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## Evaluation of an additive efficacy in broiler litter microbial level control in field: preliminary results

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#### ABSTRACT

The present study was conducted to evaluate in field the efficacy of an additive (SOP\* C POULTRY), as an agent for the control of micro-organisms in broiler litter. The Total aerobic Microbial Count (TMC), *Staphylococcus* species (spp.), Coliforms, and *Salmonella* spp. in broiler litter samples of both the Houses, 2 (H2) and 3 (H3), were determined, and also at the end of each cycle the mortality rate was recorded. The results showed significant reduction of all the microbial counts: P= 0.0078 (CMT), 0,0021 (*Staphylococcus* spp.) and 0.0541 (Coliforms), and mortality (P= 0.00106) in treated litter samples H2 and the control H3.

Key Words: Litter additive, Environment, Broiler, Aerobic bacteria, Mortality.

#### RIASSUNTO

#### VALUTAZIONE IN CAMPO DELL'EFFICACIA DI UN ADDITIVO NEL CONTROLLARE IL LIVELLO MICROBICO DELLA LETTIERA: RISULTATI PRELIMINARI

Prove di campo sono state effettuate sulla lettiera di 2 capannoni (C2, trattato, e C3, controllo) di polli da carne per valutare l'efficacia di un prodotto igienizzante di nuova concezione (SOP<sup>®</sup> C POULTRY), in grado di controllare Carica Microbica Totale aerobia (CMT), Staphilococcus spp., Salmonella spp. e Coliformi; inoltre alla fine di ogni ciclo è stata valutata la percentuale di mortalità. I risultati hanno mostrato una significativa riduzione dei valori medi di tutti i parametri microbiologici valutati nei campioni di lettiera trattata rispetto al controllo (P = 0,0078 (CMT), 0,0021 (Staphylococcus spp.), 0,0541 (Coliformi) compresa la mortalità (P = 0,00106).

Parole chiave: Additivo per lettiere, Ambiente, Polli da carne, Batteri aerobi, Mortalità.

#### Introduction

The environment in the poultry house is a combination of physical and biological factors which interact as a complex dynamic system of social interactions, husbandry system, light, temperature and the aerial environment (Sainsbury,1992; Kristensen and Wathes, 2000). The high stocking density in the modern poultry house may lead to reduced air quality with high concentrations of aerial pollutants (Curtis and Drummond, 1982; Maghirang *et al.*, 1991; Feddes and Licsko, 1993). Their concentrations in poultry houses approach, and sometimes exceed, recommended occupational limits for humans (Kristensen and Wathes, 2000). Litter is considered one of the major sources of pollutants in poultry houses, then the need to ménage it using additives has been considered since the last past years (Ivanov, 2001) but has not yet been resolved conclusively. The present study investigated the use of an additive as an agent for the control of micro-organisms in broiler litter, and also the possible effect on the mortality.

#### Material and methods

#### Planning

This study was carried out from February 2002 to March 2004 in two large commercial broiler houses, H2 and H3 of one Umbrian Company farm, in which broilers were reared at the same conditions for feed (standard pellets broiler *ad libitum*), density (16 birds per m<sup>2</sup>) to 7-8 weeks of age. The buildings were of conventional layout. The litter was cut wheat straw (about 5-10 centimetres thick). In H2 and H3 the ventilation system comprised 2 propeller fans of 40,000 m<sup>3</sup>/hour and 26,000 m<sup>3</sup>/hour capacity each, mounted on the windward side of the poultry house.

#### Treatment

Litter in H2 was treated, every 2 weeks, as follow (since the day before the arrival of the chicken): 2 grams (g.) of additive plus 25 g. of calcium carbonate per  $m^2$  (1<sup>st</sup> month), and after 1 g. per  $m^2$ until the end of the cycle.

#### Additive

The field trials were performed with Calcium sulphate (gypsum) and essential oils (lemon grass and lavender) used as carriers. By the SIRIO OPERATING PROCESS<sup>®</sup> method such components are activated by an energetic modulation process and enriched with oxygen and specific information about litter components.

#### Samples

Litter was sampled one day during the first and the seventh week of each cycle. Composite samples of about 500 g. were obtained from ten different sites within each house and placed immediately into sterile plastic bags, sealed and refrigerated until microbiological evaluation was made.

#### Microbiological analysis

Twenty-five grams of each sample was transferred into a sterile plastic bag and 225 ml of sterile 1% buffered peptone water was added. After treatment with Stomacker Circulator 400 (PBI, Milan) the samples were allowed to sit for 30-45 min at room temperature with frequent shaking. One ml of this samples (1:10 dilution) was diluted serially via 10-fold dilutions (from  $10^{-1}$  to  $10^{-8}$ ). Total aerobic bacteria, Staphylococcus spp. (Staph. spp.), Salmonella spp. and Coliforms in 1 g<sup>-1</sup> of litter were determined by plating, in duplicated, 0.1ml of appropriate dilution on SPGCA (Standard Plate Count Agar), BP (Baird Parker agar) and VRBA (Violet Red Bile Agar). The cultures were incubated at 37°C for 24-48 hr and the number of grown colonies was determined. The Salmonella spp. isolation procedure used in this study included Selenite-Cystine broth and Rappaport-Vassiliadis broth (Oxoid, Milan), as enrichment media and two plating media. The Selenite-Cystine Broth was incubated aerobically at 37°C for 24 hours and the Rappaport-Vassiliadis broth was incubated aerobically at 43°C for 24 hours. The composition of each selective medium is detailed in the Oxoid Manual. Mortality rate was recorded at the end of each eight cycles.

#### Statistical Analysis

The mean values of all parameters evaluated were compared by t-test.

#### **Results and discussion**

The results from eight cycles on treatment of litter are summarised in Table 1 and 2. Significant differences between experimental and control samples with regard both the microbial cell counts (Table 1), and the mortality (Table 2) were observed. Also the bacterial counts of the treated litter were reduced to about 70 % of the control values. Sex strains of *Escherichia coli* (*E. coli*) were isolated throughout the sampling period: 3 strains (1 from H2 and 2 from H3) during 2002-2003, and 3 strains (1 from H2 and 2 from H3) in 2004. Standard procedure were used to identify *E. coli* which do not differentiate between pathogenic and non pathogenic. The number of *E. coli* in the litter, like total aerobic bacteria, *Staphylococcus* 

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|             | Results of some<br>(treated with SC<br>(mean values fr | (treated with SOP <sup>®</sup> C POULTRY) and H3 (control)<br>(mean values from eight cycles are expressed in CFU.g <sup>-1</sup> ). |             |             |           |           |  |  |
|-------------|--|--|-------------|-------------|-----------|-----------|--|--|
| Parameters  | TMC  | TMC  | Staph. spp. | Staph. spp. | Coliforms | Coliforms |  |  |
| Dilution    | 10-8   | 10-8   | 10-8        | 10-8        | 10-6      | 10-6      |  |  |
| Houses      | treated  | control  | treated     | control     | treated   | control   |  |  |
| Mean        | 153.69   |  | 31.14       | 185.48      | 58.05     | 328.34    |  |  |
| t test (P=) | 0.0078   |  | 0.0         | 021         | 0.0541    |           |  |  |
| %           | -63.1  |  | -83         | 3.21        | -82.32    |           |  |  |
|             |  |  |             |             |           |           |  |  |

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% = reduced microbial concentration.

| Table 2. | Mortality rate (%) recorded at the end of eight cycles in H2 and H3 (treated with SOP <sup>®</sup> C POULTRY and control). |                 |                 |                 |                 |                 |                        |                 |  |
|----------|--|-----------------|-----------------|-----------------|-----------------|-----------------|------------------------|-----------------|--|
| Cycles   | 1 <sup>st</sup>  | 2 <sup>nd</sup> | 3 <sup>rd</sup> | 4 <sup>th</sup> | 5 <sup>th</sup> | 6 <sup>th</sup> | <b>7</b> <sup>th</sup> | 8 <sup>th</sup> |  |
| Treated  | 5  | 4.3             | 3.1             | 8.4             | 3.4             | 3.1             | 3.3                    | 3.9             |  |
| Control  | 9  | 4.7             | 4.3             | 10.8            | 5.7             | 3.4             | 5.1                    | 4.5             |  |

P= 0.00106

spp., and Coliforms resulted in treated litter lower than the control house litter.

The treatment of the litter proved to be effective in control of some microbial litter components. Of interest is the reduced mortality rate of broilers because in field conditions the health problems are known to be associated with litter.

Although all litter sampled was examined for *Salmonella* spp. none was found.

Several workers suggested that bird health is harmed by chronic exposure to modest burdens, especially in the presence of simultaneous challenge by respiratory pathogens (Oyetunde *et al.*, 1978; Carpenter *et al.*, 1986), but the concentration of most pollutants often rises in poultry houses as consequence of an increased generation rates from the major sources, that is the birds themselves and particularly the litter, which acts as a nutritious reservoir for microorganisms (Conceição et al., 1989).

#### Conclusions

The control of bacterial population in poultry houses is essential for better health and performance of birds.

The data of the present study seem to indicate a significant reduction of the bacteria evaluated in the treated litter. Based on the results of this field trials, it was concluded that the additive studied during this investigation has an inhibitory effect on the survivals of micro-organisms in broiler house litter.

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