

# “THE SANITATION EFFECT OF ELECTROLYZED WATER (NEUTRAL ANOLYTE -ANK) ON PATHOGEN AGENTS FROM LIVING SPACE AND FEEDSTUFFS USED IN LAYING HENS NUTRITION”

*Authors: I. SURDU<sup>1</sup>, IOANA VĂTUIU<sup>2</sup>, ȘTEFANA JURCOANE<sup>3</sup>, MARGARETA OLTEANU<sup>4</sup>, DANIELA VĂTUIU<sup>2</sup>*

*S.C SIAT S.A. Bucharest<sup>1</sup>, Applied Research & Investment Company S.A. Bucharest<sup>3</sup> Microbial Biotechnological Center Bucharest<sup>3</sup>, National Research and Development Institute for Zootechnie Balotesti<sup>4</sup>*

## ABSTRACT

*One of the most important indicators of the laying hen digestive micro flora and also with excrement contamination levels, is represented by the bacteriological water quality combined with feedstuffs and mixed diets quality. Numerous previous and preventable studies performed, against those with incidental epidemiology cases have highlighted the fact that water is a major provider of pathogen agents with negative effect, given by various bacteria, such as : Staphylococcus aureus, Escherichia coli, Cryptosporidium, Giardia lamblia, Listeria and Legionella.*

*The objective of the work consist in studying the sanitation effect of neutral anolit acid (ANK) on pathogen agents from, both living space and feedstuffs respectively, used to laying hens nutrition.*

*It has used the following solutions of neutral anolit acid in water, like:*

*I. ANK solution with an active chlorine content of 8mg/l and 12mg/l respectively, used as drinking water for laying hens;*

*II. ANK solution with an active chlorine content of 8mg/l and 12mg/l respectively used for treatment the seven types of feedstuffs (maize, barley, maize gluten maize, rice, barley, soybean meal and canola meal), three mixed feeds, and five types of eggshells, respectively. Treatments consisted in the immersion for 15' of each sample in ANK solution.*

*III. ANK solution with an active chlorine content of 36mg/l used for accommodation space treatment by washing it for 15 '.*

*Microbiological analyses were performed in order to determine the Total number of germs, Coli forms and fungi per each sample.*

*The three ANK solutions used in experiments had a total bactericidal effect on the Total Coliforms. In the same time, the total number of germ decreased until to 30 times against the control. The experiment highlights the role of active chlorine on the microbiological load in the water, feedstuffs, mixed diets, eggshells and accommodation space. As the concentration of active chlorine is much higher as more significant is the bactericidal effect of the ANK solution.*

*These experiments proved that the ANK solution could be used as disinfectant agent to control the microorganisms from water, feedstuffs, laying hens living spaces and on the surface of eggshells.*

**Key words:** *electrolyzed water, Neutral Anolyte ANK, bactericidal effect, electrochemically activated substances*

## INTRODUCTION

The most important indicators of the laying hen digestive micro flora also in relation with excrement contamination level are determined by the bacteriological water properties combined with feedstuffs and mixed diets quality. Numerous previous and preventable studies performed, against those aspects with incidental epidemiology cases have highlighted the fact that water is a major provider of pathogen agents with negative effect, given by various bacteria, such as: *Staphylococcus aureus*, *Escherichia coli*, *Cryptosporidium*, *Giardia lamblia*, *Listeria* and *Legionella*.

Pathogen agents are simple growing in feedstuffs, in living space of laying hen, on cages and on the eggshells also.

As results of studies performed by scientists from USA and Japan, the electrolyzed water became a major sanitation product used in food industry.

Electrolyzed water can play also an important role in control of pathogen microorganisms from fresh vegetables (tomatoes, cucumber, lettuce, etc). Due to it, low production cost, good bactericidal and ecological properties, in the latest years the electrolyzed water is using on large scale in all kind of animal farm.

Recent studies performed in order to reduce *Escherichia coli* and *Listeria monocytogenes* from plastic feeding tools and living space for chickens have showed that spraying the surfaces with electrolyzed water (30-50 mg/l active chlorine) has as effect the inactivation of the pathogen agents. By using, the electrostatic spraying technique of electrolyzed water on eggs has more efficiency in removing the pathogen agents from eggshell (*Salmonella Typhimurium*, *Stafilococcus aureus*, *Listeria Monocytogenes*).

Our tests performed in experimental stand with electrolyzed water from INCDZ Balotesti have as objectives evaluating the effect of electrolyzed water (neutral anolyte ANK) on microbial load from feedstuffs and mixed diets used in laying hen nutrition, from living space, cages and eggshell.

## MATERIALS AND METHODS

The sample of electrolyzed water has been produced by using an Envirolite EL 400 unit. A 25% sodium chloride solution and tap water was simultaneously pumped into unit to obtain neutral anolyte ANK with the following characteristics: pH = 7,42; ORP(Redox potential) = 845 mV, Active chlorine = 445mg/l. The active chlorine quantity from ANK was adjusted to 445 mg/l by keeping the amperage in cell to a value between 23-25 A and the pH was adjusted to 7,40 by reducing/rising the quantity of catholyte evacuated as residue.

Solutions with a content of 8mg/l, 12 mg/l and 36mg/l active chlorine were obtained by dilution the ANK with distilled water like: 18 ml ANK and 982 ml distilled water, 27 ml ANK and 973 ml distilled water respectively 81 ml ANK and 919 distilled water. The percentages of ANK in water are 1,8%, 2,7% and 8,1% ANK respectively.

ORP and pH measurements have been performed with YSI Professional Plus instrument.

Active Chlorine measurements have been performed accordingly to the Envirolite procedure, as follows:

**Method:** *Iodine titration*

**Protocol:**

Anolyte Solution: 10 ml

H<sub>2</sub>SO<sub>4</sub> 1N: 50 ml

KI: few crystals

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Titration with Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> x 5 H<sub>2</sub>O solution 0.1 N  
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Calculus of active chlorine, as concentration  
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Table nr. 1

Volume solution Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> x 5 H <sub>2</sub> O for titration (ml)	Active chlorine concentration (mg/l)
0,58	200
0,85	300
1,40	500
2,00	700
2,10	750
2,25	800
2,40	850
2,50	900
3,00	1065
3,50	1240
4,00	1420

In order to evaluate the effect of ANK on microbial load from feedstuffs and mixed diets used in laying hen nutrition, from living space, cages and eggshells were taken the following samples:

I) Feedstuffs ( 7 samples): wheat, maize, gluten maize, rice, barley, soybean meal and canola in quantity of 250 g /sample.

II) Mixed diets for laying hens ( 3 samples): mixed diet for control lot – MDC, mixed diet for lot E1 – MDE1 and mixed diet for lot E3 – MDE3;

III) Eggs samples (four eggs/lot – from five lots) like:

“Control lot” (diet MDC) – laying hens that were feeded on with tape water.

“Level 1 – E1 lot” (diet MDE1) - laying hens that were feeded on with ANK solution and a content in active chlorine of 8mg/l.

“Level 1 – E3 lot” (diet MDE3) - laying hens that were feeded on with ANK solution and a content in active chlorine of 8mg/l.

“Level 2 – E1 lot” (diet MDE1) - laying hens that were feeded on with ANK solution and a content in active chlorine of 12mg/l.

“Level 2 – E3 lot” (diet MDE3) - laying hens that were feeded on with ANK solution and a content in active chlorine of 12mg/l.

IV) Sanitation samples on the floor (4 samples): two before and two after washing for 15’ the floor with ANK, 36mg/l active chlorine.

On the feedstuffs and mixed diets, samples were performed microbiological analysis for two indicators: “Total number of germs” (NTG) respectively “Yeast and fungi”.

To determine “Total number of germs” were used the procedures from standard SR EN ISO 4833-2003.

To determine “Yeast and fungi” were used the procedures from standard SR ISO 7954-2001.

On the eggs samples were performed microbiological analysis for one indicator: “Total number of germs” (standard SR EN ISO 4833-2003)

On the sanitation samples were performed microbiological analysis for two indicators according to the actually sanitary veterinary regulations like: “Total number of germs” (standard SR EN ISO 4833-2003) and “Total coliform bacteria” (SR ISO 4831- 2006).

All samples were seeded in Petri dish, doubled, with the dilution of  $10^{-1}$ ,  $10^{-2}$ ,  $10^{-3}$  and  $10^{-4}$ .

The laboratory analyses were performed on four sorts of samples with 2-3 stages for each sort.

#### *THE FEEDSTUFFS AND MIXED DIET ANALYSIS*

In first stage were performed the initial microbial load for each sample.

In the second stage, each sample composed from 10 g substance was treated 15’ with 90 ml ANK solution containing 8mg/l active chlorine and then were performed tests to determine the two microbiological indicators.

In the third stage, each sample composed from 10 g substance was treated 15’ with 90 ml ANK solution containing 12mg/l active chlorine and then were performed tests to determine the two microbiological indicators.

#### *THE EGGSHELL ANALYSIS*

In first stage were performed the initial microbial load (NTG) for four eggs from each lot.

In the second stage, two eggs from witness lot were immersed for 15’ in 200 ml ANK solution containing 8 mg/l active chlorine than were performed tests to determine the microbiological indicator (NTG).

In the third stage, two eggs from witness lot were immersed for 15’ in 200 ml ANK solution containing 12mg/l active chlorine than were performed tests to determine the microbiological indicator (NTG).

#### *THE SANITATION TESTS ANALYSIS*

In first stage were performed the microbial load for two samples taken before washing the floor.

In the second stage were performed the microbial load for two samples taken after washing the floor.

## **RESULTS AND DISCUSSIONS**

#### *THE FEEDSTUFFS AND MIXED DIETS ANALYSIS*

In the Table nr.2 are showed the initial value of microbiological indicators for feedstuffs.

**Table nr.2**

SAMPLE Feedstuffs	MICROBIOLOGIC INDICATORS	
	Total number of germs/g	Yeast and fungi/g
1.Wheat	50000	7000
2. Maize	30000	100000
3. Maize gluten	10000	1000
4. Rice	300000	10000
5. Barley	60000	2000
6. Soybean meal	70000	3000
7. Canola meal	400000	300000

In the Table nr.3 are showed the value of microbiological indicators for feedstuffs after the treatment applied for 15’ with ANK solution containing 8mg/l and 12mg/l active chlorine.

**Table nr.3**

SAMPLE 10g feedstuff + 90ml ANK	MICROBIOLOGIC INDICATORS			
	Total number of germs/g		Yeast and fungi/g	
	ANK 8mg /l	ANK 12mg /l	ANK 8mg /l	ANK 12mg /l
1.Wheat	40000	3000	6000	5000
2. Maize	20000	10000	10000	9000
3. Maize gluten	1000	300	100	30
4. Rice	30000	20000	1000	900
5. Barley	50000	30000	200	180
6. Soybean meal	60000	50000	300	200
7. Canola meal	300000	200000	3000	2000

In the Table nr.4 are showed the initial value of microbiological indicators for mixed diets.

**Table nr. 4**

SAMPLE Mixed diets	MICROBIOLOGIC INDICATORS	
	Total number of germs/g	Yeast and fungi/g
	Lot Control – MDC	200000
Lot E1 – MDE1	250000	400000
Lot E3 – MDE3	140000	500000

In the Table nr.5 are showed the value of microbiological indicators for mixed diets after the treatment applied for 15' with ANK solution containing 8mg/l and 12mg/l active chlorine

**Table nr. 5**

SAMPLE 10g mixed diets + 90ml ANK	MICROBIOLOGIC INDICATORS			
	Total number of germs/g		Yeast and fungi/g	
	ANK 8mg /l	ANK 12mg /l	ANK 8mg /l	ANK 12mg /l
Lot Control – MDC	180000	140000	270000	200000
Lot E1 – MDE1	200000	170000	300000	230000
Lot E3 – MDE3	120000	100000	400000	350000

#### *THE EGGSHELL ANALYSIS*

In the Table nr.6 are showed the initial value of NTG for eggshells samples.

**Table nr. 6**

SAMPLE Eggshell	MICROBIOLOGIC INDICATOR
	Total number of germs/g
Witness (Control)	80000
L1 E1	50000
L1 E3	20000
L2 E1	17000
L2 E3	14000

In the Table nr.7 are showed the value of NTG for eggs from witness lot after the treatment applied for 15' with ANK solution containing 8mg/l and 12mg/l active chlorine

Table nr.7

SAMPLE	MICROBIOLOGIC INDICATOR	
	Total number of germs/g	
Eggs from witness lot	ANK 8mg /l	ANK 12mg /l
Witness (Control) lot	40000	30000

### THE SANITATION TESTS ANALYSIS

The microbiologic analysis results for sanitation tests are showed in the Table nr.8.

Table nr.8

SAMPLE	MICROBIOLOGIC INDICATORS	
	Total number of germs/cm <sup>2</sup>	Total coliform bacteria / cm <sup>2</sup>
Floor Sanitation test before washing	1000 / cm <sup>2</sup>	+
Sanitation test after washing for 15' with ANK ,36g/l active chlorine	500 / cm <sup>2</sup>	-

## CONCLUSIONS

- It was taken samples of de feedstuffs, mixed diets and eggs from INCDZ Balotesti experimental stand with electrolyzed water. For all samples were determined the initial microbial load. The initial value of NTG from feedstuffs varies in a large range, between 10000 and 400000, with the highest value for canola meal. The initial value of NTG from eggshells varies in a small range, between 14000 and 80000, with the highest value for eggs from witness lot.

- The samples were treated for 15' with ANK solution containing 8mg/l respectively 12 mg/l active chlorine.

- The two ANK solutions used in experiments had a bactericidal effect reducing the NTG from feedstuffs until thirty times.

- Feedstuffs and mixed diets with a higher microbial load need an ANK solution with high contents of active chlorine until 15mg/l.

- To the laying hens that were feeded on with ANK solution for least one month the NTG from eggshells is significantly lower than those provided by the witness lot. Te NTG value is lower and lower with the increasing the active chlorine from drinking water. In farm applications the content of active chlorine from drinking water have to be under 15mg/l. A higher content of active chlorine could affect the nutritional metabolism of laying hens. We can conclude that the ANK solution supplied to laying hens in percentage of 1,5%-3% in drinking water reduces the content of germs even from inside the laying hens' body, diminishing the potential of eggs contamination.

- It was taken samples from the floor before and after washing for 15' with ANK solution containing 36mg/l active chlorine. The reduction of NTG/cm<sup>2</sup> was for two times and the Coliforms were total removed. As technological method, ANK can be used as disinfecting agent by spraying for 15-30' weekly, the living space of laying hens. Cold fog of ANK could be applied with or without the laying hens inner. In the presence of hens, the concentration of active chlorine from ANK solution has not to be over 50mg/l to avoid breathing disorder. In order to have a maximum efficiency in the living space without hens the ANK applied as disinfectant is not diluted. Finally, we can conclude that ANK is an unpolluting and economic product that could replace a big part or total the chemical product used nowadays in poultry farm.

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