P-001 (ID 94)

THE EFFICACY OF CERTAIN FEED ADDITIVES FOR THE PREVENTION OF *CAMPYLOBACTER JEJUNI* INFECTION IN BROILER CHICKENS

Wafaa A. Abd El-Ghany*[1], M. H. Awaad[1], and Nagwa, S. R.[2]

[1] *Faculty of Veterinary Medicine, Cairo University, Egypt*
[2] *National Research Centre, Egypt*

***Corresponding author:** wafaa.ghany@yahoo.com

Summary

The efficacy of acidifiers (lactic and formic acids) and probiotics containing *Pediococcus acidilactici* (*P. acidilactici*) and *Saccharomyces boulardii* (*S. boulardii*) in reducing of *Campylobacter jejuni* (*C. jejuni*) infection in broiler chickens was investigated. Three birds out of 103 day old chick were euthanized for *C. jejuni* re-isolation. One hundred 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. Group 4 was control. At 2 weeks old, each bird was orally challenged with *C. jejuni*. Groups were kept for 3 weeks recording signs and mortalities. Faecal swabs as well as liver and intestine were collected for *C. jejuni* shedding and re-isolation; respectively. Post-mortem lesions scoring of *C. jejuni* were calculated. Results cleared more severe signs of greenish diarrhoea 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 group. In conclusion, both acidifiers and probiotic preparations greatly reduced *C. jejuni* infection in broiler chickens.

Keywords: *C. jejuni*, Acidifiers, Probiotics, Chickens, Protection

Introduction

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). Natural alternatives are widely used nowadays to control enteric infections in poultry with promising results (MOUNTZOURIS et al., 2010). Using of different organic acids to reduce *C. jejuni* intestinal colonization was studied successfully **(**SANTOS et al., 2008**)**. Competitive exclusion compounds including probiotics are used effectively to protect chicks from intestinal pathogens colonization like *C. jejuni* **(**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 *C. jejuni* infection in broiler chickens.

Materials and Methods

Three out of 103 day old, meat type chicks were euthanized to prove their freedom of *C. jejuni* infection. One hundred chicks were assigned into 4 groups each; 25 birds. Chicks of groups 1, 2 and 3 were fed on ration containing lactic and formic acids (1 kg/ton), *P. acidilactici* (100 g/ton) and *S. boulardii* (100 g/ton); respectively; from day old till 5 weeks old. Chicks of group 4 were kept as control. All groups were orally challenged with 0.5 ml containing $5X10^5$ *C. jejuni/* bird at 2 weeks old. Birds were kept for 3 weeks for signs and mortalities. Faecal swabs and liver and intestine were collected from all groups at different intervals for *C. jejuni* shedding and re-isolation; respectively. At 5 weeks old, a lesion scoring of C. jejuni after RABIE et al. (1998) was recorded. Microbiological examination of the organisms was done according to KONEMAN et al. (1995). 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

The results revealed no mortalities in all groups, but the severity of signs (depression and greenish diarrhae) was lower in treated than control birds. Figure (1) showed that the mean organ lesion score at 7, 14 and 21 days post infection was higher in acidifiers (2.04) than in *P. acidilactici* (1.40) and in *S. boulardii* (1.24) treated birds. Nevertheless, it was (3.04) in control group. Faecal swabs revealed presence of highly motile organisms. The rate of *C. jejuni* shedding decreased from 100 to 6.7% in acidifiers-treated group, from 100 to 0% in both *P. acidilactici* and *S. boulardii* treated birds, versus from 100 to 86.6% in control one (table 1). The rates of *C. jejuni* re-isolation showed descending patterns, viz. from 60 to 20% in acidifiers, from 80 to 20% in *P. acidilactici* treated groups, whereas it was 100% along 21 days post challenge in control group (figure 2).

Acidifiers and probiotics gave nearly similar degree of protection rate for *C. jejuni* infection in broilers (MOUNTZOURIS et al., 2010). PUCCI et al. (1988) mentioned that *P. acidilactici* can antagonize miceoaerophilic *C. jejuni* in their niches of intestine in which low dissolved oxygen tension exists. On the other hand, organic acids have a strong bactericidal effect on Campylobacter spp. (NASERI et al., 2012). EKLUND (1983) concluded that the un-dissociated acids diffused into the bacterial cell wall and released a proton that acidifies the cytoplasm. This result was supported by KROLL and PATCHETT (1991) and added that the cell death resulted from invisible denaturation of enzyme activity or DNA synthesis. Also, CHAVEERACH et al. (2004) showed that organic acids reduce the pH of crop to 3.8-4 and thus reduce the level of Campylobacter in the crop and the intestinal tract. However, treatments with viable probiotic bacterial cultures were effective in reducing *C. jejuni* in chickens due to bacteriocin (STERN et al., 2008). BLEHANT et al. (1989) explained the advantages of using yeast for combating enteropathogens as follows; yeast can grow at 37C, therefore be able to withstand the higher chickens body temperature, 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. Presence of mannose of *S. boulardii* on the intestinal cell wall may cause the yeast to act as a decoy for the attachment of pathogens (MILES, 1993), however RODRIGUES et al. (1996) attributed the protection to the ability of yeast to reduce the available amount of the produced toxins.

Conclusion

Both acidifiers and probiotic preparations could greatly assist in reduction and elimination of *C. jejuni* infection risk in broiler chickens.

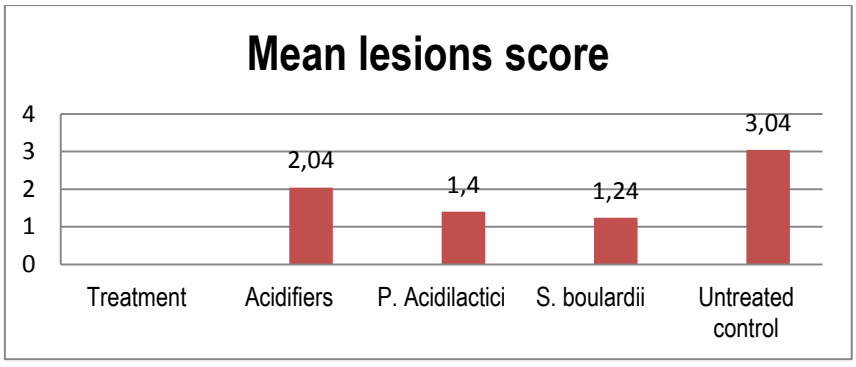

Figure (1): Post-mortem lesion scoring of *C. jejuni* in acidifiers and probiotic treated and untreated broiler chickens

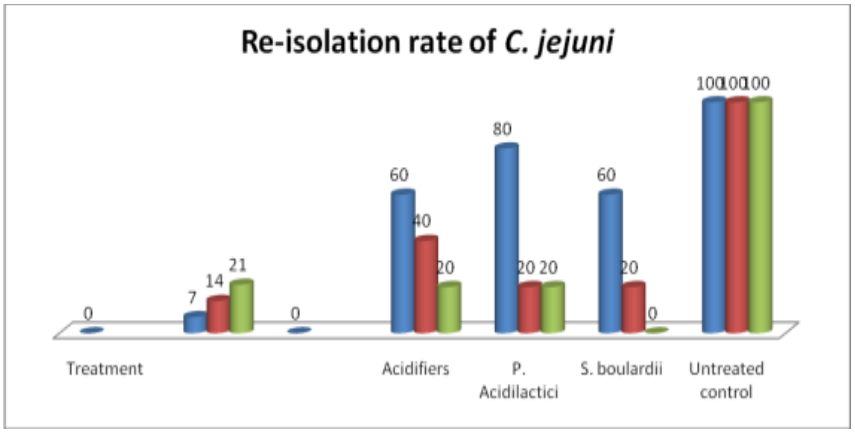

Figure (2): Re-isolation of *C. jejuni* in acidifiers and probiotic treated and untreated broiler chickens

Table (1) Shedding of *C. jejuni* in acidifiers and probiotic treated and untreated broiler chickens

Day post infection	Treatment											
	Acidifiers			*P. acidilactici*			*S. boulardii*			Untreated control		
	No. of birds	+ (%)	-	No. of birds	+ (%)	-	No. of birds	+ (%)	-	No. of birds	+ (%)	-
3	25	25 (100)	0	25	25 (100)	0	25	25 (100)	0	25	25 (100)	0
7	25	16* (64)	9	25	11* (44)	14	25	13* (52)	12	25	23 (92)	2
11	20	12* (60)	8	20	13* (13.3)	7	20	11* (55)	9	20	18 (90)	2
15	15	3* (20)	12	15	2* (13.3)	3	15	4* (26.6)	11	15	14 (93.3)	1
18	15	3* (20)	12	15	1* (6.7)	14	15	0* (0.0)	15	15	12 (80)	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

Literature

BLEHANT, M.J., J. MASSOT, G.W. ELMER, R.H. LEVY, 1989: Disposition kinetics of *Saccharomyces boulardii* in man and rat. Biopharm. Drug Disposition, **10**, 353-364.

CHAVEERACH, P., D.A. KEUZENKAMP, L.J. A. LIPMAN, F. VAN KNAPEN, 2004: Effect of organic acids in drinking water for young broilers on Campylobacter infection, volatile fatty acid production, gut microflora and histological cell changes. Poult. Sci. **83**, 330–334.

EKLUND, T. 1983: The antimicrobial effect of dissociated and undissociated sorbic acid at different pH levels. Appl. Bacteriol. **54**, 383-389.

KONEMAN, E.W, S.D. ALLEN, W.M. JANDA, W.C. WINN, 1995: Introduction to Diagnostic Microbiology. 5th ed., Lippincott Company, Philadelphia, (USA). PP. 365-398.

KROLL, R.G., R.A. PATCHETT, 1991: Biocide-induced perturbations of aspects of cells homeostasis: intracellular pH, membrane potential and solute transport. In: mechanisms of actions of chemical biocide. Pp: 189-202. S. P. Denyer and W. B. Hugal (eds). Blackwell, Oxford, England.

MILES, R.D. 1993: Manipulation of the microflora of the gastrointestinal tract: natural ways to prevent colonization by pathogens. In: Biotechnology in fed industry. Pp. 133-150. T.P. Lyons (ed) Alltech Technical populations, Nicholasville, K.Y.

MOUNTZOURIS, K.C., P. TSISTSIKOS, I. PALAMIDI, ET AL, 2010: Effects of probiotic inclusion levels in broiler nutrition on growth performance, nutrient digestibility, plasma immunoglobulins, and cecal microflora composition. Poult. Sci. **89**, 58.

NASERI, K.G., S. RAHIMI, P. KHAKI, 2012: Comparison of the effects of probiotic, organic acid and medicinal plant on *Campylobacter jejuni* challenged broiler chickens. J. Agri. Sci. Tech. **14**, 1485-1496.

PUCCI, M.J., E.R.VEDAMTHU, B.S. KUNKA, P.A. VANDENBERCH, 1988: inhibition of *Listeria monocytogens* by using bacteriocin PA-1 produced by *Pediococcus acidilactici* PAC 1.0. Appl. Environ. Microbiol. **54**, 2349-2353.

RABIE, NAGWA, S., N. KADRIA, M.A. HASSANAIN, 1998: Biological control of *Campylobacter jejuni* in chickens by *Bacillus thuringiensis* varieties. J. Egypt. Ger. Soci. Zool. **25**, 1-12.

RODRIGUES, A.C.P., R.M. NARDI, E.A. BAMBIRRE, E.C. VIEIRA, J.R. NICOLI, 1996: Effect of *Saccharomyces boulardii* against experimental oral infection with *Salmonella typhimurium* and *Shigella flexneri* in conventional and gonobiotic mice. J. Appl. Bacteriol. **81**, 251-256.

SANTOS, S. DE LOS, F. DONOGHUE, K. VENKITANARAYANAN, ET AL., 2008: Caprylic acid supplemented in feed reduces enteric *Campylobacter jejuni* colonization in ten-day-old broiler chickens. Poult. Sci. **87**, 800–804.

SHANE, S.M. 2000: Campylobacter infection of commercial poultry. Rev. Sci. Tech. Off. Int. Epiz. **19**, 376-395.

SNEDECOR, F.W., W.G. COCHRAN, 1980: Statistical methods 7th ed. Iowa State Univ. Press Ames. I.A.

STERN, N.J., B.V. ERUSLANOV, V.D. POKHILENKO, Y.N. KOVALEV, ET AL., 2008: Bacteriocins reduce *Campylobacter jejuni* colonization while bacteria producing bacteriocins are ineffective. Microb. Ecol. Hlth. Dis. **20**, 74-79.

WILLIS, W. L., L. REID, 2008: Investigating the effects of dietary probiotic feeding regimens on broiler chicken production and *Campylobacter jejuni* presence. Poult. Sci. **87**, 606-611.