Control necrotic enteritidis with the use of natural additives

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Necrotic enteritidis (NE) is one of the most common and financially devastating bacterial diseases in broiler flocks. It is caused by the anaerobic bacteria, Clostridium perfringens and results in high mortality and reduced feed intake, weight gain and flock profitability.

Production problems related to NE were formerly controlled by the use of antibiotic growth promoters (AGP). After the AGP ban in the EU and in more and more countries in Asia the use of ionophore coccidiostats, targeting both Eimeria spp. and certain Gram-positive bacteria, including Cl. perfringens, has increased in broiler chicken production and seems at present the only reliable disease control measure.

Innovative feed additives have become more prominent nowadays. The danger of antibiotic resistant pathogens developed due to the excessive use of sub-therapeutic levels of antibiotics increased research on finding alternatives to antibiotic growth promoters.

Therefore conventional growth promoters are progressively replaced with natural feed enhancers that show a positive impact on gut health and performance of animals.

Herbanoplex CP is a phytogenic feed enhancer that contains synergistic combinations of herb extracts. The product improves the growth performance of poultry through its efficiency against necrotic enteritidis.

Experiments were conducted to prove the effectiveness of a new herbal preparation developed against Cl. perfringens.

Materials and methods

Experiment 1

A standard corn-soy diet for broilers, containing growth promoters according to the specifications for each treatment was administered ad libitum.

The birds were distributed in a completely randomised design of four experimental groups with 115 birds per group and five replicates of 23 birds. The treatments were identified according to Table 1.

All treatments received diclazuril (0.2kg/t) as an anticoccidial, in the feed up to 21 days old.

Birds were inoculated with 2ml of 10^8 UFC/ml inoculum containing Clostridium perfringens on day 18, 21 and 24 via oral. All groups, except 'negative control', were inoculated. Clinical signs were evaluated daily; mortality and injury of challenged birds were evaluated after the challenge with Cl. perfringens strain up to day 42. Productive parameters were evaluated on a weekly basis.

Treatments	Doses (kg/ton)	Challenge with Clostridium perfringens
Negative control	-	-
Positive control	-	18, 21 and 24 days old
Herbanoplex CP	1.0	18, 21 and 24 days old
Zn bacitracin (30%)	0.5	18, 21 and 24 days old

Table 1. Experimental design of the four groups of birds.

Table 2. Lesion score for clostridium up to day 14 post challenge.

Day	Herbanoplex CP	Zn bacitracin	Control	
			Positive	Negative
0	0.0	0.0	0.0	0.0
3	0.0	0.0	0.0	0.0
7	0.0	0.0	0.0	0.0
10	1.2	1.8	2.2	1.6
14	1.8	2.4	2.8	1.4

The evaluation of clinical signs took into account depression, prostrate birds, diarrhoea, bloody or orange stools (dysbacteriosis), lameness, ruffled feathers, leg skin discolouration, etc.

For the evaluation of intestinal lesions five birds per treatment (one bird per replicate) were selected and randomly killed at days 22, 26, 29, 33, 26 and 40. For necrotic enteritidis the Prescott (1979) lesion score was used. The score for clostridium intestinal lesions from the duodenum to the ileum by Prescott (1979):

- 0 = no injury.
- 1 = thin or friable wall or generalised inflammation of the intestinal mucosal.
- 2 = focal necrosis or ulceration.
- 3 = multifocal necrosis.
- 4 = severe diffuse necrosis (pseudomembranes).
- 5 = death during the study with a lesion score 4 +.

For intestinal villi histopathology evaluation, approximately 2cm of intestinal portion of duodenum, jejunum and ileum of each bird per treatment were taken at day 33, 36 and 40 (other intestinal samples were taken at days 26 and 29 when estimated necessary). The samples were fixed in buffer formalin 10% and identified.

Experiment 2

Three groups (negative control, positive control and experimental; 36-36 animals) were formed with laying hens. Age of the laying hens was 32 weeks old and originated from a so-called 'problem farm' where the hens were infected with Cl. perfringens in medium level (10^4 - 10^5CFU/g Cl. perfringens).

The animals were fed ad libitum with the same layer feed. The experimental period was 10 weeks long. The feed of the experimental group was supplemented with Herbanoplex CP at 1kg/t concentration. The positive control and the trial group were artificially infected orally with Cl. perfringens ($5x10^8$ cell/dose) at day 3, 4 and 5.

Cloaca tampon-samples were collected daily, and they were washed with 1.5ml tap-water. The solution was cultured on TSC agar with Cycloserine and it was incubated at 42°C. After 24 hours the colonies were counted. During the trial, egg production parameters were observed and measured.

Results and discussion

Experiment 1

Efficacy of Herbanoplex CP was investigated parallel with Zn-bacitracin with broiler chickens in a laboratory trial. Zn-bacitracin was chosen because it is widely used outside the European Union – where its use is banned – and despite the efficiency there are problems during the application.

Animals were artificially infected with Clostridium perfringens in the positive control and the treated groups. Results of Herbanoplex CP supplementation were compared with a Zn-bacitracin treatment.

Birds in the positive control group from the 28-30 live day show the symptoms of clostridiosis.

These animals had bloody and mucus diarrhoea and because of the poor digestion undigested feed particles could be observed around the feeders. The mortality increased in

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Table 3. Post challenge histopathological evaluation of intestinal villi length (L) and width (W) (ND: Not determined).

Day	Herbanoplex CP		Zn bacitracin		Control			
					Positive		Negative	
	L	W	L	W	L	W	L	W
33	1.84	0.168	ND	ND	ND	ND	ND	ND
36	2.16	0.190	2.28	0.168	2.00	0.159	2.10	0.191
40	2.30	0.234	2.20	0.198	2.09	0.184	2.21	0.196

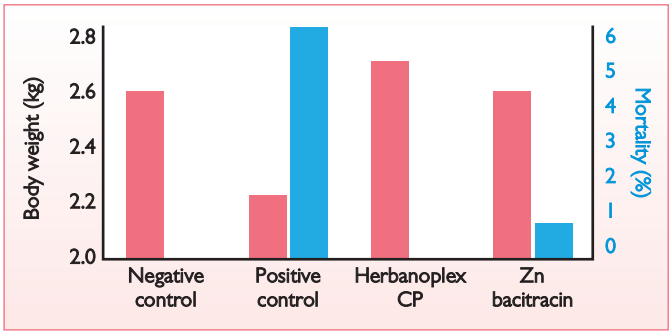

Fig. 1. Average body weight and mortality of broiler chickens at the end of the trial.

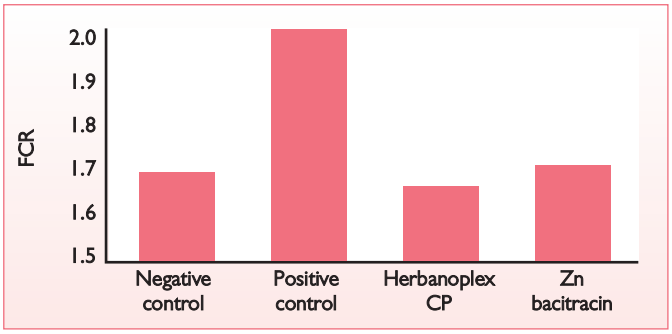

Fig. 2. Average feed conversion rate of broiler chickens after 37 days (kg/kg).

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this group and birds had nervous behaviour. The Zn bacitracin treated group showed watery faeces, but no nervous behaviour or mortality increases was found. Similar symptoms were not recognised in the other two groups. The control group and the Herbanoplex CP treated group were normal, without any sign of necrotic enteritidis.

Suggestive lesions of Clostridium, grade 1 to 3, were observed in all experimental groups 10 days post challenge. Herbanoplex CP treated group had the lowest degree of injury 10 days after challenge compared to the other experimental groups (Table 2).

At 14 days post challenge, the lowest score of injury was observed in the groups with higher body weight, Herbanoplex CP treated and negative control group, this could be related to increased sensitivity of such birds or to a greater inflammatory response induced by the growth promoters in these groups.

In the zinc-bacitracin treated group lower scores were observed compared to the positive control group but the lesions scores were higher than the Herbanoplex CP treated group. This immune response did not affect the nutrients absorption as can be seen in weight gain and feed conversion yields in these groups, compared to other groups.

Villi length and width indicate the absorption capacity of the intestinal mucosa. At day 40, the highest villi length was observed in the Herbanoplex CP treated group.

Due to the negative effect of necrotic enteritidis the villi length was lowest in the positive control group. Villi width increased in the groups treated with growth promoters. In the Zn-bacitracin treated group the villi width was the same as the negative control group, but at day 40 the highest villi width was observed in the Herbanoplex CP treated group.

As these results show, the lowest villi length and width was experienced in the positive control group (Table 3).

In accordance with the histopathological examination positive effect of the growth promoters appear in the production parameters.

The average body weight of the broiler chicken was lowest in the positive control group (Fig. 1).

Zn-bacitracin treated group produced the same body weight as the negative control group.

Average body weight was 20% higher in the Herbanoplex CP treated group compared to the positive control group (infected, but not treated) and 4% higher than the negative control and Zn-bacitracin treated group. This parameter indicates that Herbanoplex CP prevented the negative effect of necrotic enteritidis to the digestion and nutrient absorption that resulted in the increased body weight in the group.

Significant mortality (6%) appeared only in the positive control group (Fig. 1). There was 0.9% mortality in the Zn-bacitracin treated group, but it does not appear significantly different compared to the negative control and Herbanoplex CP treated group. Feed consumption can deteriorate in case of the clostridiosis as could be observed in the positive control group. Intestinal lesions caused by the Clostridia produced toxins decrease the absorption of the nutrients so the feed intake increases.

Due to the positive effect of Herbanoplex CP the FCR also improved with 7% compared to the positive control group. The Herbanoplex CP was as effective as Zn-bacitracin but the production parameters of the Herbanoplex CP treated group performed better.

● Experiment 2

In our second experiment the layers were orally challenged in three consecutive days by 1ml solution containing 10^8 CFU virulent Cl. perfringens.

Normally the number of Cl. perfringens in the intestine is low (about 10^3 CFU/g of digesta) but disorders of normal intestinal microflora may cause rapid proliferation of Cl. perfringens, increasing bacterial numbers to 10^7-10^9 CFU/g of digesta resulting in the development of clinical NE. This intestinal proliferation of Cl. perfringens was successfully reproduced in our first experiment, with no mortality, however morbidity rate was 100% in the positive control group.

Herbanoplex CP significantly reduced the number of Cl. perfringens in the intestine of the animals in the experimental group in our first experiment. The efficiency of Herbanoplex CP was studied in laboratory animal trials. 32-week-old laying hens were artificially infected with Clostridium perfringens. Symptoms of the necrotic enteritidis (depression, bloody diarrhoea, wet litter, foot pad lesion) appeared in the positive control group (without treatment), the treated group was symptomless.

The tampon-samples contained 10^6 CFU/g Cl. perfringens in the positive control group; due to the positive effect of Herbanoplex CP it was significantly lower (10^1 -10^2CFU/g) in the treated group.

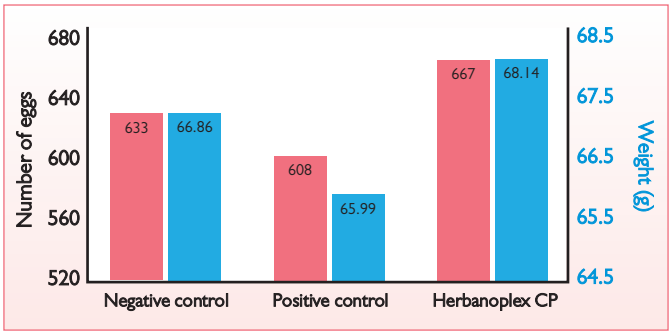
The effect of Herbanoplex CP on egg production was investigated in this trial. Our results show the necrotic enteritidis has a negative effect on egg production, because the lowest number of eggs was observed in the positive control group. In contrast, total egg number was highest in Herbanoplex CP treated group; it was 6% higher than the negative control group. There is significant difference between the negative control and Herbanoplex CP treated group that is caused by the medium level of Cl. perfringens (10^4 CFU/g) that was present in the flock from the hens originated.

Similar to the previous experiment with broiler chickens the length and width of intestinal villi was higher in the Herbanoplex CP treated group that correlates with the differences in the egg production.

Average egg weight was also highest in the Herbanoplex CP treated group (Fig. 3). Compared to the positive control group the weight of the eggs was 3% higher due to the positive effect of Herbanoplex CP.

Results show the significant adverse effect of the high Cl. perfringens infection to egg production. In the Herbanoplex CP treated group the average egg weight was 1g higher than the negative control group due to the medium level infection in the negative control group.

Fig. 3. Total egg production and the average weight of eggs.



Conclusion

Efficacy of Herbanoplex CP against necrotic enteritidis was proved in laboratory trials with laying hens and broiler chickens. This natural product is effective against Clostridium perfringens as can be observed in the results of the histopathological evaluation of the intestinal villi length and width. Our investigations parallel with Zn-bacitracin resulted that Herbanoplex CP is as effective as Zn-bacitracin against necrotic enteritidis but the production parameters of the Herbanoplex CP treated group performed better. According to the results, Herbanoplex CP ensures significant improvement in the production parameters and has a positive effect on the egg production of the laying hens that appeared in the number and the weight of the eggs.

Results of the histopathological examinations in accordance with the production parameters of our experiments indicates that a natural growth promoter, such as Herbanoplex CP, opens the door for efficient and economical production without antibiotics. ■