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Action of Antimicrobial Copper on Bacteria and Fungi Isolated from Commercial Poultry Hatcheries

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■Keywords

Antimicrobial copper, hatchery, stainless steel, copper surfaces.

ABSTRACT

Since 2008, when the US Environmental Protection Agency (EPA) registered copper and its alloys as an antimicrobial agent for contact surfaces, research has demonstrated their antimicrobial activity. The aim of this study was to evaluate the efficacy of antimicrobial copper against bacteria and fungi isolated from commercial poultry hatcheries in order to develop a microbiological control alternative in these environments. Samples were collected from the surfaces of hatcher baskets from two hatcheries. Mesophilic microorganisms and fungi/yeasts were isolated and standardized in concentration of 10^5 cells/mL. Four copper plates and four stainless steel plates were completely immersed for one minute in bacteria and fungi/yeasts solutions and left to dry for a day at room temperature. Subsequently, samples were collected from the metal plates with the aid of sterile swab and delimiter. These samples were planted onto Plate Count Agar (for mesophilic culture) and Sabouraud Dextrose Agar (for fungi and yeast culture) and incubated at 36°C for 48 hours and at 25°C for 5-7 days, respectively. After incubation, the colonies recovered from the plates were counted according to IN 62 of the Brazilian Ministry of Agriculture. Almost all contamination was eliminated from the surface of copper plates in a single day, while the stainless steel plates proved to be innocuous to the screened microorganisms. Copper, as a contact surface, proved to have important antimicrobial action on bacteria, fungi and yeasts common to hatcheries.

INTRODUCTION

The microbial contamination of hatcheries results in low-quality day-old chicks, increases early broiler mortality, causes significant economic losses throughout the production chain, and poses a risk to public health. In order to reduce this contamination, large amounts of disinfectants are used; however, many contain restricted-use substances, such as formaldehyde, known for its carcinogenic action.

Since 2008, when the US Environmental Protection Agency (EPA) registered copper and its almost 300 alloys as antimicrobial agents for contact surfaces, Copper is known worldwide and several studies have demonstrated its broad antimicrobial activity (Depner *et al.*, 2015). That registration allows copper to be used as antimicrobial based on the fact that copper kills 99.9% of bacteria within two hours (EPA, 2008). The aim of this study was to evaluate the antimicrobial efficacy of copper against bacteria and fungi isolated from commercial poultry hatcheries in order to develop a new alternative for the microbiological control of those environments.



MATERIAL AND METHODS

Hatching tray surfaces from two commercial chicken hatcheries in southern Brazil, one in Santa Catarina (A) and one in Rio Grande do Sul (B), were sampled. Approximately 144 trays from hatchery A and 36 trays from hatchery B were swabbed using a 25cm² delimiter. Hatchery A samples were placed into sterile plastic bags and hatchery B samples into sterile test tubes. Samples were submitted, under refrigeration, to the laboratory Porto Belo Serviços Veterinários, Porto Alegre, Brazil.

Mesophilic microorganisms and fungi/yeasts were isolated on Plate Count Agar (for mesophilic bacteria) and Sabouraud Dextrose Agar (for fungi and yeasts). Enterobacteriaceae, *Staphylococcus* sp., *Pseudomonas* sp., *Aspergillus* sp., and *Penicillium* sp. were identified. The concentration was standardized to 10⁵ cells/mL and diluted in 9 mL of 0.1% Peptone Water to obtain the inocula. The two inocula (mesophilic bacteria and fungi/yeast) were placed in Stomacher bag (model LABPLAS, Canada – Lot number J067894/2015-09).

The test was first carried out with the bacterial inoculum and then with the fungi/yeasts inoculum. Four C11000 copper plates containing 99.9% Cu and four stainless steel plates (6 cm x 5 cm each) were completely immersed in each inocula for 1 min, removed, placed on sterile petri dishes, and left to dry at room temperature for one day. Subsequently, samples were collected from the copper and stainless steel plates with a sterile swab and a delimiter. The samples from the copper and stainless steel plates were seeded onto Plate Count Agar and Sabouraud Dextrose Agar, and incubated at 36°C for 48 hours and at 25°C for 5-7 days, respectively. After incubation, colony forming units per square centimeter (CFU/cm²) recovered from the metal plates were counted according to the guidelines of the Normative Instruction 62 of the Brazilian Ministry of Agriculture (Brasil, 2003).

The experimental procedures were approved by the Ethics Committee on Animal Use of the Federal University of Santa Maria (UFSM), under protocol number 130/2014.

RESULTS AND DISCUSSION

Table 01 presents the microorganism counts recovered from the copper and stainless steel (control surface) plates after the inoculation with mesophilic bacteria and fungi and yeasts isolated from two commercial hatcheries. The results show that nearly all contamination was eliminated from the surface of the

copper plates in only one day, whereas the stainless steel plates did not inhibit the growth of the screened microorganisms.

Table 1 – Total counts of mesophilic microorganisms, and fungi and yeasts recovered from copper and stainless steel plates in two broilers hatcheries.

Copper plates			Stainless steel plates	
Hatchery A				
	Mesophilic microorganisms*	Fungi and yeasts*	Mesophilic microorganisms*	Fungi and yeasts*
Plate 1	Absent	Absent	Countless	Countless
Plate 2	Absent	Absent	Countless	Countless
Plate 3	2	Absent	Countless	Countless
Plate 4	Absent	Absent	Countless	75*
Hatchery B				
Plate 1	Absent	Absent	1.1X10 ⁴	4.3X10 ²
Plate 2	Absent	Absent	1.0X10 ⁵	7.2X10 ³
Plate 3	Absent	Absent	4.2X10 ⁴	4.5X10 ²
Plate 4	Absent	Absent	1.9X10 ⁴	8.7X10 ²

*(CFU/cm²)

According to Faúndez *et al.* (2004), metallic copper surfaces may be used to reduce the microbial load in areas with high exposure to contamination, such as those found in poultry production facilities. In hospital settings, copper surfaces can, in addition to the use of antibiotics, disinfectants and hand-washing practice, minimize the risk of the emergence and spread of resistant microorganisms (Mikolay *et al.*, 2010). Casey *et al.* (2010) reported that the mean number of bacteria recovered from copper-containing surfaces is 90-100% lower compared with control surfaces, which is consistent with the data found in this study.

The mechanism by which copper contact surfaces cause microbial death is called contact killing due to the tendency of this metal to alternate between cuprous (Cu¹⁺) and cupric (Cu²⁺) oxidation states. This redox cycle generates extremely reactive hydroxyl radicals that damage biomolecules. These radicals cause extensive microbial cell membrane damage allowing copper influx into the cells, where they cause oxidative damage, cell death, and DNA degradation (Depner *et al.*, 2015). According to Grass *et al.* (2011), the development of microbial resistance to killing by contact with copper surfaces is unlikely, because plasmid DNA is completely degraded after cell death by contact killing, preventing the transfer of resistance determinants between organisms; contact killing is very rapid, and microbes do not replicate on copper surfaces, and therefore to do not acquire resistance; and no bacteria resistant to copper and copper alloys have been detected after thousands of years of use of copper.



According to Virgini (2013), non-toxic additives with antibacterial and antifungal properties may be incorporated into polymers and resins at the time of injection and lamination, and maintain their activity for the entire product lifecycle. The use of such materials may aid the health control of hatcheries, which, however, that does not preclude normal cleaning and disinfection procedures. New developments with the use of copper are being researched, such as copper sheets applied in animal production equipment (feeders, drinkers, cages, etc.), and the inclusion of copper nanoparticles in fiberglass and stainless steel plates, coating of silicon surfaces with copper, etc. In addition, the use of antimicrobial copper surfaces in veterinary medicine may result in lower infection rates, limit the use of antibiotics, and reduce the risk of the development of multiresistant microorganisms, thereby promoting both animal and human health benefits (Depner *et al.*, 2015).

The results of the present study demonstrated that copper has significant action against bacteria, fungi, and yeasts commonly found in poultry hatcheries. Therefore, its application as contact surfaces or as an additive in polymers used in work benches and in hatcher baskets and setter trays may reduce contamination levels in hatcheries, improving their productivity and hatchling quality, and consequently, broiler performance. Copper application to such surfaces may also contribute to reduce the use of disinfectants, such as formaldehyde. Copper-based antimicrobial surfaces have been installed in commercial hatcheries and are being tested by this research group.

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