

***In vitro* antimicrobial activity of SILVA FEED ENC® on bacterial strains of poultry origin.**

R.GRAZIANI^{1*} G.TOSI² and R.DENTI³

¹ DVM – Via Legnago 9 – 47023 Cesena (FC) – Italy

² Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna – Sezione di Forlì – Via Marchini 1 – 47100 Forlì – Italy

³ Agronomist – SILVA TEAM – Via Torre 7 – S.Michele Mondovì (CN) - Italy

*Corresponding author: feed@silvateam.com

SILVA FEED ENC® (Silva Team – Italy) is obtained by water extraction of the chestnut wood (*Castanea sativa*). It's mainly composed by hydrolysable tannins, lignin, cellulose, hemicellulose and mineral salts. Field observations suggested a role of SILVA FEED ENC® in control of enteric disorders in different poultry (broilers, turkeys, layer hens), cattle and swine productions. Encouraged by these results we extended our studies to evaluate the *in vitro* antimicrobial activity of this compound. An *in vitro* study was carried out using the following bacterial strains isolated in poultry: *Salmonella gallinarum*, *Salmonella enteritidis*, *Salmonella typhimurium*, *Salmonella virchow*, *Escherichia coli*, *Pasteurella multocida*, *Staphylococcus aureus*, *Campylobacter jejuni* and *Clostridium perfringens* type A. In this study different concentrations (0.1%, 0.15%, 0.25% and 0.5%) of SILVA FEED ENC® were used. The following bacterial concentrations were used: 1.2X10⁹ cfu (colony forming unit) and 1.8X10⁶ cfu. Different pre-incubation times (6 and 24 hours) of SILVA FEED ENC® with bacterial strains were also carried out. The antimicrobial activity of SILVA FEED ENC® was observed on *Salmonella gallinarum*, *Pasteurella multocida*, *Staphylococcus aureus* and *Campylobacter jejuni* using the higher bacterial concentration. The *in vitro* activity on *Campylobacter jejuni* was observed after 6 hours of pre-incubation. The *in vitro* activity on *Salmonella enteritidis*, *Escherichia coli* and *Clostridium perfringens* type A was noted using the lower bacterial concentration.

Keywords: tannins, poultry, *in vitro* antimicrobial activity

Introduction

Tannins are classified into two broad groups: the hydrolysable and the condensed or non-hydrolysable tannins. The hydrolysable tannins are usually compounds containing a central core of glucose or other polyhydric alcohols esterified with gallic acid (gallotannins) or hexahydroxydiphenic acid (ellagitannins). The consumption of hydrolysable tannins results in the precipitation of a protein/tannin complex which forms a thin layer of insoluble proteins on the surface of the digestive tract. This thin layer is said to protect the mucous membrane from "irritation" and reduce the absorption of toxic substances. Consequently less fluid is eliminated in the digestive tract, reducing the danger of dehydration. Hydrolysable tannins have antimicrobial properties because of an affinity for membrane proteins and therefore they may have an undefined effect on the flora of the digestive tract. In addition tannins have the potential to aid in the control of gastrointestinal parasites in sheep and goats. SILVA FEED ENC® (Silva Team – Italy) is obtained by the hydrolyzation of the chestnut wood (*Castanea sativa*). It's mainly composed by hydrolysable tannins, lignin, cellulose, hemicellulose and mineral salts. Field observations suggested a role of SILVA FEED ENC® in control of enteric disorders in different poultry (broilers, turkeys, layer hens), cattle and swine productions. This study was undertaken to investigate the *in vitro* effects of SILVA FEED ENC® on the survival and growth of bacterial strains isolated from field cases in different poultry productions.

Materials and methods

General

The experimental work was focused on the study of the *in vitro* antimicrobial activity of SILVA FEED ENC®. For this purpose the following bacterial strains were used: *Salmonella gallinarum*, *Salmonella enteritidis*, *Salmonella typhimurium*, *Salmonella virchow*, *Escherichia coli*, *Pasteurella multocida*, *Staphylococcus aureus*, *Campylobacter jejuni* and *Clostridium perfringens* type A. Bacterial strains were isolated and identified from field cases submitted to Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna – Diagnostic laboratory of Forlì. *Salmonella gallinarum*, *Salmonella enteritidis*, *Salmonella typhimurium*, *Salmonella virchow* and *Escherichia coli* strains were cultured and titrated on Hektoen Enteric Agar. *Pasteurella multocida*, *Staphylococcus aureus* and *Clostridium perfringens* type A were cultured and titrated on Blood Agar. *Campylobacter jejuni* was cultured and titrated on Preston Agar.

Trial 1

Bacterial strains were used at a concentration of 1.2×10^9 cfu/ml. SILVA FEED ENC® was used at two different concentrations (0.1% and 0.25% corresponding to 1 kg/Ton and 2.5 kg/Ton). Before the inoculation on culture media every bacterial strain was pre-incubated with SILVA FEED ENC® for 6 and 24 hours in BHI (Brain Heart Infusion) broth at room temperature. After 24 hours of incubation at 37°C the antimicrobial activity of SILVA FEED ENC® was evaluated observing the presence or the absence of bacterial growth on specific culture media.

Trial 2

For this trial *Salmonella enteritidis* (9.2×10^5 cfu/ml), *Escherichia coli* (1.8×10^6 cfu/ml) and *Clostridium perfringens* type A (1.8×10^6 cfu/ml) strains were used. SILVA FEED ENC® was used at two different concentrations (0.15% and 0.5%). Before the inoculation on culture media every bacterial strain was pre-incubated with SILVA FEED ENC® for 6 hours in BHI (Brain Heart Infusion) broth at room temperature. After 24 hours of incubation at 37°C the antimicrobial activity of SILVA FEED ENC® was evaluated observing the presence or the absence of bacterial growth on specific culture media.

Trial 3

A poultry feed for laying hens (antibiotic and coccidiostatic free) was contaminated with a *Salmonella enteritidis* strain. Before the trial poultry feed was tested to verify the absence of *Salmonella spp.* Two batches of 100g of poultry feed were obtained: batch 1 (contaminated with 1.5×10^9 cfu/ml of *Salmonella enteritidis*) and batch 2 (contaminated with 1.5×10^9 cfu/ml of *Salmonella enteritidis* and treated with SILVA FEED ENC® at a concentration of 0.5%). After 24 hours of incubation at room temperature poultry feed of the different batches were tested for the presence of *Salmonella enteritidis* using the following isolation method: pre-enrichment in Buffered Peptone Water incubated at 37°C for 18 hours; enrichment in Rappaport-Vassiliadis medium with soya incubated at 41.5°C for 24 hours; plating on Hektoen Enteric Agar incubated at 37°C for 24 hours.

Results and Discussion

Results of the trial 1 are reported in Table 1:

Table 1: inhibitory activity against bacterial strains

STRAIN	SILVA FEED ENC® 0.1%		SILVA FEED ENC® 0.25%	
	6h of pre-incubation	24h of pre-incubation	6h of pre-incubation	24h of pre-incubation
S.gallinarum	Not inhibited	Inhibited	Inhibited	Inhibited
S.enteritidis	Not inhibited	Not inhibited	Not inhibited	Not inhibited
S.virchow	Not inhibited	Not inhibited	Not inhibited	Not inhibited
S.typhimurium	Not inhibited	Not inhibited	Not inhibited	Not inhibited
E.coli	Not inhibited	Not inhibited	Not inhibited	Not inhibited
P.multocida	Not inhibited	Inhibited	Inhibited	Inhibited
S.aureus	Not inhibited	Inhibited	Inhibited	Inhibited
C.jejuni	Inhibited	Inhibited	Inhibited	Inhibited
C.perfringens typeA	Not inhibited	Not inhibited	Not inhibited	Not inhibited

Using a very high bacterial concentration the growth of *Salmonella gallinarum*, *Pasteurella multocida*, *Staphylococcus aureus* and *Campylobacter jejuni* was inhibited using SILVA FEED ENC® at a concentration of 0.25% after 6 and 24 hours of pre-incubation. The growth of *Salmonella gallinarum* and *Campylobacter jejuni* was also inhibited using the lower SILVA FEED ENC® concentration (0.1%) and, regarding to *Campylobacter jejuni*, after 6 hours of pre-incubation only. Results of the trial 2 are reported in Table 2:

Table 1: inhibitory activity against bacterial strains

STRAIN	SILVA FEED ENC® 0.15%	SILVA FEED ENC® 0.5%
	6h of pre-incubation	6h of pre-incubation
<i>S.enteritidis</i>	Inhibited	Inhibited
<i>E.coli</i>	Inhibited	Inhibited
<i>C.perfringens</i> typeA	Inhibited	Inhibited

At lower bacterial concentration used in trial 1 the growth of *Salmonella enteritidis*, *Escherichia coli* and *Clostridium perfringens* type A was inhibited using SILVA FEED ENC® at high (0.5%) and low (0.15%) concentrations.

In trial 3 the growth of *Salmonella enteritidis* in batch 2 (infected and treated with ENC® at a concentration of 0.5%) was 90% lower than batch 2 (infected and not treated).

By the end of 2005 the European Union (EU) placed a general ban on the use of antibiotic growth promoters (AGP). Countries outside the EU have already started to reduce the levels of antibiotics in all animal feed and it is anticipated that a global ban on the use of AGP will be implemented in the future. As a result producers worldwide are looking for natural additives to maximise animal health. Enteric disorders and wet litter are serious problems in poultry production. Field observations suggest that SILVA FEED ENC® can improve the intestinal health especially in broiler production probably because less fluid is eliminated in the digestive tract. In addition SILVA FEED ENC® has a regulatory effect on the intestinal flora. Based on the results of this study SILVA FEED ENC® has an *in vitro* antimicrobial activity against bacterial strains involved in avian diseases. The anti-nutritional effects of tannins are related to condensed tannins because of their strong affinity with proteins. SILVA FEED ENC® is rich in hydrolysed tannins that react with protein at low pH and the complexes formed are reversible at the pH conditions found in most of the digestive tract. Further studies are in progress to study the exact mode of action of the SILVA FEED ENC® and his role in control of replication of enteric viruses. In conclusion it appears that SILVA FEED ENC® can be a good alternative to AGP in poultry diets. Moreover the addition of SILVA FEED ENC® will result in better intestinal integrity and will have a protective effect against common pathogens. Finally, SILVA FEED ENC® can be a useful method to control *Salmonella* spp. in animal feed.

Acknowledgments

The excellent technical assistance of Silvano Bussi (Istituto Zooprofilattico di Forlì) is greatly appreciated. Appreciation is expressed to Silva Team for providing the commercial product.

References

- Chung K.T., Wong T.Y., Wei C., Huang Y.W. and Lin Y.** (1998) Tannins and human health: a review. Critical review food science nutrition 38:421-464.
- Min B.R. and Hart S.P.** (2003) Tannins suppression of internal parasites. Journal of Animal Science 81(E.Suppl.):E102-E109.
- Scalbert A.** (1991) Antimicrobial properties of tannins. Phytochemistry 30:3875-3883.