



## Technical Article

### **Herbanoplex CP:** *Evaluation of intestinal health in broiler chickens treated with Herbanoplex® CP and challenged with clostridium perfringens*

Enteric illnesses have a strong impact on the poultry industry, because its negative effects reduce production and increase both mortality and zoonotic risks.

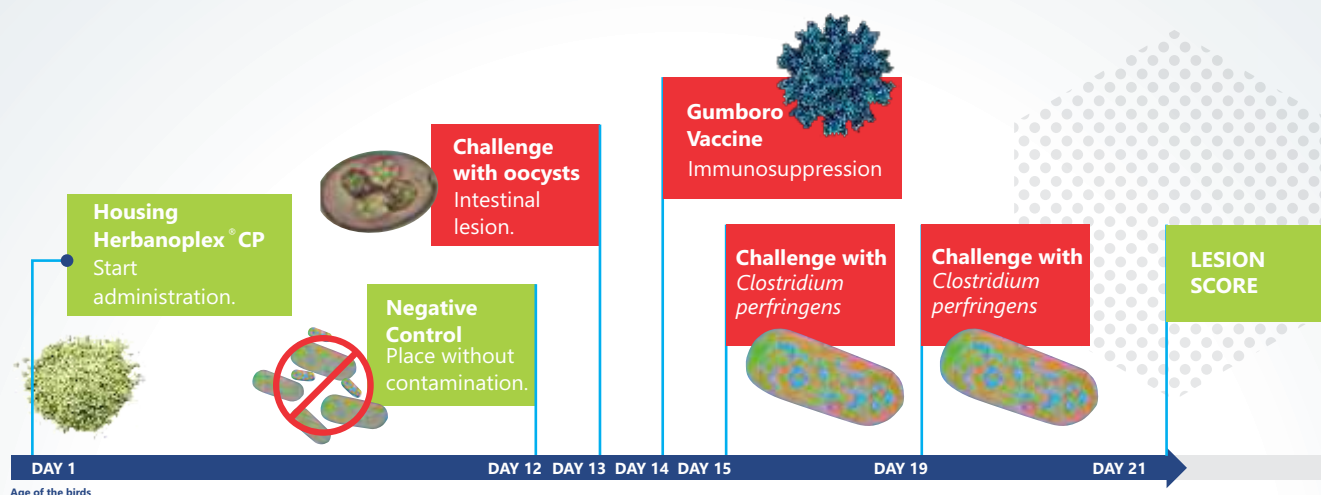
Among the most relevant pathologies, the necrotic enteritis (NE) stands out because it generates high costs for the poultry industry; annually, about 2 billion dollars are spent worldwide as a consequence of this illness that produce a growth delay and the death of birds.

Necrotic enteritis is hard to be reproduced experimentally by using *Clostridium perfringens* isolated (LEE et al., 2011). To achieve this, we must combine elements that help to develop *Clostridium perfringens* in animals' intestine, such as a high-protein diet, a high-rate of starch, immunosuppressive factors, factors that promote intestinal lesions and a sample of a viral strain of *Clostridium perfringens* for the challenges.

Necrotic Enteritis has presented a higher number of cases since the prohibition of antimicrobial growth promoters (AGPs) by the European Union in 2006 (VAN IMMERSEEL et al., 2016). Since then, different control measures have been taken in order to replace AGPs.

Vetanco, in partnership with the University of the State of Londrina (UEL), evaluated the product **HERBANOPLEX® CP** in 100 broiler chicken, male and one day-old, housed in experimental cages in the Laboratory of Infectology of the University of the State of Londrina UEL. The birds were immunosuppressed with a vaccine for Gumboro disease, challenged with oocysts of *Eimeria* and, in the sequence, challenged with *Clostridium perfringens*,  $10^8$  UFC/ml (Picture 1).

## CHALLENGE SCHEDULE AND AUXILIARY FACTORS



Picture 1

Animals were divided into five groups with specific diets according to the treatment.

The experimental diet was rich in corn and soy until 7 days of age; after that, the diet was rich in wheat (62.75%) and soy hulls (29.6%), in line with the formulation proposed by Du et al. (2016). This formulation was chosen so as to offer the proper conditions for the development of Clostridium perfringens (Table 1).

**HERBANOPLEX® CP** was added to the diet and administered to the birds since the first day of life and until the end of the experiment.

## TREATMENTS

TREATMENTS	CHALLENGE
T1 - Negative control	Without challenge
T2 - Positive control	Challenged
T3 - Herbanoplex® CP - 1kg/ton	Challenged
T4 - Herbanoplex® CP - 0,75kg/ton	Challenged
T5 - Herbanoplex® CP - 0,5kg/ton	Challenged

Table 1

The lesion score followed the method proposed by Cravens et al. (2013) with some changes. Even if there were different lesion scores published, the protocol chosen for the evaluation of the lesion produced by Clostridium perfringens was the one described above, due to the lesion profile of the test (Table 2).

## LESION SCORE ADAPTED TO THE METHOD PROPOSED BY Cravens et al. (2013)

SCALE	CHARACTERISTICS OF THE LESIONS
0	<b>NORMAL:</b> absence of necrotic enteritis lesions; the small intestine has a normal elasticity (it goes back to its position after being opened).
1	<b>MINOR:</b> the small intestine wall is thin and flaccid (it remains plane after being opened); dense mucus covering the intestinal mucosa; vessel congestion in the serous membrane; discrete mucus hyperemia.
2	<b>DISCRETE NECROTIC ENTERITIS:</b> 1 to 6 discrete necrotic enteritis pocks and/or necrosis of intestinal wall; moderate hyperemia and edema; excess of mucus and orange colored content.
3	<b>MODERATE ERUPTIONS / NECROTIC ENTERITIS AREAS:</b> more than 6 necrotic enteritis pocks and coalescent eruptions. Visible hyperemia and/or hemorrhagic areas; discrete fibrin deposit.
4	<b>SERIOUS:</b> large area of necrosis on the small intestine wall; significant hemorrhage; fibrin layers and necrotic detritus in the mucus membrane (Turkish towel appearance).
5	<b>DYING:</b> the bird is seriously weakened and will probably die within 24 hours. In the necropsy, we can notice a lesion of necrotic enteritis with a score of 2 or more.

Table 2

## RESULTS

Birds showed diarrhea with desquamation of the intestinal epithelium since the 2° day of inoculation with *Clostridium perfringens*. In the necropsy examination, it was possible to evaluate the intestinal lesion score according to the method proposed by Cravens et al. (2013).

Birds from the NEGATIVE CONTROL GROUP (T1) presented lesion scores with an average of 1.45. The lesions were, in most cases, of LEVEL 1 (Table 3).

This behavior may be due to the characteristics of the diet administered, which may have contributed to the development of the dysbacteriosis.

The profile of the diet adopted for the test was essential to determine the proper conditions of infection with *Clostridium perfringens*.

It was possible to verify that the magnitude of the lesions was increased in the groups challenged with *Clostridium perfringens*.

There was a significant difference ( $P < 0.05$ ) between the groups T3 (1Kg of HB), T4 (0.75Kg of HB) and the POSITIVE CONTROL GROUP. It was proved that the administration of **HERBANOPLEX® CP** in both concentrations produced an improvement in the intestinal health of birds (Table 3).

Most of the lesions observed in the different treatments were classified from **MINOR LEVEL-1** to **SERIOUS LEVEL-4**. The most common lesions were of **MODERATE LEVEL-3** (Picture 2). In spite of the intensity of the challenge, it is possible to notice that the necrotic enteritis administered was subclinical, because there was no mortality among the birds during the challenge and only a few animals showed severe lesions.

## RESULTS ANALYSIS

TREATMENT	BIRDS (N)	LESION SCORE						LESION SCORE AVERAGE
		0	1	2	3	4	5	
T1	20	0	12	7	1	0	0	1,45 <sup>b</sup>
T2	20	0	2	11	7	0	0	2,25 <sup>a</sup>
<b>T3</b>	<b>20</b>	<b>0</b>	<b>8</b>	<b>11</b>	<b>1</b>	<b>0</b>	<b>0</b>	<b>1,65<sup>b</sup></b>
<b>T4</b>	<b>20</b>	<b>1</b>	<b>4</b>	<b>13</b>	<b>2</b>	<b>0</b>	<b>0</b>	<b>1,80<sup>b</sup></b>
T5	20	0	3	16	0	1	0	1,95 <sup>a</sup>

Table 3: Average followed by different letters show significant differences between them by the Scott-Knott test ( $P < 0,05$ )



Picture 2. LEVEL-3 lesion

Necrotic enteritis can be both clinical and subclinical. The consequences of the clinical necrotic enteritis are large necrotic areas in the small intestine and a high mortality rate; the subclinical type presents low or no mortality rate and the lesions in the intestine are moderate. Although the subclinical type does not lead to death, it affects the zootechnic performance of animals.

## CONCLUSION

**HERBANOPLEX® CP** in two concentrations, **0.75kg/ton e 1kg/ton**, proved its action on the intestinal health of birds due to the reduction of macroscopic lesion score.

## REFERENCES

CRAVENS et al. **The effects of necrotic enteritis, aflatoxin B1, and virginiamycin on growth performance, necrotic enteritis lesion scores, and mortality in young broilers.** Poultry Science, v.92, n.8, p.1997-2004, 2013.

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DU et al. **Effects of thymol and carvacrol supplementation on intestinal integrity and immune responses of broiler chickens challenged with *Clostridium perfringens*.** Journal of Animal Science and Biotechnology, v.7; p.19, 2016.

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### **Laboratório de Medicina Aviária DMVP/CCA/UDEL**

Dra Ana Angelita S. Baptista.

Angélica Saito

Marielen de Souza

Maisa Menck

Laercio da Silva

Sergio Martins

### **Laboratório de Microbiologia NIP3/CCB/UDEL**

Dr. Gerson Nakazato

Dra Renata K.T.Kobayashi

Erick K. Nishio