The effects of a competitive exclusion product and two probiotics on *Salmonella* colonization and nutrient digestibility in broiler chickens

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Primary Audience: quality assurance personnel, veterinarians

SUMMARY

Competitive exclusion (CE) cultures, given as a single dose on the day of hatch, together with good hygienic practices has been shown to be a novel approach to control *Salmonella* in poultry. The ability of the CE product Broilact and 2 probiotics, FloraMax-B11 and Colostrum, to prevent *Salmonella* colonization in newly hatched chickens was evaluated employing a slightly modified Mead-model chicken assay. In a parallel study the effect of the 3 treatments on the production of volatile fatty acids in the ceca were determined. In the *Salmonella* study 2 separate experiments were done. In the first experiment all 3 treatment materials were given as a single dose on d 1. In the second experiment, which consisted only of Broilact and FloraMax-B11, the latter was given in the drinking water during the 3 first d after hatch. In both experiments the chicks were challenged with *Salmonella enterica* serovar Infantis on d 2. The results of the present study show that Broilact was superior to the 2 other treatment materials in protecting the newly hatched chickens against *Salmonella* colonization. The parallel study showed only minor differences among the different treatments. Based on the results of the *Salmonella* challenge study, it was concluded that Broilact was the only treatment material that was established in the gut of the newly hatched chickens in such a way that the colonization of *Salmonella* was prohibited.

Key words: competitive exclusion, broiler, *Salmonella*, bird performance

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DESCRIPTION OF PROBLEM

Competitive exclusion (CE) cultures, given as a single dose on the day-of-hatch, together with good hygienic practices has been shown to be a novel approach to control *Salmonella* in poultry [1–3]. The treatment has a beneficial effect also on those broiler flocks that have been contaminated with *Salmonella* already in the hatchery [4]. Though originally developed to control *Salmonella* infections [5], the concept has also been shown to protect chicks against chicken and human pathogenic *Escherichia coli* [6–8] and *E. coli* carrying plasmid-borne extended-spectrum β-lactamases (ESBL) or transferable class C serine β-lactamases (pAmpC enzymes) [9]. In

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addition, the effect of CE treatment against *Campylobacter* [10–12] and necrotic enteritis [13–15] has been shown both in small-scale trials and in the field. Furthermore, it has been shown that CE preparations of chicken origin also provide protection against *Salmonella* in turkey poults [16–18], quail [19], and pheasants [20].

Already at an early stage in the history of competitive exclusion, an improvement in growth rate was observed in commercial broiler flocks treated with a CE preparation [3, 12, 21, 22]. However, improvement in bird performance is probably most apparent in flocks that are suffering from a disease condition, e.g., from necrotic enteritis [15]. In a laboratory-scale study the CE product Broilact was shown to decrease ileal digesta viscosity, and feed metabolizable energy (ME) value in broilers fed wheat- and barley-based diets [23]. In another study Broilact significantly improved total feed digestibility at 35 d when the birds were fed a corn and soybean-based diet. Increases in body weight and fecal dry-matter content were also observed, as well as an improved feed conversion ratio [24].

Probiotics are used primarily to enhance the growth performance of food animals or to control conditions such as scouring. They are given in feed or water, often over a long period of time [25, 26]. Improvement in weight gain was shown when a commercial probiotic containing *Lactobacillus acidophilus* and *L. casei* was included in different broiler diets that were low in certain nutrients [27]. In another study with 2 commercial probiotics consisting of lactobacilli and fecal enterococci, no significant differences were obtained in broiler body weight, feed conversion or mortality between the probiotic treatments and control group in any of the trials [28]. As with CE products probiotics may give best result when applied to poultry flocks that are for some reason not performing well [29].

Contradictory results have been published also regarding the anti-*Salmonella* activity of probiotics [17, 30–32]. It has been suggested that the efficacy of probiotics may depend on factors such as microbial species composition (e.g., single or multistrain) and viability, administration level, application method, frequency of application, overall diet, bird age, overall farm hygiene, and environmental stress factors [33]. On the other hand, it has also been suggested that lactobacilli, which may help to confer protection against *Salmonella* when added together with the other organisms of a poultry cecal suspension, can make the situation worse when added alone [34].

Over the years, varying experimental procedures have been employed to test the efficacy of different CE products and probiotics against *Salmonella*, which has made the comparison of these 2 difficult. This study was undertaken to test the efficacy of the commercial CE product Broilact and 2 commercial probiotics, FloraMax-B11 and Colostrum, against *Salmonella* Infantis in equal circumstances using a slightly modified version of the chicken assay suggested by Mead et al. [35]. The modified assay has been described earlier by Schneitz and Hakkinen [36]. Additionally, a study was undertaken to evaluate the effect of the CE product Broilact and the 2 probiotics, FloraMax-B11 and Colostrum, on the nutrient digestibility, the AME_n of the feed, and the production of volatile fatty acids (**VFA**), showing establishment of strictly anaerobic bacteria in the chicken gut when the birds are fed a diet based on corn and soybean meal.

MATERIALS AND METHODS

Test Materials

Broilact (Orion Corporation, Espoo, Finland) is the first commercial CE product. It is a strongly selected mixture of bacteria derived from the cecal contents of one healthy adult grandparent breeder from 1988. The selection process was based on the ability of certain strictly and facultatively anaerobic bacteria to adhere to the gut wall of the bird. The inocula and final product have been tested to be free from poultry and human pathogens and other unwanted bacterial genera. Broilact is a freeze-dried product. FloraMax-B11 (Vetanco SA, Chile 33, Vicente López, Buenos Aires, Argentina) is also a freeze-dried product consisting of eleven lactic acid bacterial isolates of poultry gastrointestinal origin that belong to 5 different *Lactobacillus* species. Colostrum Liquido (BioCamp Laboratories Ltdo., Campinas, Brazil) is a broth culture containing anaerobic bacteria, bacteria of the genus *Enterococcus* and lactic acid-producing bacteria from Specific Pathogen Free (SPF)

chicks. FloraMax-B11 and Colostrum Liquido were shipped together from Brazil packed with a cooler. The storage temperatures for Colostrum Liquido and FloraMax-B11 range from +2 to +8°C and from +5 to +25°C, respectively. After arrival the products were stored in a refrigerator, and they were all within expiry when applied to the chickens. The viability of the products was not checked because no known disadvantages had occurred during transport.

Test Animals

In the *Salmonella* challenge study, in Experiment I 240 and in Experiment II 180 newly-hatched Ross 508 broiler chickens were brought from a commercial hatchery and divided randomly in groups of 10 and reared on softwood granulated bedding of aspen in solid-bottom cardboard boxes.

In the digestibility study, a total of 192 newly-hatched Ross 508 broiler male chicks were divided into 4 experimental groups, six replicates per treatment. In the beginning of the study there were 8 broiler chickens per cage and replicate. All experimental procedures were approved by the National Ethical Committee for Animal Experiments (Hämeenlinna, Finland).

Feed and Water

In the *Salmonella* challenge study, the chicks were given a commercial feed, Broiler Pikku Punaheltta (Suomen Rehu, Hankkija-Maatalous Oy, Finland). The feed did not contain any antibacterials or anticoccidials. Regular tap water was applied from watering bottles.

In the bird digestibility study, an experimental diet without growth-promoting antibiotics or coccidiostats was formulated to achieve the nutrient requirements of Ross-508 broiler chickens (Table 1). Grain ingredients of the diet were ground in a roller mill. Feeds were mixed and steam-pelleted (KAHL 33–50, AMANDUS KAHL GmbH & Co. KG, Hamburg, Germany). The pellet diameter was 4 mm. Feeds and water were available ad libitum throughout the experiments except during the fasting period. Titanium oxide (Ti) was used as an indicator in the diet to determine the digestibilities, AME_n, and retention of nitrogen.

Table 1. The composition and nutrient contents of the experimental diet in the digestibility study.

Ingredients, g/kg	
Corn	558.8
Soybean meal	350.6
Rapeseed oil	43.8
Monocalcium phosphate	19.5
Limestone	10.0
NaCl	3.8
Mineral premix[1]	2.0
Vitamin premix[2]	2.0
DL-Methionine	1.6
L-Lysine	3.1
L-Threonine	0.9
Titanium oxide	4.0
Nutrient content, g/kg DM (except DM and AME_n)	
DM, g/kg	890,9
AME, kcal/kg[3]	3487.1
Crude protein	235.5
Crude fat	79.4
Crude fiber	30.0
Ash	69.6
Lysine[4]	15.83
Methionine[4]	5.73
Threonine[4]	10.61
Calcium[4]	10.84
Phosphorus (available)[4]	5.08

[1]Provided per kilogram of the complete diet: Ca 0.63 g, iron 29.1 mg, copper 8.0 mg, manganese 50.3 mg, zinc 65.1 mg, iodine 0.51 mg, selenium 0.20 mg.

[2]Provided per kilogram of the complete diet: Ca 331 g, vitamin A 6.00 IU, vitamin D_3 2.25 IU, vitamin E 30,000 mg (α-tokoferol 27 270 mg), vitamin K_3 1,505 mg, vitamin B_1 1,257 mg, vitamin B_2 3,000 mg, vitamin B_6 2,010 mg, vitamin B_{12} 12.5 mg, biotin 75 mg, folic acid 504 mg, niacin 20,072 mg, pantothenic acid 7,506 mg.

[3]Based on chemical analysis of feed ingredients.

[4]Based on values for feed ingredients in Feed tables and nutrient requirements (MTT Agrifood Research Finland, 2012).

Experimental Design

The *Salmonella* challenge study included 2 experiments. Experiment I consisted of 3 separate trials with 80 chickens in each trial, 20 birds in 2 equal groups per treatment. Tube and syringe were used to deliver by oral gavage the dosages suggested by the manufacturers as a single dose on the day of hatch in a dose volume of 0.3 mL: 1 mg of Broilact (5 g for 5,000 chicks) in phosphate buffered peptone water, 6 mg of FloraMax-B11 (60 g for 10,000 chicks) in skimmed milk [37], and 0.01 mL of Colostrum in regular tap water (50 mL for 5,000 chicks).

Experiment II consisted also of 3 trials, but included only Broilact and FloraMax-B11. This experiment was conducted, because FloraMax-B11 was instructed to be given in the drinking water during a time period of 3 consecutive d. The dosing was, however, difficult to adjust, because it was by way of the drinking water. Thus, the total doses per chicken were 35.9, 28.3, and 30.8 mg in the 3 trials, respectively. Broilact was given per os on the day of hatch and dosed as described in Experiment 1.

Twenty-four hours after dosage, the chicks in Experiment I were challenged via oral gavage (0.5 mL) with 1,300 to 2,300 and in Experiment II with 1,400-3,600 CFU/chick of a nalidixic acid resistant derivate of *Salmonella* Infantis. The birds were humanely euthanized 5 d later with carbon dioxide gas, their ceca were removed and *Salmonella* was cultivated from their cecal contents both quantitatively and by enrichment as described by Schneitz and Hakkinen [35].

In the bird digestibility study there were 4 treatment groups: 1) untreated control; 2) 1 mg of Broilact in a dose volume of 0.3 mL per bird; 3) 6 mg of FloraMax-B11 in a dose volume of 0.3 mL per bird and; 4) 0.01 mL of Colostrum in a dose volume of 0.3 mL per bird. The products were given as a single dose on the day of hatch. Birds were randomly assigned to treatments groups. Treatment groups were placed separated in a 3-tiered battery (6 cages per group with 8 chicks in each cage). The cage wire bottoms allowed passage of feces and for total collection of the feces plates were placed under the cages.

At the ages of 12 and 23 d, 2 chicks per cage were humanly euthanized by cervical dislocation. The intestinal contents from the ileum were collected to determine ileal viscosity. Ileal digesta samples were centrifuged (12,000 $\times$ *g*, 3 minutes) and the viscosity was measured using a Brookfield DV-II+ Cone and Plate Programmable Viscometer (Brookfield Engineering Laboratories Inc., Middleboro, USA). The cone used was CPE-40.

At the age of 26 to 29 d, feces were collected from the plates under the cages to determine AME_n, total tract digestibility of organic matter and nitrogen. The 24-hour feed withdrawal period was started at the age of 29 d and after that feed was given again ad libitum. Four hours after starting the feeding the 4 remaining chicks, at the age of 30 d, from each cage were humanely killed by cervical dislocation to collect ileal and cecal contents. The ileal and cecal contents, respectively, from birds in each cage were pooled. The ileal contents were freeze-dried and stored in a refrigerator for later use. The ileal digestibility of protein and organic matter were measured from ileal contents and the digesta pH and concentrations of VFA and lactic acid were measured from the fresh cecal contents.

Chemical Analysis

Feed samples for analysis from the ready-mixed batch were taken of the basal experimental diet, and passed through a hammer mill fitted with a 1-mm mesh. Dry matter content, crude fat and ash were determined by standard methods [38]. Crude fiber was determined with the modified method AOAC (method 962.09) using glass wool instead of a ceramic fiber filter. Nitrogen content was analyzed using the Leco FP 428 nitrogen analyzer (Leco Corporation, St. Joseph, MI). Crude protein content was calculated by multiplying the nitrogen content by 6.25 [protein is 16% nitrogen (100/16 = 6.25)]. Energy value in Table 1 (kcal/kg AME) is based on the chemical analysis of feed ingredients. The gross energy (GE) of the feed and later mentioned feces was measured with Parr 6200 Oxygen Bomb Calorimeter (Parr Instrument Co. Moline, IL 61265). The pH was determined with Mettler Toledo 345 pH meter (Mettler-Toledo AG, Schwerzenbach, Switzerland), but before that, the cecal sample was diluted 1:3 (weight/weight) with deionized water. To exclude crude material, the sample was centrifuged at 3,000 rpm for 10 minutes (Heraeus Multifuge). The determination of lactic acid was performed colorimetrically from the supernatant [39, 40]. For determination of VFA, the supernatant was filtered (chromafil GF/PET-20/25) and further diluted 1:5 with deionized water and mixed. Two hundred microliter of the diluted supernatant, 50 μL of formic acid (98%) and 50 μL of saturated mercuric chloride (8 g/100 mL) were mixed in a 25-mL measuring bottle. The determination was done using a gas chromatograph (model 6890, Hewlett-Packard, Wilmington, DE) with an automatic injector HP 7683, FID detector, split injection port, and a silica

capillary column (10 m × 0.53 mm). The carrier gas was helium (flow 7 ml/min). In the determination of the results, an external standardization was used.

Calculations and Statistical Analysis

The following formula was used to calculate apparent digestibility of the diet [23]:

$$\text{Digestibility (\%)} = 100 - [100 \times (\text{Dietary titanium oxide content} / \text{Fecal or ileal titanium oxide content} \times \text{Fecal or ileal nutrient content} / \text{Dietary nutrient content})]$$

AME was calculated according to Amerah et al. [41] using the followed formula:

$$\text{AME (kcal/kg diet DM)} = (\text{feed intake} \times \text{GE}_{\text{diet}}) - (\text{excreta output} \times \text{GE}_{\text{excreta}}) / \text{feed intake}$$

where, GE = The gross energy of diet or excreta

AME_n values were determined by correction for zero nitrogen retention by simple multiplication with 8.73 kcal/g of nitrogen retained in the body [41].

The results of bird digestibility study were calculated using the ANOVA in GLM Procedure of SAS (SAS Institute Inc., Cary, NC, USA). The parameters presented in Tables 4 and 5 were analyzed using the following model: $Y_{ij} = \mu + t_i + \varepsilon_{ik}$, where Y_{ij} = observation, μ = the general mean, t_i = the effect of the treatment (i = 1,. . . .4), and ε_{ijk} = the experimental error term. The Tukey's range test was used as a single-step multiple comparison procedure and as a statistical test to compare differences between means (ls-means). $P \leq 0.05$ was considered to be significant.

RESULTS AND DISCUSSION

Mead et al. [35] described a chick assay to standardize the method used to evaluate the efficacy of CE preparations against *Salmonella*. Newly-hatched chicks are treated orally on d 1, challenged orally 24 h later with *Salmonella* and examined five d post challenge to determine both the proportion of positive birds in treated and control groups and the levels of *Salmonella* carriage in infected individuals. The efficacy of the treatment is determined by calculating an Infection Factor (**IF**) value, which is the geometric mean of the number of *Salmonella* organisms per gram of cecal contents for all chicks in a particular group (IF = $\log_{10}$CFU g^{-1}). A Protection Factor (**PF**) value is obtained by dividing the IF value for the control group by that for the treated group [35, 42]. A PF value of 4.0 has been suggested as the lowest limit for acceptance of a CE preparation for use in the field. However, a better way to evaluate the efficacy of a treatment material may be to use the difference between the IF values of control and treated groups (difference = **ΔIF** value) [36]. In this study we examined the results in the same way, as described by Schneitz and Hakkinen [36], because statistical evaluation is not needed when the differences in the efficacy between the different treatment groups is manifest.

The results of the *Salmonella* challenge study are presented in Tables 2 and 3. Each IF value presented in Tables 2 and 3 is a mean of 20 chicks (2 groups of 10 chicks each). In Experiment I (Table 2), 3 chickens in trials 2 and 3, and in Experiment II (Table 3) 2 chickens in trials 2 and 3, died during the rearing period as presented in the columns infected/all.

The chicks treated with Broilact were well protected against the challenge organism, the ΔIF values in Experiment 1 being 5.3, 5.0 and 5.0 in the 3 trials, respectively. In Experiment II the ΔIF values in the 3 trials were 4.8, 5.3 and 6.1. When FloraMax-B11 and Colostrum were given as a single dose on d 1 (Experiment I, Table 2), no effect could be seen by these 2 treatments. In contrast, there were higher loads of *Salmonella* in 2 trials treated with FloraMax-B11 and in one trial with Colostrum than in the corresponding *Salmonella* control groups.

In Experiment II when FloraMax-B11 was given for 3 d in the drinking water, there was a slight reduction of *Salmonella* in the treated groups compared to the controls, the ΔIF values being 1.2, 1.1 and 0.5, respectively.

Table 2. Experiment I: The efficacy of Broilact, FloraMax-B11 and Colostrum against *Salmonella* Infantis when the treatment materials were given as a single dose to day-old chicks.

	Treatment materials									*Salmonella*	
	Broilact			FloraMax-B11			Colostrum			control	
Trial	IF[1]	ΔIF[2]	inf/all[3]	IF	ΔIF	inf/all	IF	ΔIF	inf/all	IF	inf/all
1	0.3	5.3	1/20	5.4	0.2	20/20	5.4	0.2	20/20	5.6	20/20
2	0.4	5.0	3/20	5.6	−0.2	19/19[4]	5.9	−0.5	20/20	5.4	20/20
3	0.2	5.0	2/20	5.8	−0.6	20/20	4.9	0.3	19/19[4]	5.2	19/19[4]

[1]Infection Factor (IF) is the logarithmic number of colony forming units of *Salmonella* Infantis per gram of cecal contents (IF = $\log_{10}$CFU/gram).

[2]ΔIF is the difference between the IF of the control group and that of the treated group.

[3]The column shows the number of *Salmonella*-positive birds per all in that group.

[4]One chick died during the trial period.

Table 3. Experiment II: The efficacy of Broilact and FloraMax-B11 against *Salmonella* Infantis when Broilact was given as a single dose to day-old chicks, and FloraMax-B11 was given in the drinking water for 3 d consecutively.

	Treatment materials							
	Broilact			FloraMax-B11			*Salmonella* control	
Trial	IF[1]	ΔIF[2]	inf/all[3]	IF	ΔIF	inf/all	IF	inf/all
1	0.0	4.8	0/20	3.6	1.2	19/20	4.8	20/20
2	0.0	5.3	0/19[4]	4.2	1.1	18/20	5.3	20/20
3	0.9	6.1	7/20	6.5	0.5	19/19[4]	7.0	20/20

[1]Infection Factor (IF) is the logarithmic number of colony forming units of *Salmonella* Infantis per gram of cecal contents (IF = $\log_{10}$CFU/gram).

[2]ΔIF is the difference between the IF of the control group and that of the treated group.

[3]The column shows the number of *Salmonella*-positive birds per all in that group.

[4]One chick died during the trial period.

However, seeing the high doses of FloraMax-B11 consumed by the chickens, the result is surprisingly poor. One explanation may be that neither FloraMax-B11 nor Colostrum was able to block the potential attachment sites on the gut epithelia well enough to provide protection. This lack of attachment is evident also from Experiment II, in which the chicks were given FloraMax-B11 in the drinking water for 3 d consecutively and the results improved only marginally. On the other hand, as suggested by Barnes and Impey [34], lactobacilli may provide protection against *Salmonella* together with the other organisms of the cecal suspension, but can also make the situation worse when added alone. In our experience, lactobacilli, when isolated from their native environment, lose properties such as the ability to attach to intestinal epithelial cells [43]. This loss of wild-type properties seems to be the case also with complex, pure-culture preparations that in the beginning have been equal in efficacy to mixed cecal cultures [44, 45]. It has also been shown that the best protection against *Salmonella* is achieved by pure-culture preparations containing between 28 and 50 strains from 10 different genera [46, 47]. The poor results with FM-B11 and especially Colostrum Liquido are difficult to explain and may be at least to some extent due to unknown misfortune during transport. However, in Experiment II, FM-B11 showed some efficacy against *Salmonella* when the product was fed continuously for 3 d in high doses.

Because of varying testing methods, the efficacy of different products against *Salmonella* is difficult to compare. The efficacy of FloraMax-B11 against *Salmonella* has been shown in a chicken assay model in which the birds were

Table 4. The effects of Broilact, FloraMax-B11, and Colostrum on pH value and concentrations of VFA (μmol/g) and lactic acid (μmol/g) in the cecal contents at 30 d of age.

	Control	Broilact	FloraMax-B11	Colostrum	SEM
pH	6.2	6.4	6.4	6.3	0.07
Acetic acid	55.0	59.0	53.8	49.5	3.06
Propionic acid	2.2^{b}	7.7^{a}	2.5^{b}	2.0^{b}	0.60
Isobutyric acid	0.3^{b}	0.5^{a}	0.4^{a,b}	0.2^{b}	0.06
Butyric acid	10.9^{a,b}	12.9^{a}	11.0^{a,b}	8.6^{b}	0.71
Isovaleric acid	0.3^{b}	0.6^{a}	0.4^{a,b}	0.2^{b}	0.07
Valeric acid	0.8	1.1	0.9	0.8	0.08
Total VFA	69.4^{a,b}	81.8^{a}	67.3^{b}	59.7^{b}	3.78
Lactic acid	0.2	0.1	0.2	0.2	0.03

^{a,b}Means in the same row followed by the same letters do not differ; $P > 0.05$ (Tukey's test).

first challenged with *Salmonella* and treated 1 h later with the probiotic and their cecal contents are checked for *Salmonella* 24 and/or 72 h post challenge [48, 49]. When the chicks were first treated with FloraMax-B11 and challenged 24 h later with *Salmonella* and killed 24 h after challenge, 2 out of 4 trials failed to work [37]. Higgins et al. [37] suggest that the timing of the challenge is crucial. On the other hand, the nature of this kind of product is prophylactic rather than therapeutic, so in that sense they should work, especially in situations where the challenge comes after treatment, though the concept of CE has been shown to work also in situations where the birds have been contaminated already in the hatchery [4].

Table 4 shows the pH and VFA and lactic acid concentrations in cecal contents from the bird digestibility study (samples taken at 30 d of age). The cecal pH values did not differ between the 3 treatments. Broilact increased ($P \leq 0.05$) the concentrations of cecal propionic, isobutyric, and isovaleric acids, as well as the concentration of total VFA, compared to the control, but that difference was not significant (Table 4). Compared to FloraMax-B11 and Colostrum, Broilact increased the concentration of propionic acid and total VFA ($P \leq 0.05$). Further, compared to Colostrum, Broilact increased the cecal concentrations of isobutyric, butyric, and isovaleric acids ($P \leq 0.05$). There was no increase in the concentration of total VFA in the cecal contents of chicks treated with either FloraMax-B11 or Colostrum compared to the control. Because bacterial fermentation mainly occurs in the cecum [50], only the cecal contents were taken for analysis. In the normal intestinal flora, VFA are produced mainly as a result of the metabolism of sporing and non-sporing anaerobic bacteria, and the increase in the concentrations of VFA is considered to be a clear indicator of the establishment and growth of anaerobic bacteria in the chicken gut [51, 52]. Those VFA that are inhibitory to *Salmonella* include acetic, propionic, and butyric acids [24]. Corrier et al. [53] noticed a considerable increase in propionic acid and total VFA concentrations in the cecal contents of CE treated chicks compared with the controls at 3 d of age, 2 d after treatment, indicating that VFA-producing bacteria present in the treatment material were rapidly established in the ceca after treatment on the day-of-hatch. In another study Nisbet et al. [54] showed that CE cultures that increased ($P \leq 0.05$) cecal propionic acid in 3-day-old chicks, decreased ($P \leq 0.05$) cecal *Salmonella* colonization in 10-day-old chicks compared with the untreated controls, and CE cultures that failed to increase ($P \leq 0.05$) cecal propionic acid concentrations in 3-day-old chicks, failed also to protect the chicks against cecal *Salmonella* colonization in 10-day-old chicks. Similar correlation between increasing levels of cecal propionic acid concentrations and decreasing incidence of *Salmonella* in the cecal contents was also reported by Martin et al. [55].

Table 5 shows the AME_n and the apparent digestibilities and retention of nutrients. The different treatments had only minor effects on the nutrient utilization and digesta viscosity. Broilact increased ($P \leq 0.05$) the total tract digestibility of organic matter compared to that of FloraMax-B11. In addition, total tract digestibility of nitrogen was increased ($P \leq 0.05$) in the

Table 5. The effects of Broilact, FloraMax-B11, and Colostrum on the nitrogen-corrected AME (AME_n) (kcal/kg DM) digestibility of the feed (%), the viscosity of the ileal contents (cPs) and fecal dry matter (%).

	Control	Broilact	FloraMax-B11	Colostrum	SEM
AME_n MJ/kg DM	3,343.8	3,391.6	3,343.8	3,343.8	14.33
Total tract digestibility of organic matter, %	74.2[a,b]	74.9[a]	73.5[b]	74.1[a,b]	0.31
Total tract digestibility of nitrogen, %	61.2[a]	60.4[a,b]	58.0[b]	61.3[a]	0.80
Ileal digestibility of protein, %	82.1	83.3	82.3	82.1	0.50
Ileal digestibility of organic matter, %	73.8	74.2	73.9	73.5	0.70
[1]Ileal viscosity, [2]cPs, at 12 d of age	4.1	4.0	4.0	3.1	0.26
Ileal viscosity, [2]cPs, at 23 d of age	4.4	4.2	3.9	3.0	0.40
Fecal dry matter, %	27.3	23.5	24.8	26.8	1.27

[1]Treatment 1: N = 5, SEM = 1,115*SEM.

[2]cPs = Centipoise.

[a,b]Means in the same row followed by the same letters do not differ $P > 0.05$ (Tukey's test).

Colostrum treated birds but also in the untreated controls compared to FloraMax-B11. In the previous study by Schneitz et al. [23] Broilact treatment decreased the ileal viscosity, but in the current study there were no effects on viscosity by the different treatments. On the other hand, the ileal viscosity in all treatment groups was much lower than in the previous study. Intestinal viscosity is known to be a major factor limiting bird performance [56]. Increasing viscosity reduces the mixing and feed passage rate [57]. The composition of the feed is known to affect the viscosity in the small intestine. Soluble arabinoxylans in rye and wheat and β-glucans in barley have shown to give rise to highly viscous conditions in the small intestine of chicks [56, 58, 59]. According to Rodriguez et al. [60] digesta viscosity at the jejunal level was significantly higher in birds receiving the diet based on wheat and barley than in birds fed corn based diet.

The intestinal microflora of the chick changes with age, many of the strictly anaerobic strains appearing only after wk 2 and wk 3 of life [61–63]. The retarded development of the intestinal microflora makes the young chicken vulnerable to enteropathogens such as *Salmonella* [5]. It has been shown, that newly-hatched chickens are relatively well protected against an oral *Salmonella* challenge, already a couple of hours after treatment with a CE culture [64, 65]. Because the effect is so rapid, protection is thought to be primarily physical [66]. The native microflora blocks the potential attachment sites on the gut epithelia, thus increasing resistance to *Salmonella*. The results of the current study are in agreement with the fact that mixed CE cultures like the commercial CE product Broilact are superior in preventing *Salmonella* colonization in the gastrointestinal tract of broiler chickens. The significant increase in the VFA concentrations, especially in that of propionic acid at 30 d of age, indicates that, either the strictly anaerobic bacteria in Broilact remained in the ceca of the test chickens or Broilact enhanced the establishment of strictly anaerobic bacteria in the ceca of the test chickens compared to the other treatments.

CONCLUSIONS AND APPLICATIONS

1. The results of the *Salmonella* challenge study indicate that only Broilact of the 3 treatment materials tested, became established in the gut of the newly hatched chickens in such a way that the colonization of *Salmonella* was prohibited.
2. The significant increase in the VFA concentrations, especially that of propionic acid at the end of the trial period, further indicates colonization of strictly anaerobic bacteria in the ceca of the Broilact-treated chicks.
3. The bird digestibility study showed only minor improvements among the different treatments which may at least partly depend on the composition of the feed.

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