

EFFECTS OF DIVERCIN AS7 IN *CLOSTRIDIUM PERFRINGENS* CHALLENGED BROILER CHICKENS

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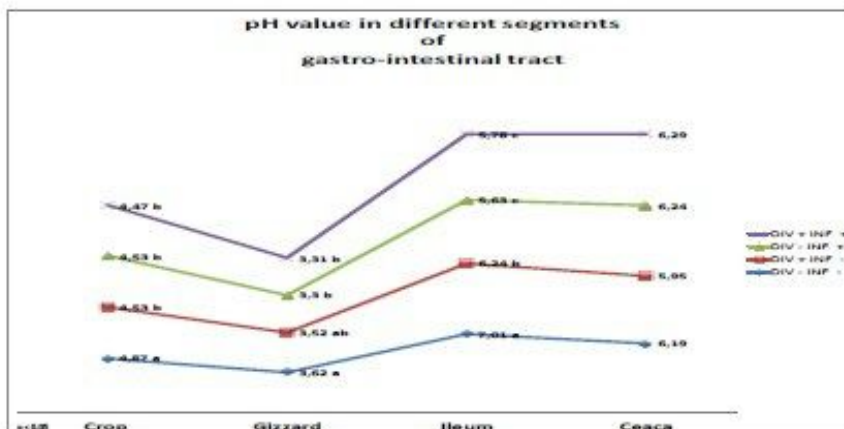
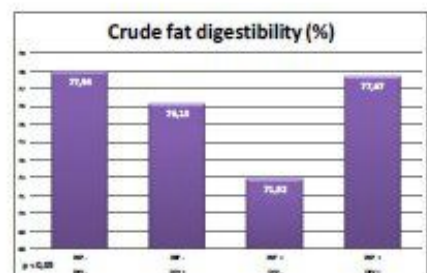
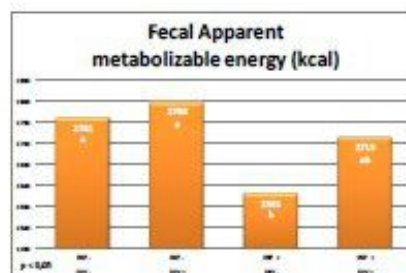
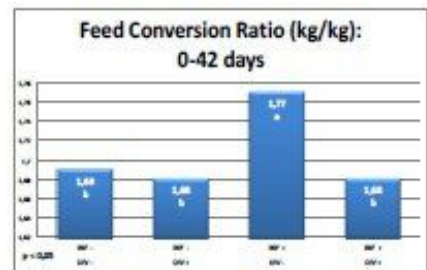
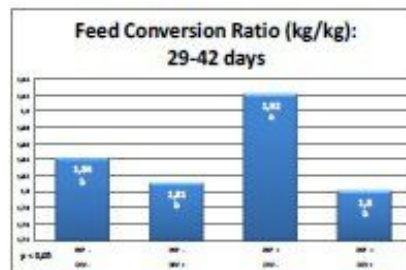
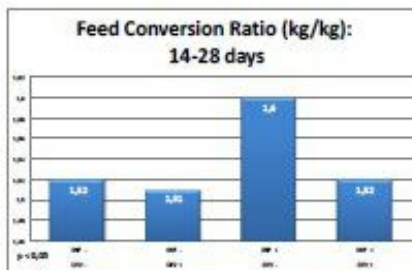
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The aim of the present study was to investigate the effects of a divercin AS7 preparation on performance, nutrient digestibility and pH value of the digesta in broiler chickens challenged with *Clostridium perfringens*. The effect of the divercin AS7 supplementation was evaluated in a growth performance experiment with broiler chickens kept in floor pens over an experimental period of 42 days.

A total of 480 1-d-old male ROSS 308 chicks were randomly distributed to 4 dietary treatments using 12 replicate pens per treatment and 10 birds per pen. The control diet was offered without or with divercin AS7, 200 AU divercin/mL (DIV) and the birds were either not infected or infected (INF) from 18 to 21d of birds age with three *Clostridium perfringens* strains isolated from chickens suffering from necrotic enteritis.



CONCLUSION:

On basis of current trial it could be stated that dietary supplementation of the *Carnobacterium divergens* bacteriocin in *Clostridium perfringens* challenged birds may have some beneficial effects on the chicken performance.

P-152 Effects of divercin AS7 in *Clostridium perfringens* challenged broiler chickens

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Abstract

Carnobacterium divergens produces the subclass IIa bacteriocins consisting of 30 to 60 amino acids and so far, three major bacteriocins produced by this species have been identified; divercin V41, divercin AS7 and divergicin M35. The aim of the present study was to investigate the effects of a divercin AS7 preparation on performance, nutrient digestibility and pH value of the digesta in broiler chickens challenged with *Clostridium perfringens*. The effect of the divercin AS7 supplementation was evaluated in a growth performance experiment with broiler chickens kept in floor pens over an experimental period of 42 days. A total of 480 1-d-old male ROSS 308 chicks were randomly distributed to 4 dietary treatments using 12 replicate pens per treatment and 10 birds per pen. The control diet was offered without or with divercin AS7, 200 AU divercin/mL (0.2 mL/kg) and the birds were either not infected or infected (from 18 to 21d of birds age) with three *Clostridium perfringens* strains isolated from chickens suffering from necrotic enteritis. In the infected birds the divercin AS7 supplementation improved feed conversion ratio (1.80 vs 1.92) from 29 to 42 days and in the entire trial (1.68 vs 1.77, $p=0.005$). No effects of divercin or infection were observed on body weight gain of the chickens throughout the trial. In the divercin-supplemented groups, the faecal apparent metabolisable energy content was higher (2715 vs 2581Kcal, $p=0.030$). The divercin supplementation decreased the pH value of the digesta in crop and ileum during infection.

Key words: *Clostridium perfringens*; bacteriocin; gastrointestinal tract; broiler chicken

Introduction

Clostridium perfringens is usually a part of the intestinal microflora of healthy chickens and does not do any harm to the host bird. However overgrowth of this bacterium may lead to gas gangrene with lesions found mainly in the small intestine. This may give way to the development of necrotic enteritis (NE), a disease considered to be the most economically devastating for the broiler industry all over the world. There are numerous feed additives proposed to have beneficial effects in controlling NE, however, the results of different studies are contradictory. A new strategy to reduce *Clostridium perfringens* colonization might be the dietary supplementation of bacteriocins, which are small peptides, lethal to bacteria other than the producing strain (Grilli et al 2009). Therefore the aim of the present experiments was to evaluate the effect of dietary divercin AS7 in broiler chickens infected with field strains of *Clostridium perfringens*.

Materials and Methods

A total of 480 1-d-old male ROSS 308 chicks were randomly distributed to 4 dietary treatments using 12 replicate pens per treatment and 10 birds per pen. The control (C) diet was unsupplemented or supplemented (DIV) with lyophilized divercin AS7 preparation, 200 AU divercin/g (0.2 g/kg) and infected (CI) or not (DIVI) with three field isolates of *Clostridium perfringens*. On day 18, 19 and 20, the birds from CI and DIVI treatments were individually orally infected twice a day with 1 ml of a overnight culture (10^8 bacteria /mL) of *C. perfringens*. The other treatments received at the same time 1 ml of sterile water. Till 14

days of age all birds were fed the same basal diet without divercin supplementation. The divercin activity was quantified by 2-fold dilutions and expresses in activity unit (AU) per milliliter as previously described by Ennahar (2001). The divercin AS 7 preparation was produced as described in details by Józefiak et al (2010). The feed intake and body weight of the chickens were measured on days 28 and 42. At the end of the trial (42d) 21 randomly picked chickens from each experimental group (3 chickens from 7 pens), were killed by cervical dislocation. For pH analyses of gastrointestinal contents, the digesta from crop, ileum and caeca from 3 birds per pen were pooled (7 replicate digesta samples of approximately 10g). The digestibility was evaluated as described in details by Józefiak et al. (2010). Statistical analysis of the results was performed using the General Linear Models procedure (GLM) of the SAS[®] (SAS Institute, 1988) according to the following general model In cases where the overall effect was significant ($p < 0.05$), means were compared pair wise (pdiff). Results are given as the least square means with pooled standard error of the mean (SEM).

Results and Discussion

The negative effects associated with the *C.perfringens* colonization are well documented. Thus every year in the literature papers are appearing focusing on new strategies to control this pathogen infection and colonization process. There are also many protocols to reproduce NE under laboratory conditions (Gholamiandehkordi et al 2007). The performance results (Table 1) of the present experiment are in agreement with other challenge studies where inoculation of the broiler chickens with *C.perfringens* impaired feed utilization. While divercin AS7 supplementation in infected birds improved FCR to the level observed in non-challenged birds. Similar to the work of Olkowski et al (2006) and Gholamiandehkordi et al (2007) we did not induce mortality of the animals. In contrast to (Wu et al 2011), who used similar amounts of the fishmeal in the diet (2.5%) but was challenging birds with a combination of *C.perfringens* and *Eimeria* sp. induced 9.33% mortality. In general the, in the present trial pH value (Table 2) of the digesta is in agreement with work conducted by the other authors. However to our knowledge this is first observation in the available literature showing that challenge of the broiler chickens with *Clostridium perfringens* may affect pH throughout the GIT, reducing its value in the upper parts (crop, gizzard, ileum) and increasing it in lower segments as caeca. This finding may be helpful in interpretation of some negative effects of *Clostridium perfringens* colonization as impaired feed utilization (Table 3). While all endogenous, as well as exogenous enzymes need optimal pH to catalyze biochemical reactions during absorption of the nutrients from the diet. It could be stated that the observed lower pH value particularly in the ileum after challenge with *Clostridium perfringens* (5.63 vs 7.01) might negatively affect these processes thus impairing broiler performance. Thus it could be concluded that the observed lower AMEn as well fat digestibility values caused by *C.perfringens* challenge are partially combined with lower pH of the digesta in the GIT. In contrast to our earlier studies (Józefiak et al 2010) the dietary divercin AS7 also lowered pH probably due to usage of the cellulose carrier, while it is documented that insoluble dietary fiber fractions may affect fermentation in birds GIT. In the divercin-supplemented groups, the faecal apparent metabolizable energy content was higher (2715 vs 2581Kcal, $P=0.030$) and this is in agreement with our earlier studies (Józefiak et al 2010).

On basis of current trial it could be stated that dietary supplementation of the *Carnobacterium divergens* bacteriocin in *Clostridium perfringens* challenged birds may have some beneficial effects on the chicken performance.

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Table 1. Effects of Divercin AS7 in *Clostridium perfringens* on broiler performance

Treatment		Performance								
		14-28 days			29-42 days			0-42 days		
Divercin	Infection	BWG	FI	FCR	BWG	FI	FCR	BWG	FI	FCR
-	-	1041	1584 ^b	1.52	1279	2345	1.84 ^b	2319	3923	1.69 ^b
+	-	1008	1519 ^b	1.51	1296	2343	1.81 ^b	2305	3860	1.68 ^b
-	+	1042	1664 ^a	1.60	1244	2388	1.92 ^a	2285	4047	1.77 ^a
+	+	1005	1522 ^b	1.52	1320	2381	1.80 ^b	2326	3897	1.68 ^b
Pooled SEM		9.705	15.2	0.01	10.9	15.7	0.01	15.01	26.51	0.01
P-value		0.384	0.003	0.328	0.124	0.679	0.0002	0.829	0.114	0.005

^{a,b}means in the same row for each parameter with different superscripts are significantly different (p<0.05)

Table 2. Effects of Divercin AS7 in *Clostridium perfringens* on pH values of the digesta in different GIT segments

Treatment		pH			
Divercin	Infection	Crop	Gizzard	Ileum	Ceaca
-	-	4.87 ^a	3.62 ^a	7.01 ^a	6.19
+	-	4.53 ^b	3.52 ^{ab}	6.24 ^b	5.95
-	+	4.53 ^b	3.30 ^b	5.63 ^c	6.24
+	+	4.47 ^b	3.31 ^b	5.78 ^c	6.29
Pooled SEM		0.044	0.045	0.131	0.053
P-value		0.001	0.017	<.0001	0.093

^{a,b,c}means in the same row for each parameter with different superscripts are significantly different (p<0.05)

Table 3. Effects of Divercin AS7 in *Clostridium perfringens* on nutrient digestibility

Treatment		Nutrient digestibility	
Divercin	Infection	Crude fat digestibility	Fecal AMEn
-	-	77.94	2761 ^a
+	-	76.13	2798 ^a
-	+	71.92	2581 ^b
+	+	77.67	2715 ^{ab}
Pooled SEM		1.285	29.017
P-value		0.306	0.030

^{a,b}means in the same row for each parameter with different superscripts are significantly different (p<0.05)