



In order to validate the added value that this product has, a study will be carried out by the Institute of nutrition and food technology INTA. This study consists of 2 protocols, which are detailed below:

1. Protocol 1,

- Isolation and characterization of the pathogenic flora.
- Identification and characterization of non-pathogenic flora.
- In Vitro determination of the susceptibility to copper sulfate of pathogens and native microflora
- Determination of plasmids in fusobacterium strains.

2. Protocol 2,

- Comparison of pathogenic and non-pathogenic flora in shod animals with and without alloy.
- Determination of copper in tissue and serum of animals shod with and without alloy.
- Clinical monitoring of shod animals with and without alloy.



FIRST PROGRESS REPORT

PROJECT: STUDY OF THE ANTIBACTERIAL ACTIVITY OF COPPER HORSESHOES IN THE INFECTIOUS PATHOLOGY OF THE HORSE.

According to the proposed schedule of activities, the sampling began on June 17, 2002. The performance of this activity was coordinated with Dr. Mario Acuña. It was decided to carry out 1 weekly sampling on June 17, 23 and 30. The activities carried out to date are:

1. Acquisition of inputs and culture media
2. Taking samples of horse hooves shod with copper and iron
3. Taking blood and tissue samples
4. Hoof microflora isolation (under development)
5. Identification of isolated bacterial species (in development)
6. Determination of copper in blood and tissue.

Sampling.

As of the date of this Report, the sampling activity has been successfully completed. The 40 samples necessary for both the microbiological study and for the determination of Copper in blood and tissues were obtained.

Bacteriological study.

20 samples of hoofs of horses shod with Iron (100%) and 20 of horses shod with Copper (100%) and copper/zinc alloy (85/15%) were obtained. The 40 samples were cultured in specific media for aerobic and anaerobic microorganisms and are currently in the genus and / or species identification phase.

The microbiological results showed a significantly lower microbial presence in the hooves of horses shod with copper (pure or in alloy) compared to horses shod with iron (Annex 1). So far these results have not been correlated with the time of exposure of horses to copper shoes.

Determination of Copper in tissue and serum.

Samples of 10 horses shod with Copper and 10 shod with Iron were taken to the INTA Microminerals Laboratory to be processed. The determination of copper levels in tissue and serum are in process, so the results are estimated to be available in the second week of July, so they will be incorporated in the second progress report.

Prof. Guillermo Figueroa G., Head of the Microbiology Laboratory INTA-Universidad de Chile

Annex 1. Comparison of the number of colonies obtained in cultures from horse hooves shod with copper (pure or alloy) and iron. Similar culture media and similar incubation times were used.



Photo 1. Sample CH-4. COPPER Horseshoe in MYP medium for detection of *Bacillus* sp



Photo 2. Sample CH-13. IRON Horseshoe in the medium MYP



Photo 3. Shows CH-16. COPPER Horseshoe in Baird Parker medium for *Staphylococcus*



Photo 4. Sample CH-18. IRON Horseshoe in medium Baird Parker



SECOND PROGRESS REPORT

PROJECT: STUDY OF THE ANTIBACTERIAL ACTIVITY OF COPPER HORSESHOES IN THE INFECTIOUS PATHOLOGY OF THE HORSE.

This report describes the results obtained in bacteriological studies of hoof samples and determination of copper in tissue and serum samples.

Bacteriological study.

40 samples of horse hooves were evaluated, of which 20 corresponded to horses shod with iron, 9 with 85.15% copper alloy and 11 with 100% copper.

The results showed that in general, in the hoof samples obtained from horses shod with iron, high bacterial counts and a high frequency of polymicrobial cultures were observed, both of aerobic and anaerobic flora. Unlike the samples from horses shod with copper, in which the counts were low and only involved a few bacterial species (figure 1).

Specifically, regarding the potential pathogens investigated, it was observed:

I. Anaerobic microorganisms

Fusobacterium necrophorum: It was not isolated in any of the samples. *Clostridium*: The presence of species of the genus *Clostridium* were detected only in an experimental sample and in a control, respectively. *Bacteroides*: Species of the genus *Bacteroides*, were also detected only in an experimental and control sample respectively. Regarding anaerobic microorganisms, 24 strains remain to be confirmed.

II. Aerobic microorganisms

Streptococcus β -hemolytic: In neither of the two groups was this pathogen identified.

Staphylococcus aureus: In the group of horses shod with iron, it was isolated in one case.

Enterobacteriaceae: In 100% (20/20) of the samples of horses shod with iron, the abundant presence of various bacterial species was demonstrated, including *E. coli*, *Klebsiella*, *Enterobacter*, *Citrobacter* and *Proteus*. On the other hand, only in 15% (3/20) of the samples of horses shod with copper, little development of enterobacteria was detected.



Bacillus: Of the samples of horses shod with iron, 75% (15/20) found high counts of species of the genus Bacillus, while for the group of horses shod with copper, only 45% (9 / 20) and in lower counts.

III. Filamentous and yeast fungi

The presence of filamentous fungi was demonstrated at similar rates (50%), where if significant differences were detected it was in the bacterial counts, in fact the counts of the horses shod with iron were higher than those shod with copper, in fact in these last only few colonies were observed. Yeast fungi were not detected in any of the animals sampled from both groups.

The identification of the native microflora is in process, initially the presence of a large and varied range of opportunistic bacteria is observed in iron-shod horses.

Determination of copper in tissue and serum.

The copper content analysis was performed on 20 samples (10 from horses shod with copper and 10 shod with iron), the results are summarized as follows:

Serum samples did not show differences in copper content, that is, horses shod with this material and horses shod with iron had similar copper averages, 97.5 and 97 ug / dl respectively.

The analyzes carried out on the surface hull samples (corresponding to the second layer), showed important differences regarding their copper content. This is how horses shod with copper (pure or in alloy) showed on average a higher concentration of this metal in their hoof tissue (270.98mg / 100g) than those shod with iron (14.24mg / 100g).

The determinations made in samples of deep hoof (corresponds to the fourth layer, 1 cm below the superficial sample) of horses shod with copper revealed a higher concentration of this metal (39mg v / s 0.86mg / 100g) despite showing a great variability between different animals.

These results will be correlated later with the exposure time of each of the horses, once the information regarding the date on which they were shod is delivered.



Figure 1. Bacterial development in the Mc medium, a representative plate of the differences observed in hoofs of horses shod with copper (left) and iron (right) is shown.

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ANTIBACTERIAL ACTIVITY OF COPPER HORSESHOES (Antibacterial activity of copper horseshoes) **Rivas P**, Acuña M, Figueroa A, Troncoso M, Cárdenas M, Ruiz M and Figueroa G. Lab. Of Microbiology, INTA, Universidad de Chile - patocarolina@yahoo.es

Infectious pathologies can affect the hoof of horses. Recent studies show that metallic copper or its alloys exert an antibacterial effect on *E. coli* O157: H7, *S. enterica* and *C. jejuni*. Objective: To evaluate the antibacterial activity of copper horseshoes on the agents that cause pathologies in the hoof. Methods: 40 horse hoof tissue samples, 20 copper-shod experimentals and 20 iron-shod controls were evaluated. The presence of aerobic and anaerobic agents was qualitatively identified and quantified. The in vitro susceptibility to SO_4Cu of 11 strains from the experimental group and 12 from the control group was determined. The broth dilution technique was used with SO_4Cu concentrations between 62.5 and 16000 μM . Results: In the group braided with copper there was little microbial development, only 2/20 (10%) had *Klebsiella* while in the control group it was abundant, 19/20 (95%) showed the presence of one or more *Enterobacteriaceae*, with *E. coli* and *Proteus* (60%). In the case of *Bacillus* sp, it was detected in the experimental group in 9/20 (45%) in a small quantity, while in the control group it was isolated in 15/20 (75%) with abundant development. *S. aureus* was only detected in the iron group. With respect to the anaerobes, *C. novyi* was isolated in the experimental group in a small quantity, while, in the control group, *C. perfringens* in abundant quantity. *B. gracilis* was identified in both groups, with less development in the group with copper. The MICs for copper ranged between 4000 and 8000 μM without showing differences between the two groups. Conclusion: The scarce bacterial development as well as the absence of polymicrobial cultures in the group of horses shod with copper, unlike the control group, suggest that this metal exerts a local antibacterial action on the pathogens.

Area: Veterinary Microbiology

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