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Effects of *Euphorbia hirta* and Acidifiers Supplement on Resistance of Broiler Chickens against *Salmonella enteritidis* Infection: Oral Challenge Model

Research Article

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ABSTRACT

The main purpose of this study was to investigate the effects of *Euphorbia hirta* and acidifiers supplement on resistance of broiler chickens against *Salmonella enteritidis* infection. A total of 120 day-old male broiler chicks were randomly assigned to 3 dietary treatments. 1) basal diet, 2) basal diet supplemented with 2 g/kg organic acid (OA) and 3) basal diet supplemented with 7.5 g/kg *E. hirta* (EH7.5). At d 3, all chicks were inoculated by 1 mL *S. enteritidis* (1.5×10^8 cfu/bird). Cloacal swabs were taken from the inoculated chicken on d 2, 10, 17, 23 and 28 post inoculation and the chicks were killed for microbiological examination on d 7, 14 and 28 after challenge. At seven and 14 days after challenge, the number of *S. entritidis* was significantly ($P<0.05$) higher in control group than the other groups and the rate of ceca enumeration dropped significantly ($P<0.05$) after 14 days in birds fed with OA compared to the EH7.5 diet. At 10 d after challenge, EH7.5 and OA treatments were significantly reduced in *S. entritidis* shedding compared with the control group ($P<0.05$). Cloacal *S. entritidis* shedding was not observed after 17 days post challenge in OA, as well as 23 days post challenge in OA and EH7.5 groups. In conclusion, this study clearly demonstrated that feeding of both EH7.5 and OA could successfully control *S. enteritidis* infection in broilers. However, based on *S. entritidis* cecal colonization and *S. entritidis* cloacal swabs, it is inferred that OA appeared to be more effective than EH7.5 inclusion in the diet on controlling salmonelosis in broilers.

KEY WORDS broiler, *Euphorbia hirta,* herbal plant, organic acid, Salmonella.

INTRODUCTION

Food-borne diseases are still a major problem in the world, even in well-developed countries (Mead *et al.* 1999). So far, many pathogenic microorganisms, such as *Salmonella enteritidis* (SE), *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Listeria monocytogenes* and *Campylobacter jejuni* have been reported as the causal agents of foodborne diseases and/or food spoilage (Betts *et al.* 1999; Deak *et al.* 1996). *Enterobacteriaceae* of the genus *Salmonella* are an economically and biologically significant food pathogen and have been identified by the Center for disease control and prevention (CDC) as one of the four food pathogens of greatest concern. From population-based studies, CDC estimates that between 800000 and 4 million illnesses from the more than 2400 strains of *Salmonella* occur each year in the United States and between 800 and 4000 people die each year from illness caused by *Salmonella* (USGAO, 1996; Bailey and Maurer, 2001). *S. enteritidis*, *S. typhimurium* and *S. heidelberg* have been implicated in

approximately 50% of the foodborne salmonellosis outbreaks in the United States. *Salmonella enterica* serovar *Enteritidis* is the leading cause of human food-borne infections associated with the consumption of chicken eggs and meat (Kimura *et al.* 2004; Marcus *et al.* 2007). Scientific literature indicates an increase of over 85% in the number of infections caused by *S. entritidis* during the last few years from products of poultry origin (Altekruse *et al.* 2006). Since 2003, all members of the European Union have to put into practice monitoring programs to control these pathogens (European Parliament and European Council, 2003). On the other hand, the extensive use of antibiotics in animal farms has led to an imbalance of the beneficial intestinal flora and the appearance of resistant bacteria. The use of probiotics, prebiotics and acidifier in order to competitively exclude the colonization of intestinal pathogens has been projected in poultry (Hashemi *et al.* 2010; Hashemi *et al.* 2011; Pascual *et al.* 1999). Acidifiers have been used extensively in recent years and apparently have the ability to reduce Salmonella shedding in feces by changing the bowel pH. But, as the antibiotics, some bacteria strains can develop resistance against acidifiers (Heres *et al.* 2004). More recently a new group of feed additives was introduced in poultry farms based on the antibacterial *in vitro* effects such as volatile short-chain fatty acids (VSCFA; C4) (Fernández-Rubio *et al.* 2009) and phytogenic components (Cowan, 1999; Yang *et al.* 2009) on gram-negative bacteria. Furthermore, numerous reports have shown antibacterial properties of herbs and herb derivatives against *C. sporogenes* (Dorman and Deans, 2000) and other bacteria such as *E. coli*, *S. aureus* and *S. typhimurium* (Juven *et al.* 1994; Cosentino *et al.* 1999; Hashemi *et al.* 2008a; Zulkifli *et al.* 2012) and *S. entritidis* (Al-Turki, 2007). Although there are many results demonstrating *in vitro* the antibacterial activity of herbal plants overall, the available literature suggests that, at least for broilers, an overall antimicrobial potential of phytogenic compounds *in vivo* cannot generally be ruled out. Therefore more research that could prove the effectiveness of raised herbal plant and acidifiers as an alternative to antibiotics is needed to control foodborne pathogens in poultry industry. Thus the objective of the present study was to determine the effect *E. hirta* supplementation and acidifier on gut colonization and organ invasion by *S. enteritidis* in broilers.

MATERIALS AND METHODS

Chickens

A total of 120 day-old male broiler chicks (Cobb 500) from a commercial hatchery were screened and found free of Salmonella contamination. Screening was performed by randomly selecting droppings from the paper liners of the package boxes the chicks were transported in and subjecting the droppings to a XLT-4 (CM1061; XLT-4 Selective Supplement SR0237, Oxoid) culture examination.

Experimental design

Commencing from day one of age, the chicks were wing-banded and randomly assigned to 1 of 3 treatment groups. Three dietary treatments were compared:

1. Basal diet (Control)
2. Basal diet + 2 g/kg organic acid (OA)
3. Basal diet + 7.5 g/kg *E. hirta* (EH7.5)

Each treatment group contained 8 replicates with 5 birds each. Chicks were raised in battery cage with wire floor and housed in a conventional open-sided house with cyclic temperatures (minimum, 24 ˚C; maximum, 34 ˚C). The relative humidity was recorded between 75 to 90%.

The acidifier (Orgacids™) manufactured by Sunzen Corporation Sdn Bhd. Malaysia and consisted of formic, phosphoric, lactic, tartaric, citric and malic acid. *Euphorbia hirta* was collected in the Universiti Putra Malaysia surrounding areas. The plant was identified and authenticated by the Institute of Bioscience, Universiti Putra Malaysia. The whole plant was washed and dried at 50 ˚C and then ground into powder using a Wiley mill (Thomas® Wiley Cutting Mill Model 4) through a 1 mm screen and stored at 4 ˚C till further use. Birds were allowed free access to water or feed and an unmedicated corn-soybean-based mash form diet, which met or exceeded NRC requirements (NRC, 1994). The composition of the experimental basal diets is shown in Table 1. Proximate analyses confirmed formulated values for all critical nutrients in the diets that were fed. The feed was assayed for Salmonella content before the experiment following an enrichment procedure with some modifications (Barrow, 1991). Briefly, 10g of feed samples were diluted 1:10 and pre-enriched in buffered peptone water (BPW) overnight at 37 ˚C, 100 µL were then transferred to 10 mL rappaport-vassiliadis R10 (RV) (CM0669, Oxoid) enrichment broth and incubated overnight at 37 ˚C. The RV enrichment broth was analyzed for growth of *S. entritidis*on XLT-4 agar after incubation of the plates for 24 hrs at 37 ˚C. Feed and water were provided *ad libitum* and the birds were under continuous lighting.

Salmonella source for challenge and inoculation procedure

A primary poultry isolate of *Salmonella enteritidis*, bacteriophage type 13A, was obtained from the Department of Veterinary Pathology and Microbiology, Universiti Putra Malaysia. This isolate was resistant to novobiocin (NO) (SR0181, Oxoid) and nalidixic acid (NA) (N8878; Sigma-Aldrich). Inocula were prepared in Phosphate buffered saline (PBS). A bacterium was spectrophotometrically quanti-

tated to 1.5 × 108 cfu/mL in PBS and actual challenge concentration was confirmed by colony counts of dilutions spread plated on XLT-4 agar plate containing 20 µg nalidixic acid (NA) and 25 µg novobiocin (NO) to inhibit the growth of other bacteria (McElroy *et al.* 1994).

Table 1 Ingredients and main nutrient composition of basal diets

Item	Starter 1 to 21 d	Finisher 22 to 42 d
Ingredient (g/kg as-is)		
Maize	494.6	582.6
Soybean meal (480 g CP/kg)	350.1	364.3
Palm oil	60	60
Fishmeal (600 g CP/kg)	60	62
NaCl	3.0	3.0
Di-calcium phosphate	14	12
Limestone	9.0	9.0
DL-Methionine (980 g/kg)	1.5	1.5
Lysine (980 g/kg)	2.0	-
Choline-HCl (700 g/kg)	0.8	0.6
Vitamin-mineral premix[a]	5.0	5.0
Calculated nutrient composition (g/kg)		
Calculated[b]		
Dry matter	892.3	911.2
Crude protein	228.0	200.0
Metabolizable energy (kcal/kg)	3103	3205
Lysine	16.0	12.3
Methionine + cysteine	9.0	7.0
Calcium	9.7	9.2
Available phosphorus	5.0	4.6

[a] Supplied per kilogram of diet: vitamin A: 1500 IU; Cholecalciferol: 200 IU; vitamin E: 10 IU; Riboflavin: 3.5 mg; Pantothenic acid: 10 mg; Niacin: 30 mg; Cobalamin: 10 µg; Choline chloride: 1000 mg; Biotin: 0.15 mg; Folic acid: 0.5 mg; Thiamine: 1.5 mg; Pyridoxine: 3.0 mg; Iron: 80 mg; Zinc: 40 mg; Manganese: 60 mg; Iodine: 0.18 mg; Copper: 8 mg and Selenium: 0.15 mg.
[b] Based on NRC (1994) feed composition table.
CP: crude protein.

At d3, all chicks were inoculated by 1mL *S. enteritidis* (1.5×10^8cfu/bird) with a curved and blunted needle at the pharyngeal side of the tongue. All chicks were observed at least once daily throughout the study and any clinical abnormalities noted. Any dead or moribund birds were removed.

S. entritidis shedding

In order to check *S. entritidis* shedding after inoculation, cloacal swabs were taken from the inoculated birdson d2, 10, 17, 23 and 28 post-inoculation. Swabs were directly enriched in RV at 41 ˚C for 24 hrs, mixed thoroughly and plated onXLT-4 agar for 24 hrs at 37 ˚C to screen positive *S. entritidis* samples.

Sampling protocol for *S. entritidis* organ invasion and *S. entritidis* concentration in cecal contents

The chicks were killed for microbiological examination on d7, 14 and 28 after challenge. On each occasion, 10 challenged birds were chosen (2 birds/cage) at random from each of the eight test groups and killed by cervical dislocation. From each bird entire crop, liver, gall bladder, spleen and apex of heart were removed aseptically and transferred to RV enrichment broth for organ invasion and at the same time, the ceca (one of the horns) from each bird were collected into sterile pill tube. Care was taken to avoid cross-contamination of samples and non-disposable post-mortem instruments used for removing organs were immersed in ethanol and flame sterilized after each bird.

Briefly, for organ invasion, crop, liver, gall bladder, spleen, and apex of heart were enriched in RV enrichment broth for 24 hrs at 41 ˚C. Following enrichment, the broth was streaked on XLT-4 agar plate containing 25 µg/mL NO and 20 µg/mL NA, incubated for an additional 24 hrs at 37 ˚C and examined for the presence or absent of *S. entritidis* colonies. Typical black or black-centered with a yellow periphery colonies developing on the plates were recorded as *Salmonella spp*.

Additionally, for enumeration of total colony-forming units of Salmonella in ceca, a 0.25 g of cecal contents was placed into sterile tube containing 2.25 mL of buffered peptone water (BPW) (CM0509, Oxoid). Serial dilutions of each sample were performed using 1 mL of the sample and placed into 9 mL of BPW for final concentrations of 10, 100, 1000 cfu/mL. One hundred microliters from each dilution tube was placed onto a XLT-4 plate and spread plated with a bacterial cell spreader. All of the plates were incubated for 24 hrs at 37 ˚C and determined the numbers of Salmonella colony-forming units and expressed as $\log_{10}$ Salmonella per gram of cecal content (Corrier *et al.* 1993; Corrier *et al.* 1995). Cecal contents in which *S. entritidis* were not detected at a 10-fold dilution on XLT-4 plates or after RV enrichment broth and XLT-4 plating for 24hrs at 37 ˚C were scored as 0 cfu. Cecal contents that were negative a 10-fold dilution on XLT-4 plates but were positive after RV enrichment and XLT-4 plating were arbitrarily assigned log 0.95 cfu of *S. entritidis* per gram of cecal contents. Suspect colonies were confirmed by biochemical tests on triple sugar-iron agar and lysine-iron agar and further identified as *S. entritidis* serologically using Salmonella O antiserum group D, factors 1, 9 and 12.

Statistical analysis

A completely randomized design (CRD) with 3 treatments and 8 replicates and 5 birds per replicate was employed. Statistical analyses were performed using the procedure inthe SAS statistical package (SAS, 2005). Chi-squared analysis was used to determine significant differences among treatment groups for incidences of *S. entritidis* colonization of the crop, liver, spleen, gall bladder and heart apex (Woodward *et al.* 2005). Salmonella colony-forming units were logarithmically transformed prior to analysis to

achieve homogeneity of variance and were expressed as $\log_{10}$ colony-forming units. Log colony-forming units of *S. entritidis* count among treatment groups were determined by ANOVA using the GLM procedures. Significant differences were further separated using Duncan's multiple range test (Duncan, 1995). Statistical significance was considered at $P \leq 0.05$.

RESULTS AND DISCUSSION

Cecal colonization by *S. entritidis*

Enumeration of *S. entritidis* colonization of the ceca is shown in Table 2.

Table 2 The effect of *Euphorbia hirta* (EH) and acidifier (OA) supplementation on *Salmonella enterica* serovar *Enteritidis* cecal colonization of broiler (Mean±SEM)[1,2]

Treatments	Days after challenge		
	7 days	14 days	28 days
Control	2.12±0.07[a]	1.64±0.13[a]	0.00
EH7.5	1.68±0.12[b]	0.95[b]	0.00
OA	1.56±0.10[b]	0.00[c]	0.00

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
[1] Values represent the mean of 8 broilers per treatment.
[2] Broilers were administered by crop gavage with 1.5×10^8 cfu of *S. enteritidis* at 3 days of age.

Seven days after challenge, the number of *S. entritidis* was significantly higher in control group than the other groups. A significant decrease in *S. entritidis* enumeration from birds receiving OA and EH7.5 was observed. There were no differences between OA and EH7.5 treatments in ceca enumeration. At 14 days after challenge, significantly higher number of *S. entritidis* colony-forming units per gram was observed in control treatment. The rate of ceca enumeration dropped significantly after 14 days in OA compared to the EH7.5 treatments. Irrespective of treatment, on 28 days of age *S. entritidis* was not detected.

Organ colonization by *S. entritidis*

The results of *S. entritidis* colonization in the crop, liver, spleen, gall bladder and heart apex are shown in Table 3. Seven days after challenge, the liver of *S. entritidis* positive birds fed with OA exhibited significant reduction compared with the EH7.5 and control groups. Reduction in liver *S. entritidis* incidence was 50% compared with the EH7.5 and control groups. The use of OA decreased *S. entritidis* colonization in spleen compared to the incidence level in EH7.5 and control groups ($P<0.05$). There was no significant difference between EH7.5 and control groups in spleen *S. entritidis* colonization. There were no significant differences among treatment groups for crop, gall bladder and heart apex *S. entritidis* colonization. There were no differences in organ *S. entritidis* colonization among treatments following 14 days of challenge.

At 28 days of age *S. entritidis* was not detected in crop, liver, bile bladder and heart apex between treatments.

Cloacal swabs *S. entritidis* enumeration

The cloacal swabs taken before inoculation were free of *S. entritidis* in all groups. The results of *S. entritidis* shedding after inoculation are shown in Table 4. All chicks infected on d3 were positive for *S. entritidis* at 2 days after challenge. Cloacal shedding of *S. entritidis* was affected by treatments at 10 d after challenge. *S. entritidis* shedding in EH7.5 and OA were significantly reduced compared with the control group. There were no significant differences between EH7.5 and OA groups.

On 17 d after challenge, the highest contamination rate was observed in control group and there were no significant differences between EH7.5 and OA groups. Cloacal *S. entritidis* shedding was not observed after 17 days post challenge in OA, as well as 23 days post challenge in OA and EH7.5 groups. Generally, the average time of shedding is longer in the control group than in the OA and EH7.5 treatments throughout the duration of the experiment.

The present results clearly suggest that feeding EH7.5 to broilers resulted in a decrease *S. enteritidis* colonization and this finding is in coincidence with the observation of Zulkifli *et al.* (2012). The antimicrobial effect of *E. hirta* has been well documented by Vijaya *et al.* (1995) and Oglubie *et al.* (2007). The antibacterial properties of *E. hirta* could be associated with phytochemical compounds like alkaloids, tannins, saponins and steroid (Hashemi *et al.* 2008b). Phytochemicals exert their antimicrobial activity through different mechanisms, tannins for example act by iron deprivation, hydrogen bounding or non specific interactions with vital proteins such as enzymes (Scalbert, 1991). Chung *et al.* (1993) showed that tannic acid inhibits the growth of intestinal bacteria such as *Bacteroides fragilis*, *Clostridium perfringens*, *E. coli* and *Enterobacter cloacae*. Sawer *et al.* (2005) demonstrated that the main indoloquinoline alkaloid, cryptolepine, causes cell lysis and morphologicalchanges of *S. aureus*, but the antimicrobial effects of the alkaloid may be through another mechanism, since the compound is known to be a DNA intercalator and an inhibitor of DNA synthesis through topoisomerase inhibition (Karou *et al.* 2006). Alkaloids such as berberine and harmane are effective against a broad range of bacteria and attributed to its ability to intercalate with DNA and suppress DNA and protein synthesis in microorganism cells (Phillipson and O'Neill, 1987; Wang *et al.* 2009). Saponins are a major family of secondary metabolites that occur in a wide range of plant species. The main mechanism by which saponins display an antimicrobial activity is based on their ability to form complexes with sterols present in the membrane of microorganisms.

Table 3 The effect of *Euphorbia hirta* (EH) and acidifier (OA) supplementation on *Salmonella enterica* serovar Enteritidis (SE) colonization of the crop, liver, spleen, bile bladder and heart apex of broilers[1,2]

Treatments	SE culture-positive %									
	Crop		Liver		Spleen		Bile bladder		Heart apex	
7 days after challenge										
Control	(3/8) 37.5%		(7/8)^{a} 87.5%		(5/8)^{ab} 62.5%		(0/8)	0%	(0/8)	0%
EH7.5	(0/8)	0%	(7/8)^{a} 87.5%		(6/8)^{a} 75%		(1/8) 12.5%		(0/8)	0%
OA	(0/8)	0%	(3/8)^{b} 37.5%		(2/8)^{b}	25%	(1/8) 12.5%		(0/8)	0%
14 days after challenge										
Control	(6/8) 75%		(4/8) 50%		(4/8) 50%		(1/8) 12.5%		(0/8)	0%
EH7.5	(6/8) 75%		(3/8) 37.5%		(5/8) 62.5%		(2/8)	25%	(0/8)	0%
OA	(5/8) 62.5%		(5/8) 62.5%		(5/8) 62.5%		(0/8)	0%	(0/8)	0%
28 days after challenge										
Control	(0/8)	0%	(0/8)	0%	(2/8) 25%		(0/8)	0%	(0/8)	0%
EH7.5	(0/8)	0%	(0/8)	0%	(0/8)	0%	(0/8)	0%	(0/8)	0%
OA	(0/8)	0%	(0/8)	0%	(0/8) 0%		(0/8)	0%	(0/8)	0%

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
[1] Values represent the mean of 8 broilers per treatment.
[2] Broilers were administered by crop gavage with 1.5 ×10^{8} cfu of *S. enteritidis* at 3 days of age.

Table 4 The effect of *Euphorbia hirta* (EH) and acidifier (OA) supplementation on *Salmonella enterica* serovar *Enteritidis* (SE) cloacal swab in broilers[1]

Treatments	Days after challenge				
	Days 2	Days 10	Days 17	Days 23	Days 28
Control	(40/40) 100%	(22/32)^{a} 68.75%	(7/24)^{a} 29.16%	(5/24)^{a} 20.83%	(0/16) 0%
EH7.5	(40/40) 100%	(12/32)^{b} 37.5%	(4/24)^{ab} 16.16%	(0/24)^{b} 0%	(0/16) 0%
OA	(40/40) 100%	(8/32)^{b} 25%	(0/24)^{b} 0%	(0/24)^{b} 0%	(0/16) 0%

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
[1] Broilers were administered by crop gavage with 1.5 ×10^{8} cfu of *S. enteritidis* at 3 days of age.

This causes damages in the membrane and the consequent collapse of cells (Morrissey and Osbourn, 1999). Findings also suggested that EH7.5 may lower the pH of crop, gizzard, jejunum, ileum and caecum (Zulkifli *et al.* 2012). According to IMPGC (Indian Medicinal Plants Growers Consortium), PROTA (Plant Plant Resources of Tropical Africa) and Adedapo *et al.* (2005), *E. hirta* consist of several organic acid such ellagic, gallic, tannic, maleic, tartaric and coumaric acid. The pH level in specific areas of the gastrointestinal tract is a factor which establishes a specific microbial population. Most of the pathogens grow in a pH close to 7 or slightly higher. In contrast, beneficial microorganisms live in an acidic pH and compete with pathogens (Ferd, 1974). At low pH, more of the organic acids will be in the undissociated form and are lipophilic. They can diffuse across cell membranes, including those of bacteria and molds (Partanen, 1999). Once in the bacterial cell, the higher pH of the cell contents will disrupt enzymatic reactions and nutrient transport systems (Cherrington *et al.* 1991).

In addition, the process of transporting the free proton out of the cell requires energy, which will contribute to reduced energy availability for proliferation, resulting some degree of bacterostatic. Antimicrobial action in the crop is an important part of EH7.5 benefit, because the crop is a major site of colonization for *E. coli* and Salmonella (Ewing *et al.* 1994).

It is also critical for the antimicrobial action to persist into the lower gut where many of the anaerobic opportunistic pathogens are found. Lower microbial profileration in the ileum is also important because it reduces the competition of the microflora with the host for endogenous nitrogen lost into the gut lumen by pancreatic and gut epithelial secretions and by enterocyte attrition and shedding. It was demonstrated that irrespective of dosage, feeding of *E. hirta* increased ileal Lactobacillus population in broiler chickens (Zulkifli *et al.* 2012). Russell and Diez-Gonzalez (1998) indicated that lactic acid bacteria are able to grow at relatively lower pH more than other bacterial species, such as *E. coli* and Salmonella.

Competitive exclusion is one of the main mechanism by which Lactobacilli prevent colonization of the intestine by pathogen such as Salmonella and *E.coli. Lactobacillus animalis* has been demonstrated to inhibit growth, *in vitro* adhesion of various Salmonella strains as well as the production of antimicrobial substances (Kankaanpaa *et al*. 2004). Inhibition of adherence of enterotoxigenic *E. coli*, enteropathogenic *E. coli* and *S. typhimurium* to intestinal enterocytes, by strains of Bifidobacteria and Lactobacillus has been shown (Briandet *et al*. 1999).

Furthermore, Lactobacilli have also been widely reported to produce antibacterial compounds called bacteriocins. Higgins *et al.* (2008) hypothesized that bacterocins to be the mechanism by which Lactobacilli exert cytotoxic affects *in vivo*.

Individual acids and blends of several organic acids have been commonly used in swine diets as alternatives to antibiotics with more consistent results than those of poultry (Dibner and Buttin, 2002). Byrd *et al.* (2001); Boling-Frankenbach *et al.* (2001); Jarquin *et al.* (2007) and Fernández-Rubio *et al.* (2009) reported that OA supplementation significantly lowered gastrointestinal tract pH and reduced Salmonella colonization and fecal shedding. The finding of reduction in colonization of Salmonellais also observed in present study. Van Immerseel *et al.* (2006) reviewed the mode of action of organic acids in controlling Salmonella in poultry and indicated that medium-chain fatty acids are more antibacterial against Salmonella than short-chain fatty acids.

Further they concluded that if diets can be designed to stimulate organic acid production in caecum, it may be possible to control *Salmonella spp* via even easier and more cost-effective measures, compared with addition of acids to feed or drinking water. In the present study it is interesting to note that OA supplementation appears to be more effective than EH7.5 in controlling *S. enteritidis* in broiler chickens. This result is also consistent with that of Zulkifli *et al.* (2012).

CONCLUSION

This study clearly demonstrated that feeding of both EH7.5 and OA could successfully control *S. enteritidis* infection in broilers However, based on *S. entritidis* cecal colonization and *S. entritidis* cloacal swabs, it is concluded that OA appeared to be more effective than EH7.5 inclusion in the diet on controlling Salmonelosisin broilers.

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