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# Dietary Divercin Modifies the Gastrointestinal Microbiota and Improves Growth Performance of Broiler Chickens

Source: D. Józefiak, A. Sip, M. Rawski, A. Rutkowski & S. Kaczmarek - University of Life Sciences in Poznan, Poland; O. Højberg, B. B. Jensen & R. M. Engberg - Aarhus University, Tjele, Denmark

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## Introduction

Even though bacteriocins have been used in human nutrition for decades (Cleveland et al. 2001; Joerger 2003; Leisner et al. 2007), only limited information is available regarding the effects of directly fed bacteriocins on broiler chickens. A few recent publications do however indicate that different bacteriocins administered with the feed may inhibit colonization of Campylobacter jejuni and Salmonella enteritidis (Stern et al. 2005; Line et al. 2008) in the gastrointestinal tract (GIT).

Carnobacterium divergens produces the subclass IIa bacteriocins consisting of 30 to 60 amino acids (Leisner et al. 2007) and so far, three major bacteriocins produced by this species have been identified; divercin V41 (Rihakova et al. 2009), divercin AS7 (Sip et al. 1998) and divergicin M35 (Tahiri et al. 2004). Apart from the data from our preliminary study (Józefiak et al. 2010b), no information is so far available on the use of bacteriocins produced by Carnobacterium divergens as feed additives for broiler chickens. The aim of the present study was therefore to investigate the effects of a divercin AS7 preparation on broiler chicken performance, nutrient digestibility, counts of coliform bacteria (indicator of potential pathogens) and lactic acid bacteria (LAB, representative commensals) as well as on the microbial activity in the GIT expressed by the levels of pH and organic acids (short chain fatty acids + lactic and succinic acid) in lumen content.

## Study design and analyses

The effect of the divercin AS7supplementation was evaluated in a growth performance experiment with broiler chickens kept in floor pens over an experimental period of 35 days. A total of 450 day-old male ROSS 308 chicks were randomly distributed to 3 dietary treatments using 15 replicate pens per treatment and 10 birds per pen. The positive control (PC) diet was supplemented with an ionophore coccidiostat (salinomycin, 60 mg/kg), the negative control diet (NC) did not contain any additive and the divercin diet (DIV) was supplemented with 200 AU divercin/mL (0.2 ml/kg). In each treatment, excreta and digesta samples were taken for apparent metabolizable energy (AMEn) and nutrient digestibility. The pH of pooled digesta in different GIT sections (crop, ileum and ceca) and plate counting of coliform bacteria and lactic acid bacteria were performed immediately after slaughter. The concentration of short-chain fatty acids and lactic acid in the same segments was determined by gas chromatography as described earlier (Jensen et al. 1995; Canibe et al. (2007). The study was set up as a completely randomized design, and data were tested using the GLM procedure of SAS (Sas 1990). Means were separated using a Duncan's multiple range test. All statements of significance are based on $P<0.05$.

## Results and discussion

Birds fed diets supplemented with salinomycin showed the best performance results (Table 1). Compared with the non-supplemented control, the divercin supplementation increased the body weight gain in the starter period ($P=0.006$) and at the end of the trial ($P<0.001$) and tended to improve total feed conversion ratio as well as feed intake in each growth stage. The levels of apparent metabolizable energy (AMEn) and ileal digestible energy (Table 2) increased after salinomycin supplementation ($P<0.001$ and $P=0.016$, respectively). However, there was no difference between PC and DIV treatments with respect to the estimated ileal digestible energy. The observed growth promoting effect of salinomycin is in good agreement with earlier studies on broiler chickens (Engberg et al. 2000), ruminants (Spears 1990) and pigs (Moore et al. 1986). In poultry, many of the applied ionophore coccidiostats do not only target Eimeria species but also certain Gram-positive GIT bacteria; the coccidiostats have thus been observed to reduce the numbers of e.g., Clostridium perfringens (Bjerrum et al. 2005) and LAB, including species like Lactobacillus salivarius, previously reported to be dominating in the broiler GIT (Engberg et al. 2000).

In the present trial, the dietary supplementation with salinomycin numerically increased the counts of LAB bacteria (Table 3), however only significantly in the ceca (P=0.015). Divercin tended to reduce the LAB population in the contents of crop (P=0.14). However, in ileum (P=0.015) and ceca (P=0.006), the reduction in LAB was significant. The counts of coliform bacteria in the different intestinal segments were not influenced by the dietary treatments (P>0.05).

Compared with the non-supplemented controls, the digesta pH in crop and ileum was higher when birds were fed salinomycin and divercin (P=0.011 and P=0.001, respectively). In the ceca, the pH was highest in the non-supplemented control (P=0.043).

In crop contents, the dominant organic acids were lactic acid and acetic acid the (Table 4), further, succinic acid and butyric acid were detected in low concentrations. Compared with the non-supplemented diet, the addition of salinomycin and divercin reduced lactic acid, acetic acid and succinic acid concentrations by approximately 50% (P>0.001) in crop contents. In ileal content, the most abundant organic acids were also lactic and acetic acid, whereas succinic acid was detected only in low concentrations (Table 4). The concentrations of lactic acid was influenced by the dietary treatment (P<0.001) and was lowest in ileal contents of birds supplemented with salinomycin (34.5 ug/g) as compared to divercin (72.4 ug/g) and to the non-supplemented control diet (114.0 ug/g). The concentration of acetic acid in ileal contents was not influenced by the dietary treatment (P>0.05). In cecal contents, the organic acid profile was much more diverse with acetic acid, butyric acid and propionic acid being the dominating organic acids (Table 4). Low concentrations of isobutyric, isovaleric and valeric acids were detected in cecal contents as well, whereas lactic acid was not detected in this GIT segment. In general, divercin reduced the microbial fermentation (organic acid production) to the same extent as salinomycin (Table 4). There was, however, no significant difference between treatment groups with respect to the production of organic acids in the ceca (P>0.05).

To our knowledge, the present study is the first attempt to use a Carnobacterium divergens bacteriocin as a feed supplement and to demonstrate positive growth promoting and antibacterial effects of divercin in broiler chickens. Though consistent with our preliminary studies (Józefiak et al. 2010), the results presented here should be followed up by further investigations in order to provide more detailed information on e.g., broiler growth performance in response to different divercin doses as well as the specific bacteria being targeted by the bacteriocin. Finally, it could be attempted to increase the effectiveness of divercin in the broiler GIT by protective encapsulation of the peptide.

**Table 1.** *Performance results*

| Item | Treatment | | | SEM[d] | *P*[e] |
|---|---|---|---|---|---|
| | PC[a] | NC[b] | DIV[c] | | |
| Feed conversion ratio | | ---------------(g feed/g gain)------------- | | | |
| 1 – 14 d | 1.48$^{y}$ | 1.54$^{x}$ | 1.49$^{y}$ | 0.01 | 0.001 |
| 14 – 35 d | 1.60 | 1.67 | 1.67 | 0.02 | 0.140 |
| 1 – 35 d | 1.58 | 1.65 | 1.61 | 0.01 | 0.059 |
| Body weight gain | | ------------------(g/bird)----------------- | | | |
| 1 – 14 d | 328$^{x}$ | 304$^{y}$ | 320$^{x}$ | 3.37 | 0.006 |
| 14 – 35 d | 1474$^{x}$ | 1360$^{y}$ | 1413$^{xy}$ | 14.25 | 0.003 |
| 1 – 35 d | 1801$^{x}$ | 1664$^{x}$ | 1733$^{y}$ | 15.83 | <0.001 |
| Feed intake | | ------------------(g/bird)----------------- | | | |
| 1 – 14 d | 484 | 469 | 476 | 3.77 | 0.236 |
| 14 – 35 d | 2357 | 2274 | 2349 | 18.99 | 0.138 |
| 1 – 35 d | 2838 | 2737 | 2790 | 18.21 | 0.074 |

[a]The positive control (PC) diet contained 60mg/kg of the salionomycin

[b]The negative control (NC) diet did not contain any additives

[c]The divercin supplemented diet contained with 200 AU of divercin/mL (0.2mL/kg)

[d]SEM – standard error of the mean

[e]*P* – probability

**Table 2.** *Ileal digestibility of nutrients, energy, nitrogen retention and AMEn content.*

| Item | Treatment | | | SEM[4] | P[5] |
|---|---|---|---|---|---|
| | PC[1] | NC[2] | DIV[3] | | |
| | (kcal) | | | | |
| AMEn | 13.6[x] | 12.1[y] | 12.1[y] | 0.211 | <0.001 |
| Ileal digestible energy | 12.9[x] | 10.3[y] | 11.6[xy] | 0.440 | 0.016 |
| | (%) | | | | |
| Ileal crude protein digestibility | 0.74 | 0.62 | 0.70 | 0.021 | 0.074 |
| N Retention | 0.49[x] | 0.37[y] | 0.40[xy] | 0.021 | 0.048 |
| Ileal crude fat digestibility | 0.92 | 0.67 | 0.69 | 0.055 | 0.118 |
| Total tract fat digestibility | 0.90[x] | 0.76[y] | 0.72[y] | 0.028 | 0.013 |

[1]The positive control (PC) diet contained 60mg/kg of the salionomycin

[2]The negative control (NC) diet did not contain any additives

[3]The divercin supplemented diet contained with 200 AU of divercin/mL (0.2mL/kg)

[4]SEM – standard error of the mean

[5]P – probability

**Table 3.** *Counts of coliform and lactic acid bacteria (log cfu /g digesta) and pH in crop, ileal and cecal digesta*

| Item | Treatment | | | SEM[4] | P[5] |
|---|---|---|---|---|---|
| | PC[1] | NC[2] | DIV[3] | | |
| Crop | | | | | |
| Coliformes | 6.98 | 7.08 | 6.55 | 0.110 | 0.112 |
| Lactic acid bacteria | 7.92 | 7.93 | 7.41 | 0.124 | 0.140 |
| pH | 4.33[x] | 3.94[y] | 4.31[x] | 0.189 | 0.011 |
| Ileum | | | | | |
| Coliformes | 6.47 | 6.29 | 6.24 | 0.144 | 0.785 |
| Lactic acid bacteria | 7.90[x] | 7.72[y] | 7.03[x] | 0.142 | 0.015 |
| pH | 6.28[x] | 5.14[y] | 5.55[y] | 0.376 | 0.001 |
| Caeca | | | | | |
| Coliformes | 7.88 | 7.71 | 7.13 | 0.191 | 0.253 |
| Lactic acid bacteria | 9.24[x] | 8.53[y] | 8.00[y] | 0.180 | 0.006 |
| pH | 5.49[y] | 5.78[x] | 5.52[y] | 0.181 | 0.043 |

[1]The positive control (PC) diet contained 60mg/kg of the salionomycin

[2]The negative control (NC) diet did not contain any additives

[3]The divercin supplemented diet contained with 200 AU of divercin/mL (0.2mL/kg)

[4]SEM – standard error of the mean

[5]P – probability

**Table 4.** *Concentrations of organic acids (µmol/g digesta) in the contents of crop, ileum and ceca of broiler chickens.*

| Item | Treatment | | | | |
|---|---|---|---|---|---|
| | PC[a] | NC[b] | DIV[c] | SEM[d] | P[e] |
| Crop | | | | | |
| Acetic acid | 11.3$^{y}$ | 26.8$^{x}$ | 11.4$^{y}$ | 2.23 | <0.001 |
| Butyric acid | 0.2 | 0.7 | 0.7 | 0.16 | 0.456 |
| Lactic acid | 55.3$^{y}$ | 119.0$^{x}$ | 52.0$^{y}$ | 9.59 | <0.001 |
| Succinic acid | 2.8$^{y}$ | 8.7$^{x}$ | 3.2$^{y}$ | 0.79 | <0.001 |
| Total organic acids | 69.7$^{y}$ | 155.0$^{x}$ | 67.3$^{y}$ | 12.36 | <0.001 |
| Ileum | | | | | |
| Acetic acid | 5.8 | 6.1 | 4.2 | 0.49 | 0.241 |
| Lactic acid | 34.5$^{z}$ | 114.0$^{x}$ | 72.4$^{y}$ | 10.37 | <0.001 |
| Succinic acid | 0.2$^{y}$ | 2.0$^{x}$ | 0.7$^{y}$ | 0.27 | 0.006 |
| Total organic acids | 40.7$^{z}$ | 122.0$^{x}$ | 77.2$^{y}$ | 10.69 | 0.001 |
| Ceaca | | | | | |
| Acetic acid | 86.5 | 92.0 | 79.6 | 3.34 | 0.32 |
| Propionic acid | 18.4 | 16.8 | 18.4 | 1.02 | 0.78 |
| Isobutyric acid | 0.9 | 1.2 | 1.0 | 0.10 | 0.49 |
| Butyric acid | 19.8 | 23.1 | 19.4 | 1.44 | 0.53 |
| Isovaleric acid | 0.5 | 0.6 | 0.5 | 0.08 | 0.95 |
| Valeric acid | 3.4 | 2.9 | 2.7 | 0.14 | 0.20 |
| Succinic acid | ND[f] | 4.35 | 1.32 | - | - |
| Total organic acids | 129.5 | 141.0 | 123.0 | 4.87 | 0.31 |

[a]The positive control (PC) diet contained 60mg/kg of the salinomycin

[b]The negative control (NC) diet did not contain any additives

[c]The divercin supplemented diet contained with 200 AU of divercin/mL (0.2mL/kg)

[d]SEM – standard error of the mean

[e]P – probability

[f]ND – not detected

For further information on this research, feel free to contact:

**Damian Jozefiak, Ph.D.**
Department of Animal Nutrition and Feed Management
University of Life Sciences in Poznań
ul.Wołyńska 33
60-637 Poznań
Poland
E-mail: **damjo@up.poznan.pl**