Pediocin A improves growth performance of broilers challenged with *Clostridium perfringens*[1]

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ABSTRACT The aim of this study was to investigate the efficacy of the anticlostridial pediocin A from *Pediococcus pentosaceus* FBB61 to contain negative effects associated to *Clostridium* proliferation in broilers, through 2 subsequent investigations. In the first study, 36 Ross 508 broilers were divided into 3 groups and fed for 21 d as follows: the control diet (CTR), the control diet supplemented with supernatant filtrate of a culture of *P. pentosaceus* FBB61-2 (Bac−, isogenic mutant nonproducing pediocin A), and the control diet supplemented with supernatant filtrate of a culture of *P. pentosaceus* FBB61 (Bac+) containing pediocin A. Birds were challenged with 10^6 cells of *Clostridium perfringens*. In the second study, 216 Ross 508 broilers were allocated in 18 pens and divided into 3 groups fed the same diet for 42 d: a control group (CTR), a group challenged with 10^8 cells of *C. perfringens* (CP), and a group challenged with 10^8 cells of *C. perfringens* and receiving the control diet supplemented with *P. pentosaceus* FBB61 and pediocin A (PA). Broiler BW, ADG, ADFI, and feed conversion rate were measured throughout the studies. At the end of both experiments, an appropriate number of birds was killed and analyzed for necrotic enteritis lesions and microbiological examinations. In the first study, on d 9, ADG and BW were 20% higher for Bac+ compared with CTR and Bac−; on d 14, ADG was higher for Bac+ (+23%, $P < 0.05$), whereas BW was higher for Bac+ and Bac− compared with CTR (+23 and +14%, respectively; $P < 0.05$). In the second study, on d 14, ADG and BW were higher for PA compared with CTR and CP (+15% on average; $P < 0.05$), whereas between 15 and 42 d, there was only a tendency toward a higher ADG for PA when compared with the CP group (+4%, $P = 0.08$). Diet supplementation with pediocin A improved broiler growth performance during the challenge with *C. perfringens* and tended to restore the ADG depletion during the 42-d period.

Key words: broiler, *Clostridium perfringens*, necrotic enteritis, pediocin A

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INTRODUCTION

Pediococcus pentosaceus FBB61 was isolated in 1953 from cucumber fermentation, and it was shown to produce the bacteriocin pediocin A (Costilow et al.,1956; Etchells et al., 1964; Fleming et al., 1975; Rueckert, 1979), associated to plasmid pMD136, that when cured resulted in the loss of immunity to the bacteriocin and its production (Daeschel and Klaenhammer, 1985). Pediocin A was shown to belong to the class III bacteriocins according to Klaenhammer (1993) and to have a molecular weight of 80 kDa and a broad range of activity against gram-positive bacteria (Piva and Headon, 1994), whereas *P. pentosaceus* FBB61 was described as the most effective strain against clostridia (Daeschel and Klaenhammer, 1985; Okereke and Montville, 1991).

Necrotic enteritis (**NE**) is a multifactorial disease in poultry with high economical cost, and it has become increasingly prevalent in the European Union after the ban of antibiotic growth promoters and animal proteins (McDevitt et al., 2006). Clinical signs of NE include depression, decrease of feed ingestion, diarrhea, and severe necrosis of the intestinal tract (Ficken and Wages, 1997). Several predisposing factors, such as dietary components, immunosuppression, mechanical irritation of the gut, and sudden gut microflora changes appear to contribute to this syndrome (McReynolds et al., 2004). The occurrence of necrotic lesions in the intestinal tract is associated with the proliferation of *Clostridium perfringens*, which can also be detected in the intestinal tract of healthy birds (Novoa-Garrido et al., 2006; Olkowski et al., 2006). Conditions that pro-

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mote excessive growth of *C. perfringens* in the chicken intestine lead to α-toxin production, which causes mucosal lesions and clinical disease (Rehman et al., 2006). Necrotic enteritis occurs most commonly in broilers (2 or 5 wk of age) but is also observed in pullets, layers, and turkeys (Collier et al., 2003; Crespo et al., 2007). The aim of this study was to investigate the efficacy of the anticlostridial pediocin A from *P. pentosaceus* FBB61 (WO/2004/087189; Piva and Casadei, 2004) as a tool to contain negative effects, in terms of health and loss of performance, associated to *Clostridium* proliferation in the intestine of broilers in the absence of antibiotic growth promoters.

MATERIALS AND METHODS

Bacterial Cultures and Growth Conditions

Throughout the studies, *P. pentosaceus* FBB61 (ATCC 43200) and its isogenic mutant were revitalized and subcultured in M17 broth (Oxoid Ltd., Basingstoke, UK) supplemented with 1% (wt/vol) glucose at 34°C for 18 h, whereas *C. perfringens* type A (ATCC 13124) was revitalized and subcultured in reinforced clostridial broth (Oxoid Ltd.) in anaerobic conditions at 37°C for 24 h.

Expression and Purification of Pediocin A

Semipurified pediocin A for antimicrobial assay was obtained as described previously by Casadei et al. (2009). For the in vivo studies, pediocin A preparation was obtained as follows. A *P. pentosaceus* FBB61 cultural broth was collected to define the sample titer of pediocin A and bacteria concentration. The so-obtained broth was used in the appropriate amount for the floor pen study, whereas for the pilot study, the cultural broth underwent a purification step through centrifugation at 16,500 × *g* at 4°C for 10 min. Supernatant was collected and filtered with Stericup (Millipore Corporation, Bedford, MA) through a membrane with pores of 0.45-μm diameter and then titered for pediocin A.

Titration of activity of pediocin A was performed through an agar spot test technique. Briefly, from each supernatant filtered produced, 20-μL aliquots were delivered into wells of a M17 agar plate previously seeded with 20 μL of *P. pentosaceus* FBB61-2 (Daeschel and Klaenhammer, 1985) fresh overnight culture and incubated at 39°C overnight. The sample titer [activity units (**AU**)/mL] was defined as the reciprocal of the highest dilution showing definite inhibition of the indicator lawn.

Antimicrobial Assay

An antimicrobial assay was conducted to assess the most effective dose of pediocin A against *C. perfringens* to be used in in vivo challenge studies. The minimal inhibitory concentration (**MIC**) of pediocin A against *C. perfringens* type A (ATCC 13124) was determined using a broth dilution method. The test was performed in disposable tubes containing 2-fold dilutions (320 to 0.6 AU/mL) of semipurified pediocin A in a reinforced clostridial broth (Oxoid Ltd.). An overnight culture of *C. perfringens* was prepared and adjusted so that the final concentration in each tube was approximately 10^4 cfu/mL. The tubes were incubated anaerobically at 37°C for 24 and 48 h. Bacterial growth was indicated by the presence of turbidity in the tube and measured by optical density (**OD**) at 600 nm (UltroSpec 3000, Pharmacia Biotech, Biochrom, Ltd., Cambridge, UK). The MIC was determined as the first tube, in ascending order, where the OD was 0.00. To confirm MIC, 100 μL of broth from each tube after 48 h of incubation was plated onto reinforced clostridial medium agar. After 24 h of incubation, the growth of viable cells was observed. The MIC was the lowest concentration that resulted in a significant decrease in OD values where >99.9% or more of the initial inoculum was killed (Cosentino et al., 1999).

Pilot Study

Birds and Diets. Thirty-six female Ross 508 broilers (44.8 ± 1.8 g) were obtained at 1 d of age, and 12 chickens were placed in each of 3 isolation units (0.9 m^2, 0.075 m^2/bird) equipped with bedding straw, drinkers, heating lamps, and a filtered air supply; the lighting program was 16L:8D. Each isolation unit was assigned to 1 of 3 experimental groups: a negative control (**CTR**), fed basal diet (Table 1); a positive control, fed the same diet supplemented with supernatant filtrate of a cultural broth of the isogenic mutant *P. pentosaceus* FBB61-2 devoid of pediocin A expression (**Bac−**); and a treated group, fed the control diet supplemented with supernatant filtrate of a cultural broth of *P. pentosaceus* FBB61 (**Bac+**). In the Bac+ group, pediocin A was provided at 80 AU/g of feed. All birds were challenged with *C. perfringens* and fed ad libitum for 21 d.

The study was conducted in the facilities of the University of Bologna, whose ethical committee reviewed and approved the experimental protocol.

Microbial Challenge. On d 9, birds were challenged by intracrop administration of vaccinal oocysts (Paracox 5, Intervet Italia srl, Schering-Plough Animal Health, Kenilworth, NJ) at a dose 50 times higher than the recommended one, to produce a mild intestinal coccidiosis, and favor *C. perfringens* infection (Shane et al., 1985).

A fresh overnight culture of *C. perfringens* type A was administered by mouth on d 14, 15, and 16, twice daily (10^6 cfu/bird).

Evaluation of Growth Performance. On d 0, 9, 14, and 21 from the beginning of the study, BW and ADFI were recorded. Average daily gain and feed conversion rate (**FCR**) during the periods of 0 to 9, 0 to 14, 15 to 21, and 0 to 21 d were calculated.

Table 1. Basal diet composition and chemical analysis (% as-fed basis)

Item	Pilot study	Floor pen study	
		Phase I (0 to 14 d)	Phase II (15 to 42 d)
Ingredients			
Soft wheat meal	35.0	39.0	40.05
Soybean meal	30.0	40.09	35.0
Corn meal	27.2	13.9	16.5
Soybean oil	3.0	3.2	5.0
Dicalcium phosphate	2.0	1.9	1.9
Calcium carbonate	1.3	0.6	0.5
Vitamins and minerals[1]	0.5	0.5	0.5
Salt (NaCl)	0.3	0.2	0.2
Lysine HCl	0.3	0.16	—
DL-Methionine	0.3	0.3	0.2
Sodium bicarbonate	0.1	0.15	0.15
Analyzed nutrients			
DM	88.69	87.91	89.55
CP	22.22	25.08	21.71
Ether extract	5.43	5.30	6.75
Crude fiber	3.08	2.85	2.65
Ash	6.19	5.99	5.76
ME, kcal/kg	2,990	2,838	2,920

[1]Providing per kilogram: vitamin A, 2,500.000 IU; cholecalciferol, 15 mg; vitamin E, 15,000 IU; vitamin K, 1,200 mg; vitamin B_1, 400 mg; vitamin B_2, 1,600 mg; pantothenic acid, 2,500 mg; vitamin B_6, 1,200 mg; biotin, 30 mg; folic acid, 250 mg; vitamin C, 20,000 mg; vitamin PP, 8,000 mg; vitamin B_{12}, 6 mg; Cu, 1,000 mg; Fe, 10,000 mg; Mn, 30,000 mg; Se, 40 mg; Zn, 15,000 mg; I, 200 mg; Co, 40 mg.

Fecal and Intestinal Sampling. One pool of feces was collected from each isolator at 11, 12, 13, and 14 d, to perform oocyst counts with an optical microscope. At 21 d, 5 birds per group were killed, analyzed for intestinal lesions, and ileal contents were sampled and plated on to violet red bile, Rogosa, and tryptose-sulfite-cycloserine agar (Oxoid Ltd.) for enumeration of coliforms, lactic acid bacteria (**LAB**), and *C. perfringens*, respectively.

Statistical Analysis. The isolator was the experimental unit for ADFI and FCR calculations, and for oocyst shedding. These data did not undergo statistical analysis, whereas each bird was the experimental unit for BW, ADG calculations, and microbiological counts, and was analyzed by 1-way ANOVA followed by Tukey post hoc test (Graphpad Software 4.1, San Diego, CA). Differences were stated as significant at $P < 0.05$.

Floor Pen Study

Birds and Diets. Two-hundred sixteen male 1-d-old Ross 508 broilers (34.7 ± 2.2 g) were randomly allocated in 18 pens divided in 3 experimental groups (d 0): a negative control group (CTR) fed the basal diet (Table 1), a group inoculated with *C. perfringens* (**CP**), a group inoculated with *C. perfringens* and fed a diet supplemented with *P. pentosaceus* FBB61 (10^7 cfu/g) and pediocin A (**PA**), providing 60 and 40 AU/g fed in the first and second phase diets (0 to 14 d; 15 to 42 d), respectively. All diets were formulated as isoproteic and isoenergetic, without antibiotic growth promoters and coccidiostat drugs. Birds were reared in 1.95-m^2 pens (0.16 m^2/bird) with a lighting program of 16L:8D and were fed ad libitum for 42 d.

The study was conducted in the research facilities of the Research Centre for Animal Production and Environment (CERZOO), which is a Good Laboratory Practices-certified facility. The ethical committee of the University of Bologna reviewed and approved the experimental protocol.

Microbial Challenge. An overnight culture of *C. perfringens* type A was administered by mouth on d 11, 12, and 13 twice daily (10^8 cfu/bird). The CTR group received only water.

Evaluation of Growth Performance. On d 0, 14, and 42 since the beginning of the study, BW and ADFI were recorded. Average daily gain and FCR during the periods of 0 to 14, 15 to 42, and 0 to 42 d were calculated.

Fecal and Intestinal Sampling. Excreta from 5 birds/pen were sampled on d 11, 12, and 13, and on each day, the 5 birds/pen samples were pooled to have 1 sample per pen. Samples were then plated on to violet red bile, Rogosa, and tryptose-sulfite-cycloserine agar for the enumeration of coliforms, LAB, and *C. perfringens*, respectively. On d 14, twelve birds per treatment (2 birds/pen) were killed and analyzed for intestinal lesions and *C. perfringens* colonic counts. On d 42, twenty-four birds per treatment (4 birds/pen) were slaughtered, and ileum and cecum were sampled for the enumeration of coliforms, LAB, and *C. perfringens* as described previously.

Statistical Analysis. The pen was the experimental unit for growth performance and fecal counts, whereas each bird was the experimental unit for intestinal counts. Data were analyzed with 1-way ANOVA, followed by Fisher's post hoc test to compare the means of groups (SAS software, release 2002-2003, SAS In-

stitute Inc., Milan, Italy). Differences were stated as significant at $P \leq 0.05$.

RESULTS

Pilot Study

The MIC of pediocin A against 10^4 cfu/mL of *C. perfringens* was 20 and 40 AU/mL after 24 and 48 h of incubation, respectively.

Health Status of Birds, Intestinal Lesions, Oocyst Shedding, and Bacterial Counts. Only 1 bird died in the CTR group, but the cause of death was not associated with NE, but with colibacillosis. Macroscopic evaluation of intestinal mucosa showed specks of blood with focal distribution throughout the intestine, as well as hemorrhage areas. Generally, there was a lack of marked differences in lesions among groups. No brownish or diphtheric pseudo-membranes were observed. Gram-stained smears of intestinal mucosa demonstrated rod-shaped bacteria with typical *C. perfringens* morphology. Oocyst shedding was less pronounced for the Bac+ and Bac− groups than for CTR throughout the collection days. There were no statistical differences among treatments for coliforms, LAB, and *C. perfringens* in ileal samples.

Growth Performance. Data are shown in Table 2. During the first period (0 to 9 d), ADG was significantly higher for Bac+ versus CTR and Bac− (+31 and +21% vs. CTR and Bac−, respectively; $P < 0.01$), and consequently, BW was higher for Bac+ versus CTR and Bac− (+24 and +17% vs. CTR and Bac−, respectively; $P < 0.01$). On d 14, ADG was still higher for Bac+ compared with CTR (+23%, $P = 0.02$), and BW was higher for Bac+ and Bac− compared with CTR (+23 and +14%, respectively; $P = 0.02$). No statistical differences were found among treatments for growth performance on d 21.

Floor Pen Study

Health Status of Birds, Intestinal Lesions, and Bacterial Counts. Mortality was 4.2 and 6.9% for CP and PA groups, respectively, whereas it was null for the CTR unchallenged birds. Although in dead birds there were no clinical signs and typical lesions of NE, it could be presumed that the bacterial challenge may have had a negative influence on broiler health status. The necroscopic examination of birds killed at 14 and 42 d did not show typical lesions of NE. No statistical differences were found among treatments for coliforms, LAB, and *C. perfringens* in excreta, colon, ileum, and cecum counts.

Growth Performance. Data are shown in Table 3. No statistical differences were found among treatments for ADFI between 0 and 14 d. During the same period, ADG was significantly higher for PA versus CTR and CP (+14 and +15%, respectively; $P = 0.01$), and FCR ratio tended to be numerically lower in PA versus CTR and CP (−23 and −20%, respectively; $P = 0.08$). At 14 d, BW was significantly higher for PA versus CTR and CP (+17 and +18%, respectively; $P < 0.01$). In the second phase, ADG of PA tended to be higher compared with the CP group (+4%, $P = 0.08$) and equal to the unchallenged birds. Overall, ADFI was significantly lower in the PA group compared with the CTR group, with no significant changes in overall ADG, FCR, and final BW.

Table 2. Body weight, ADG, ADFI, and feed conversion rate (FCR) of broilers in the pilot study

	Treatment[1]			Statistic[2]	
Item	CTR	Bac−	Bac+	SEM	*P*-value
Initial BW, g	44.8	43.2	45.4	1.60	0.24
0 to 9 d					
ADFI, g	27.41	23.70	27.41	—	—
ADG, g	15.45[b]	16.79[b]	20.31[a]	1.85	<0.01
FCR	1.34	1.10	1.08	—	—
9-d BW, g	183.8[b]	194.3[b]	228.3[a]	14.24	<0.01
0 to 14 d					
ADFI, g	34.29	33.45	33.69	—	—
ADG, g	21.20[b]	24.20[ab]	26.07[a]	2.28	0.02
FCR	1.62	1.38	1.29	—	—
14-d BW, g	296.8[b]	338.8[a]	365.0[a]	27.29	0.02
14 to 21 d					
ADFI, g	73.11	70.24	69.23	—	—
ADG, g	44.24	40.55	39.77	8.58	0.75
21-d BW, g	606.5	622.7	642.9	44.89	0.59
Overall 0 to 21 d					
ADFI, g	42.43	42.74	44.03	—	—
ADG, g	26.75	27.59	28.47	2.86	0.71
FCR	1.60	1.44	1.44	—	—

[a,b]Means with different superscript within the same row differ significantly ($P < 0.05$).

[1]CTR = basal diet; Bac− = basal diet with supernatant filtrate of the isogenic mutant of *Pediococcus pentosaceus* FBB61-2 nonproducing pediocin A; Bac+ = basal diet with supernatant filtrate of *P. pentosaceus* FBB61 producing pediocin A.

[2]For BW and ADG, n = 12; for feed intake and FCR, n = 1.

DISCUSSION

After the ban of antibiotic growth promoters, as of January 1, 2006, clostridial enteritis is an urgent issue in livestock production, primarily because of the loss of performance of affected animals. *Clostridium perfringens* is the main causative agent of NE, a worldwide-spread multifactorial disease that can affect birds both in acute and subclinical forms. Acute NE may cause 1% of daily mortality up to 30% of mortality for the entire production cycle (Van Immerseel et al., 2004; Dahiya et al., 2006). Subacute enteritis is very frequent, with great economic implication associated with lower digestion and absorption, feed efficiency, and impairment of growth performance (Kaldhusdal et al., 2001; Løvland and Kaldhusdal, 2001; Hofacre et al., 2003; Van Immerseel et al., 2004).

This study aimed to induce subacute NE in broiler chickens, to understand if the supplementation of pediocin A and its producer strain *P. pentosaceus* FBB61 could restore growth performance to regular levels.

In the pilot study, broilers challenged with *C. perfringens* showed lesions typical of NE, whereas in the floor pen study, broilers challenged with *C. perfringens* did not produce clinical signs of NE and there was no mortality directly associated to *C. perfringens* exposure. The same results were shown by the other authors, who observed no clinical signs of NE after *Clostridium* challenge in broiler chickens (Drew et al., 2004; Olkowski et al., 2006). Even though there is plenty of literature on NE challenge models and management, limited information is available about the addition of bacteriocins to bird diets, thus outlining the uniqueness of the present study. Lactic acid bacteria and their bacteriocins have been long studied as food preservatives and starters because they improve food shelf-life and organoleptic and nutritional value (Wood and Holzapfel, 1995; Leroy and De Vuyst, 2004; De Vuyst and Leroy, 2007); the most popular example of a LAB-derived bacteriocin is nisin, which is found to be a generally recognized as safe ingredient by the Food and Drug Administration since 1988. By definition, bacteriocins are typically active against closely related bacteria, limiting their use to a selected number of pathogenic strains; pediocin A was in fact described to have a broad spectrum of activity against numerous strains of gram-positive bacteria and foodborne pathogens such as *Listeria* and *Bacillus cereus.* Piva and Headon (1994) found 133 AU of pediocin A to be effective in killing 100 cells of each strain. In the present study, we demonstrated that pediocin A had strong inhibitory capacities against *C. perfringens* type A in an in vitro antimicrobial assay. Because *C. perfringens* is the organism involved in the pathogenesis of poultry enteritis, pediocin A application in feeds seems to be innovative. Few data are available about the use of bacteriocins in feeding poultry as a method to counteract intestinal pathogen development and shedding, even though in recent times there has been growing interest in such a field. Cole et al. (2006), as well as Stern et al. (2005), described the efficacy of bacteriocins derived from a strain of *Lactobacillus salivarius* against *Campylobacter coli* colonization in turkeys, but no studies with *C. perfringens* are currently available.

In these experiments, we studied the effect of feeding a partially purified bacteriocin, pediocin A, and its *P. pentosaceus* producer strain to birds challenged with high doses of *C. perfringens* (10^6 to 10^8 cfu). The MIC of pediocin A against *C. perfringens* was 40 AU/mL after 48 h, and although equal versus higher doses were used in the in vivo experiments (80 AU/g of feed in the pilot study and 40 to 60 AU/g feed in the floor pen study), no effect was found in the number of viable *C. perfringens* cells in the intestines of killed chickens. This might

Table 3. Body weight, ADG, ADFI, and feed conversion rate (FCR) of broilers in the floor pen study

	Treatment[1]			Statistic[2]	
Item	CTR	CP	PA	SEM	*P*-value
Initial BW, g	34.7	35.2	34.7	0.18	0.12
Phase I (0 to 14 d)					
ADFI, g	39.4	37.5	34.5	2.16	0.31
ADG, g	16.7[b]	16.5[b]	19.0[a]	0.59	0.01
FCR	2.38	2.30	1.83	0.17	0.08
14-d BW, g	269[b]	266[b]	315[a]	7.63	<0.01
Phase II (15 to 42 d)					
ADFI, g	139.4	133.3	130.5	3.39	0.20
ADG, g	71.1	68.0	70.7	0.97	0.08
FCR	1.96	1.96	1.85	0.06	0.34
Final BW, g	2,266	2,244	2,296	27.11	0.41
Overall (0 to 42 d)					
ADFI, g	101.9[a]	96.8[ab]	93.1[b]	2.32	0.05
ADG, g	50.7	48.4	50.6	0.93	0.17
FCR	2.01	2.01	1.85	0.07	0.19

[a,b]Means with different superscript within the same row differ significantly ($P < 0.05$).

[1]CTR = negative control group; CP = *Clostridium perfringens*-challenged control group; PA = *C. perfringens*-challenged group fed diet supplemented with *Pediococcus pentosaceus* and pediocin A.

[2]n = 6.

be explained by considering the diluting effect of in vivo intestinal microbial content. It is well known that the intestine of birds hosts a very large bacterial population (10^4 to 10^5 cfu/g and 10^{10} to 10^{11} cfu/g in the small and large intestine, respectively), among which a large portion is made of gram-positive bacteria (Bjerrum et al., 2006). Because gram-positive organisms are the target of pediocin A, it is possible that there was a sparing effect on *C. perfringens* due to the high competitiveness of the environment. Nevertheless, birds fed with pediocin A and *P. pentosaceus* had better performance in both experiments. In the pilot study, during the first period (0 to 9 d, before challenge) and at 14 d (after challenge), birds fed with pediocin A had higher ADG by 20 and 19%, respectively, when compared with untreated birds. Despite the inherent differences between the pilot and the floor pen studies associated to the different exposure to coccidia and the use of pediocin A along with the *P. pentosaceus* cells, the results of the first experiment were confirmed in the floor pens study, with a larger number of replicates and birds. In fact, birds fed a pediocin A-containing diet had higher ADG by 14 and 15% when compared with a negative and a positive control, respectively, during the period 0 to 14 d immediately after challenge ($P = 0.01$). During the second phase (15 to 42 d), after the bacterial challenge, the difference in ADG between infected birds with or without pediocin treatment was less pronounced (4%) without reaching significant levels ($P = 0.08$).

These results can be explained by the fact that even if we were not able to measure appreciable reduction of *C. perfringens* counts, pediocin A might have targeted other gram-positive species, thus revealing a possibility for pediocin A to beneficially modulate bacterial balance, by favoring beneficial bacteria at the site where noxious bacterial overgrowth occurs during enteritis (Casadei et al., 2009). Previous data showed that pediocin A was able to reduce the extent of fermentation in vitro, resulting in a long-lasting utilization of fermentable energy sources, and to control intestinal microbial metabolism (Casadei et al., 2009). Even though the mechanisms of action of antibiotic growth promoters is not still fully understood, they are known to depress microflora growth, thereby increasing performance by 5 to 10%. Pediocin A may act similarly as a growth-promoting analog.

Even though *C. perfringens* is the main causative agent of NE, there are many factors that can contribute to the development of pathology, and *C. perfringens* itself is not enough to cause NE, even when administered at 10^8 cfu/d. It is likewise evident that such a high number of *C. perfringens* cells in the intestine may disturb microflora metabolism, alter the intestinal fermentation pattern, and impair digestion and absorption. In both experiments, pediocin A helped birds to restore weight to levels comparable to unchallenged birds. Moreover, in the second experiment, along with pediocin A, we added a *P. pentosaceus* producer strain to the diet, which could have had exerted probiotic effects, growing in the intestine of birds, and then activating in situ pediocin A production. This observation is supported by results from a previous study, in which *P. pentosaceus* FBB61 added to an in vitro cecal fermentation system significantly modified the extent of fermentation by reducing ammonia concentration and isoacid molar proportions due to cecal pediocin A secretion from the added *P. pentosaceus* FBB61 (Piva et al., 1995). Recently, Lee et al. (2007) demonstrated the growth-promoting and protecting effect of a *Pediococcus*-based probiotic in *Eimeria*-challenged birds, reporting higher ADG and a decrease in fecal oocyst shedding in birds fed with this probiotic when compared with challenged birds without probiotic supplementation. Mountzouris et al. (2007) found that feeding broiler chickens with a multibacterial species probiotic, containing a *Pediococcus* strain, significantly improved growth performance and, to an extent, cecal microflora composition when compared with avilamycin.

In the floor pen study, we could not determine the extent of loss of activity of pediocin A due to digestive processes nor the in situ synthesis by *P. pentosaceus* FBB61. A microencapsulated gastroprotected form of pediocin A would allow us to gain additional insight in the possible application of bacteriocin to modulate intestinal microflora.

In conclusion, our data demonstrated that both pediocin A alone and the combination with its producer strain *P. pentosaceus* have in vivo growth-promoting effects, further substantiating previous in vitro results (Casadei et al., 2009), and that pediocin A allowed optimal growth in birds challenged with enteropathogenic *C. perfringens*.

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