J. Agr. Sci. Tech. (2012) Vol. 14: 1485-1496

Comparison of the Effects of Probiotic, Organic Acid and Medicinal Plant on *Campylobacter jejuni* Challenged Broiler Chickens

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ABSTRACT

***Campylobacter* is known to be one of the most common causes of human intestinal disorders. Since poultry are known to be the main reservoirs for this pathogen, decreasing this bacterium in intestinal tract could be beneficial in reducing contamination of poultry products. The effects of probiotic (PrimaLac®), medicinal plant (Sangrovit®) and organic acid (Selko-pH®) as broiler feed additives on cecal colonization, and fecal excretion of broilers were studied. Other parameters such as performance, immune response and intestinal morphology were also determined. A total of 300 broiler chicks (Cobb 500) were divided into 5 groups. Groups consisted of unsupplemented feed (negative and positive controls), probiotic, medicinal plant and drinking water containing organic acid mixture. Except for the negative control group, all chickens were orally challenged with (10^9 cfu mL^{-1}) *Campylobacter jejuni* at day 21. Cecal and fecal samples were collected for *Campylobacter* count. Body weight (BW), feed intake (FI) and feed conversion ratio (FCR) were determined weekly and cumulatively. BW and FI in the probiotic treated group were higher (P< 0.05) than the positive control group. On day 49 all supplemented treatments showed a reduction of *Campylobacter* colonization in cecal contents (P< 0.05). Fecal samples showed reductions (P< 0.05) on day 35 and 42. Villi height of duodenum and jejunum in the probiotic and medicinal plant treated groups were improved (P< 0.05). Immune response was significantly higher in these two groups (P< 0.05). These effects could be due to the antibacterial effects of the used feed supplements. Our results indicate that these feed additives could be potential treatments for reducing *Campylobacter* in the intestine of broilers. Probiotic and medicinal plant improve growth performance of these birds.**

Keywords: Broiler, Campylobacter, Herbs, Organic acids, Probiotic ®.

INTRODUCTION

Campylobacter jejuni is known to be the leading cause of acute human bacterial intestine infection both in the western and developing countries (Anonymous, 2008). Poultry can easily colonize *Campylobacter* spp. in their intestinal tract and are known to be the main reservoirs for these bacteria. The use of antibiotics as feed additive is under discussion in regard to human food safety because of the potential development of antibiotic resistant bacteria. Antibiotic resistant strains of *C. jejuni* and *C. coli* from broilers have also been discovered (Jorgensen *et al*., 2002). Therefore, there is an urgent demand to search for alternative strategies to control *Campylobacter* both in humans and chickens. Because of the vast number of pathogens in feces, leaking

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[DOR: 20.1001.1.16807073.2012.14.7.15.7]

intestinal content during the slaughter process frequently contaminates poultry carcasses with *Campylobacter* spp. (Rosenquist *et al*, 2006). Using alternative methods to prevent colonization of these bacteria in the intestinal tract of flocks may help control the transmission of these bacteria from food to humans.

Among the candidates for replacement of antibiotics are organic acids, enzymes, probiotics, prebiotics and plant extracts, which have been suggested to control intestinal microbial growth (Higgins *et al*., 2008).

Organic acids have long been used as food additives and for extending the shelf life of perishable food ingredients. Fatty acids, especially medium-chain fatty acids, have been reported to possess antimicrobial activities against a wide range of microorganisms (Van Immerseel *et al*., 2004). Selko-pH® is a pH-adjusting solution consisting of a proprietary mixture of formic acid, acetic acid, ammonium format, mono- and diglyceride of unsaturated fatty acids and copper acetate. It has been shown that use of caprilic acid in feed (Santos *et al*., 2008) and mono caprin in drinking water and feed (Hilmarsson *et al*., 2006) reduce enteric colonization of *Campylobacter* in chicks. It has also been reported that using organic acids (lactic acid) in drinking water during feed withdrawal reduces *Campylobacter* population in crop and carcasses (Byrd *et al*., 2001).

Probiotics are live microbacteria and their fermentation products beneficially reduce the undesirable microflora population in the gastrointestinal tract of chicks (Chiang and Hsieh, 1995). Decreasing the pH of the intestine also produces bacteiocins that are believed to be natural antibiotics, thus suppressing the growth of harmful bacteria (Nava *et al*., 2005). Different studies have shown that the administration of probiotics in broiler diets increases growth performance. Willis and Reid (2008) found that chickens receiving probiotics early in life were significantly less colonized with *C. jejuni* than chickens in the control group.

The use of herbs as natural antimicrobials is well accepted by the general public as well as by countries that restrict the imports of products derived from animals fed with antibiotics. Herbal supplements have shown to have beneficial effects on broiler performance and carcass quality (Schleicher *et al*., 1998). Sangrovit® is a phytogenic feed additive that is extracted from some plants including *Sanguinaria canadensis*. Sanguinarine is a quaternary ammonium salt from the group of benzylisoquinoline alkaloids. It has been shown that sanguinarine suppresses the growth of some bacteria that cause gastrointestinal distress (Mahadria *et al*., 2003), enhances appetite and feed intake, and promotes growth (Tschirner *et al*., 2003).

The objective of this study was to investigate the effects of probiotic, prebiotic, organic acids and Sangrovit® on reducing cecal colonization and fecal excretion of *Campylobacter jejuni*. Growth performance, immune response, and histomorphology of intestine of the broilers have also been considered.

MATERIALS AND METHODS

Birds

Three hundred day-old male broiler chicks (Cobb 500) were obtained from a local commercial hatchery. The initial weight of the birds was 49.6-50.2 g. The chicks were divided into 5 experimental groups (n= 60 per treatment group). Each treatment group was divided into 4 replicates (n=15 per replicate). The dietary treatments were comprised of 5 groups of birds: 2 groups (negative and positive control) were fed with an unsupplemented basal diet (Table 1), probiotic (PrimaLac®) (0.1 g kg^{-1}), organic acid (Selko-pH®) in drinking water (1 mL in 1 liter for 2 weeks, and 7-8 hours for the rest of the growth period) and Plant extract (Sangrovit®) (20-50 g $tone^{-1}$). The experimental diets were formulated to meet NRC (1994) nutrient

[DOR: 20.1001.1.16807073.2012.14.7.15.7]

Table 1. The composition and nutrient values of basal diet (%).

Ingredients	Starter	Grower	Finisher
Corn	60.12	63.27	66.82
Soybean meal (48%)	26.76	24.81	23.81
Fish meal	7.11	5.00	2.22
Vegetable fat	3.00	4.00	4.00
Limestone	1.59	1.28	1.23
Dicalcium phosphate	0.68	0.90	1.18
Mineral and Vitamin premixa*	0.40	0.40	0.40
Salt	0.24	0.25	0.27
DL-Met	0.10	0.07	0.07
L-Lys	0.00	0.014	0.006
Calculated analysis (per kg of diet)			
ME (mg)	12.4	12.7	12.7
CP (g)	220.0	200.0	180.0
Fat (g)	61.07	70.30	68.7
Met and Cys (g)	9.5	8.50	7.70
L-Lys (g)	13.7	12.0	10.0

* Each g contains: Vitamin A-82= 500IU; Vitamin B_2= 50 mg; Vitamin D_3= 12,000 IU; Vitamin K= 10 mg; Vitamin B_1= 4mg; Vitamin B_6= 8mg; Vitamin B_{12}= 40 mg; Vitamin E= 40 mg; Calcium D Pantothenate= 40mg, Niacin= 60mg.

requirements for broilers. Birds from 1 to 10 days old were fed a diet containing 3,000 kcal kg^{-1} ME and 21% crude protein; from 11 to 22 days old 3,100 kcal kg^{-1} ME and 20% crude protein and from 23 to 42 days old 3,200 kcal kg^{-1} ME and 19% crude protein. The broilers were vaccinated at day one with Infectious Bronchitis Vaccine (Poulvac IB Primer 1000, spray), at day 10 with Newcastle Disease Vaccine (B1, ocular inoculation), day 14 with Infectious Bursal Disease Vaccine (in drinking water) and at day 18 the vaccination against Newcastle was repeated with La Sota (Clone 30, in drinking water). Feed and water were provided *ad libitum*.

Bacteria Preparation and *Campylobacter* Challenge

C. jejuni ATCC 33291 strain which was used for inoculation of the birds was stored frozen at -80°C in an 80% glycerol solution. The culture was prepared for the challenge experiment by rapidly sub culturing it on blood agar and incubating the plates for 48 hours at 42°C under microaerobic conditions (5% O_2, 10% CO_2, and 85% N_2). After 48 hours of incubation at 42°C, the majority of *C. jejuni* strains produced grey, moist, flat, and spreading growth on agar. The bacteria were harvested and diluted in PBS to the specific viable concentration of 10^9 cfu mL^{-1} according to the method explained by Lamb-Rosteski *et al.* (2008). Inoculum concentration was estimated by MacFarlane tubes. The inoculum was kept on ice for less than 1 h before oral gavage (inoculation in crop) of chicks. On day 21 except the negative control group, that received 1.0 mL of sterile PBS, the rest of the birds were orally challenged with a 1.0 mL dose of the inoculum (Lamb-Rosteski *et al*., 2008).

Selective Blood Medium

This was prepared to the following formulation *Campylobacter* Agar Base (DifcoTM) at pH 7.4. The medium was sterilized by autoclaving at 121°C for 15 min. Saponin lysed sheep red blood cells (SRBC) 5%, polymixin sulfate 5000 IU L^{-1},

[DOR: 20.1001.1.16807073.2012.14.7.15.7]

vancomicin 10 mg L^{-1}, and Fungyzone 5 mg L^{-1} were added to the cooled molten agar before pouring into the petri dishes (Takahashi *et al.,* 1982).

Measurements

Microbiological Analysis

On days 28 and 49 after inoculation, three birds of each replicate were euthanized by cervical dislocation. *Campylobacter jejuni* presence in cecal contents (1 g) was investigated. On days 35 and 42, fecal samples were taken from five birds, mixed together with a sterile swab and 1 gram of the mixed sample was transported to the laboratory. Samples were cultured on selective Campylo blood agar plates and incubated at 42°C for 48 hours under microaerobic conditions (5% O_2, 10% CO_2, and 85% N_2) (Axelsson-Olsson *et al*., 2005).

Immunity and Some Blood Parameters Assay

Injections of SRBC antigen were done intramuscularly for the evaluation of immune system responses at days 21 and 39. Two birds from each replicate were randomly selected and blood samples were taken via wing vein at days 28 and 49. Thereafter, anti-body titration against SRBC was done by hemaglutination inhibition (HI) test. Immunoglobulin M and G (IgM and IgG) contents were determined by using 2-Mercaptoethanol (Martin *et al.,* 1989). Three broilers from each replicate were randomly selected and blood samples were taken via wing vein at days 28 and 49.

Weight Assessment

The body weight of birds per replicate was recorded on the individual basis at weekly intervals and cumulatively. Feed consumption per replicate was also recorded on a weekly basis and cumulatively. Feed conversion ratio per replicate was worked out at weekly intervals by taking into consideration the weekly body weight gain and the feed consumption of the respective replicate.

Morphology

For the histomorphological examination of intestine, the tissue samples from duodenum, jejunum, and ileum were collected from the euthanized birds and fixed in 10% buffered formalin saline. Tissues were dehydrated by immersing through a series of alcohols with increasing concentrations (from 70% to absolute), infiltrated with xylene, and embedded in paraffin. Casting of blocks was carried out in L-molds (two L shaped pieces) which facilitated the manipulation of size as per the requirement. A rotary type microtome was used for cutting the paraffin sections. The blocks were properly trimmed and sections of 5 mm thickness were cut. The morphometric variables measured included villi height, crypt depth, and villi width at the top and the base. The mean from 10 villi per sample was used as the average value for further analysis. Continuous ribbons (6-7 inches long) of the material were cut and laid on the surface of constant temperature water bath (about 55°C). The sections were separated with a heated scalpel after they spread completely. The cut sections were mounted on the clean glass slides using Mayer's egg albumin as the section adhesive. The mounted slides were dried in paraffin oven at 60°C for one hour (Beçak and Paulete 1976).

Statistical Analysis

The data were analyzed using GLM procedure of SAS (2004). Significant differences among treatments were

[DOR: 20.1001.1.16807073.2012.14.7.15.7]

determined by using Duncan's multiple range tests.

RESULTS

Mean values of weekly BW, FI and FCR are shown in Table 2. There was no significant difference in FI, BW and FCR among any groups on day 1-21. However, in day 22-49, and day 1-49 periods positive control (CN+) versus negative control (CN-) showed differences (P< 0.05) in FI and FCR. There was a significant difference between FI and BW of supplemented treatments. No difference between FI and BW in probiotic and Sangrovit® was observed in these intervals. However, FI and BW in the organic acid supplemented group were decreased (P< 0.05) in experimental periods. In general, the organic acid (Selko-pH®) and positive control groups had the lowest (P< 0.05) weight gain when compared with the supplemented groups.

No significant difference was observed in FCR among supplemented groups. In the entire experimental period, the positive and negative control groups had the highest and lowest FCR, respectively (P< 0.05).

Table 3 shows the number of birds classified according to cecal and fecal bacteria counts of *Campylobater jejuni* (log-scaled intervals). On day 49 and 42 the positive control group, which received no supplement in feed, was heavily colonized with *Campylobacter* in cecal and fecal contents containing 7.47 and 7.21 $\log_{10}$ cfu g^{-1}, respectively. On day 28 however, the positive control group had the highest *Campylobacter jejuni* numbers in the cecal contents. There was no significant difference among any of the treatments. Except for day

Table 2 Effect of probiotic, organic acid and medicinal plant on growth performance of *Campylobacter* infected broilers.

Performance	Feed intake (kg)			Weight gain (kg)			Feed conversion ratio		
Age (days)	1-21	22-49	1-49	1-21	22-49	1-49	1-21	22-49	1-49
CN+ [a]	1188.7	4782.6^{bc}	5971.4^{ab}	773.2	1928.2^{c}	2701.4^{c}	1.5	2.5^{a}	2.2^{a}
CN- [b]	1229.6	4948.7^{a}	6178.3^{a}	772.3	2172.3^{a}	2944.6^{a}	1.6	2.2^{c}	2.0^{b}
PRO [c]	1215.8	4899.7^{b}	6013^{ab}	782. 5	2080.9^{b}	2863.4 ^{ab}	1.5	2.3^{ab}	2.1^{ab}
OA [d]	1203.2	4721.3^{c}	5924.5^{c}	769.8	2004.8^{bc}	2774.6^{bc}	1.5	2.3^{ab}	2.1^{ab}
SA [e]	1236.7	4898 ^{ab}	6134.7^{ab}	780.4	2107.8^{b}	2888.3^{ab}	1.5	2.3^{ab}	2.1^{ab}
SEM [f]	14.87	27.64	34.49	2.251	21.84	21.11	0.019	0.025	0.014

[a] Positive control (septic); [b] Negative control (unseptic); [c] Probiotic (Primalac®); [d] Organic acid (Selko-pH®); [e] Medicinal plant (Sangrovit®); [f] Standard error of means, [a–b-c] Means in a row with no common superscript differ significantly (P< 0.05).

Table 3. Cecal and fecal yield of *Capmylobacter jejuni* in broilers Viable *Campylobacter* count (Log 10 cfu mL^{-1}).

	Cecal content		Fecal content	
Treatment	d 28	d 49	d 35	d 42
CN+ [a]	4.2	7.5^{a}	6.3^{a}	7.2^{a}
CN- [b]	3.6	5.7^{c}	4.0^{c}	5.3^{c}
PRO [c]	4.1	6.6^{b}	5.4^{b}	6.4^{ab}
OA [d]	4.0	6.2^{cb}	4.7^{c}	5.6^{bc}
SA [e]	4.2	6.34^{cb}	5.5^{b}	6.5^{ab}
SEM [f]	0.107	0.164	0.193	0.182

[a] Positive control (septic); [b] Negative control (unseptic); [c] Probiotic (Primalac®); [d] Organic acid (Selko-pH®); [e] Medicinal plant (Sangrovit®); [f] Standard error of means, [a–b-c] Means in a row with no common superscript differ significantly (P< 0.05).

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28, there was always a significant difference (P< 0.05) between the positive and negative control groups. All samples taken from supplemented groups in days 35, 42 and 49 showed significant reduction (P< 0.05) in *Campylobacter jejuni* in intestinal contents.

Addition of probiotic to diet reduced (P< 0.05) *Campylobacter jejuni* in cecal samples on day 49. The reduction of this bacterium between organic acid and Sangrovit® had no significant difference compared to the negative control and probiotic groups. Furthermore, when the mean log10 number of bacteria of fecal samples from probiotic and Sangrovit ® treatments taken on days 35 and 42 were compared, no significant difference was observed, indicating a similar reduction in the total bacteria. Moreover, organic acids and the negative control groups on day 35 showed no significant difference in number of this bacterium.

The villi height, crypt depth, and villi surface area and villi height: crypt depth ratio (VH: CD) of broiler duodenum, jejunum, and ileum at 49 days of age are presented in Table 4. Data show that supplemented groups had improved villi height in duodenum and jejunum (P< 0.05).

In the duodenum, there was a significant increase in the height of the villi (P< 0.05). As expected, the positive control group had the lowest height of villi whereas the negative control, probiotic and Sangrovit® treatment groups had higher villi (P< 0.05). VH:CD of the probiotic supplemented group was significantly higher (P< 0.05) in this part of the intestine. The other treatments had slightly improved VH:CD, but the difference was not significantly as compared with the positive control group.

Probiotic, Sangrovit® and the negative control groups had significantly higher villi compared to the positive control group in the jejunum. Similar to the duodenum, adding probiotic to the diet significantly improved VH:CD compared to the positive control group. There was no significant difference among other supplemented treatments. There was no significant effect on the ileum parameters.

Table 5 shows the immune response of broilers to different feed additives. No significant difference in the first post immunization was observed. In the second post immunization differences (P< 0.05) were observed between total titer, IgG and IgM of the positive control and negative control groups. Supplemented groups increased immune response in chickens. Total titer and IgM of probiotic and Sangrovit® were significantly improved compared with the positive control group. In these supplemented treatments IgG was higher than the positive control group. But a

Table 4. Effects of feed additives on the morphology of the intestinal mucosa at different sites in the small intestine.

	Duodenum (µm)		Jejunum (µm)		Ileum (µm)	
Dietary treatment	Villi height	Villi height: Crypt depth	Villi height	Villi height: Crypt depth	Villi height	Villi height: Crypt depth
CN+ [a]	974.6[b]	5.162[b]	767.1[b]	5.224[b]	671.6	5.0465
CN- [b]	1080.5[a]	5.318[ab]	863.1[a]	5.602[ab]	679.5	5.2488
PRO [c]	1075.6[a]	6.018[a]	882.4[a]	6.119[a]	694.0	5.0961
OA [d]	1038.4[ab]	5.325[ab]	826.1[ab]	5.430[ab]	691.8	5.0098
SA [e]	1054.9[a]	5.859[ab]	876.5[a]	5.759[ab]	689.1	5.2945
SEM [f]	15.7	0.15	14.54	0.12	6.7	0.16

[a] Positive control (septic); [b] Negative control (unseptic); [c] Probiotic (Primalac®); [d] Organic acid (Selko-pH®); [e] Medicinal plant (Sangrovit®); [f] Standard error of means, [a–b-c] Means in a row with no common superscript differ significantly (P< 0.05).

[DOR: 20.1001.1.16807073.2012.14.7.15.7]

Table 5. Effects of feed additives on the immune response of broilers (1/log 2)**.**

	Post first immunization		Post second immunization	
Treatment	IgM	Total tier	IgM	Total titer
CN+ [a]	1.70	3.16	2.00[b]	3.55[b]
CN- [b]	2.0	4.0	3.50[a]	5.31[a]
PRO [c]	1.83	3.83	4.43[a]	5.12[a]
OA [d]	1.66	3.33	2.33[ab]	4.15[ab]
SA [e]	1.83	3.83	3.33[a]	5.16[a]
SEM [f]	0.138	0.162	0.203	0.226

[a] Positive control (septic); [b] Negative control (unseptic); [c] Probiotic (Primalac®); [d] Organic acid (Selko-pH®); [e] Medicinal plant (Sangrovit®); [f] Standard error of means, [a–b-c] Means in a row with no common superscript differ significantly (P< 0.05).

significant difference was observed only in the probiotic group. Moreover, improvement of immune response observed in organic acid group was not significantly higher than the positive control group.

DISCUSSION

Controlling the growth of intestinal microflora is important for improving the well-being of the host. Good intestinal health will lead to a better growth rate and feed efficiency in poultry (Montagne *et al*., 2003). Many bacteria compete with the host for nutrients in the gastrointestinal tract. Antimicrobial agents are known to reduce the intestinal microbial load and this reduces the amount of toxin levels in the intestine (Bedford, 2000). Dietary supplements such as organic acids, probiotics and plant extracts are known to be antimicrobial agents (Mountzouris *et al*., 2010).

No significant difference in FI, BW and FCR was observed in the first three weeks of the experiment. Results of this experiment are in agreement with findings of Mohan *et al*. (1996). The present study revealed that although supplementation of organic acids in water causes a reduction in FI and BWG, it also improves FCR. Our finding is in agreement with the results of Pinchasov and Elmalich (2000), indicating that the application of acetic acid in diets depressed FI and BW in broilers.

The results of this study are in contradiction to the finding of Islam *et al*. (2008) who reported improvements in FI and BW in broilers due to supplementation of organic acids in diet. Although a reduction in FI and BW occurred with the use of organic acids, the FCR demonstrates that these birds were as efficient in feed utilization as other supplemented treatment groups.

The improvement in performance and feed efficiency of probiotics fed broiler chickens (Kabir *et al*., 2004; Mountzouris *et al*., 2007) is known to be via retention of beneficial microbial population in the digestive tract and improving feed digestion and absorption (Fuller, 1989). The positive effects of probiotics on broiler performance in this experiment are in agreement with studies of Kalavathy *et al*. (2003) and Kabir *et al*. (200-4). Results of studies by Tschirner *et al*. (2003) and Vieira *et al*. (2008) indicated beneficial effects of using Sangrovit® in broilers and swine diets. These data support the positive effect of Sangrovit® on BW and FCR also observed in our study.

Short-chain fatty acids (SCFA) have been widely used to prevent pathogenic bacteria in food products. Fatty acids diffuse the bacteria in the undissociated form and dissociate in the protoplasm, causing intracellular acidification (Sun *et al*., 1998). Drinking water is known to be the most important factor in spreading *Campylobacter* infection in broiler flocks (Gibbens *et al*., 2001). A recent study has shown that using organic

[DOR: 20.1001.1.16807073.2012.14.7.15.7]

acids in drinking water can reduce bacteria in fecal content of pigs (De Busser *et al*., 2010). Chaveerach *et al*. (2004) reported that administration of organic acids in drinking water kept the water free from *Campylobacter* bacteria. The results of this study showed that organic acid (Selko-pH®) had the greatest effect in reducing *Campylobacter jejuni* in broilers intestine. Unlike the administration of organic acids in feed, addition of organic acids to drinking water has the greatest effect on the upper part of the intestine, and a minor effect on lower intestine (Thompson and Hinton, 1997). Reduction of *Campylobacter* could be due to the fact that Selko-pH® reduced the 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.

As mentioned before, Sangrovit® is a commercial product containing quaternary benzophenanthridine alkaloids (QBA's), predominantly sanguinarine. QBAs have antimicrobial activity (Lenfeld *et al*, 1981). It is suggested that sanguinarine inhibits the growth of some bacteria that cause gastrointestinal upset (Mahadria *et al*., 2003). Niewold (2007) suggested that growth promoting effects of antimicrobials on animals are in fact mediated by anti-inflammatory mechanisms. Sangrovit® treatments have increased lysozyme concentrations in pig serum (Gudev *et al*., 2004). Lysozyme is capable of hydrolyzing the cell wall of certain bacteria. The present study showed that probiotics reduced the number of *C. jejuni* in intestinal contents. These findings are similar to previous studies performed by Willis and Reid (2008).

In the present study, significant effects of dietary treatment on intestine morphology were observed at day 49. As reported in some other studies, probiotic (Chichlowski *et al*., 2007) organic acids (Garcıa *et al*., 2007) and medicinal plants (Tataral *et al*., 2008) have promoting effects on intestinal morphology of monogastric animals.

Our results are in agreement with previous studies, indicating an improvement in villi height due to the administration of probiotic and Sangrovit® in broiler diets. Organic acid treatment did not show a significant difference with the control groups, although all the measurements were higher than the positive control group. Unlike additional organic acids in feed, organic acids in drinking water have greatest effect on the upper intestine, and a minor effect on lower intestine (Thompson and Hinton, 1997). Gunal *et al*. (2006) reported that the increments of VH and VH:CD in jejunum and ileum of probiotic-fed broilers were greater compared with the control groups. In a study conducted by Chichlowski *et al*. (2007) it has been stated that a probiotic containing lactobacilli *Bifidobacterium thermophilum* and *Enterococcus faecium* increased the jejunum VH and decreased the VD compared with the control group. The positive effect of Sangrovit® on villi height can be rationalized by the positive effects of QBA's on intestine.

It is believed that feed supplements can enhance the immune response in broilers. Sangrovit® is known to have immunomodulatory effects (Chaturverdi *et al*., 1997). It has been reported that Sangrovit® stimulates phagocytic activity and thus promotes host protective responses (Gudev *et al*., 2004). Addition of *Lactobacillus acidophilus* (0.1% in drinking water) (Hanamanta and Narayana, 2010) and probiotic in feed (Koenen *et al*., 2004) increased immune response in broiler chickens.

In conclusion, it can be stated that the results of this study indicate that supplementation of organic acid (Selko-pH®) in drinking water, probiotic (Primalac®) and plant extract (Sangrovit®) to broiler feed may reduce the incidence of Campylobacter infection in these birds.

ACKNOWLEDGEMENTS

The authors acknowledge the support of grants from Tarbiat Modares University.

[DOR: 20.1001.1.16807073.2012.14.7.15.7]

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مقایسه اثر پروبیوتیک، اسید آلی و داروی گیاهی در جوجه‌های چالش داده شده با کمپیلوباکتر ژژونی

ک. غریب ناصری، ش. رحیمی، و پ. خاکی

چکیده

کمپیلوباتر به عنوان یکی از عوامل اصلی اختلالات روده‌ای در انسان شناخته شده است. از آنجایی که طیور مخزن اصلی این باکتری به شمار می‌روند، کاهش این باکتری در روده آنها می‌تواند راهی برای کاهش آلودگی محصولات طیور با این باکتری باشد. در مطالعه حاضر تاثیر پروبیوتیک (پریمالاک)، گیاه دارویی (سانگروویت) و ترکیب اسید آلی (سالکو-پی‌اچ) به عنوان افزودنی‌های خوراکی بر کاهش کلنیزه شدن این باکتری در سکوم و دفع آن از روده مورد بررسی قرار گرفت. همچنین عملکرد این پرندگان، سیستم ایمنی و مورفولوژی روده آنها نیز اندازه‌گیری شده است. بدین

[DOR: 20.1001.1.16807073.2012.14.7.15.7]

منظور ۳۰۰ جوجه گوشتی (کاب ۵۰۰) به ۵ گروه تقسیم شدند. گروه‌ها شامل خوراک بدون افزودنی (گروه‌های کنترل مثبت و منفی)، پروبیوتیک، گیاه دارویی و گروه حاوی ترکیب اسید آلی در آب آشامیدنی بودند. به جز گروه کنترل منفی، در روز ۲۱ تمام جوجه‌ها با کمپیلوباکتر ژژونی ($cfu/mL۱۰^{۹}$) به صورت دهانی گاواژ شدند. نمونه‌های محتویات سکوم و مدفوع برای شمارش کمپیلوباکتر ژژونی جمع‌آوری شدند. وزن بدن، خوراک مصرفی و ضریب تبدیل به صورت هفتگی مورد ارزیابی قرار گرفت. وزن بدن و خوراک مصرفی در گروه پروبیوتیک بیش از سایر گروه‌ها بود ($P < ۰/۰۵$). در روز ۴۹ تمام گروه‌های دارای افزودنی کاهش معنی‌داری را در تعداد باکتری‌های کلنیزه شده در سکوم نشان دادند ($P < ۰/۰۵$). نمونه‌های مدفوع نیز این کاهش ($P < ۰/۰۵$) را در روزهای ۳۵ و ۴۲ نشان دادند. طول ویلی‌های دئودنوم و ژژونوم روده در گروه‌های پروبیوتیک و گیاه دارویی بهبود یافته ($P < ۰/۰۵$) و پاسخ ایمنی در این دو گروه به طور معنی‌داری بیشتر از سایر گروه‌ها بود ($P < ۰/۰۵$) که این امر می‌تواند به خاصیت ضد باکتری این مواد مربوط باشد. از مطالعه اخیر می‌توان چنین نتیجه گرفت که افزودنی‌های فوق در کاهش کلنیزه شدن کمپیلوباکتر ژژونی در روده جوجه‌های گوشتی مؤثرند. همچنین پروبیوتیک و گیاه دارویی باعث بهبود عملکرد می‌شوند.

[DOR: 20.1001.1.16807073.2012.14.7.15.7]