are available on the behaviour of SE and *E. coli* when inoculated into the eggs as mixed cultures. In other biological systems, like pig slurry treatment, these bacteria have negative correlation coefficient (Schmidt and Cardoso, 2003).

This research aimed to obtain information about SE and *E. coli* behavior in egg yolk and albumen when submitted to different storage temperatures.

MATERIAL AND METHODS

Mutant pathogenic *S*E phage type 4 and a non-pathogenic *E. coli*, both resistant strains to nalidixic acid and novobiocin (100µg/mL each), isolated from commercial chicken, were used in this study. Both organisms were cultured in tryptic soy broth (TSB) at 35°C for 20 hours, when the population counts reached 9.0 log colony forming units (CFU)/mL. Then, they were inoculated separately and mixed into the albumen and yolk preparations using sterile pipettes.

Less than 24 hours after chicken eggs were laid, they were disinfected by immersion in an ethyl alcohol (70°GL) solution for 10 minutes and allowed to dry in a laminar flow chamber. The eggs were broken and the albumen and the yolk were aseptically separated, placed in sterile plastic bags (*pool* of 15 eggs/each experiment), and gently hand homogenized before and after inoculation. The count for both microorganisms was 2.0 log CFU per gram of egg preparation. The plastic bags were sealed and incubated at 30°C and 8°C for fourteen days. The tested eggs came from a free *Salmonella* farm.

Before each analysis, the egg contents of the plastic bags were hand homogenized and samples (5mL) were taken using sterile pipettes. These samples were first suspended in 45mL of buffered peptone water (BPW), serial decimal dilutions prepared in BPW and 0.1mL of each dilution surface-plated onto McConkey agar containing nalidixic acid and novobiocin (100µg/mL of culture media). Colonies that grew on the agar were counted after 24-48 hours of incubation at 35°C.
The samples were taken after 0, 2, 6, 12, 16, and 24 hours when the incubation temperature was 30°C; and after 0, 6, 12, 16, and 24 hours when the incubation temperature was 8°C. In addition, further samples were taken after 48, 72, 168, and 336 hours at incubation temperatures of 8°C and 30°C.

The pH values of the samples of albumen and yolk were measured using pH paper[1] every 24 hours, before the enumeration procedures.

The data for bacterial growth were submitted to variance analysis, using Statistica 5.5 (Datasoft Inc.)

RESULTS AND DISCUSSION

Fig. 1 shows the results for the growth of SE and *E. coli* at 30°C, both separately and together. The growth of each microrganism was not statistically affected when mixed cultures were inoculated into the albumen ($P<0.05$).

The counts of SE reached 5.0 to 6.0 log CFU/g and did not alter for the 336h observation period. On the other hand, *E. coli* counts only decreased to 1.0 log CFU/g and there were no viable cells at the end of the same period. Thus, it seems that the cells of this specific strain of SE were much more resistant to the antimicrobial substances present in the albumen than those of *E. coli*.

The antimicrobial activity of albumen is more effective against Gram-positive bacteria, although there is some activity against Gram-negative ones (Yadav and Vadehra, 1977; Tranter and Board, 1982; Tranter and Board, 1984; Baron et al., 1997a; Gast and Holt, 2001). This experiment showed that SE is resistant to the unfavourable conditions of the albumen.

These results agree with Baron et al. (2004) who showed that the growth of *Salmonella* sorovars in albumen at 30°C was unaffected by those conditions, including their pathogenic characteristics. *Salmonella* can overcome the deficiency of iron by secreting siderophors that allow their growth in albumen, using the iron for

[1]Neutralit pH 5.0-10.0 - Merck – São Paulo, Brazil.

their metabolism (Baron et al., 1997b). *E. coli* showed a different pattern. Albumen was an unfavourable medium for this organism at 30°C and no viable cells were found at the end of the experiment. At this temperature, the antimicrobial characteristics can be effective against some Gram-negative bacteria. There is no consensus about the behavior of *E. coli* in albumen at 30°C. Yadav and Vadehra (1977) studied a strain of *E. coli* and found that this microorganism was capable of growing in albumen at 30-32°C. Tranter and Board (1984) found that *E. coli* could not grow in albumen when incubated at this same temperature, and could die. They believed that the albumen had more antimicrobial activity at this temperature than when it was incubated at lower temperatures.

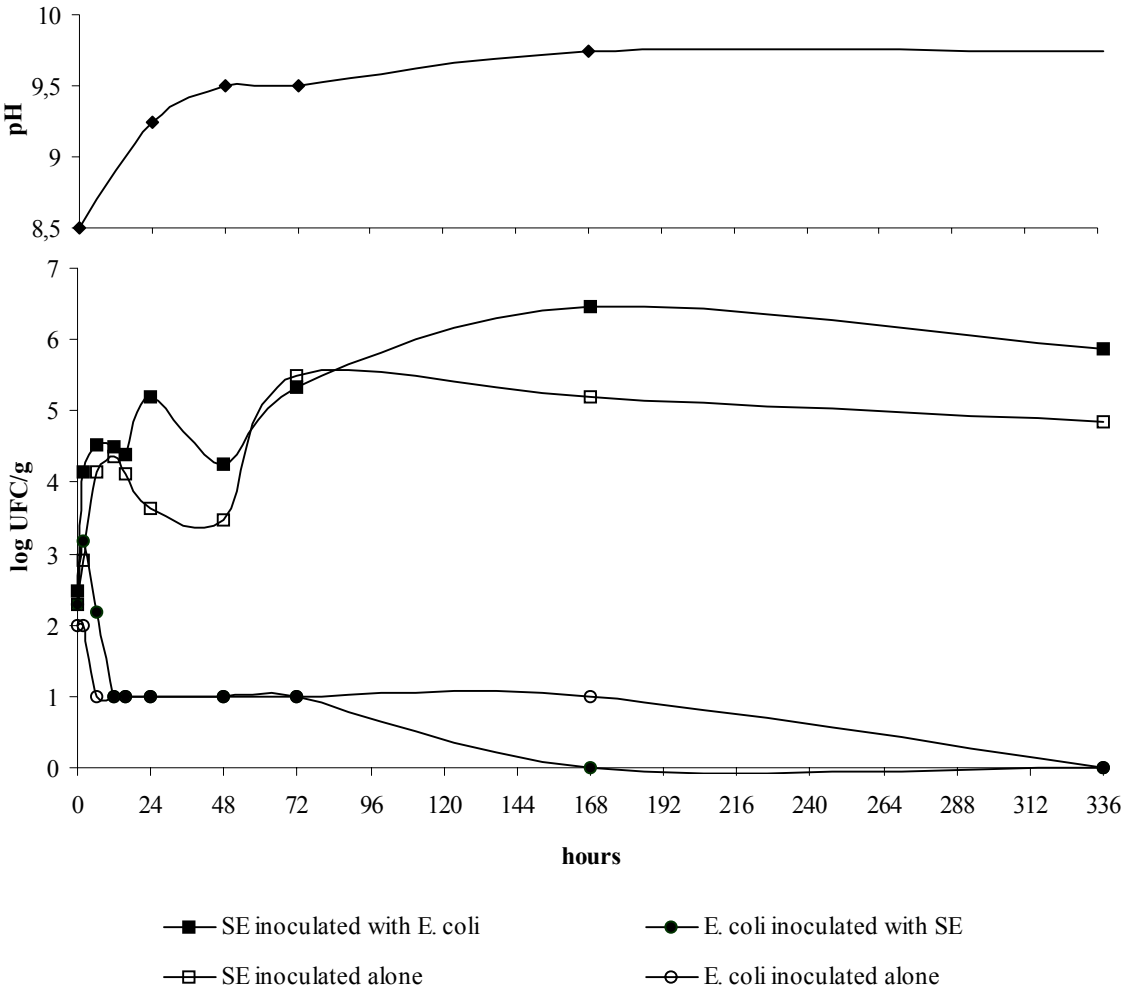

Figure 1. *Salmonella* Enteritidis and *Escherichia coli* behavior in albumen incubated at 30°C.

The pH value increased from 8.5 to 9.5 during the first 48 hours and there were no further changes. The inoculation of both bacteria did not change the pH (Figure 1).

Fig. 2 shows the results for the growth of SE and *E. coli* at 8°C. At this temperature, the SE count declined but maintained its viability until the end of the experiment. It shows that the temperature affects the behaviour of SE. There was also statistical difference between the behaviors of this bacteria at 8 and 30°C ($P<0.05$).

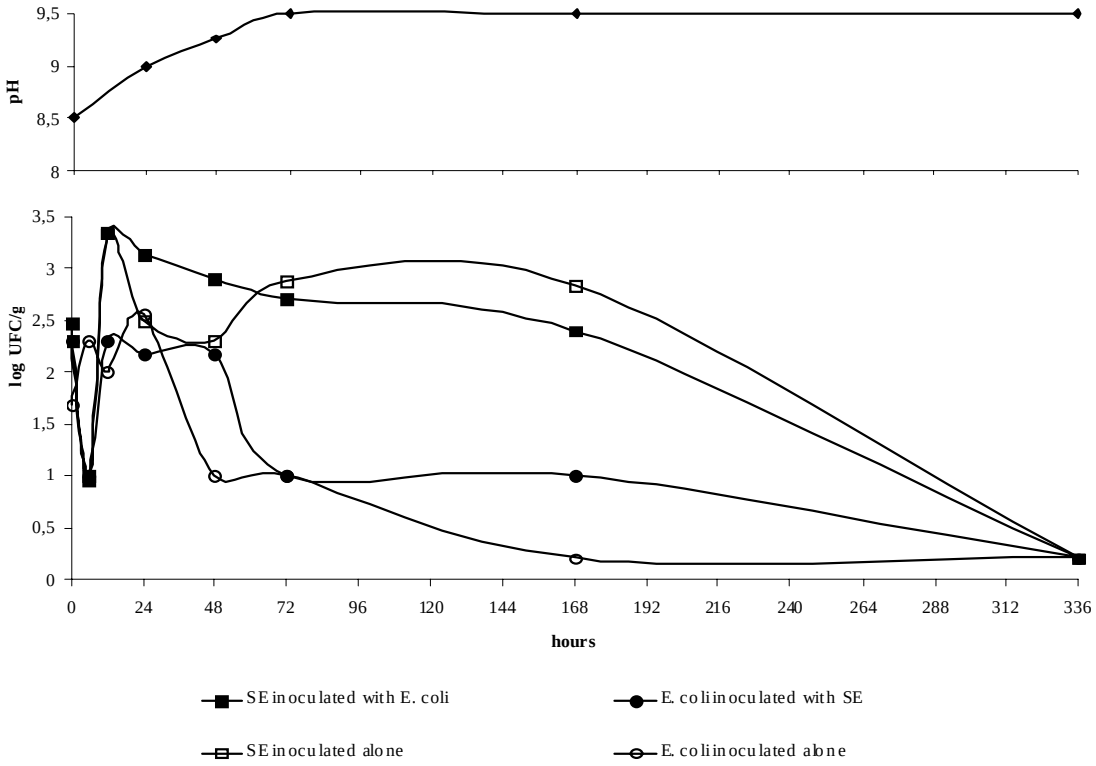

Figure 2. *Salmonella* Enteritidis and *Escherichia coli* behavior in albumen incubated at 8°C.

Gast and Holt (2000) found little variability in SE growth at different temperatures (10, 17.5, and 25°C). At refrigeration temperatures, the number of viable cells rose slowly and the risk of disease was lower than when stored at higher temperatures. The success of the cooling process depends on the time lapse between the start of the temperature decrease and the minimum inhibitory temperature (Gast and Holt, 2001). Otherwise, *Salmonella* can grow in food stored at low temperatures such as 2°C (Angelotti et al., 1961; Matches and Liston, 1968; Palumbo 1986; D'Aoust, 1991). However, Gibson et al. (1988) had difficulty in fitting the growth curves at low temperatures. This is why it is so difficult to anticipate bacterial behavior at refrigeration temperatures.

E. coli cells were viable in the albumen preparation stored at 8°C for 336h, until the end of the experimental period. This pattern was different from that at 30°C, when no viable cells were found at the end. Lower temperatures allowed bacterial viability. Albumen can be more toxic to bacterial cells at higher temperatures (Tranter and Board, 1984). In the current study, it was noticed that *E. coli* was more fragile than SE in the albumen at 8ºC. This is explained by the capacity of SE to reach the yolk and multiply, giving rise to outbreaks of foodborne disease.

The storage temperature affected the pH curve in the albumen preparations. At 8ºC, the pH rose slower than at 30°C (Fig. 2), reaching the value 9.5 after 72 hours, 24 hours later than at 30°C. The highest pH level was the same at both storage temperatures. These results are similar to those of Sabrani and Payne (1978). The inoculated bacteria did not affect albumen alkalinity ($P<0.05$).

Both SE and *E. coli* grew in the egg yolk preparations and reached 7.5-8.7 log CFU/g at 30°C in the first 24 hours after inoculation. Growth slowed down after this period and became stable after 48 hours (Fig. 3). Both bacteria showed the same growth pattern regardless of the inoculation conditions as a single or mixed culture, with no statistical difference ($P<0.05$).

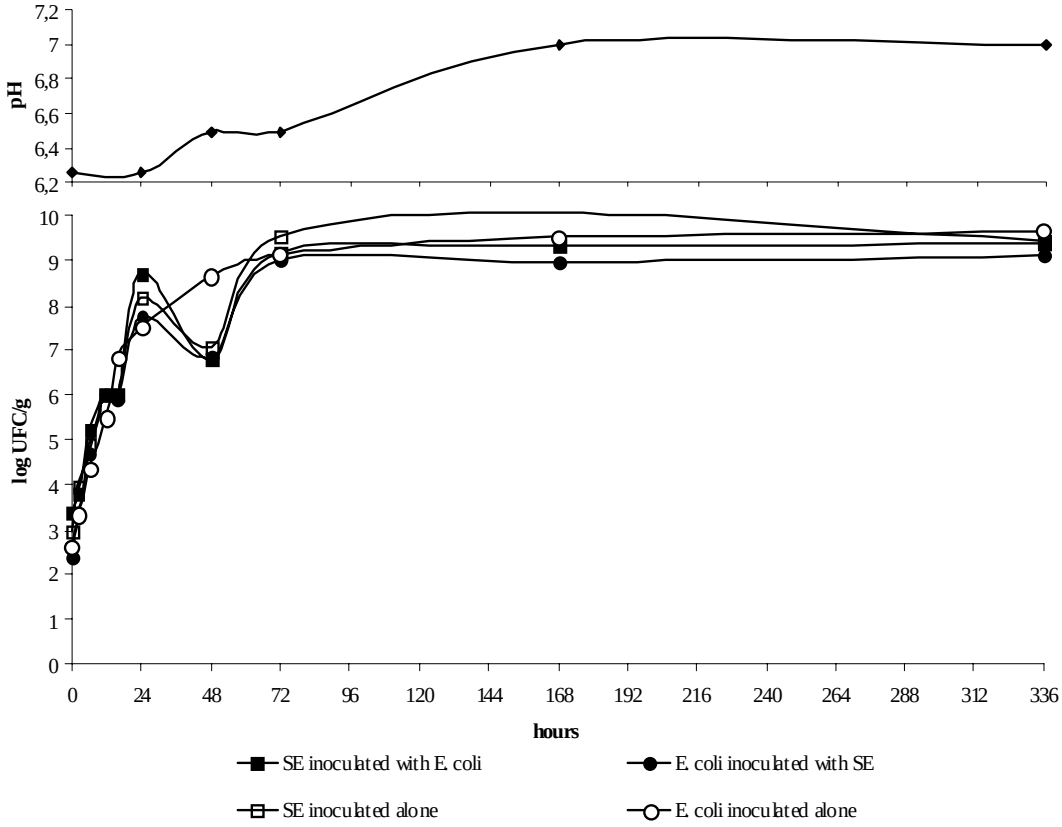

Figure 3. *Salmonella* Enteritidis and *Escherichia coli* behavior in egg yolk incubated at 30°C.

Yolk, unlike other egg structures, contains no anti-bacterial growth elements (Tranter and Board, 1982). This is why bacteria can reach high counts in yolk. These results correlated with other reports, in which different phage types of SE (PhT4, 8, 13a, and 14b) were inoculated into egg yolk and incubated at 25°C, all showed similar growth behavior (Gast and Holt, 2001).

Egg yolk is an excellent culture media, and even a small contamination can reach high levels (8.0 log CFU/g), leading to foodborne diseases. SE, in high counts, can survive any heat or cooking treatment (Humphrey et al., 1989). Viable cells of SE have been found in 44% of all fried, scrambled, hard-cooked eggs, and omelettes prepared with contaminated eggs (Mahdi Saeed and Koons, 1993).

The storage temperature affected the pH curve in the egg yolk preparations, but the inoculation condition of being a single bacteria or a mixed culture did not ($P<0.05$). The yolk pH rose slightly throughout the experimental period. The pH rose slower at lower temperatures than at higher ones.

The storage temperature had a marked effect on the growth of SE and *E. coli* in the egg yolk preparations. At 8°C (Fig. 4), SE remained viable and the growth of *E. coli* was inhibited. After the 336h of the experimental period, the SE counts had risen to 4.5 and 4.8 and the *E. coli* counts had reduced to 0.20 log CFU/g. SE can grow in egg yolk preparations kept at refrigeration temperatures (10°C), but not at lower temperatures (<7.2°C). The present research showed that SE could be a health problem even when egg yolk is stored at low temperatures, because it keeps its viability and it can reach high counts as the temperature increases.

The behavior of these microorganisms is also affected by the pH, that has different values in albumen and yolk. The pH of the yolk is more adequate to bacterial growth.

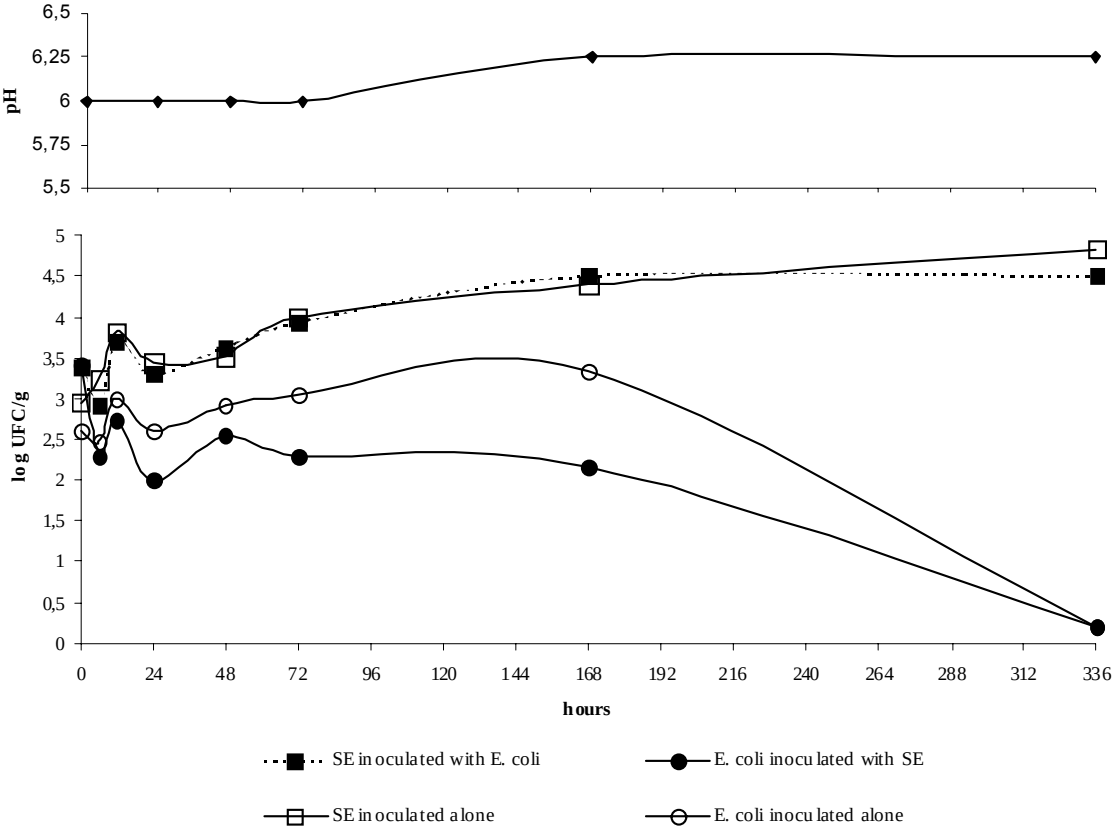

Figure 4. *Salmonella* Enteritidis and *Escherichia coli* behavior in egg yolk incubated at 8°C.

CONCLUSIONS

Despite of SE and *E. coli* belong to the same family (*Enterobactereaceae*), they have different behavior in egg. SE showed resistance against the antimicrobial activity of the albumen even under high temperatures (30°C), while *E. coli* had the development inhibited. In albumen, cooling temperatures (8°C) allowed the survival of both bacteria at the end of the experiment. In egg yolk, *Salmonella* can grow even at low temperatures, while *E. coli* is inhibited.

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REFERENCES

ANGELOTTI, R.; FOTER, M.J.; LEWIS, K.H. Time-temperature effect on salmonellae and staphylococci in foods. I. Behavior in refrigerated foods. *Am. J. Public Health*., v.51, p.76-83, 1961.

BARON, F.; GAUTIER, M.; BRULE, G. Factors involved in the inhibition of growth of *Salmonella enteritidis* in liquid egg white. *J. Food Prot.*, v.60, p.1318-1323, 1997a.

BARON, F.; GAUTIER, M.; BRULE, G. Growth inhibition of *Salmonella enteritidis* in liquid egg white. In: CONGRESS SALMONELLA AND SALMONELLOSIS, 1997, Ploufragan. *Proceedings*... Ploufragan, 1997b. p.347-350.

BARON, F.; BRIANDET, R.; LESNE, J. et al. Influence of a nonfavorable environment, egg white, on resistance to heat and disinfectant, adhesion, and virulence of *Salmonella* Enteritidis. *J. Food Prot.*, v.67, p.2269-2273, 2004.

D'AOUST, J.Y. Psychrotrophy and foodborne *Salmonella*. *Int. J. Food Microbiol.*, v.13, p.207-216, 1991.

GAST, R.K.; HOLT, P.S. Influence of the level and location of contamination on the multiplication of *Salmonella enteritidis* at different storage temperatures in experimentally inoculated eggs. *Poult. Sci.*, v.79, p.559-563, 2000.

GAST, R.K.; HOLT, P.S. Multiplication in egg yolk and survival in egg albumen of *Salmonella enterica* serotype Enteritidis strains of phage types 4, 8, 13a, and 14b. *J. Food Prot.*, v.64, p.865-868, 2001.

GIBSON, A.M.; BRATCHELL, N.; ROBERTS, T.A. Predicting microbial growth: growth responses of salmonellae in a laboratory medium as affected by pH, sodium chloride and storage temperature. *Int. J. Food Microbiol.*, v.6, p.155-178, 1988.

GUIDELINES on Prevention and Control of Salmonellosis. Geneve: WHO, 1983.

HUMPHREY, T.J.; GREENWOOD, M.; GILBERT, R.J. et al. The survival of salmonellas in shell eggs coocked under simulated domestic conditions. *Epidemiol. Infect.*, v.103, p.34-45, 1989.

MAHDI-SAEED, A.; KOONS, C.W. Growth and resistance of *Salmonella enteritidis* in refrigerated and abused eggs. *J. Food Prot.*, v.56, p.927-931, 1993.

MATCHES, J.R., LISTON, J. Low temperature growth of *Salmonella*. *J. Food Sci.*, v.33, p.641-645, 1968.

NUNES, I.A.; HELMUTH, R.; SCHROETER, A. et al. Phage typing of *Salmonella* Enteritidis from different sources in Brazil. *J. Food Prot.*, v.66, p.324-32, 2003.

PALUMBO, S.A. Is refrigeration enough to restrain foodborne pathogens? *J. Food Prot.*, v.49, p.1003-1009, 1986.

SABRANI, M.; PAYNE, C.G. Effect of oiling on internal quality of eggs stored at 28 and 12ºC. *Br. Poult. Sci.*, v.19, p.567-571, 1978.

SCHMIDT, V.; CARDOSO, M. Sobrevivência e perfil de resistência a antimicrobianos de *Salmonella* sp. isoladas em um sistema de tratamento de dejetos de suínos. *Cienc. Rural*, v.33, p.881-888, 2003.

TAVECHIO, A.T.; GHILARDI, A.C.R.; PERESI, J.T.M. et al. *Salmonella* serotypes isolated from nonhuman sources in São Paulo, Brazil, from 1996 through 2000. *J. Food Prot.*, v.65, p.1041-1044, 2002.

TRANTER, H.S.; BOARD, R.G. The antimicrobial defense of avian eggs: biological perspective and chemical basis. *J. Appl. Biochem.*, v.4, p.295-338, 1982.

TRANTER, H.S.; BOARD, R.G. The influence of incubation temperature and pH on the antimicrobial properties of hen egg albumen. *J. Appl. Microbiol.*, v.56, p.53-61, 1984.

YADAV, N.K.; VADEHRA, D.V. Mechanism of egg white resistance to bacterial growth. *J. Food Sci.*, v.42, p.97-99, 1977.