

SUMMARY

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Poultry is one of the most consumed foods both nationally and internationally.

Quality of poultry depends on several factors, such as applied growth technology, insured food, microclimate conditions, etc.

For this reason, in the doctoral thesis entitled "***Contributions to the improvement of the decontamination technology of halls for intensive breeding of broiler chickens***", we intend to study the effectiveness of decontaminants frequently used in poultry farms in Romania, in order to improve the current decontamination protocol, according to the results of in vitro analyzes.

Several objectives were considered in the research:

The objective number 1 was to know the effectiveness of the decontamination protocol applied in the Romanian poultry units (the standard protocol); the particularities of the decontamination protocol working steps and the decontaminants used (concentrations and contact times applied) were taken into account.

The following decontamination substances were used in the standard decontamination protocol:

- DM Cid S in a concentration of 0.5%, contact time of 30 minutes;
- CID 2000 at a concentration of 0.25%, contact time of 30 minutes;
- Virocid at 0.5% concentration, contact time 30 minutes;
- Virkon'S at 1% concentration, contact time 30 minutes;
- CID 20 in a concentration of 0.5%, 30 minutes contact time.

All decontamination steps involving thermonelation were performed at an ambient temperature of $+18^{\circ}\text{C} + 20^{\circ}\text{C}$, as recommended by the manufacturing companies.

During research, the levels of microclimate factors and productive performances of puppies studied were studied:

- the microbial load of the chicken litter areas after the decontamination process, as well as at different growth ages (9, 21 and 35 days, respectively);
- microbial air load in the hall after decontamination and at various ages of broiler chickens;
- the productive performance of the chickens in the studied lots; in order to achieve this goal, they were pursued:

- body weight;
- growth gain in weight;
- consumption of feed;
- feed conversion index;
- mortality.

Results obtained from sanitation tests harvested after decontamination: No evidence has produced a positive result for bacteria of the genus *Staphylococcus spp.*, coliform bacteria and *Enterobacteriaceae*.

Instead, NTG positive samples were found to be 4% for the feed line, 6% for feed bunkers and 5% for the walls of the halls. For NTF, positive samples (5%) were found only at the wall level.

Analysis of the 9-day-old chicks tests indicated that positive samples for *Staphylococcus spp.* Were in a proportion ranging from 4% (feed lines) and 10% (hall walls), those for coliform bacteria between

0% (hall walls) and 10% (watering lines), and for *Enterobacteriaceae* between 5% (hall walls) and 13% (watering lines).

For NTG, the lowest positive samples were 4% (feed bunkers) and the maximum (30%) on the watering lines, while for NTF, most positive samples were on feed bunkers (36%) and the smallest on the watering lines (16.66%).

The results obtained at the age of 21 days in chickens revealed that positive samples were in the proportion of 12-20% for *Staphylococcus spp.*, 10-24% for coliform bacteria, 22-36% for *Enterobacteriaceae*, 23-45% for NTG and 56-85% for NTF.

At 35 days of chickens, the proportion of positive samples collected on the watering lines was 38% for coliform bacteria, 60% for staphylococci, 42% for enterobacteriaceae, 48% for NTG, and 100% for NTF; at the feed lines, the percentages of positive samples were between 42% for coliform bacteria and 100% for NTF, while for the bunker fodder target a lower percentage of positive samples was found except for NTF that was 100% positive samples.

Most positive samples were harvested from the walls of the halls, between 45% (enterobacteriaceae) and 100% (NTF).

The results obtained from analyzes carried out to establish the microbial air load showed that, after decontamination, 10% of the samples had a positive result for both NTG and NTF.

Analyzes for determining the microbial load of air from the growth hall at different ages (9, 21 and 35 days) showed that for both NTG and NTF all samples were positive at each of the three ages where the harvests were carried out.

As far as the productive performances of the chickens are concerned, they were appropriate, exceeding the performance specified in the Ross-308 Hybrid Growth Guide.

Thus, the weight of the chickens in the Lm-1 group at the end of the growing period was 3019.48 ± 16.71 g, and the total growth rate was 2974.32 g/period, resulting in a daily average increase of 70.81 g/head/day; the value of the feed conversion index was 1.633 kg n.c./kg growth.

At the end of the growth period, the total mortality rate reached 2.69%.

Objective number 2 aimed at "in vitro" verification of decontaminants commonly used in poultry houses in our country.

To achieve this, a bacterial strain of *Staphylococcus spp.* and a fungal fungus of *Aspergillus brasiliensis* was used.

The first step was to investigate the bactericidal activity of the decontaminants used, following the contamination of 9 surfaces with a bacterial suspension of *Staphylococcus aureus*, decontamination with the five substances was performed at set concentrations, allowing the substances to act for 10 minutes, 20 minutes and 30 minutes, respectively.

The concentrations used were:

- Virkon'S 0.5%, 1% and 1.5%;
- DM Cid S, 0.25%, 0.5% and 1%;
- CID-2000, 0.25%, 0.5% and 1%;
- CID-20 0.25%, 0.5% and 1%;
- VIROCID 0.25%, 0.5% and 1%.

Analyzing the data obtained, it was found that four of the decontaminants used had a very good decontamination effect at all three prepared concentrations and pre-contact times; Virkon's, Virocid, DM Cid S and CID-2000 respectively.

In contrast, the CID 20 decontaminant, at 0.25% concentration and 10 minute contact time, had an ineffective bacterial activity; at 20 and 30 minute contact times, bacterial activity was appropriate, the specific media plate being sterile (absence of microbial growth); the same corresponding result was also observed at concentrations of 0.5% and 1%.

In the second stage, the fungicidal activity of the decontaminants was verified: after the contamination of the 100 cm² surfaces with the fungal suspension of *Aspergillus brasiliensis*, the decontamination with the five decontaminants prepared at the same concentrations was carried out; the environmental contact time of the substances was 10 minutes, 20 minutes and 30 minutes, respectively.

After analyzing the results obtained, it was found that Virkon'S solution at the 0.5% concentration had ineffective fungicidal activity for all three contact times; In contrast, Virkon'S at a concentration of 1% and 1.5%, respectively, had a corresponding fungicidal activity, even at the shortest contact time (10 minutes).

The other decontaminated substances had adequate results for all concentrations used and for all contact times.

Objective 3 was directed to the production-based verification of the optimized decontamination protocol based on in vitro results, using the following substances:

- ▶ DM Cid S at a concentration of 0.25%, contact time 10 minutes;
- ▶ CID 2000 in a concentration of 0.25%, contact time 10 minutes;
- ▶ Virocid at a concentration of 0.25%, contact time 10 minutes);
- ▶ Virkon'S 1% in 1% concentration, contact time 10 minutes;
- ▶ CID 20 concentration 0.5%, contact time 10 minutes.

The differences between standard decontamination protocol and optimized protocol were as follows:

- changing the concentration of Virocid and DM Cid S solutions from 0.5% to 0.25%;
- reducing the contact time from 30 minutes to 10 minutes;
- increase of the temperature in the hall, from +18°C to +25°C, which causes the fog resulting from thermonebulisation to persist for longer (+18°C, mist falls faster on the surface of the floor, contact with airborne microorganisms is shorter in time).

The results of the tests collected after the decontamination showed that all samples analyzed for the identification of *Staphylococcus* genus, coliform bacteria and enterobacteriaceae had negative results regardless of where they were harvested.

In contrast, for NTG, positive samples were 1.66% for watering lines, 2% for feed lines, 4% for bunk feed and 5% for samples taken from walls of halls; the identification of the total number of fungi revealed positive samples only for the walls of the halls (5%).

The samples collected at the age of 9 days for the identification of bacteria of *Staphylococcus spp.* Showed that the percentage of positive samples ranged between 2% (feed lines) and 5% (watering and walls), for coliform bacteria between 4% fodder lines) and 8.33% (watering lines) and samples for bacteria in the *Enterobacteriaceae* family between 4% (feed lines) and 10% (watering lines).

NTG had the highest percentage of positive samples (25%) on watering lines and the smallest (4%) at feed bunkers, while NTF recorded a maximum of positive samples at feed lines (36%), and a minimum (5%) for watering lines.

At 21-day-old chicks, the share of positive samples for *Staphylococcus spp* oscillated between 10% (feed bunkers) and 20% (hall walls), for coliforms the variation limits were 10% (the walls of the hall)

and respectively, 20% (feed lines), while for *Enterobacteriaceae* positive samples were found between 15% (hall walls) and 38.33% (water lines).

In the case of NTG, the proportion of positive samples ranged between 28% (feed bunkers) and 40% (the walls of the hall), and for NTF, between 45% (the walls of the hall) and 83,33% (watering lines).

The results obtained at the age of 35 days in chickens showed that the percentage of positive samples reached a maximum of 50% (watering lines) for *Staphylococcus spp.*, 74% (feed bunkers) for coliform bacteria and 40% (lines of fodder) for *Enterobacteriaceae*; NTG exhibited 60% positive samples (hall walls), while NTF had 90% positive sample values (hall walls).

Analyzes performed to establish the microbial air load in the hall showed a significant decrease after completion of the decontamination process, 90% of the analyzed samples having a negative result for NTG, while no positive test was recorded for the NTF parameter; on the other hand, the results of the samples collected at the three pups' ages indicated a 100% positive sample for both NTG and NTF.

In the chickens of the Lexp-1 group, raised in the decontaminated hall according to the optimized protocol, the mean weight at the end of the series was 3028.83 ± 15.07 g.

The total weight gain was 2983.63 g/cap/period, and the daily average gain of 71.03 g/cap/day.

The feed conversion rate achieved by chickens in this batch was 1,647 kg n.c./kg growth.

For the total study period (1-42 days), the share of puppies in the Lexp-2 lot was 2.57%.

Objective number 4 was designed to compare the performances of the chickens reared in decontaminated halls after the standard protocol (Lm-2) with those of the chicks housed in decontaminated halls after the optimized protocol (Lexp-2).

The results obtained in the tests carried out after decontamination showed that the percentage of positive samples was 0% for staphylococci, coliforms and enterobacteriaceae in both batches.

At the Lm-2 lot, NTG and NTF had positive results for all 4 tested targets, with limits between 1.67% (watering lines) and 6% (feed bunkers) for NTG and respectively between 2 % (feed bunkers) and 10% (hall walls) in the NTF.

The results of the sanitation tests analyzed for the Lexp-2 lot house indicate lower percentages of positive samples against the Lm-2 group; thus positive samples were found for the NTG parameter only on feed bunkers (2%), while for the NTF parameter the number of positive samples was zero.

As a result of the tests carried out on the 9 day old chicks, the maximum positive samples for *Staphylococcus spp.* Were 20% (hall walls) in the Lm-2 group and 15% (hall walls) in the Lexp lot -2.

For coliform bacteria, the maximum percentage of positive samples was 15% (hall walls) in the Lm-2 group and 6.67% (watering lines) at Lexp-2; bacteria in the *Enterobacteriaceae* family had a maximum positive sample of 20% (hall walls) in the Lm-2 group and 11.67% (watering lines) at Lexp-2.

Regarding NTG, 12% positive samples (feed lines) and 20% (watering lines) were identified in the Lm-2 lot, while in the Lexp-2 lot the limits were between 10% (the bunkers feed) and 15% (watering and walls).

For the NTF parameter, positive sanitation samples were between 25% (watering and walls) and 40% (feed lines) in the Lm-2 group and between 6% (feed lines) and 30% (hall walls) in the Lexp-2 lot.

The results obtained in the 21-day-old chicks tests showed that the percentage of positive samples increased for all five parameters analyzed; thus, 16-25% samples were positive for *Staphylococcus spp.* in lot Lm-2 and 8.34-20% at Lexp-2; 16.67-26% positive samples for coliform bacteria in the Lm-2 group and 13.34-18% for the Lexp-2 lot; for *Enterobacteriaceae* the positive samples varied between 10-20% in the Lm-2 lot and 12-20% in the Lexp-2 lot.

For NTG, a maximum percentage of positive samples of 50% (hall walls) in the Lm-2 group and 45% (hall walls) were obtained in the Lexp.-2 lot, while the minima were 28.34% (lines watering) at Lm-2 and 11.67% (watering lines), respectively, at the Lexp-2 lot.

For NTF, a maximum percentage of positive samples of 75% (watering lines) in the Lm-2 group and 73.34% (watering lines) in the Lexp-2 lot were identified.

Assays at 35-day-old chicks showed a maximum of 70% (house walls) in Lm-2 and 61.67% (water line) at Lexp-2 for *Staphylococcus spp.*

For coliform bacteria, a maximum of 70% (hall walls) positive samples for Lm-2 were found, and 55% for Lexp.-2 (hall walls); *Enterobacteriaceae* reached 42% positive samples (feed line) for Lm-2 and 36% positive samples (feed line) for Lexp-2.

NTG in the Lm-2 lot hall had a maximum of 60% positive (hall walls), and for Lexp-2 55% (hall walls); the smallest values were found on feed bunkers, 42% in Lm-2 and 38% in L-exp-2.

NTF recorded a very high proportion of positive samples, with levels of 86-96.67% for the Lm-2 group and 75-95% for the Lexp-2 group respectively.

The results of analyzes performed to establish the microbial air load indicated 10% positive samples for NTG and 8% positive samples for NTF in lot Lm-2 and 9% positive NTG positive samples and 6% positive samples for NTF at the Lexp-2 lot.

From the first sampling of air samples (9-day-old chicks), all the results were positive, a phenomenon valid for the 21st day of chickens and 35th tests respectively; this is a consequence of the fact that the decontamination of maintenance is not practiced in the hall.

Both batches analyzed showed 100% positive samples at all three age ranges at which sampling was performed.

The mean weight at the end of the growth period (42 days) was 3017.78 ± 16.68 g in the chickens of the Lm-2 lot and 3018.35 ± 14.76 g in the Lexp-2 lot.

The feed conversion index had values of 1.43 kg n.c./kg increase in chickens belonging to the Lm-2 lot and 1.42 kg n.c./kg growth in the Lexp-2 lot.

Batch outflows were below the maximum allowed by the Ross-308 Growth Guide (5%), being only 2.92% in chickens in the Lm-2 group and 2.78% in the Lexp -2 group.

Based on the results of the four series of experiments, some recommendations for poultry practice were formulated as follows:

- applying an optimized decontamination protocol to the chickens for raising broiler chickens because, in addition to good antimicrobial efficacy, it reduces the amount of substances used and working times (for DM Cid S and Virocid concentrations of 0.25% versus 0, 5% as recommended, for all substances contact times of 10 minutes versus 30 minutes as recommended);
- providing a temperature of +25°C during the decontamination of the halls (thermoneblowing substances) as it prolongs the "fog effect" favorable to the antimicrobial and antifungal activity;
- pre-laboratory testing of decontaminants to be purchased, as the manufacturer's recommended concentrations are safe, without taking into account the economic efficiency of farms; in addition, the use of lower amounts/concentrations of chemicals reduces the degree of contamination of the product (poultry meat) and protects the environment;
- regularly performing maintenance decontamination in broiler chambers to ensure good health and the possibility of externalizing their productive potential.