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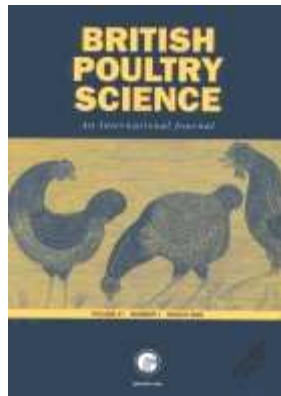
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**Dietary divercin modifies gastrointestinal microbiota and improves growth performance in broiler chickens**

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Running title: Divercin in broiler chicken diets

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5 21 **Abstract** 1. The aim of the present study was to investigate the effects of dietary  
6  
7 22 administration of a divercin AS7 liquid preparation on broiler chicken performance,  
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9 23 nutrient digestibility, counts of lactic acid bacteria (LAB) and coliform bacteria, as  
10  
11 24 well as on the microbial activity in the gastrointestinal tract (GIT) as expressed by  
12  
13 25 digesta pH and concentrations of short-chain fatty acids and lactic acid.  
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15  
16 26 2. A total of 450 1-d-old male Ross 308 chickens were randomly distributed to three  
17  
18 27 dietary treatments, with 15 pens per treatment and 10 birds per pen. The dietary  
19  
20 28 treatments consisted of a positive control (PC) supplemented with 60 mg/kg  
21  
22 29 salinomycin, a negative control (NC) without any additives, and the divercin (DIV)  
23  
24 30 supplemented diet containing 0.2 ml/kg of the liquid divercin AS7 preparation.  
25  
26  
27 31 3. The dietary divercin AS7 supplementation significantly increased body weight gain  
28  
29 32 at 35 d compared to the NC group. Moreover, the pH of crop contents was higher and  
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31 33 that of caecal contents lower in birds fed on the divercin supplemented diets.  
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33  
34 34 4. Significantly lower counts of LAB were observed in the crops and caeca of the birds  
35  
36 35 treated with divercin. Further, the divercin supplementation decreased lactic and  
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38 36 succinic acid concentrations in the crop and ileum.  
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40  
41 37 5. The present study demonstrates that the use of divercin supplemented diets can  
42  
43 38 influence composition and activity of the microbiota in the broiler chicken GIT even in  
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45 39 the lower parts that should otherwise not be targeted due to the peptide structure of the  
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47 40 bacteriocin.  
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52 41 INTRODUCTION  
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5 42 In recent years, a number of studies have demonstrated a close relationship between  
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7 43 broiler performance, health and the gastrointestinal tract (GIT) microbiota (Engberg *et*  
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9 44 *al.*, 2002; Maisonnier *et al.*, 2003; Engberg *et al.*, 2004; Choct 2009). Taking the  
10  
11 45 complexity and biodiversity of the GIT micro-ecosystem into consideration, a  
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13 46 manipulation of the GIT microbiota in a way that is profitable for the host-bird and for  
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15 47 the farmer, however, seems a difficult goal to achieve. Several studies have  
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17 48 demonstrated that the protective potential of the native commensal microbiota may  
18  
19 49 easily be disrupted by different nutritional factors (Dahiya *et al.* 2006; Choct 2009),  
20  
21 50 thereby compromising one of the suggested ways for the commensal microbiota to  
22  
23 51 support performance and health of broilers; namely elimination of pathogenic bacteria  
24  
25 52 from the GIT. On the other hand, it is also well recognised that the commensal  
26  
27 53 microbiota, no matter how protective it may be, does impose a burden upon the host  
28  
29 54 that goes beyond simply competing for feed components (Engberg *et al.*, 2000), *via*  
30  
31 55 more specific mechanisms such as compromising fat digestibility (Knarreborg *et al.*  
32  
33 56 2002). A mere reduction in the commensal microbial load of the GIT may therefore, in  
34  
35 57 a short term perspective, represent a growth promoting factor for the host, though  
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37 58 admittedly, it may at the same time render the host more susceptible to pathogen  
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39 59 invasion.

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47 60 Probiotics with potentially beneficial effects on broiler growth and health have  
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49 61 gained considerable interest (Choct 2009) and have been shown to stimulate the  
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51 62 immune system and to reduce the growth and intestinal colonisation of pathogenic  
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53 63 bacteria by competitive exclusion (Rijkers *et al.* 2010). Further, some probiotic  
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5 64 bacteria produce different types of peptides, *e.g.* bacteriocins, with direct antimicrobial  
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7 65 effects (Joerger 2003). Even though bacteriocins have been used in human nutrition for  
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10 66 decades (Cleveland *et al.*, 2001; Joerger 2003; Leisner *et al.*, 2007), only limited  
11  
12 67 information is available regarding the effects of directly fed bacteriocins on broiler  
13  
14 68 chickens. A few recent publications do however indicate that different bacteriocins  
15  
16 69 administered with the feed may inhibit GIT colonisation by *Campylobacter jejuni* and  
17  
18 70 *Salmonella enteritidis* (Stern *et al.*, 2005; Line *et al.*, 2008).

21  
22 71 Among bacteria capable of producing bacteriocins, much attention has been  
23  
24 72 paid to the lactic acid bacteria, LAB (Stern *et al.*, 2006; Svetoch *et al.*, 2008).  
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26 73 *Carnobacterium* is a genus of LAB that can be isolated from various sources such as  
27  
28 74 the GIT of fish, dairy products and alpine permafrost (Leisner *et al.*, 2007). The  
29  
30 75 *Carnobacterium* genus comprises nine described species, of which *Carnobacterium*  
31  
32 76 *divergens* and *Carnobacterium maltaromaticum* have been most widely studied, due to  
33  
34 77 their role in the inhibition of many pathogenic and food spoilage bacteria such as  
35  
36 78 *Clostridium*, *Bacillus*, *Brochothrix*, *Streptococcus*, *Staphylococcus* and *Micrococcus*.  
37  
38 79 They do, however, also possess antimicrobial activity against fellow LAB of the genera  
39  
40 80 *Lactobacillus*, *Leuconostoc*, *Carnobacterium*, *Pediococcus* and *Enterococcus* (Sip *et*  
41  
42 81 *al.*, 1998; Leisner *et al.*, 2007); a feature potentially related to *Carnobacterium*  
43  
44 82 bacteriocin production.

45  
46 83 *Carnobacterium divergens* produces the subclass IIa bacteriocins consisting of  
47  
48 84 30 to 60 amino acids (Leisner *et al.* 2007) and so far, three major bacteriocins produced  
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50 85 by this species have been identified; divercin V41 (Rihakova *et al.* 2009), divercin AS7  
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5 86 (Sip *et al.* 1998) and divergicin M35 (Tahiri *et al.* 2004). Apart from the data from our  
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7 87 preliminary study (Józefiak *et al.*, 2010), no information is so far available on the use  
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10 88 of bacteriocins produced by *Carnobacterium divergens* as feed additives for broiler  
11  
12 89 chickens. The aim of the present study was therefore to investigate the effects of a  
13  
14 90 divercin AS7 preparation on broiler chicken performance, nutrient digestibility, counts  
15  
16 91 of coliform bacteria (indicator of potential pathogens) and LAB (representative  
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18 92 commensals) as well as on the microbial activity in the GIT expressed by the levels of  
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20 93 pH and short-chain fatty acids.  
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22

## 23 24 MATERIALS AND METHODS

### 25 26 **Birds and housing**

27  
28 96 The effect of the divercin AS7 supplementation was evaluated in a growth performance  
29  
30 97 experiment with broiler chickens kept in floor pens (1.58 × 1.16 m) over an  
31  
32 98 experimental period of 35 d. A total of 450 1-d-old male ROSS 308 chicks were  
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34 99 randomly distributed to three dietary treatments using 15 replicate pens per treatment  
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38 100 and 10 birds per pen. The birds were given 23 h light and 1 h dark during the first  
39  
40 101 week, and then 19 h light and 5 h dark from d 7 to 21. From 22 to 42 d of age, there  
41  
42 102 was 23 h light and 1 h dark. The positive control (PC) diet was supplemented with an  
43  
44 103 ionophore coccidiostat (salinomycin, 60 mg/kg), the negative control diet (NC) did not  
45  
46 104 contain any additive and the divercin diet (DIV) was supplemented with 200 AU  
47  
48 105 divercin/ml (0.2ml/ kg).  
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### 50 51 52 **Diets and feeding programme**

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54 107 The compositions of the experimental diets are presented in Table 1. The divercin  
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5 108 activity was quantified by two-fold dilutions, and expressed in activity units (AU) per  
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7 109 millilitre as previously described by Ennahar (2001). The divercin AS7 preparation  
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9  
10 110 was produced according to the technology elaborated by Sip *et al.* (1999) and was  
11  
12 111 provided in liquid form. In the last 5 d of the experiment, 0.2% of the wheat was  
13  
14 112 replaced by titanium oxide as an internal marker for digestibility analyses. Table 1 near here

### 113 **Data collection**

114 The feed intake and body weight of the chickens were measured on d 14 and 35.  
115 Mortality was registered throughout the entire experiment. At the end of the trial (35 d)  
116 from each experimental group, 21 randomly picked chickens (three chickens from 7  
117 pens) were killed by cervical dislocation. For analyses of gastrointestinal contents  
118 (enumeration of bacteria, pH and organic acid concentrations), the contents of crop,  
119 ileum and caecum from three birds per pen were pooled (7 replicate digesta samples of  
120 approximately 10 g). Approximately 5 g of the sample were used freshly for the  
121 culturing of intestinal bacteria and for pH analyses. The rest of the samples were  
122 immediately frozen and stored at -20°C for the analysis of organic acids by gas  
123 chromatography.

124 For the evaluation of apparent metabolisable energy (AME<sub>N</sub>) and nutrient  
125 digestibility, a further 15 randomly chosen birds per treatment (one bird per pen) were  
126 killed on d 35 and ileal contents were collected. The ileum was removed and digesta  
127 were flushed from the terminal ileum (15 cm adjacent to the ileo-caecal junction). To  
128 provide sufficient material for chemical analysis, the fresh ileal samples were pooled  
129 into 5 replicates of approximately 60 g. From the same pens excreta samples of approx.

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5 130 100 g were collected at 35 d. The digesta and excreta samples were immediately  
6  
7 131 frozen, freeze-dried and ground before further analyses.  
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10 132 **Chemical analyses**

11  
12 133 Feed samples were analysed in duplicate for crude protein, crude fat, and crude fibre  
13  
14 134 using AOAC (2005) methods 976.05, 920.39 and 2002.04, respectively. For all  
15  
16 135 chemical analysis, samples were ground to pass through a 0.5 mm sieve. Titanium  
17  
18 136 dioxide was determined according to the method described by Short *et al.* (1996), and  
19  
20 137 samples were prepared according to the procedure proposed by Myers *et al.* (2004).  
21  
22 138 Gross energy was determined using an adiabatic bomb calorimeter (KL 12Mn,  
23  
24 139 Precyzja-Bit PPHU, Poland) standardised with benzoic acid. Nitrogen content was  
25  
26 140 analysed by the Kjel Foss Automatic 16210 (A/S N. Foss Electric, Denmark) and the  
27  
28 141 crude protein content was calculated using a multiplication factor of 6.25. Fat content  
29  
30 142 was determined using Soxhlet System HT 1043, Extraction Unit (Foss Tecator  
31  
32 143 Denmark).  
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38 144 **Microbiological analyses**

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40 145 The pH of pooled digesta from the crop, ileum and caecum was measured immediately  
41  
42 146 after slaughter using a combined glass and reference electrode. The rest of the sample  
43  
44 147 (2.5 g), was diluted in 30 ml of sterile pre-reduced salt medium (Miller and Wolin  
45  
46 148 1974). For enumeration of bacteria, the suspension was homogenised for two minutes  
47  
48 149 in CO<sub>2</sub>-flushed plastic bags using a stomacher homogeniser (Interscience, France).  
49  
50 150 Subsequently, the samples were serially diluted in 10-fold steps using pre-reduced salt  
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52 151 medium according to the technique of Miller and Wolin (1974). Lactic acid bacteria  
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5 152 were enumerated by spread-plating on de Man, Rogosa and Sharp (MRS) agar (Merck  
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7 153 1.10660) incubated for 48 h at 38°C in anaerobic jars (Anaerocult® A, Merck  
8  
9  
10 154 1.13829). Coliform bacteria were enumerated by spread-plating on MacConkey agar  
11  
12 155 (Merck 1.05465) incubated aerobically for 24 h at 38°C.

13  
14 156 The concentration of short-chain fatty acids and lactic acid in the contents of  
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16  
17 157 the different gastrointestinal segments was determined by gas chromatography as  
18  
19 158 described earlier (Canibe *et al.*, 2007) using a Hewlett Packard gas chromatograph  
20  
21 159 (Model 6890, Hewlett Packard, Agilent Technologies, Naerum, Denmark) equipped  
22  
23 160 with a flame-ionisation detector and a 30-m ZB-5 column with an internal diameter of  
24  
25 161 0.32 mm and coated with 5%-phenyl 95%-dimethylpolysiloxane with a film thickness  
26  
27 162 of 0.25 µm. Total concentration of organic acids is expressed as the sum of acetic,  
28  
29 163 propionic, butyric, isobutyric, valeric, succinic and lactic acids.

### 33 164 **Digestibility calculations**

34  
35 165 The following equations were used to calculate the apparent digestibility of the protein,  
36  
37 166 fat (using titanium dioxide and fat digestibility (dig.) calculation as an example) and  
38  
39 167 AME<sub>N</sub> content of experimental diets:  
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$$Fat\ dig.\ (%) = \{1 - [(TiO_2\%_{diet}/TiO_2\%_{digesta/excreta}) \times (fat\%_{digesta/excreta}/fat\%_{diet})]\}$$
  
46  
47 170 
$$\times 100$$

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$$AME_N\ (kJ/kg) = GE_{kJ/kg\ diet} - [GE_{kJ/kg\ excreta} \cdot (TiO_2\%_{diet}/TiO_2\%_{excreta})] - 34.39 \cdot \{N\%_{diet}$$
  
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$$- [N\%_{excreta} \cdot (TiO_2\%_{diet}/TiO_2\%_{excreta})]\}$$

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6  
7 175 Where GE is gross energy, N is nitrogen, TiO<sub>2</sub> is titanium oxide, and 34.39 is the  
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10 176 energy equivalent of uric acid nitrogen (i.e., 34.39 kJ/kg uric acid nitrogen).

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12  
13 177 **Statistical analysis**

14  
15 178 The study was set up as a completely randomised design, and data were tested using  
16  
17 179 the GLM procedure of SAS (SAS 1990). Means were separated using a Duncan's  
18  
19 180 multiple range test. All statements of significance are based on  $P < 0.05$ .

20  
21 181 **RESULTS**

22  
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24 182 Throughout the trial, the mortality was below 2% without any signs of viral or bacterial  
25  
26 183 infections. Birds fed on diets supplemented with salinomycin showed the best  
27  
28 184 performance results (Table 2). Compared with the non-supplemented control, the  
29  
30 185 divercin supplementation increased the body weight gain in the starter period  
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32 186 ( $P = 0.006$ ) and at the end of the trial ( $P < 0.001$ ), and tended to improve total feed  
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34 187 conversion ratio as well as feed intake in each growth stage.

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Tables 2 & 3 near here

188 The levels of apparent metabolisable energy (AME<sub>N</sub>) and ileal digestible  
189 energy (Table 3) increased after salinomycin supplementation ( $P < 0.001$  and  $P = 0.016$ ,  
190 respectively). However, there was no difference between PC and DIV treatments with  
191 respect to the estimated ileal digestible energy. Although the ileal crude fat digestibility  
192 of birds fed the PC diet was approximately 24 percentage points higher than that of the  
193 other groups (0.92 vs. 0.68), no statistical significant difference was found ( $P = 0.118$ ).  
194 Total tract fat digestibility was significantly higher ( $P = 0.013$ ) in the PC group (Table

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5 195 3). The lowest ileal crude protein digestibility, as well as total nitrogen retention, was  
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7 196 observed in the birds fed the NC diet. Table 4 near here

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10 197 In the present trial, the dietary supplementation with salinomycin numerically  
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12 198 increased the counts of LAB bacteria (Table 4), but only significantly in the caecum  
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14 199 ( $P=0.015$ ). Divercin tended to reduce the LAB population in the contents of the crop  
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16 200 ( $P=0.14$ ). However in the ileum ( $P=0.015$ ) and caecum ( $P=0.006$ ) the reduction was  
17  
18 201 significant. The counts of coliform bacteria in the different intestinal segments were  
19  
20 202 not influenced by the dietary treatments ( $P>0.05$ ).

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24 203 Compared with the non-supplemented controls, the digesta pH in crop and  
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26 204 ileum was higher when birds were fed salinomycin and divercin ( $P=0.011$  and  
27  
28 205  $P=0.001$ , respectively). In the caecum, the pH was highest in the non-supplemented  
29  
30 206 control ( $P=0.043$ ). Table 5 near here

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32  
33 207 In crop contents, the dominant organic acids were lactic acid and acetic acid  
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35 208 (Table 5), while succinic acid and butyric acid were detected in small concentrations.  
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37 209 Compared with the non-supplemented diet, the addition of salinomycin and divercin  
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39 210 reduced lactic acid, acetic acid and succinic acid concentrations by approximately 50%  
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41 211 ( $P>0.001$ ) in crop contents. Also in ileal content, the most abundant organic acids were  
42  
43 212 lactic and acetic acid, whereas succinic acid was detected only in low concentrations  
44  
45 213 (Table 5). The concentration of lactic acid was influenced by the dietary treatment  
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47 214 ( $P<0.001$ ) and was lowest in ileal contents of birds supplemented with salinomycin  
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49 215 ( $34.5 \mu\text{g/g}$ ), as compared with divercin ( $72.4 \mu\text{g/g}$ ) and the non-supplemented control  
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51 216 diet ( $114.0 \mu\text{g/g}$ ). The concentration of acetic acid in ileal contents was not influenced  
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5 217 by the dietary treatment ( $P>0.05$ ). In caecal contents, the organic acid profile was  
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7 218 much more diverse with acetic acid, butyric acid and propionic acid being the  
8  
9 219 dominant organic acids (Table 5). Low concentrations of isobutyric, isovaleric and  
10  
11 220 valeric acids were detected in caecal contents, whereas lactic acid was not detected at  
12  
13 221 this location. In general, divercin reduced the microbial fermentation to the same extent  
14  
15 222 as salinomycin (Table 5). There was no difference between groups with respect to the  
16  
17 223 production of organic acids in the caecum ( $P>0.05$ ).  
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20

## 21 224 DISCUSSION

22  
23  
24 225 The observed growth promoting effect of salinomycin is in good agreement with  
25  
26 226 earlier studies on broiler chickens (Engberg *et al.*, 2000), ruminants (Spears 1990) and  
27  
28 227 pigs (Moore *et al.*, 1986). In poultry, many of the applied ionophore coccidiostats do  
29  
30 228 not only target *Eimeria* species but also certain Gram-positive GIT bacteria. These  
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32 229 substances have been observed to reduce the numbers of, for example, *Clostridium*  
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34 230 *perfringens* (Bjerrum *et al.*, 2005) and LAB, including species like *Lactobacillus*  
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36 231 *salivarius*, previously reported to be dominating in the broiler GIT (Engberg *et al.*,  
37  
38 232 2000). The improvement of bird growth and feed utilisation imposed by salinomycin  
39  
40 233 may be explained by a limitation of microbial growth and activity *per se*, alleviating  
41  
42 234 the direct competition with the host for nutrients. More specifically, by targeting  
43  
44 235 bacteria responsible for deconjugation of bile salts (Knarreborg *et al.* 2002; Guban *et*  
45  
46 236 *al.* 2006), salinomycin treatment may also improve fat digestibility (Knarreborg *et al.*  
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48 237 2004). In accordance with this, the present study demonstrated an increased fat  
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50 238 digestibility and AME<sub>N</sub> content after dietary salinomycin supplementation. The AME<sub>N</sub>  
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5 239 content was actually higher than the calculated energy value of the diets (13.6 vs. 13.0  
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7 240 MJ/kg). Compared with the NC diet, the ileal digestible energy increased after  
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9  
10 241 divercin supplementation (10.3 vs. 11.6 MJ/kg), being in between the NC and the PC  
11  
12 242 diet. Both additives also improved ileal crude protein digestibility as well as nitrogen  
13  
14 243 retention. The present results thus suggest that the divercin-medicated improvement in  
15  
16 244 broiler growth performance ( $P=0.001$ ) and feed utilisation ( $P=0.059$ ) may be directly  
17  
18 245 linked to an improved nutrient digestibility, due to a reduction of the GIT microbial  
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21 246 load and activity..  
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24 247 Dietary supplementation with different bacteriocins has been shown to suppress  
25  
26 248 different pathogenic bacteria. Cole *et al.* (2006) demonstrated significant reduction of  
27  
28 249 *Campylobacter jejuni* in turkey poultlets after dietary addition of bacteriocin B602 from  
29  
30 250 *Paenibacillus polymyxa* (NRRL B-30509), and bacteriocin OR7 from *Lactobacillus*  
31  
32 251 *salivarius* (NRRL B-35014). *Enterococcus faecium* strain J96 isolated from the crop of  
33  
34 252 a chicken showed protective effects on chickens infected with *Salmonella pullorum*  
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36 253 (Audisio *et al.*, 1999). Mountzouris *et al.* (2010) examined avilamicin and a probiotic  
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38 254 product based on 5 bacterial species isolated from the crop (*Lactobacillus reuteri* DSM  
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40 255 16350), jejunum (*Enterococcus faecium* DSM 16211), ileum (*Bifidobacterium*  
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42 256 *animalis* DSM 16284), and caecum (*Pediococcus acidilactici* DSM 16210, and  
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44 257 *Lactobacillus salivarius* DSM 16351) of healthy adult chickens. These bacteria are all  
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46 258 capable of producing bacteriocins (Cleveland *et al.*, 2001; Joerger 2003; Diez-  
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48 259 Gonzalez, 2007; Nazef *et al.*, 2008), and it has thus been concluded that some of the  
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50 260 growth promoting effects were linked to antimicrobial activity of those peptides.  
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5 261 However, the highest ileal apparent digestibility coefficients of crude protein and ether  
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7 262 extract were observed in avilamycin supplemented treatment, followed by the  
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9 263 probiotic-supplemented group (Mountzouris *et al.*, 2010).

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11 264 Grilli *et al.*, (2009) showed that pediocin A from *Pediococcus pentosaceus*  
12  
13 FBB61, belonging to the class III bacteriocins, significantly improved growth  
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15 265 performance of broiler chickens challenged with *Clostridium perfringens*. In the  
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17 266 present study, we did not enumerate specific pathogens. However, the experimental  
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19 267 diets provided animal fat sources (lard and tallow) known to stimulate the intestinal  
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21 268 growth of *Clostridium perfringens* (Knarreborg *et al.*, 2004). It cannot be excluded  
22  
23 269 therefore, that some of the observed salinomycin and divercin effects may be linked to  
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25 270 a suppression of *C. perfringens*; a very active player in bile acid de-conjugation  
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27 271 (Knarreborg *et al.*, 2002).  
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33 273 Lactobacilli are among the most abundant bacteria in the chicken GIT and  
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35 274 typically dominate in the upper GIT segments (crop, gizzard and ileum). Lu *et al.*  
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37 275 (2003) thus reported approx. 70% of the sequences in an ileum-derived 16S rRNA  
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39 276 gene library to be allocated to the *Lactobacillus* genus. In the caecum, on the other  
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41 277 hand, where strict anaerobes proliferate readily, lactobacilli only accounted for approx.  
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43 278 8% of the library sequences (Lu *et al.*, 2003). Lactic acid bacteria are usually  
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45 279 considered beneficial for the host bird due to their production of, for example, lactic  
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47 280 and acetic acids, leading to pH reduction (Engberg *et al.*, 2004), as well as their  
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49 281 production of certain bacteriocins (Nazef *et al.*, 2008). On the other hand, there is also  
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51 282 certain evidence that some small intestine LAB, *e.g.* *Lactobacillus salivarius* and  
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5 283 *Enterococcus faecium* might have negative effects on broiler performance (Engberg *et*  
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7 284 *al.* 2000; Guban *et al.* 2006).

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10 285 In general, the observations of the present study suggest salinomycin as well as  
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12 286 divercin can suppress the overall microbial activity of the broiler GIT; the  
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14 287 concentration of organic acids was thus highest in the non-supplemented control group  
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16 288 in each GIT segment (Table 5), though not significant for the caecum, and the observed  
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18 289 levels of total and individual organic acids were in good agreement with our earlier  
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20 290 studies (Józefiak *et al.*, 2006; Józefiak *et al.*, 2007). The suppression of the microbial  
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22 291 activity by salinomycin and divercin is clearly reflected in the pH levels of the upper  
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24 292 GIT segments, but not in the caecum; an apparent discrepancy also observed earlier  
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26 293 (Engberg *et al.*, 2000). More specifically, the LAB activity also seemed to be  
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28 294 suppressed by salinomycin and divercin, as indicated by the lower lactic acid  
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30 295 concentrations and higher pH in the upper parts of the GIT (crop and ileum), compared  
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32 296 with the control group. Surprisingly, however, significant reduction of the LAB (MRS)  
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34 297 counts was only observed in the ileum of the divercin treated broilers, whereas  
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36 298 salinomycin actually increased caecal LAB counts; a discrepancy that cannot be readily  
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38 299 explained, but similar trends have been observed before (Engberg *et al.*, 2000) and it  
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40 300 emphasises the complexity of the mechanisms involved. In particular, the question of  
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42 301 which species of broiler GIT lactic acid bacteria may be targeted by divercin and  
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44 302 salinomycin remains unanswered, as no detailed identification of the involved LAB has  
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46 303 been conducted.

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54 304 A recent study by Shin *et al.* (2008) showed that the broiler chicken GIT is inhabited  
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5 305 by many bacteriocin-producing bacteria with antibacterial activity against pathogens.  
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7 306 Even though carnobacteria are relatively widespread in nature, however, there is so far  
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10 307 no evidence that they are present in the GIT of broiler chickens (Leisner *et al.*, 2007); a  
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12 308 fact that may explain the strong effects of divercin AS7 introduction on bacterial  
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14 309 fermentative activity. The fact that divercin AS7 in the present study tended to  
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16 310 suppress LAB even in the caecum indicates that this bacteriocin, even in an  
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18 311 unprotected form, may continue to be active in the lower segments of the broiler GIT,  
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20 312 although, as a protein, it is prone to hydrolysis in the upper intestine.  
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24 313 To our knowledge, the present study is the first attempt to use a  
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26 314 *Carnobacterium divergens* bacteriocin as a feed supplement and to demonstrate  
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28 315 positive growth promoting and antibacterial effects of divercin in broiler chickens.  
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30 316 Although consistent with our preliminary studies (Józefiak *et al.* 2010), the results  
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32 317 presented here should be followed up by further investigations in order to provide  
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34 318 more detailed information on broiler growth performance, for example, in response to  
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36 319 different divercin doses; as well as the specific bacteria being targeted by the  
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38 320 bacteriocin. Finally, it could be attempted to increase the effectiveness of divercin in  
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40 321 the broiler GIT by protective encapsulation of the peptide.  
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469 **Table 1.** *Composition of the basal diets and their calculated nutritive value*

Ingredients ( $g\ kg^{-1}$ )	Diet (0 – 35 d)
Wheat	326.8
Barley	250.0
Soyabean meal	215.4
Beef tallow	30.0
Pig lard	53.7
Double zero rapeseed meal	60.0
Fish meal	30.0
Monocalcium phosphate	11.0
Mineral-vitamin premix <sup>a</sup>	5.0
Limestone	4.2
L-Lysine -HCl	2.8
DL-Methionine	2.1
L-Threonine	0.3
Sodium carbonate ( $Na_2CO_3$ )	1.0
Salt (NaCl)	2.6
Titanium oxide ( $TiO_2$ ) <sup>b</sup>	2.0
<i>Calculated nutritive value (<math>g\ kg^{-1}</math>)</i>	
ME ( $MJ\ kg^{-1}$ )	12.95
Crude protein	220.0
Crude fat	100.0
Crude fibre %	34.50
Calcium – Ca %	8.50
Lysine %	13.0
Methionine %	5.5
Methionine + Cystine %	9.3
Threonine %	8.1
P available.	4.20
<i>Analysed composition (<math>g\ kg^{-1}</math>)</i>	
Crude protein	214.0
Crude fibre	39.2
Crude fat	101.2
ME ( $MJ\ kg^{-1}$ )	12.89

470 <sup>a</sup>Providing the following per kilogram of diet: vitamin A (retinol), 11.166 IU; cholecalciferol, 2.500 IU;  
 471 vitamin E (alpha tocopherol), 80 mg; menadione, 2.50 mg; cobalamin, 0.02 mg; folic acid, 1.17 mg;  
 472 choline, 379 mg; D-pantothenic acid, 12.50 mg; riboflavin, 7.0 mg; niacin, 41.67 mg; thiamin, 2.17 mg;  
 473 D-biotin, 0.18 mg; pyridoxine, 4.0 mg; ethoxyquin, 0.09 mg; Mn ( $MnO_2$ ), 73 mg; Zn ( $ZnO$ ), 55 mg; Fe  
 474 ( $FeSO_4$ ), 45 mg; Cu ( $CuSO_4$ ), 20 mg; I ( $CaI_2O_6$ ), 0.62 mg; Se ( $Na_2SeO_3$ ), 0.3 mg.

475 <sup>b</sup>Replaced corresponding amount of the wheat in each diet, from 30-35 d of broiler growth



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**Table 2. Performance results**

Item	Treatment			SEM <sup>d</sup>	P <sup>e</sup>
	PC <sup>a</sup>	NC <sup>b</sup>	DIV <sup>c</sup>		
	Feed conversion ratio (g feed/g gain)				
1 – 14 d	1.48 <sup>y</sup>	1.54 <sup>x</sup>	1.49 <sup>y</sup>	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 <sup>x</sup>	304 <sup>y</sup>	320 <sup>x</sup>	3.37	0.006
14 – 35 d	1474 <sup>x</sup>	1360 <sup>y</sup>	1413 <sup>xy</sup>	14.25	0.003
1 – 35 d	1801 <sup>x</sup>	1664 <sup>z</sup>	1733 <sup>y</sup>	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

478 <sup>a</sup>The positive control (PC) diet contained 60 mg/kg of salinomycin479 <sup>b</sup>The negative control (NC) diet did not contain any additives480 <sup>c</sup>The divercin supplemented diet contained 200 AU of divercin/ml (0.2 ml/kg)481 <sup>d</sup>SEM – standard error of the mean482 <sup>e</sup>P – probability483 <sup>x, y, z</sup> Within rows, mean values without a common superscript differ significantly.

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**Table 3.** *Ileal digestibility of nutrients and energy, nitrogen retention and AME<sub>N</sub>*

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*content.*

Item	Treatment			SEM <sup>d</sup>	P <sup>e</sup>
	PC <sup>a</sup>	NC <sup>b</sup>	DIV <sup>c</sup>		
	(kcal)				
AMEn	13.6 <sup>x</sup>	12.1 <sup>y</sup>	12.1 <sup>y</sup>	0.211	<0.001
Ileal digestible energy	12.9 <sup>x</sup>	10.3 <sup>y</sup>	11.6 <sup>xy</sup>	0.440	0.016
Ileal crude protein digestibility	0.74	0.62	0.70	0.021	0.074
N Retention	0.49 <sup>x</sup>	0.37 <sup>y</sup>	0.40 <sup>xy</sup>	0.021	0.048
Ileal crude fat digestibility	0.92	0.67	0.69	0.055	0.118
Total tract fat digestibility	0.90 <sup>x</sup>	0.76 <sup>y</sup>	0.72 <sup>x</sup>	0.028	0.013

492 <sup>a</sup>The positive control (PC) diet contained 60 mg/kg of salinomycin493 <sup>b</sup>The negative control (NC) diet did not contain any additives494 <sup>c</sup>The divercin supplemented diet contained 200 AU of divercin/ml (0.2 ml/kg)495 <sup>d</sup>SEM – standard error of the mean496 <sup>e</sup>P – probability497 <sup>x, y, z</sup> Within rows, mean values without a common superscript differ significantly.

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**Table 4.** *Counts of coliform and lactic acid bacteria (log cfu/g digesta) and pH in crop, ileal and caecal digesta*

Item	Treatment			SEM <sup>d</sup>	P <sup>e</sup>
	PC <sup>a</sup>	NC <sup>b</sup>	DIV <sup>c</sup>		
	Crop				
Coliforms	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 <sup>x</sup>	3.94 <sup>y</sup>	4.31 <sup>x</sup>	0.189	0.011
	Ileum				
Coliforms	6.47	6.29	6.24	0.144	0.785
Lactic acid bacteria	7.90 <sup>y</sup>	7.72 <sup>y</sup>	7.03 <sup>x</sup>	0.142	0.015
pH	6.28 <sup>x</sup>	5.14 <sup>y</sup>	5.55 <sup>x</sup>	0.376	0.001
	Caecum				
Coliforms	7.88	7.71	7.13	0.191	0.253
Lactic acid bacteria	9.24 <sup>x</sup>	8.53 <sup>y</sup>	8.00 <sup>y</sup>	0.180	0.006
pH	5.49 <sup>y</sup>	5.78 <sup>x</sup>	5.52 <sup>y</sup>	0.181	0.043

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<sup>a</sup>The positive control (PC) diet contained 60 mg/kg of salinomycin

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<sup>b</sup>The negative control (NC) diet did not contain any additives

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<sup>c</sup>The divercin supplemented diet contained 200 AU of divercin/ml (0.2 ml/kg)

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<sup>d</sup>SEM – standard error of the mean

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<sup>e</sup>P – probability

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<sup>x, y, z</sup> Within rows, mean values without a common superscript differ significantly.

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**Table 5.** Concentrations of organic acids ( $\mu\text{mol/g}$  digesta) in the contents of crop, ileum and caecum of broiler chickens.

Item	Treatment			SEM <sup>d</sup>	P <sup>e</sup>
	PC <sup>a</sup>	NC <sup>b</sup>	DIV <sup>c</sup>		
	Crop				
Acetic acid	11.3 <sup>y</sup>	26.8 <sup>x</sup>	11.4 <sup>y</sup>	2.23	<0.001
Butyric acid	0.2	0.7	0.7	0.16	0.456
Lactic acid	55.3 <sup>y</sup>	119.0 <sup>x</sup>	52.0 <sup>y</sup>	9.59	<0.001
Succinic acid	2.8 <sup>y</sup>	8.7 <sup>x</sup>	3.2 <sup>y</sup>	0.79	<0.001
Total organic acids	69.7 <sup>y</sup>	155.0 <sup>x</sup>	67.3 <sup>y</sup>	12.36	<0.001
	Ileum				
Acetic acid	5.8	6.1	4.2	0.49	0.241
Lactic acid	34.5 <sup>z</sup>	114.0 <sup>x</sup>	72.4 <sup>y</sup>	10.37	<0.001
Succinic acid	0.2 <sup>y</sup>	2.0 <sup>x</sup>	0.7 <sup>y</sup>	0.27	0.006
Total organic acids	40.7 <sup>z</sup>	122.0 <sup>x</sup>	77.2 <sup>y</sup>	10.69	0.001
	Caecum				
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 <sup>f</sup>	4.35	1.32	-	-
Total organic acids	129.5	141.0	123.0	4.87	0.31

526 <sup>a</sup>The positive control (PC) diet contained 60 mg/kg of salinomycin527 <sup>b</sup>The negative control (NC) diet did not contain any additives528 <sup>c</sup>The divercin supplemented diet contained 200 AU of divercin/ml (0.2m/kg)529 <sup>d</sup>SEM – standard error of the mean530 <sup>e</sup>P – probability531 <sup>f</sup>ND – not detected532 <sup>x, y, z</sup> Within rows, mean values without a common superscript differ significantly.

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