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Efficacy of Certain Feed Additives for the Prevention of *Campylobacter jejuni* Infection in Broiler Chickens

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ABSTRACT

In this study, the efficacy of acidifiers (lactic and formic acids) and probiotic preparations containing *Pediococcus acidilactici* (*P. acidilactici*) and *Saccharomyces boulardii* (*S. boulardii*) in reducing of *Campylobacter jejuni* (*C. jejuni*) infection in broiler chickens was investigated. One hundred and three day old broiler chicks were used. Three birds were euthanized for *C. jejuni* re-isolation at day old. One hundred chicks were assigned into 4 equal groups each, 25 birds. Groups 1, 2 and 3 were fed on ration containing acidifiers, *P. acidilactici* and *S. boulardii*, respectively, from day old till 5 weeks old. Chicks of group 4 were fed on plain ration (control). At 2 weeks of age, each bird was orally challenged with *C. jejuni*. Groups were kept for 3 weeks recording signs and mortalities. Faecal swabs were collected at different intervals for *C. jejuni* shedding. Liver and intestine were collected weekly for *C. jejuni* re-isolation. At 5 weeks of age, all birds were subjected for post-mortem lesions scoring of *C. jejuni*. Results cleared more severe signs of greenish diarrhea in control than treated birds with no mortalities in all groups. Significant ($p<0.05$) reduction of *C. jejuni* shedding and re-isolation rates as well as lesions scoring was seen in groups treated with *S. boulardii* and *P. acidilactici* followed by acidifiers over control birds. In conclusion, both acidifiers and probiotic preparations greatly reduced and eliminated *C. jejuni* infection in broiler chickens.

Key words: *Campylobacter jejuni*, acidifiers, probiotics, chickens, protection

INTRODUCTION

Campylobacter spp. colonizes the intestinal tract of poultry as they are considered as the main reservoir of the organism. *Campylobacter jejuni* (*C. jejuni*) infection is considered as, one of the most important bacterial disease causing acute human enteric infection in developing countries (Shane, 2000). Control of such infection in poultry is very important to reduce the health hazard in human. *Campylobacter* resistant strains were developed due to misuse of antimicrobials in poultry field (Jorgensen *et al*., 2002). Natural alternatives are widely used nowadays to control enteric infections in poultry with promising results (Higgins *et al*., 2008; Mountzouris *et al*., 2010). Using of different organic acids either in feed or water to reduce *C. jejuni*, intestinal colonization was studied (Byrd *et al*., 2001; Hilmarsson *et al*., 2006; De Los Santos *et al*., 2008) successfully. Competitive exclusion compounds including, probiotics are used effectively, to protect chicks from intestinal pathogens colonization like *C. jejuni* (Schoeni and Doyle, 1992; Mead *et al*., 1996; Willis and Reid, 2008).

The aim of this study was to investigate the efficacy of acidifiers (lactic and formic acids) and probiotic preparations containing *Pediococcus acidilactici* (*P. acidilactici*) and *Saccharomyces boulardii* (*S. boulardii*) in reducing of *Campylobacter jejuni* (*C. jejuni*) infection in broiler chickens.

MATERIALS AND METHODS

Challenge bacteria: Local strains of *C. jejuni* representing biotype 1 and 2 were used as challenging bacteria. At 2 weeks of age, each bird in all groups was orally challenged with 0.5 mL containing 5×10^5 *C. jejuni* in thioglycolate broth.

Acidifiers: An acidifiers consisting of lactic acid and formic acid produced by INVI, Belgium was added at a level of 1 kg t^{-1} feed from day old till the end of the experiment (5 weeks old).

Probiotic preparations: *Pediococcus acidilactici* (*P. acidilactici*) MA18/5M and *Saccharomyces boulardii* (*S. boulardii*) produced by Lallemand, France were used as probiotics. Each was added at the level of 100 g t^{-1} feed. Both compounds were used along the entire of the experiment starting from one day old.

Experimental design: One hundred and three day old meat type chicks were used. Three birds were euthanized and subjected to *C. jejuni* re-isolation at day old to prove their freedom of infection. The reminder one hundred chicks were randomly assigned into four equal groups each; 25 birds and housed in thoroughly cleaned and disinfected houses and provided with feed and water *ad libitum*. Chicks of groups 1, 2 and 3 were fed on ration containing lactic and formic acids, *P. acidilactici* and *S. boulardii*, respectively, from day old till the end of the experiment (5 weeks old). Chicks of group 4 were fed on plain ration free from additives (control). Ration contained coccidiostate semduramicin at concentration of 25 ppm. No antibiotics were added to the ration or water. The chicks were vaccinated by eye drop against Newcastle disease using Hitchner B1 vaccine at 7 days old and La Sota vaccine at 21 days old, against infectious bursal disease using D78 at 12 days old and against infectious bronchitis using H 120 at 17 days old. Avian Influenza (AI) inactivated H5N2 vaccine was given subcutaneously (S/C) at 7 days old.

Measured parameters

Signs and mortalities: All groups were kept under observation for 3 weeks post challenge recording signs and mortalities.

Shedding rate: Faecal swabs were collected from all groups at 3, 7, 11, 15, 18 and 21 days post challenge for exploring the frequency of *C. jejuni* shedding.

Re-isolation rate: Liver and intestine of euthanized birds were collected weekly (7, 14 and 21 days) after challenge for *C. jejuni* re-isolation.

Post-mortem lesions scoring: At 7, 14 days and all birds at the end of observation period were subjected for post-mortem examination for lesions scoring of *C. jejuni* after Nagwa *et al*. (1998).

Bacteriological examination: Faecal swabs were cultured on semisolid thiol broth, incubated at 37°C for 48 h, examined directly under phase contrast microscope for viability, characteristic motility and morphology of *C. jejuni* organism (Smibert, 1974). Specimens from the liver and intestine were collected from sacrificed birds were streaked on Skirrow's agar plates and incubated at 37°C for 48 h under microaerophilic atmosphere of 5% O_2, 10% CO_2 and 85% N_2 (Holt *et al*., 1994). Films were made from specific colonies and stained with Gram's stain to demonstrate characteristic morphology of *Campylobacter* organisms. Biochemical identification of the organisms was done according to Koneman *et al*. (1997).

Statistical analysis: All data were statistically analyzed and means were compared by one way ANOVA ($p<0.05$) using Post Hoc test according to Snedecor and Cochran (1980).

RESULTS AND DISCUSSION

Antibiotics were previously and extensively used to control enteric pathogens but there is a problem of possible adverse effect on the human health and development of resistant bacteria. The European Union prohibited the use of antibiotic growth promoters in 2006, so alternatives like probiotics that stabilize the gut microflora and control the multiplication of pathogens "competitive exclusion" as well as acids are used now days with successful promising results (Dankowiakowska *et al*., 2013). In this study, the protective effect of certain acidifiers and probiotic preparations on the prevention of *C. jejuni* infection was investigated in broiler chickens.

The results revealed that there were no mortalities in all treated and untreated control groups but the severity of signs (depression and greenish diarrhea) was lower in treated chickens than in un-treated control birds.

Figure 1 shows the mean organ lesion score at 7, 14 and 21 days post infection. It was relatively higher in acidifiers treated group (2.04) than in *P. acidilactici* treated one (1.40) and in *S. boulardii* treated birds (1.24). Nevertheless, all treated groups had lower mean scores than in control group (3.04).

On the other hand, culturing followed by microscopical examinations of faecal swabs collected from different groups revealed presence of highly motile organisms with cork-screw like motion. The rate of *C. jejuni* shedding in the treated groups showed significant decrease during 21 days post infection as compared with the untreated control group (Table 1). Thus the percentage of shedding decreased from 100-6.7% in acidifiers-treated group, from 100-0% in both *P. acidilactici* and *S. boulardii* treated birds, versus from 100-86.6% in untreated control ones.

Table 1: Shedding of *Campylobacter jejuni* in acidifiers and probiotic treated and untreated broiler chickens

	Treatments															
	Acidifiers				*Pediococcus acidilactici*				*Saccharomyces boulardii*				Untreated control			
Day post infections	No. of birds	(+)	(%)	(-)	No. of birds	(+)	(%)	(-)	No. of birds	(+)	(%)	(-)	No. of birds	(+)	(%)	(-)
3	25	25	100.0	0	25	25	100.0	0	25	25	100.0	0	25	25	100.0	0
7	25	16*	64.0	9	25	11*	44.0	14	25	13*	52.0	12	25	23	92.0	2
11	20	12*	60.0	8	20	13*	13.3	7	20	11*	55.0	9	20	18	90.0	2
15	15	3*	20.0	12	15	2*	13.3	3	15	4*	26.6	11	15	14	93.3	1
18	15	3*	20.0	12	15	1*	6.7	14	15	0*	0.0	15	15	12	80.0	3
21	15	1*	6.7	14	15	0*	0.0	15	15	0*	0.0	5	15	13	86.6	2

*Significant decrease over their untreated control group ($p<0.05$), +: Positive, -: Negative

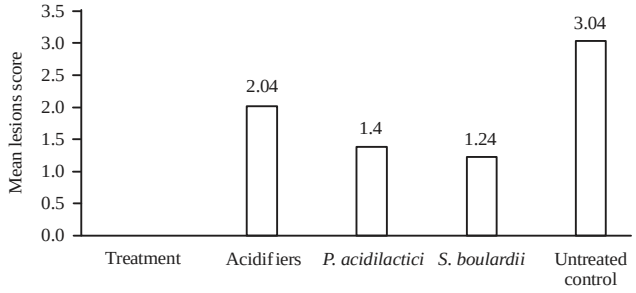

Fig. 1: Post-mortem lesion scoring of *Campylobacter jejuni* in acidifiers and probiotic treated and untreated broiler chickens

Liver and intestinal specimens taken from sacrificed broiler chickens showed small rounded grey or puffy brownish colonies after culturing onto Skirrow's media. Gram staining of colonies revealed Gram negative, short, curved and spiral with (S) or comma shaped rods. Biochemical identification of the re-isolated organisms showed that they are catalase and oxidase positive, grow in 1% glycine, don't grow in 3.5% NaCl, produce H_2S on lead acetate paper not on TSI agar, negative urease, reduce nitrate, positive hippurate hydrolysis and grow in 0.02% cysteine.

The rates of *C. jejuni* re-isolation from both liver and intestine in weekly sacrificed treated groups during 21 days after experimental infection also showed descending patterns, viz., from 60-20% in acidifiers treated chickens, from 80-20% in *P. acidilactici* treated group, whereas in control untreated birds the rate was 100% along the whole 21 days post challenge (Fig. 2).

Both acidifiers and probiotic preparations reduced the risk of *C. jejuni* infection in broiler chickens. The probiotic *S. boulardii* succeeded in complete elimination of *C. jejuni* from birds within 3 weeks post infection. This finding was supported by the previous report of Line *et al*. (1997), who mentioned that treatment of 6 week old broilers subjected to transport stress with *S. boulardii* led to significant reduction in caecal *Salmonella* and *Campylobacter* population.

Acidifiers and probiotics gave nearly similar degree of protection rate for *C. jejuni* infection in broilers. Speck (1976) supported the usage of *P. acidilactici* organism as a probiotic in microbiological control of enteropathogens for many reasons; it is non pathogenic and part of normal chicken intestinal flora, thus capable to colonize the gut. Also, Daeschel and Klaenhamner (1985) concluded that strains of *P. pentosaceus* produced bacteriocin associated with plasmid DNA, this pediocin is active against several strains of Gram negative bacteria like *C. jejuni*. Moreover, Pucci *et al*. (1988) mentioned that *P. acidilactici* is able to grow under both aerobic and anaerobic condition, so it can serve to antagonize the microaerophilic *C. jejuni* in these niches of intestine in which low dissolved oxygen tension exists.

On the other hand, numerous studies demonstrated that organic acid have a strong bactericidal effect on *Campylobacter* species (Schulenberg *et al*., 1996; Chaveerach *et al*., 2002; Naseri *et al*., 2012). Eklund (1983) discussed the mechanism by which organic acids inhibit the growth of enteropathogens and concluded that the undissociated acids diffused into the bacterial cell wall and released a proton that acidifies the cytoplasm. Cherrington *et al*. (1991) and Kroll and Patchett (1991) supported this result and added that the cell death initiated by formic acid (the shortest chain organic acid) was probably caused by the undissociated form which diffused into the cell and resulted in invisible denaturation of enzyme activity or DNA synthesis. Contrary results were obtained by Wieliczko (1995), who found high amount of *C. jejuni* in birds received formic and lactic acids.

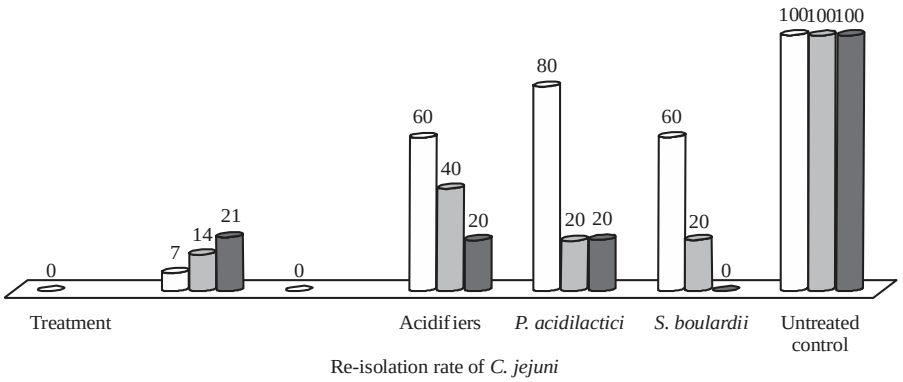

Fig. 2: Re-isolation of *Campylobacter jejuni* in acidifiers and probiotic treated and untreated broiler chickens

Supplementation with organic acids and probiotics in chickens feed had been proved to be effective in reducing pathogenic microorganisms (Mountzouris *et al*., 2010).

Chaveerach *et al*. (2004) showed that organic acid had the greatest effect in reducing *C. jejuni* in broilers intestine and this could be due to the fact that reduction of pH of crop to 3.8-4 and gavaged *Campylobacter* were affected by a pH shock in the crop and reduced the amount of inoculated bacteria in GI tract.

The present study showed that probiotics reduced the number of *C. jejuni* in intestinal contents. Similar results were found by Willis and Reid (2008). Stern *et al*. (2008) demonstrated that treatments with viable probiotic bacterial cultures were ineffective in reducing *C. jejuni* in chickens, while bacteriocin treatment from these corresponding bacteria substantially reduced *C. jejuni* colonization in the live birds.

Blehaut *et al*. (1989) explained the advantages of using yeast as a competitive exclusion product for combating enteropathogens as follows; east can grow at 37C, therefore be able to withstand the higher chickens body temperature, also it is able to survive in low pH of the proventriculus and gizzard to reach the intestine and caeca where it can survive either aerobically and anaerobically and finally yeast is easily to be administered.

May researchers discussed the mechanisms by which *S. boulardii* protects the birds from GIT infection. Miles (1993) referred this protection to the presence of mannose on the intestinal cell wall which may cause the yeast to act as a decoy for the attachment of pathogens. Another opinion was discussed by Rodrigues *et al*. (1996) who attributed the protection to the ability of yeast to reduce the available amount of the produced toxins.

CONCLUSION

In conclusion, both acidifiers and probiotic preparations could greatly assist in reduction and elimination of *C. jejuni* infection risk in broiler chickens.

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