

## Therapeutic Alternatives to Antibiotic Administration in Veterinary Medicine

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### Abstract

Starting from ONE HEALTH concept of integrated medicine in which human, animal and environmental health are approached together, this paper presents the attempts of ROMVAC Co. to reduce the risk of the occurrence and spread of antibiotic-resistant germs (ARGs) originating from the digestive tract of broiler chickens and mammary glands of dairy cows. The purpose is to demonstrate the efficacy of alternative veterinary medicines to antibiotic therapy in avian enteritis and dairy cow mastitis.

*Enterococcus faecium* NCIMB 11181 culture at a minimum concentration of  $1 \times 10^8$  CFU / ml; Ross 308 broiler chickens (250,000 heads); automatic dosing system in water flow - Dosatrom. The product was administered during the first 3 days of life and the body weight evolution was monitored at 0, 7, 14, 21, 28 and 35 days. Oils with strong antibacterial and antiviral effects (oregano, thyme, pine etc.) were used by ROMVAC to produce three medicines: a) Greenvet mastirat - gel, curative, b) Ugeroclean - S, liquid soap and c) Ugeroclean - gel applied after milking. The products were administered locally to mammalian animals (cattle, sheep, goats) daily for 7 days. Udder edema, milk production, dermal reaction to the product (erythema, nodules, pustules, etc.) and clinical status of the animals were daily monitored.

Chickens that received probiotic had a weight gain of 68 grams / chicken (over 6 tons per 100,000 chickens) and a lower mortality rate of 0.78% (1794 chickens). *E. coli* and *Salmonella germs* have completely disappeared from the digestive tract.

Vegetable oil products improved clinical symptoms of mastitis, were well tolerated by animals, decreased the intensity of erythema, improved the clinical condition of animals, maintained milk production, very few cases requiring antibiotic treatment. The product is also an insect repellent. The in vitro antiviral activity of vegetable oils on CDV paramyxovirus has also been demonstrated. Replacement of antibiotic treatment in veterinary medicine with alternative therapies avoids the occurrence and transmission to the human consumer of ARGs via chicken and milk.

**Keywords:** ONE HEALTH concept, antibiotic resistance, avian enteritis, cow mastitis

### Резюме

Във връзка с концепцията на интегративната медицина за ЕДИННО ЗДРАВЕ, в която се предприема единен подход към здравето на човека, животните и околната среда, настоящата статия представя опитите на ROMVAC Co. да намали риска от появата и разпространението на резистентни към антибиотици микроорганизми, произхождащи от храносмилателния тракт на бройлерни пилета и млечните жлези на крави. Целта е да се демонстрира ефикасността на ветеринарни лекарства, алтернативни на антибиотичната терапия на птичия ентерит и мастита на кравите. Култури от *Enterococcus faecium* NCIMB 11181 при минимална концентрация  $1 \times 10^8$  CFU / ml; бройлерни пилета Ross 308 (250,000), автоматична дозираща система във воден поток Dosatrom. Продуктът се прилага през първите 3 дни от живота и се следи развитието на телесното тегло на 0, 7, 14, 21, 28 и 35

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ден. ROMVAC използват етерични масла със силни антибактериални и антивирусни ефекти (риган, мащерка, бор и др.), за производството на три лечебни препарата: а) Greenvet mastirat - gel, лечебен, б) Ugeroclean - S, течен сапун и с) Ugeroclean – гел за приложение след доене. Продуктите се прилагат локално върху бозайници (говеда, овце, кози) ежедневно в продължение на 7 дни. Ежедневно се прави проверка за едема на вимето, млекопродукция, кожни реакции към продукта (едема, нодули, обриви и т.н.). Пилетата, които са получили пробиотика, наддават на тегло 68 г/пиле (повече от 6 тона за 100 000 пилета) и са с по-ниска смъртност - 0.78% (1794 пилета). От храносмилателния им тракт напълно са изчезнали *E. coli* и *Salmonella*.

Продуктите от зеленчукови етерични масла подобряват клиничните симптоми на мастита, добре се понасят от животните, намаляват интензивността на еритемата, подобряват клиничното състояние на животните, поддържат продукцията на мляко и само в единични случаи се налага приложението на антибиотици. Освен това продуктът е репелент за насекоми. Демонстрирана е също *in vitro* антивирусната активност на зеленчуковите масла върху CDV парамиксовирус. Чрез заместване на антибиотичното лечение във ветеринарната медицина с алтернативни терапии се избягва появата и разпространението чрез пилетата и млякото на антибиотик-резистентни микроорганизми към консумиращите хора.

## Introduction

Antibiotic resistance is such a worrying phenomenon worldwide that it is already being talked of a post-antibiotic era (Alanis, 2005). One of the many causes of antibiotic resistance is the excessive and unjustified use of antibiotics in intensive animal husbandry. Daily man-made products, such as meat and milk, can be sources of multi-resistant bacteria that arise from repeated exposure to a wide range of antibiotics.

Devoted to the “ONE HEALTH” concept, in which human, animal and environmental health forms a whole, ROMVAC Co. has initiated a program to create new products that reduce the need for antibiotics in animal husbandry. This paper presents two distinct solutions dedicated to reducing the use of antibiotics in the control of intestinal infections in broiler chickens and ruminants’ mastitis, the major diseases that cause widespread use of antibiotics in these species (Pol and Ruegg, 2007). ROMVAC Co. has developed the BIOENTEROM product, a probiotic containing live bacteria of the genus *Enterococcus faecium* strain NCIMB 11181, usable from the first day of life of broiler chickens, for the rapid colonization of the digestive tube with a beneficial flora, with a multiplication rate of 18 minutes/generation, capable to block cellular receptors in the gut for pathogenic bacteria. The Greenvet series was also produced. It is intended for the prevention and treatment of mastitis in ruminants and consists of three products: Greenvet mastirat - curative gel, b) Ugeroclean - S, liquid soap and c) Ugeroclean - protective gel, applicable after milking. They are based on very strong antibacterial vegetable oils (oregano, thyme, pine, etc.).

Starting from ONE HEALTH concept, this paper presents the attempts of ROMVAC Co. to reduce

the risk of the occurrence and spread of antibiotic-resistant germs (ARGs) originating from the digestive tract of broiler chickens and mammary glands of dairy cows. The purpose is to demonstrate the efficiency of using these products as an alternative in veterinary medicine to the excessive use of antibiotics in the treatment of avian enteritis and mastitis in ruminants.

## Materials and Methods.

### *Bioenterom products*

The BIOENTEROM product was tested in an industrial farm, on 234,000 Ross 308 broiler chickens, compared to a similar flock benefiting from the classic probiotic free growth technology. The chickens are raised in halls with the capacity of 18,000 heads/hall, and the population density was 18 chickens/square meter. For the test lot, cultures of *Enterococcus faecium* NCIMB 11181, with a minimum concentration of  $1 \times 10^8$  CFU / ml, were administered in drinking water in a concentration of 0.2-2%, using DOSATRON, an automatic drug dosing system for flow water. The product was administered daily in the first 3 days of life. In the control group, for the first 3 days of life, chickens received Enrofloxacin 10% (1ml/l) and Colicrid (0.5ml/l) in drinking water. Average body weights were recorded on the first day and then at 7, 14, 21, 28 and 35 days. The mortality rate was also recorded and bacterial examinations of intestinal flora were carried out in dead chickens.

### *Greenvet products*

In order to obtain GREENVET products, a three-stage process was designed: a) establishing the bacteriological and/or fungal profile of mastitis in some ruminant species; b) *in vitro* testing of substances with antibacterial activity (Aiemsraad *et al.*, 2011); and c)

clinical testing of products resulting from the combination of effective components in in vitro testing.

There were ruminants (bovine, ovine and goats) with mammalian clinical signs from which milk was harvested. The samples were examined bacteriologically and mycologically. Isolated bacterial strains were identified based on morphological and biochemical characteristics using the APIWEB (Biomerieux) and ABIS online software (www.tgw1916.net) and tested for antibiotic sensitivity determination. Antibiotics against which sensitivity was assessed were: Gentamycin, Neomycin, Enrofloxacin, Colistin Sulfate, Amoxicillin, Ampicillin, Oxitetracycline, Doxycycline, Erythromycin, Sulfamethoxazole + Trimethoprim, Florfenicol, Novobiocin, Lincomycin + Spectinomycin, Phosphomuscine. Anti-septic oils (pine, juniper, cypress, thyme, oregano, peppermint oil), salvia extract, honey, propolis, kaolin, collagen, vitamin D2, have been used as antibacterial and healing substances. Of these, 8 combinations were performed which were subjected to in vitro testing of the bactericidal effect. The final products were based on the most effective formulas.

## Results and Discussion

### Bioenterom products

Comparative analysis of the results revealed differences in weight and mortality rates. The data are represented in the tables and diagrams below.

Comparative analysis of body weight gain of chickens show, at 35 days, an average increase of 68 g in the group that received BIOENTEROM compared to the control group, which, for a number of 100,000 chickens, is an increase of more than 6 tons of meat (Table 1, Fig. 1).

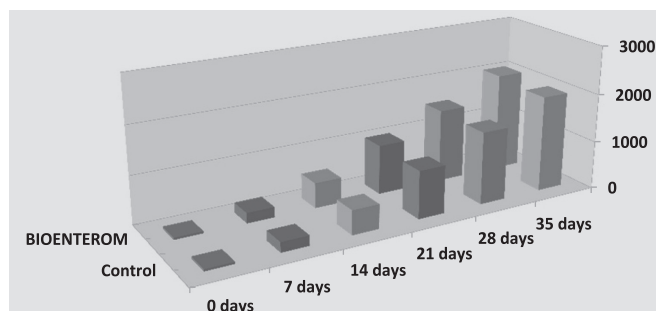
**Table 1.** Evolution of average body weight of chickens

Variant	0 days	7 days	14 days	21 days	28 days	35 days
Control	41.5	208	505	992	1500	1984
BIOENTEROM	41.5	216	526	1031	1521	2052

There is a reduction in mortality in the group receiving the BIOENTEROM probiotic by 0.78% compared to the control group. Also, the bacteriological examination revealed that the BIOENTEROM group did not show germs of the genus *Salmonella* and enteropathogens *Escherichia coli* (Table. 2, Fig. 2).

### Greenvet products

The results obtained from the bacteriological, mycological and antibiotic sensitivity examinations are presented in Table 3.



**Fig. 1.** Evolution of average body weight of chickens (ordinate – average body weight in grams; abscissa – age of broilers in days at the time of body weight measurement).

Of the 12 isolated bacterial strains, 3 species are known as pathogens (*P. aeruginosa*, *E. coli*, *S. chromogenes*) and 4 are opportunistic pathogens (*S. xylosus*, *S. lugdunensis*, *B. licheniformis*, *K. varians*). Following the mycological examination, *Penicillium* spp. was isolated from a bovine milk sample and *Penicillium* spp. and *Cladosporium* spp. were isolated from another bovine milk sample. In the rest of the samples (10) mycological examination was negative.

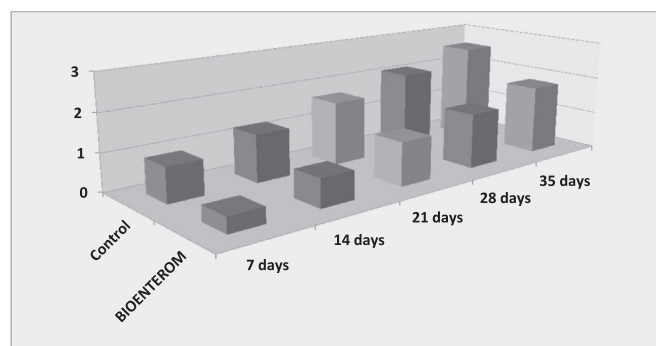
### In vitro testing of formulations obtained

Antimicrobial activity testing of the 8 combinations of active substances (P1-P8 products) was performed against 6 isolated microbial species

**Table 2.** Evolution of broiler mortality

Variant	7 days	14 days	21 days	28 days	35 days
Control	0.92%	1.25%	1.69%	2.12%	2.58%
BIOENTEROM	0.41%	0.74%	1.15%	1.45%	1.81%

from ruminant mastitis, using the dilution method. Minimum Inhibitory Concentration (MIC) is the concentration at which an antibacterial agent



**Fig. 2.** Evolution of broiler mortality (ordinate – rate of mortality (%); abscissa – age of broilers in days at the time of mortality measurement).

completely inhibits the growth of the test microorganism. Serial dilutions of the antibacterial agent were performed in the liquid culture medium (BHI

**Table 3.** Results of bacteriological, mycological and antibiotic sensitivity examinations

No.	Bacteriological exam	Antibiotic sensitivity chart	Mycological exam
1.	<i>Staphylococcus</i> spp., non-hemolytic	Sensitive: FOS, FFC, CN, LS, TY, SXT Moderate: ENR, NV, AML Resistant: DOX, OT, E	negative
2.	<i>S. lugdunensis</i>	Sensitive: LS, CN, FFC, FOS, ENR, SXT, TY Moderate: N, AML Resistant: OT, DOX, E, AMP, AML, NV, E	negative
3.	<i>Staphylococcus</i> spp., non-hemolytic	Sensitive: SXT, AMP, E, CN, ENR, FFC, N, DOX, LS, E Moderate: NV Resistant: AML	negative
4.	<i>S. xylosus</i>	Sensitive: E, AML, AMP, SXT, CN, FFC, ENR, LS, OT, DOX, N Moderate: Resistant: FOS, NV	negative
5.	<i>S. chromogenes</i>	Sensitive: E, AML, AMP, SXT, CN, FFC, FOS, ENR, LS, DOX, N Moderate: Resistant: NV	negative
6.	<i>S. chromogenes</i>	Sensitive: CN, FOS, FFC, ENR, NV, SXT Moderate: LS Resistant: OT, DOX, N, AML, AMP, E	<i>Penicillium</i>
7.	<i>E. coli</i>	Sensitive: CN, FOS, ENR, OT, DOX, N, CT, AML Moderate: FFC Resistant: LS, AMP, E, SXT	negative
8.	<i>Bacillus licheniformis</i>	Sensitive: CN, FFC, FOS, ENR, OT, LS, DOX, N, AML Moderate: NV Resistant: E, AMP	negative
9.	<i>S. xylosus</i>	Sensitive: AML, E, SXT, LS, FFC, FOS, ENR, OT, LS, DOX, N Moderate: Resistant: NV, AMP	<i>Penicillium, Cladosporium</i>
10.	<i>Staphylococcus</i> spp., hemolytic	Sensitive: CN, FFC, FOS, ENR, LS, DOX, N, SXT, NV Moderate: AML Resistant: OT, AMP, E	negative
11.	<i>P. aeruginosa</i>	Sensitive: CN, FOS, ENR, LS, CT Resistant: OT, AMP, AML, DOX, N, SXT, NV	negative
12.	<i>Kocuria varians</i>	Sensitive: ENR, OT, FOS, DOX, AML, AMP, SXT, G, FFC Moderate: N Resistant: NV	negative

**Explanation:** CN - Gentamicin, N - Neomycin, P - Penicillin, ENR - Enrofloxacin, AML - Amoxicillin, AMP - Ampicillin, CT - Colistin, DOX - Doxycycline, OT - Oxytetracycline, SXT - Sulfamethoxazole + Trimethoprim, LS - Lincomycin + Spectinomycin, FOS - Fosfomycin, FFC - Florfenicol, NV - Novobiocin.



glucose broth, red-phenol), to which a suspension of microorganisms was added. After an incubation of 18-24 h, the MIC was identified as the minimal antibacterial agent concentration at which bacterial growth did not occur. The MIC is considered to be the concentration of the antimicrobial agent in the last tube, where there was no change in color from

product with external application, curative in the treatment of ruminant mastitis - especially for the incipient forms previously treated with various drugs containing antibiotics. The product contains: essential oils (pine, thyme, oregano, and mint), kaolin, collagen, vitamin D<sub>2</sub>, natrosol, deionized water. The following formula - at work - will additionally con-

**Table 4.** Results of antimicrobial activity testing in vitro

Microorganisms	MIC of antimicrobial active substances						
	P1	P2	P3	P4	P6	P7	P8
<i>E. coli</i>	1/16	0	1/8	1/16	1/16	1/16	1/16
<i>S. aureus</i>	1/16	0	1/4	1/4	1/16	1/16	1/4
<i>Streptococcus agalactiae</i>	1/16	0	1/16	1/16	1/16	1/32	1/16
<i>Enterobacter cloacae</i>	1/16	0	1/8	1/8	1/16	1/16	1/16
<i>Streptococcus uberis</i>	1/16	0	1/16	1/16	1/16	1/16	1/4
<i>B. licheniformis</i>	0	0	0	0	0	0	0

red to yellow. The results are shown in Table 4.

P1 and P6 products showed high and constant antibacterial activity (1/16 dilution) versus *E. coli*, *S. aureus*, *S. agalactiae*, *S. uberis* and *E. cloacae*. P3 and P4 had a relatively high antibacterial activity, more intense against *S. agalactiae* (1/16), averaging against *Enterobacter* (1/8) and lower than *S. aureus* (1/4). P7 showed high activity against the tested microorganisms (except *B. licheniformis*), the highest activity (1/32) against to *Streptococcus agalactiae*. P8 showed higher activity (1/16) than *E. coli*, *E. cloacae* and *S. agalactiae*, lower (1/8) than *S. aureus* and *S. uberis*, and absent from *B. licheniformis*. None of the products had activity against *B. licheniformis*. Product P2 did not have microbial activity against any tested species.

The P1, P6, P7 products (but also P3, P4, P8) could be used to prevent/control infections with microorganisms resistant to Oxitetracycline, Neomycin, Gentamycin, Erythromycin or other antibiotics.

Clinical testing of the products obtained compared to classical treatment formulas

From the list of volatile oils tested in the laboratory, it was chosen those with a pronounced antibacterial activity (oregano, thyme, pine etc.) from which ROMVAC Co. produced three new drugs: a) a curative medicinal product - Greenvet mastitrat - gel, b) liquid soap - Ugeroclean - S, used to clean the udder before milking and c) protective gel - Ugeroclean - G, a formula applied after milking to form a protective layer at the nipple level (Adukwu *et al.*, 2012).

1. Greenvet mastitrat - gel - is a veterinary

tain different amounts of honey and propolis.

The product restores the elasticity of the udder, prevents its inflammation, moisturizes and revitalizes the skin, improves circulation, prevents irritation during milking, reduces edema and hematoma, also causes a significant increase in milk production. It is recommended for the treatment of incipient forms - curative or advanced - adjuvant, for classical treatment of cattle, buffaloes, goats, sheep, swine, canines etc.

Apply to the entire breast skin or only to the affected quarter, whenever it is needed or after each milking, for 2-3 days as such or complementary to the usual treatment depending on the condition of the condition as mentioned above.

2. Ugeroclean S - liquid soap is a veterinary product with external application. In milking hygiene, an important role is given to the washing and disinfection solution before milking. There are a large number of washing and disinfecting agents on the market before milking. These agents are based on chemicals having a disinfecting or surfactant role for washing. Over time, chemicals are losing their effectiveness against increasingly resistant bacterial strains. For this reason, GREENVET has attempted to test formulations of washing agents based on herbal extracts and volatile oils.

The product contains volatile oils with antiseptic role (pine, juniper, and cypress), sage extract, flaxseed oil, honey, propolis extract, and surfactants. Also, honey and propolis extract have a very good antiseptic action.

3. Ugeroclean G - is a veterinary product with external application after milking, designed to form a protective layer at the nipple level, in order to maintain the health of the udder. These substances make a protective film, stopping the penetration of pathogenic microorganisms inside the udder. The Ugeroclean-G composition consists of: volatile oils (pine, juniper, and cypress), natrosol, menthol, sage infusion, honey, propolis extract. It is recommended to apply a thin film to the nipple, which has antiseptic activity and contributes to the healing of the cutaneous micro-lesions occurring during the milking.

The products were tested in the farm on a herd of 345 cattle, of which: milk cows -190 heads, pregnant heifers - 30 heads, youth 12 - 18 months - 35 heads, youth 6 - 12 months - 30 heads, calves 0 - 6 months - 60 heads.

Approximately 8 to 10% of dairy cows had udder problems. Among the causes were local microbism, mechanical milking system failures, chemicals used for disinfection, lack of movement, environmental factors (rainy weather and lower or very high temperatures) as well as high milk production. At the end of the administration period, the clinical status of most animals was found to improve. In the early mastitis forms, it is effective when administered in a timely manner, with respect to indications, and successfully replaces the use chemotherapy or antibiotics. Thanks to its composition of essential oils, the product also possesses repellent activity for insects reducing the risk of disease spread by insect biting.

## Conclusions

The preventive use of the BIOENTEROM probiotic in broiler ensures rapid colonization of the digestive tract with beneficial flora, blocks the development of pathogenic bacteria, effectively combats bacterial enteritis, removing *E. coli* and *Salmonella* from the digestive tract. Also, protecting the intestinal mucosa by forming a biofilm at this level reduces the absorption of toxins in the digestive tract. The use of BIOENTEROM result-

ed in higher weight gains, lower mortality losses, reduced the spread of pathogenic microorganisms in the environment, and the development of antibiotic-resistant strains.

GREENVET products, applied according to recommendations, produced positive results. The products are effective in maintaining the healthy condition of the udder and protect the animal from insects biting. Products are well tolerated by the animal body with no side effects on the skin. Milk production was not adversely affected after administration.

Replacement of antibiotic treatment in veterinary medicine with alternative therapies is not only possible (Duval, 1995), but gives the advantage to avoid the occurrence and transmission of ARGs to the human consumer via chicken and milk.

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## References

- Adukwu E. C., S. C. H. Allen, C. A. Phillips (2012). The anti-biofilm activity of lemongrass (*Cymbopogon flexuosus*) and grapefruit (*Citrus paradisi*) essential oils against five strains of *Staphylococcus aureus*. *J. Appl. Microbiol.* **113** (5): 1217–1227.
- Aiemsard J., S. Aiumlamai, C. Aromdee, S. Taweechaisupapong, W. Khunkitti (2011). The effect of lemongrass oil and its major components on clinical isolate mastitis pathogens and their mechanisms of action on *Staphylococcus aureus* DMST 4745. *Res. Vet. Sci.* **91** (3): e31-e37.
- Alanis A. J. (2005). Resistance to antibiotics: are we in the post-antibiotic era? *Arch. Med. Res.* **36** : 697–705.
- Duval J. (1995). Treating mastitis without antibiotics. Ecological Agriculture Projects. AGRO-BIO - 370 - 11E, McGill University Canada. <http://eap.mcgill.ca/agrobio/ab370-11e.htm>.
- Pol, M., P. L. Ruegg (2007). Treatment practices and quantification of antimicrobial drug usage in conventional and organic dairy farms in Wisconsin. *J. Dairy Sci.* **90**: 249-261.