Technical Article

Herbanoplex CP ®: a unique pathosupressor phytocompound for preventing necrotic enteritis

Introduction

Antibiotic free poultry production can be realized with the application of alternative gut health stabilizers. For gut stabilization, different feed additives are applied during poultry rearing:

- organic acids are the most commonly used additives for gut stabilization in the broilers diet, with well-defined antimicrobial activities and also a pH reducing ability,
- trace elements and minerals can also be used, which possess antibacterial activities and can improve the immune status of the birds resulting in higher feed conversions,
- probiotics can be added to the diet, which help in establishing a normal flora,
- prebiotics can be used, which enhance the growth of the normal microbiome,
- synbiotics can be applied, which are a combination of probiotics and prebiotics,
- phytocompounds can be implemented into the broilers diet, which possess antimicrobial activities, meaning that in case of all phytocompounds, minimal inhibition concentrations (MICs) and killing dosages (CIDs) can be defined.

All the above mentioned feed additive categories have important place in the broiler industry. In many cases they are used in combinations, taking advantage of the attributes of each group. For example, in the case of synbiotics, these compounds can increase the individually obtained beneficial effect of both probiotics and prebiotics. However, phytocompounds seem to be the sole feed additive category that may reduce the use of antibiotics if their inclusion in the broiler's diet becomes more widespread. This also means that phytocompounds alone or in combination with other feed additives represent true alternatives to antibiotics.

Antibiotic resistance is a growing problem in both animal husbandry and human healthcare. Therefore, governments - with subsidies and/or prohibitions - are calling for a reduction in antibiotic use. Most recent studies show a strong correlation between the antibiotic load in animal husbandry and the human antibiotic usage: namely reducing the antibiotic usage in animal husbandry also results in reduced human antibiotic applications.

Research

Multidrug-resistant *Clostridum perfringens (Cp)* isolates are reported worldwide. *Cp* field isolates

originating from Europe, Asia and America were collected from necrotic enteritis (NE) outbreaks and monitored for their antibiotic susceptibility. For this purpose, a quick microplate test kit was developed, using 5 commonly available farm antibiotics (Doxycycline, Enrocin, Spectinomycin, Tylosin and Zinc-bacitracin) in their concentration ranges employed on the field. Screening of various field isolates of *Cp* revealed that more than 80% of the tested Cp strains were resistant towards Zinc-bacitracin, while 27% were resistant towards Enrocin (Fig.1). Among the tested antibiotics, only Tylosin was the one that inhibited the growth of *Cp* isolates at the field concentrations in all cases. These results strongly suggest, that the currently applied antibiotics are no longer effective in case of a NE outbreak worldwide.

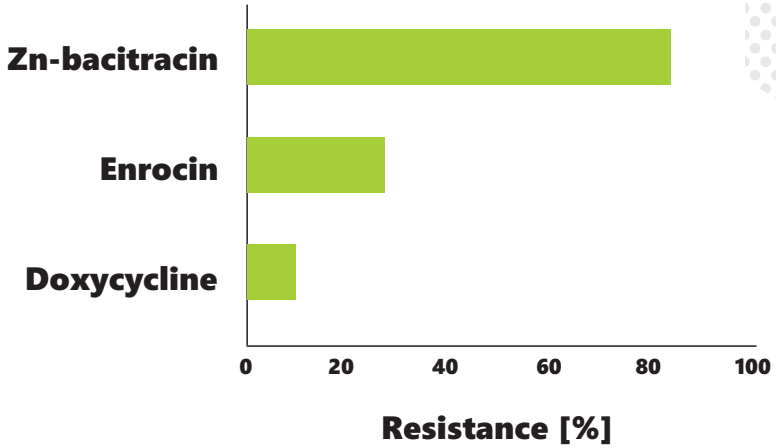

Fig.1. Resistance profile of *Clostridum perfringens* isolates originating from field outbreaks

The developed method for screening antibiotic susceptibility was adopted and used for screening various plant extracts and oils for their selective antimicrobial properties towards *Cp*. The aim of the research was to find combinations of phytocompounds that selectively prevent the multiplication of the causative agent of necrotic enteritis (NE) at an economical price. Fig.2. shows the screening results for selected plant extracts

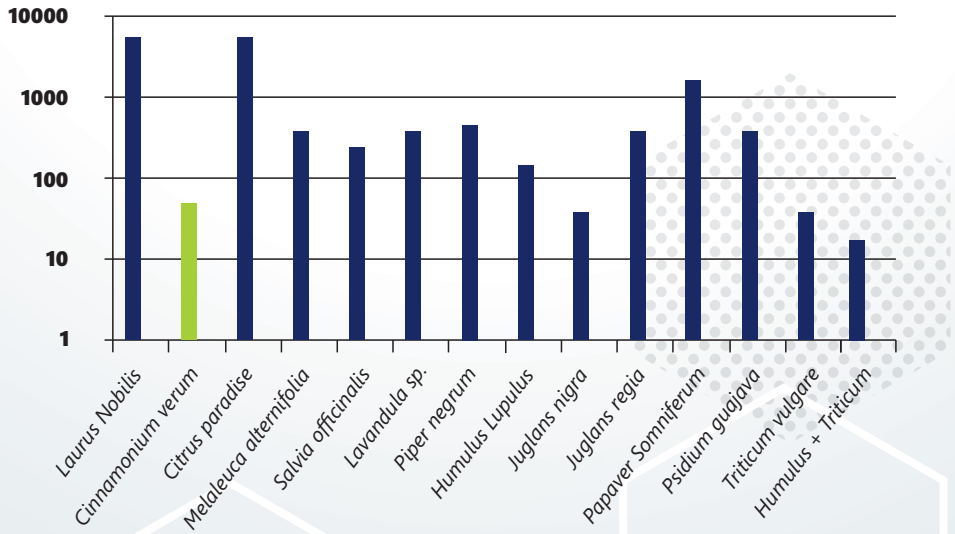

Fig.2. Minimum inhibitory concentrations of the phytocompounds. Of the more than hundred screened phytocompounds, only few of the most commonly used and some interesting plants are included on the figure.

The results revealed, that inhibition of Cp varies in a range of 10-10,000 ppm concentrations, based on the type of tested phytocompound.

Our *in vitro* experiments also concluded, that the biological activities of plant extracts and oils vary largely based on the geographical origin, on the harvesting season and on the extraction conditions of the phytocompounds. In addition, phytocompounds may also be inactivated /digested by microbial activities. In case of *Cinnamonium verum* for example, three different sources were screened, and the obtained biological activities varied between 40-160 ppm, which supports the fact, that the biological activities of phytocompounds vary significantly based on their origin (Fig.3), harvesting time and/or extraction conditions (Zs. Bata, IPPE, Atlanta, 2018).

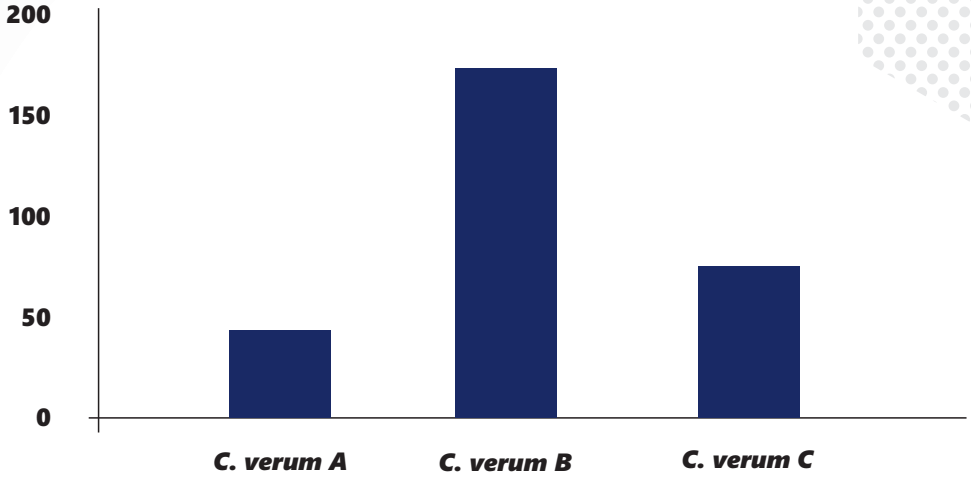

Fig.3. Variation in biological activities of different geographical regions

Extensive screening of more than a hundred phytocompounds resulted in the development of an optimal phytocompound combination. This combination, **Herbanoplex CP®**, shows antimicrobial properties towards Cp and towards additionally present pathogens, such as *Enterococcus spp*. or *Staphylococcus sp*. in 1 kg/t concentration. In contrast, in vivo experiments showed, that only 10-15 times higher dosage of **Herbanoplex CP®** limits the growth of the normal microbiome (Table 1.).

Table 1. MIC values of Herbanoplex CP (kg/t) of different pathogen and non-pathogen strains

Microorganismo	Herbanoplex CP (kg/t)
Clostridium perfringens	1
Enterococcus spp.	1
Staphylococcus aureus	0.8
Lactobacillus acidophilus	15
Lactococcus lactis	10
Bifidobacterium spp.	10-13

Our research also revealed, that upon combining phytocompounds with trace minerals, the stabilities of these extracts and oils can be increased and the variation within the biological activities (geographical or seasonal) can be minimized. Therefore, the developed phytocompound,

Herbanoplex CP® contains a mixture of drug extracts combined with trace elements, and maintains its selective anticlostridial activity in the recommended 1 kg/t feed concentration.

The efficacy of **Herbanoplex CP®** was tested in 16 different European trials with non-defined challenge. Challenge was performed with the addition of litter filtrate containing defined CFU numbers of *Cp* and *E. coli*. During the trials the following conditions varied (Fig.4.): good quality day old chicks with no litter filtrate challenge (red trials, with healthy microbiom), good quality day old chicks with the addition of litter filtrate (blue trials) or bad quality day old chicks without litter filtrate challenge (green trials), where in both cases the pathobiom was developed and the shift in the microbiom composition was detectable; and bad quality day old chicks with the addition of litter filtrate (yellow trials).

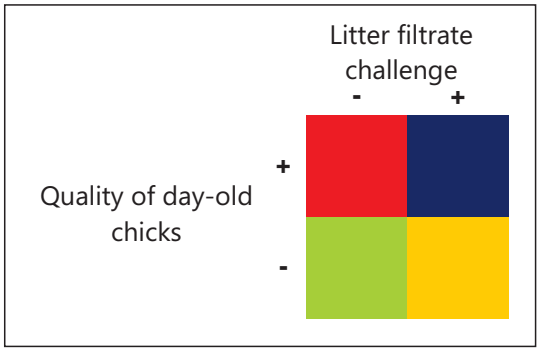

Fig.4. Variation of the trial conditions

During our in vivo trials the effect of **Herbanoplex CP®** on the production parameters were tested. At the beginning of the trial, flock density was 20% higher than the technology recommendations. At 5d of age, 20% of the smallest birds were removed and examined via hystopathology. The quality of the day old chicks was categorized based on the followings: if 25% or more of the removed birds were diagnosed as weak day old chicken and E. coli infection was also detected among the selected chicks, birds were considered to be low quality (unhealthy microbiom), while if less than 25% of the 5d old birds were determined to be underdeveloped, birds were considered to be high/good quality.

The stress treatment arising from the technology were modelled with the addition of litter filtrate, on 4th, 6th, 10th, 14th and 18th days of life. The stress treatment imitated the installation of the day old chicks on used litter and also the strong pathogen exposure during rearing. Litter filtrates in general contained coliforms with 4-5 x 10^6 CFU numbers, *E. coli* in 10^6 CFU concentration, *Cp* in 10^5 CFU and 10^3 oocysts.

Results of the efficacy of **Herbanoplex CP®** on the body weight at 5 weeks of age are presented on Fig.5. In each case, the body weight results of the trial group are compared to the positive control group, which is represented with 100% relative body weight (red line).

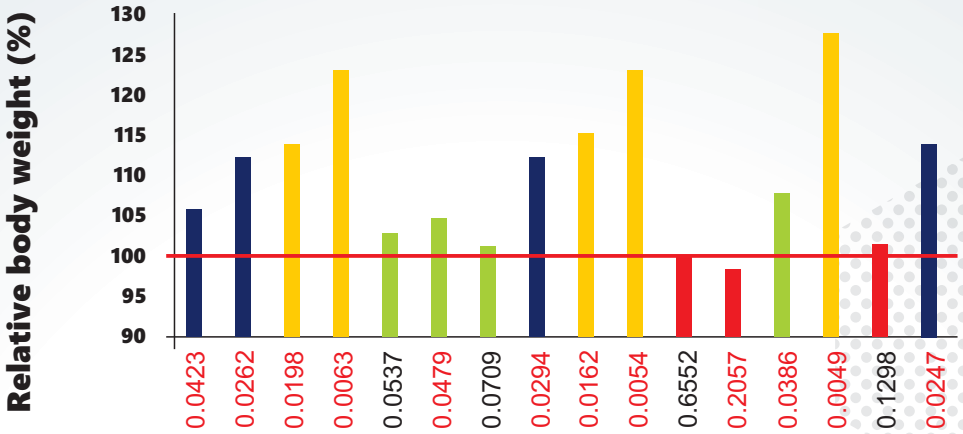

Fig.5. Results of the 16 European trials performed with non-defined challenge. Statistical analysis was performed with GLM/nested ANOVA design

Results revealed that in those cases, where the microbiome is healthy and no pathogen challenge is present (red trials), no statistical differences can be seen between the trial and the control groups (with p values of 0.6552, 0.2057 and 0.1298). Nonetheless, the trial groups did show a more homogeneous body weight distribution.

In those cases, where either the quality of the day old chicks is low (green trials) or the challenge is applied (blue trials), the trial groups result in significantly higher body weights in 66,7% of the cases. The obtained p values were 0.0537; 0.0479; 0.0709 and 0.0386 in the green trials with 2-8 % body weight gain increase, while the p values were 0.0423; 0.0262; 0.0294 and 0.0247 in the blue trials with 6-13% higher body weight gain.

Highest significant differences in the body weights between the trial and the control groups were obtained in those cases where both the quality of the day old chicks were low and the non-defined challenge model was applied (yellow trials), with p values of 0.0198; 0.0063; 0.0162; 0.0054 and 0.0049. In these yellow trials the pathogen load was the highest resulting in significantly higher body weights in all cases in the trial groups with 14-28% body weight gain increase.

Conclusion

Herbanoplex CP® has a selective antimicrobial property *in vitro* towards the pathobiom *Cp*. In contrast, it only inhibits the growth of the normal microflora in 10-15 times higher dosage.

Addition of **Herbanoplex CP®** into the diet improves the production parameters with 2-28% , depending on the farm conditions. In case, the keeping conditions are exceptional and the quality of the birds are excellent (red trials), the addition of **Herbanoplex CP®** to the diet results in a more homogeneous flock.

As soon as the keeping conditions become problematic or the quality of the birds (weak day old birds) differ from the ideal, implementation of **Herbanoplex CP®** to the diet results in significantly higher body weights in 66.7% of the cases. The effect of rearing technology results in higher loss in the production parameters compared to the effect of the quality of the day old chicks (see Fig.5. green or blue trials).

When both the keeping conditions and the quality of the birds do not meet the standard, significantly higher production parameters are observed in all cases. The obtained production parameters are equal to the production parameters that can be obtained with suitable antibiotic applications.

Phytocompounds therefore provide real alternatives to antibiotics and can be used in the prevention of NE. The combination of phytocompounds with trace minerals increases the microbial stability of the plant extracts or oils and reduces the variation in the biological activities originating from either geographical or seasonal differences. **Herbanoplex CP®** is a unique combination of phytocompounds and trace elements, which within 1 kg/t concentration limits the growth of *Cp* and prevents the outbreak of NE. The efficacy of **Herbanoplex CP®** was proven in 16 different trials performed in Europe, with different keeping conditions. The incorporation of the developed pathosupressor phytocompound is highly recommended where the rearing conditions do not meet the standard.